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ABSTRACT

Background: Prothrombotic and inflammatory variables decrease after obesity surgery. The contact activation system may be a common denominator of these changes.
Objective: To characterize the contact system before and 6 months after Roux-en-Y gastric bypass (RYGB) and to evaluate associations with changes (post-surgery minus pre-surgery) in metabolic variables.
Methods: Women (n = 42) and men (n = 18) with obesity underwent RYGB, and measures of kallikrein generation, factor XII (FXII), prekallikrein, high molecular weight kininogen (HK), and C1 esterase inhibitor (C1-inh) were determined before and 6 months after surgery. Associations were evaluated using correlation and multivariate regression analyses.
Results: After RYGB, the endogenous kallikrein potential (EKP), peak kallikrein generation, FXII, and prekallikrein were reduced, and kallikrein generation lag time was prolonged (all $p < 0.0005$). Before and after RYGB, absolute values of EKP, lag time, and peak kallikrein generation correlated consistently with contact system proteins (range of correlation coefficients (r_S): −0.43 to −0.28 and 0.24 to 0.45 (pre-surgery); −0.43 to −0.30 and 0.28 to 0.50 (post-surgery)). RYGB-associated changes in EKP correlated with C1-inh ($r_S = -0.29$, $p = 0.025$), but also with triglycerides ($r_S = 0.34$, $p = 0.007$) and cholesterol ($r_S = 0.28$, $p = 0.029$), and independently associated with changes in C1-inh ($\beta = -0.40$) and triglycerides ($\beta = 0.39$). Changes in C1-inh associated with reductions in body weight ($\beta = -0.39$) and HbA1c ($\beta = 0.38$).
Conclusion: The contact system was affected 6 months after RYGB. Absolute values of kallikrein generation before and after RYGB correlated with contact system proteins, whereas changes after RYGB associated with changes in C1-inh and metabolic variables.

1. Introduction

The prevalence of obesity continues to increase worldwide [1,2] and is accompanied by an increased risk of cardiovascular disease (CVD) [3,4]. Numerous studies have shown that the obesity-associated increased CVD risk can be reduced by obesity surgery [5,6]. In line with this, the hemostatic balance is affected in an antithrombotic direction [7,8]. Obesity is also associated with low-grade inflammation [9] which is alleviated by obesity surgery [10].

There is a well-known interplay between coagulation, inflammation, and the contact system [11,12]. However, no studies have yet examined whether obesity surgery affects the contact system, which is initiated by activation of coagulation factor XII (FXII) and propagated and regulated by the action of high-molecular weight kininogen (HK), prekallikrein, and C1 esterase inhibitor (C1-inh) [11,12].

Interestingly, increased platelet activation has been observed in

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obesity [13], and obesity is also associated with endoplasmic reticulum (ER) stress [14–16], both with an inborn capacity to initiate the contact system *in vivo* via increased levels of polyphosphates (PolyP) from activated platelets [11,17] and misfolded proteins in ER stress [17,18]. Thus, targeting the contact system in obesity might have an anti-thrombotic and anti-inflammatory potential by interfering with activation of coagulation and inflammation [11,17].

Obesity surgery lowers the concentration of many liver-derived hemostatic proteins [7,8,10], and we therefore anticipated that obesity surgery might reduce concentrations of contact system proteins as well. The aim of the present study was to characterize the contact system before and 6 months after Roux-en-Y Gastric bypass (RYGB) and to evaluate associations with changes in previously reported reductions in metabolic variables [10,19]. We hypothesize that RYGB affects the contact system and that RYGB-related changes are associated with reductions in metabolic variables.

2. Materials and methods

The present sub-study is part of a larger study investigating the effects of supervised physical training following RYGB on weight loss [19], muscle strength and aerobic capacity [20], physical activity and quality of life [21], and markers of CVD [8,10]. Results reported in this paper are based on secondary outcome variables and include samples before and 6 months after bariatric surgery during ongoing weight loss. All subjects gave oral and written informed consent, and the study was conducted according to the Helsinki Declaration, approved by the local Ethics Committee (Project-ID: S-20120112), and registered at http://www.clinicalTrials.gov (NCT01690728).

