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Clinical Research Article

Effect of Breastfeeding Duration on Coagulation in Women With and Without History of Gestational Diabetes Mellitus

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Abstract

Context: Breastfeeding is associated with a reduced maternal risk for cardiovascular diseases (CVDs).

Objective: Since the underlying mechanisms are still poorly understood, we here examined the effect of breastfeeding on the plasmatic coagulation system in women with and without history of gestational diabetes mellitus (GDM).

Methods: A total of 76 participants of the German Gestational Diabetes Study (PREG; NCT04270578) were examined 14 months (interquartile range [IQR], 12-26 months) after delivery with a 5-point oral glucose tolerance test. Global coagulation tests, prothrombotic coagulation proteins (FII/FVII/FVIII/FIX), antithrombotic proteins (antithrombin, protein C/S), and endothelial markers (von Willebrand factor and plasminogen activator inhibitor 1) were determined. The Framingham risk score was used to estimate the 10-year CV risk. The effect of breastfeeding duration on coagulation was analyzed using multivariable linear models.

Results: The mean duration of breastfeeding was 11 months (IQR, 7-14 months). Overall, longer duration of breastfeeding was associated with lower CV risk (Framingham risk score; $P = .05$) and was negatively associated with FIX ($P = .018$). We detected an interaction between previous GDM and breastfeeding duration for FIX ($P_{\text{Interaction}} = .017$): Only in women with GDM history was the duration of breastfeeding negatively associated with FIX activity ($P = .016$). This association persisted in statistical models adjusted for age, body mass index, insulin sensitivity, and C-reactive protein. The duration of breastfeeding was not associated with anticoagulant proteins and endothelial markers.

Conclusion: Longer duration of breastfeeding is associated with lower CV risk and an improved coagulation profile. Women with GDM history appear to benefit particularly from prolonged breastfeeding.

Key Words: gestational diabetes, hemostasis, coagulation, cardiovascular risk, Framingham risk score, breastfeeding

Abbreviations: aPTT, activated partial thromboplastin time; BMI, body mass index; BP, blood pressure; CRP, C-reactive protein; CVD, cardiovascular disease; GDM, gestational diabetes mellitus; GEE, generalized estimating equation; HbA_{1c}, glycated hemoglobin A_{1c}; HDL, high-density lipoprotein; INR, international normalized ratio; NGT, normal glucose tolerance; PAI-1, plasminogen activator inhibitor 1; PREG, German Gestational Diabetes Study.

Breastfeeding has many well-documented short- and long-term advantages for the child. Cumulating evidence indicates that breastfeeding also has beneficial effects for the mother. Meta-analyses have demonstrated robust associations of breastfeeding and reduced maternal risks for type 2 diabetes, breast cancer, and ovarian cancer (1, 2). Moreover, a recent meta-analysis demonstrated that breastfeeding is associated with reduced maternal risk for cardiovascular diseases (CVD) (3). Longer duration of breastfeeding is linked to decreased maternal risk for various CV events, including coronary heart disease, stroke, and fatal CVD (4, 5). Women with history of gestational diabetes mellitus (GDM) are at increased risk not only for type 2 diabetes but also for CVD. In these women, breastfeeding appears to particularly reduce maternal CV risk (6). Several studies indicate that women with GDM history benefit from breastfeeding in term of

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reduced CV risk factors, such as body mass index (BMI), hypertension, dyslipidemia, and in terms of reduced risk for CV events (6).

Several underlying mechanisms for the epidemiological link between breastfeeding and reduced CV risk have been proposed (3). Hormones that are increased during lactation, such as prolactin and oxytocin, may improve blood pressure (BP) with subsequent benefits for maternal CV risk (7, 8). Another hypothesis is that breastfeeding itself increases energy turnover and thereby leads to a significant weight loss that could improve the CV risk profile. Arguing against a major contribution of this are inconclusive or contradictory results on the effect of breastfeeding on body weight (9). Furthermore, changes in the maternal postpartum metabolism are also discussed as causes of the improved CV profile of breastfeeding women (10). Despite a number of theories, specific links explaining the beneficial effects of breastfeeding for maternal CVD risk are still poorly understood.

