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Clinical Research Article

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# Lifestyle Intervention Improves Prothrombotic Coagulation Profile in Individuals at High Risk for Type 2 Diabetes

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**Abbreviations:** aPTT, activated partial thromboplastin time; CRP, C-reactive protein; CVD, cardiovascular disease; F, coagulation factor; OGTT, oral glucose tolerance test; PAI-1, plasminogen activator inhibitor-1; PLIS, Prediabetes Lifestyle Intervention Study; PT%, percentage activity of prothrombin time; VTE, venous thromboembolism; vWF, von Willebrand factor.

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## Abstract

**Context:** Patients with obesity and insulin resistance are at higher risk for arterial and venous thrombosis due to a prothrombotic state.
**Objective:** The present study addressed whether this is reversible by lifestyle intervention and elucidated potential underlying associations.
**Methods:** A total of 100 individuals with impaired glucose tolerance or impaired fasting plasma glucose participated in a 1-year lifestyle intervention, including precise metabolic phenotyping and MRS-based determination of liver fat content as well as a comprehensive analysis of coagulation parameters before and after this intervention.
**Results:** During the lifestyle intervention, significant reductions in coagulation factor activities (II, VII, VIII, IX, XI, and XII) were observed. Accordingly, prothrombin time (PT%)



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and activated partial thromboplastin time (aPTT) were slightly decreased and prolonged, respectively. Moreover, plasminogen activator inhibitor-1 (PAI-1), von Willebrand factor (vWF), and also protein C and protein S decreased. Fibrinogen, antithrombin, D-dimer, and FXIII remained unchanged. Searching for potential regulators, especially weight loss, but also liver fat reduction, improved insulin sensitivity, and decreased low-grade inflammation were linked to favorable changes in hemostasis parameters. Independent of weight loss, liver fat reduction (FII, protein C, protein S, PAI-1, vWF), improved insulin sensitivity (protein S, PAI-1), and reduced low-grade inflammation (PT%, aPTT, FVIII/IX/XI/XII, vWF) were identified as single potential regulators.

**Conclusion:** Lifestyle intervention is able to improve a prothrombotic state in individuals at high risk for type 2 diabetes. Besides body weight, liver fat content, insulin sensitivity, and systemic low-grade inflammation are potential mechanisms for improvements in hemostasis and could represent future therapeutic targets.

**Key Words:** prediabetes, hemostasis, lifestyle intervention, thrombosis, fibrinolysis

Patients with obesity and insulin resistance are at increased risk for arterial and venous thrombosis and subsequent cardiovascular disease (CVD) due to a prothrombotic state and systemic low-grade inflammation (1-3). Thrombosis may result from an imbalance between coagulation and fibrinolysis. The coagulation cascades depend on circulating coagulation factors that activate each other. These cascades result in the conversion of fibrinogen to fibrin and end up in the formation of a fibrin clot. Inhibitors, such as antithrombin or protein C and protein S, limit this process. The fibrin formation is antagonized by the fibrinolysis system that degrades the fibrin clot. This is under the control of plasmin, which builds up from its precursor plasminogen, a process regulated by plasminogen activators and inhibitors such as plasminogen activator inhibitor-1 (PAI-1).

Higher activities of procoagulant parameters, such as vitamin-K dependent coagulation factors (FII, FVII, FIX, and FX), FVIII, or von Willebrand factor (vWF) are well established in obesity and insulin resistance (4-6). These are independent risk factors for venous thromboembolism (VTE), such as deep venous thrombosis and pulmonary embolism (7). Besides the prothrombotic imbalance, hypofibrinolysis is present in obesity and insulin resistance (8, 9). Increased PAI-1 is a marker for hypofibrinolysis and represents a major risk factor for VTE (10, 11). Recently, metabolic syndrome was identified as a high risk condition for VTE recurrence (12). Of note, risk of VTE recurrence increases with the number of these features present in a patient (12). Although the underlying causes for these associations are still under debate, several mechanisms have been proposed that link obesity, insulin resistance, and metabolic syndrome with VTE. Liver steatosis and adipose tissue are assumed contributors to the hemostatic imbalance (2, 13). Indeed, liver steatosis correlates with increased activities of the procoagulant parameters FVIII, FIX, FXI, FXII, and vWF (14, 15). Adipose tissue may directly contribute to the increased risk of VTE by secreting antifibrinolytic parameters (eg, PAI-1) and proinflammatory molecules (eg, interleukin-6). These proinflammatory molecules may stimulate hepatic inflammation and thereby promote secretion of procoagulant factors from the liver (eg, FVIII) (2, 16).