2.1. Participants and study design

The study design is described in detail elsewhere [19]. In the present study, we included 42 women and 18 men, mean age 42.3 years, who were eligible for RYGB according to the national and international guidelines, as well as Danish criteria regulating free access to bariatric surgery (BMI > 35 kg/m^2 with obesity-related disease or BMI > 50 kg/m^2 with obesity-related social or physical complications). Major comorbidities were hypertension (n = 27), type 2 diabetes (n = 18), and dyslipidemia (n = 12), and medications therefore included antihypertensives (n = 27), antidiabetics (n = 18), and lipid lowering drugs (n = 11). Nineteen patients did not receive any medication [8]. Only non-smoking patients were included. Laparascopic RYGB surgery was performed at the Department of Surgery, University Hospital of Southern Denmark, Esbjerg, with a 20–30 ml gastric pouch, a 60 cm biliopancreatic limb, and a Roux limb of 150 cm [19]. Blood samples were obtained before and 6 months after RYGB. We excluded patients who received anticoagulant medication or hormone replacement therapy/oral contraceptives.

The subjects' characteristics before and 6 months after obesity surgery are presented in Table 1. Previously published results relevant for the present sub-study show that measures of body weight, blood lipids, and glucose [19] decrease after surgery.

A normal-weight control group matched on age and gender with the medication-free patients (mean BMI 22.5 kg/m^2, 17 women and 2 men, mean age 38.8 years) was included in the present study.

2.2. Blood sampling

Venous blood samples were collected between 7.45 and 8.30 in the morning after at least 10 h of fasting and 15 min of rest in a supine position. For the present study, all samples were collected in trisodium citrate tubes (0.109 M Na_3-citrate, Becton Dickinson, Plymouth, UK, ref: 363048). Immediately after sampling, platelet poor plasma was prepared by centrifugation for 20 min at 2000 ×*g* (20 °C). Plasma was rapidly frozen and stored at −80 °C until analyzed.

Table 1
Subjects' characteristics before and 6 months after obesity surgery.

Variable	Pre-surgery (n = 60)	Post-surgery (n = 60)
Body weight (kg)	126.6 (121.2–132.0)	99.2 (94.4–103.9)***
BMI (kg/m^2)	43.0 (41.4–44.5)	33.7 (32.2–35.2)***
Total cholesterol (mM)	4.48 (4.21–4.75)	3.94 (3.74–4.14)***
LDL cholesterol (mM)	2.88 (2.65–3.11)	2.32 (2.15–2.49)***
HDL cholesterol (mM)	1.03 (0.97–1.09)	1.14 (1.08–1.19)***
Triglycerides (mM)	1.38 (1.22–1.53)	0.99 (0.90–1.08)***
Glucose (mM)	6.22 (5.84–6.60)	5.52 (5.26–5.78)***
Insulin (pM)	159.4 (131.6–187.4)	66.9 (55.2–78.6)***
HbA1c (mM)	38.3 (35.9–40.8)	34.5 (33.1–35.8)***

Mean values (95% confidence intervals) before surgery and 6 months after surgery were compared with a paired *t*-test. BMI, body mass index; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; HbA1c, hemoglobin A1c. Subjects' characteristics were previously reported in Mundbjerg et al. [19] and Stolberg et al. [8,10].
*** $p < 0.001$.

2.3. Analytical methods

Samples were thawed in a water bath at 37 °C and analyzed in one series for each patient before and after RYGB. Plasma kallikrein generation was determined as described by Biltoft et al. [22]. In brief, undiluted citrate plasma was activated with silica (Sigma, Brøndby, Denmark) diluted in activated partial thromboplastin time (APTT) reagents (Triolab, Brøndby, Denmark), and FXII-dependent kallikrein generation was measured with the fluorogenic substrate Boc-Leu-Lys-Arg-AMC (Bachem, Bubendorf, Switzerland). Affinity purified human kallikrein (Enzyme Research Laboratories, Swansea, UK) was used as a calibrator. Fluorescence was read in a Fluoroskan Ascent microplate fluorometer (Fischer Scientific, Slangerup, Denmark) with a 390/460 nm filter set. The Thrombinoscope Software (Thrombinoscope BV, Maastricht, The Netherlands) was used to generate kallikrein formation curves. These curves display kallikrein generation lag time, peak kallikrein generation, and area under the curve (endogenous kallikrein potential, EKP) using the fluorogenic substrate to enable continuous measurement of kallikrein formation. This assay follows the same principles as the well-known thrombin generation assay [23], although in the kallikrein generation assay undiluted citrate plasma is activated with silica thereby activating FXII-dependent kallikrein generation over time.

