

Assessment of the Effect of Dietary Modifications and Bioenteric Intra-gastric Balloon Treatment on the Changes of Some Morphological and Biochemical Parameters in Obese Patients

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Keywords

Obesity · Metabolic diseases · Diet · Bioflavonoids · EFA-3 · Probiotics

Abstract

Obesity is the most common disease of affluence of the XXI century. According to WHO (World Health Organization), it is defined as a chronic metabolic disorder manifested by excessive accumulation of adipose tissue with high tendency for familial occurrence. According to WHO, obesity reaches epidemic proportions in many countries. High BMI (Body Mass Index) correlates with coexisting diseases. Traditional dietetic treatment often does not bring any results. A form of conservative (non-surgical) support for patients in fighting with obesity is the reduction of stomach volume by bioenteric intra-gastric balloon (BIB) treatment. The aim of the work was to develop a diet with anti-inflammatory properties, well-tolerated by the patients after BIB treatment. An American diet was modified by changing the composition of fatty acids, increasing anti-oxidative potential and adding synbiotics for patients treated with BIB. Chemical analysis of reconstructed food ratios of recommended diet was per-

formed, analysing the content of micronutrients, composition of fatty acids, antioxidative capacity, reducing power and the content of polyphenols. Improvement in anthropometric measurement results and satisfying body weight loss were obtained, while preserving fat-free body mass. Improvement in the parameters of lipid metabolism was also observed, that is, decrease in total CH (cholesterol) and TG (triglycerides), and normalized concentration of HDL (high density lipoproteins) and LDL (low density lipoproteins) fractions. Reduced concentration of glucose in blood and lower blood pressure was also noted. Performed study confirms the effectiveness of complex treatment with BIB and properly adjusted individualized diet. Observations and own experience allow to deduce that patients who resign from systematic contact with a dietician cannot maintain reduced body weight. Abandoning previous habits is the only way to maintain the effect of weight loss. Most importantly, the change in patients' awareness and consequent behaviour in the future are crucial. Even though genes may contribute to obesity, environmental factors mainly determine the possibility of the disease to occur. Therefore, the change of patients' lifestyle after body weight reduction will decide on their fate.

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Introduction

Obesity is the most common disease of affluence of the XXI century. According to WHO, it reaches epidemic proportions in many countries. In studies concerning Europeans it was shown that it is most common among the residents of Malta (60% of obese), whereas it is least common in France (44% of the population) [1]. Obesity is regarded as a health problem affecting not only adults but also children and adolescents [2].

Excessive amount of fat tissue is not only an aesthetic problem. The patients were also diagnosed with type 2 diabetes, coronary artery disease, hypertension, osteoarthritis, digestive tract diseases (non-alcoholic fatty liver disease, cholelithiasis), obstructive sleep apnoea, arteriosclerotic vascular disease, hormonal disorders, stroke, and even some types of cancer, including not only those localized in the digestive tract but also in liver, breast, endometrium and urinary system [3]. Psychological problems may lower the quality of life leading to disturbed social and family relations [4]. Economical problem may also be important.

A type of conservative (non-surgical) support for patients in fighting with obesity is the reduction of stomach volume by bioenteric intragastric balloon (BIB) treatment. Bariatric procedures are linked to risks of micro- and macro-nutrient deficiencies. In Poland, American dietetic recommendations are applied for patients after BIB treatment [5, 6].

BIB is a very effective, non-invasive and the safest method among all bariatric procedures, as it is fully reversible. It is, however, linked to the risk of micro- and macro-nutrients deficiencies. In Poland, there have not been any dietetic recommendations developed so far for treating the patients after BIB. The aim of the work was to compose an appropriate diet with anti-inflammatory properties through the modification of the composition of fatty acids and increasing the content of synbiotics, bioflavonoids, vitamins A, E, C and folic acid, for patients treated with BIB.

Materials and Methods

Test Group

The study involved the patients treated for 2 years in Medical Center Sonomed in Szczecin. Initially, a group of 150 obese people with accompanying diseases was included in the analyses. Due to the lack of contact and cooperation with some patients, the final group comprised of 90 people. All the patients were additionally diagnosed with type 2 diabetes, dyslipidaemia and hypertension.

The observation period lasted 6–8 months. All the analyses were performed twice – at the beginning and at the end of the observation. The project received a positive opinion of the Bioethical Committee of the District Chamber of Physicians in Szczecin OIL-Sz/Mf/KB/452/06/05/2015.

The patients were qualified for the bariatric procedure during the initial visit, after the consultation with the attending doctor. The average age was 38 ± 8.1 for women and 36 ± 9.8 for men. The average BMI was 35.25 ± 5.46 kg/m² for women and 37.34 ± 8.2 kg/m² for men. All the patients had a history of unsuccessful trials of body weight loss with typical methods, that is, diet. A dietician and a psychologist's care were provided to all the patients.

Anthropometrical Measurements

Obese patients were subjected to body composition analysis during the initial visit. Anthropometrical measurements were performed with body composition analyser based on bioelectrical impedance analysis using tetra-polar technique with 8 electrodes. The IOI 353 analyser is certified (CEO 123) and meets the MDD 93/42/EEC directives for medical devices. The measurements were taken twice – before and after the procedure of the balloon removal after 6–8 months.

BIB Procedure

The patients were qualified according to the recommendations of the American Gastroenterological Association and the European Association for Endoscopic Surgery. Patients were prepared for the BIB treatment with ORBERA™ system using the standard gastroscopy protocol, after the endoscopic analysis of oesophagus and stomach. ORBERA™ system is dedicated to support body mass loss through the induction of satiety centre by partial filling of the stomach. The balloon of ORBERA™ system is placed in a stomach and filled with saline, which inflates it to a ball. The filled balloon reduces stomach volume. Inflatable construction of ORBERA™ balloon makes it possible to regulate its volume from 400 to 700 cm³ when it is introduced. The system is used together with the diet supervised by a dietician and with a lifestyle changing program, which aims at increasing the chances of long-time maintenance of reduced body weight.

