

# Seasonal and Longitudinal Variation of Thrombotic and Thrombolytic Factors in Humans by Gender and Age

Student: Erin Patrick Murray

Faculty Mentor: Margaret Field

## **Background Information**

It is well known that there is a delicate balance in the clotting and anti-clotting factors which control blood coagulation. If this balance is disturbed in either direction, the resulting pathology can result in excessive clotting or hemorrhage. Recent research indicates that clotting times vary by season but not much is known about why or how this happens. Suggested causes for the variation, such as change in temperature and resulting increase in viscosity seem reasonable at first glance, but recent studies have refuted the previously plausible explanations leaving many mechanisms to be explained. New techniques for examining clotting proteins and information from other species give us an opportunity to investigate the changes in a number of haemostatic proteins in human subjects.

There has been a fair amount of recent research into the seasonal variation of a few

clotting factors but none of these projects have put together all the necessary components to draw conclusions about what kind of role this variation plays in the haemostatic system in the human body. A number of studies have shown temperature has no effect on the variations of studied factors including fibrinogen and Tissue Plasminogen Activator (TPA), Tissue Plasminogen Activator Inhibitor (TPAi) and Antithrombin III (ATIII), which suggests there is no seasonal variation in those observed factors. (Stout and van der Bom). However, a separate study, which measured the activated clotting time (ACT) of patients in Ankara, Turkey, seems to contradict these results of Stout and van der Bom. This study reports longer ACTs in the winter with respect to the summer which seems to indicate an increase in clotting factor concentration in the summer with respect to the winter (Undar). Early work on turtles suggested increased levels of plasma heparin

could be responsible for the reduced clotting in cold-torpor (Kupchella). More recent studies using heparin also suggest that there may need to be a seasonal variation on pre-surgical dosing of heparin. (Hodoglugil)

Other studies have attempted to analyze the effects of age on the haemostatic system. One study's findings showed a variation in the concentration of Fibrinogen by approximately 10mg/dl per decade (Abbate). However, this study didn't analyze the effects of seasonal variation or variation of other clotting factors which may or may not rise along with the concentration of fibrinogen. Another study suggested that rather than a seasonal rise or fall in a particular factor, elderly people simply lose their ability to balance both sides of the haemostatic system leading to an increase in clotting after the age of 55 years old. (Sagripan).

There were 3 thrombotic factors (clotting) and 3 thrombolytic factors (anti-

clotting) which were measured over the course of this project. The thrombotic factors which were studied were Fibrinogen, the precursor to the Fibrin mesh formed in a clot, Factor VII (FVII) which is sometimes administered to people as a treatment for people with hemophilia, TPAi which doesn't directly form a clot, but inhibits the ability of TPA to break down clots and Factor X (FX) which is the link between the extrinsic and intrinsic clotting pathways in the clotting cascade and is activated to increase clotting. The thrombolytic factors which were studied were AT III, and TPA which both work in similar methods to dismantle clots after they have been formed.

## **Methods**

There were 45 samples obtained over the course of 1 year from a plasmaphoresis center in Massachusetts in order to ensure a more dramatic change in seasonal climate than could be found in the San Francisco Bay Area. 24 of those samples were from

the summer with 9 of those samples being female and the other 15 male. The remaining 21 samples were obtained from the winter with 9 female and 12 male samples. Three samples from each age group were obtained to replicate results from multiple subjects. Due to a freezer malfunction, the original samples from the winter were lost which were replaced with samples which the plasmaphoresis center had available. Unfortunately, the oldest male age group was unavailable so it was eliminated from the winter group of samples. Also, when the samples were replaced a number were duplicates from the same patient drawn on a different date.

Samples were distributed into aliquots and frozen down to approximately -50°C. Once a sample was thawed for the second time it was discarded to avoid degradation of proteins due to excess freeze-thaw cycles. Samples were grouped by season, age and gender with the female

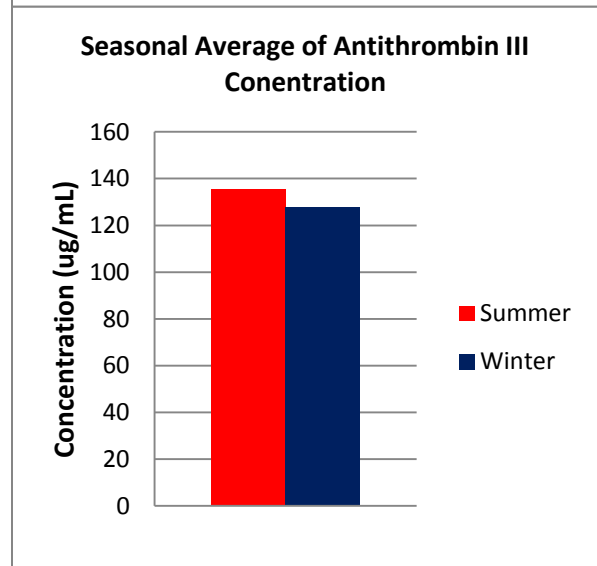
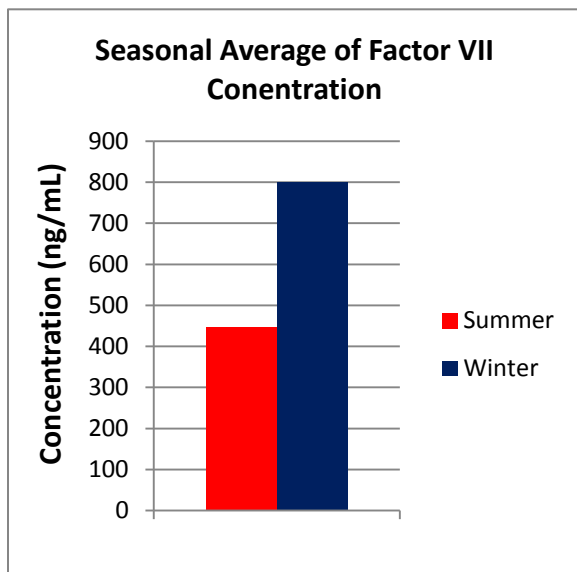
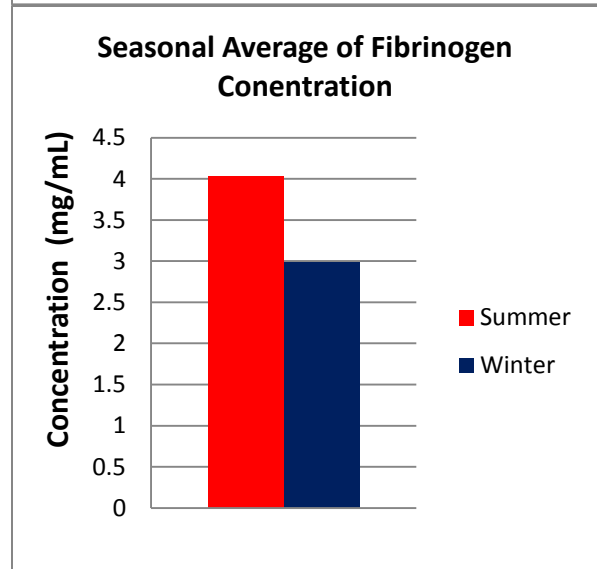
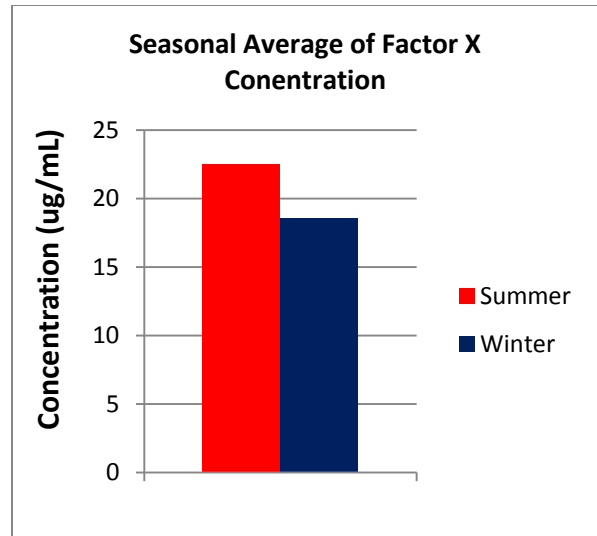
samples restricted to premenopausal, perimenopausal, and postmenopausal groups.