We used ELISA's to determine concentrations of FXII [24], prekallikrein [25], and HK [26]. Concentrations of C1-inh were determined with commercial C1-inh antibodies (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany) using the BN II analyzer (Siemens Healthcare Diagnostics Products GmbH). Analytical methods used for previously reported variables (Table 1) are described elsewhere [19].

2.4. Statistics

Sample size was determined for the primary effect variable body weight, as previously presented [19]. Effects of RYGB were analyzed using a paired *t*-test for normally distributed variables (presented as mean and 95% confidence interval) and a Wilcoxon test for skewed variables (presented as median and interquartile range). Pre-surgery and control values were compared with a Mann-Whitney test.

Associations between absolute values (before and after surgery) of contact system proteins and kallikrein generation and between changes (post-surgery minus pre-surgery) in contact system proteins and kallikrein generation were assessed using Spearman's rank correlation coefficient (r_S) due to non-normally distributed variables (prekallikrein and C1-inh). Spearman's correlation coefficient was also used to estimate associations between changes in metabolic variables and changes in contact system proteins and kallikrein generation. Only body weight, total cholesterol, and HbA1c were included in the correlation analyses

among highly correlated variables (body weight/BMI, total cholesterol/LDL-cholesterol and glucose/HbA1c).

The correlation analyses were followed by standard multivariate regression analyses with changes in contact systems proteins and metabolic measures as independent variables and changes in measures of kallikrein generation as dependent variables. Only variables with $p < 0.05$ in the correlation analyses were included in the multivariate analyses which were adjusted for age, sex (female = 0, male = 1), diabetes (no diabetes = 0, diabetes = 1), and medication (use = 0/no use of any type of medication = 1) before surgery.

A p-value of less than 0.05 was considered significant. The SPSS program (version 24; IBM SPSS Inc., Chicago, IL, USA) was used for all statistical analyses.

3. Results

We observed highly significant reductions in EKP, peak kallikrein generation, FXII, and prekallikrein 6 months after RYGB compared with pre-surgery. Kallikrein generation lag time was prolonged, and C1-inh and HK were unchanged (Table 2). These results were confirmed in the subgroup of 19 patients who did not receive any medication (not shown). Only C1-inh was significantly lower in normal-weight individuals than in individuals with obesity before RYGB (Table 2).

Table 3 presents the results of the correlation analyses between contact system proteins and kallikrein generation. Before and after surgery, EKP correlated positively with HK and prekallikrein and negatively with C1-inh, whereas kallikrein generation lag time correlated negatively with FXII. Also, peak kallikrein generation correlated significantly with contact system proteins before and after surgery. Among surgery-associated changes, only C1-inh correlated with EKP (Table 3).

Table 4 presents correlations between changes in metabolic variables and changes in contact system biomarkers. Reductions in total cholesterol and triglycerides were positively associated with decreased EKP, whereas changes in C1-inh correlated negatively with reductions in body weight and positively with reductions in HbA1c.

The multivariate regression analyses, adjusted for age, gender, medication, and diabetes, showed that the reduction in EKP associated with changes in C1-inh ($\beta = -0.40$, $p = 0.002$) and triglycerides ($\beta = 0.39$, $p = 0.008$), with the multivariate model explaining 34% ($p = 0.002$) of the reduction in EKP after surgery. Reduced body weight ($\beta = -0.39$, $p = 0.003$) and HbA1c ($\beta = 0.38$, $p = 0.003$) associated with changes in C1-inh, and the model explained 34% ($p = 0.001$) of the change in C1-inh after surgery.

Table 2
Biomarkers of the contact system before and 6 months after obesity surgery and in normal-weight controls.