Blood Pressure Measurement

Blood pressure was measured on an outpatient basis by a nurse using Korotkoff's method. Blood pressure measurement is the most commonly used technique for hypertension diagnosis and treatment. The patients with recognised hypertension registered their measurements every day, which were available for viewing during subsequent visits.

Blood Collection

The blood was collected in laboratory conditions from all the patients just below the elbow crease before BIB procedure and again on the day of balloon removal. The blood for further analytic tests was collected by a nurse. Some tests were performed on whole blood. The remaining material was centrifuged at 2,000 rpm for 5 min at 4 °C to separate serum from blood cells, and then the samples were frozen at –20° until further analyses.

Division of the test group and characteristics of the diet
The patients were divided into 3 groups:

Table 1. Qualitative analysis of 100 g of daily diet of patients from group III

Protein, %		Fat, %		Carbohydrates, %		Ash, %		Water, %	
mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
17	0.42	8.4	0.16	14	0.4	0.8	0.01	59.8	0.6

Group I – control group was composed of 30 patients not adhering to dietary requirements;

Group II was composed of 30 patients adhering to dietary recommendations of American specialists from the University of Nevada School of Medicine;

Group III was composed of 30 patients adhering to authors' modifications of American recommendations. The diet contained increased amounts of bioflavonoids, vitamins A, E and C and mainly n-3 EFAs. The composition of the diet was developed to increase the anti-inflammatory effect. Moreover, the patients from this group consumed once a day, every second month during the study, a synbiotic composed of: probiotics – *Bifidobacterium lactis*, *Lactobacillus acidophilus*, *Lactobacillus paracasei*, *Lactobacillus plantarum*, *Lactobacillus salivarius* and *Lactobacillus lactis*, and prebiotics – fructooligosaccharides and inulin. The number of live lactic acid bacteria in the preparation was counted as CFU/g by plating the probiotics on solid medium. The total number of colony forming units (CFU) was 1×10^9 /g.

Patient's Treatment

After the procedure, all the patients were hydrated intravenously with physiological multi-electrolyte isotonic fluid PWE with osmolarity 295 mOsmol/L. During the first 5–7 days, due to the bad tolerance of the balloon resulting in nausea and stomach pains (reported in 90% of the patients), the recommended oral consumption of fluids was reduced to 60 mL at a time, at the frequency preventing from dehydration, to 1,500 mL per day; thus for 5–7 days the diet was only liquid. It was recommended to slowly drink still water, broth, natural yoghurts, cow's milk, soy milk, fruit and vegetable juices and stewed fruit drinks. During those days, the stomach mucous membrane adapted to the presence of foreign body of the balloon. This period varied in length, but it did not last longer than a few days and depended on the condition of the patient and lasted until vomiting stopped.

The next stage of the diet was the introduction of semi-liquid diet. It was a transition period and lasted for a short time – ca. 3–5 days. The patients were recommended to consume mild cream soups with mixed lean cooked meat (lean poultry or fish). At this stage cooked and mixed root vegetables were also suggested. Additionally, yoghurts, puddings, jellies, jelly desserts and stewed fruits drinks without fruit peels were introduced. The volume of one meal should not exceed 150 mL.

It is known that long lasting low consumption of protein leads to reduced concentration of albumins, decreased muscle mass, fatigue, bad state of hair and nails and anaemia. No such symptoms were observed in patients after the BIB procedure was performed because the period of using diets with changed consistency was shorter than that in case of invasive procedures.

Next, during ca. 3 weeks, the introduced diet had mushy consistency. Initial diet energy load was ca. 400 kcal, thus it did not

deliver all nutrients in sufficient amounts. The intake of proteins during that period was 60–80 g/day. One meal had an average volume of 200 mL. The sources of protein were eggs, low fat dairy products, fish and poultry. The amount of fluids per day was ca. 2,000 mL.

In subsequent weeks, the meals of 200 mL changed their consistency. They were not mixed but slowly and carefully chewed. The food was cooked, steamed, stewed or roasted without fat. The meals fulfilled the recommendations of easily digested diet. The intake of additional liquids was suggested 30 min before or 1 h after a meal. Daily diet included 3 main meals, containing complete protein at the amount of ca. 85 g/day (Table 1), and 3 additional meals, composed of cocktails or mousses based mainly on vegetables that is spinach, kale, broccoli, cauliflowers, beetroots, carrots or pumpkins, supplemented with smaller amounts of apples, raspberries, chokeberries, berries, cherries and blackberries, and outside the season – frozen fruits or oranges, pineapples, lemons, pomegranates or grapefruits. Cocktails were enriched with parsley leaves and ginger due to anti-inflammatory and anti-vomiting effect (ginger contains gingerol). Moreover, it is known that ginger has a hyperglycaemic, hypolipemic, anti-oxidative and anti-aggregative activity [7–10]. Many of the cocktails were based on fermented milk drinks. Daily food ratio did not exceed 1,000 kcal. The amount of carbohydrates in the diet was limited to 70 g/day (Table 1).

Monosaccharides and saturated fats were eliminated. Higher amounts of dietary fibre were badly tolerated by many patients, and thus it was recommended not to exceed 30 g/day. Proposed diet differed from the recommendations of American specialists from the University of Nevada School of Medicine (Kulick et al. [5]) mainly by the content of antioxidants, synbiotics and n-3 acids (Table 2, 3).

