Lifestyle intervention improves prothrombotic coagulation profile in individuals at high-risk for type 2 diabetes

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Abstract

Aims: Patients with obesity and insulin resistance are at higher risk for arterial and venous thrombosis due to a prothrombotic state. If this is reversible by lifestyle intervention was addressed in the current study and potential underlying associations were elucidated.

Subjects and methods: One-hundred individuals with impaired glucose tolerance or impaired fasting plasma glucose participated in a 1-year lifestyle intervention, including precise metabolic phenotyping and MRT-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%) and activated partial thromboplastin time (aPTT) were slightly decreased and prolonged, respectively. Moreover, PAI-1, 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.

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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.

Conclusions: 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

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Introduction

Patients with obesity and insulin resistance are at increased risk for arterial and venous thrombosis and subsequent cardiovascular disease 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 by plasmin which is build 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, the 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

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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 anti-fibrinolytic parameters (e.g. PAI-1) and proinflammatory molecules (e.g. interleukin-6). These proinflammatory molecules may stimulate hepatic inflammation and thereby promote secretion of procoagulant factors from the liver (e.g. 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 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.

Methods

Participants

The present study is a sub-study of the prediabetes lifestyle intervention study (PLIS), a stratified randomized multi-center trial, including individuals with prediabetes in Germany (NCT01947595) (18). In the PLIS trial, prediabetes as inclusion criteria was defined as impaired plasma glucose tolerance and/or increased fasting plasma glucose according to

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criteria of the American Diabetes Association (19). Further inclusion criteria for the present analyses were age between 18 - 75 years and BMI <45 kg/m^2. Participants were stratified in 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 into the present analysis was that participants received an intensive lifestyle intervention (description see below). Participants randomized into 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 to receive antihypertensive drugs (N=42), lipid-lowering drugs (N=15), antiplatelet drugs (N=10), estrogens (N=3) or direct oral anticoagulants (N=2). Data from two 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 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.

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Lifestyle intervention

The principal aim of the 1-year lifestyle intervention program was to reach a body weight reduction of >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), 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 counselling 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, Nümbrecht, Germany) according to standard operating procedures at the beginning of the study and after one 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 (i.e. triglycerides, total cholesterol, low-density

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lipoprotein-cholesterol (LDL-cholesterol), high-density lipoprotein-cholesterol (HDL-cholesterol), creatinine (enzymatic method), urine protein and urine albumin) were measured using lithium-heparin containing plasma on an ADVIA XPT Clinical Chemistry System (Siemens Healthineers, Eschborn, Germany). Glycated hemoglobin (HbA1c) was determined on a Tosoh G8 HPLC Analyzer (Tosoh Bioscience, Griesheim, Germany).

Plasminogen activator inhibitor-1 (PAI-1) concentration was determined in citrate containing plasma samples using a commercially available (R&D Systems, Wiesbaden, Germany) 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 (%): 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; von Willebrand factor (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 that is certified by the German accreditation council and regularly participates at internal and external quality

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assessments and proficiency testing. All laboratory analyses were performed by thoroughly trained technicians according to local standard operating procedures and manufacturer's instructions.

Oral glucose tolerance test

Blood glucose concentrations were determined using sodium fluoride containing tubes (Sarstedt) from all participants during a standardized oral glucose tolerance test 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 quantification of liver fat content was performed after an overnight fast on a 3 T whole-body imager (Magnetom Vida, Siemens Healthineers, Erlangen, Germany) in the posterior part of segment 7 applying a single-voxel STEAM technique. Liver fat content was determined in % by the ratio of lipids (methylene+methyl) and water+lipids.

Statistical methods

All data are presented as median and interquartile range (1^{st} and 3^{rd} quartile). To compare results before and after the lifestyle intervention, the non-parametric paired Wilcoxon-signed rank test was used. Changes of anthropometric, metabolic and hemostasis parameters were calculated as percentage changes of median results after lifestyle

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intervention and before lifestyle intervention. OGTT-derived insulin sensitivity was calculated using results of 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 dependent variable and changes (after lifestyle intervention /before lifestyle intervention) of metabolic parameter as effect variables. 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 (VIF). A VIF <5 indicates that there is no collinearity between the investigated variables. All analyses were performed using JMP software (SAS Institute, Cary, United States).

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 out of the PLIS cohort (table 2 shows characteristics before and after the program). Median age was 58 years (IQR 52 – 64). During the study they lost 9.2% body weight, 59.6% liver fat content and C-reactive protein concentration decreased by 41.2%. Fasting glucose (-8.3%), glycated hemoglobin (-4.9%), 2h-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

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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 figure 1).