Variable	Pre-surgery (n = 60)	Post-surgery (n = 60)[a]	Controls (n = 19)[b]
EKP (nM×min)	1439 (1345–1534)	1245 (1162–1327)***	1483 (1235–1783)
KG lag time (min)	0.79 (0.72–0.87)	0.93 (0.84–1.02)***	0.80 (0.60–1.00)
Peak KG (nM)	767 (695–839)	636 (573–699)***	892 (583–1083)
FXII (mg/l)	38.9 (36.9–40.9)	36.3 (34.6–38.1)***	36.2 (32.6–43.6)
HK (%)	115 (109–121)	115 (110–121)	108 (95–117)
PK (μg/ml)[#]	38.0 (31.3–42.3)	32.8 (27.5–40.8)***	33.6 (30.8–39.6)
C1-inh (g/l)[#]	0.23 (0.22–0.26)	0.24 (0.22–0.25)	0.20 (0.20–0.22)***

EKP, endogenous kallikrein potential; KG, kallikrein generation; FXII, factor XII; HK, high-molecular weight kininogen; PK, prekallikrein; C1-inh, C1 esterase inhibitor.

[a] Post-surgery compared with pre-surgery: Mean values and 95% confidence intervals were compared with a paired t-test.

[#] Median values and interquartile ranges were compared with a Wilcoxon test.

[b] Controls compared with pre-surgery: Median values and interquartile ranges were compared with a Mann-Whitney test.

*** $p < 0.001$.

4. Discussion

In the present study, we demonstrated that EKP, peak kallikrein generation, concentrations of FXII, and prekallikrein were decreased 6 months after RYGB compared with pre-surgery, and kallikrein generation lag time was prolonged. Contact system proteins correlated with measures of kallikrein generation before and after surgery. The RYGB-associated decrease in EKP associated with changes in C1-inh, but also with reductions in triglycerides. Reductions in body weight and HbA1c associated with changes in C1-inh.

This is the first study focusing on the contact system after obesity surgery. Contact system proteins were consistently associated with EKP, lag time, and peak kallikrein generation before and after surgery. A recent study has demonstrated that certain drugs reduce the plasma kallikrein generation capacity, and that concentrations of C1-inh and prekallikrein are independently associated with EKP [26]. We observed that surgery-related changes in contact system proteins were only weakly associated with changes in kallikrein generation indicating that other factors affect changes in kallikrein generation.

It is of interest to note that lowering of triglycerides after RYGB associated with the reduction in EKP. Triglycerides have previously been linked to contact system proteins as it has been demonstrated that negatively charged components from the lipolysis of triglyceride-rich lipoproteins provide a contact surface that supports the activation of FXII [27,28]. Also, a strong positive association has been reported between triglycerides and levels of kallikrein [29] and FXIIa [30], and patients with hyperlipidemia have increased concentrations of FXIIa [31]. In another study, however, FXIIa, kallikrein, and FXIa in complex with C1-inh were unchanged after 12 weeks of triglyceride-lowering treatment [32]. Over the years, various methods have been used to assess contact system biomarkers, and more studies are needed to confirm our findings that lowering of *in vivo* triglyceride levels contribute to the reduced capacity of plasma to generate kallikrein.

Levels of contact system proteins were lower in participants with normal-weight than in obesity, although only significant for C1-inh. The net effect was a comparable kallikrein generation capacity in the two groups. In contrast, kallikrein generation was impaired after RYGB because C1-inh was at the same elevated level 6 months after surgery compared with pre-surgery levels. The reason for this persistently elevated level of C1-inh is unknown. Surprisingly, the reduction in body weight associated with an increase in C1-inh after RYGB. This negative association was also observed in medication-free patients ($\beta = -0.43$), ruling out medication discontinuation as a confounder. Other confounders related to obesity surgery are the inevitably accompanying metabolic improvements and changes in comorbidity, diet, and exercise. The observed associations between changes in metabolic variables and changes in C1-inh are difficult to translate into physiological relevance, because we observed no significant change in the median concentration of C1-inh after RYGB. To our knowledge, no previous studies have found associations between changes in body weight or HbA1c and changes in C1-inh.

The clinical impact of the impaired kallikrein generation potential following obesity surgery remains to be elucidated. However, the role of the contact system in health and disease has achieved growing interest during recent years. FXII-deficient patients have a normal hemostatic capacity and do not suffer from spontaneous or injury-related bleeding, but studies in FXII-deficient mice have shown defective thrombus growth which can be restored after FXII-infusion [33,34]. In humans, FXII can be activated *in vivo* by PolyP from activated platelets thereby driving coagulation within the thrombus, but also affecting inflammation [11,17]. Further, *in vivo* activation of FXII induced by misfolded proteins is demonstrated in patients with hereditary angioedema (HAE)

Table 3
Correlations between contact system proteins and measures of kallikrein generation.