Given these unfavorable alterations that cause substantial morbidity and mortality, there is an urgent need for approaches that are able to revert this prothrombotic state. Several studies investigated the effects of exercise, weight loss, and diet modifications on individual aspects of the hemostasis system (17). However, these data are often restricted to investigations of single parameters and are sometimes contradictory. Furthermore, underlying mechanisms that change hemostasis parameters during lifestyle intervention have been sparsely investigated.

We therefore investigated in individuals at high-risk for type 2 diabetes effects of lifestyle intervention on major components of hemostasis and elucidated potential underlying mechanisms.

## Materials and Methods

### Participants

The present study is a substudy of the Prediabetes Lifestyle Intervention Study (PLIS), a stratified, randomized, multicenter trial, including individuals with prediabetes in Germany (NCT01947595) (18). In the PLIS trial, prediabetes as an inclusion criterion was defined as impaired plasma glucose tolerance and/or increased fasting plasma glucose according to criteria of the American Diabetes Association (19). Further inclusion criteria for

the present analyses were age between 18 and 75 years and body mass index less than 45 kg/m$^2$. Participants were stratified as high-risk and low-risk individuals for the development of type 2 diabetes based on definitions used in previous reports (20, 21). High-risk participants showed reduced insulin sensitivity and insulin secretion as well as increased liver fat content. These individuals participated in either a conventional or an intensive lifestyle intervention. A prerequisite for inclusion in the present analysis was that participants received an intensive lifestyle intervention (see description as follows). Participants randomly assigned to the conventional lifestyle intervention group were excluded from the analysis. Data from 100 participants at high risk for type 2 diabetes who were exclusively included at the study center in Tübingen were randomly selected for the present analysis. None of the included participants reported a previous history of arterial or venous thrombosis (exclusion criteria are listed in Table 1). Participants reported receiving antihypertensive drugs (N = 42), lipid-lowering drugs (N = 15), antiplatelet drugs (N = 10), estrogens (N = 3), or direct oral anticoagulants (N = 2). Data from 2 participants receiving direct oral anticoagulants were excluded from the analyses.

All participants underwent a screening procedure including an oral glucose tolerance test (OGTT) as well as a determination of liver fat content and insulin sensitivity. OGTT and determination of liver fat content was carried out at the beginning of the study and after one year of lifestyle intervention (details see below). Written informed consent was obtained from all participants according to the Declaration of Helsinki. The local ethics committee of the medical faculty of the University of Tübingen approved the study.

**Table 1.** Exclusion criteria for participants included in the present analysis

| Diabetes mellitus type 1 and 2 |
|---|
| Acute coronary syndrome |
| Symptomatic coronary artery disease |
| Chronic kidney disease |
| Active malignant disease |
| Systemic infection |
| Severe mental illness |
| Drug abuse |
| Pregnancy |
| Lactation |
| Elevated liver transaminases (3× above upper limit of reference range) |
| Pulmonary embolism |
| Deep vein thrombosis |

## Lifestyle Intervention

The principal aim of the 1-year lifestyle intervention program was to reach a body weight reduction of more than 5% in all study participants by changing dietary habits as follows: reduction of total fat intake (< 30% of total energy intake), reduction of saturated fat intake (< 10% of total energy intake), and increase in fiber intake (> 15 g/1000 kcal of total energy intake). All participants included in the present analyses were instructed to perform 6 hours of exercise weekly. Each participant received 16 coaching sessions including dietary counseling during the intervention. Adherence to diet and exercise goals was graded according to results of accelerometers and diet protocols.

## Sample Collection and Laboratory Measurements

Blood sample collection was performed using sodium fluoride, lithium heparin, clot activator, and citrate-containing tubes (all from Sarstedt) according to standard operating procedures at the beginning of the study and after 1 year of lifestyle intervention. Blood samples were collected in the morning after an overnight fast and were immediately stored on ice and centrifuged within 30 minutes. Supernatants were subsequently stored at –80 °C degrees. Determination of glucose concentrations and wide range C-reactive protein (CRP) concentrations was performed using a hexokinase method or immunoturbidimetric assay on the ADVIA XPT clinical chemistry analyzer, respectively. Insulin and C-peptide concentrations were determined using the ADVIA Centaur XPT chemiluminescence immunoassay system (all instruments from Siemens Healthineers). Further clinical chemistry parameters (ie, triglycerides, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, creatinine [enzymatic method], urine protein, and urine albumin) were measured using lithium-heparin containing plasma or urine samples on an ADVIA XPT Clinical Chemistry System (Siemens Healthineers). Glycated hemoglobin was determined on a Tosoh G8 HPLC Analyzer (Tosoh Bioscience).

