Estrogen, Protein S, and Obesity Contribute to Thrombosis in Premenopausal Obese Women Who Use Oral Contraceptives

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Introduction

Louisiana is the fourth most obese state in the United States with 36.8% of the state's population being obese (Table 1). This is a significant recurring problem because obesity leads to many other health problems. One prominent condition of obesity is the increase in risk of thrombosis by 2-2.5-fold (1). Blood coagulation occurs by a finely tuned cascade of enzymatic reactions that result in fibrin formation. Central to this process is a complex of a vitamin K-dependent proteases, factor IXa (FIXa), and factor VIIIa (FVIIIa), assembled on a phospholipid-containing membrane (Figure 1). The FXa/FVIIIa complex is the kinetically significant activator of factor X (FX). During thrombin formation by activated FX (FXa), several anticoagulant reactions prevent systemic activation of coagulation. Impairment of these anticoagulant activities increases the risk of venous thrombosis. Common causes of high-risk venous thrombosis are hereditary and acquired deficiencies of the plasma anticoagulant Protein S (PS) (2). PS, vitamin K-dependent protein, negatively regulates coagulation by inhibiting FIXa, thereby limiting factor FXa and thrombin formation. PS is synthesized in the liver, which becomes hypoxic in obese individuals. Hypoxia causes hypoxia inducible factor 1 alpha to downregulate PS expression in obese individuals; this effect explains why obesity increases the risk of thrombosis. Additionally, the risk of thrombosis increases by as much as 24-fold in obese individuals who use oral contraceptive agents (3). The female hormone estrogen also decreases plasma PS levels by 2-3 fold (4). Thus, women who use estrogen-based oral contraceptive agents experience reductions in PS abundance, resulting in the greater risk for thrombosis. Estrogen suppresses PS levels by inhibiting PS gene transcription; estrogen receptor α and transcription factor SP1 mediate this transcriptional inhibition (4). Importantly, the combination of obesity and estrogen-based oral contraceptives dramatically increases thrombotic risk. These combined conditions are prominent in Louisiana because women are at a greater risk for obesity at the premenopausal age (Tables 2 and 3). In this project, our goal is to determine how oral contraceptive agents and obesity synergize to reduce PS levels by measuring free and bound PS levels in plasma and measuring thrombin generation with the plasma of obese premenopausal women.

Blood Coagulation Pathway

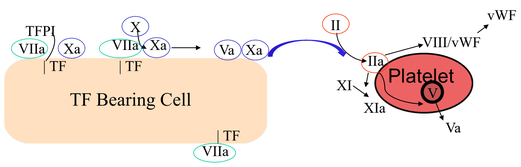

Figure 1. Initiation of Coagulation. Tissue factor is expressed in the damaged vessel (TF bearing cell), which binds to FVIIa. FVIIa activates FIX and FX. FXa via FX binds to FII to form thrombin.

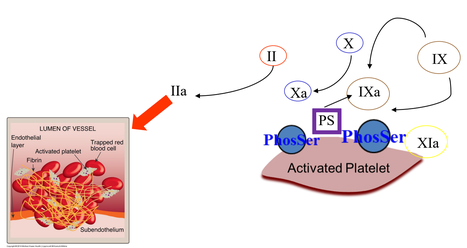

Figure 2. Propagation of coagulation. PS binds to the enzyme FIXa in the presence of Phosphatidyl serine (PhosSer). Therefore, PS inhibits FXa generation in the presence and absence of VIIIa. After thrombin generation is amplified, the platelet is activated by factors V and VIII. This serves as a cofactor in prothrombinase complex and accelerates activation in the initiation step. This allows continuous generation of thrombin and fibrin in order to form sufficient clots.

Obesity Statistics

Average Obesity Prevalence by State
5 Highest States vs. 5 Lowest States
Source: Centers for Disease Control and Prevention

Rank	State	Obesity Rate (95% Confidence Interval)
1	West Virginia	39.5% (CI 37.8-41.3)
1	Mississippi	39.5% (CI 37.8-41.2)
3	Arkansas	37.1% (CI 35.1-39.1)
4	Louisiana	36.8% (CI 34.9-38.7)
5	Kentucky	36.6% (CI 34.9-38.4)
46	California	25.8% (CI 24.8-26.9)
47	New Jersey	25.7% (CI 23.3-28.2)
47	Massachusetts	25.7% (CI 24.3-27.2)
49	Hawaii	24.9% (CI 23.6-26.3)
50	Colorado	23.0% (CI 21.9-24.0)

Table 1. Average Obesity Prevalence by State. The Centers for Disease Control and Prevention reports Louisiana as the fourth most obese state with 36.8% of the population being affected by obesity. This shows how significant the problem of obesity being a common risk factor for increased risk of thrombosis is in the state of Louisiana.

