

# Increased tissue factor activity promotes thrombin generation at type 1 diabetes onset in children

Hildegard Jasser-Nitsche<sup>1</sup>  | Harald Haidl<sup>1</sup>  | Gerhard Cvirn<sup>2</sup> | Sina Pohl<sup>1</sup> | Siegfried Gallistl<sup>1</sup> | Elke Fröhlich-Reiterer<sup>1</sup> | Axel Schlagenhauf<sup>1</sup>

<sup>1</sup>Department of Paediatrics and Adolescent Medicine, Division of General Paediatrics, Medical University of Graz, Graz, Austria

<sup>2</sup>Otto Loewi Research Center, Physiological Chemistry, Medical University of Graz, Graz, Austria

## Correspondence

Axel Schlagenhauf, Division of General Pediatrics, Department of Pediatrics and Adolescent Medicine, Medical University of Graz, Auenbruggerplatz 34/2, 8036 Graz, Austria.

Email: axel.schlagenhauf@medunigraz.at

## Abstract

**Objective:** In type 1 diabetes (T1D), a prothrombotic status due to elevated coagulation factors coincides with metabolic derailment. In a previous study, we discovered altered thrombin generation profiles in children with T1D. These alterations are potentially most pronounced at T1D onset and ameliorated after insulin treatment. We tested this hypothesis in a longitudinal study, measuring thrombin generation together with coagulation parameters in children at T1D onset and during follow-up.

**Materials and methods:** Twenty-three children (12 female, age: 9.4 [2.7-17.3] years; median [range]) were tested at T1D onset and after long-term insulin treatment. Thrombin generation was measured using calibrated automated thrombography. Tissue factor (TF) activity and tissue factor pathway inhibitor (TFPI) activity were measured using enzyme-linked immunosorbent assay (ELISA).

**Results:** A procoagulant shift was observed in thrombin generation traces at T1D onset compared to follow-up (time to peak: 5.67 [4.11-7.67] min vs 6.39 [4.89-10.44] min,  $P < .001$ ). These alterations at T1D onset coincided with increased TF activity (5.18 [0.01-12.97] pmol/L vs 2.67 [0.04-10.41] pmol/L,  $P < .05$ ) and increased TFPI activity (0.051 [0.038-0.074] U/mL vs 0.035 [0.026-0.056] U/mL,  $P < .05$ ).

**Conclusion:** The procoagulant shift in thrombin generation at T1D onset is a result of increased TF activity, but this effect is partially counterbalanced by increased TFPI levels. Elevated TF and TFPI levels hint to a fragile hemostatic balance at the endothelial lining of blood vessels. Additional prothrombotic stimuli may tip over this balance explaining the increased thrombotic risk of children with T1D.

## KEYWORDS

endothelial damage, T1D, thrombin generation

## 1 | INTRODUCTION

Type 1 diabetes (T1D) is a chronic metabolic disease and one of the most common autoimmune diseases in childhood. Hallmarks of the disease are hyperglycemia and the need for a lifelong

Hildegard Jasser-Nitsche and Harald Haidl contributed equally to this study.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2020 The Authors. Pediatric Diabetes published by John Wiley & Sons Ltd.

insulin replacement.<sup>1</sup> At diabetes onset, dysbalances of the hemostatic system might occur. Hypercoagulability and thrombosis are well-known complications at the onset of disease or at ketoacidotic derailment.<sup>2</sup> Longstanding diabetes with poor metabolic control can be associated with micro- and macrovascular diseases, which are accompanied or sustained by altered coagulation factors.<sup>3</sup>

Endothelial dysfunction is well documented in T1D-patients and may be a precursor of vascular diseases. Disturbances in the coagulation system secondary to this dysfunction can contribute to long-term consequences of T1D and promote atherosclerosis.<sup>4,5</sup>

Numerous abnormalities of coagulation factors have been reported in diabetes patients. Most of the research in this field investigated patients with type 2 diabetes,<sup>6</sup> but in T1D, different coagulation factors, fibrinolytic factors, and activation markers are altered as well: increased plasma levels of von Willebrand factor,<sup>7,8</sup> fibrinogen, Factor VII, ADAMTS13, tissue-plasminogen activator activity, plasminogen activator inhibitor-1 activity, D-Dimer, and thrombin-antithrombin (TAT) complexes have been observed.<sup>9,10</sup>

Some coagulation factors and inhibitors correlate with the degree of metabolic control. Factor VIII levels and tissue factor pathways inhibitor (TFPI) are elevated in children with T1D and poor metabolic control, whereas the latter has been shown to normalize with the improvement of metabolic control.<sup>11,12</sup>