Alterations in the coagulation system are closely linked to CVD and metabolic diseases (11). A prothrombotic tendency favors the development of CVDs (12, 13). In particular, there is a close relationship between the coagulation system and the risk for atherothrombosis. Coagulation proteins and markers of endothelial dysfunction (eg, von Willebrand factor [vWF]) play a central role in the progression of atherothrombosis and may thereby contributing to an increased CVD risk (14, 15). Furthermore, metabolic disturbances like insulin resistance, obesity, and subclinical inflammation, which are well-established CV risk factors, predispose individuals to a prothrombotic state (16).

Therefore, we aimed to clarify the effect of breastfeeding on the plasmatic coagulation system of women with and without history of GDM.

Materials and Methods

Study Design and Participants

The present study is part of the German Gestational Diabetes study (PREG), a prospective multicenter cohort study (NCT04270578) (17). In brief, the aim of this study is to precisely characterize women with and without GDM for metabolic and phenotypically variables during and repeatedly after pregnancy. For the present analyses, 80 breastfeeding women were randomly selected from study participants who were recruited at the study center in Tübingen, Germany. Results were obtained from clinical and laboratory examinations at the first available follow-up visit after pregnancy. Due to clots in the coagulation tube, 4 participants were additionally excluded from the analyses.

Informed written consent was obtained from all study participants before study enrollment. The study was conducted according to the Declaration of Helsinki and approved by the local ethics committee of the University Hospital Tübingen (protocol No. 218/2012BO2).

Clinical and Laboratory Examinations

GDM was diagnosed according to recommendations of the German Diabetes Association and the World Health Organization (18, 19). All participants underwent a 5-point, 2-hour oral glucose tolerance test using 75-g glucose intake at the beginning. Venous blood samples were taken at 0, 30, 60, 90, and 120 minutes. Insulin sensitivity was assessed according to the formula by Matsuda and DeFronzo (20). The Framingham risk score was calculated using clinical (age [validated range, 30-79 years], sex, smoking, use of antihypertensive drugs, and systolic BP) and laboratory parameters (total cholesterol and high-density lipoprotein [HDL] cholesterol) with the R library CV risk (21, 22). Weight and height of participating women were measured using a calibrated scale (Seca GmbH & Co KG) and a wall-mounted stadiometer. Percentage body fat was determined by bioimpedance measurement with an Akern BIA101 and calculated with BodyGramPRO software (both from SMT medical GmbH & Co). Assessment of breastfeeding duration and intensity was performed retrospectively with a questionnaire described in (17). BP was measured with an automated sphygmomanometer (BOSO Carat Professional, Bosch + Sohn GmbH & Co). The level of physical activity was assessed by the habitual physical activity score (23).

Blood samples were collected using sodium-fluoride, lithium-heparin, clot activator, as well as citrate-containing tubes (all from Sarstedt) after an overnight fast. All samples were immediately stored on ice, centrifuged within 30 minutes, and subsequently analyzed. All laboratory analyses were performed according to manufacturer instructions by trained laboratory technicians: Glucose and C-reactive protein (wide range CRP; Siemens catalog No. 02230141, RRID:AB_2909499) concentrations were determined using a hexokinase method and an immunoturbidimetric assay on an ADVIA XPT clinical chemistry analyzer (Siemens Healthineers), respectively. Insulin (Siemens catalog No. 02230141, RRID:AB_2909499) and C-peptide (Siemens catalog No. 02230141, RRID:AB_2909499) concentrations were analyzed on an ADVIA Centaur XPT chemiluminescence immunoassay system (Siemens Healthineers). Further parameters (ie, total cholesterol, low-density lipoprotein-cholesterol, HDL cholesterol, triglycerides, nonesterified fatty acids, creatinine [enzymatic method]) were measured using lithium-heparin or sodium-fluoride–containing plasma on an ADVIA XPT Clinical Chemistry System. Glycated hemoglobin A_{1c} (HbA_{1c}) was determined using a high-performance liquid chromatography method on a Tosoh G8 Analyzer (Tosoh Bioscience).

