

Computational Analysis of the Effects of Reduced Temperature on Thrombin Generation: The Contributions of Hypothermia to Coagulopathy

Alexander Y. Mitrophanov, PhD,* Frits R. Rosendaal, MD, PhD,† and Jaques Reifman, PhD*

BACKGROUND: Hypothermia, which can result from tissue hypoperfusion, body exposure, and transfusion of cold resuscitation fluids, is a major factor contributing to coagulopathy of trauma and surgery. Despite considerable efforts, the mechanisms of hypothermia-induced blood coagulation impairment have not been fully understood. We introduce a kinetic modeling approach to investigate the effects of hypothermia on thrombin generation.

METHODS: We extended a validated computational model to predict and analyze the impact of low temperatures (with or without concomitant blood dilution) on thrombin generation and its quantitative parameters. The computational model reflects the existing knowledge about the mechanistic details of thrombin generation biochemistry. We performed the analysis for an “average” subject, as well as for 472 subjects in the control group of the Leiden Thrombophilia Study.

RESULTS: We computed and analyzed thousands of kinetic curves characterizing the generation of thrombin and the formation of the thrombin–antithrombin complex (TAT). In all simulations, hypothermia in the temperature interval 31°C to 36°C progressively slowed down thrombin generation, as reflected by clotting time, thrombin peak time, and prothrombin time, which increased in all subjects ($P < 10^{-5}$). Maximum slope of the thrombin curve was progressively decreased, and the area under the thrombin curve was increased in hypothermia ($P < 10^{-5}$); thrombin peak height remained practically unaffected. TAT formation was noticeably delayed ($P < 10^{-5}$), but the final TAT levels were not significantly affected. Hypothermia-induced fold changes in the affected thrombin generation parameters were larger for lower temperatures, but were practically independent of the parameter itself and of the subjects’ clotting factor composition, despite substantial variability in the subject group. Hypothermia and blood dilution acted additively on the thrombin generation parameters.

CONCLUSIONS: We developed a general computational strategy that can be used to simulate the effects of changing temperature on the kinetics of biochemical systems and applied this strategy to analyze the effects of hypothermia on thrombin generation. We found that thrombin generation can be noticeably impaired in subjects with different blood plasma composition even in moderate hypothermia. Our work provides mechanistic support to the notion that thrombin generation impairment may be a key factor in coagulopathy induced by hypothermia and complicated by blood plasma dilution. (Anesth Analg 2013;117:565–74)

From the *DoD Biotechnology High-Performance Computing Software Applications Institute, Telemedicine and Advanced Technology Research Center, U.S. Army Medical Research and Materiel Command, Fort Detrick, Frederick, Maryland; and †Departments of Clinical Epidemiology and Thrombosis and Haemostasis, Leiden University Medical Center, Leiden, The Netherlands.

Accepted for publication April 22, 2013.

Funding: AYM and JR were supported by the Military Operational Medicine Research Program of the U.S. Army Medical Research and Materiel Command, Fort Detrick, Frederick, MD, as part of the U.S. Army’s Network Science Initiative. The Leiden Thrombophilia Study, completed previously (FRR), was funded by the Netherlands Heart Foundation (89-063).

The authors declare no conflicts of interest.

Disclaimer: The opinions and assertions contained herein are private views of the authors and are not to be construed as official or as reflecting the views of the U.S. Army or the U.S. Department of Defense. This paper has been approved for public release with unlimited distribution.

Reprints will not be available from the authors.

Address correspondence to Alexander Y. Mitrophanov, PhD, or Jaques Reifman, PhD, DoD Biotechnology High-Performance Computing Software Applications Institute, Telemedicine and Advanced Technology Research Center, U.S. Army Medical Research and Materiel Command, ATTN: MCMR-TT, 504 Scott St., Fort Detrick, Frederick, MD 21702. Address e-mail to alex@bhsai.org (A.Y.M.) or jaques.reifman.civ@mail.mil (J.R.).

Copyright © 2013 International Anesthesia Research Society
DOI: 10.1213/ANE.0b013e31829c3b22

Hypothermia, a state of abnormally low body core temperature ($<36^{\circ}\text{C}$ in trauma patients), is one of the major factors contributing to coagulopathy of trauma.^{1–3} Heavy bleeding may result in a decreased blood volume and associated tissue hypoperfusion, which, in turn, leads to insufficient heat generation in the tissues due to a reduced rate of oxygen-dependent adenosine triphosphate synthesis.⁴ Transfusion of blood products and resuscitation fluids, which are stored at low temperatures or even in a frozen state, further contribute to the development of hypothermia and coagulopathy.^{2,3} Body rewarming techniques are generally expected to mitigate the adverse effects of hypothermia,^{1,3} but they might require time to take effect when rapid action is needed for a patient’s survival. Moreover, in trauma situations, hypothermia-induced coagulopathy may be complicated by other factors (such as blood dilution),⁵ which cannot be corrected by rewarming. To develop new, broad-spectrum therapeutic strategies for reversing traumatic and surgical coagulopathy,⁶ it is thus necessary to investigate the effects of hypothermia on blood clotting at the molecular level.

The impairment of blood clotting under hypothermic conditions is well recognized.⁵ However, the mechanisms of

Report Documentation Page				Form Approved OMB No. 0704-0188	
Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.					
1. REPORT DATE SEP 2013		2. REPORT TYPE		3. DATES COVERED 00-00-2013 to 00-00-2013	
4. TITLE AND SUBTITLE Computational Analysis of the Effects of Reduced Temperature on Thrombin Generation: The Contributions of Hypothermia to Coagulopathy				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S)				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) US Army Medical Research and Materiel Command, DoD Biotechnology High Performance Computing Software Applications Institute, Telemedicine and Advanced Technology Research Center, Fort Detrick, MD, 21702				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; distribution unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT Same as Report (SAR)	18. NUMBER OF PAGES 10	19a. NAME OF RESPONSIBLE PERSON
a. REPORT unclassified	b. ABSTRACT unclassified	c. THIS PAGE unclassified			

such impairment have not been firmly established. Some of the existing experimental evidence indicates that hypothermia noticeably impacts the generation of thrombin, a central enzymatic component of the biochemical blood coagulation network.^{7–10} Yet, other reports suggest that thrombin generation biochemistry is practically unaffected by mild and moderate hypothermia and emphasize the role of hypothermia-induced effects that are not attributed to thrombin generation deficiency.^{11–14} The use of different experimental systems and conditions by different laboratories, which can quantitatively impact thrombin generation kinetics,^{15,16} has hampered definitive conclusions.

The ambiguity regarding the effects of temperature on thrombin generation suggests that experimental results should be analyzed and interpreted using new, complementary approaches.¹⁵ Here, we apply computational methods to help understand and predict temperature modulation of thrombin kinetic curves. This approach, based on extending the validated Hockin–Mann computational model of thrombin generation,^{17,18} was motivated by recent applications of this model to study the impairment of thrombin generation in dilution-induced coagulopathy¹⁹ and to investigate the therapeutic effects of factor VIIa¹⁵ and prothrombin complex concentrates²⁰ on thrombin accumulation. Moreover, the Hockin–Mann model has been of considerable utility in the analysis of other clinically relevant blood clotting problems, such as causes for thromboembolic complications^{21,22} and the action of anticoagulants.^{23,24} The model reflects the current mechanistic view, albeit simplified, of the biochemical thrombin generation network in human blood plasma, and captures the typical kinetics of thrombin generation *in vitro*.