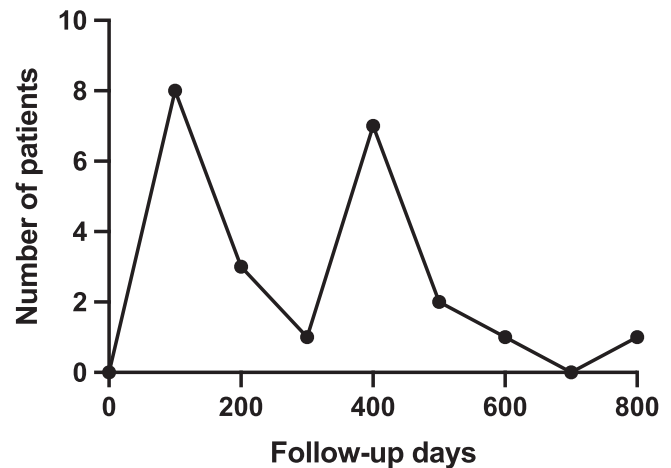
Studies employing functional coagulation assays to investigate the hemostatic status of T1D patients are inconclusive. A study by Binay et al, investigating thromboelastometry profiles of children with T1D, proposed a tendency for hypercoagulability, which was, however, not confirmed by Tran et al.<sup>13,14</sup>

While results from thromboelastometry measurements are contradictory, thrombin generation measurements may provide a more refined picture of the coagulation changes occurring in T1D patients. Thrombin is a key enzyme in plasmatic coagulation and its generation is a result of interactions between several procoagulatory factors and their inhibitors. Changes in thrombin generation represent subtle shifts in the hemostatic balance. The measurement of thrombin generation is sensitive to clotting factor deficiencies and detects the effect of anticoagulant drugs as well as deficiencies in antithrombin (AT), protein C or protein S, and activated protein C resistance.<sup>15-18</sup>

Our group compared children with T1D with age-matched healthy controls in a previous case-control study. Examinations in a pediatric cohort minimize the presence of preexisting vascular damage or other pathologies that possibly interact with coagulation changes triggered by diabetes. We found faster onsets of thrombin generation and altered thrombin generation profiles in the patient cohort without correlation with metabolic control or diabetes duration.<sup>19</sup>

Therefore, we hypothesize that these alterations in thrombin generation are secondary effects of the elevated blood glucose and that insulin treatment ameliorates these changes. The faster onset of thrombin generation might be attributable to higher plasmatic tissue factor activity caused by endothelial decay, which may subside with metabolic control.

In this longitudinal study, we intended to test this hypothesis by measuring the thrombin generation in children at the onset of T1D



**FIGURE 1** Frequency distribution of the follow-up recruitment (100-day intervals)

and during follow-up. Furthermore, we wanted to investigate whether the aforementioned alterations in the thrombin generation are associated with increased plasmatic tissue factor activity.

## 2 | MATERIALS AND METHODS

The study was approved by the local ethics committee (EK-No. 26-277 ex 13/14). Inclusion criteria were age 1 to 18 years and newly diagnosed T1D. Exclusion criteria consisted of evidence of diseases secondary due to T1D and missing informed consent of the patient or their legal guardian. In total 41 children and adolescents with newly diagnosed T1D, who had been admitted to our hospital, were included in the study. In 23 patients—12 female, 11 male—a follow-up examination was possible. The time between the initial and the follow-up examination was 276 days in median (55-754 days) (Figure 1). The demographic and T1D-specific data are depicted in Table 1.

After the informed written consent was given, blood was drawn immediately after the diagnosis before starting therapy. In case of a follow-up, blood was obtained within the routine blood check. The blood was drawn into precipitated S-Monovette premarked tubes (3 mL) from Sarstedt (Nümbrecht, Germany), containing 0.30 mL of a 0.106 mol/L trisodium citrate solution. Platelet-poor plasma (PPP) was obtained by centrifugation (1600g, 10 minutes, 20°C) and stored at -80°C for further examination.

### 2.1 | Thrombin generation experiments

Thrombin generation assays were performed using calibrated automated thrombography as reported previously with a Fluoroskan Ascent plate reader (Thermo Labsystems, Helsinki, Finland) and the Thrombinoscope software (Thrombinoscope BV, Maastricht, the Netherlands).<sup>20</sup> Twenty microliters of activating reagent (5 pM or 1 pM tissue factor, 4 μM phospholipids; final concentration) or calibrator (both Thrombinoscope BV, Maastricht, the Netherlands) were placed into