For coagulation measurements, supernatants of citrate containing samples were stored at −80 °C until analysis. Citrate plasma samples were only once thawed and the following reagents were used: Dade Innovin: prothrombin time (%); Actin FS: activated partial thromboplastin time (aPTT); Innovance D-Dimer (Siemens Healthineers catalog No. OPBP03, RRID:AB_3083793): D-Dimer; Berichrom protein C (chromogenic): protein C activity; Innovance free protein S antigen (Siemens Healthineers catalog No. OPGL023, RRID:AB_3083792): free protein S antigen; Innovance antithrombin assay: antithrombin activity; Dade Thrombin: fibrinogen concentration; Innovance vWF assay (Siemens Healthineers catalog No. OPHL03, RRID:AB_3083790): vWF activity; FVIII chromogenic: FVIII activity; Dade Innovin plus respective coagulation factor poor plasma: FII and FVII; Actin FS plus respective coagulation factor poor plasma: FIX (all reagents from Siemens Healthineers). All coagulation measurements were performed on an Atellica COAG 360 coagulation analyzer (Siemens Healthineers). Plasminogen activator inhibitor 1 (PAI-1; R and D Systems catalog No. DSE100, RRID:AB_3083789) concentrations were measured in citrate-containing plasma samples using a commercially available enzyme-linked immunosorbent assay (R&D Systems).

Statistical Analysis

Results are presented as means ± SDs for normally distributed variables or as means and interquartile ranges (IQRs) for nonnormally distributed variables. Statistical analyses were performed using R version 4.1.0. Associations between breastfeeding duration, Framingham risk score and levels of coagulation parameters were analyzed using multiple linear regression analyses and generalized estimating equation (GEE) models (R packages gee version 4.13-20 and geepack version 1.3-2). Nonnormally distributed results of laboratory parameters were log-transformed prior to analysis. *P* values less than .05 were considered statistically significant.

Results

Clinical Characteristics of Study Participants

A total of 76 breastfeeding women were included in the final analysis. Clinical and laboratory characteristics of all participants were obtained 14 months (IQR, 12-26 months) after pregnancy and are shown in Table 1. Among them, 42 women (55%) had a history of GDM. The mean duration of breastfeeding was 11 months (IQR, 7-14 months). Women with a history of GDM had a significantly shorter mean duration of breastfeeding (9 months [6-13 months]) compared to women with normal glucose tolerance in pregnancy (NGT, 12 months [9-16 months]). Furthermore, women with a history of GDM were older (36.3 ± 4.9 years) compared to NGT women (33.3 ± 5.1 years; $P = .010$). BMI, percentage body fat, HbA_{1c}, insulin sensitivity, and CRP concentrations were not significantly different between women with or without history of GDM. Moreover, there were no differences in smoking, educational level, and physical activity between women with and without a history of GDM (Table 2). Evaluating the plasmatic coagulation profile, no differences were observed between both groups in any of the assessed coagulation parameters (data not shown).

Effect of Breastfeeding Duration on Cardiovascular Risk and Coagulation

In a first step, the relation between the duration of breastfeeding and CV risk, assessed as Framingham risk score, was analyzed: Longer duration of breastfeeding was associated with a lower Framingham risk score in the total cohort (N = 66; $P = .05$, Fig. 1A). Women with a history of GDM had a significantly higher median Framingham risk score (2.63) compared to women with in-pregnancy NGT (1.90; $P = .031$, see Table 2). Analyzing the components of the Framingham risk score, age was the variable that was significantly different between both groups ($P = .020$).

Next, the association between the duration of breastfeeding and coagulation parameters was analyzed. Longer durations of breastfeeding were significantly associated with reduced activities of coagulation factor FIX in the total cohort ($P = .018$, see Fig. 1C). Moreover, a history of GDM was found to interact with the duration of breastfeeding on FIX activity ($P_{interaction} = .017$; see Fig. 1D): A statistically significant association between longer duration of breastfeeding and reduced activities of coagulation factor FIX was present only in breastfeeding women with a history of GDM ($P = .016$), but not in women with in-pregnancy NGT ($P = .242$). Adjusting for the potential confounders age, BMI, CRP, and insulin sensitivity, the significant association in breastfeeding women with GDM history persisted (Table 3).