PAI-1 concentration was determined in citrate-containing plasma samples using a commercially available (R & D Systems) enzyme-linked immunosorbent assay.

All coagulation parameters were measured using only once-thawed and thoroughly mixed citrate-containing plasma samples on an Atellica COAG 360 coagulation platform. Samples obtained before and after the lifestyle intervention were processed together. The following reagents were used for coagulation measurements: prothrombin time (PT%): Dade Innovin; activated partial thromboplastin time (aPTT): Actin FS; D-dimer: Innovance D-dimer; protein C activity: Berichrom protein C (chromogenic);

free protein S antigen: Innovance free protein S antigen; antithrombin activity: Innovance antithrombin assay; fibrinogen concentration: Dade Thrombin; vWF activity: Innovance vWF assay; FVIII activity: FVIII chromogenic; FII and FVII: Dade Innovin plus respective coagulation factor poor plasma; FIX, FXI, and FXII: Actin FS plus respective coagulation factor poor plasma; FXIII activity: Berichrom FXIII (chromogenic; instrument and all reagents from Siemens Healthineers).

Clinical chemistry and hemostasis parameters were measured at the Institute for Clinical Chemistry and Pathobiochemistry of the University Hospital Tübingen, which is certified by the German accreditation council and regularly participates in internal and external quality assessments and proficiency testing. All laboratory analyses were performed by thoroughly trained technicians according to local standard operating procedures and manufacturers' instructions.

### Oral Glucose Tolerance Test

Blood glucose concentrations were determined using sodium fluoride–containing tubes (Sarstedt) from all participants during a standardized OGTT before and at 30, 60, 90 and 120 minutes after the ingestion of 75-g glucose (Accu-Check Dextro O.G.T.; Roche).

### Magnetic Resonance Spectroscopy

Magnetic resonance spectroscopy for the quantification of liver fat content was performed after an overnight fast on a 3 Tesla whole-body imager (Magnetom Vida, Siemens Healthineers) in the posterior part of segment 7 applying a single-voxel STEAM technique. Liver fat content was determined as a percentage by the ratio of lipids (methylene + methyl) and water + lipids.

### Statistical Methods

All data are presented as median and interquartile range (first to third quartile). To compare results before and after the lifestyle intervention, the nonparametric paired Wilcoxon-signed rank test was used. Changes of anthropometric, metabolic, and hemostasis parameters were calculated as percentage changes of median results after the lifestyle intervention and before the lifestyle intervention. OGTT-derived insulin sensitivity was calculated using the results of the glucose and insulin measurements during the OGTT (22). Associations between metabolic parameters and hemostasis parameters were calculated using multiple regression analyses. Analyses were performed using change (after lifestyle intervention/before lifestyle intervention) of hemostasis parameter as the dependent variable and change (after lifestyle intervention/before lifestyle intervention) of the metabolic parameter as effect variable. Additionally, age, sex, and baseline levels of the investigated parameters were added to the analyses. Prior to analyses, data were transformed using a logarithmic function. Multicollinearity was assessed by using the variation inflation factor. A variation inflation factor of less than 5 indicates there is no collinearity between the investigated variables. All analyses were performed using JMP software (SAS Institute).

## Results

### Anthropometric and Clinical Characteristics

For this analysis 98 individuals at high risk for type 2 diabetes who participated in the 1-year lifestyle intervention were randomly selected from the PLIS cohort (Table 2 shows characteristics before and after the program). Median age was 58 years (IQR, 52-64 years). During the study participants lost 9.2% body weight, 59.6% liver fat content, and CRP concentration decreased by 41.2%. Fasting glucose (–8.3%), glycated hemoglobin (–4.9%), 2-hour glucose (–16.5%), fasting insulin (–22.0%), and fasting C-peptide concentrations (–25.0%) decreased and OGTT-derived insulin sensitivity (+45.7%) improved. Renal function and urinary albumin excretion did not change. The majority of the participants achieved the body weight (66%), exercise (57%), and fiber intake (64%) intervention goals. In contrast, decrease in total fat intake (34%) and saturated fat intake (25%) was achieved by only a small number of participants (adherence to lifestyle goals is shown in Fig. 1).