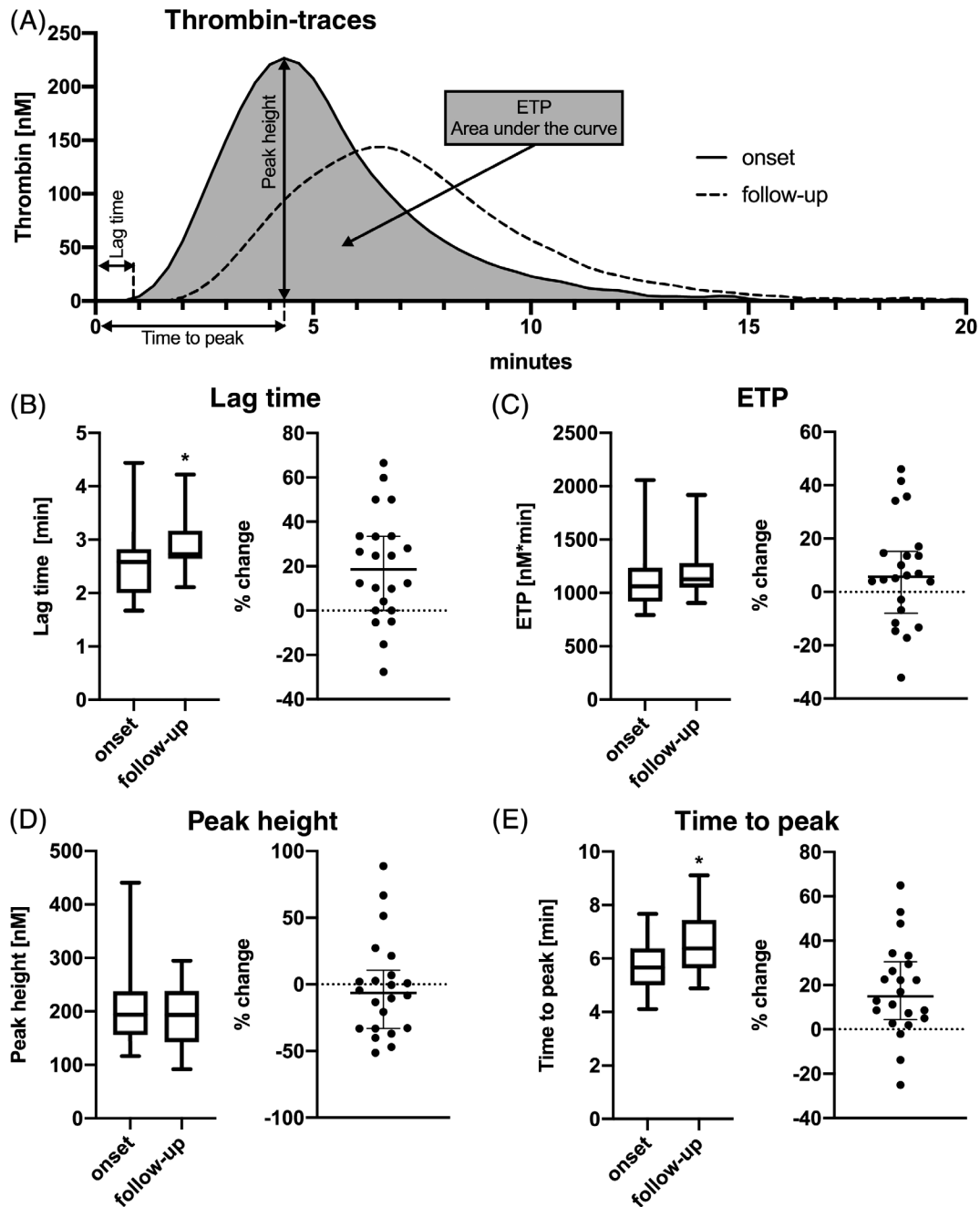
Parameter	T1D onset	Follow-up	P
N (female)	23 (12)	23 (12)	—
Age (y)	9.4 (2.7-17.3)	10.5 (3.6-18.4)	—
BMI (kg/m <sup>2</sup> )	15.2 (12.84-23.44)	17.7 (14.3-26.1)	<.0001**
HbA1c (mmol/mol)	102 (34-156)	59 (41-135)	.0002**
HbA1c (%)	11.48 (5.26-16.42)	7.55 (5.90-14.50)	.0002**

**TABLE 1** Demographic data of T1D population at onset and follow-up

Note: Data depicted in median (range).

Abbreviation: T1D, type 1 diabetes.

\*\*P < .001 for differences between the two samples points.



**FIGURE 2** Thrombin generation parameters with 5 pM exogenous tissue factor. A, Representative thrombin traces of one patient at onset and during follow-up. B-F, Absolute values of thrombin generation parameters at onset and follow-up depicted in boxplots (left) and relative changes for each patient with median and IQR (right). ETP, endogenous thrombin potential. \*P < .05

the respective wells of a 96-well-plate (Immulon 2 HB, Thermo Scientific), and then 80  $\mu$ L of PPP was added. The measurement was started by the automatic dispensation of 20  $\mu$ L fluobuffer-CaCl<sub>2</sub>, containing a fluorogenic substrate (Z-Gly-Gly-Arg-amino-methyl-coumarin, Bachem, Bubendorf). The thrombin generation profiles were recorded in triplicates. Parameters derived from the thrombin generation traces were lag time, time to peak, endogenous thrombin potential (ETP), and peak height (Figure 2A). The velocity index was calculated with the formula: (peak height/[time to peak–lag time]).

## 2.2 | Activation markers

Prothrombin fragments 1 + 2 (F1 + 2) and TAT were determined as in vivo markers for the generated thrombin, using commercially available enzyme-linked immunosorbent assay (ELISA) systems (Enzygnost F1 + 2 and Enzygnost TAT, Siemens Healthcare Diagnostics, Erlangen, Germany).

## 2.3 | Coagulation factors and inhibitors

TFPI was measured in PPP samples using the Actichrome TFPI activity assay (American Diagnostica, Greenwich, CT). The TFPI antigen levels were determined using the Immubind Total TFPI ELISA Kit (American Diagnostica, Stamford, CT). The tissue factor activity was determined with the ActichromeTF activity assay (Biomedica Diagnostic Inc, North Vancouver, Canada). Factor II was determined with a one-stage clotting assay using an ACL Top 350 coagulation analyzer with

Hemosil reagents (Instrumentation Laboratory, Bedford, MA). AT was measured using the “Hitachi 917” chemistry analyzer (Boehringer Mannheim GmbH, Mannheim, Germany) with the Antithrombin III reagent (Roche/Hitachi, Holliston, MA).