Table 1. Clinical characteristics of study participants

	Total cohort (N = 76)	History of NGT in pregnancy (N = 34)	History of GDM (N = 42)	*P*
Age, y	34.9 (5.2)	33.3 (5.1)	36.3 (4.9)	**.010**
BMI	27.3 (5.4)	27.1 (4.9)	27.4 (5.9)	.857
Body fat, %	37.2 (9.8)	37.9 (9.0)	36.7 (10.5)	.614
Breastfeeding duration, mo	11.0 (7.0-14.0)	12.0 (9.0-15.5]	9.0 (6.0-12.75)	**.024**
Time after delivery, mo	13.9 (12.5-25.4)	13.7 (12.4-16.6)	15.1 (12.5-45.1)	.204
Parity				
1st child	35 (46.1)	22 (64.7)	13 (31.0)	**.007**
2nd^{+} child	41 (53.9)	12 (35.3)	29 (69.0)	
Glycemic state at current examination				
NGT	56 (73.7)	25 (73.5)	31 (73.8)	.527
IFG	9 (11.8)	3 (8.8)	6 (14.3)	
IGT	10 (13.2)	6 (17.6)	4 (9.5)	
IGF + IGT	1 (1.3)	0 (0.0)	1 (2.4)	
HbA_{1c}, %	5.47 (0.3)	5.42 (0.30)	5.51 (0.30)	.214
Insulin sensitivity (ISI-Matsuda)	12.06 (8.38-16.32)	12.93 (7.48-17.29)	11.98 (8.91-14.66)	.484
C-reactive protein	0.08 (0.03-0.39)	0.08 (0.04-0.3)	0.1 (0.03-0.42)	.863
Smoking (%)	6 (7.9)	1 (2.9)	5 (11.9)	.311
Higher education (%)	36 (47.4)	18 (52.9)	18 (42.9)	.519
Habitual physical activity	8.31 (1.22)	8.61 (1.30)	8.07 (1.11)	.055

Group differences were tested with *T* test for normally distributed variables, Kruskal-Wallis-test for nonnormally distributed variables and χ^2 test for categorical variables. Bold values indicate statistical significance.
Abbreviations: BMI, body mass index; GDM, women with history of gestational diabetes mellitus; HbA_{1c}, glycated hemoglobin A_{1c}; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; ISI, insulin sensitivity index; NGT, normal glucose tolerance.

Global coagulation parameters (Quick/international normalized ratio and aPTT), fibrinogen, D-dimer, the antithrombotic parameters antithrombin, protein C, and protein S, and the endothelial markers vWF and PAI-1 showed no statistically significant associations with breastfeeding duration (see Table 3).

Table 2. Framingham Risk Score and its components in women with and without history of gestational diabetes mellitus (n = 66)

	History of NGT in pregnancy (N = 28)	History of GDM (N = 38)	*P*
Framingham Risk Score	1.90 (1.10)	2.63 (1.48)	**.031**
Age, y	34.6 (4.6)	37.2 (4.2)	**.020**
BMI	27.7 (5)	27.8 (6)	.955
HDL cholesterol, mg/dL	54 (12)	53 (11)	.639
Total cholesterol, mg/dL	178 (28)	177 (35)	.869
$BP_{systolic}$	123 (16)	126 (13.2)	.362
Smoking, %	0 (.0)	4 (10.5)	.212
Antihypertensive medication, %	1 (3.6)	1 (2.6)	≥.999

Group differences were tested with *T* test for normally distributed variables, Kruskal-Wallis-test for nonnormally distributed variables and χ^2-test for categorical variables. Bold values indicate statistical significance.
Abbreviations: BMI, body mass index; BP, blood pressure; GDM, women with history of gestational diabetes mellitus; HDL, high-density lipoprotein; NGT, normal glucose tolerance.