### Changes of Hemostasis Parameters During Lifestyle Intervention

Detailed results of the hemostasis parameters are presented in Table 3. The PT% decreased (–4.4%) and aPTT was slightly prolonged (+0.02%). Activities of FII, FVII, FVIII, FIX, and FXI significantly decreased between –9.3% and –2.0%. Furthermore, activities of vWF, protein C, and protein S significantly decreased between –10.3% and –5.7%. PAI-1 concentrations decreased by 44.2%. In contrast, fibrinogen and D-dimer concentrations and activities of antithrombin and FXIII showed no significant changes in response to the lifestyle interventions.

**Table 2.** Characteristics of study participants before the study and after 1-year lifestyle intervention

| | Before | After 1-y lifestyle intervention | Change, % | *P* |
|---|---|---|---|---|
| Sex, n, F/M | 59/39 | | | |
| Age, y | 58 (52-64) | 59 (53-65) | | |
| Body weight, kg | 89.3 (77.4-105.9) | 81.1 (69.5-95.7) | –9.2 | < .001 |
| Body mass index | 31.4 (27.5-34.7) | 28.7 (25.9-32.3) | –8.6 | < .001 |
| $HbA_{1c}$, mmol/mol | 41 (38-43) | 39 (36-41) | –4.9 | < .001 |
| Creatinine, mg/dL | 0.8 (0.7-0.8) | 0.8 (0.7-0.8) | 0.0 | .69 |
| Urine albumin, mg/L | 11 (11-19) | 11 (10-15) | 0.0 | .18 |
| Urine protein, g/L | 0.07 (0.06-0.12) | 0.07 (0.06-0.11) | 0.0 | .08 |
| Fasting glucose, mmol/L | 6.0 (5.7-6.4) | 5.5 (5.1-5.9) | –8.3 | < .001 |
| 2h-glucose, mmol/L | 7.9 (6.9-8.8) | 6.6 (5.6-8.0) | –16.5 | < .001 |
| Liver fat content, %[a] | 8.9 (5.1-14.7) | 3.6 (1.8-7.3) | –59.6 | < .001 |
| C-peptide, fasting, pmol/L[b] | 672 (542-848) | 504 (394-720) | –25.0 | < .001 |
| Insulin, fasting, pmol/L | 82 (59-112) | 64 (45-87) | –22.0 | < .001 |
| OGTT-derived insulin sensitivity[a] | 7.0 (4.8-9.5) | 10.2 (6.6-14.3) | 45.7 | < .001 |
| C-reactive protein, mg/dL[b] | 0.17 (0.06-0.38) | 0.10 (0.03-0.26) | –41.2 | < .001 |

Data are presented as median and interquartile range (first to third). Results before and after the 1-year lifestyle intervention were compared using the paired Wilcoxon-signed rank test.

Abbreviations: 2h-glucose, 2-hour glucose during 75-g oral glucose tolerance test; F, female; $HbA_{1c}$, glycated hemoglobin; M, male; OGTT, oral glucose tolerance test.

Data were available from [a]84 and [b]96 individuals.

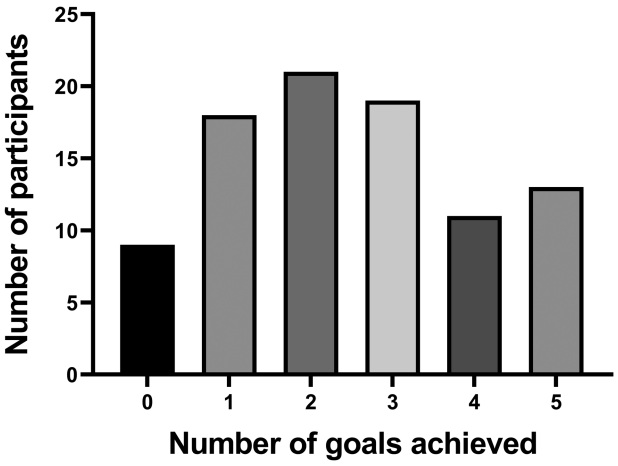


**Figure 1.** Lifestyle intervention goals achieved by the participants. Lifestyle intervention goals were achieved if the indicated goal was achieved on at least half the days during the 1-year lifestyle intervention. Goals of the lifestyle intervention were defined as body weight reduction greater than 5% of initial body weight, reduction of total fat intake (< 30% of total energy intake), reduction of saturated fat intake (< 10% of total energy intake), increase of fiber intake (> 15 g/1000 kcal of total energy intake), and increase in daily exercise (> 8000 meters/d).