## 2.4 | Statistical Analysis

SPSS (Vers. 25) was used for performing calculations, Graphpad Prism 6.0 (Graphpad Software, San Diego, CA) for creating figures. The summarized results are given as medians with ranges. The data of all newly diagnosed T1D were used for calculating correlations. The Wilcoxon test was chosen for comparing the values at the onset of T1D with the ones at the respective follow-up.

## 3 | RESULTS

### 3.1 | Thrombin generation experiments

The results from the thrombin generation experiments varied with the amount of exogenous tissue factor (TF) employed as a trigger. When 5 pM TF were used, differences were found between the samples from T1D patients at the onset and the samples taken at the follow-up. The thrombin trace showed an observable procoagulant shift at the onset (Figure 2A). This procoagulant shift was manifested in a significantly shorter lag time and time to peak at the initial manifestation compared to the follow-up (Table 2, Figure 2B,E). There was an observable tendency for a higher peak height and a higher velocity

**TABLE 2** Thrombin generation parameters—lag time, ETP, peak height, time to peak, velocity index—of T1D patients with 5 pM exogenous TF at onset and follow-up

Parameter	T1D onset	Follow-up	P
Lag time (min)	2.58 (1.67-4.44)	2.73 (2.11-4.22)	.0028*
ETP (nM $\times$ min)	1089 (792-2058)	1125 (906-1918)	.2756
Peak height (nM)	198 (116-441)	194 (92-295)	.2479
Time to peak (min)	5.67 (4.11-7.67)	6.39 (4.89-10.44)	.0006**
Velocity index (nM/min)	62.35 (27.56-210.03)	55.46 (15.92-101.91)	.0917

Abbreviations: ETP, endogenous thrombin potential; T1D, type 1 diabetes; TF, tissue factor.

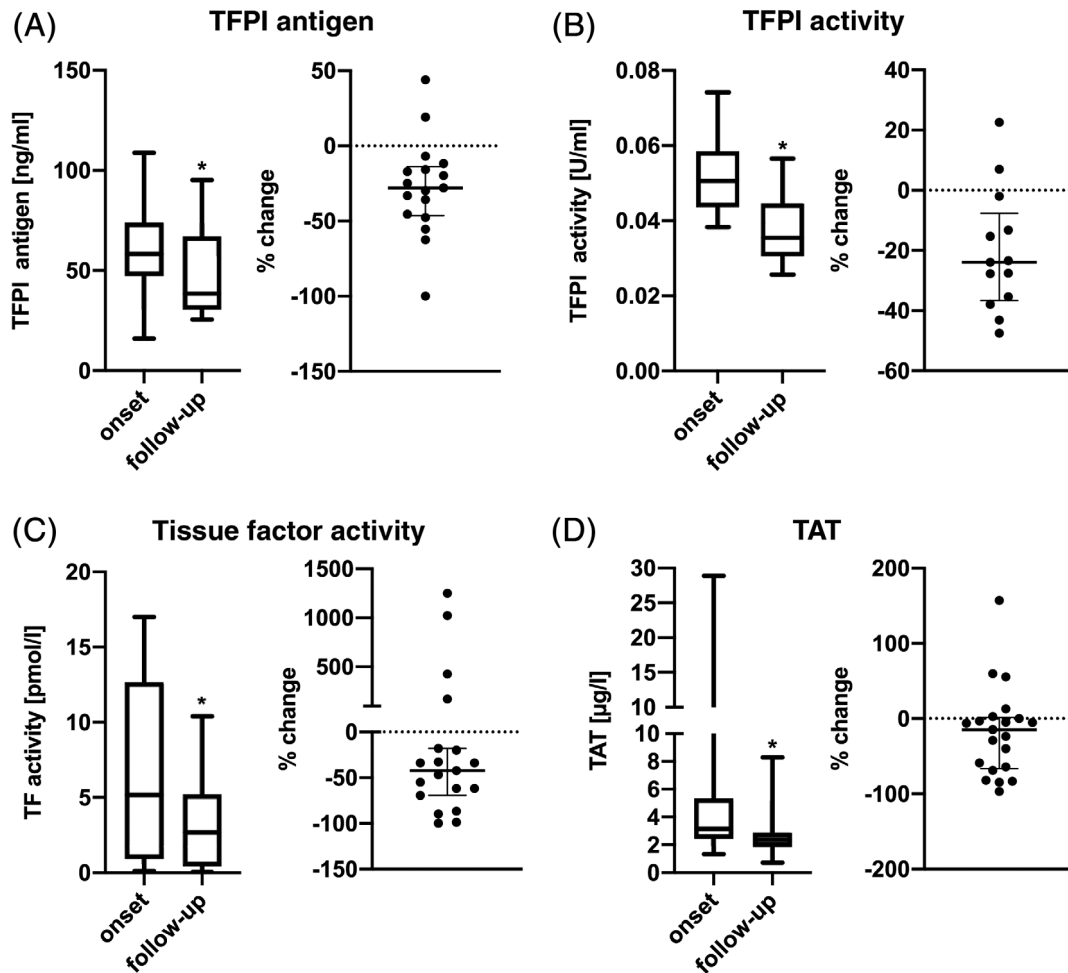
\* $P < .05$ .