Changes of hemostasis parameters during lifestyle intervention

Detailed results of hemostasis parameters are presented in table 3. The percentage activity of prothrombin time decreased (PT%; -4.4%) and activated partial thromboplastin time slightly prolonged (aPTT; +0.02%). Activities of FII, FVII, FVIII, FIX and FXI significantly decreased between -9.3% and -2.0%. Furthermore, activities of von-Willebrand-Factor (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 lifestyle intervention.

Potential metabolic modulators of improved hemostasis after lifestyle intervention

Weight loss was associated with 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 decrease in FVIII and vWF, with the latter not observed for any other tested parameter. Improved fasting glucose was associated with decrease in FII, FIX, FXI, protein S and PAI-1 in response to lifestyle intervention. The sum of achieved lifestyle goals was associated with decrease in PAI-1.

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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 decrease in FII, protein C, protein S, vWF and PAI-1. Similarly, improvement of insulin sensitivity was associated with decrease in protein S and PAI-1. Finally, decrease in CRP concentrations was associated with 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 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 important determinant of improvements in hemostasis parameters, but additional, weight loss independent effects were found. In particular, reduced liver fat content, improved insulin sensitivity and reduced subclinical inflammation may contribute to the beneficial effects of lifestyle intervention.

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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 current results, indicating improved fibrinolysis. Two large studies in individuals with impaired glucose tolerance showed no change (24) or only modest decrease (23) in fibrinogen concentration during lifestyle interventions. This is in line with our results showing no significant change in fibrinogen concentration. Some other studies focused on more specific isolated hemostasis parameters including single parameters (e.g. 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 due to different study designs with diverse numbers of participants, variable intervention protocols and most importantly non-standardized coagulation assays, thus lacking a comprehensive hemostasis assessment (17,35). Our unique approach allows the simultaneously 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 pre-analytical and analytical methodology.

We addressed potential metabolic modulators and underlying mechanisms for improved hemostasis in response to lifestyle intervention. Decrease in liver fat content appears to be an important contributor, as it is tightly linked to the decrease in FII and might also impact on 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 NAFLD show higher activities of FVIII, FIX, FXI and FXII compared to controls. Higher PAI-1 concentrations, independently of other metabolic parameters, have been reported in non-

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alcoholic steatohepatitis (NASH) (38). Although data about the association of the hemostasis system and liver fat content are sometimes contradictory, it is assumed that patients with NAFLD have a procoagulant imbalance (37,39), resulting in increased CVD risk (40). Our results underline the relation between liver fat accumulation and 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 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, decrease in vWF and PAI-1 by lifestyle intervention suggests improved endothelial function. However, since vWF is only associated with liver fat in the weight loss adjusted analysis and mainly secreted by endothelial cells the precise molecular associations that link liver fat accumulation and hemostasis on the cellular level needs 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 current findings. Inflamed adipose tissue is thought to be crucial as it secrets major procoagulant and anti-fibrinolytic parameters (e.g. 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

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inflammation thereby propagating the hepatic secretion of procoagulant parameters (e.g. 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 major determinant of 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 resolve a prothrombotic state (43). Our study adds that even moderate weight loss without surgery has comparable effects. Furthermore, weight loss clearly correlates with 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 current 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, decrease in protein C and protein S was 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

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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 resolve insulin resistance, as state closely linked to diabetes and cardiovascular risk (45). Our current results underline that improvements in insulin sensitivity can ameliorate hemostasis. We found that improved insulin sensitivity was strongly associated with 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, that 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, considering the link between diabetes and venous thromboembolism data 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 modest or not associated with an increased VTE risk after adjusting for confounders (50).

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The study is also subject to some limitations. First, the focus of the present analyses was on plasmatic coagulation. However, there are further important regulators of the hemostasis system which need to be considered: platelets, i.e. platelet dysfunction, neutrophil extracellular traps (NET) and extracellular vesicles (EV). These are also important contributors to the pathogenesis of arterial and venous thrombosis (2,51-53). The second limitation includes the lack of a non-treated 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 current 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. Besides 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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Figure legends

Figure 1: Lifestyle intervention goals achieved by the participants. Lifestyle intervention goals were achieved if the indicated goal was achieved at least on half of the days during the 1-year lifestyle intervention. Goals of the lifestyle intervention were defined as: body weight reduction >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 (>8000m/day).