### Potential Metabolic Modulators of Improved Hemostasis After Lifestyle Interventions

Weight loss was associated with a decrease in PT%, FII, FVII, FIX, FXI, protein C, protein S, and PAI-1 (details are presented in Table 4). Comparable results were found for improvements in liver fat content, insulin sensitivity, and CRP. Reduction in CRP was also associated with prolonged aPTT and a decrease in FVIII and vWF, with the latter not observed for any other tested parameter. Improved fasting glucose was associated with a decrease in FII, FIX, FXI, protein S, and PAI-1 in response to the lifestyle interventions. The sum of achieved lifestyle goals was associated with a decrease in PAI-1.

### Potential Metabolic Modulators Independent of Weight Loss

To address this, multivariate regression models were performed, adjusting for weight loss as a covariate (see Table 5). Independent of weight loss, reduction of liver fat content was associated with a decrease in FII, protein C, protein S, vWF, and PAI-1. Similarly, improvement of insulin sensitivity was associated with a decrease in protein S and PAI-1. Finally, a decrease in CRP concentrations was associated with a decrease in PT%, aPTT, FVIII, FIX, FXI, FXII, and vWF.

## Discussion

The present study provides insights into beneficial effects on hemostasis parameters in response to lifestyle intervention by comprehensively investigating major parameters of the plasmatic coagulation system. Our results indicate that lifestyle intervention can improve the prothrombotic state in individuals at high-risk for type 2 diabetes. Procoagulant and hypofibrinolytic parameters and also markers of endothelial dysfunction significantly decreased in response to lifestyle intervention. In line, the

**Table 3.** Results of hemostasis measurements before and after 1-year lifestyle intervention in individuals at high risk for type 2 diabetes

| | Before | After 1-y lifestyle intervention | Change, % | *P* |
|---|---|---|---|---|
| PT, % | 91 (82-99) | 87 (80-96) | –4.4 | .007 |
| aPTT, s | 27 (25-29) | 27 (26-30) | 0.02 | < .001 |
| FII, % | 100 (91-110) | 98 (88-107) | –2.0 | < .001 |
| FVII, % | 105 (88-125) | 96 (76-113) | –8.6 | < .001 |
| FVIII, % | 107 (88-123) | 97 (80-111) | –9.3 | < .001 |
| FIX, % | 103 (88-117) | 98 (85-114) | –4.9 | < .001 |
| FXI, % | 106 (97-116) | 102 (92-112) | –3.8 | < .001 |
| FXII, % | 94 (74-109) | 94 (73-107) | –0.01 | .04 |
| FXIII, % | 133 (109-152) | 136 (113-150) | 2.2 | .76 |
| Protein S, % | 105 (93-116) | 97 (89-106) | –7.6 | < .001 |
| Protein C, % | 122 (108-138) | 115 (102-126) | –5.7 | < .001 |
| D-dimer, µg/mL FEU | 0.67 (0.39-0.90) | 0.61 (0.44-0.79) | –9.0 | .25 |
| Antithrombin, % | 98 (93-103) | 98 (91-103) | 0.0 | .34 |
| Fibrinogen, mg/dL | 286 (254-320) | 273 (239-318) | –4.5 | .06 |
| PAI-1, ng/mL | 4.3 (3.1-7.2) | 2.4 (1.8-4.7) | –44.2 | < .001 |
| vWF activity, % | 116 (85-147) | 104 (71-136) | –10.3 | < .001 |

Data are presented as median and interquartile range (first to third). Results before and after the 1-year lifestyle intervention were compared using the paired Wilcoxon-signed rank test.

Abbreviations: aPTT, activated partial thromboplastin time; F, factor; FEU, fibrinogen equivalent units; PAI-1, plasminogen activator inhibitor-1; PT, prothrombin time; vWF, von Willebrand factor.