Relationship Between Cardiovascular Risk and Coagulation Parameters in Breastfeeding Women

Next, we tested the relationship between coagulation parameters and Framingham risk score. We found significant associations between the Framingham risk score and coagulation factor IX, free protein S antigen, and PAI-1 (Table 4). In detail, elevated activities of coagulation factor IX and increased concentrations of free protein S antigen and PAI-1 were associated with increased Framingham risk scores in breastfeeding women (Fig. 2). In a final model, adjusting for relevant confounders, only protein S revealed a statistically significant association with Framingham risk score.

Discussion

We investigated the link between breastfeeding and CVD risk by testing the effect of breastfeeding duration on the plasmatic coagulation system in women with and without a history of GDM.

First, we found that longer duration of breastfeeding was related to a lower estimated 10-year CV risk. This suggests beneficial health effects of longer breastfeeding for the maternal CVD risk profile. Second, longer duration of breastfeeding was associated with reduced plasmatic activity of prothrombotic coagulation factor IX, suggesting an

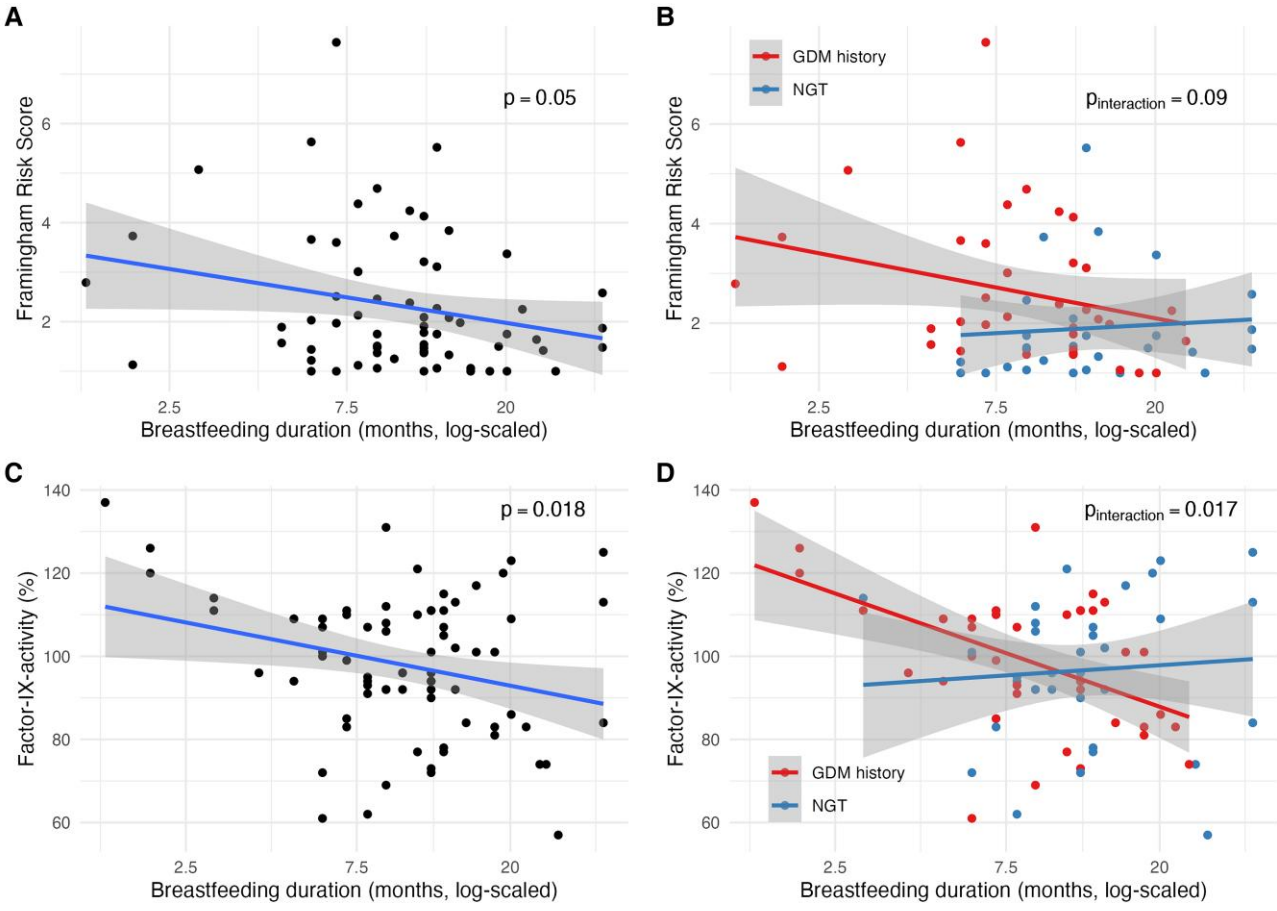

Figure 1. Association of breastfeeding duration with Framingham Risk Score and factor IX activity in the total cohort (A and C, N = 66) and separately for breastfeeding women with a history of gestational diabetes mellitus (GDM) and women with a normoglycemic pregnancy (NGT; B and D).

Table 3. Associations of coagulation parameters with duration of breastfeeding and Framingham Risk Score in women with history of gestational diabetes mellitus or normal glucose tolerance in pregnancy

	Breastfeeding duration, GDM	Breastfeeding duration, NGT
Quick	−2.660	0.598
INR	0.023	−0.009
aPTT	0.834	−0.497
Fibrinogen	−16.487	8.809
Antithrombin	1.468	4.501
D-dimer	−0.115	0.268
Factor II	−5.703	−1.929
Factor VII	−2.672	1.096
