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Influence of fasting and sample collection time on 38 biochemical markers in healthy children: A CALIPER substudy

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ABSTRACT

Objectives: Fasting samples can be difficult to obtain in the pediatric setting, particularly in neonates. As part of the Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER), we aimed to determine if there are differences in serum concentrations of pediatric biochemical markers measured at fasting, postprandial, and random time points throughout the day.

Design and methods: Blood was drawn from 27 healthy children and adolescents (aged 4–18) with informed consent at 4 time points: after overnight fast, mid-morning after breakfast, within 2 h after lunch, and late afternoon. The effect of fasting on 38 chemistries was evaluated by paired, two-tailed student's t-tests. Analysis of the effect of time of day was done using paired, repeated-measures ANOVA.

Results: Fasting significantly affected 22 analytes, with HDL cholesterol being the most highly affected. Values tended to decrease postprandially, except for five analytes, including triglycerides, which increased. By ANOVA, 28 chemistries significantly differed across times of day tested.

Conclusions: Fasting is necessary for analysis of certain chemistries in pediatric subjects. Pediatricians should consider diurnal factors when ordering non-fasting tests and interpreting test results.

Introduction

Pediatric laboratory medicine is a challenging area of clinical chemistry due to the difficulty in obtaining appropriate blood samples from small children and the considerable physiological and metabolic changes occurring during childhood and adolescence that influence many of the biochemical markers measured in the laboratory. Drawing blood is the first step in evaluating health status in hospitalized patients, and the results have a direct impact on medical decisions. The process of blood drawing can be plagued by many errors and interferences, making it a key pre-analytical variable [1]. It is common practice for blood specimens to be collected after an overnight fast (8–12 h), as there is evidence that fasting blood collection decreases variability between samples, particularly for analytes (for example, triglycerides) that differ significantly between the fasting and non-fasting states [2,3]. Although feasible in the adult population, difficulties can arise in obtaining fasting samples from pediatric patients, particularly in infants, toddlers, and young children, who are normally fed at short and regular intervals throughout the day. Thus, pediatric hospitals strive to improve and tailor their practices to the population at hand.

A Canada-wide project, the Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER), was established in 2004 to define the unique age and gender-specific reference intervals for various biomarkers in the pediatric population [4]. Current gaps in pediatric reference intervals can result in serious consequences for patients, including excessive and inappropriate testing, lengthier hospital stays, incorrect treatment, or delayed diagnosis of disease [5]. Through the collaboration of various groups across the country, a large amount of data has already been generated from more than 3500 healthy pediatric subjects on a variety of biomarkers, and using different analytical platforms [6–14]. The CALIPER initiative also includes a number of sub-projects aimed at addressing other issues in pediatric laboratory medicine. One of these issues is the determination of whether fasting and/or diurnal factors affect the measurement of common analytes in the clinical laboratory. Few studies have evaluated the effects of non-fasting chemistry results on medical decision making, particularly in the pediatric setting.

Commonly ordered blood tests with known differences between the fasting and non-fasting states include total cholesterol, triglycerides, HDL, and glucose [2,15]. Other biomarkers change diurnally (eg. renin, cortisol, growth hormone) [16,17] or with a child's developmental stage (eg. alkaline phosphatase, sex hormones) [18–20]. It is thus convenient, when a panel of tests are being ordered, that a patient has fasted prior to blood collection and that samples are drawn at the appropriate time of day. With regards to neonates, who are fed every 2 to 3 h during their first years of life, a 'fasting' blood sample is obtained by sampling immediately prior to the next scheduled feeding.

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Table 1
Demographics of the 27 study participants.

Age	4–18 years (mean, 13 years)
Gender	11 males; 16 females
Ethnicity	10 Caucasian
	9 South Asian
	3 Arab
	2 Black
	2 Middle Eastern
	1 Korean

In this study we aimed to evaluate the effects of fasting and sample collection time on the measurement of 38 routine clinical chemistry analytes and to determine if values change significantly depending on the fasting state or time of day. No previous studies have been done to evaluate such an array of analytes, and more specifically, this has not been done in the pediatric setting. Determining the effects of fasting and time of day can have important implications for medical assessment of pediatric patients and for studies into pediatric reference intervals.

