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Effect of a 3-Week Low-Calorie Diet and Balneological Treatment on Selected Coagulation Parameters in Morbidly Obese Patients

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Abstract

Background. The most common cause of death among people with obesity are cardiovascular complications as a result of a hypercoagulability state.

Objectives. The purpose of the study was to assess the potential of coagulation system activation depending on the tissue factor and to analyze of the influence of a 3-week low-calorie diet and balneological treatment on selected coagulation parameters in morbidly obese patients.

Material and Methods. The study included 36 patients (28 females and 8 males; mean age 46) with the value of BMI > 40 kg/m². The study was designed in two stages: baseline and after 21-days. The evaluation of tissue factor (TF), tissue factor pathway inhibitor (TFPI), von Willebrand factor (vWF), fibrinogen, D-dimer, thrombin-antithrombin complexes (TAT), and the activity of antithrombin (AT) was performed in patients before and after the treatment. The control group consisted of 24 healthy volunteers (16 females and 8 males) at a mean age of 39 with BMI ≤ 24.9 kg/m².

Results. There were significantly higher levels of TF, TFPI, fibrinogen, TAT complexes and D-dimer in the study group as compared to the controls. Moreover, there were no significant changes in the parameters studied before and after the treatment. In the group of obese patients, there were significant positive correlations between the concentrations of vWF and BMI and BMI changes and a significant negative correlation between the WHR changes and TFPI concentration.

Conclusions. The study confirmed that morbidly obese patients represent a high risk of hypercoagulability state, despite no clinical evidence, which could be due to the great inhibitory potential of TFPI in suppressing the extrinsic pathway of the coagulation system. However, the lack of effect of the 3-week exposure to the LCD and balneological treatment in morbidly obese subjects indicates that substantial fat mass must be reduced before adequate hemostasis is re-established (*Adv Clin Exp Med* 2016, 25, 4, 755–761).

Key words: morbid obesity, low-calorie diet, balneotherapy, tissue factor, tissue factor pathway inhibitor.

According to the World Health Organization (WHO), obesity is related to an excessive accumulation of adipose tissue, which may result in health deterioration [1]. This occurs when energy input

is greater than energy expenditure, causing both the hyperplasia and the hypertrophy of adipocytes. Adipocyte hypertrophy has negative consequences connected with insulin resistance and chron-

ic low-grade inflammation [2, 3]. Moreover, metabolic abnormalities occur depending on fat distribution rather than on the total amount of the fat accumulated [3].

Obesity, in particular morbid obesity (BMI > 40 kg/m²), results in a number of serious complications, such as type 2 diabetes mellitus, peripheral vascular disease, disorders of the musculoskeletal system and obstructive sleep apnea syndrome, leading to a significant decrease in quality of life. Others, such as cancer, ischemic heart disease with myocardial infarction or cerebral stroke, pose a serious life threat. The last two complications are major causes of death of morbid obese subjects [2, 4].

The most life-threatening obesity complication occurring in the cardiovascular system is associated with coagulation system hyperactivity. The state of hypercoagulability can often be observed in people with morbid obesity in the form of symptoms of thrombotic disease, frequently resulting from minor injuries or skin damage [5]. The cause is an increase in the concentration and activity of plasma coagulation factors such as hyperfibrinogenemia, VII, VIII and von Willebrand factors, platelet hyperactivity and, indirectly, endothelium damage as an independent factor contributing to the risk of developing thrombosis [6, 8, 9]. Tissue factor is the main activator of the extrinsic coagulation process, which plays a crucial role in thrombin creation and thrombi generation, and it is directly connected to thrombosis and vascular dysfunction. TF overproduction as well as increased procoagulant risk are observed in obesity [7–9].

The applicable literature proposes many approaches to limiting fat mass for the treatment of obesity-related risk and consequences, associated with a decreased secretion of pro-inflammatory adipocytokines and improved glucose homeostasis [2]. In recent years, the role of tissue factor as the major initiator of the extrinsic coagulation pathway has been stressed. In the absence of data related to disorders of the coagulation system in morbid obesity, the objective of the study was to assess the potential of coagulation system activation depending on the tissue factor and to analyze the influence of a 3-week low-calorie diet and balneological treatment on selected coagulation parameters in morbidly obese patients.