Here, we extend the capabilities of the Hockin–Mann model and introduce a rigorous and general computational strategy to model the effects of changing temperature on the kinetics of biomolecular systems. We use this strategy to analyze the temperature dependence of thrombin generation kinetics. The contributions of this work are 2-fold. First, we characterize the effects of hypothermia (alone and in combination with blood dilution) on the 5 standard quantitative thrombin generation parameters, which has not been previously done. Second, we establish how the effects of hypothermia on thrombin generation are impacted by inter-subject variability.

METHODS

Study Group

Because this work is a computational study that uses previously obtained experimental data, no IRB approval was necessary. We performed computational analyses for a subject characterized by average values of the concentrations of blood coagulation components in normal human blood plasma (the “average” subject),^{15,18} as well as for 472 individual subjects in the control group of the Leiden Thrombophilia Study (LETS).²⁵

Computational Modeling

The Hockin–Mann model is a system of mathematical equations where the unknown variables are the concentrations of the biochemical species in the thrombin generation network.^{17,18} The equations reflect the chemical reactions in the

network (listed in Table A1, see Appendix) and link the concentrations with their rates of change. The solutions of the equations represent the temporal dynamics of the concentration variables. The model is parameterized by the values of 44 constants (termed kinetic constants) that define the rates of the reactions in the thrombin generation network. The input of the model is the initial concentrations of the proteins that participate in the reactions, and the model’s output is the resulting kinetic trajectories for the protein concentrations computed over a specified time interval (50 minutes). The default model input is the average coagulation factor concentrations in normal human plasma, and this input can be changed to represent alternative blood compositions, such as those in the LETS subjects. The LETS dataset contains relative levels of blood coagulation factors for each of the subjects. We rescaled them using the known average concentration values in absolute units and used the resulting concentrations as the model inputs to simulate thrombin generation in individual subjects. In our simulations for diluted blood plasma, the default inputs were modified according to the dilution scenarios.^{19,20}

The extension of the Hockin–Mann model proposed in this work concerns the effects of changing temperature on biochemical kinetics. Because accurate values of the parameters (termed temperature coefficients [TCs]) that govern those effects have not been determined, our approach is based on random sampling of TC sets and using the Hockin–Mann model to compute a thrombin curve (i.e., a kinetic trajectory for the concentration of thrombin) for each of such sets. Groups of thrombin curves computed for many sampled TC sets define the predicted kinetic curve ranges (or, “corridors”) to which the true hypothermic thrombin curves are expected to belong.

A typical procedure for modeling temperature dependence can be described as follows. For any given hypothermic temperature T_2 (all temperatures are expressed in degrees Celsius) and each of the 44 kinetic constants in the model, we generated a number, M , of random TCs (see Appendix for details on TC sampling).^{26,27} (The use of TCs is grounded in physical chemistry, being an approximation of the well-known Arrhenius equation.^{26,28}) M was equal to 5000 for most simulations. As a result of such random sampling, we obtained M sets, each set containing 44 TC values. In every set, each TC was used to calculate the hypothermic value of 1 of the 44 kinetic constants as follows: $k_2 = k_1 \times \gamma^{(T_2 - T_1)/10}$, where k_1 and k_2 denote the values of the kinetic constant at temperatures T_1 and T_2 , respectively; γ denotes the TC; and $T_1 = 37^\circ\text{C}$ represents the normal body temperature.^{26,27} For each of the M sets, the equations constituting the Hockin–Mann model were solved numerically, and a thrombin trajectory was generated. The M resulting trajectories were analyzed using statistical methods, and predictions of thrombin generation at the hypothermic temperature T_2 were obtained from those analyses. Additional details about our computational procedures are given in the Appendix.

Statistical Analysis

Groups of simulated thrombin curves and thrombin–antithrombin complex (TAT) curves were analyzed using

descriptive statistics. (These curve groups were generated by TC randomization, as described in the previous subsection.) The thrombin or TAT raw-output kinetic curves from the computational model were discretized into 500 evenly spaced points. Each of these points corresponded to a given time moment within the default simulation time interval. For each of these time moments, we analyzed the statistical sample of thrombin or TAT levels constituted by the corresponding curve values from every curve in the group. For each of the resulting 500 samples, we calculated the range (i.e., maximum variation), interquartile range, and the median, which were plotted against time to represent their temporal changes (Figs. 1 and 2).

For groups of model-generated thrombin and TAT curves, we calculated and statistically analyzed quantitative parameters, such as clotting time (CT) and thrombin peak height (PH) (see Results). In these analyses, the curves in a group either were generated for a given subject via TC randomization or were generated for every subject in the LETS subject group in the absence of TC randomization. For

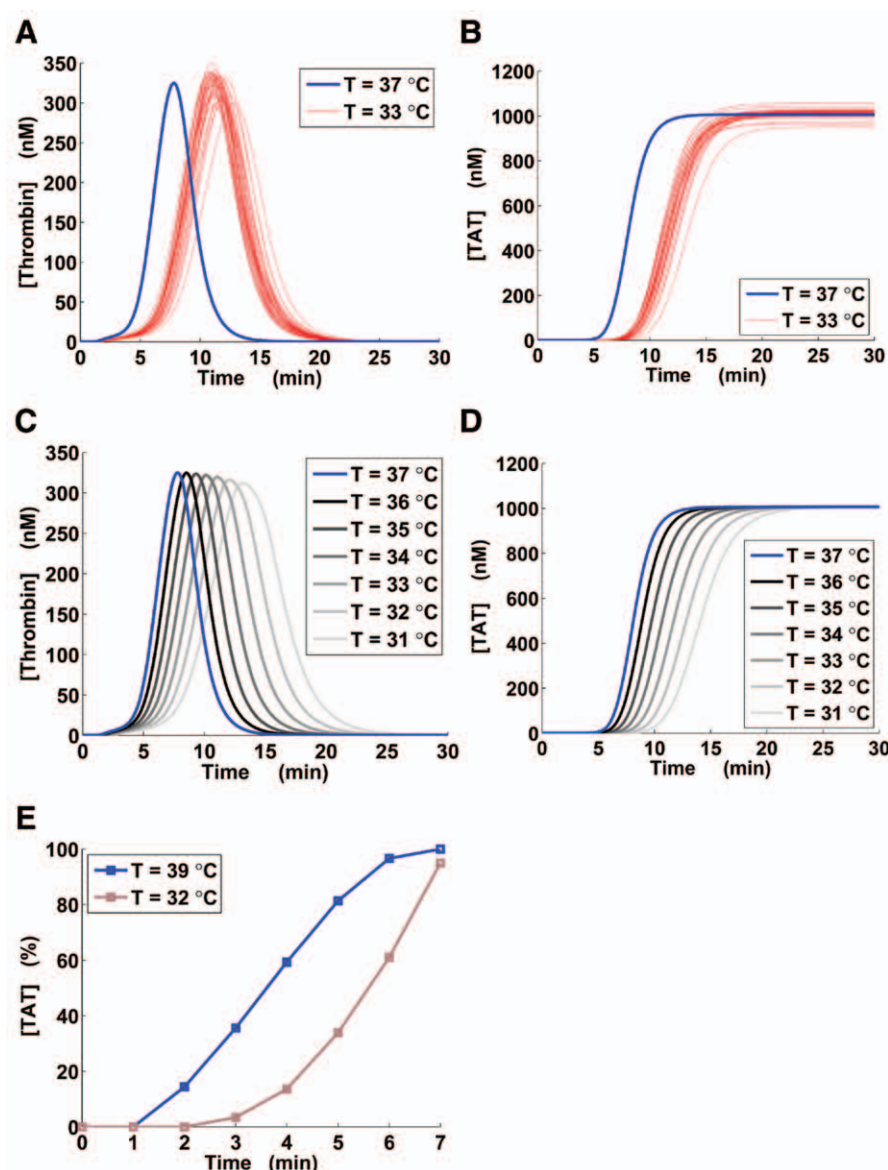
each curve in the group, the quantitative parameters were calculated. The parameter groups constituted the statistical samples for which we calculated interquartile ranges and means and performed sample comparisons using nonparametric hypothesis testing. Kinetic curve parameter samples were compared using Wilcoxon rank sum test when TCs were randomized (in the computations for the “average” subject) or Wilcoxon signed rank test when no TC randomization was performed (in the computations for the LETS subjects). The resulting *P* values were Bonferroni-corrected to account for multiple comparisons of a thrombin parameter distribution for the normal temperature with the distributions of the same parameter for different hypothermic temperatures (for a fixed dilution scenario).