Samples were analyzed using sandwich ELISA techniques. All ELISA kits were purchased from Molecular Innovations except the Fibrinogen kit which was from Cell Science. Protocol, including plasma and antibody dilutions, for each ELISA was provided in each individual kit and the plates were read at 495nm in a BioRad Benchmark plate reader.

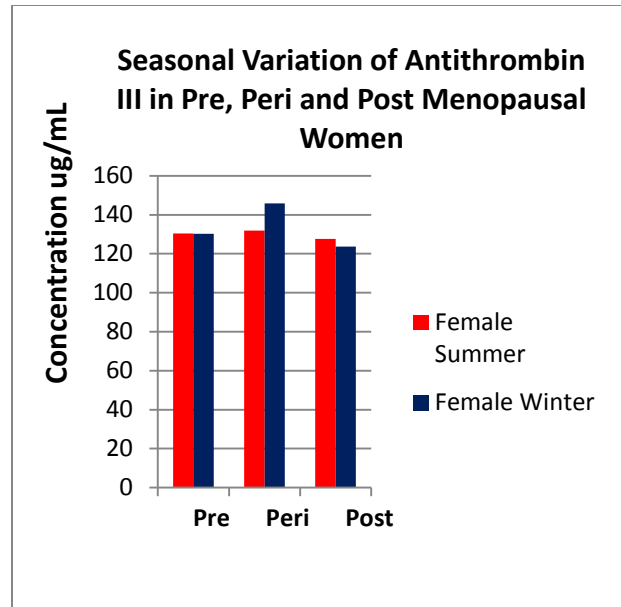
## **Results**

Statistically significant seasonal variation ( $P$  value > 0.95) was observed in Fibrinogen, and FVII. Seasonal variation was also observed in FX, but there weren't enough data points for it to be considered significant at the 95% confidence level. The average FVII concentration was 446.6409ng/mL in the summer and 799.7882ng/mL in the winter. The average

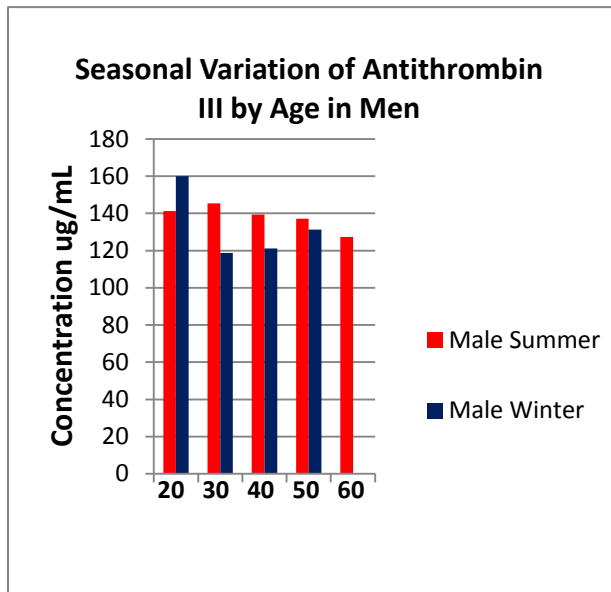
Fibrinogen concentration was 4.02542821mg/mL in the summer and 2.98034556mg/mL for the winter. FX average summer concentration was 22.506779ug/mL while the average winter concentration was 18.603515ug/mL. No seasonal variation was observed in ATIII and TPAi. The TPA assay was unable to obtain measurements for the samples because the TPA concentration in human plasma fell below the sensitivity of the plate which was designed for measurement of TPA in people with genetic defects which produce more TPA than normal values.

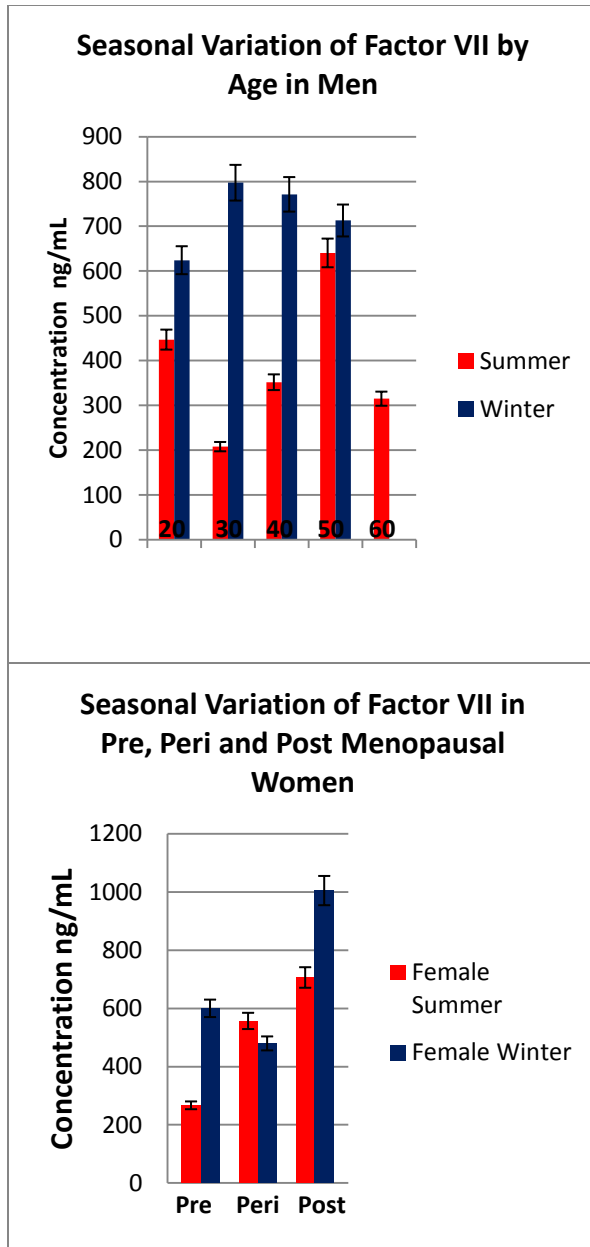


While there were not enough samples from each age group to establish statistically significant longitudinal variation, some preliminary trends did appear. For ATIII, after the initial drop from 25-29 year olds to 35-39 year olds the concentration rose gradually as the subjects aged for men, and women experienced a peak in the peri-menopausal women in the winter. In general, however, ATIII showed the least variation among all the examined factors.