Material and Methods

Sixty subjects, 36 suffering from morbid obesity (BMI > 40 kg/m²) and 24 healthy, normal-weight individuals (BMI < 24.9 kg/m²), were recruited from the Ciechocinek-based Clinic of Balneology and Physical Medicine of the Nicolaus

Copernicus University in Toruń, Collegium Medicum in Bydgoszcz. A written informed consent was obtained from each participant before entering the study. The study was permitted by the Bioethics Committee of the Nicolaus Copernicus University in Toruń, Collegium Medicum in Bydgoszcz, Poland (KB/138/2009).

Thirty-six patients [(28 females aged 28–69 (mean age 48) and 8 males aged 20–56 (mean age 43)] were enrolled in the study. The mean duration of obesity was 20 years. The average adipose mass in the female group accounted for 54.59%, and 52% in the male group. Seven percent of women (2) had smoked cigarettes in the past but had stopped at least 5 years ago, and the male group was 100% non-smokers.

The standard interview conducted by the internal medicine doctor showed information about the general condition of the patients and co-existing diseases. The family history of 86% of the patients indicated that their first- and second-degree relatives were obese. In 19% of the patients, dyslipidemia occurred, whereas 70% of the patients suffered from hypertension and in 9 patients an impaired glucose tolerance was observed. Among all the patients, 26 had taken hypertension drugs (angiotensin-converting enzyme inhibitor drugs). The exclusion criteria included receiving anticoagulant, thrombolytic therapy and antiplatelet drugs, thromboembolic disease or pulmonary embolism less than 6 months before, and surgical procedure less than 3 months before the beginning of the experiment (Table 1).

Twenty-four healthy normal-weight volunteers constituted the control group ([16 females and 8 males]; mean age 39) the BMI ranged from 22.06 ± 1.79 kg/m², WHR – 0.83 ± 0.05, body fat – 33.17 ± 4.84% (20.18 ± 3.45 kg). The exclusion criteria for the controls were hypertension, hyperlipidemia, hyperglycemia, current smoking, acute and chronic infection and symptoms of metabolic syndrome.

The study was developed in two stages: baseline and after 3 weeks. The patients were informed about the study formula: During the 3-week observation they should obey an unchanging lifestyle. The patients were on a low-calorie diet (LCD): 1000 kcal/day for 7 days (five small, balanced meals, with minimal carbohydrate intake, 2–3 portions of vegetables and fruit), followed by an inserted very low-calorie diet: 400 kcal/day for 4 days and return to LCD to the end of the intervention period. The menu was prepared and controlled by a professional nutritionist. The meals were served at a resort restaurant. The daily exercise program included 1 h of brisk walking (5–6 km) and 45 min of morning gymnastics. Balneotherapy was a 3-week program which covered mineral water bubble bathing [4% NaCl (sodium chloride)] at 37°C for 15 min, min-

Table 1. Selected clinical data of patients with morbid obesity depending on gender

Parameters (units) (references values)	Females (n = 28) (mean ± SD)	Males (n = 8) (mean ± SD)
Mean age (years)	48 ± 10	43 ± 14
Body mass (kg)	116.5 ± 19	121 ± 11
BMI (kg/m ²)	44.4 ± 5.3	44 ± 4
WHR	0.90 ± 0.05	0.98 ± 0.04
Hypertension (BP > 140/90 mm Hg)	71%	62%
LDL-cholesterol (< 130 mg/dL)	142 ± 36	142 ± 41
HDL-cholesterol (> 65 mg/dL)	52 ± 9	50 ± 6
Total cholesterol (< 190 mg/dL)	223 ± 31	222 ± 41
Fasting glucose (mg/dL) (70–99 mg/dL)	97.3 ± 7	96.5 ± 11

Values of anthropometric and biochemical parameters are presented as mean (X) ± standard deviation (SD); BMI – body mass index; WHR – waist-hip ratio.

eral water pool exercises at 31°C for 40 min, and warm mud baths at 40°C for 30 min.