	EKP (nM×min)			KG lag time (min)			Peak KG (nM)		
	Pre	Post	Change	Pre	Post	Change	Pre	Post	Change
	r_S	r_S	r_S	r_S	r_S	r_S	r_S	r_S	r_S
FXII (mg/l)	0.20	0.03	0.18	−0.43**	−0.43**	−0.02	0.37**	0.37**	0.10
HK (%)	0.45***	0.50***	0.04	−0.08	0.08	0.14	0.27*	0.20	−0.01
PK (μg/ml)	0.45***	0.28*	0.22	−0.07	0.04	−0.01	0.24*	0.17	0.04
C1-inh (g/l)	−0.38**	−0.30*	−0.29*	0.14	0.13	−0.06	−0.28*	−0.21	−0.07

Pre, before surgery; Post, after surgery; Change, after surgery minus before surgery; EKP, endogenous kallikrein potential; KG, kallikrein generation; FXII, factor XII; HK, high-molecular weight kininogen; PK, prekallikrein; C1-inh, C1 esterase inhibitor; r_s, Spearman's rank correlation coefficient.
*** $p < 0.001$.
** $p < 0.01$.
* $p < 0.05$.

Table 4
Correlations between *changes* in metabolic variables and *changes* in contact system biomarkers. Significant correlations are marked in bold.

Variables	ΔBody weight (kg)		ΔTotal chol (mM)		ΔHDL chol (mM)		ΔTriglycerides (mM)		ΔHbA1c (mM)		ΔInsulin (pM)	
	r_S	p	r_S	p	r_S	p	r_S	p	r_S	p	r_s	p
ΔEKP (nM×min)	0.10	0.441	**0.28**	**0.029**	0.04	0.762	**0.34**	**0.007**	−0.20	0.131	−0.07	0.624
ΔKG lagtime (min)	0.12	0.345	−0.06	0.657	0.14	0.282	−0.25	0.054	0.17	0.207	0.19	0.160
ΔPeak KG (nM)	0.04	0.744	0.18	0.164	−0.04	0.755	0.17	0.186	−0.20	0.125	−0.21	0.107
ΔFXII (mg/l)	0.03	0.838	0.02	0.884	0.07	0.615	0.21	0.114	0.22	0.097	0.08	0.573
ΔHK (%)	0.20	0.134	0.15	0.268	0.13	0.331	0.00	0.998	−0.10	0.447	0.05	0.690
ΔPK (μg/ml)	−0.05	0.721	0.24	0.068	0.21	0.103	0.25	0.053	0.10	0.456	0.05	0.715
ΔC1-inh (g/l)	**−0.27**	**0.039**	0.17	0.208	−0.16	0.210	0.12	0.352	**0.48**	**<0.001**	−0.06	0.651

Chol, cholesterol; HDL, high-density lipoprotein; HbA1c, hemoglobin A1c; EKP, endogenous kallikrein potential; KG, kallikrein generation; FXII, factor XII; HK, high-molecular weight kininogen; PK, prekallikrein; C1-inh, C1 esterase inhibitor; r_s, Spearman's rank correlation coefficient.

[25], thereby selectively driving inflammation and not coagulation [17,18]. Consequently, the properties of the contact system activators determine whether the coagulation or inflammatory pathways (or both) are affected.

A strength of our study is the major effect on the contact system after a surgical intervention thereby providing the possibility of addressing associations with intra-individual changes after weight loss and not only absolute values in a cross-sectional design. Study limitations are the high number of drugs and co-morbidities affecting the patients, although medication/no medication was not significant in the multivariate analyses, and the surgery-associated changes were confirmed in patients not receiving any medications.

In conclusion, the contact system was affected 6 months after RYGB. Absolute values of kallikrein generation before and after RYGB were associated with contact system proteins, whereas surgery-related changes were associated with changes in C1-inh and metabolic variables.

CRediT authorship contribution statement

The authors' contributions to the present study were: E. M. Bladbjerg, C. R. Stolberg, and L. H. Mundbjerg: data collection; E. M. Bladbjerg: data analysis; E. M. Bladbjerg, J.J. Sidelmann, J. B. Gram, and Y. Palarasah: data interpretation; E. M. Bladbjerg: drafting of the manuscript. All authors were involved in the concept and design of the study, critical revision of the manuscript, and final approval of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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