**Table 4.** Associations between changes of metabolic and hemostasis parameters during 1-year lifestyle intervention

| | Δ Body wt, kg | Δ Liver fat content, %[a] | Δ OGTT-derived insulin sensitivity[b] | Δ fasting glucose, mg/dL | Δ fasting C-peptide, pmol/L[b] | Δ fasting insulin, pmol/L | Δ C-reactive protein, mg/dL[b] | Sum of achieved lifestyle goals[c] |
|---|---|---|---|---|---|---|---|---|
| Δ PT, % | 0.20[d] | 0.25[d] | –0.15[d] | 0.07 | 0.25[e] | 0.23[d] | 0.32[f] | 0.02 |
| Δ aPTT, s | –0.16 | –0.13 | 0.20 | –0.14 | –0.14 | –0.25[d] | –0.29[e] | –0.07 |
| Δ FII, % | 0.44[f] | 0.55[f] | –0.40[f] | 0.26[d] | 0.44[f] | 0.43[f] | 0.23[d] | –0.14 |
| Δ FVII, % | 0.35[f] | 0.23[d] | –0.26[e] | 0.17 | 0.31[e] | 0.31[e] | 0.33[e] | –0.09 |
| Δ FVIII, % | –0.06 | 0.10 | –0.12 | 0.08 | 0.07 | 0.17 | 0.27[d] | 0.10 |
| Δ FIX, % | 0.48[f] | 0.39[f] | –0.33[e] | 0.31[e] | 0.46[f] | 0.36[f] | 0.51[f] | –0.16 |
| Δ FXI, % | 0.35[f] | 0.26[d] | –0.36[f] | 0.23[d] | 0.26[e] | 0.40[f] | 0.31[e] | –0.03 |
| Δ FXII, % | 0.18 | 0.08 | –0.12 | 0.06 | 0.12 | 0.15 | 0.31[e] | 0.00 |
| Δ Protein S, % | 0.37[f] | 0.42[f] | –0.43[f] | 0.28[e] | 0.38[f] | 0.39[f] | 0.08 | –0.02 |
| Δ Protein C, % | 0.37[f] | 0.48[f] | –0.25[d] | 0.12 | 0.35[f] | 0.27[d] | 0.26[d] | –0.08 |
| Δ PAI-1, ng/mL | 0.48[f] | 0.48[f] | –0.51[f] | 0.38[f] | 0.56[f] | 0.55[f] | 0.25[d] | –0.45[f] |
| Δ vWF activity, % | –0.12 | 0.20 | 0.05 | –0.10 | –0.05 | 0.03 | 0.22[d] | 0.16 |

Data were adjusted for age, sex, and baseline levels of the indicated parameters.

Abbreviations: aPTT, activated partial thromboplastin time; F, factor; OGTT, oral glucose tolerance test; PAI-1, plasminogen activator inhibitor-1; PT, prothrombin time; vWF, von Willebrand factor.

Data were available for [a]84, [b]96, and [c]91 participants.

Shown are effect sizes ($\beta_{std}$) for each association. Statistical significance: [d]*P* less than or equal to .05, [e]*P* less than or equal to .01, [f]*P* less than or equal to .001.

global coagulation tests PT% and aPTT decreased and were prolonged, respectively. Fibrinogen, antithrombin, D-dimer, and FXIII remained unchanged. Interestingly, the anticoagulant proteins, protein C and protein S, also decreased. Focusing on the underlying associations, not only weight loss was identified as an important determinant of improvements in hemostasis parameters, but additionally, weight loss–independent effects were

**Table 5.** Associations between metabolic parameters and hemostasis parameters during 1-year lifestyle intervention independent of weight loss

| | Δ Liver fat content, %[a] | Δ OGTT-derived insulin sensitivity[b] | Δ C-reactive protein, mg/dL[b] |
|---|---|---|---|
| Δ PT, % | 0.09 | –0.04 | 0.28[d] |
| Δ aPTT, s | 0.04 | 0.12 | –0.25[c] |
| Δ FII, % | 0.36[c] | –0.16 | 0.07 |
| Δ FVII, % | –0.05 | –0.04 | 0.19 |
| Δ FVIII, % | 0.13 | –0.25 | 0.30[d] |
| Δ FIX, % | 0.13 | –0.02 | 0.40[e] |
| Δ FXI, % | 0.06 | –0.20 | 0.22[c] |
| Δ FXII, % | –0.08 | 0.01 | 0.27[c] |
| Δ Protein S, % | 0.35[d] | –0.34[d] | –0.06 |
| Δ Protein C, % | 0.35[c] | –0.01 | 0.13 |
| Δ PAI-1, ng/mL | 0.36[c] | –0.28[c] | 0.06 |
| Δ vWF activity, % | 0.42[d] | –0.07 | 0.30[c] |