Venous blood (4.5 mL) for tests of TF, TFPI, vWF, fibrinogen, D-dimer, thrombin-antithrombin complexes and activity of antithrombin was collected in a fasting state into cooled tubes (Becton Dickinson Vacutainer® System, Plymouth, UK) containing 0.13 mol/L trisodium citrate (the final blood:anticoagulant ratio was 9:1) after 30 min of rest between 7:30 and 9:30 am and after a 12 h overnight fast. The blood samples were immediately mixed and centrifuged at 3000 × g at +4°C for 20 min. The plasma was divided into 200 µL Ependorf-type tubes and then the samples were frozen at –80°C (according to the manufacturer's procedures) until assayed, however no longer than six months. To determine the concentration of lipid profile, blood was collected in a 4.5 mL tube without anticoagulant. It was centrifuged at 3000 × g for 20 min at +4°C and subjected to further analytical procedures. To measure fasting glucose, blood was collected in a 4.5 mL tube with sodium fluoride EDTA (ethylenediaminetetraacetic acid). The plasma was centrifuged at 2000 × g for 10 min at +4°C and subjected to further analytical procedures.

Hemostatic Assays

The concentrations of TF and TFPI were determined by Enzyme Linked Immunosorbent Assay (ELISA) (IMUBIND® total TF, IMUBIND® total TFPI, respectively; American Diagnostica inc., Greenwich, USA). The TAT concentration was measured by ENZYGNOST® TAT micro (Behring, Marburg, France). D-dimer and anti-

gen of vWF were estimated by ASSERACHROM® D-DI and ASSERACHROM® VWF:Ag (Diagnostica Stago, Asnieres, France), respectively. However the concentration of fibrinogen and the activity of antithrombin were measured in an automated coagulometer CC-3003 apparatus and the reagents were produced by Bio-Ksel Co. (Grudziądz, Poland). The parameters of lipid profile and fasting glucose were determined using specific tests for the Abbott Clinical Chemistry Analyzer® Architect c8000.

Statistical Analysis

The statistical analysis was performed using the statistics program STATISTICA v. 10.0 software. The Shapiro-Wilk test was applied to assess the normality of the distribution, which facilitated the use of the independent t-test; for those variables, mean (X) and standard deviations (SD) were determined. The U-Mann-Whitney rank-sum test was used when the distribution was not normal, the median (Me), lower quartile (Q1) and upper quartile (Q3) were determined to describe variables. To analyze the differences in the concentration of selected coagulation parameters evaluated twice (baseline and after 3-weeks), the Wilcoxon test and the dependent t-test were applied. To assess the correlation between the parameters, the Spearman's coefficient (R) was used. The p-values < 0.05 were considered significant.

Results

Table 2 presents the concentrations of TF, TFPI, fibrinogen, TAT complexes, D-dimer and AT

Table 2. Comparison of the analyzed parameters in the study group and in the control group

Parameters	Study group N = 36	Control group N = 24	p-values
	X ± SD/Me(Q1;Q3)	X ± SD/Me(Q1;Q3)	
TF (pg/mL)	178.90 98.40/272.84	126.24 79.88/161.25	0.02
TFPI (ng/mL)	147.90 120.30/229.80	47.88 37.80/53.54	0.0001
Fibrinogen (g/L)	3.20 ± 0.92	2.96 ± 0.71	0.04
TAT (ng/mL)	3.30 2.32/13.85	1.70 1.09/2.94	0.003
AT (%)	101.08 95.47/108.80	99.20 87.04/105.99	0.104
D-dimer (ng/mL) FEU	361.15 244.57/475.74	190.88 119.55/377.93	0.0105

FEU – fibrinogen equivalent units; the values of selected coagulation parameters are shown as medians (Me) and lower (Q1)/upper (Q3) quartile and mean (X) ± standard deviation (SD).

activity in morbidly obese subjects relative to the controls. Significantly higher levels of TF, TFPI, fibrinogen, TAT complexes and D-dimer were noted ($p = 0.02, p = 0.0001, p = 0.04, p = 0.003, p = 0.0105$). Table 3 shows the concentration of TF, TFPI, fibrinogen, vWF, TAT complexes, D-dimer and AT activity in the study group before and after the treatment. There was no effect observed of the 3-week

exposure to the LCD and balneological treatment in morbidly obese subjects. Table 4 shows the relationships between BMI, BMI changes, WHR and WHR changes after the treatment in morbidly obese subjects. In the group of patients, there were significant positive correlations between the concentration of vWF and BMI and BMI changes and a significant negative correlation between the WHR changes and TFPI concentration.