\*\* $P < .001$  for differences between the two sample points.

**TABLE 3** Thrombin generation parameters—lag time, ETP, peak height, time to peak, velocity index—of T1D patients with 1 pM exogenous TF at onset and follow-up

Parameter	T1D onset	Follow-up	P
Lag time (min)	6.4 (4.01-14.00)	7.11 (4.00-12.36)	.4198
ETP (nM $\times$ min)	729 (321-1715)	611 (230-1675)	.7262
Peak height (nM)	67.3 (26.7-173.0)	50 (16-199)	.4826
Time to peak (min)	11.60 (9.33-20.00)	12.89 (7.67-19.94)	.1982
Velocity index (nM/min)	13.24 (4.21-38.60)	7.96 (2.12-52.91)	.3535

Abbreviations: ETP, endogenous thrombin potential; T1D, type 1 diabetes; TF, tissue factor.



**FIGURE 3** Plasmatic coagulation parameters with significant differences at onset and at follow-up. Absolute values at onset and follow-up depicted in boxplots (left) and relative changes for each patient with median and IQR (right). TAT, thrombin-antithrombin complex; TFPI, tissue factor pathway inhibitor. \* $P < 0.05$

index at the onset, but this trend was not statistically significant (Figure 2D, Table 2). The overall amount of thrombin generated, represented by the ETP, was not significantly different between onset and follow-up (Figure 2C).

With only 1 pM exogenous TF employed to trigger thrombin generation, no shift was observed in the thrombin trace at onset compared to the follow-up. The experiments with this lower TF concentration resulted in a higher variability of lag time, time to peak, peak height, ETP, and velocity index between patients. There were no significant differences between onset and follow-up for any of these parameters (Table 3).

### 3.2 | Plasmatic coagulation and activation markers

The samples from patients at the onset of T1D exhibited a significantly higher TF activity compared to the respective samples taken at the follow-up (Figure 3, Table 4). Equivalently, TFPI antigen and activity were higher at onset than at the follow-up. Factor II and AT levels were not significantly different in the two sample points. The levels of

prothrombin fragments 1 + 2 were not elevated, while the TAT-levels were significantly increased during onset. The duration between initial manifestation and follow-up did not affect any of the aforementioned parameters.

## 4 | DISCUSSION

T1D onset puts a tremendous strain on the metabolic system. Upon initial manifestation, most therapeutic efforts are centered on the control of blood glucose and the acid-base metabolism. Later on, the prevention of micro- and macrovascular complications come into view. Long-term alterations in coagulation have been investigated in patients with diabetes and are discussed to be involved in the development of angiopathy, atherosclerosis, and associated diseases.<sup>21</sup> However, not much is known about the impact of T1D on coagulation at an early stage of the disease. In this study, we investigated the hemostatic balance in a cohort of children with T1D at the time of diagnosis and after a period of time under insulin treatment.

**TABLE 4** Plasmatic coagulation parameters—AT, TFact, TFPI antigen and activity, TAT, F1 + F2—of T1D patients at onset and follow-up

Parameter	T1D onset	Follow-up	P
AT (%)	104.5 (81.0-140.0)	106.0 (75.0-129.0)	.3071
Factor II (%)	94 (57-202)	99 (73-122)	.7657
TFact (pmol/L)	5.18 (0.01-12.97)	2.67 (0.04-10.41)	.0230*
TFPI antigen (ng/mL)	58.4 (16.0-108.8)	38.4 (25.6-95.2)	.0448*
TFPI activity (U/mL)	0.051 (0.038-0,074)	0.035 (0.026-0.056)	.0046*
TAT (µg/L)	3.15 (1.35-28.87)	2.38 (0.72-18.50)	.0177*
F1 + F2 (pmol/L)	151.2 (44.5-437.4)	125.4 (55.6-335.0)	.5028

Abbreviations: AT, antithrombin; ETP, endogenous thrombin potential; F1 + F2, prothrombin fragments 1 + 2; T1D, type 1 diabetes; TAT, thrombin-anti-thrombin complex; TFact, tissue factor activity; TFPI, tissue factor inhibitor.

\* $P < .05$  for differences between the two sample points.

The main finding of our thrombin generation experiments is a procoagulant shift at the onset of the disease with 5 pM TF activation. To exclude an effect of glucose itself on the thrombin generation, we tested standard plasma with increasing glucose levels (50-500 mg/dL) and found no correlative dependency (data not shown). Neither did we find a correlation with pH-levels in our experiments which is in line with findings by White et al, who investigated acidosis dependent alterations in thromboelastometry.<sup>22</sup> Hence, these changes hint to either a decrease of inhibitors of thrombin generation or an elevation of procoagulant triggers.

