The Effects of Cortisol on Blood Coagulation in Zebrafish

B.A. Bashara and D. M. Baker
Department of Biological Sciences, University of Mary Washington, Fredericksburg, VA

INTRODUCTION

- The coagulation cascade is a series of reactions in which an inactive factor becomes enzymatically active following contact with previous enzymes in their activation pathway (Gorbet and Sefton 2004) (Fig 1).
- The cascade can be activated via the tissue factor (extrinsic) or contact (intrinsic) pathway (Smith et al 2015).
- In humans, increased cortisol can lead to pathological increases in blood coagulation and lead to thrombotic events such as deep vein thrombosis and pulmonary emboli (Trementino et al 2010).

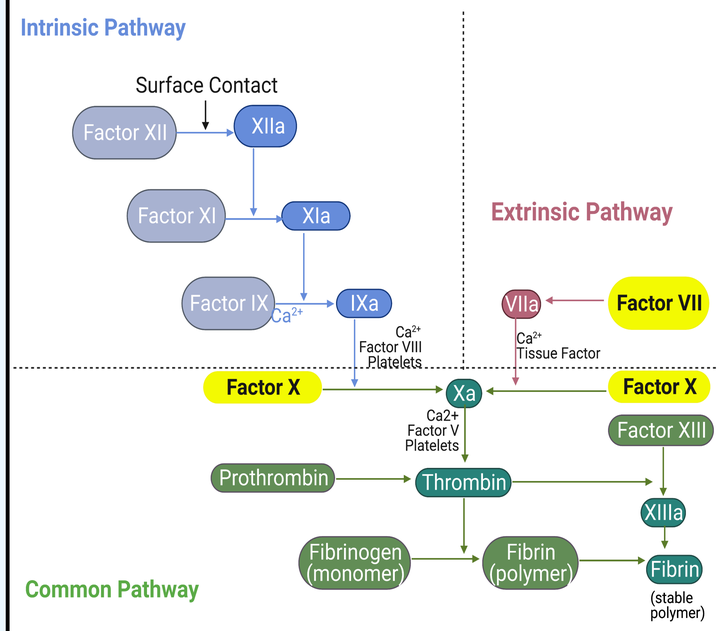

Fig. 1. Overview of the coagulation cascade pathways (adapted from Gorbet and Sefton 2004), created with BioRender.com. The extrinsic pathway is activated by Factor VII, and intrinsic by Factor XII. Both pathways converge through Factor X.

- Zebrafish, *Danio rerio*, (Fig 2) are a teleost species used as a genetic model.
- If zebrafish exhibit the same pathological increases, they could be used as a model organism for testing of anticoagulant medication.

Fig. 2. Male zebrafish (top) and female zebrafish (bottom).

OBJECTIVES

To investigate the effects of cortisol on blood coagulation in zebrafish, via effects on transcription of the genes encoding coagulation factors (Table 1) in chronically stressed zebrafish.

Table 1: Coagulation factor genes measured in this study.

Gene	Protein Encoded	Protein Function
f7	Coagulation Factor VII	Initiation of the contact pathway
f10	Coagulation Factor X	Conversion of prothrombin into thrombin (tissue factor pathway)

METHODS

1 Stress Events

To induce chronic stress, 18 male and 18 female adult zebrafish were subjected to acute stressors repeatedly for 7 days (Table 2). 18 male and 18 female zebrafish were not subjected to chronic stress events as a control.

Table 2: Schedule of Stress Events

Treatment Day	Treatment Time	Stressor
Day 1	11am 4pm	Net Chase Low Water Levels
Day 2	10am 5:45pm	Crowding Net Capture
Day 3	9am 3pm	Net Chase Crowding
Day 4	7:30am 2pm	Net Capture Low Water Levels
Day 5	9:45am 5pm	Net Chase Net Capture
Day 6	8am 2:30pm	Low Water Levels Net Capture
Day 7	12pm 4pm	Net Chase Crowding

2 Sample Collection

24 hours after completion of the last stress event, the zebrafish were euthanized and dissected. Livers were transferred to TriReagent and stored at -80°C for RNA extraction.

3 Sample Preparation

RNA Extraction: RNA was isolated from each liver using the Zymo Research MicroPrep Direct-zol RNA kit.

Reverse Transcription: Complementary DNA (cDNA) was synthesized from the isolated RNA using Maxima H Minus cDNA synthesis kit.

4 Measurement of Gene Expression

qPCR: Transcript levels of the coagulation factor genes (Table 1) and the housekeeping gene β-actin were measured in triplicate for all cDNA samples with SYBR Green Master Mix.

Data Analysis

- Transcript levels were normalized to β-actin and calculated using the ΔΔCt method.
- A t-test was used to test for differences in relative transcript levels between stressed and control groups, and between sexes (α= 0.05).