Table 3. Effect of the treatment on TF, TFPI, fibrinogen, vWF, AT, TAT complexes and D-dimer identified in the study group

Parameters	Before treatment (baseline) X ± SD/Me (Q1;Q3)	After treatment (3-week later) X ± SD/Me (Q1;Q3)	p-values
TF (pg/mL)	178.90 98.40/272.84	182.82 120.54/288.35	0.9929
TFPI (ng/mL)	147.90 120.30/229.80	138.48 83.04/229.28	0.2210
Fibrinogen (g/L)	3.20 ± 0.92	3.30 ± 0.64	0.9375
vWF (%)	116.66 102.26/133.90	121.89 92.04/143.61	0.5540
TAT (ng/mL)	3.30 2.32/13.85	4.29 2.07/6.99	0.8225
AT (%)	101.08 95.47/108.80	104.36 97.34/112.78	0.6487
D-dimer (ng/mL) FEU	361.15 244.57/475.74	378.14 230.03/469.57	0.8094

FEU – fibrinogen equivalent units; the values of selected coagulation parameters are shown as medians (Me) and lower (Q1)/upper (Q3) quartile and mean (X) ± standard deviation (SD).

Table 4. Spearman (R) correlation coefficients of the parameters analyzed with BMI, BMI changes, WHR and WHR changes in patients with morbid obesity

Parameters/units	BMI	BMI changes	WHR	WHR changes
TF (pg/mL)	-0.2684	-0.0209	0.1126	0.2836
TFPI (ng/mL)	-0.1080	-0.2453	-0.2196	-0.5373*
Fibrinogen (g/L)	0.1714	0.2373	0.3360	0.0143
vWF (%)	0.5359*	0.6058*	0.3164	-0.0159
TAT (ng/mL)	-0.0421	0.3198	-0.0205	0.2829
AT (%)	-0.1259	-0.2050	0.1393	-0.2041
D-dimers (ng/mL) FEU	-0.0364	-0.1156	-0.1030	-0.1617

* p < 0.05.

Discussion

The state of hypercoagulability is one of the most serious complications in morbidly obese patients. In the present study, we observed significantly higher levels of TF, TFPI, fibrinogen, TAT complexes and D-dimer in the study group as compared to the controls. This observation is consistent with the studies of Ay et al. [10] and Darvall et al. [11], which may indicate the activation of the coagulation system, leading to an increase in thrombin generation. El-Hagracy et al. presented a positive correlation between TF and BMI, elevating thrombotic tendency [12].

In physiological conditions, the endothelium expresses TF in trace amounts, insufficient to trigger the cascade of blood coagulation. However, in response to various stimuli, such as inflammation, hypertension, oxidative stress or hyperglycemia, TF secretion may be stimulated. It is well-established that obesity is associated with over-expression of pro-inflammatory cytokines secreted by the adipocytes, in particular IL-6 and TNF- α [13–15], which may suggest that low-grade inflammation in obese patients may activate extrinsic coagulation pathway. This thesis is confirmed by the observations made by Szotowski et al.; after provoking endothelial cells with IL-6 and TNF- α , TF is released by the endothelium [16]. Together with increased synthesis of TF, IL-6 and TNF- α , the risk of hypercoagulability grows rapidly. However, at the same time, its inhibitor, the tissue factor pathway inhibitor (TFPI), is released to control the coagulation activity or as a result of endothelial damage [16], which is in line with the results of the current study.

Lipid disorders with an increase in the concentration of VLDL and LDL cholesterol occur in patients with obesity, in particular with morbid obesity associated with TFPI over-expression. Lipids

have a strong affinity for binding TFPI. Lipoprotein-associated TFPI presents fewer anticoagulant properties than free TFPI [12, 17]. On the other hand, an increase in the expression of pro-inflammatory adipocytokines stimulates the release of TFPI [17]. An increase in the concentration of TFPI in obese subjects has been observed by Radziwon et al. [18]. Also, El-Hagracy et al. found an increase in the TFPI level in patients with hyperlipidemia [12]. According to the authors, an increase in the concentration of TFPI signifies damage to the endothelium, as the TF inhibitor is mainly connected with the surface of the endothelium. This may also result from an increase in the concentration of cholesterol in morbidly obese patients. However, Kopp et al. noted a significantly high TF and VII but lower TFPI among morbidly obese diabetic subjects [19].