Factor VIII	0.088	0.044
Factor IX	**−6.132**[a]	4.629
Protein C	−2.871	−0.915
Protein S	−0.501	−2.411
vWF activity	6.065	4.603
PAI-1	−0.047	0.191

Shown are effect sizes of multivariate linear models adjusted for age, body mass index, C-reactive protein, and insulin sensitivity.
Abbreviations: aPTT, activated partial thromboplastin time; GDM, women with history of gestational diabetes mellitus; INR, international normalized ratio; NGT, normal glucose tolerance; PAI-1, plasminogen activator inhibitor 1; vWF, von Willebrand factor.
[a]Statistical significance: *P* less than or equal to .05.

Table 4. Associations of coagulation parameters with Framingham Risk Score

	Framingham Risk Score (univariate)	Framingham Risk Score (GEE model)
Quick	0.015	0.002
INR	−1.955	0.195
aPTT	−0.014	0.051
Fibrinogen	0.003	5.36×10^{-4}
Antithrombin	−0.016	−0.014
D-dimer	−0.120	0.517
Factor II	0.011	−0.003
Factor VII	0.008	−0.005
Factor VIII	−0.537	−0.008
Factor IX	**0.025**[a]	0.003
Protein C	0.009	−0.008
Protein S	**0.043**[b]	**0.026**[a]
VWF activity	−0.001	−0.201
PAI-1	**0.640**[b]	0.248

Shown are effect sizes (β) for each association. The GEE model was adjusted for age, body mass index, C-reactive protein, insulin sensitivity, breastfeeding duration, and gestational diabetes mellitus status.
Abbreviations: aPTT, activated partial thromboplastin time; GEE, generalized estimating equation; INR, international normalized ratio; NGT, normal glucose tolerance; PAI-1, plasminogen activator inhibitor 1; vWF, von Willebrand factor.
Statistical significance: [a]*P* less than or equal to .05; [b]*P* less than or equal to .01.

improved coagulation profile in breastfeeding women. Of note, women with a history of GDM particularly benefit from longer duration of breastfeeding compared to women with in-pregnancy NGT. Third, the plasmatic coagulation system was closely related to Framingham risk score, suggesting a link between the coagulation system and CVD risk in breastfeeding women.