For FVII, after decreasing from 25-29 year olds to 35-39 year olds in the concentration increased consistently as the subjects aged into the 55-59 year old age group in the summer and decreased after 35-39 years old in the winter for the male subjects. FVII displayed an increase in age with women in the summer and displayed its lowest concentration in the peri-menopausal women and the highest in the post-menopausal women in the winter





**Discussion:**

Of the factors which were examined, 2 were found to have statistically significant seasonal variation, Fibrinogen, and FVII. FVII, a member of the thrombotic cascade in

blood coagulation, displayed an average concentration of 446.6409ng/mL in the summer and 799.7882ng/mL in the winter. This trend of high concentrations in the winter followed by a decrease of concentration in the summer was consistent with the literature observed prior to this study (Meade). The longitudinal variation of FVII was not found to be significant, although it did show a vague trend of increasing concentration with age. This trend was also seen by Meade with a greater increase in Women as they progress through menopause and similar yet less significant change in men. Fibrinogen, another member of the thrombotic cascade, had an average concentration of 4.02542821mg/mL in the summer and 2.98034556mg/mL for the winter. Fibrinogen had no significance in the longitudinal data and also displayed no real trend with concentrations varying significantly from one age group to another. This trend of high summer concentrations

may or may not be consistent over a large range of thrombotic factors involved in blood coagulation, but the amount of sample which were incorporated in this study are not enough to draw sufficient conclusions. FX, another thrombotic factor, displayed a similar trend as when compared to fibrinogen, an increased concentration in the summer with respect to the winter and could represent the beginnings of a widespread trend across many thrombotic factors. The data from FX failed to reach a value of  $P > 0.95$ , but it did display a clear trend and may become a statistically significant portion of data as the number of samples increases. The longitudinal study of FX was also insignificant and seemed to show little to no trend except for possibly trending to decrease with age but more samples would be needed to make any kind of definitive conclusion about FX. TPA<sub>i</sub> was analyzed but large spikes in the 25-29 year old males puts the rest of the data from that plate into

question and renders it virtually unusable for males. TPA<sub>i</sub> in female did trend to show a decrease in concentration as the subjects aged but this too, was not statistically significant with the number of samples used in this study.

None of the thrombolytic factors which were seen showed statistical significance for seasonal variation or longitudinal variation. The plate used to analyze TPA didn't yield any information about the concentrations of TPA, because the concentrations fell below the lower measurable limit of the plate. This is reasonable as this plate was designed to analyze the concentrations of TPA in individuals who have higher concentrations of TPA than a normal individual. ATIII was the only thrombolytic factor which data was obtained and this showed a relatively constant concentration for the seasonal analysis and the longitudinal analysis. These results may indicate a trend in thrombotic



factors in general, but no such trend can be seen in any thrombolytic factors studied. All graphs, including those not used in results/discussion sections can be found in the Appendix.

## **Conclusion**

We conclude that there is significant seasonal variation in at least two clotting factors, although these cannot account for an increase in thromboembolic disease seen in the winter. Rather than a decrease in clotting factors our results show the measured thrombotic factors were decreased in the winter in Fibrinogen, the primary precursor to the Fibrin which makes up a significant portion of a clot. While sparse, some literature suggest that clotting factors should decrease however our results are consistent with the study performed by Undar, which showed clots took longer to form in the winter with respect to the summer and implying a decrease in clotting

factor concentration in the winter. We also conclude that the thrombolytic factors examined do not show seasonal or longitudinal variation. No specific patterns were found with respect age and gender at a 95% confidence level.

Future work will include a much larger sample population and will concentrate primarily on the seasonal variations found. We hope to add Protein C, a clotting factor which has been reported to have seasonal variation in Stout, and heparin, a glycosaminoglycan which functions in the thrombolytic pathway which appears to have a seasonal variation as shown by Hodoglulil and by Kupchella in a cold-blooded model.