Additionally, the present study revealed no significant changes in the selected parameters of coagulation before and after the treatment. In 131 obese individuals randomly assigned to one of three diets (high monounsaturated fat diet, low-fat diet and control-diet) Bladbjerg et al. observed that TFPI was not affected during the 6-month study [20]. In morbidly obese patients after a weight loss induced by bariatric surgery, Ay et al. also identified a significant reduction in the TF concentration but no influence on TFPI and fibrinogen levels [10]. Our results and those reported by Ay et al. and by Bladbjerg et al. are inconsistent with the results by Kopp et al., who noted a significant decrease in TF concentration, total and free TFPI, factor VII and fibrinogen in morbidly obese patients after the weight loss induced by gastropasty [10, 19, 20]. The results suggest that the relevant adipose tissue must be reduced before adequate hemostasis is re-established, which indicates that moderate weight loss in morbidly obese patients does not protect them from high cardiovascular risk.

In the group of obese patients after weight loss, there was a significant negative correlation noted between the WHR changes and TFPI concentration. It suggests that the weight loss and also the reduction of waist-to-hip ratio lead to an increased TFPI level.

Thromboembolic complications are the major causes of sudden cardiac death (SCD) in the cases of patients with morbid obesity. An important endogenous inhibitor of the coagulation system is antithrombin, which mainly inactivates factor Xa and thrombin. In the present study, in obese subjects the antithrombin activity was similar as compared to healthy controls. However, according to Batist et al., together with the reduction in body mass expressed by a decrease in BMI, there was an increase in AT activity [21]. Bowles et al. have shown a positive correlation between BMI and antithrombin activity [22]. Antithrombin, by binding thrombin, endogenously inactivates the key enzyme of the cascade of blood coagulation. It is believed that the formation of the thrombin-antithrombin complex (TAT) signifies the intensity of thrombin formation, which is reflected in the research carried out by Ten Cate [23]. In the available literature, there is little data relating to the concentration of TAT in the blood of people with morbid obesity. The present study has shown an increased TAT complexes level in morbidly obese patients, which may confirm the high activity of the coagulation process and hypercoagulability state in obesity.

Obesity is characterized by hyperfibrinogenemia, which, through increased platelet aggregation, changing the rheological properties of the blood and the clot structure, impairs blood flow in

the microcirculation and the fibrinolytic systems [8, 9]. Our work has revealed an increase in the concentration of fibrinogen in patients with morbid obesity. Since hyperfibrinogenemia is an independent factor in the development of thrombosis, the results are strong evidence of a prothrombotic tendency in morbidly obese subjects. The results are consistent with the studies made by Ay et al. [10] and Kopp et al. [19]. Fanari et al. have also observed a positive correlation between increased BMI and the concentration of fibrinogen in plasma [24].

In the group of obese patients, there were significant positive correlations between the concentrations of vWF and BMI and BMI changes. Obesity as well as weight loss expressed by BMI changes are associated with an increase in vWF level, which suggests endothelial dysfunction, irrespective of the body mass, possibly connected with a persistent systemic low-grade pro-inflammatory state. Combining these facts, one can see that elevated levels of vWF are associated with a high prevalence of cardiovascular incidents in this group of patients [24].

In conclusion, morbidly obese patients are in a high-risk hypercoagulability state (an increase in the concentration of TF, TAT complexes, D-dimer and fibrinogen), despite no clinical evidence, which could be due to the great inhibitory potential of TFPI in suppressing the extrinsic pathway of the coagulation system. However, the lack of effect of the 3-week exposure to the LCD and balneological treatment in morbidly obese subjects indicates that the substantial fat mass must be reduced before adequate hemostasis is re-established.

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Conflict of interest: None declared

Received: 14.03.2015

Revised: 14.04.2015

Accepted: 24.04.2015