SAMUEL LUNENFELD RESEARCH INSTITUTE MOUNT SINAI HOSPITAL

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

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ABSTRACT

The measurement of disease relevant analytes in blood can vary as a function of differences in sample handling and processing. In the present series of studies, we investigated sample handling and processing variables that could impact the accurate measurement of disease relevant analytes in plasma and serum such as insulin, leptin, insulin growth factor-1(IGF-1), insulin growth factor binding protein-3 (IGFBP-3), and vitamin D. We investigated the following variables: processing of samples at room temperature compared to on-ice; time to extraction of serum and/or plasma (30 minutes compared to 2 hours); time to cyrostorage (0 hours compared to 2 hours). Blood samples were collected from 30 healthy female volunteers (18-65) on each of two separate occasions under informed consent. The blood samples were processed according to experimental conditions and analytes were measured using either singleplex or multiplexed immunoassays according to vendor protocols. The primary dependent measure was the measured concentration of each analyte as a function of sample processing condition, time to extraction of plasma and serum, and time to cryopreservation. Results showed that for the moderate-to-high abundant analytes such as insulin and leptin, there was minimal impact of sample processing and handling variables on the measured concentrations. However, for these analytes, significant differences in measured concentrations were observed between plasma and serum. For insulin and leptin, higher concentrations were measured in plasma as compared to serum, while the reverse was the case for IGF-1 and IGFBP-3 and for Vitamin D. In addition, delay in processing of the samples resulted in detection of higher concentrations for Vitamin D, with this effect significant for serum samples. Taken together, these experiments show that serum and plasma yield different levels of these proteins, and that careful consideration must be given to sample handling and processing at least for some of these analytes.

INTRODUCTION

Advances in the identification and quantitation of proteins from body fluids hold significant promise for the discovery and application of biomarkers to aid in the development of safer and more effective medicines to treat patients; a biomarker is a cellular or molecular indicator. Identifying and quantitating proteins in body fluids are complex tasks. Proteins are a heterogeneous class of biological macromolecules that vary in stability, particularly when they are obtained from biological samples such as plasma or serum. The handling and processing of serum and plasma samples is therefore critical to the protein biomarker discovery. However, there is significant inter-laboratory variations in sample handling and processing that could lead to inconsistencies in protein identification and quantitation. It is critical to determine the sample handling and processing variables that impact on the stability of proteins in order to standardize these processes to minimize sources of error variance in biomarker discovery and development research.

OBJECTIVES

Evaluate 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

Evaluate the effect of sample handling on the measurement of proteins of interest in plasma versus serum

Analytes of Interest

Insulin, Leptin, Insulin-like growth factor binding protein 3 (IGFBP-3), Insulin-like growth factor 1 (IGF-1), Vitamin D (Experiment 2 only)

EXPERIMENT 1

STUDY DESIGN

A 2x2x2factorial, with the following within-subjects variables:
1.Matrix (plasma vs. Serum)
2.Time to sample procesing (30 minutes vs 2 hours)
3.Time to cryostorage (0 hours vs 2 hours)

Figure 1: Schematic of the design of experiment 1.

METHODS

Participants

Thirty healthy, female volunteers , between 18-65 years of age, were recruited for each of 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^{TM} P100 tubes containing EDTA and protease inhibitors (plasma).

Sample handling is summarised in Figure 1. Prior to centrifugation, the EDTA tubes were placed in a refrigerator at 4˚C for either 30 minutes or 2 hours. The SST tubes were kept at room temperature (RT) for either 30 minutes or 2 hours. BD^{TM} 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 remained at RT for 2 hours prior to freezing.

RESULTS

Experiment 1

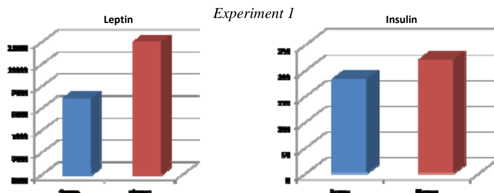

Figure 1 : Concentration of leptin measured in serum and plasma. Plasma levels were significantly higher than serum levels, $F_{1,29} = 13.32$, $p = 0.001$.

Figure 2: Concentration of insulin measured in serum and plasma. There was a trend towards higher levels of insulin in plasma vs serum, although this was not statistically significant, $F_{1,29} = 2.564$, $p = 0.12$.

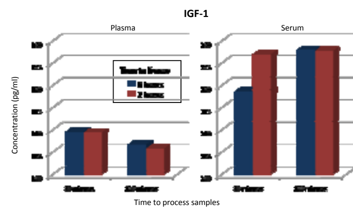

Figure 3 : Concentration of insulin growth factor 1 (IGF-1) as a function of sample type, time to process samples, and time to freeze samples. Overall, IGF-1 levels were significantly lower in plasma than in serum. Plasma levels decreased with a delay in sample processing. In contrast, serum levels increased when there was either a delay in sample processing or a delay in cryostorage, $F_{1,29} = 12.797$, $p = 0.001$.

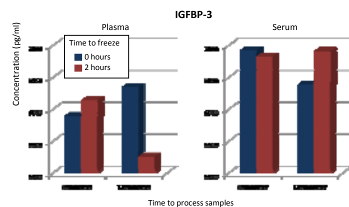

Figure 4 : Concentration of insulin growth factor binding protein-3 (IGFBP-3) as a function of sample type, time to process samples, and time to freeze samples. There was a significant 3-way interaction, $F_{1,29} = 12.797$, $p = 0.001$.