Data were adjusted for age, sex, baseline levels of the indicated parameters, and baseline body weight and change of body weight.
The variation inflation factor was less than 2.5 for all analyses.
Abbreviations: aPTT, activated partial thromboplastin time; F, factor; OGTT, oral glucose tolerance test; PAI-1, plasminogen activator inhibitor-1; PT, prothrombin time; vWF, von Willebrand factor.
Data were available for [a]84 and [b]96 participants.
Shown are effect sizes ($\beta_{std}$) for each association. Statistical significance: [c]*P* less than or equal to .05, [d]*P* less than or equal to .01, [e]*P* less than or equal to .001.

found. In particular, reduced liver fat content, improved insulin sensitivity, and reduced subclinical inflammation may contribute to the beneficial effects of lifestyle intervention.

Effects of lifestyle intervention on isolated aspects of the hemostasis system have been studied before (5, 17, 23-26). The most widely investigated parameter PAI-1 decreased during various lifestyle intervention programs (24, 25, 27-34), a finding that is replicated by our present results, indicating improved fibrinolysis. Two large studies in individuals with impaired glucose tolerance showed no change (24) or only a modest decrease (23) in fibrinogen concentration during lifestyle interventions. This is in line with our results showing no significant change in fibrinogen concentration. Other studies focused on more specific isolated hemostasis parameters including single parameters (eg, FVII and vWF) (17, 26, 30). These studies suggest that lifestyle intervention may reduce activities of procoagulant and hypofibrinolytic parameters, which is also supported by our present results. However, these studies are hardly comparable because of different study designs with diverse numbers of participants, variable intervention protocols, and most important, nonstandardized coagulation assays, thus lacking a comprehensive hemostasis assessment (17, 35). Our unique approach allows the simultaneous investigation of a large number of hemostasis parameters in a very well-defined cohort of individuals with an appropriate sample size using state-of-the-art preanalytical and analytical methodology.

We addressed potential metabolic modulators and underlying mechanisms for improved hemostasis in response to lifestyle intervention. A decrease in liver fat content appears to be an important contributor because it is tightly linked to the decrease in FII and might also affect FIX and PAI-1. These findings extend previous reports from cross-sectional studies on the connection between liver fat and hemostasis parameters (14, 36, 37). Individuals with nonalcoholic fatty liver disease show higher activities of FVIII, FIX, FXI, and FXII compared to controls. Higher PAI-1 concentrations, independent of other metabolic parameters, have been reported in nonalcoholic steatohepatitis (38). Although data about the association of the hemostasis system and liver fat content are sometimes contradictory, it is assumed that patients with nonalcoholic fatty liver disease have a procoagulant imbalance (37, 39), resulting in increased CVD risk (40). Our results underline the relation between liver fat accumulation and the prothrombotic state by demonstrating that reduction of liver fat is indeed linked to improved hemostasis. This was often assumed but has not been tested before.

Importantly, liver fat reduction was found to be associated with a decrease in PAI-1 concentration and vWF activity independent of weight loss. These findings emphasize that the beneficial effects of lifestyle intervention may include mechanisms involving liver metabolism. Furthermore, both parameters are well-established markers of endothelial dysfunction, a key mechanism contributing to the increased cardiovascular risk in obesity and insulin resistance (40, 41). Consequently, a decrease in vWF and PAI-1 by lifestyle intervention suggests improved endothelial function. However, since vWF is associated only with liver fat in the weight loss–adjusted analysis and is mainly secreted by endothelial cells, the precise molecular associations that link liver fat accumulation and hemostasis at the cellular level need to be addressed in future studies.

While subclinical inflammation is a well-known connector between obesity/insulin resistance and cardiovascular risk, the mechanistic link is still not fully understood (13). Altered hemostasis is suspected to play an important role (41), a hypothesis supported by our present findings. Inflamed adipose tissue is thought to be crucial because it secrets major procoagulant and antifibrinolytic parameters (eg, tissue factor and PAI-1) (2) and adipokines, which might also play an important role in the activation and regulation of hemostasis (13). For example, proinflammatory molecules like interleukin-6 induce hepatic inflammation

thereby propagating the hepatic secretion of procoagulant parameters (eg, FVIII) (16). Findings in our study support this pathophysiological link by showing strong associations between systemic low-grade inflammation and changes in FVIII activity. The investigated inflammatory marker, CRP, was not only the most important contributor to the change of FVIII during lifestyle intervention, but was also identified as a major determinant of the decrease in FIX, FXII, and vWF. These parameters are well-recognized risk factors for VTE (7). Consequently, reduction of systemic low-grade inflammation by lifestyle intervention is an important mechanism of reduced risk for arterial and venous thrombosis (2).