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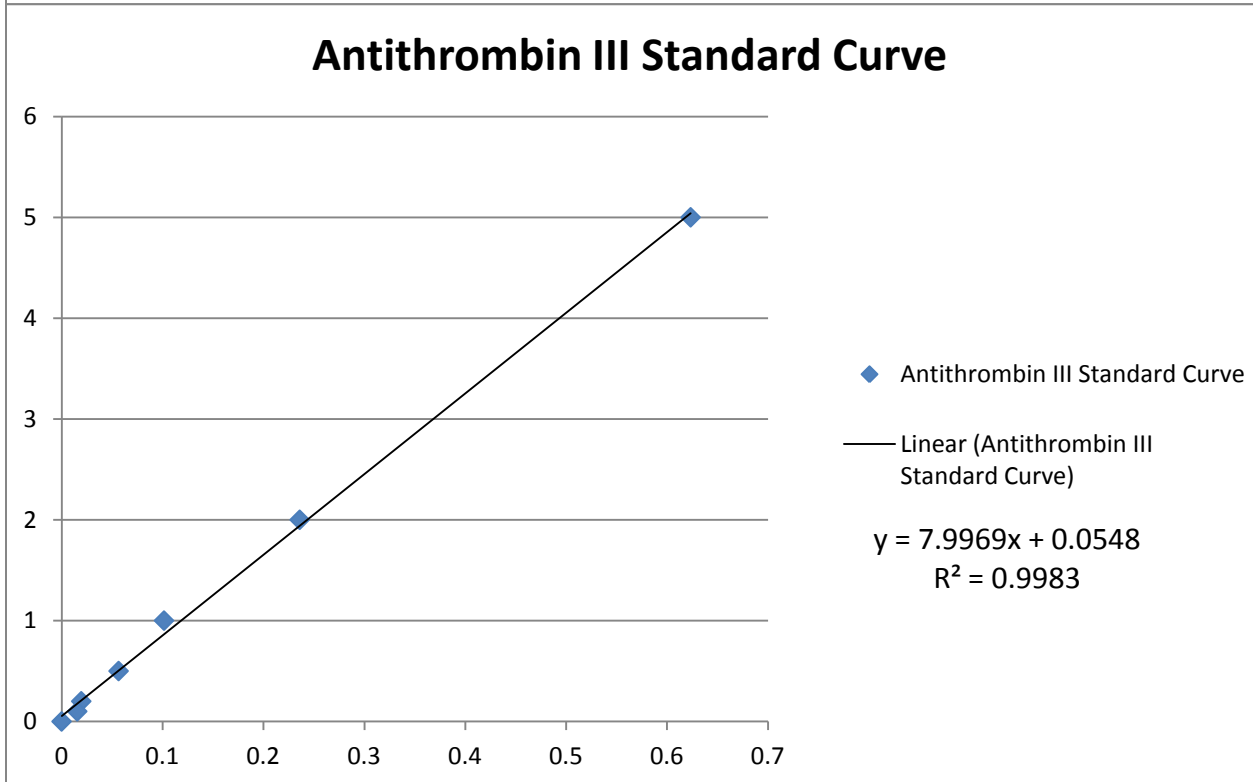
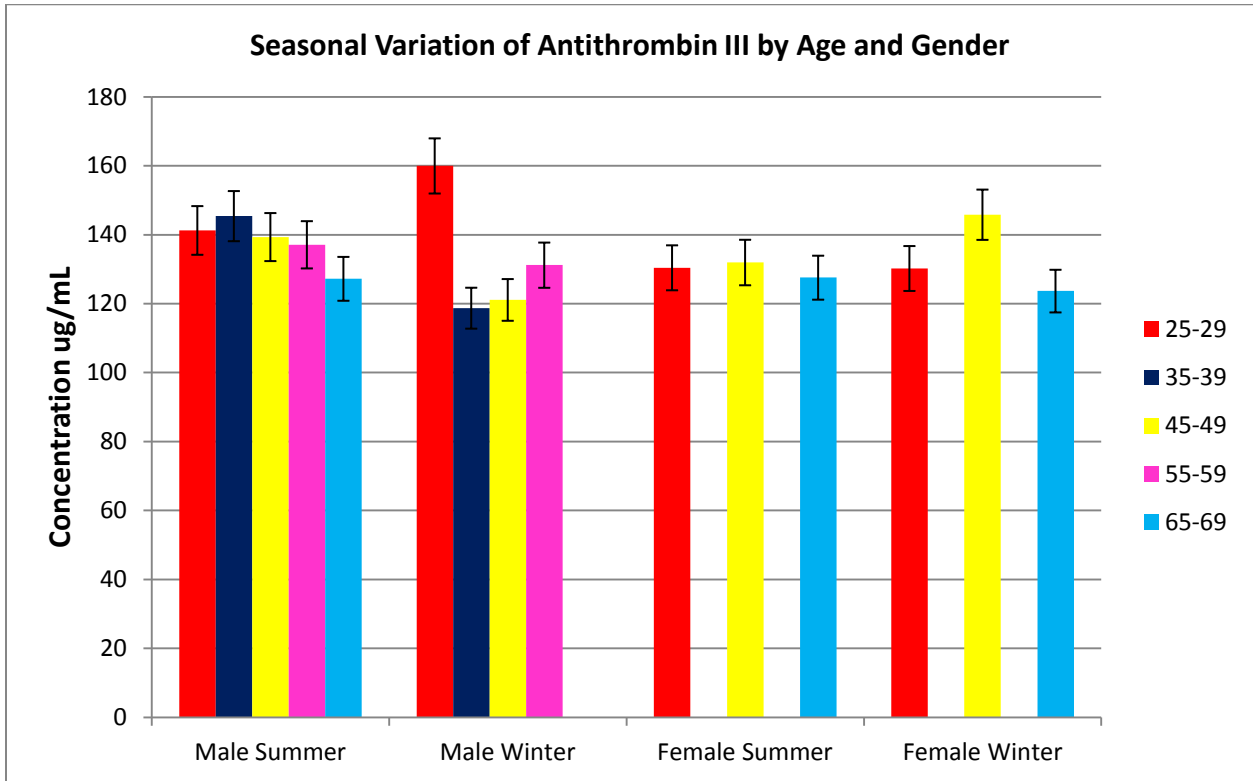
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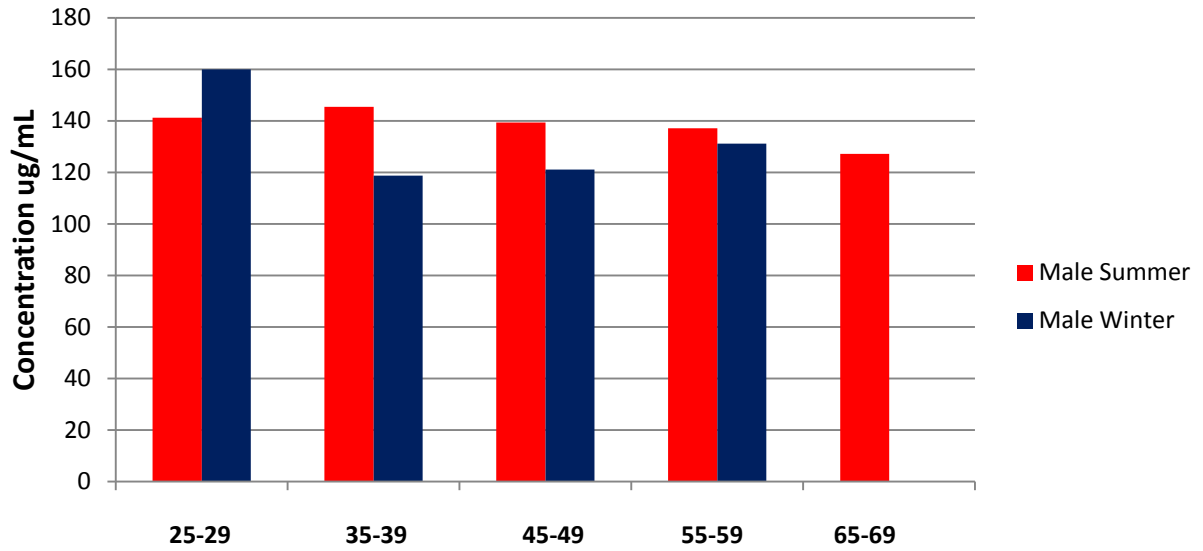
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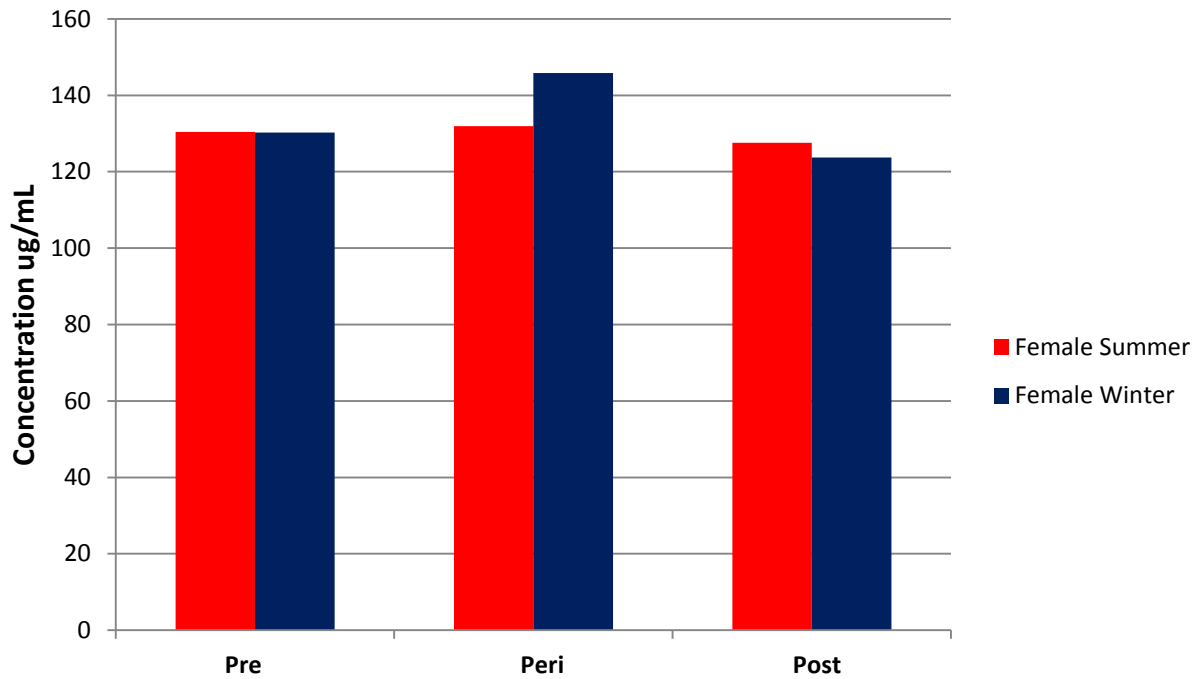
## Appendix