RESULTS

Temperature Modulation of Thrombin Curves

For each of the considered hypothermic temperatures (31°C, 32°C, 33°C, 34°C, 35°C, and 36°C), we independently

Figure 1. Thrombin generation under normal and hypothermic conditions. TAT designates thrombin–antithrombin complexes, and brackets designate concentration. A and B, Model-generated thrombin curves for the “average” subject under normal temperature (blue) and hypothermic temperature (red) conditions. Each red curve corresponds to 1 of 30 randomly generated sets of temperature coefficients (TCs). C and D, Model-generated thrombin curves for the average subject under normal temperature (blue) and median thrombin curves for simulated hypothermia. (Five thousand random TC sets were generated independently for each hypothermic temperature. The resulting thrombin and TAT curves were used to calculate the median thrombin and TAT values at each of the time points that discretize the considered time interval.) E, Normalized data on hypothermia induced in vivo in a porcine model; data taken from Martini et al.²⁹



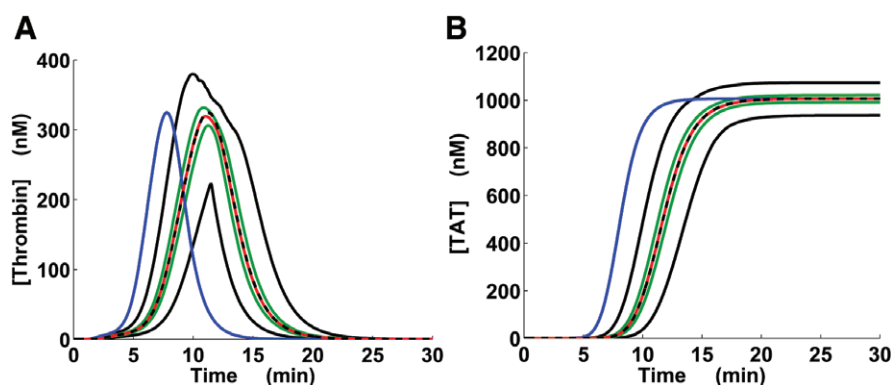


Figure 2. Simulated thrombin generation ranges for hypothermic temperatures in the “average” subject. A, Thrombin curves; B, Thrombin–antithrombin complex (TAT) curves. Blue curves correspond to normal temperature. Solid black, green, and red curves correspond to the range, interquartile range, and median, respectively, calculated from the hypothermic curves generated for 5000 random temperature coefficient (TC) sets. The dashed black lines correspond to hypothermic curves calculated using a model for which all TCs were assigned the value 2.5 (no randomization). All hypothermic thrombin and TAT curves were generated for 33°C.

generated 5000 random sets of TCs, and for each TC set we calculated thrombin and TAT curves for the average subject. Because the generated curves depended on the TCs, there were variations between the curves within each curve group (with 1 group corresponding to 1 hypothermic temperature). Yet, for all the model-generated hypothermic thrombin curves, CT (time to 10 nM thrombin) and thrombin peak time (PT) were larger than the average subject values at normal temperature, i.e., 37°C. The same result held for the 50% activation time (the time it takes the curve to reach 50% of its final plateau value, a standard measure of timing for the activation of biological processes)³⁰ for all hypothermic TAT curves (Fig. 1, A–D). These results are consistent with a number of works reporting hypothermia-delayed clotting measured by thromboelastography and related techniques in human blood,^{7,9,31,32} as well as with TAT measurements for a porcine model of hypothermia-induced coagulopathy (Fig. 1E).

Localization of Predicted Thrombin Curves Under Hypothermic Conditions

We investigated how well the median curves describe the behavior of groups of model-generated thrombin and TAT curves at each hypothermic temperature. For every group of 5000 curves described in the previous subsection, we calculated the curves corresponding to the range and interquartile range. The plots for 33°C are shown in Figure 2; the plots for all other considered hypothermic temperatures were similar (results not shown). While the calculated ranges for the hypothermic curves could have substantial width (due to the sensitivity to outliers), the interquartile ranges for all predicted hypothermic thrombin and TAT curves were quite narrow (Fig. 2). Therefore, if the sample medians were estimated accurately, they could serve as a proxy for the behavior of the hypothermic curves for the “true” (unknown) TCs.

To assess the accuracy of estimation of the median curves, we repeated all described simulations for a larger sample size (10,000 random sets of TCs, generated independently for every considered hypothermic temperature). The resulting curves for the medians and interquartile ranges practically coincided, and the curves for the ranges nearly coincided, with the corresponding curves for sample size

5000 (results not shown). Thus, sample size 5000 was used in all subsequent analyses involving TC randomization.

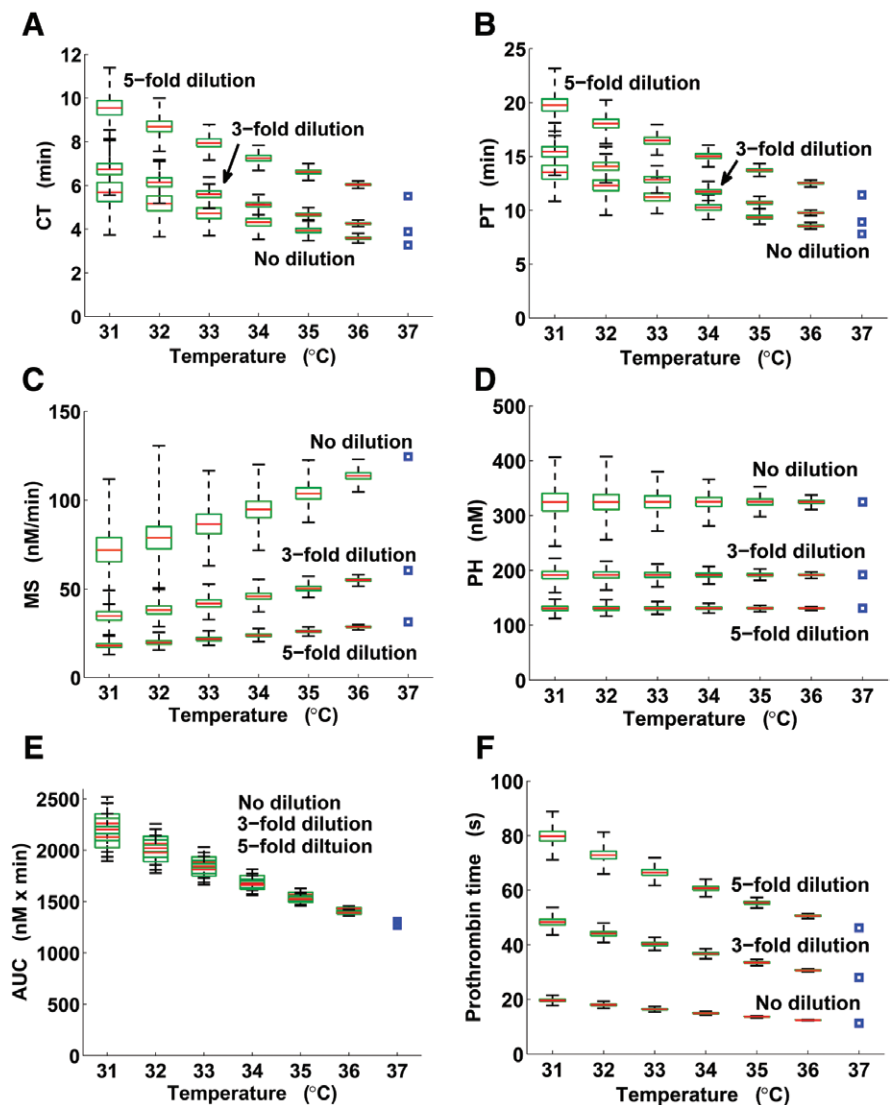
Because generation and analysis of thousands of thrombin curves are computationally expensive, we attempted to simplify the method. We hypothesized that hypothermic thrombin and TAT kinetic curves calculated for the average subject with each TC equal to 2.5 (middle of the sampling interval; see Appendix) could serve as a good estimate for the median curves calculated with TC randomization. Comparison of the curves showed a very good correspondence for all considered temperatures (the case of 33°C is shown in Fig. 2).