Besides these specific links, weight loss is the best investigated factor in regards to hemostasis. The weight loss goal was achieved by the majority of the participants. In our study, weight loss was associated with improvements in major hemostasis parameters, including procoagulant parameters FVII and FVIII, and also FII, FIX, FXI, and FXII, which have not been studied before in detail during lifestyle intervention. Extreme weight loss by bariatric surgery was found to decrease FVII (42), which could contribute to resolving the prothrombotic state (43). Our study adds that even moderate weight loss without surgery has comparable effects. Furthermore, weight loss clearly correlates with a decrease in PAI-1 in our study, which is in line with previous reports on modest as well as significant weight loss (24, 30, 33). Our present study extends these findings on the relation of weight loss and hemostasis as we addressed FII, FIX, and FXI and found marked improvements. All these findings underline the beneficial role of weight loss on the prothrombotic state and thrombotic risk in obesity. Interestingly, decreases in protein C and protein S were also significantly associated with weight loss. Both anticoagulant proteins were reported to be increased in obesity, and this may reflect a compensatory mechanism of the prothrombotic state (44). Additionally weight loss–independent effects were observed for protein C (liver fat and insulin sensitivity) and protein S (liver fat). The underlying mechanisms linking protein C, protein S, and liver fat/insulin sensitivity need to be addressed in future studies.

Lifestyle intervention is one of few approaches to resolving insulin resistance, a state closely linked to diabetes and cardiovascular risk (45). Our present results underline that improvements in insulin sensitivity can ameliorate hemostasis. We found that improved insulin sensitivity was strongly associated with a decrease in PAI-1. Previous data indicate that insulin may directly stimulate PAI-1 synthesis and that improved insulin sensitivity may be causally linked to reduced PAI-1 concentration (24, 46, 47). Subcutaneous adipose tissue was found to be a significant contributor of increased PAI-1 synthesis in obesity and insulin resistance (46). Moreover, insulin and C-peptide concentrations, which both reflect systemic insulin sensitivity, highly correlate with PAI-1 concentration in men with coronary heart disease (48). This indicates a link between insulin resistance, reduced fibrinolysis, and CVD. Improved insulin sensitivity in response to lifestyle intervention was associated with reduced activity of protein S. Although details of the regulation of protein S by metabolism are unknown, a link between insulin sensitivity and protein S was reported in mice (49). Improved insulin sensitivity was further associated with reduced procoagulant parameters (FII, FVIII, FIX, and FXI), demonstrating that improved insulin sensitivity not only has beneficial effects on fibrinolysis but also reduces the procoagulant state in individuals with impaired glucose tolerance. However, data linking diabetes and VTE are still controversial. Several studies have shown that the risk of VTE is increased in diabetes, but other studies have reported that diabetes is only very modestly or not associated with an increased VTE risk after adjusting for confounders (50).

This study also has some limitations. First, the focus of the present analyses was on plasmatic coagulation. However, there are further important regulators of the hemostasis system that need to be considered: platelets, that is, platelet dysfunction, neutrophil extracellular traps, and extracellular vesicles. These are also important contributors to the pathogenesis of arterial and venous thrombosis (2, 51-53). The second limitation includes the lack of a nontreated control group. We studied individuals at high risk for type 2 diabetes receiving an intensive lifestyle intervention. Since lifestyle intervention is the standard treatment of individuals at increased risk for the development of type 2 diabetes (54), a high-risk control group without lifestyle intervention would not be justified. Third, changes of single hemostasis parameters observed in the present study are mainly modest, suggesting a minor clinical relevance. However, considering the sum of all individual changes, a significant clinical impact may be assumed.

In conclusion, this study provides novel insights into the regulation of hemostasis by lifestyle intervention in individuals at high risk for type 2 diabetes. In addition to the well-recognized reduction of diabetes risk, lifestyle intervention has beneficial effects on hemostasis along multiple paths, suggesting that lifestyle intervention improves the prothrombotic state and may thereby reduce the risk for venous and atherothrombotic diseases.

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***Data Availability:*** Restrictions apply to the availability of some or all data generated or analyzed during this study to preserve patient confidentiality or because they were used under license. The corresponding author will on request detail the restrictions and any conditions under which access to some data may be provided.

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