The balance between the two antagonists Factor II and AT is known to influence thrombin generation.<sup>23</sup> We did not find significant differences between onset and follow-up in those two factors (Table 4). Thus, they cannot be the underlying cause for the observed alterations in thrombin generation. Both, Factor II and AT, are liver-derived, which militates against a liver involvement in the hemostatic aberrations during the metabolic derailment.

TFPI is an inhibitor of coagulation with a strong influence on thrombin generation, particularly in the early phase of thrombin generation. We found levels above the pediatric reference range at the onset of the disease and a significant reduction after the beginning of treatment.<sup>24</sup> A similar result was found by Rigla et al, who tested young adults with insufficiently controlled T1D and demonstrated reduced TFPI levels after optimization of the treatment. However, this finding is contradictory to a shortened lag time/time to peak since a heightened TFPI level is supposed to elongate lag time and time to peak. We considered a decrease of TFPI activity despite high antigen levels due to a nonspecific glycosylation, but our parallel measurement of TFPI antigen and activity did not show a relevant divergence (Table 4, Figure 3A,B). A short lag time/time to peak can be the result of an increased turnover in the initiation phase of coagulation. In this phase, TF is one of the main procoagulant triggers and TFPI is its counterpart. Coinciding with the observed TFPI progression, we found a higher TF activity at onset than after the beginning of treatment. We postulate that increased endogenous TF activity adds to the exogenous TF added in our experiments and shortens the onset of the thrombin generation. An elevated TFPI activity may counterbalance the higher TF activity, but this compensatory effect is only observable

at low exogenous TF concentrations, since high TF concentrations blunt the effect of TFPI. This may be the reason why the results of thrombin generation experiments diverge, depending on whether 1 pM or 5 pM of exogenous TF were used.

TF and TFPI are mainly located and stored in the endothelium. The close adjacency of the pro- and anticoagulant factors prevents a premature onset of coagulation. In case of a nonspecific damage of the endothelium, elevated levels of both, pro- and anticoagulant proteins, can be found in the bloodstream. High blood glucose levels seem to exhibit deleterious effects on the endothelium, either directly or indirectly via oxidative stress, advanced glycation end-products (AGE), or osmotic forces. The endothelial release of hemostatic factors is not specific to glucose and can be seen with various other agents as well.

The simultaneous release of pro- and anticoagulant factors sustains the hemostatic balance, but this balance is fragile and seems to be easily shifted toward a prothrombotic state. Thus, additional prothrombotic factors, for example, the insertion of a central venous catheter, can quickly lead to a thrombotic event. We found significantly elevated levels of TAT, which is in line with previous studies and represents an indicator for an enhanced thrombin turnover.<sup>9,19,25</sup>

A hypercoagulable, prothrombotic state caused by endothelial dysfunction is considered a pathophysiological precursor of atherosclerosis. Diverse mechanisms for the development of a prothrombotic state during hyperglycemic episodes have been proposed before: increased transcription of coagulation proteins, AGE, destruction of the glycocalyx, and enhanced interaction with platelets.<sup>26</sup> In this study, we observed a novel aspect of the aforementioned prothrombotic state, which is mainly associated with an endothelial perturbation.

Potential hemostatic alterations due to hyperglycemia are not solely present in patients with diabetes. Various findings argue for a prothrombotic condition secondary to endothelial perturbation: Studies wherein healthy volunteers have been exposed to hyperglycemia and/or hyperinsulinemia resulted in elevated levels of activation markers of coagulation.<sup>27,28</sup> Stegenga et al measured elevated TAT and TF activity starting from 3 hours after the beginning of the continuous glucose administration, which hints to an acute phase reaction.<sup>27</sup> Studies by Liao et al and Tala et al demonstrate the impact of blood

glucose levels on the outcome of severely ill patients and the relations between hyperglycemia and thrombosis.<sup>29-31</sup> The effects of sugar-sweetened beverages via an increased glycemic load on the endothelial function are broadly discussed.<sup>32</sup>

Limitations of our study lie in the potentially different duration of symptoms before T1D was diagnosed. In addition, the follow-up period after the initial manifestation differs broadly, but we argue that all patients were under long-term treatment at the second blood draw with a metabolic situation beyond the point of onset and hospitalization. Still, the efficacy of disease control varied as can be seen in HbA1c values (Table 1).

Summing up, our research provides additional evidence that hyperglycemia at the onset of T1D coincides with an elevated TF activity, which is only partially compensated by an increased TFPI activity. In combination with additional hemostatic triggers, an elevated TF activity tends to induce the hypercoagulability in children and adolescents. This fragility of the TF/TFPI balance explains the higher risk for thrombosis in diabetic patients and may be one of the initial and recurrent hemostatic peculiarities that promote the development of atherosclerosis.

#### CONFLICT OF INTEREST

The authors declare no potential conflicts of interest.

#### AUTHOR CONTRIBUTIONS

All authors have read and approved the final manuscript. Hildegard Jasser-Nitsche, Elke Fröhlich-Reiterer, Siegfried Gallistl, and Harald Haidl designed the study; Hildegard Jasser-Nitsche and Harald Haidl were responsible for acquisition of blood samples and wrote the paper. Sina Pohl and Axel Schlagenhauf performed the assays. Elke Fröhlich-Reiterer, Siegfried Gallistl, Gerhard Cvirn, and Axel Schlagenhauf gave relevant expert input and revised the manuscript.

#### PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/pedi.13086>.

#### ORCID

Hildegard Jasser-Nitsche  <https://orcid.org/0000-0002-1992-7565>

Harald Haidl  <https://orcid.org/0000-0003-4026-1507>

#### REFERENCES

- Atkinson MA, Eisenbarth GS, Michels AW. Type 1 diabetes. *Lancet*. 2014;383(9911):69-82. [https://doi.org/10.1016/S0140-6736\(13\)60591-7](https://doi.org/10.1016/S0140-6736(13)60591-7).
- Bialo SR. Rare complications of pediatric diabetic ketoacidosis. *World J Diabetes*. 2015;6(1):167-174. <https://doi.org/10.4239/wjd.v6.i1.167>.
- Forbes JM, Cooper ME. Mechanisms of diabetic complications. *Physiol Rev*. 2013;93(1):137-188. <https://doi.org/10.1152/physrev.00045.2011>.
- Loeffen R, Spronk HMH, ten Cate H. The impact of blood coagulability on atherosclerosis and cardiovascular disease. *J Thromb Haemost*. 2012;10(7):1207-1216. <https://doi.org/10.1111/j.1538-7836.2012.04782.x>.
- Seon MJ, Chung IN, Sei WY, et al. Endothelial dysfunction and microvascular complications in type 1 diabetes mellitus. *J Korean Med Sci*. 2008;23(1):77-82. <https://doi.org/10.3346/jkms.2008.23.1.77>.
- Grant PJ. Diabetes mellitus as a prothrombotic condition. *J Intern Med*. 2007;262:157-172. <https://doi.org/10.1111/j.1365-2796.2007.01824.x>.
- Silveira AMV, Sjöberg S, Blombäck M, Östman J. von Willebrand factor antigen in plasma and urine in patients with type I (insulin-dependent) diabetes mellitus with and without nephropathy. *J Diabetes Complications*. 1992;6(2):89-95. [https://doi.org/10.1016/1056-8727\(92\)90017-F](https://doi.org/10.1016/1056-8727(92)90017-F).
- Domingueti CP, Dusse LMS, Fóscolo RB, et al. Von willebrand factor, ADAMTS13 and D-dimer are correlated with different levels of nephropathy in type 1 diabetes mellitus. *PLoS One*. 2015;10(7):e0132784. <https://doi.org/10.1371/journal.pone.0132784>.
- Carmassi F, Morale M, Puccetti R, et al. Coagulation and fibrinolytic system impairment in insulin dependent diabetes mellitus. *Thromb Res*. 1992;67(6):643-654. [https://doi.org/10.1016/0049-3848\(92\)90068-L](https://doi.org/10.1016/0049-3848(92)90068-L).
- Adly AAM, Elbarbary NS, Ismail EAR, Hassan SR. Plasminogen activator inhibitor-1 (PAI-1) in children and adolescents with type 1 diabetes mellitus: relation to diabetic micro-vascular complications and carotid intima media thickness. *J Diabetes Complications*. 2014;28(3):340-347. <https://doi.org/10.1016/j.jdiacomp.2014.01.011>.
- Muntean WE, Borkenstein MH, Haas J. Elevation of factor VIII coagulant activity over factor VIII coagulant antigen in diabetic children without vascular disease. A sign of activation of the factor VIII coagulant moiety during poor diabetes control. *Diabetes*. 1985;34(2):140-144. <https://doi.org/10.2337/diabetes.34.2.140>.
- Rigla M, Mateo J, Fontcuberta J, Souto JC, De Leiva A, Pérez A. Normalisation of tissue factor pathway inhibitor activity after glycaemic control optimisation in type 1 diabetic patients. *Thromb Haemost*. 2000;84(2):223-227. <https://doi.org/10.1055/s-0037-1614000>.
- Binay C, Bozkurt Turhan A, Simsek E, Bor O, Akay OM. Evaluation of coagulation profile in children with type 1 diabetes mellitus using rotational Thromboelastometry. *Indian J Hematol Blood Transfus*. 2017;33(4):574-580. <https://doi.org/10.1007/s12288-017-0793-0>.
- Tran TH, Al-Harfi I, Harle CC, Kahr WHA, Morrison GC, Kornecki A. Coagulation assessment in children with diabetic ketoacidosis. *Pediatr Crit Care Med*. 2013;14(3):256-260. <https://doi.org/10.1097/PCC.0b013e318271fcb7>.
- Hemker HC, Giesen P, Al Dieri R, et al. Calibrated automated thrombin generation measurement in clotting plasma. *Pathophysiol Haemost Thromb*. 2003;33(1):4-15. <https://doi.org/10.1159/000071636>.
- Cimenti C, Koestenberger M, Leschnik B, Haidl H, Muntean W. The respective and combined anticoagulant effects of recombinant human activated protein C, melagatran and heparins using CAT. *Thromb Res*. 2007;119(3):361-367. <https://doi.org/10.1016/j.thromres.2006.03.004>.
- Hemker HC, Al Dieri R, De Smedt E, Béguin S. Thrombin generation, a function test of the haemostatic-thrombotic system. *Thromb Haemost*. 2006;96(5):553-561. <https://doi.org/10.1160/TH06-07-0408>.
- Fritsch P, Cvirn G, Cimenti C, et al. Thrombin generation in factor VIII-depleted neonatal plasma: nearly normal because of physiologically low antithrombin and tissue factor pathway inhibitor. *J Thromb Haemost*. 2006;4(5):1071-1077. <https://doi.org/10.1111/j.1538-7836.2006.01947.x>.
- Cimenti C, Schlagenhauf A, Leschnik B, et al. Only minor changes in thrombin generation of children and adolescents with type 1 diabetes mellitus—a case-control study. *Thromb Res*. 2016;148:45-49. <https://doi.org/10.1016/j.thromres.2016.10.016>.