Combined Effects of Temperature and Dilution on Standard Quantitative Parameters of Thrombin Generation

For the generated groups of 5000 thrombin curves for each hypothermic temperature, we calculated the 5 standard thrombin curve parameters^{15,19}: CT, PT, maximum slope of the thrombin curve (MS), PH, and the area under the thrombin curve (AUC). Moreover, we used the same strategy to generate thrombin curves and calculate the quantitative parameters for each hypothermic temperature when the blood plasma in the average subject was diluted 3- or 5-fold. The resulting parameter distributions are shown in Figure 3, A to E. For each considered temperature, the medians and interquartile ranges of the parameter distributions followed a pattern that was consistent with our previous results for normal temperature¹⁹: dilution increased CT and PT, decreased MS and PH, and left AUC almost unchanged; these effects were more pronounced for 5-fold than for 3-fold dilution. As the temperature decreased, CT and PT monotonically increased, MS monotonically decreased, and PH remained almost unchanged (Fig. 3, A–D). Remarkably, AUC demonstrated a robust increase for decreasing temperature (Fig. 3E).

To model the effects of hypothermia on prothrombin times (typically reported in experimental studies), we performed similar calculations for CT when thrombin generation was activated by tissue factor (TF) at a concentration of 17 nM rather than the default value of 5 pM. We chose this TF concentration because, in the average subject under

Figure 3. Model-predicted quantitative parameters of thrombin generation for the “average” subject under the normal and hypothermic temperature conditions with and without dilution. A, Clotting time (CT) (time to 10 nM thrombin); B, Thrombin peak time (PT); C, Maximum slope of the thrombin curve (MS); D, Thrombin peak height (PH); E, Area under the thrombin curve (AUC); F, Prothrombin time, calculated as CT for initial tissue factor concentration = 17 nM. The blue squares correspond to single values for the normal temperature, whereas the boxplots show thrombin parameter distributions corresponding to groups of thrombin curves calculated for randomly generated sets of temperature coefficients (5000 random sets were generated independently for each hypothermic temperature value and each dilution scenario). In the boxplots, the whiskers, green horizontal lines, and red lines correspond to the range, interquartile range, and median of the parameter distributions, respectively. Statistical significance of hypothermia-induced differences (for each parameter, except PH, tested independently): normal temperature parameter values versus hypothermic values for the same parameter, $P = 0$ to machine precision.



normal temperature, this TF concentration gave a CT of 11.38 seconds, which can be considered a representative value for normal prothrombin time in humans.³³ Our results for thus estimated prothrombin time (Fig. 3F) were qualitatively similar to those obtained for CT (Fig. 3A). Our computational predictions for both prothrombin time and CT were consistent with experimental results reported in the literature,^{7,9,10} including results on combined effects of hypothermia and blood dilution.⁸

To identify the thrombin generation parameters that were affected by hypothermia the most, we calculated the fold changes (FCs) between the normal temperature values of the parameters and the corresponding median hypothermic values (for the same dilution scenario). The FC was defined for the hypothermic value (HV) and the normal value (NV) of a parameter as follows: $FC = HV/NV$ if $HV > NV$, and $FC = NV/HV$ otherwise.¹⁵ For all dilution scenarios and all temperatures, the FC in PH was 1.00. For any other parameter (including prothrombin time) and dilution scenario, the FCs for 31°C, 32°C, 33°C, 34°C, 35°C, and 36°C were approximately 1.73, 1.58, 1.44, 1.31, 1.20, and 1.10, respectively.

Temperature Effects and Intersubject Variability

Intersubject differences in normal levels of coagulation factors result in considerable intersubject differences in thrombin curves (at 37°C, Fig. 4A), which also exist for hypothermic temperatures (e.g., at 33°C, Fig. 4B). We thus investigated how differences in coagulation factor composition impact the influence of low temperatures on the quantitative parameters of thrombin generation. To this end, we applied our simulation strategy to the group of LETS subjects. We initially performed full TC-randomization simulations (with randomly generated sets of 5000 TCs) for 2 LETS subjects having the minimum and maximum normal PT values in the group. These simulations demonstrated that interquartile ranges for predicted thrombin and TAT kinetics can be sufficiently narrow even for subjects with quantitatively very different thrombin generation kinetics (Fig. 4, C and D). Thus, in our subsequent simulations with the LETS subjects, we focused on the behavior of median thrombin curves, which we approximated by calculating thrombin generation kinetics with each TC set to 2.5 (no randomization).

In each subject, decreasing the temperature caused a monotonic increase in CT, PT, AUC, and prothrombin time,