Adult Obesity Prevalence by Gender
Source: Centers for Disease Control and Prevention

Gender	Louisiana Population (95% Confidence Interval)	National Population (95% Confidence Interval)
Women	39.5% (CI 36.9-42.1)	31.3% (CI 30.8-31.7)
Men	34.1% (CI 31.4-36.9)	30.6% (CI 30.1-31.0)

Table 2. Adult Obesity Prevalence by Gender. The Centers for Disease Control and Prevention shows women are more likely to be obese than men. Since women have higher levels of estrogen, specifically while using oral contraceptive agents, they are at a greater risk for thrombosis as a result of the combination of obesity and oral contraceptive use.

Obesity Prevalence by Age Group
Sources: Centers for Disease Control and Prevention/ State of Childhood Obesity

Age Group	Louisiana Population (95% Confidence Interval)	National Population (95% Confidence Interval)
10-17	20.8%	15.3%
18-24	27.9% (CI 22.2-34.5)	18.1% (CI 17.2-18.9)
25-34	33.9% (CI 28.8-39.3)	29.5% (CI 28.7-30.4)
35-44	40.6% (CI 35.9-45.5)	34.5% (CI 33.6-35.3)
45-54	45.8% (CI 41.6-50.1)	36.9% (CI 36.1-37.7)
55-64	40.2% (CI 36.2-44.2)	35.1% (CI 34.4-35.8)
65+	31.8% (CI 28.6-35.2)	28.9% (CI 28.4-29.5)

Table 3. Obesity Prevalence by Age Group. The Centers for Disease Control and Preventions and the State of Childhood Obesity data suggests that adults between 25-64 are most likely to be obese. This age group is where premenopausal women categorize into.

Methods

In this project, we used a large collection of plasma samples (n=75) from control and obese women who either do or do not use oral contraceptive agents. We used ELISA assays to measure the amounts of total and free PS in plasma from non-obese and obese individuals based on BMI. We also measured the free PS levels in obese individuals who used oral contraceptives (Figure 3). Finally, we used a specific thrombin generation assay to measure thrombin formed by these plasma samples (Figure 4). We measured free PS levels in males but did not find any significant changes in obese men compared to the controls (Figure 5). We also performed a parallel study with mice using the same measurements and thrombin generation assays for the same subject group (Figure 6).

Results

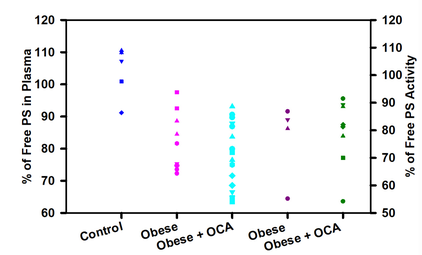

Figure 3. Free PS levels/PS activity. Free PS levels were measured by CYROcheck Clot S™ from Precision Biologic (CCS-30). Obese + oral contraceptive users show less PS activity and PS levels in plasma than the obese group who shows less than the control group.

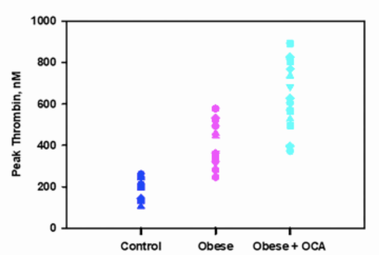

Figure 4. Peak thrombin generation. Obese individuals and obese individuals who used oral contraceptive agents showed higher risk of thrombosis.

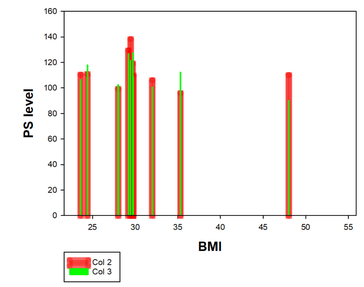

Figure 5. Male PS levels. Males did not show any significant changes in PS levels related to their BMI. We will expand this study to a larger population of obese men with higher estrogen levels.

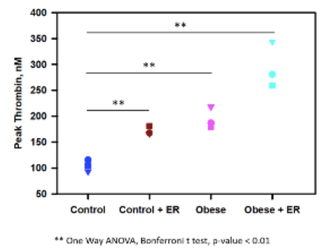

Figure 6. Levels of peak thrombin generation from diet-induced obese mice in the presence and absence of estrogen. Obesity and oral contraceptive agents reduce plasma PS levels and increase thrombin generation in mice.

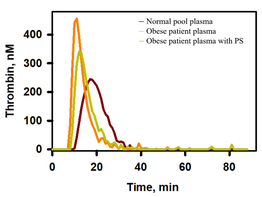

Figure 7. Peak thrombin generation in citrated plasma from obese individuals with and without PS (150 nM) along with normal subjects. PS supplementation reduces thrombin generation in obesity.

Conclusions

We observed that obesity and estrogen, individually and synergistically, decrease the plasma PS levels. Therefore, premenopausal, obese women on oral contraceptives have greater thrombin generation potential compared with obese women who do not use oral contraceptives.

In further research, we will focus on 1) determining the molecular mechanism by which hypoxia, associated with obesity, and estrogen, from contraceptives, affect PS level and 2) investigate therapies to elevate PS level in obese ± estrogen premenopausal women (Figure 7).

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