According to the European and American guidelines, from the 3rd day after an invasive bariatric surgery, the daily intake of the following supplements is recommended:

- A set of various minerals and vitamins: 1–2 pills/day;
- Calcium preparation (citrate preferably): 1,200–2,000 mg/day with vitamin D₃: 400–800 µg/day;
- Iron preparation containing 150–200 mg elementary iron per day (iron sulphate/fumarate/gluconate);
- Folic acid 400 µg/day (usually is included in multivitamin preparations);
- Vitamin B₁₂ in form of pills 1,000 µg/day or intramuscularly 1,000 µg/month or 3,000 µg/6 months [6].

In authors' diet prepared for the patients after BIB procedure (endoscopic, non-invasive) there were no reasons to recommend vitamin and mineral supplementation (Table 1, 4, 5). All the patients were examined with respect to their nutritional status before the BIB procedure to exclude the risk of vitamins' and bioelements' deficiencies.

Table 2. Composition and contents of fatty acids in food ratios (%)

Systematic name of fatty acid	Lipid numbers	Fatty acid content, %	SD
Tetradecanoic	C14:0	2.862	0.09
Pentadecanoic	C15:0	0.163	0.02
Hexadecanoic	C16:0	19.986	0.15
	C16:1	4.339	0.11
Octadecanoic	C18:0	3.94	0.09
	C18:1	38.156	0.21
Octadecadienoic	C18:2 n-6	15.63	0.18
	C18:3 n-3	6.65	0.12
Octatetraenoic	C18:4 n-3	0.777	0.02
Docosanoic	C22:1	2.382	0.08
Eicosatetraenoic	C20:4 n-6	2.571	0.03
	C20:4 n-3	0.10	0.01
Eicosapentaenoic	C20:5 n-3	1.10	0.04
Tetracosanoic	C24:1	0.101	0.01
Docosapentaenoic	C22:5 n-3	0.20	0.03
Docosahexaenoic	C22:6 n-3	1.05	0.07
	SFA	26.95	
	MUFA	44.98	
	n-3 PUFA	9.87	
	n-6 PUFA	18.20	
	(n-6)/(n-3) PUFA	1.84	
Fat content		8.4	0.16

Data in rows marked with the same letter does not differ significantly, Tukey's test, $p \leq 0.05$.

Assessment of Nutritional Value of Author's Diets

To assess the correctness of the composition of recommended authors' diet, the content of the following compounds were analysed on reconstructed food ratios (Table 1): water, protein, fat, carbohydrates and ash. The composition of solid meals was also analysed – those containing quark, avocado, linseed oil, fish, jellied turkey, eggs and bread.

Water content was measured according to AOAC 2004, (926.06). Humidity (%) was analysed using type EB53/E2 dryer (WTB Binder, Germany). The analyses were carried out at a temperature of 105 °C, drying until stable weight of the sample was obtained. Weight of a sample was measured before and after the drying (scales WPA/180/C "RADWAG", Radom) with 0.0001 g accuracy. Total ash content was determined according to AACC 2004 (920.153), by heating crumbled 3 g samples in muffle furnace FCF 7SHM ("Czyłok", Jastrzębie Zdrój) at the temperature of 900 ± 10 °C. Total nitrogen content was analysed with the Kjeldahl method according to AOAC 2004, (46-08, N × 5.75). The analysis was performed using Kjeltac System 1026 ("Tecator", Sweden). The weight of the heated sample was 0.5 g. The digestion was performed in glass flasks, in the presence of sulphuric acid (10 mL) with the addition of selenite mixture, in Digestion System 6 1007 Digester ("Tecator"), at a temperature of 365 °C until obtaining a clear solution.

Total fat content was determined with the Soxhlet method according to AOAC 2004, (32.2). The analysis was performed using

the extraction method on Soxhlet apparatus, which was based on extracting the fat in a dried sample with naphthyl ether, removal of the solvent residues through evaporation and drying and weight determination of fat content.

The analysis of peroxide value was performed according to BN-74 8020-07. The method is based on the oxidation of ferrous ions to ferric ions by peroxides present in rancid fat, and colorimetric measurement of the intensity of colour of compound ions formed by ferric ions in the presence of ammonium thiocyanate.

Anisidine value and total oxidation value (Totox) of fat were determined according to PN-93 A-86926 (Table 6).

Moreover, in food ratios, the composition of fatty acids was determined (Table 2) according to AOAC 2004, (Aa 9-86). Methyl esters of fatty acids were analysed with gas chromatography (AOAC 991.39) using gas chromatography-mass spectrometry. The conditions of the analysis: column SP™ -2560, 100 × 0.25 cm³/min, Split 1:50, injector temperature 220 °C; oven temperature: initial 140 °C, temperature growth from 140 to 240 °C/4 min, final temperature 240 °C/10 min.

Sensory Evaluation of Oils

A 5-point sensory analysis was performed according to PN-ISO 4121:1998 in case of proposed diet supplements rich in n-3 EFAs, that is, Budwig unrefined linseed oil ("LenVitol" Olfarm Sp.z.o.o., Pietrzykowice), evening primrose oil ("Wiesiołek" Olfarm Sp.z.o.o., Pietrzykowice) and Icelandic cod-liver oil ("LY-SIHF" Reykjavik, Iceland), before they were administered to patients, in order to select appropriate oil source. The discriminants assessed during the evaluation were colour, taste and smell. The evaluation was carried out in a sensory laboratory at the West Pomeranian University of Technology in Szczecin. Sensory profile analysis of taste and colour was performed according to PN-ISO 11036:1999, PN-ISO 6658:1998 and PN-ISO 6564:1999. Sensory evaluation was made by a panel of 10 assessors trained in oil quality analysis.