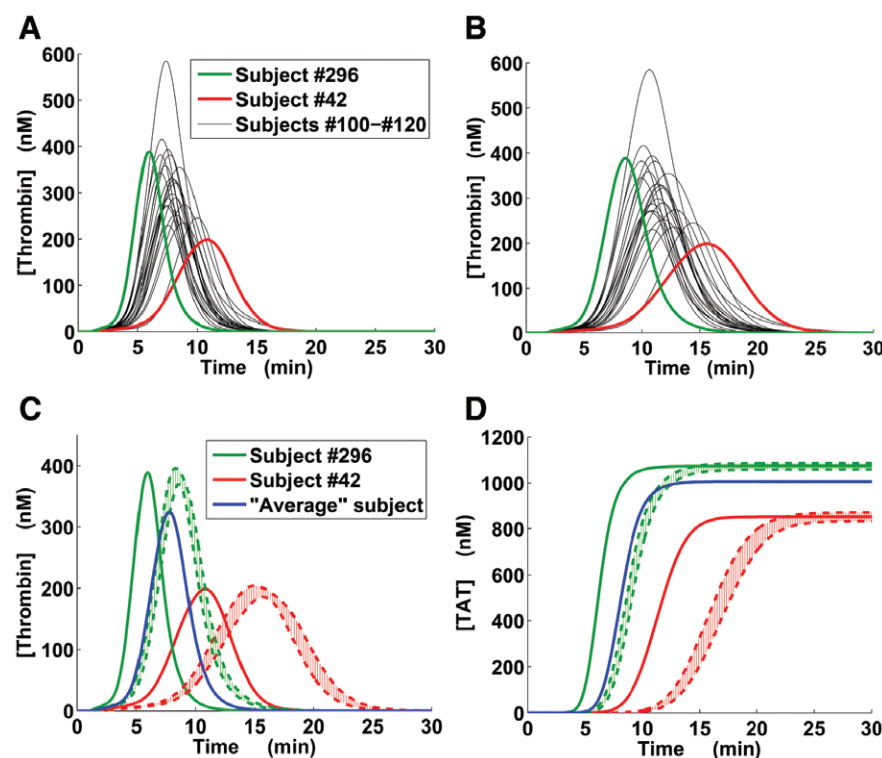


Figure 4. Intersubject variability in the Leiden Thrombophilia Study (LETS) subject group. A and B, Thrombin generation in individual subjects. A, Normal temperature (37°C); B, Hypothermic temperature (33°C). The green and red curves correspond to the subjects characterized by the smallest and the largest normal peak time, respectively. The hypothermic curves were calculated by setting all temperature coefficients (TCs) to 2.5 (no randomization). C and D, Temperature dependence for the subjects with extreme thrombin peaks in the LETS subject group. C, Thrombin curves; D, Thrombin-antithrombin (TAT) curves. Blue curves correspond to the "average" subject; green and red curves correspond to the LETS subjects with extreme thrombin peaks (A and B). Solid lines correspond to normal temperature; dashed lines correspond to hypothermic temperature (33°C). The upper and lower dashed lines of the same color designate the interquartile range (highlighted by fill of the same color) for a group of thrombin curves calculated for 5000 random TC sets.

and a monotonic decrease in MS (Table 1). PH was practically unaffected by temperature in all subjects. Interestingly, despite the presence of noticeable intersubject variability in parameter values at each of the temperatures (Table 1), the FCs in the thrombin parameters (and prothrombin time) for every subject followed a quantitative pattern very similar to that for the average subject (see the Combined Effects of Temperature and Dilution on Standard Quantitative Parameters of Thrombin Generation). Our results for the thrombin curve parameters are consistent with analogous calculations for the TAT curve parameters. Indeed, in our LETS subject group, 50% activation times for the TAT curves were systematically increased, and maximum slopes were decreased, whereas the TAT plateau was practically unaffected by hypothermia (Table 2).

DISCUSSION

During the initial phase of hemostasis, blood from a disrupted vessel comes in contact with TF-bearing cells in the surrounding tissues; this triggers the generation of thrombin, which converts fibrinogen into fibrin, the main structural component of blood clots.^{34,35} Here, we extended the validated Hockin-Mann kinetic model^{17,18} to systematically investigate how thrombin generation is affected by (systemic and possibly local) hypothermia, alone and in combination with blood dilution. Our computational strategy was based on the physically justified assumption that hypothermia impacts thrombin generation by reducing the rates of the biochemical reactions in the thrombin generation network (see Methods and Appendix). Our results suggest that thrombin generation is progressively delayed at hypothermic temperatures (Fig. 1, A–D). While this delay might be slight (and, therefore, hard to detect experimentally) for

mild hypothermia (34°C–36°C for trauma patients³), it is noticeably larger for moderate hypothermia (32°C–34°C for trauma patients³) (Figs. 1–3; Table 1). Our randomization-based computational strategy gives narrowly localized predictions for thrombin generation kinetics and can allow one to avoid TC randomization for certain analyses (Fig. 2).

In recent studies, *in vitro* thrombin generation kinetics are often characterized by the standard thrombin curve parameters: CT, PT, MS (the timing parameters), PH, and AUC (the amount parameters).^{15,16,21} Together, these parameters provide a more complete description of all temporal phases of thrombin generation than the traditional indicators, such as prothrombin time. However, a comprehensive experimental study of hypothermia-induced effects on these parameters has not yet been reported. As a key finding of this study, our computational analysis of thrombin generation in the average subject suggested that CT, PT, and AUC (as well as prothrombin time) are increased in hypothermia, whereas MS is decreased, and PH is practically unaffected (Fig. 3). Moreover, we established that the effects of dilution on thrombin generation at hypothermic temperatures are analogous to their effects at normal temperature (Fig. 3) and are fully consistent with the results of our earlier study.¹⁹ We extended current knowledge regarding multifactorial coagulopathy by showing that hypothermia and dilution act in an additive fashion to affect the parameters of thrombin generation (Fig. 3).

Our computations demonstrated that, for the average subject, the magnitude of the hypothermia-induced FC in every parameter affected by hypothermia depends on the temperature, but not on the parameter itself. By applying our computational strategy to the LETS subject group we showed that, despite considerable intersubject variability

Table 1. Thrombin Generation Parameters in the Leiden Thrombophilia Study (LETS) Subject Group Under Temperature Variation

Temperature, °C	CT, min	PT, min	MS, nM/min	PH, nM	AUC, nM × min	Prothrombin time, s
37 ^a	3.31 (2.99–3.73)	7.90 (7.43–8.49)	121.28 (98.89–145.28)	320.34 (276.06–368.27)	1304.27 (1153.38–1481.32)	11.51 (10.85–12.21)
36	3.63 (3.28–4.08)	8.66 (8.14–9.31)	110.66 (90.23–132.56)	320.34 (276.06–368.27)	1429.42 (1264.06–1623.47)	12.61 (11.90–13.38)
35	3.98 (3.60–4.48)	9.49 (8.92–10.20)	100.93 (82.33–120.95)	320.34 (276.06–368.27)	1566.59 (1385.36–1779.25)	13.82 (13.03–14.66)
34	4.36 (3.94–4.91)	10.40 (9.78–11.18)	92.13 (75.12–110.36)	320.34 (276.06–368.27)	1716.92 (1518.29–1949.98)	15.16 (14.28–16.08)
33	4.78 (4.32–5.38)	11.40 (10.72–12.25)	84.07 (68.55–100.70)	320.34 (276.06–368.27)	1881.67 (1663.99–2137.10)	16.60 (15.64–17.62)
32	5.23 (4.73–5.89)	12.50 (11.75–13.43)	76.71 (62.54–91.88)	320.34 (276.06–368.27)	2062.23 (1823.66–2342.17)	18.19 (17.14–19.30)
31	5.74 (5.19–6.46)	13.69 (12.87–14.71)	69.99 (57.07–83.84)	320.34 (276.06–368.27)	2260.12 (1998.65–2566.92)	19.93 (18.81–21.13)

The thrombin curves for hypothermic temperatures (<37°C) were computed with all temperature coefficients set to 2.5 (no randomization). The data are shown as median (interquartile range). Prothrombin time for each subject was calculated as CT for an initial TF concentration of 17 nM.

Statistical significance of hypothermia-induced differences for each parameter, except PH, tested independently; normal temperature parameter values versus hypothermic values for the same parameter, $P < 10^{-5}$. The P -value computations were performed as follows. We analyzed 1 thrombin generation parameter (corresponding to 1 Table column) at a time; PH, due to the obvious insensitivity to temperature (see the Table), was excluded from analysis. For each of the remaining 5 parameters, we considered 7 statistical samples. Each of those samples corresponded to 1 temperature level and comprised the 472 parameter values computed for the 472 LETS subjects for that temperature (the medians and interquartile ranges of each of those samples are shown in the corresponding cells of the Table). For the 7 samples, we performed 6 statistical comparisons using Wilcoxon signed rank test (MATLAB function SIGNRANK): the sample for normal temperature was compared with each of the samples for the hypothermic temperatures. Each such comparison returned a P value characterizing the difference between the samples for the given 2 temperatures. Because we tested whether the normal temperature sample was different from all 6 hypothermic temperature samples, and performed 6 tests for that, the 6 P values were Bonferroni-corrected via multiplying by 6. Each of thus corrected P values was $<10^{-5}$.