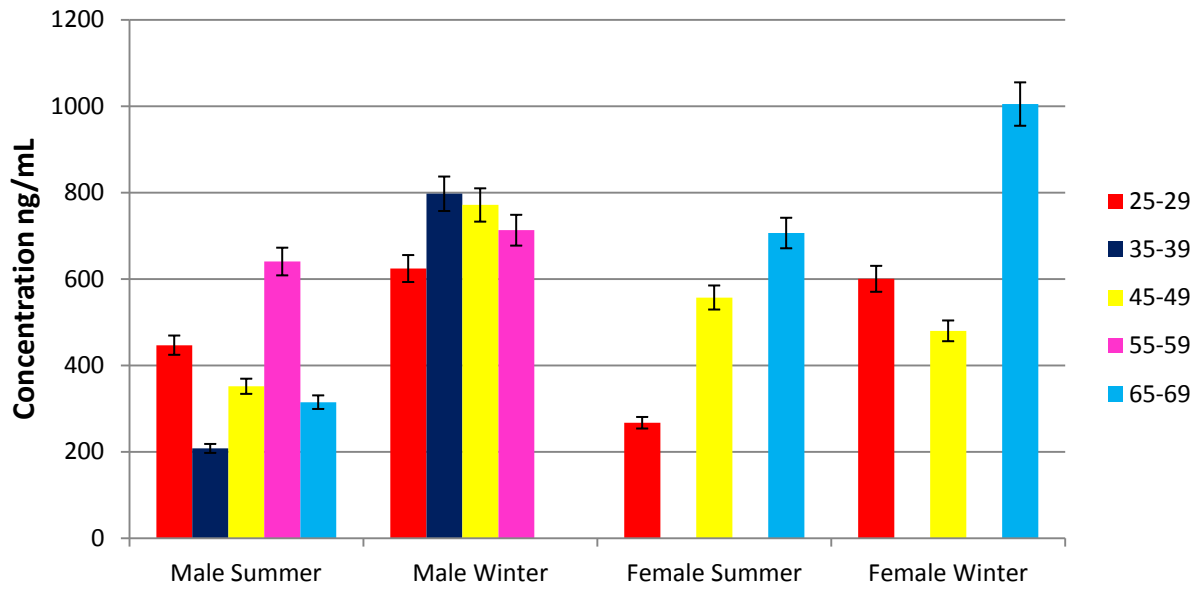
## Seasonal Variation of Antithrombin III by Age in Men



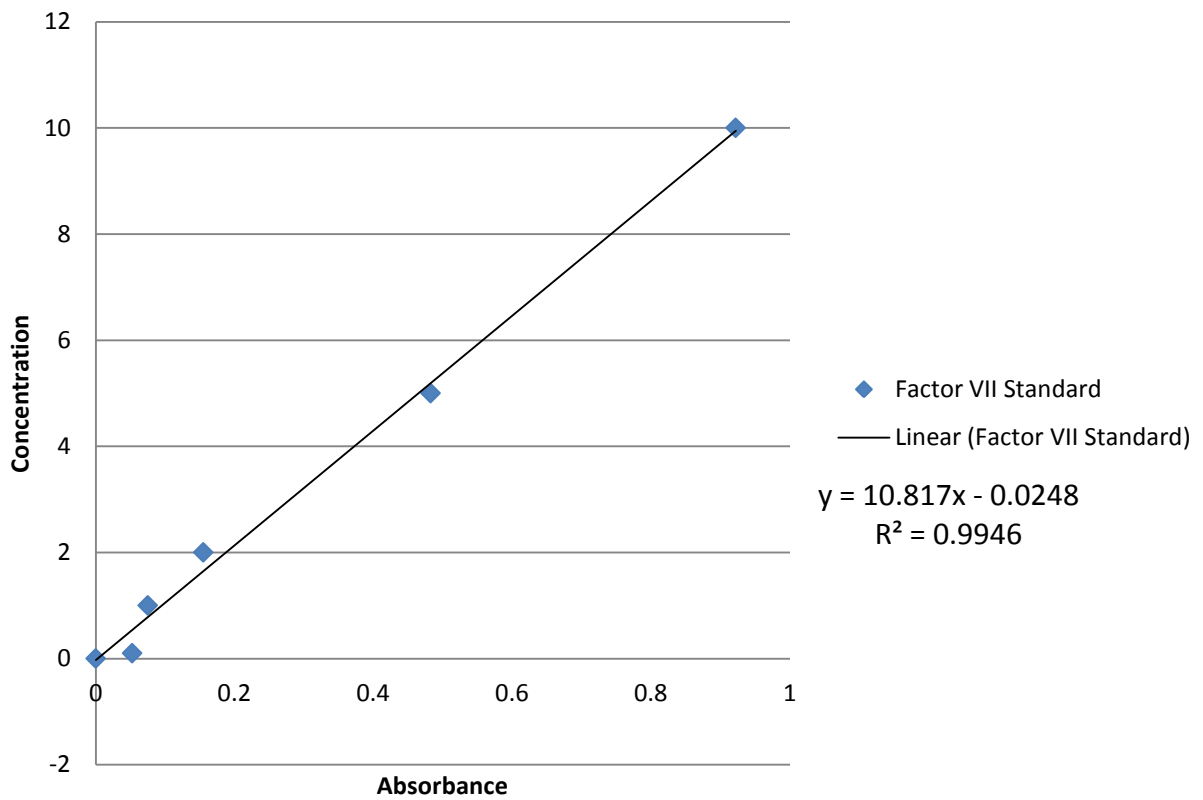
## Seasonal Variation of Antithrombin III in Pre, Peri and Post Menopausal Women



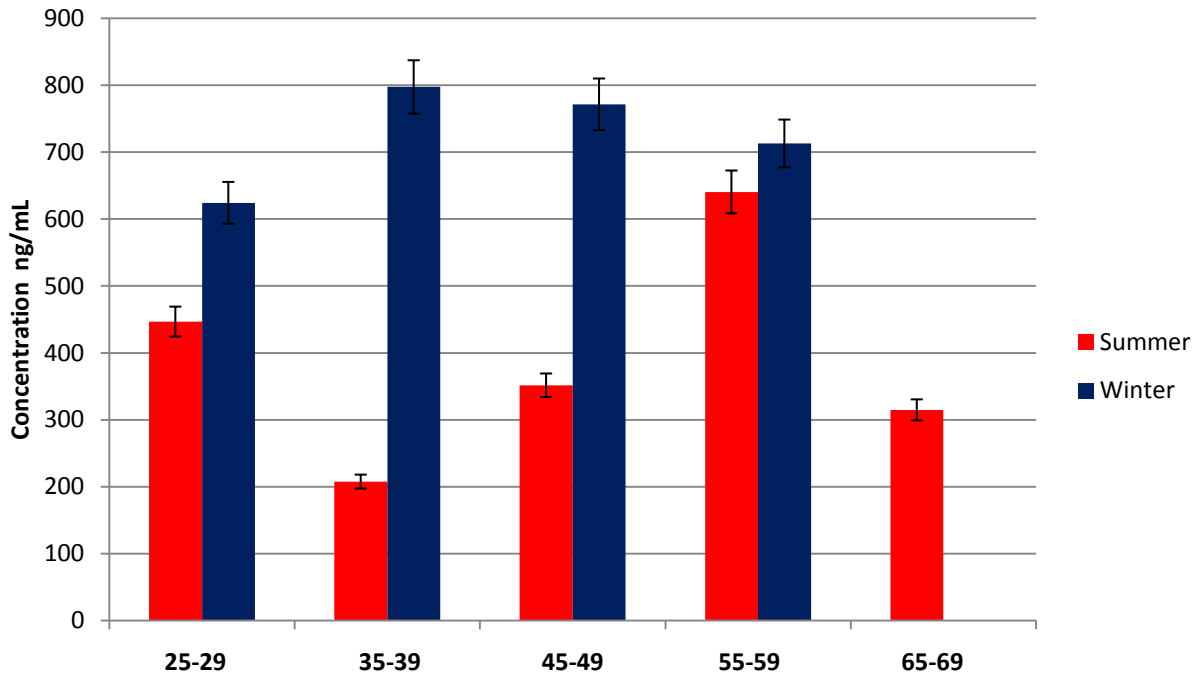
## Seasonal Variation of Factor VII by Age and Gender