The following rating scale was used:

- 5 points – very good,
- 4 points – good,
- 3 points – satisfactory,
- 2 points – barely satisfactory,
- 1 point – unsatisfactory.

Total assessment for the samples was calculated on the basis of the average score obtained for analysed determinants (Table 7).

Analysis of Antioxidative Properties

It was assumed that appropriate composition of bioactive compounds will give the diet exceptional antioxidative properties. Reproduced meals and cocktails were mixed in a multi-functional device Thermomix TM 5 at 10,700 rpm. Obtained homogenous mass was diluted 20 times with distilled water, shaken for 1 min and then filtered. Such obtained filtrates were the samples for the analyses of antioxidative properties.

Antioxidant capacity Trolox Equivalent Antioxidant Capacity (TEAC) according to Re et al. [11]. The analysis of antioxidative activity of drinks with TEAC method was based on the spectrophotometric measurement of the decolorization of ABTS (2,2'-azino-bis[3-ethylbenzothiazoline-6-sulphonic acid]) radicals by antioxidants contained in the sample, at wavelength of 734 nm. The results are presented in mMTE per 100 g of a meal or per 100 mL of a cocktail.

Table 3. Antioxidative capacity (TEAC), reducing power (FRAP) and total polyphenols content in cocktails and meals

Sample	TEAC		FRAP		Total polyphenols	
	mean, $\mu\text{M TE}/100\text{ g or }100\text{ mL}$	SD	mean, $\mu\text{M TE}/100\text{ g or }100\text{ mL}$	SD	mean, mg catechin/ 100 g or 100 mL	SD
1	480.02	13.12	195.23	10.12	120.51	3.47
2	969.21	58.21	737.41	66.43	250.51	18.97
3	611.13	24.34	452.21	44.21	159.49	2.22
4	433.22	24.18	78.50	2.23	80.00	3.35

Table 4. Analysis of body composition using bioelectrical impedance (mean \pm SD)**a.**

	Metabolic age		Mass of body fat, kg		Visceral fat area, cm^2		Body mass index, kg/m^2	
	before	after	before	after	before	after	before	after
Group 1	43.2 \pm 7.16	42.6 \pm 6.96	40.7 \pm 8.53	37.9 \pm 8.77	144.3 \pm 46.02	135.4 \pm 46.86	35.7 \pm 5.44	34.6 \pm 5.52
Group 2	43.8 \pm 10.54	41.4 \pm 10	38.7 \pm 9.9	26.6 \pm 11.78	172 \pm 55.31	146.6 \pm 50.57	35.5 \pm 5.85	32.2 \pm 6.02
Group 3	43.4 \pm 7.4	38.3 \pm 6.77	40.0 \pm 16.78	23.5 \pm 9.51	185.5 \pm 98.44	97.6 \pm 41.1	36.7 \pm 7.19	28.3 \pm 5.38

b.

	Waist to hip ratio		Minerals in body, kg		Proteins in body, kg		Total body water, kg	
	before	after	before	after	before	after	before	after
Group 1	0.9 \pm 0.06	0.9 \pm 0.06	5.8 \pm 1.09	4.4 \pm 1.22	11.6 \pm 2.63	9.4 \pm 3.15	44.8 \pm 9.37	42.9 \pm 9.69
Group 2	0.96 \pm 0.06	0.90 \pm 0.06	5.7 \pm 0.74	4.6 \pm 0.66	11.7 \pm 1.81	9.5 \pm 0.66	44.8 \pm 5.75	41.5 \pm 7.15
Group 3	0.98 \pm 0.11	0.82 \pm 0.07	6.1 \pm 1.57	5.3 \pm 1.31	12.8 \pm 3.39	11.8 \pm 2.75	49.0 \pm 12.54	43.2 \pm 12.11

Table 5. Morphology and biochemical analysis of blood

Patients parameter	Group I		Group II		Group III	
	before	after	before	after	before	after
HGB, g/dL	13.55 \pm 0.98	12.24 \pm 1.21	13.78 \pm 0.17	12.78 \pm 0.24	14.44 \pm 0.26	14.16 \pm 0.21
Iron, $\mu\text{g}/\text{dL}$	83.5 \pm 5.68	81.18 \pm 5.43	91.85 \pm 1.42	88.13 \pm 1.45	75.36 \pm 3.94	75.75 \pm 3.41
Sodium, mmol/L	144.63 \pm 2.88	142.13 \pm 2.36	155.13 \pm 12.12	151.13 \pm 12.92	136.13 \pm 6.92	132.25 \pm 4.8
Potassium, mmol/L	3.78 \pm 0.13	3.56 \pm 0.35	3.72 \pm 0.13	3.55 \pm 0.14	3.4 \pm 0.4	3.9 \pm 0.39
Calcium, mmol/L	2.35 \pm 0.06	2.15 \pm 0.07	2.17 \pm 0.16	2.01 \pm 0.18	2.33 \pm 0.3	2.15 \pm 0.28
Copper, $\mu\text{g}/\text{dL}$	79.00 \pm 1.6	76.03 \pm 0.28	71.11 \pm 1.75	59.05 \pm 3.43	72.5 \pm 3.42	72.88 \pm 2.3
Zinc, $\mu\text{g}/\text{dL}$	70 \pm 1.51	65.69 \pm 2.55	71.63 \pm 7.71	60.13 \pm 1.25	72.38 \pm 2.92	77.75 \pm 4.2
Vitamin B ₁ , $\mu\text{g}/\text{L}$	56.13 \pm 1.36	49.13 \pm 2.03	49.63 \pm 2.45	44.5 \pm 1.6	45.13 \pm 3.31	45.88 \pm 3.76
Vitamin B ₆ , $\mu\text{g}/\text{dL}$	13.93 \pm 0.29	13.16 \pm 0.68	12.03 \pm 0.63	11.43 \pm 1.12	14.39 \pm 0.26	12.8 \pm 0.34
Vitamin B ₁₂ , pg/mL	352.88 \pm 7.32	350.63 \pm 4.98	284.75 \pm 23.03	281.13 \pm 14.37	318.75 \pm 5.68	309.13 \pm 6.92
Folic acid, ng/mL	6.33 \pm 0.27	3.96 \pm 0.41	5.6 \pm 0.4	4.4 \pm 0.36	8.38 \pm 0.67	12.59 \pm 0.86
Vitamin A (retinol), $\mu\text{g}/\text{L}$	501.00 \pm 16.38	478.63 \pm 14.62	319.25 \pm 8.75	299 \pm 5.63	444.38 \pm 21.62	441.25 \pm 12.42
Vitamin D ₃ 25(OH) D, pg/mL	20.13 \pm 1.73	17.00 \pm 1.2	16.93 \pm 2.38	16.03 \pm 0.58	17.86 \pm 2.26	27.6 \pm 4.79