CT = clotting time (time to 10 nM thrombin); PT = thrombin peak time; MS = maximum slope of the thrombin curve; PH = thrombin peak height; AUC = area under the thrombin curve.

^aData for 37°C (except prothrombin time) have been published previously,²⁰ and are given here for comparison purposes.

in absolute parameter values (Fig. 4; Table 1), the relative effect of hypothermia on each parameter for every subject was nearly the same as observed for the average subject. While intersubject variability in hypothermia-induced effects on thrombin generation has not been studied in laboratory experiments, based on our findings, we anticipate that experimentally measured relative effects of hypothermia will only mildly depend on intersubject differences. In all subjects, the impact of hypothermia on thrombin generation predicted by our model was substantial. Indeed, at 33°C, the CT and PT were approximately 44% larger than the corresponding normal values. This suggests that noticeable hypothermia-induced thrombin generation delays for moderate or severe (<32°C for trauma patients³) hypothermia should be expected.

Our study has limitations that follow from the simplified representation of the blood clotting process and our choice of methodology. First, this is a computational study that does not account for blood flow, as well as for some of the components of the blood coagulation system, such as fibrin(ogen), thrombomodulin, and proteins C and S. Yet, this model reflects experimental setups that are frequently used to study thrombin generation in vitro.^{15,18,19,24} Moreover, the moderate complexity of the model allows for tractable analyses that may elucidate major trends to be subsequently tested in more complete (and complex) systems. Second, our modeling strategy does not explicitly represent the effects of temperature on the cells (such as platelets) whose surfaces support thrombin generation.³⁴ However, it is reasonable to assume that, because many of the underlying processes in cell activation are governed by biochemical reactions, the dependence of cell activation on temperature can be implicitly represented by modulating the kinetic constants in the Hockin–Mann model (see Methods and Appendix). Finally, a lack of published experimental data precluded direct testing of some of our computational predictions regarding hypothermia-induced effects on thrombin and TAT curve parameters. Particularly, the hypothermia-induced increase in AUC, predicted by our model and experimentally detected for murine blood plasma,³⁶ remains a hypothesis in need of experimental validation for human blood. Being a systematic study of the effects of hypothermia on thrombin generation, our work sets a direction for future experimental investigations. Such investigations should be aimed at measuring the effects of in vitro-induced hypothermia on thrombin generation parameters in human blood plasma, as well as the effects of in vivo-induced hypothermia on thrombin generation in porcine models. Moreover, future investigations should focus on the multifactorial nature of traumatic coagulopathy and supplement our results on hypothermia and dilution with an analysis of acidosis and acute traumatic coagulopathy.

Our results complement the existing experimental studies and provide further evidence that thrombin generation impairment is a major cause of hypothermia-induced abnormalities in blood coagulation. A significant practical consequence of this conclusion is the possibility to develop pharmacological strategies that reverse the effects of hypothermia by restoring normal thrombin generation. The feasibility of this approach is supported by the ability of

Table 2. Thrombin–Antithrombin Complex (TAT) Curve Parameters in the Leiden Thrombophilia Study (LETS) Subject Group Under Temperature Variation

Temperature, °C	50% activation time, min	MS_TAT, nM/min	Plateau level, nM
37	8.27 (7.75–8.88)	288.76 (249.67–323.14)	999.71 (901.51–1092.40)
36	9.06 (8.49–9.73)	263.48 (227.81–294.84)	999.71 (901.51–1092.40)
35	9.93 (9.31–10.66)	240.41 (207.86–269.03)	999.71 (901.51–1092.40)
34	10.88 (10.20–11.68)	219.36 (189.66–245.47)	999.71 (901.51–1092.40)
33	11.93 (11.18–12.80)	200.15 (173.06–223.98)	999.71 (901.51–1092.40)
32	13.07 (12.25–14.03)	182.63 (157.91–204.37)	999.71 (901.51–1092.40)
31	14.32 (13.43–15.38)	166.64 (144.08–186.48)	999.71 (901.51–1092.40)

The TAT curves for hypothermic temperatures (<37°C) were computed with all temperature coefficients set to 2.5 (no randomization). Fifty percent activation time is the time to 50% of the plateau level. The plateau level is estimated as the TAT level at the end of the considered time interval (50 min). The data are shown as median (interquartile range).

Statistical significance of hypothermia-induced differences (for 50% activation time and MS_TAT tested independently): normal temperature parameter values versus hypothermic values for the same parameter, $P < 10^{-5}$. The P -value computations were performed similarly to our analysis for the data in Table 1. We analyzed 1 TAT curve parameter at a time; the plateau level was insensitive to temperature (see the Table) and was therefore excluded from analysis. For each of the remaining 2 TAT curve parameters, we considered 7 samples, each corresponding to 1 temperature level and comprising the 472 parameter values for the 472 LETS subjects for that temperature. For the 7 samples, we performed 6 statistical comparisons using Wilcoxon signed rank test: the normal temperature sample was compared with each of the hypothermic temperature samples. Each such comparison returned a P value, which were Bonferroni-corrected via multiplying by 6. Each of thus corrected P values was $<10^{-5}$.

MS_TAT = maximum slope of the TAT curve.

recombinant factor VIIa, which acts at the level of thrombin generation, to mitigate the adverse effects of hypothermia on blood coagulation.^{32,37,38} Yet, it is known that other factors, such as suggested impairment of platelet function^{14,39} and reduction in fibrinogen availability,⁴⁰ may contribute to hypothermia-induced coagulopathy. The relative contributions and interdependencies of all these factors should be addressed in future investigations. ■■

APPENDIX

Supplemental Methods

In mathematical terms, the Hockin–Mann model is a system of 34 nonlinear ordinary differential equations that are based on the mass action law of chemical kinetics. We implemented the updated version¹⁷ of the model in the SimBiology toolbox of the MATLAB software suite (MathWorks, Natick, MA). All computations were performed in MATLAB 2012a. Our general modeling methodology followed our recent work.^{15,19,20} The initial tissue factor (TF) concentration had a default standard value of 5 pM, unless stated otherwise, and was not affected by dilution.^{19,20}

Temperature coefficients (TCs) in our modeling strategy were sampled uniformly and independently from the interval (2–3), which can be suggested as a reasonable choice of sampling interval.²⁶ TC randomization was deemed necessary because the simplifying assumption of equal or similar TCs throughout a biochemical network may not account for true system kinetics.²⁷ Randomization of model parameters is often used to assess model behavior in the absence of accurate estimates for kinetic constants.^{30,41} Indeed, deriving TCs from experimental measurements reported in the literature was not possible because the experimentally measured values of the kinetic constants were adjusted in the process of model development,¹⁸ and no experimental temperature-dependence data on these model-specific kinetic constants are currently available. The use of a uniform sampling distribution is supported by a statistical and information-theoretic principle (known as the Maximum Entropy Principle) according to which, in the absence of detailed information, one should use the distribution that is characterized by maximum entropy under given constraints. In the case of

a continuous distribution on a finite interval, the distribution with maximum entropy is the uniform distribution.⁴² The intuition behind this principle is that making the most “neutral” choice of distribution may allow one to minimize possible biases.