20. Haidl H, Pohl S, Leschnik B, Gallistl S, Muntean W, Schlagenhauf A. Neonatal thrombocytopenia: thrombin generation in presence of reduced platelet counts and effects of rFVIIa in cord blood. *Sci Rep*. 2019;9(1):8014. <https://doi.org/10.1038/s41598-019-44199-y>.
21. Carr ME. Diabetes mellitus: a hypercoagulable state. *J Diabetes Complications*. 2001;15(1):44-54. [https://doi.org/10.1016/S1056-8727\(00\)00132-X](https://doi.org/10.1016/S1056-8727(00)00132-X).
22. White H, Bird R, Sosnowski K, Jones M. An in vitro analysis of the effect of acidosis on coagulation in chronic disease states—a thromboelastograph study. *Clin Med*. 2016;16(3):230-234. <https://doi.org/10.7861/clinmedicine.16-3-230>.
23. Kremers RMW, Peters TC, Wagenvoort RJ, Hemker HC. The balance of pro- and anticoagulant processes underlying thrombin generation. *J Thromb Haemost*. 2015;13(3):437-447. <https://doi.org/10.1111/jth.12798>.
24. Sosoithikul D, Kittikalayawong Y, Aungbamnet P, Buphachat C, Seksarn P. Reference values for thrombotic markers in children. *Blood Coagul Fibrinolysis*. 2012;23(3):208-211. <https://doi.org/10.1097/MBC.0b013e328350294a>.
25. Reverter JL, Reverter JC, Tässies D, et al. Thrombomodulin and induced tissue factor expression on monocytes as markers of diabetic microangiopathy: a prospective study on hemostasis and lipoproteins in insulin-dependent diabetes mellitus. *Am J Hematol*. 1997;56(2):93-99. [https://doi.org/10.1002/\(SICI\)1096-8652\(199710\)56:2<93::AID-AJH4>3.0.CO;2-W](https://doi.org/10.1002/(SICI)1096-8652(199710)56:2<93::AID-AJH4>3.0.CO;2-W).
26. Lemkes BA, Hermanides J, Devries JH, Holleman F, Meijers JCM, Hoekstra JBL. Hyperglycemia: a prothrombotic factor? *J Thromb Haemost*. 2010;8(8):1663-1669. <https://doi.org/10.1111/j.1538-7836.2010.03910.x>.
27. Stegenga ME, Van Der Crabben SN, Blümer RME, et al. Hyperglycemia enhances coagulation and reduces neutrophil degranulation, whereas hyperinsulinemia inhibits fibrinolysis during human endotoxemia. *Blood*. 2008;112(1):82-89. <https://doi.org/10.1182/blood-2007-11-121723>.
28. Rao AK, Chouhan V, Chen X, Sun L, Boden G. Activation of the tissue factor pathway of blood coagulation during prolonged hyperglycemia in young healthy men. *Diabetes*. 1999;48(5):1156-1161. <https://doi.org/10.2337/diabetes.48.5.1156>.
29. Dungan KM, Braithwaite SS, Preiser JC. Stress hyperglycaemia. *Lancet*. 2009;373(9677):1798-1807. [https://doi.org/10.1016/S0140-6736\(09\)60553-5](https://doi.org/10.1016/S0140-6736(09)60553-5).
30. Liao WI, Wang JC, Lin CS, et al. Elevated glycemic gap predicts acute respiratory failure and in-hospital mortality in acute heart failure patients with diabetes. *Sci Rep*. 2019;9(1):6279. <https://doi.org/10.1038/s41598-019-42666-0>.
31. Tala JA, Silva CT, Pemira S, Vidal E, Faustino EVS. Blood glucose as a marker of venous thromboembolism in critically ill children. *J Thromb Haemost*. 2014;12(6):891-896. <https://doi.org/10.1111/jth.12583>.
32. Loader J, Meziat C, Watts R, et al. Effects of sugar-sweetened beverage consumption on microvascular and macrovascular function in a healthy population. *Arterioscler Thromb Vasc Biol*. 2017;37(6):1250-1260. <https://doi.org/10.1161/ATVBAHA.116.308010>.

**How to cite this article:** Jasser-Nitsche H, Haidl H, Cvirn G, et al. Increased tissue factor activity promotes thrombin generation at type 1 diabetes onset in children. *Pediatr Diabetes*. 2020;21:1210–1217. <https://doi.org/10.1111/ pedi.13086>