Our results are in line with our results previous reports demonstrating beneficial effects of breastfeeding on maternal CVD risk (3, 24). Short- as well as long-term beneficial health effects of breastfeeding have been demonstrated and several studies particularly addressed the effects of longer durations of breastfeeding (3, 25). However, the mechanistic link between breastfeeding and CVDs is still unclear. There is evidence that breastfeeding beneficially affects CV risk factors such as hypertension, metabolic syndrome, and type 2 diabetes. Underlying mechanisms for these effects include endocrine changes, energy turnover, and postpartum metabolic changes (26-28). Furthermore, subclinical atherosclerosis was found to be inversely associated with breastfeeding as the risk for aortic calcification and coronary calcification was lower in women who breastfed compared to women who did not breastfeed (29). Atherosclerotic CVDs are closely linked to alterations in the coagulation system, providing a potential link between breastfeeding and CVD (14, 16). Our present findings support this hypothesis. We detected lower activity of coagulation factor FIX in case of longer duration of breastfeeding. To the best of our knowledge, this is the first study addressing the link between breastfeeding and the coagulation system. Coagulation factor FIX belongs to the prothrombotic coagulation proteins that are essential for the clotting process. Besides this role in plasmatic coagulation, several studies demonstrated that increased coagulation factor FIX activity is linked to a higher risk for CV events and thrombotic diseases (30, 31). Interestingly, we found a significant interaction between duration of breastfeeding and history of GDM on coagulation factor FIX. Only in women with a history of GDM was longer breastfeeding linked to lower FIX activity while breastfeeding was not related to FIX in women with in-pregnancy NGT. In general, women who suffered from GDM have a higher subsequent risk for the development of type 2 diabetes and CVDs (32, 33). Even in pregnancy, studies suggest a prothrombotic tendency in case of GDM (34, 35). Recently, we were able to demonstrate that lifestyle intervention is able to improve a prothrombotic coagulation profile in individuals at high metabolic risk (36). This was mainly modulated by improvements in body weight, insulin sensitivity, and subclinical inflammation. Since it has been shown that breastfeeding is associated with postpartum weight loss and a lower risk for type 2 diabetes, women with GDM may particularly benefit more from breastfeeding compared to women with NGT with respect to coagulation (24).

Furthermore, the present study supports previous findings in nonbreastfeeding individuals of a link between the coagulation system and CV risk. In our breastfeeding women, there was a close link between coagulation proteins and Framingham risk score. In addition to the well-established associations between the prothrombotic coagulation factor IX and PAI-1 with increased CV risk (37-39), the antithrombotic protein, free protein S antigen, was also positively associated with increasing Framingham risk score. However, in multiple linear regression analyses adjusted for relevant confounders only the significant association of protein S with the Framingham risk score persisted. In line with this finding,