Model Parameter Analysis

To elucidate the model parameters that influence the magnitude of temperature effects the most, we performed parameter sensitivity analysis in the small vicinity of the default parameter set in the “average” subject. To this end, we increased the value of each of the 44 parameters (i.e., the kinetic constants) by $X\%$ (one at a time), and for each of such perturbations we calculated $|\Delta FC|$, which is the absolute difference between the fold change (FC) value returned by the perturbed model and the FC value for the unperturbed model. The FC values were computed for clotting time (CT), as described in the Results section, with hypothermic temperature equal to 32°C. Here, we chose to focus on CT because hypothermia-induced change in CT may be regarded as a typical effect of hypothermia, which can be detected both computationally and experimentally. The core temperature of 32°C is a critical temperature threshold indicating the onset of severe hypothermia for trauma patients.

The resulting 44 values of $|\Delta FC|$ (each corresponding to 1 perturbed parameter) were sorted in descending order to elucidate the most “sensitive” parameters. While the outcome of such ordering generally depended on the value of X , for X ranging from $1 \times 10^{-6}\%$ to $1 \times 10^{-4}\%$, the top 5 most sensitive parameters were those with indices 9, 4, 10, 2, and 14 (in order of decreasing $|\Delta FC|$). The corresponding biochemical reactions are given in Table A1. These reactions characterize the interactions of factor VII (FVII) and factor VIIa (FVIIa) and their complexes with TF and other clotting factors. These results are consistent with a previously performed sensitivity analysis of the Hockin–Mann model at normal temperature¹⁷ and indicate that the reactions involving FVII, FVIIa, and TF may have the largest influence on the effects of temperature on thrombin generation.

Table A1. Biochemical Reactions Represented in the Mathematical Model of Thrombin Generation¹⁷

Biochemical reactions

TF + FVII (1) ↔ (2) TF:FVII
TF + FVIIa (3) ↔ (4) TF:FVIIa
TF:FVIIa + FVII → (5) TF:FVIIa + FVIIa
FXa + FVII → (6) FXa + FVIIa
FIIa + FVII → (7) FIIa + FVIIa
TF:FVIIa + FX (8) ↔ (9) TF:FVIIa:FX → (10) TF:FVIIa:FXa
TF:FVIIa + FXa (11) ↔ (12) TF:FVIIa:FXa
TF:FVIIa + FIX (13) ↔ (14) TF:FVIIa:FIX → (15) TF:FVIIa + FIXa
FXa + FII → (16) FXa + FIIa
FIIa + FVIII → (17) FIIa + FVIIIa
FVIIIa + FIXa (18) ↔ (19) FIXa:FVIIIa
FIXa:FVIIIa + FX (20) ↔ (21) FIXa:FVIIIa:FX → (22) FIXa:FVIIIa + FXa
FVIIIa (23) ↔ (24) FVIIIa1-L + FVIIIa2
FIXa:FVIIIa:FX → (25) FVIIIa1-L + FVIIIa2 + FX + FIXa
FIXa:FVIIIa → (25) FVIIIa1-L + FVIIIa2 + FIXa
FIIa + FV → (26) FIIa + FVa
FXa + FVa (27) ↔ (28) FXa:FVa
FXa:FVa + FII (29) ↔ (30) FXa:FVa:FII → (31) FXa:FVa + mFIIa
mFIIa + FXa:FVa → (32) FIIa + FXa:FVa
FXa + TFPI (33) ↔ (34) FXa:TFPI
TF:FVIIa:FXa + TFPI (35) ↔ (36) TF:FVIIa:FXa:TFPI
TF:FVIIa + FXa:TFPI → (37) TF:FVIIa:FXa:TFPI
FXa + AT → (38) FXa:AT
mFIIa + AT → (39) mFIIa:AT
FIXa + AT → (40) FIXa:AT
FIIa + AT → (41) FIIa:AT
TF:FVIIa + AT → (42) TF:FVIIa:AT
FIXa + FX → (43) FIXa + FXa
mFIIa + FV → (44) mFIIa + FVa

The reacting biochemical species are blood coagulation factors and their inactive precursors, whose description can be found in the original publication that introduced the model¹⁸ and references cited therein. The numbers in parentheses pointed at by the arrows in the reaction equations designate the kinetic constant indices for those reactions, e.g., A (x) ↔ (y) B denotes the reaction A → B with rate y and the reverse reaction with rate x. TF = tissue factor; AT = antithrombin; TFPI = tissue factor pathway inhibitor.

DISCLOSURES

Name: Alexander Y. Mitrophanov, PhD.

Contribution: Alexander Y. Mitrophanov designed and conducted the study, analyzed the data, and prepared the manuscript.

Attestation: Alexander Y. Mitrophanov approved the final manuscript. Alexander Y. Mitrophanov attests to the integrity of the original data and the analysis reported in this manuscript. Alexander Y. Mitrophanov is the archival author.

Name: Frits R. Rosendaal, MD, PhD.

Contribution: Frits R. Rosendaal contributed to data collection and analysis.

Attestation: Frits R. Rosendaal approved the final manuscript.

Name: Jaques Reifman, PhD.

Contribution: Jaques Reifman designed the study, analyzed the data, and edited the manuscript.

Attestation: Jaques Reifman approved the final manuscript. Jaques Reifman attests to the integrity of the original data and the analysis reported in this manuscript.

This manuscript was handled by: Jerrold H. Levy, MD, FAHA.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Kenneth Mann, Dr. Saulius Butenas, and Ms. Fania Szlam for valuable discussions, and to 3 anonymous reviewers whose comments have helped to improve the paper.