RESULTS

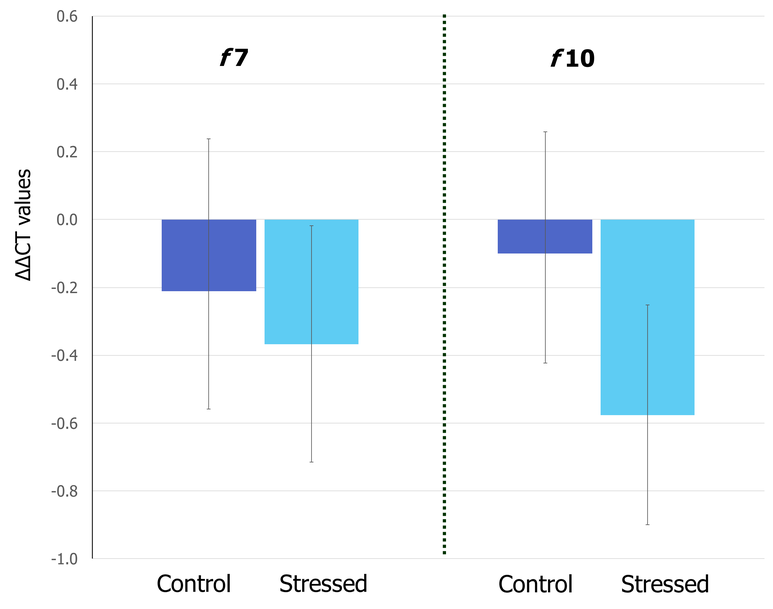

Fig 3. Mean (±SE) ΔΔCT values normalized to housekeeping gene, β-actin. All groups (n=34). Mean transcript levels were not significantly different for either gene.

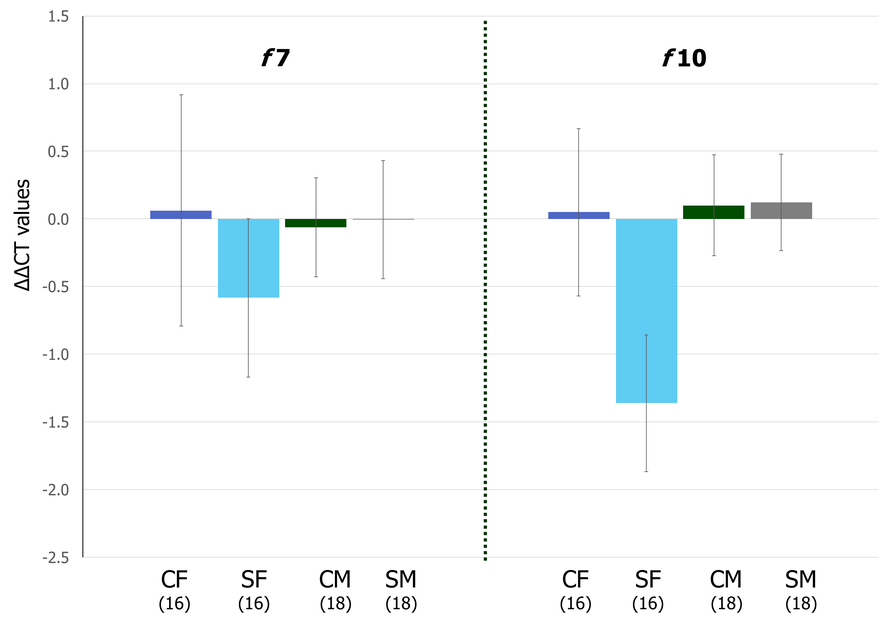

Fig 4. Mean (±SE) ΔΔCT values were compared for each sex independently. CF= control females (n=16); SF = stressed females (n=16); CM = control males (n=18); and SM = stressed males (n=18). Mean transcript levels were not significantly different between sexes.

CONCLUSIONS

- Transcript levels did not differ significantly for either gene between stressed and control groups (Fig 3) or between sexes (Fig 4).
- There is no evidence that increased cortisol causes differences in the transcription of the genes coding for these two coagulation factors.
- High variation among sample resulted in low statistical power.
- Further experimentation could be performed using a longer period of stress events or investigating the transcription of different coagulation factors.

ACKNOWLEDGMENTS

Funding for this project was provided by the UMW Undergraduate Research Funds and the UMW Honors Program.

LITERATURE CITED

Gorbet, M. and Sefton, M. Biomaterial-Associated thrombosis: roles of coagulation factors, complement platelets and leukocytes. *Biomaterials.* 2005; 25(26): 5681-5703.

Smith, S., Travers, R., and Morrissey, J. How it all starts: initiation of the clotting cascade. *Crite Rev Biochem Mol Biol.* 2015; 50(4): 326-336.

Trementino, L., et al. Coagulopathy in Cushing's syndrome. *Neuroendocrinology.* 2010; 92: 55-59.