Elevated Risk of Thrombosis from Synergistic Downregulation of Protein S by Obesity and Estrogen

Ashley L. Paysse MS, Ma. Lorena C. Duhaylungsod MS, Narender Kumar PhD, Mohammad Mohammad PhD, Rinku Majumder PhD

LSUHSC Department of Interdisciplinary Oncology

NIH 5RO1HL151613

Introduction

Protein S (PS) is an essential natural anticoagulant whose deficiency is a major contributor to acquired hypercoagulability. The alteration in PS activity leads to myocardial infarction, stroke, and deep vein thrombosis.

Studies showed that the female sex hormones such as estrogen and progesterone lower PS, thus increasing the risk of thrombosis by 3-fold. Decreased plasma PS is also associated with obesity which elevates the risk of thrombosis by 2.5-fold. However, the risk of thrombosis drastically increases by 24-fold in obese subjects who use estrogen-based oral contraceptive pills (Bloemenkamp et al. 2000; Suzuki et al. 2010).

Aim

This project aims to determine how obesity and estrogen synergistically downregulate PS concentration and thereby dramatically elevate thrombotic risk.

Methods

Blood was collected using aseptic venipuncture in sodium citrate tubes, and the plasma was isolated immediately by centrifugation at 1500 x g at 20 °C, and the following assays were preformed:

i. Activated partial thromboplastin time (aPTT)
ii. Thrombin generation assay (TGA)
iii. Western blotting

Study Outline

Pre-menopausal women aged 18-45 years were enrolled in this study based on four categories:

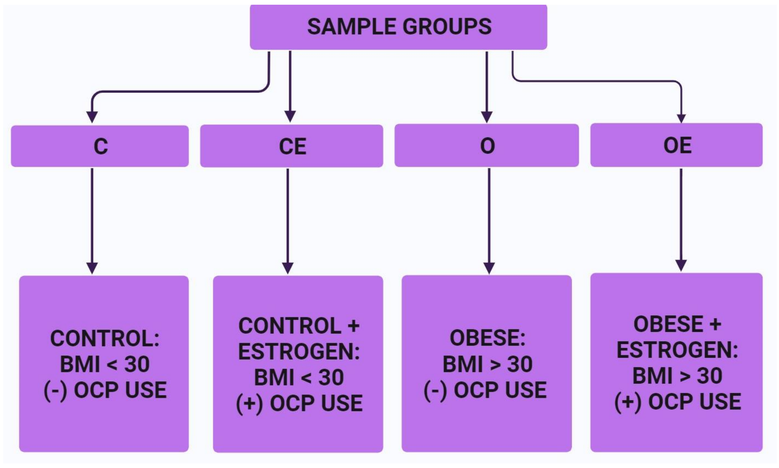


Figure 1

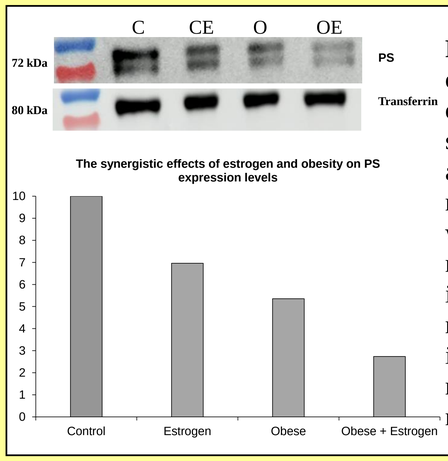


Figure 1. The synergistic effects of estrogen and obesity on PS expression levels. Purified plasma samples were separated by SDS-PAGE and transferred onto a nitrocellulose membrane. The membrane was blocked with 5% non-fat milk in TBST for 60 mins, followed by overnight incubation in PS antibody. The following day, the membrane was washed with TBST and incubated in secondary antibody for 60 mins directed the against the species raised in the primary antibodies.

Figure 2

Figure 2. Thrombin Generation Assay (TGA). TGA buffer was added to a 96-well plate and incubated at 37°C for 5 minutes. Plasma samples were then added to the wells and incubated for an additional 5 minutes. Technothrombin Reagent B was added and incubated for 2 minutes. Finally, 1.2mM fluorogenic substrate Z-Gly-Gly-Arg-AMC was added and read immediately. Statistics were performed by One-way ANOVA ($p<0.001$).

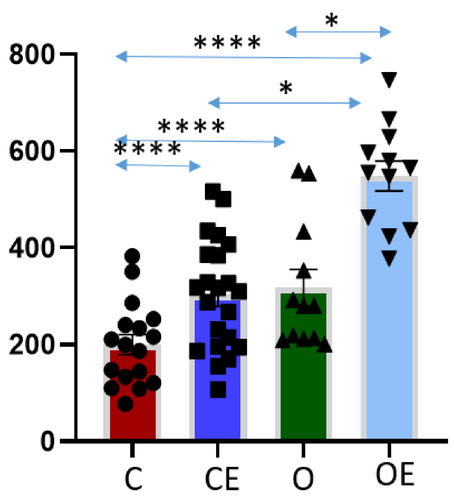
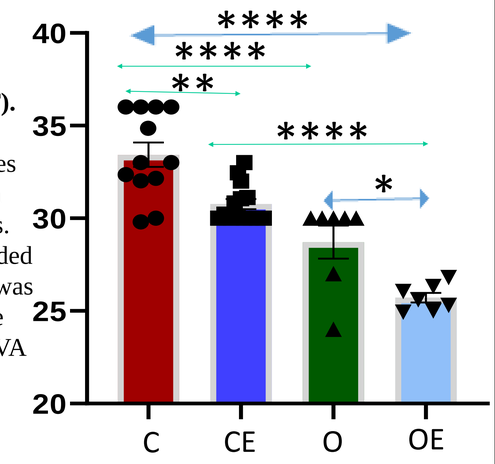


Figure 3

Figure 3. Activated partial thromboplastin time. (aPTT). Plasma samples and Kontact solution were added to cuvettes and incubated in a Ceveron m series at 37°C for 180 seconds. 25mmol/L $CaCl_2$ was then added to initiate the reaction. aPTT was then measured. Statistics were performed by One-way ANOVA ($p<0.001$).



Results

- Quantification of WB bands using ImageJ software showed a significant reduction in PS expression levels in OE samples compared to CE or O participants.
- Formation of thrombin in OE samples was higher compared to control samples which also coincide with the observed low PS expression levels.
- Clotting time was greatly decreased in OE samples compared to control, CE, and O samples.

Conclusion

Increased risk of thrombosis can be directly correlated to the downregulation of PS observed in OE premenopausal women. This can be seen in the inverse relationship between low PS expression and high thrombin generation. This is further proven by decreased clotting time in women who are OE. Efforts are underway collecting more samples to increase the power of this study, including ELISA experiments.

References

1- Bloemenkamp KW, et al., Higher risk of venous thrombosis during early use of oral contraceptives in women with inherited clotting defects. Arch Intern Med, 2000. 160(1): p. 49–52.

2-Suzuki, A., et al., Down regulation of PROS1 gene expression by 17beta estradiol via estrogen receptor alpha ERalpha Sp1 interaction recruiting receptor interacting protein 140 and the corepressor HDAC3 complex. J BiolChem, 2010. 285(18): p. 13444 53.