Table 6. Indicators of fat quality in patients' diet

pH		PV mgO/100 g fat		AV		Totox
mean	SD	mean	SD	mean	SD	
5.4	0	0.86	0.1	0.9668	0.1	1.1922

Table 7. Sensory evaluation of oils

Oil	Discriminants				
	appearance	colour	taste	smell	total score
Linseed oil	4.50	4.20	1.80	2.80	3.33
Evening primrose oil	4.00	4.00	1.25	1.80	2.76
Icelandic cod-liver oil	3.80	4.00	1.00	1.00	2.45

Determination of reducing power Ferric Reducing Antioxidant Power (FRAP) according to Benzie and Strain [12]. Determination of antioxidant capacity (reducing antioxidants) with FRAP assay is based on spectrophotometric measurement of reducing power – the transition of the complex $\text{Fe}^{3+} \pm \text{TPTZ}$ ($\text{Fe}^{3+} \pm \text{tripyriddyliotriazine}$) into $\text{Fe}^{2+} \pm \text{TPTZ}$ at the wavelength of 593 nm. The results are presented in mMTE per 100 g of a meal or per 100 mL of a cocktail.

Determination of total polyphenols according to Cheung et al. [13]. The assay was based on the reaction of a phenol group with the Folin-Ciocalteu reagent which, after the reaction with phenol group, formed a blue-grey complex that could be determined colorimetrically using the absorbance measurement at 765 nm. The results were calculated as mg of catechin per 100 g of a meal or 100 mL of a cocktail.

TE – Trolox Equivalent – equivalent of trolox, a synthetic analogue of vitamin E.

Morphological and Biochemical Analyses Performed on the Patients: A complete blood count was performed for all the patients in all test groups using standard methods. The following concentrations were tested in blood serum: vitamin D₃ – using chemiluminescence, vitamins E and A – using liquid chromatography (HPLC), vitamin B₁₂ and folic acid – using electro-chemiluminescence, Ca and Fe – using colorimetry, and Na and K – using indirect potentiometry (Table 5). Total CH, HDL, LDL and TG were analysed with colorimetric method, and glucose was determined using colorimetric method with hexokinase in blood on fasting. The analyses were performed at 6 months intervals before the treatment and after dieting process.

Results

Assessment of Body Mass and Some Anthropometrical Parameters

As a result of a complex care, the average body mass reduction in all the patients reached 15.76 kg, which cor-

responded to the reduction of BMI from 36.3 kg/m² to the final 31 ± 4.9 kg/m².

Detailed data comparing the average results of body composition analysis in 3 examined groups are presented in Table 4.

In patients from group III, using the diet rich in flavonoids, vitamins, minerals, n-3 EFA and synbiotics, the highest body mass reduction was observed – from 107 to 77.5 kg, as compared to group II – from 100.9 to 85.2 kg, and group I – from 102.9 to 98.3 kg. Moreover, using bioelectrical impedance analysis, it was shown that the most effective diet was the authors' diet used in group III. The highest fat tissue mass reduction and visceral fat surface reduction were observed together with the improvement in metabolic age of the patients and with simultaneously the lowest loss of muscle mass (Table 4). The composition of the diet for group III allowed for maintaining proper concentration of mineral compounds and protein in blood (Table 4).

Using non-parametric Wilcoxon signed-rank test, statistically significant differences were shown in body mass reduction, and hence in BMI, between the control group I and group III. At the same time, there were statistically significant differences in reduction of visceral fat area VFA between the groups II and III. In case of the reduction of total adipose tissue, statistically significant differences were noted between groups I and II.

There were no significant differences between the groups in fat-free body mass.

Assessment of Systolic and Diastolic Blood Pressure

The mean systolic and diastolic pressure measurements of the patients before and after the procedure are presented in Table 8. The analyses show that in all the patients, there were changes in both systolic and diastolic pressure. Only in groups II and III, after half a year of using a diet and BIB treatment, the pressure could be regarded as desired.