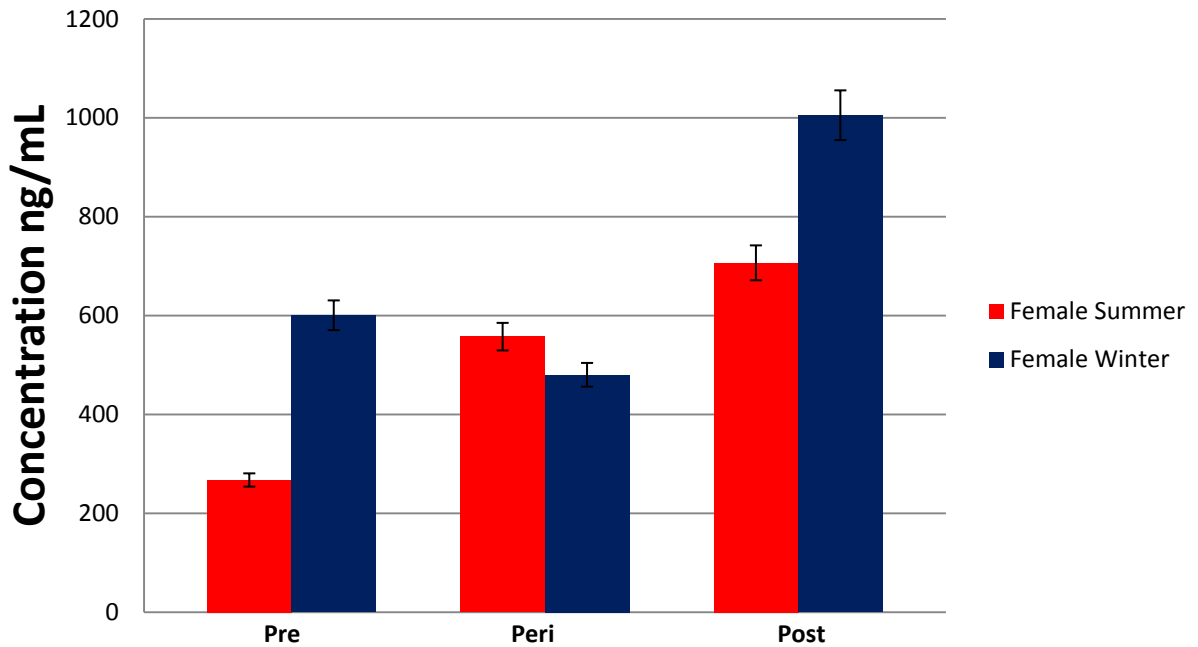
### Factor VII Standard Curve



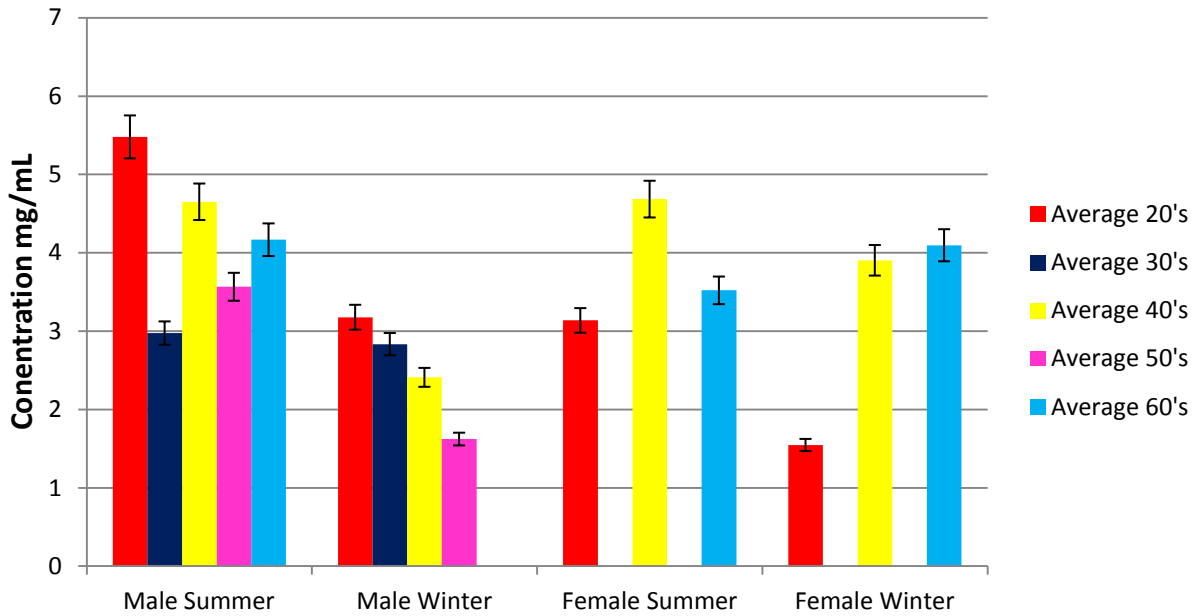
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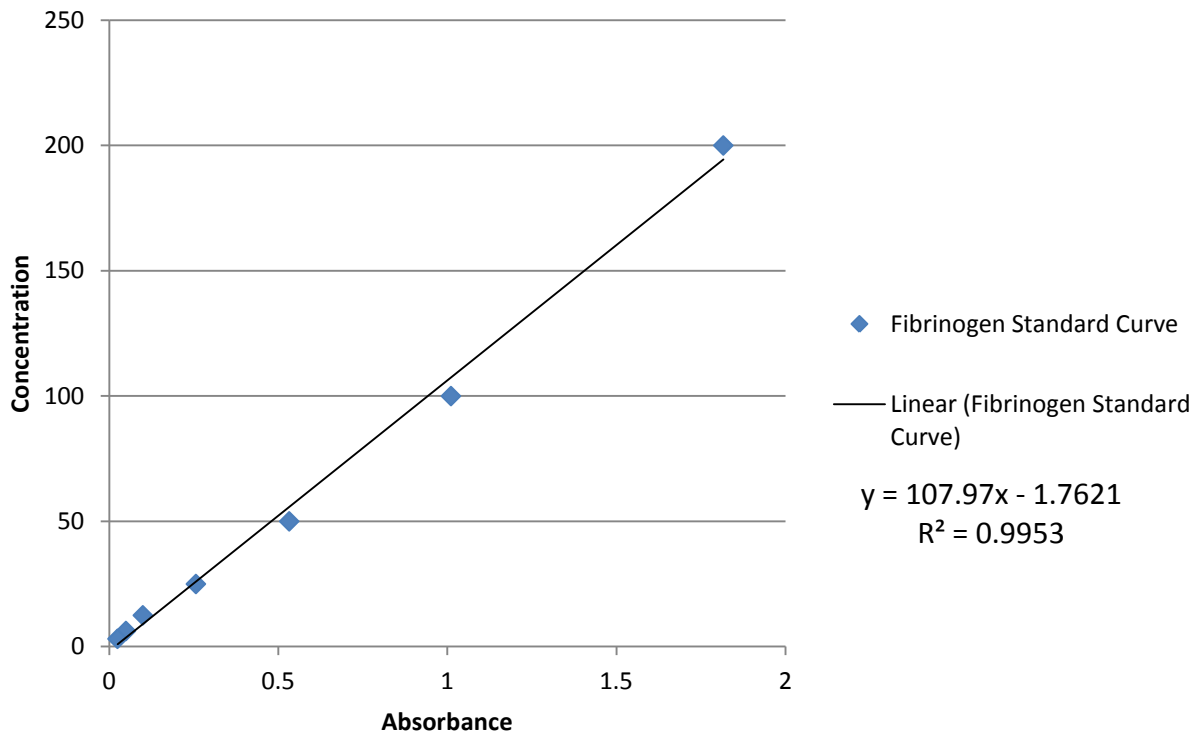
### Seasonal Variation of Factor VII in Pre, Peri and Post Menopausal Women