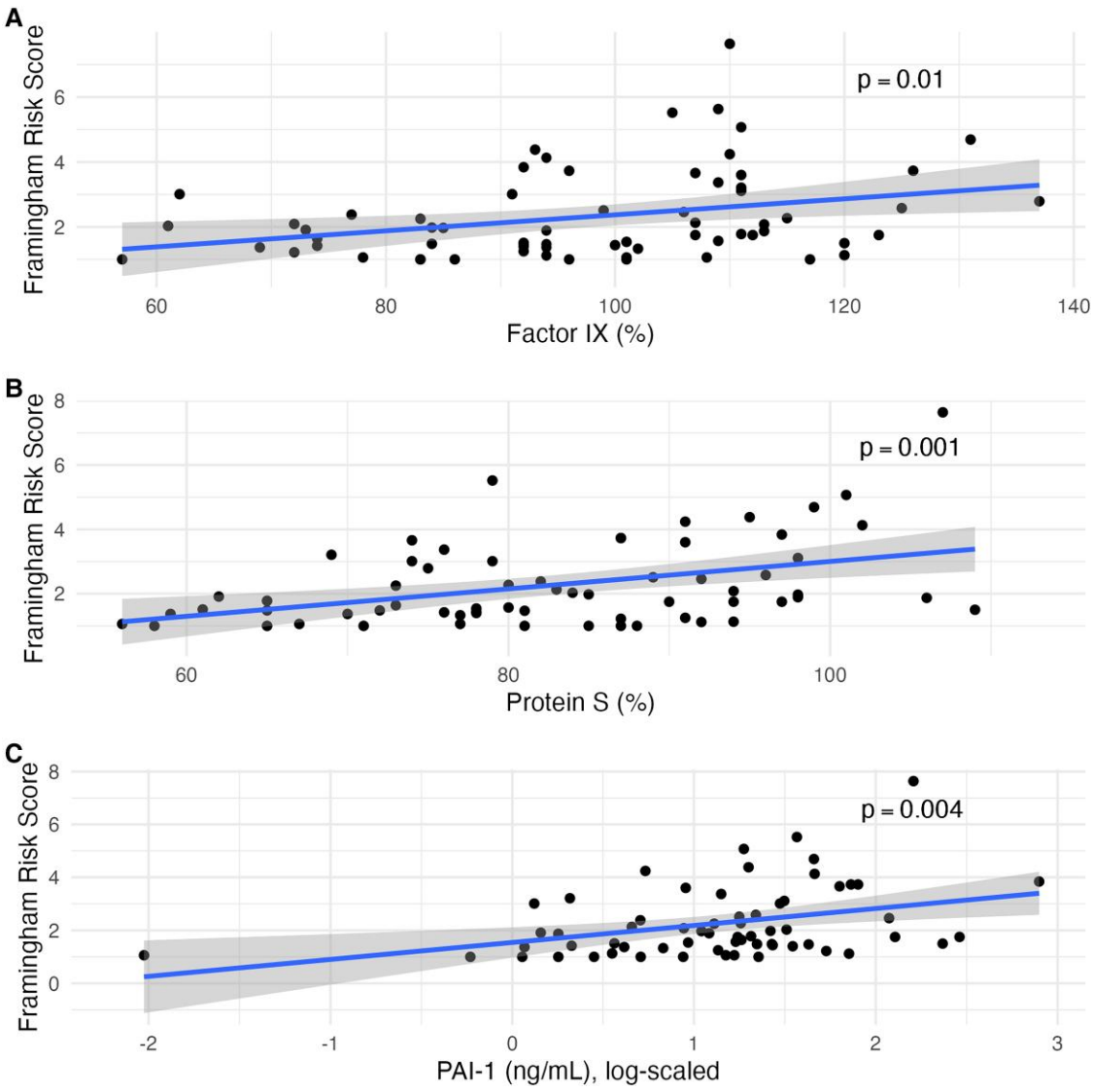

Figure 2. Relationship of Framingham Risk Score and A, factor IX; B, protein S; and C, plasminogen activator inhibitor 1 (PAI-1).

other studies reported a significant association of increased concentrations of free protein S and future risk of CVDs (40, 41). Since protein S is an anticoagulant, this might represent a compensatory mechanism in response to a prothrombotic tendency. Alternatively, protein S availability could be regulated independently of its coagulation function, for example, by its binding protein C4b–binding protein, which is closely linked to inflammation processes (42). Coagulation factor IX and PAI-1 were mainly regulated by confounders, such as insulin sensitivity and markers of inflammation (ie, CRP) as has already been demonstrated in individuals with high metabolic risk (16). Consequently, these findings emphasize that the link between CVDs and coagulation is complex and possible context-dependent (15). Clearly, future studies including follow-up visits later in life are needed to obtain more insights into these associations.

This study also has some limitations. First, the small number of participants limits the power of the study. Additionally, the Framingham risk score analysis had to be limited to a smaller subgroup of breastfeeding women due to age restrictions in the Framingham risk score calculation. Furthermore, coagulation measurements before breastfeeding were not available, which limits the prospective interpretation of the results. Therefore, larger studies are needed to clarify the significance of the findings and to draw definitive conclusions.

In summary, our study introduces plasmatic coagulation as a new player in the link between breastfeeding and the risk of CVDs. Longer duration of breastfeeding appears to have beneficial effects on coagulation profile, especially in women with a history of GDM. Thus, breastfeeding could lower maternal CV risk profile at least in part through the coagulation system.

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Disclosures

The authors have nothing to disclose.

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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