Assessment of Blood Glucose Level

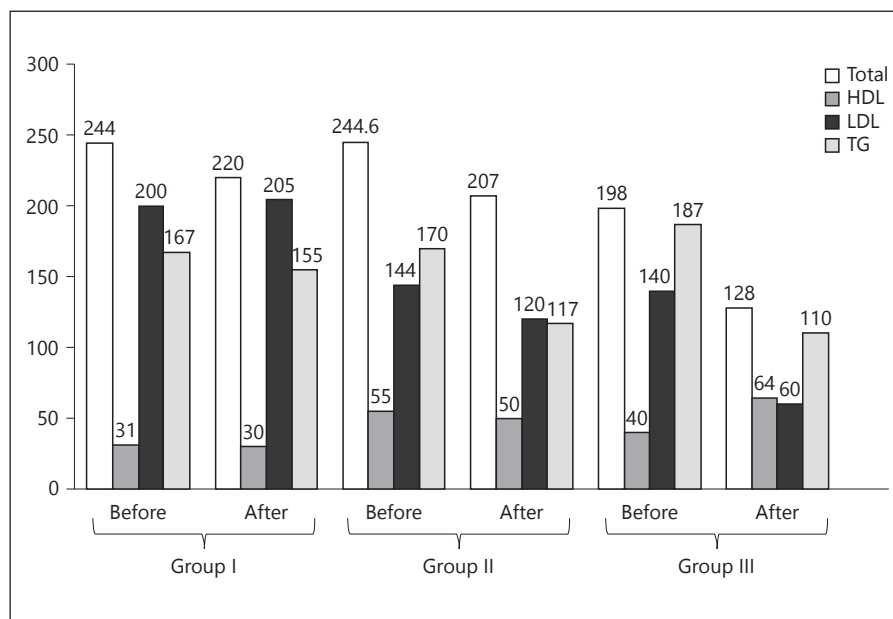
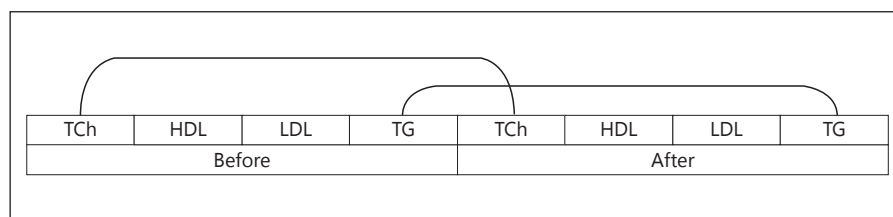
According to the clinical recommendations of the Polish Diabetes Association, appropriate levels of glucose in blood on fasting are those below 99 mg/dL. Both groups II and III, adhering to American diet and authors' dietetic recommendations during the treatment with OBERA method, showed significant improvement of glycaemia (Table 9).

Table 8. Blood pressure measurements

Patients	Mean systolic and diastolic pressure before the procedure	Mean systolic and diastolic pressure after the procedure
Group I	150±15/90±5	140±10/90±6
Group II	145±15/90±6	139±10/87±5
Group III	147±12/85±7	129±11/80±5

Table 9. Average blood glucose levels

Patients	Average blood glucose level before the procedure	Average blood glucose level after the procedure
Group I	117±8	115.8±4
Group II	120±5	87±4
Group III	125±6	77±5

**Fig. 1.** Mean values of lipid profile in patients before and after the procedure.**Fig. 2.** Assessment of the probability of the occurrence of statistically significant differences between obtained lipid profile results in group II.

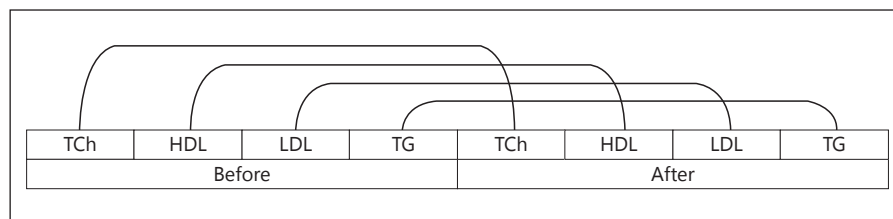
Assessment of Lipid Profile

According to the standards of the Polish Cardiac Society, the desirable total cholesterol level should be 150–190 mg/dL, LDL – below 115 mg/dL, HDL – above 35 mg/dL in men and above 40 mg/dL in women, triglycerides – 35–150 mg/dL (Fig. 1). The decrease in total cholesterol level was observed in all the patients from all the groups but the best results, in case of the reduction of total cholesterol level, LDL and triglycerides, were obtained in patients treated with authors' diet, that is, group III.

The results were compared by determining the probability of the occurrence of statistically significant differences (Fig. 2, 3).

A non-parametric Wilcoxon signed-rank test with $p < 0.05$ was used for the results of lipid profile analyses, and the data obtained before the procedure and after the procedure was compared. There are no statistically significant differences in group I. In group II, there were statistically significant differences in the levels of total cholesterol and triglycerides (Fig. 2).

Fig. 3. Assessment of the probability of the occurrence of statistically significant differences between obtained lipid profile results in group III.



In group III, statistically significant differences were shown between all the fractions of lipid profile (Fig. 3).

Assessment of Blood Morphology and the Concentration of Selected Bioelements and Vitamins

Blood morphology and biochemical parameters in collected blood samples were analysed twice. Mean values are presented in Table 5.

Statistical analysis of obtained results of blood parameters in patients showed significant differences in case of: copper in group II, zinc and folic acid in all the groups, vitamins A and B₁ in groups I and II, vitamin B₁₂ in group III, and vitamin D₃ in groups I and III.

Assessment of Diets Value

Linseed oil was selected as a diet supplement during organoleptic evaluation of oils, as a result of sensory analysis (Table 7). Diets of patients from group III were supplemented by 20 mL of linseed oil per day, as the main source of n-3 EFAs.

Based on the analyses, the composition of fatty acids was determined in diets of patients from group III. A favourable, pro-healthy composition of fatty acids and appropriate proportions of n-6/n-3 fatty acids was observed (Table 2).

Protein content in authors' diets was 80–85 g/day, carbohydrates 70 g/day and total fat 42 g/day. Fat content in daily food ratio was influenced by food produce and linseed oil used as a supplement (20 mL/day).