## Seasonal Variation of Fibrinogen by Age and Gender

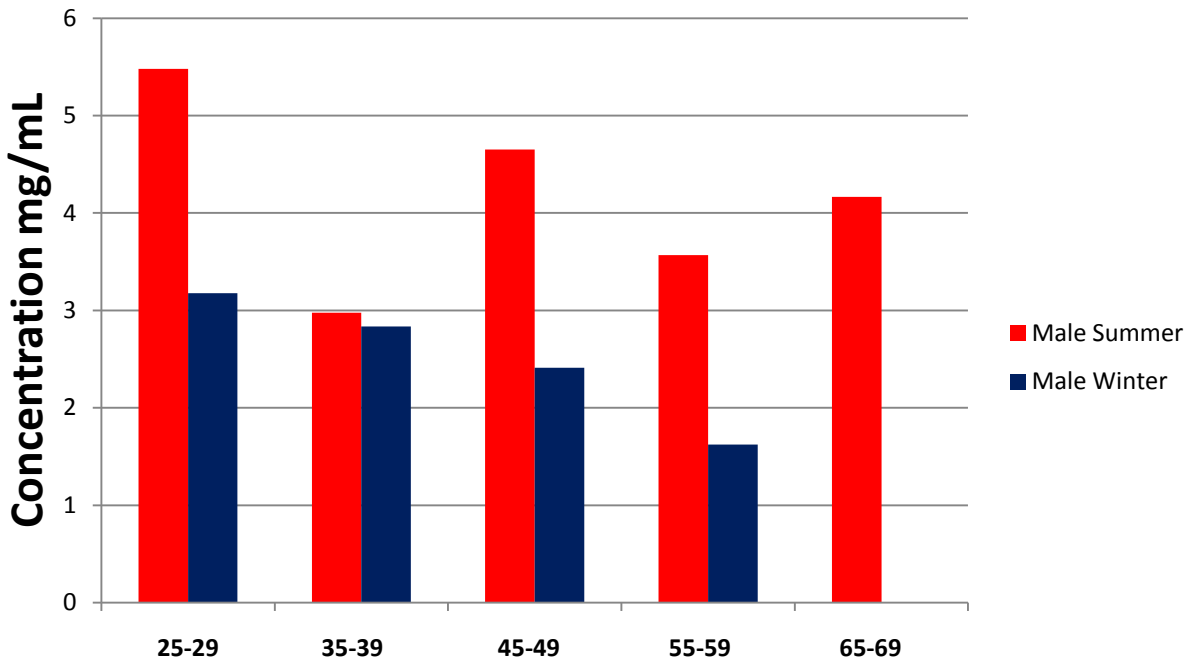


## Fibrinogen Standard Curve

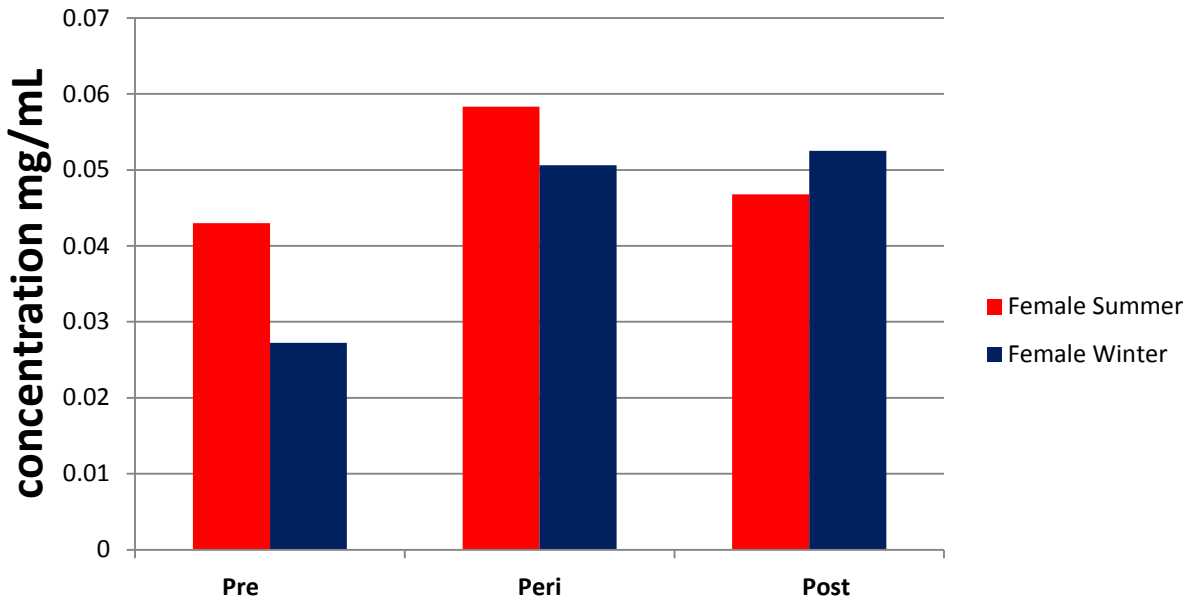




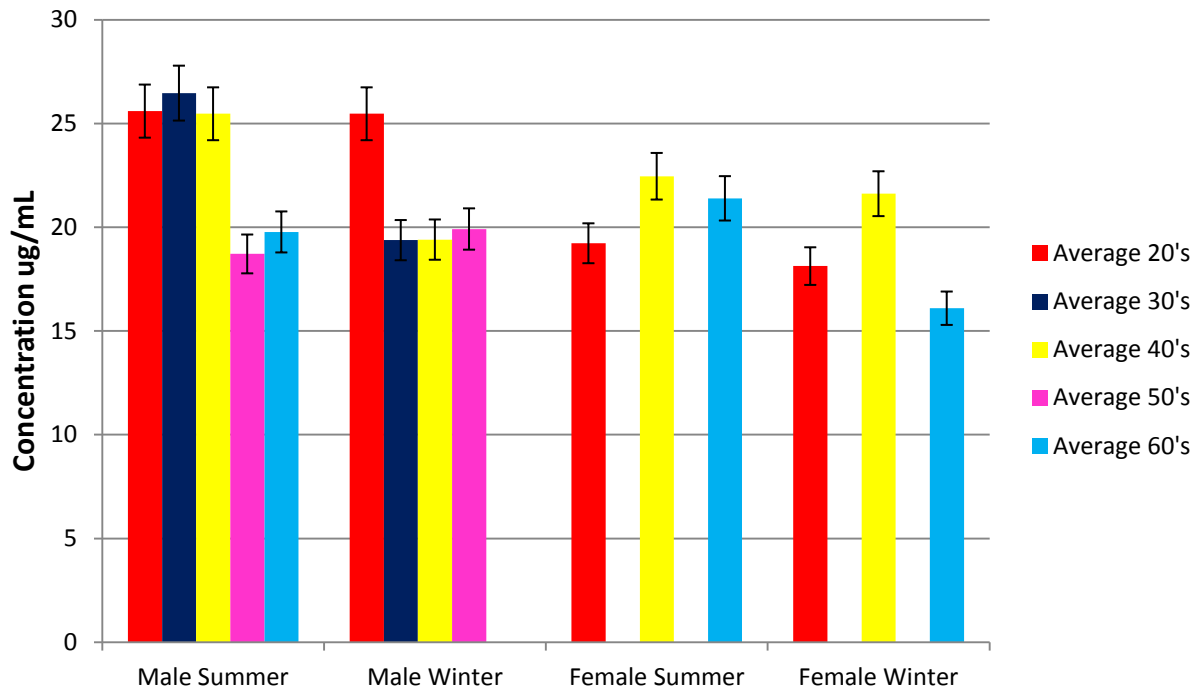
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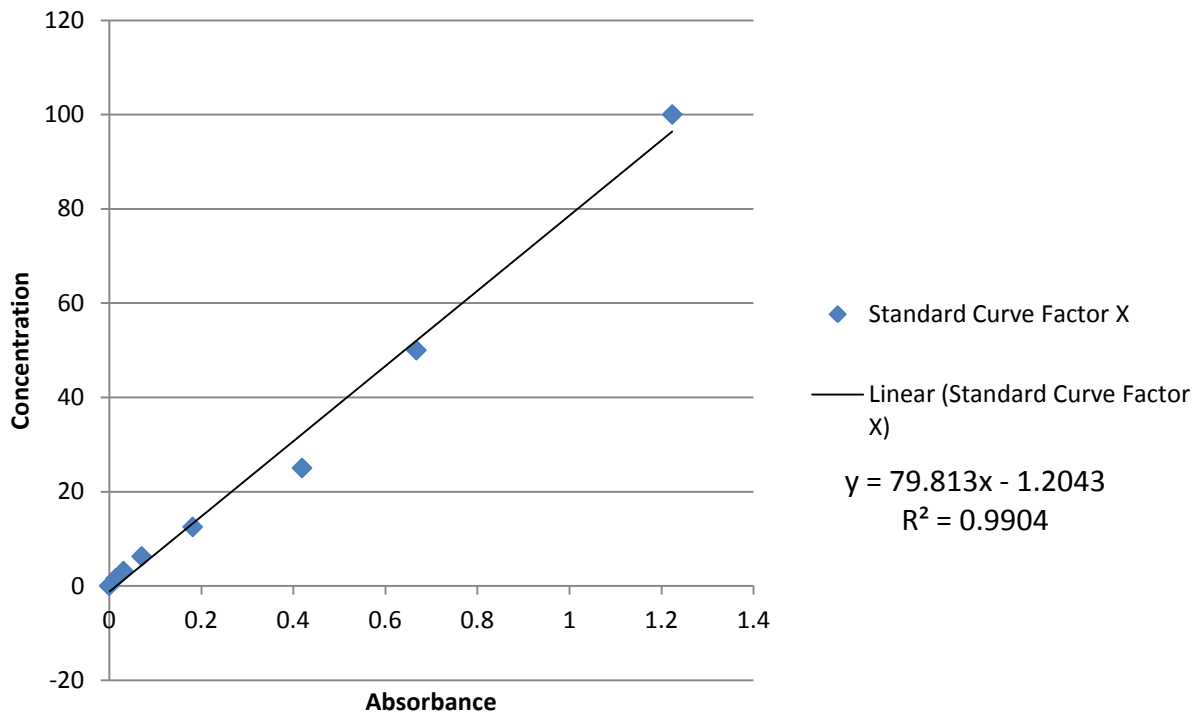
