A 2x3x3 factorial design with the following within-subjects variables: 1. Time (plasma vs. Serum) 2. Time to process samples (30 minutes vs 2 hours) 3. Temperature (4°C vs. Room temperature)

### EXPERIMENT 1

**RESULTS**

1. There were clear differences between serum and plasma levels for all analytes measured. Leptin and insulin levels were higher in plasma than serum while the reverse was true for IGF-I and IGFBP-3.
2. The impact of time to process samples and time to freeze samples was minimal for leptin and insulin. Similar stability for these analytes has been reported in the literature (1–3).
3. There was some impact on sample handling on IGF-1 and IGFBP-3.
   - For IGFBP-3, delaying sample processing resulted in a reduction in measured levels of IGFBP-3, while serum, a delay in sample processing resulted in a significant increase in IGFBP-3 levels.
   - For IGF-I, delaying the processing and freezing of the plasma samples resulted in a significant increase in the measured levels of this analyte. Finally, for both insulin and IGF-I, serum levels were significantly higher than plasma levels.

**CONCLUSIONS – EXPERIMENT 1**

- There were slight decreases in IGF-I levels in plasma when there was a delay in processing samples at 4°C; the reverse was true for processing samples at room temperature.
- 2 hours. The SST tubes were kept at room temperature (RT) for either 30 minutes or 2 hours. BD P100 tubes containing EDTA and preservative inhibitors (plasma).

- The purpose of Experiment 2 was to determine whether the differences in measured levels of the analytes observed in experiment 1 could be accounted for by the differences in temperature during the initial processing of the samples. In experiment 1, the plasma samples were kept cold prior to and during sample processing, while the serum samples were maintained and processed at room temperature. Therefore, in experiment 2, plasma samples were maintained at 4°C or at room temperature prior to sample processing.

- An additional purpose of experiment 2 was to assess the impact of the sample handling protocols on the measurement of Vitamin D (25-hydroxyvitamin D) in plasma and serum.

### EXPERIMENT 2

**METHODS**

Participants

There were 60 female volunteers, between 18-65 years of age, were recruited for each of the two studies. Blood samples were acquired under informed consent. Volunteers were asked to fast for a minimum of eight hours overnight, and all blood was drawn in the morning.

Sample Acquisition

Experiment 1

- Approximately 22.5 mL of blood was drawn per participant into five vacutainer tubes, 2x3mL lavender-topped tubes containing EDTA (plasma), 2x4mL gold-topped SST tubes (serum), 1x6.5mL BD P100 tubes containing EDTA and preservative inhibitors (plasma).

- Sample handling is summarized in Figure 1. Prior to centrifugation, the EDTA tubes were placed in a refrigerator at 4°C for either 3 hours or 2 hours. The SST tubes were kept at room temperature (RT) for either 30 minutes or 2 hours. BD P100 tubes were kept at RT for 2 hours prior to centrifugation. Samples were centrifuged at 1,500g for 15 minutes at 4°C. Aliquots were prepared immediately following centrifugation, and the aliquots were either frozen at −80°C or maintained at RT for 2 hours prior to freezing.

- 2 hours after the blood draw.

**RESULTS**

1. There was a slight decrease in IGF-I levels in plasma when there was a delay in processing samples at 4°C; the reverse was true for processing samples at room temperature.
2. Serum levels of IGF-I were significantly higher than plasma levels.
3. IGF-1 levels were significantly higher in serum than in plasma when plasma samples were processed at 4°C. None of the other analytes showed a significant difference in IGF-1 levels between plasma samples processed at 4°C and at room temperature.

**CONCLUSIONS – EXPERIMENT 2**

- The results of experiment 2 were consistent with those of experiment 1.

- There were significant differences in serum and plasma levels for all of the analytes measured. For insulin and leptin, plasma levels were significantly higher than serum levels, while the reverse was true for IGF-I and IGFBP-3. For both insulin and IGF-I, serum levels were significantly higher than plasma levels.

### GENERAL CONCLUSIONS

1. There were significant differences in serum and plasma levels for all of the analytes measured. For insulin and leptin, plasma levels were significantly higher than serum levels, while the reverse was true for IGF-I and IGFBP-3.
2. Insulin and leptin were minimally impacted by differences in sample handling and processing.
3. Differences in serum and plasma levels of IGF-I and IGFBP-3 may be accounted for, in part, by the different environmental conditions under which serum and plasma samples were processed.

### EFFECTS OF SAMPLE HANDLING AND STORAGE VARIABLES ON THE MEASUREMENT OF DISEASE-RELEVANT ANALYTES IN PLASMA AND SERUM.

- Evaluation of the effect of sample handling and storage conditions on the measurement of a series of blood-borne factors potentially relevant to breast cancer prognosis in women ages 18-65 years of age.

- The handling and processing of samples and plasma samples were processed. Differences in serum and plasma levels were observed when plasma was processed at room temperature (F1,29 = 13.32, p = 0.001).

- For IGF-I and IGFBP-3, there was some impact on sample handling on IGF-1 and IGFBP-3.

- The impact of time to process samples and time to freeze samples was minimal for insulin and leptin. Similar stability for these analytes has been reported in the literature (1–3).

- There was some impact on sample handling on IGF-1 and IGFBP-3.

- For IGFBP-3, delaying sample processing resulted in a reduction in measured levels of IGFBP-3, while serum, a delay in sample processing resulted in a significant increase in IGFBP-3 levels.

- IGFBP-3 resulted in room temperature resulted in significantly higher levels of IGFBP-3 than when plasma samples were processed at 4°C.

- 2 hours. The SST tubes were kept at room temperature (RT) for either 30 minutes or 2 hours. BD P100 tubes containing EDTA and preservative inhibitors (plasma).

- 2 hours after the blood draw.

**METHODS**

The methods were the same as for experiment 1 with the following exceptions:

- EDTA plasma were kept either at room temperature or at 4°C prior to centrifugation.

- All aliquots were frozen immediately; there was no delay condition.