CONCLUSIONS - EXPERIMENT 1

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-1 and IGFBP-3
2.The impact of time to processing of samples and time to freezing of samples was minimal for insulin and leptin. Similar stability for these analytes have been reported in the literature (1-3).
3.There was some impact on sample handling on IGF-1 and IGFBP-3.

- For IGF-1, delaying sample processing resulted in a reduction in measured levels of IGF-1, while for serum, either a delay in sample processing or in sample cryostorage resulted in increased levels of IGF-1.
- For IGFBP-3, delaying the processing and freezing of the plasma samples resulted in a significant reduction in the measured levels of this analyte. Finally, for both IGF-1 and IGFBP-3, serum levels were significantly higher than plasma levels.

EXPERIMENT 2

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 4^0C or at room temperature prior to sample processing.

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

STUDY DESIGN

A 2x2 factorial with the following within-subjects variables:
1.Processing condition (room temperature or at 4^0C)
2.Time to sample processing (30 minutes vs 2 hours)

In addition, serum samples were analyzed following sample processing at either 30 minutes or 2 hours after the blood draw.

METHODS

The methods were the same as for experiment 1 with the following exceptions:
1.The BD^{TM} P100 tube condition was not included
2.EDTA plasma were kept either at room temperature or at 4^0C prior to centrifugation
3.All aliquots were frozen immediately; there was no delay condition

RESULTS

Experiment 2

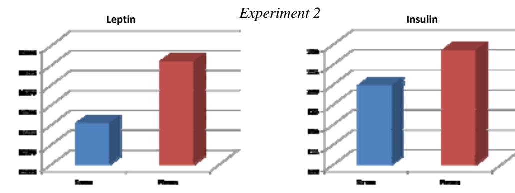

Figure 5 : Concentration of leptin measured in serum and plasma (processed at room temperature). Plasma levels were significantly higher than serum levels, $F_{1,29} = 12.401$, $p = 0.001$.

Figure 6: Concentration of insulin measured in serum and plasma (processed at room temperature). Plasma levels were significantly higher than serum levels,, $F_{1,29} = 4.894$, $p = 0.035$.

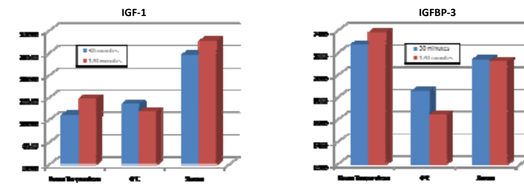

Figure 7. IGF-1 levels were significantly higher in serum as compared to plasma, $F_{1,29} = 56.23$, $p < 0.001$. For plasma, delaying the processing of samples at room temperature resulted in a slight increase in levels of IGF-1, while delaying the processing of samples at 4°C resulted in a slight decrease in levels, $F_{1,29} = 10.993$, $p < 0.002$.

Figure 8. IGFBP-3 levels were significantly higher in plasma processed at room temperature as compared to plasma processed at 4°C, $F_{1,29} = 7.138$, $p = 0.012$. Serum levels of IGFBP-3 were not significantly different from plasma levels when plasma was processed at room temperature, $F_{1,29} < 1.0$.

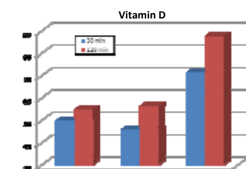

Figure 9. Overall serum levels of Vitamin D were significant higher than plasma levels (room temperature), $F_{1,29} = 52.687$, $p < 0.001$; delaying processing the samples also resulted in higher levels, $F_{1,29} = 21.007$, $p < 0.001$. For plasma samples, there was a significant interaction between processing condition and time to processing of samples, $F_{1,29} = 5.045$, $p = 0.032$, although the differences across conditions were modest.

CONCLUSIONS – EXPERIMENT 2

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

Insulin and leptin:
1.No significant impact of processing plasma samples under different environmental conditions (i.e., room temperature vs. 4^0C)
2.No significant impact of time to process samples (30 minutes vs 120 minutes)
3.For both insulin and leptin, higher levels were measured in plasma as compared to serum.

IGF-1:
1.There was a slight decrease in IGF-1 levels in plasma when there was a delay in processing samples at 4^0C; the reverse was true for processing samples at room temperature.
2.Serum levels of IGF-1 were significantly higher than plasma levels.

IGFBP-3:
1.IGFBP-3 resulted in room temperature resulted in significantly higher levels of IGFBP-3 than when plasma samples were processed at 4^0C
2.No significant difference between serum levels of IGFBP-3 and plasma levels of IGFBP-3 when plasma was processed at room temperature

Vitamin D
1.There was a modest impact sample handling variables on the measurement of Vitamin D in plasma samples; lower levels were observed when samples were processed at 30 minutes as compared to 120 minutes, and this difference was lessened somewhat by processing the samples at 4^0C
2.The major difference was observed when comparing Vitamin D levels measured in serum and plasma; serum levels of Vitamin D 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-1, IGFBP-3, and Vitamin D
2.Insulin and leptin were minimally impacted by differences in sample handling/processing.
3.Some effect of sampling handling/processing conditions on the measurement of IGF-1, IGFBP-3, and Vitamin D, although the effect was modest in each case.
4.The differences observed between plasma and serum levels of IGFBP-3 may be accounted for, in part, by the different environmental conditions under which serum and plasma samples were processed. Differences in serum and plasma levels were observed when plasma was processed at 4^0C and serum at room temperature. A similar difference was observed when plasma was processed at room temperature as compared to 4^0C

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