### Seasonal Variation of Fibrinogen in Pre, Peri and Post Menopausal Women



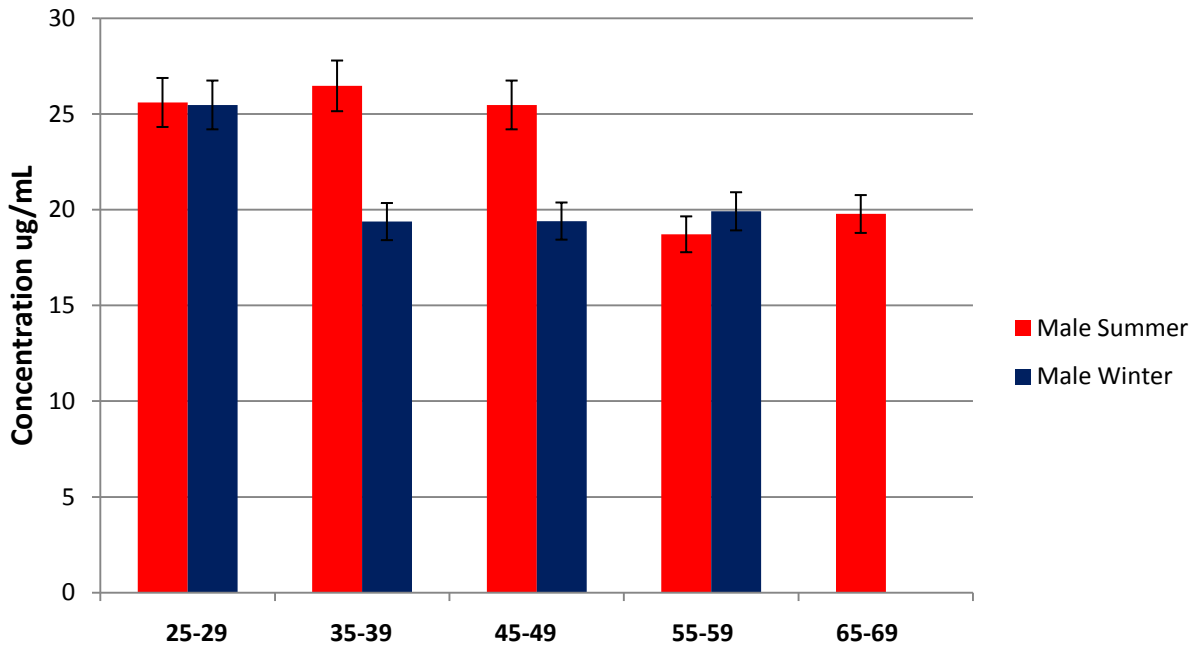
## Seasonal Variation of Factor X by Age and Gender



## Standard Curve Factor X



### Seasonal Variation of Factor X by Age in Men



### Seasonal Variation of Factor X in Pre, Peri and Post Menopausal Women