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Tables

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 (3x above the upper limit of the reference range)
Pulmonary embolism
Deep vein thrombosis

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

	Before	After 1-year lifestyle intervention	Change	p-value
Sex (n, f/m)	59/39			
Age (years)	58 (52 – 64)	59 (53 – 65)		
Body weight (kg)	89.3 (77.4 – 105.9)	81.1 (69.5 – 95.7)	-9.2%	<0.0001
Body-Mass-Index (kg/m^2)	31.4 (27.5 – 34.7)	28.7 (25.9 – 32.3)	-8.6%	<0.0001
Glycated hemoglobin (HbA1c, mmol/mol)	41 (38 – 43)	39 (36 – 41)	-4.9%	<0.0001
Creatinine (mg/dl)	0.8 (0.7 – 0.8)	0.8 (0.7 – 0.8)	0.0%	0.687
Urine albumin (mg/l)	11 (11 – 19)	11 (10 – 15)	0.0%	0.1838
Urine protein (g/l)	0.07 (0.06 – 0.12)	0.07 (0.06 – 0.11)	0.0%	0.0771
Fasting glucose (mmol/l)	6.0 (5.7 – 6.4)	5.5 (5.1 – 5.9)	-8.3%	<0.0001
2h-glucose (mmol/l)	7.9 (6.9 – 8.8)	6.6 (5.6 – 8.0)	-16.5%	<0.0001
Liver fat content (%) [A]	8.9 (5.1 – 14.7)	3.6 (1.8 – 7.3)	-59.6%	<0.0001
C-peptide, fasting (pmol/l) [B]	672 (542 – 848)	504 (394 – 720)	-25.0%	<0.0001
Insulin, fasting (pmol/l)	82 (59 – 112)	64 (45 – 87)	-22.0%	<0.0001
OGTT-derived insulin sensitivity [A]	7.0 (4.8 – 9.5)	10.2 (6.6 – 14.3)	45.7%	<0.0001
C-reactive protein (mg/dl) [B]	0.17 (0.06 – 0.38)	0.10 (0.03 – 0.26)	-41.2%	<0.0001

Data were available from the following numbers of individuals: [A] 84, [B] 96.

Data are presented as median and interquartile range (1st – 3rd).

Results before and after the 1-year lifestyle intervention were compared using the paired Wilcoxon-signed rank test.

Abbreviations: f: female; m: male; 2h-glucose: 2h-glucose during 75g-oral glucose tolerance test; OGTT: oral glucose tolerance test.

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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-year lifestyle intervention	Change	p-value
PT [%]	91 (82 – 99)	87 (80 – 96)	-4.4%	0.0073
aPTT [s]	27 (25 – 29)	27 (26 – 30)	0.02%	0.0003
FII [%]	100 (91 – 110)	98 (88 – 107)	-2.0%	<0.0001
FVII [%]	105 (88 – 125)	96 (76 – 113)	-8.6%	<0.0001
FVIII [%]	107 (88 – 123)	97 (80 – 111)	-9.3%	<0.0001
FIX [%]	103 (88 – 117)	98 (85 – 114)	-4.9%	<0.0001
FXI [%]	106 (97 – 116)	102 (92 – 112)	-3.8%	0.0002
FXII [%]	94 (74 – 109)	94 (73 – 107)	-0.01%	0.0371
FXIII [%]	133 (109 – 152)	136 (113 – 150)	2.2%	0.7569
Protein S [%]	105 (93 – 116)	97 (89 – 106)	-7.6%	<0.0001
Protein C [%]	122 (108 – 138)	115 (102 – 126)	-5.7%	<0.0001
D-Dimer [µg/ml FEU]	0.67 (0.39 – 0.90)	0.61 (0.44 – 0.79)	-9.0%	0.2500
Antithrombin [%]	98 (93 – 103)	98 (91 – 103)	0.0%	0.3382
Fibrinogen [mg/dl]	286 (254 – 320)	273 (239 – 318)	-4.5%	0.0627
PAI-1 [ng/ml]	4.3 (3.1 – 7.2)	2.4 (1.8 – 4.7)	-44.2%	<0.0001
vWF activity [%]	116 (85 – 147)	104 (71 – 136)	-10.3%	0.0002

Data are presented as median and interquartile range (1^{st} – 3^{rd}).

Results before and after the 1-year lifestyle intervention were compared using the paired Wilcoxon-signed rank test.

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

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

	Δ Body weight [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*	0.25*	-0.15*	0.07	0.25**	0.23*	0.32***	0.02
Δ aPTT [s]	-0.16	-0.13	0.20	-0.14	-0.14	-0.25*	-0.29**	-0.07
Δ FII [%]	0.44***	0.55***	-0.40***	0.26*	0.44***	0.43***	0.23*	-0.14
Δ FVII [%]	0.35***	0.23*	-0.26**	0.17	0.31**	0.31**	0.33**	-0.09
Δ FVIII [%]	-0.06	0.10	-0.12	0.08	0.07	0.17	0.27*	0.10
Δ FIX [%]	0.48***	0.39***	-0.33**	0.31**	0.46***	0.36***	0.51***	-0.16
Δ FXI [%]	0.35***	0.26*	-0.36***	0.23*	0.26**	0.40***	0.31**	-0.03
Δ FXII [%]	0.18	0.08	-0.12	0.06	0.12	0.15	0.31**	0.00
Δ Protein S [%]	0.37***	0.42***	-0.43***	0.28**	0.38***	0.39***	0.08	-0.02
Δ Protein C [%]	0.37***	0.48***	-0.25*	0.12	0.35***	0.27*	0.26*	-0.08