REFERENCES

- Lier H, Krep H, Schroeder S, Stuber F. Preconditions of hemostasis in trauma: a review. The influence of acidosis, hypocalcemia, anemia, and hypothermia on functional hemostasis in trauma. *J Trauma* 2008;65:951–60
- Thorsen K, Ringdal KG, Strand K, Søreide E, Hagemo J, Søreide K. Clinical and cellular effects of hypothermia, acidosis and coagulopathy in major injury. *Br J Surg* 2011;98:894–907
- Tsuei BJ, Kearney PA. Hypothermia in the trauma patient. *Injury* 2004;35:7–15
- Kheirbek T, Kochanek AR, Alam HB. Hypothermia in bleeding trauma: a friend or a foe? *Scand J Trauma Resusc Emerg Med* 2009;17:65
- Hess JR, Brohi K, Dutton RP, Hauser CJ, Holcomb JB, Kluger Y, Mackway-Jones K, Parr MJ, Rizoli SB, Yukioka T, Hoyt DB, Bouillon B. The coagulopathy of trauma: a review of mechanisms. *J Trauma* 2008;65:748–54
- Alam HB, Bice LM, Butt MU, Cho SD, Dubick MA, Duggan M, Englehart MS, Holcomb JB, Morris MS, Prince MD, Schreiber MA, Shults C, Sondeen JL, Tabbara M, Tieu BH, Underwood SA; Hemostatic Resuscitation Research Group. Testing of blood products in a polytrauma model: results of a multi-institutional randomized preclinical trial. *J Trauma* 2009;67:856–64
- Dirkmann D, Hanke AA, Görlinger K, Peters J. Hypothermia and acidosis synergistically impair coagulation in human whole blood. *Anesth Analg* 2008;106:1627–32
- Gubler KD, Gentilello LM, Hassantash SA, Maier RV. The impact of hypothermia on dilutional coagulopathy. *J Trauma* 1994;36:847–51
- Rundgren M, Engström M. A thromboelastometric evaluation of the effects of hypothermia on the coagulation system. *Anesth Analg* 2008;107:1465–8
- Staab DB, Sorensen VJ, Fath JJ, Raman SB, Horst HM, Obeid FN. Coagulation defects resulting from ambient temperature-induced hypothermia. *J Trauma* 1994;36:634–8
- Kheirabadi BS, Crissey JM, Deguzman R, Holcomb JB. *In vivo* bleeding time and *in vitro* thrombelastography measurements are better indicators of dilutional hypothermic coagulopathy than prothrombin time. *J Trauma* 2007;62:1352–9
- Martini WZ, Cortez DS, Dubick MA, Park MS, Holcomb JB. Thrombelastography is better than PT, aPTT, and activated clotting time in detecting clinically relevant clotting abnormalities after hypothermia, hemorrhagic shock and resuscitation in pigs. *J Trauma* 2008;65:535–43
- Ramaker AJ, Meyer P, van der Meer J, Struys MM, Lisman T, van Oeveren W, Hendriks HG. Effects of acidosis, alkalosis, hyperthermia and hypothermia on haemostasis: results of point-of-care testing with the thromboelastography analyser. *Blood Coagul Fibrinolysis* 2009;20:436–9
- Wolberg AS, Meng ZH, Monroe DM 3rd, Hoffman M. A systematic evaluation of the effect of temperature on coagulation enzyme activity and platelet function. *J Trauma* 2004;56:1221–8
- Mitrophanov AY, Reifman J. Kinetic modeling sheds light on the mode of action of recombinant factor VIIa on thrombin generation. *Thromb Res* 2011;128:381–90
- Wolberg AS. Thrombin generation assays: understanding how the method influences the results. *Thromb Res* 2007;119:663–5
- Danforth CM, Orfeo T, Mann KG, Brummel-Ziedins KE, Everse SJ. The impact of uncertainty in a blood coagulation model. *Math Med Biol* 2009;26:323–36
- Hockin MF, Jones KC, Everse SJ, Mann KG. A model for the stoichiometric regulation of blood coagulation. *J Biol Chem* 2002;277:18322–33
- Mitrophanov AY, Rosendaal FR, Reifman J. Computational analysis of intersubject variability and thrombin generation in dilutional coagulopathy. *Transfusion* 2012;52:2475–86
- Mitrophanov AY, Rosendaal FR, Reifman J. Therapeutic correction of thrombin generation in dilution-induced coagulopathy: computational analysis based on a data set of healthy subjects. *J Trauma Acute Care Surg* 2012;73:S95–S102
- Brummel-Ziedins K, Undas A, Orfeo T, Gissel M, Butenas S, Zmudka K, Mann KG. Thrombin generation in acute coronary syndrome and stable coronary artery disease: dependence on plasma factor composition. *J Thromb Haemost* 2008;6:104–10

22. Undas A, Gissel M, Kwasny-Krochin B, Glusko P, Mann KG, Brummel-Ziedins KE. Thrombin generation in rheumatoid arthritis: dependence on plasma factor composition. *Thromb Haemost* 2010;104:224–30
23. Adams TE, Everse SJ, Mann KG. Predicting the pharmacology of thrombin inhibitors. *J Thromb Haemost* 2003;1:1024–7
24. Orfeo T, Butenas S, Brummel-Ziedins KE, Gissel M, Mann KG. Anticoagulation by factor Xa inhibitors. *J Thromb Haemost* 2010;8:1745–53
25. van der Meer FJ, Koster T, Vandenbroucke JP, Briët E, Rosendaal FR. The Leiden Thrombophilia Study (LETS). *Thromb Haemost* 1997;78:631–5
26. DeAngelis DL. Strategies and difficulties of applying models to aquatic populations and food webs. *Ecol Modell* 1988;43:57–73
27. Moehren G, Markevich N, Demin O, Kiyatkin A, Goryanin I, Hoek JB, Kholodenko BN. Temperature dependence of the epidermal growth factor receptor signaling network can be accounted for by a kinetic model. *Biochemistry* 2002;41:306–20
28. Winzor DJ, Jackson CM. Interpretation of the temperature dependence of equilibrium and rate constants. *J Mol Recognit* 2006;19:389–407
29. Martini WZ, Pusateri AE, Uscilowicz JM, Delgado AV, Holcomb JB. Independent contributions of hypothermia and acidosis to coagulopathy in swine. *J Trauma* 2005;58:1002–9
30. Mitrophanov AY, Groisman EA. Response acceleration in post-translationally regulated genetic circuits. *J Mol Biol* 2010;396:1398–409
31. Bladbjerg EM, Jespersen J. Activity of recombinant factor VIIa under different conditions *in vitro*: effect of temperature, pH, and haemodilution. *Blood Coagul Fibrinolysis* 2008;19:369–74
32. Kheirabadi BS, Delgado AV, Dubick MA, Scherer MR, Fedyk CG, Holcomb JB, Pusateri AE. *In vitro* effect of activated recombinant factor VII (rFVIIa) on coagulation properties of human blood at hypothermic temperatures. *J Trauma* 2007;63:1079–86
33. Millenson MM, Bauer KA, Kistler JP, Barzegar S, Tulin L, Rosenberg RD. Monitoring “mini-intensity” anticoagulation with warfarin: comparison of the prothrombin time using a sensitive thromboplastin with prothrombin fragment F1+2 levels. *Blood* 1992;79:2034–8
34. Hoffman M, Monroe DM. Coagulation 2006: a modern view of hemostasis. *Hematol Oncol Clin North Am* 2007;21:1–11
35. Mann KG, Brummel K, Butenas S. What is all that thrombin for? *J Thromb Haemost* 2003;1:1504–14
36. Tchaikovski SN, VAN Vlijmen BJ, Rosing J, Tans G. Development of a calibrated automated thrombography based thrombin generation test in mouse plasma. *J Thromb Haemost* 2007;5:2079–86
37. Klemcke HG, Delgado A, Holcomb JB, Ryan KL, Burke A, DeGuzman R, Scherer M, Cortez D, Uscilowicz J, Macaitis JM, Bliss J, Wojtaszczyk J, Christensen S, Currier H, Pusateri AE. Effect of recombinant FVIIa in hypothermic, coagulopathic pigs with liver injuries. *J Trauma* 2005;59:155–61
38. Martinowitz U, Holcomb JB, Pusateri AE, Stein M, Onaca N, Freidman M, Macaitis JM, Castel D, Hedner U, Hess JR. Intravenous rFVIIa administered for hemorrhage control in hypothermic coagulopathic swine with grade V liver injuries. *J Trauma* 2001;50:721–9
39. Scharbert G, Kalb ML, Essmeister R, Kozek-Langenecker SA. Mild and moderate hypothermia increases platelet aggregation induced by various agonists: a whole blood *in vitro* study. *Platelets* 2010;21:44–8
40. Martini WZ. Coagulopathy by hypothermia and acidosis: mechanisms of thrombin generation and fibrinogen availability. *J Trauma* 2009;67:202–8
41. Mitrophanov AY, Jewett MW, Hadley TJ, Groisman EA. Evolution and dynamics of regulatory architectures controlling polymyxin B resistance in enteric bacteria. *PLoS Genet* 2008;4:e1000233
42. Nguyen HT, Kreinovich V, Wu B, Xiang G. Computing Statistics Under Interval and Fuzzy Uncertainty: Applications to Computer Science and Engineering. Berlin, Germany: Springer, 2012