To assess the quality of fat in the diet, the total oxidation value (Totox) of fat was determined (Table 6). Totox conventionally is used to describe total oxidation of oils and fats, characterizing primary decomposition products as peroxide value PV (mg/100 g) together with anisidine value AV, which refers to the presence of secondary oxidation products. Totox is calculated by the formula: $\text{Totox} = 0.26 \times \text{PV} + \text{AV}$. The values obtained indicate very high quality of lipids in patients' diet.

According to Achremovicz and Szary-Sworst [14], PUFA, called vitamin F, should cover 1/3 of daily

demand on fat in human diet, with the ratio of n-3 acids to n-6 acids from 2:1 to 3:4.1. Many authors suggest [15, 16] that changes undergoing in fats have unfavourable character. Radicals formed by oxidation are the cause of many diseases and secondary oxidation products are toxic for humans [17, 18]. The actual oxidation status of fat can be judged by anisidine value and can describe its stability (Table 6). It is regarded that the value below 3 units shows on good quality of fat [19].

Analysis of Antioxidative Capacity, Reducing Power and Polyphenols Content

Antioxidative potential was analysed in 4 samples in 3 repetitions (Table 3). First cocktail was composed of spinach, kale, orange, parsley leaves, apple and kiwi, with the addition of linseed. Second cocktail was a combination of raspberries, forest fruits, pumpkin and pomegranate juice. Third cocktail contained beetroot, ginger, apple and orange. Fourth sample included the components of solid meals and contained quark, avocado, linseed oil, fish, jellied turkey, eggs and bread. Analysed food ratios showed high but various antioxidative properties. Cocktails were richer in bioactive compounds than meals. According to their bioactive properties, the cocktails could be arranged in the following way: cocktail 2 based on raspberry and pumpkin > cocktail 3 with beetroot > green cocktail 1 (Table 3).

Discussion

Reaction of an organism to the presence of ORBERA™ balloon in the stomach may vary depending on the overall condition of a patient, own motivation, knowledge, appropriate care, diet and support, and the type and intensity of physical activity. A complex care over the patients was provided to obtain satisfactory results of body mass reduction and to avoid vitamins and minerals deficiencies and destabilization of water and electrolyte balance due to vomiting.

The treatment and dietary care were aimed at maintaining a negative energy balance by providing ca. 400 kcal/day in initial period (for approximately a month) to 1,000 kcal/day in subsequent months, thus obtaining recommended body mass reduction. A very quick weight loss was observed during the first 3 months and afterwards the process slowed down and body weight became stable.

Performed study confirmed the effectiveness of BIB treatment, which was efficient if a patient adhered to the diet. There were cases when patients did not use the recommendations and gained weight.

The treatment resulted in the reduction of fat tissue mass, while maintaining fat-free body mass. Attention should be paid to the reduction of the surface of visceral adipose tissue, which highly correlates with the development of metabolic diseases [20]. Similar profits from using BIB treatment were observed by scientists from other countries [21–23]. The consequences of obesity for the function of cardiovascular system mainly depend on the distribution of adipose tissue. It was reported that obesity characterized by the development of subcutaneous adipose tissue localized near hips, buttocks and thighs is not linked to higher risk of cardiovascular diseases [24]. It was suggested that it results from high activity of lipoprotein lipase in adipocytes, which determines effective capture of lipids from blood and accumulating them as metabolic energy storage [25]. However, people with an excessive amount of abdominal adipose tissue are characterized by insulin resistance, atherogenic dyslipidaemia with high concentration of TG, apolipoprotein B and LDL and low levels of HDL. In this group of people, there are also observed biochemical parameters of chronic inflammatory state, higher tendency to intravascular coagulation and impaired functionality of endothelium [24, 26].

There are many diets promising rapid effects, often with unhealthy consequences due to improper limited composition. The progress in understanding the effect of a diet, which has a favourable influence on an organism, made it possible to develop diets with pro-healthy properties. Therefore, the aim was to compose an “authors” diet and supplementation with fatty acids due to their pro-healthy effect, for example, reduction of the levels of triglycerides and cholesterol, especially LDL, or anti-inflammatory activity and prevention of cardiac arrhythmia [27]. Via enzymatic pathways, according to Zagrodzki [28], a human organism obtains max ca. 5–10% EPA and ca. 1% DHA, which is EPA precursor. Taking into consideration age-related decrease in the activity of de-

aturase, essential for metabolism of fatty acids, there is a need to increase the amount of fatty acids in everyday diet. Much improved lipid metabolism was observed in the group of patients given daily dose of 20 mL of linseed oil. High consumption of fat and improper composition of fatty acids in a diet is one of the causes of diseases of affluence, obesity, atherosclerosis, metabolic syndrome and others [29, 30]. In our studies, it was observed that in a whole daily food ratio, the content of unsaturated fatty acids of n-6 family was 18.20% and that of n-3 family was at the level of 9.87%. The dominating n-6 fatty acid was linoleic acid (C 18:2 n-6), which amounted to 15.63% of all fatty acids, and the prevailing n-3 fatty acid was α -linolenic acid (C 18:3 n-3), which made up 6.65% of daily consumed fatty acids. The quality of lipids in daily food ratio was on a very high level. Total oxidation value of fat was 1.1922. Appropriate diet contributed to the reduction of total cholesterol from 198 to 128 mg/dL, HDL increased from 40 to 64 mg/dL, LDL decreased from 140 to 60 mg/dL and triglycerides decreased from 187 to 110 mg/dL.