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Δ PAI-1 [ng/ml]	0.48***	0.48***	-0.51***	0.38***	0.56***	0.55***	0.25*	-0.45***
Δ vWF activity [%]	-0.12	0.20	0.05	-0.10	-0.05	0.03	0.22*	0.16

Data were available from the following number of participants: [A] 84, [B] 96, [C] 91.

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

Shown are effect sizes ($ß_{std}$) for each association; Asterisks indicate statistical significance: *p≤0.05, **p≤0.01, ***p≤0.001.

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

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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**
Δ aPTT [s]	0.04	0.12	-0.25*
Δ FII [%]	0.36*	-0.16	0.07
Δ FVII [%]	-0.05	-0.04	0.19
Δ FVIII [%]	0.13	-0.25	0.30**
Δ FIX [%]	0.13	-0.02	0.40***
Δ FXI [%]	0.06	-0.20	0.22*
Δ FXII [%]	-0.08	0.01	0.27*
Δ Protein S [%]	0.35**	-0.34**	-0.06
Δ Protein C [%]	0.35*	-0.01	0.13
Δ PAI-1 [ng/ml]	0.36*	-0.28*	0.06
Δ vWF activity [%]	0.42**	-0.07	0.30*

Data were available from the following number of participants: [A]84, [B]96.

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

Shown are effect sizes ($ß_{std}$) for each association; Asterisks indicate statistical significance: *p≤0.05, **p≤0.01, ***p≤0.001.

The variance inflation factor (VIF) was <2.5 for all analyses.

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

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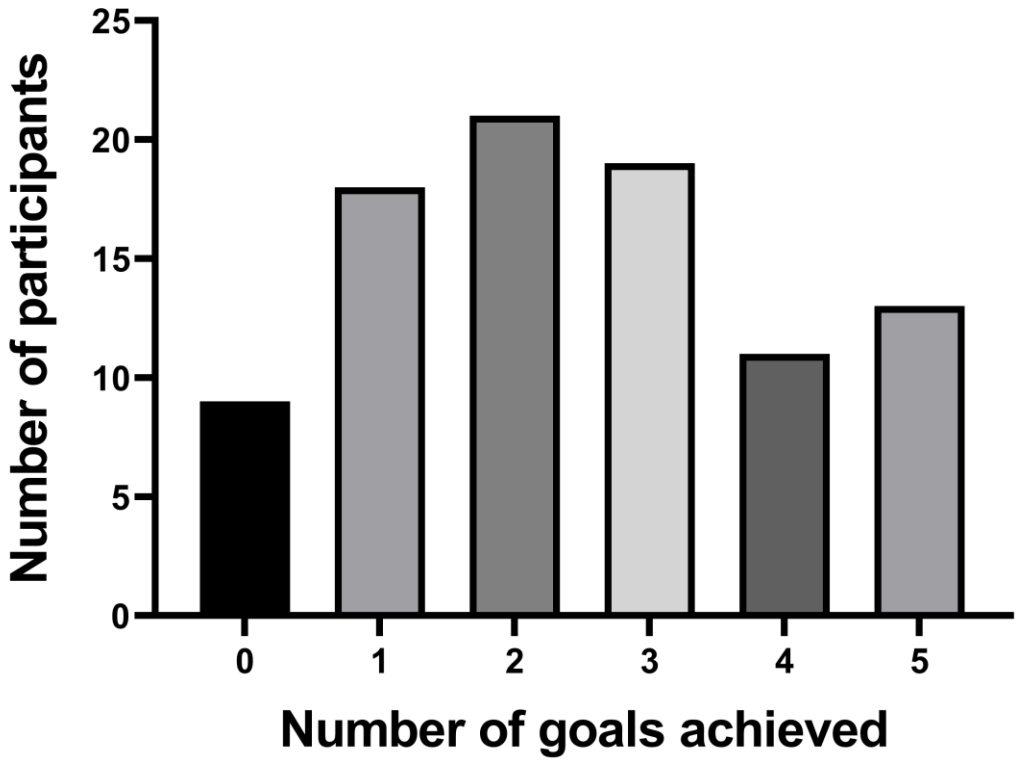
Figure 1



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