The prevention of diseases resulting from inefficiency of blood antioxidative systems relies on appropriate intake of exogenous antioxidants with a diet [31, 32]. Based on obtained data and the knowledge on frequency and size of food ratios, we determined the daily intake of bioactive compounds in the diet of patients from group III. Daily intake of antioxidative compounds, depending on the type of consumed cocktails, was within the range 5479,44 – 8414,58 μ M TE/day in case of antioxidant capacity (TEAC), 1642,38 – 4896,44 μ M TE/day in case of reducing power, and 1.20–2.23 g of catechin equivalents per day in case of the content of total polyphenols. These values are high, taking into consideration that they do not include bioactive compounds coming from drinks, including tea, which according to Ilow et al. [33] may provide 60% of the total antioxidative potential of a diet. In the study of Ilow, the total antioxidative potential was estimated, based on ORAC tables, to ca. 9000 μ M TE/day (ca. 3500 μ M TE/day without considering tea) [33]. Total polyphenols content was also significantly higher than that reported by Wilczyńska and Retel, who estimated it at the level of 0.441 g polyphenols/day [34].

The main goal of obesity treatment was to reduce the risk of accompanying diseases and ensure that there is an improvement of metabolic disorders and the reduction of body weight to an appropriate level. Diet rich in bioactive compounds made it possible to normalize blood pressure. Desirable measurements of blood pressure were achieved in patients using authors’ diet packed with anti-inflam-

matory compounds. Parameters of lipid metabolism were also improved, that is, reduced total CH, TG and normalized levels of HDL and LDL. Glucose level in blood was also reduced. Recommendations of ADA and the Polish Diabetes Association point at lowering the concentration of LDL to the level below 100 mg/dL as the main aim in the treatment of lipid disorders. This result was obtained only in case of patients using authors' diet. Numerous studies confirm the effectiveness of bariatric methods in the reduction of body mass and glucose level in blood. Moreover, the improvement in heart and metabolic profile in obese children was also reported [21–23].

Stimac et al. [35] in prospective studies involving 171 patients after BIB confirmed the effectiveness of the procedure as a method of weight reduction, at the same time obtaining substantial improvement in blood pressure, glycaemia and triglycerides level. However, the reduction of total cholesterol and low-density lipoproteins, and the increase in high density lipoproteins were not observed. Mafort et al. [36] also confirmed positive effects of BIB treatment. Through the reduction of body weight, they observed significant improvement in lungs functions and biochemical parameters in patients with metabolic syndrome [35, 36].

Bariatric procedures are linked to the risk of macro- and micronutrients deficiencies. It is advisable to frequently monitor nutritional status of a patient due to observed deficiencies in protein, iron, vitamin B₁₂, folic acid, calcium, potassium, fat soluble vitamins: A, D₃ and E [37–40]. In own study, due to proper diet composition, no deficiencies in vitamins or bioelements were observed.

Endoscopic placement of a balloon in a stomach causes the prolonged stimulation of hydrochloric acid secretion, which can lead to the formation of erosions or ulcers. To prevent this, patients were treated with proton pump inhibitors. A change of pH in a stomach towards alkaline may lead to too low concentration of intrinsic factor IF, called Castle factor, which can contribute to the deficiency in vitamin B₁₂. The risk of B₁₂ deficiency is described in the course of treatment after bariatric surgeries [41, 42]. Taking into consideration the short time of BIB treatment and reversibility of the procedure, maintained levels of vitamin B₁₂ were assessed as proper and safe [5, 43].

The results of studies from recent years have shown that intestinal microflora plays an important role in food absorption and regulation of energetic processes. Overweight people have distorted proportion between Bacteroidetes and Firmicutes [44]. A diet rich in protein and poor in carbohydrates can result in deficiencies in Bifido-

bacterium [45]. Such dysbiosis can lead to endotoxemia caused by bacterial translocation through damaged intestinal epithelium, which activates the elements of immune system and visceral adipose tissue. This results in chronic inflammatory state, insulin resistance, type 2 diabetes, non-alcoholic fatty liver disease and obesity. For such reasons the regulation of intestinal microflora, especially in people with improper BMI and accompanying metabolic diseases, should be a part of the therapy [46–48]. Patients who were given synbiotic achieved normalized lipid profile and glucose level and satisfactory reduction of body mass, in comparison to groups I and II.

Many specialists confirm that surgical treatment of extreme obesity is the only effective way of fighting with this disease [49, 50]. Such procedures lead to stable body weight reduction, lower mortality and cure or reduce the symptoms of accompanying diseases, which contributes to improved quality of life, remission of depression and other psychic disorders and prolongs life of obese patients [51–53]. BIB treatment is a form of conservative (non-surgical) support for patients fighting with obesity. It is very effective, non-invasive, totally reversible and the safest method among all bariatric procedures [54, 55]. Endoscopic bariatric treatment may be an alternative to pharmacological treatment of obesity and provides higher efficiency with lower risk in comparison to conventional surgical procedures [56]. A very crucial element of the treatment of bariatric patients is the change of eating habits for the rest of patients' lives [57, 58]. Enriching the diet with bioflavonoids and other compounds reduces oxidative stress and the concentration of pro-inflammatory cytokines, and increases the concentration of oxidative stress enzymes in blood (article in press). This was also confirmed by other authors [59–61]. Observations and own experience enable to deduce that patients who resign from systematic contact with a dietician cannot maintain reduced body weight. Abandoning previous habits is the only way to maintain the effect of weight loss. Most importantly, the change in patients' awareness and consequent behaviour in the future are crucial. Even though genes may contribute to obesity, environmental factors mainly determine the possibility of the disease to occur [62–66]. Therefore, the change of patients' lifestyle after body weight reduction will decide their future quality of life.

Disclosure Statement

The authors declare that they have no conflicts of interest to disclose.

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