Methods

Subject selection and sample collection

The study was conducted at The Hospital for Sick Children in Toronto, Canada, with institutional ethics board approval. 27 healthy (based on questionnaire responses), ethnically-diverse pediatric subjects ranging in age from 4–18 years (Table 1) were recruited to participate with informed consent. Subjects were asked to fast overnight (8 h), and to visit the hospital the next morning. Upon their arrival, blood was drawn into serum separator tubes and sent to the clinical chemistry laboratory; this fasting sample is designated as "time point" 1 (T1). Next, subjects were asked to have breakfast, after which another blood sample was drawn (T2), which reflected a postprandial specimen. A third specimen drawn 2 h later, after lunch (T3), also reflected a postprandial specimen. Later in the afternoon (~5 PM), participants gave a final blood sample (T4), which was to reflect a sample drawn at random. All together, blood was drawn at 4 different time points for each participant, with an average of about 2.5 h between each time point. Care was taken to ensure that the timing of sampling at each time point was consistent across subjects in order to minimize variability in timing. Sampling from the 27 subjects was done over a period of two days, spanning two different weekends. All samples were aliquoted and stored at −80 °C until batch testing, at which time they were subjected to one freeze–thaw cycle.

Analytical systems and assays

Samples were tested on either the Cobas Integra 400 (Roche), or the VITROS 5,1 FS Chemistry Systems Analyzer (Ortho Clinical Diagnostics). In total, 38 analytes were tested, and all 4 samples from each patient were run on the same analyzer. Each instrument was calibrated prior to use using the manufacturer's reagents and calibrators. In addition to using purchased quality control materials, two patient samples of known analyte concentrations were tested with each batch of samples. All assays were performed according to the manufacturer's instructions. Each specimen was evaluated for endogenous interferents such as

Table 2
Results of paired, two-tailed, student *T*-test analyses on the 27 subjects. Comparisons were done between fasting and postprandial values for each analyte, for each individual subject tested. For analytes that were significantly different between the fasting and postprandial states ($P<0.05$), the mean change is indicated as a percentage of the fasting value.

Test name	Instrument	Biochemical principle	P-value (T1-T2)	Mean % change
Alanine aminotransferase	Vitros 5,1 FS	Enzyme-coupled reaction with alanine & alpha-ketoglutarate substrates	0.2	
Albumin	Vitros 5,1 FS	Bromcresol green (BCG)	<0.05	−1.8
Alkaline phosphatase	Vitros 5,1 FS	Enzymatic activity using para-nitrophenyl phosphate	<0.05	−7.0
Alpha-1 acid glycoprotein	Cobas Integra	Immunoturbidimetric	0.1	
Alpha-1 antitrypsin	Cobas Integra	Immunoturbidimetric	0.6	
Amylase	Vitros 5,1 FS	Dyed starch substrate	<0.05	−3.0
Aspartate aminotransferase	Vitros 5,1 FS	Enzyme-coupled reaction with alanine & alpha-ketoglutarate substrates	<0.05	−4.4
Bilirubin, total	Vitros 5,1 FS	Dyphylline to dissociate from albumin	<0.05	−5.1
Bilirubin, unconjugated	Vitros 5,1 FS	Binding to cationic mordant	0.06	
C3	Cobas Integra	Immunoturbidimetric	0.7	
C4	Cobas Integra	Immunoturbidimetric	0.07	
C reactive protein	Cobas Integra	Immunoturbidimetric	0.07	
Calcium	Vitros 5,1 FS	Arsenazo III indicator dye	<0.05	−1.3
Carbon dioxide, total	Vitros 5,1 FS	Enzymatic	<0.05	+3.8
Ceruloplasmin	Cobas Integra	Immunoturbidimetric	0.2	
Chloride	Vitros 5,1 FS	Ion-selective electrodes	0.8	
Cholesterol	Vitros 5,1 FS	Enzymatic	<0.05	−2.5
Creatine kinase	Vitros 5,1 FS	Enzymatic	0.1	
Creatinine	Vitros 5,1 FS	Enzymatic	<0.05	+2.8
Gamma glutamyl transferase	Vitros 5,1 FS	Enzyme-coupled reaction	<0.05	−2.5
Glucose	Vitros 5,1 FS	Glucose oxidase	0.3	
Haptoglobin	Cobas Integra	Immunoturbidimetric	0.2	
HDL cholesterol	Vitros 5,1 FS	Enzymatic	<0.05	−4.3
Immunoglobulin A	Cobas Integra	Immunoturbidimetric	<0.05	−2.0
Immunoglobulin G	Cobas Integra	Immunoturbidimetric	<0.05	−1.7
Immunoglobulin M	Cobas Integra	Immunoturbidimetric	<0.05	−3.2
Iron	Vitros 5,1 FS	Immunoturbidimetric	0.4	
Lactate dehydrogenase	Vitros 5,1 FS	Enzyme-coupled reaction	0.7	
Magnesium	Vitros 5,1 FS	Formazan dye	<0.05	−1.7
Phosphate	Vitros 5,1 FS	Reduction reaction by p-methylaminophenol sulfate	<0.05	−3.0
Potassium	Vitros 5,1 FS	Ion-selective electrodes	<0.05	−4.1
Sodium	Vitros 5,1 FS	Ion-selective electrodes	<0.05	+0.7
Soluble transferrin receptor	Cobas Integra	Immunoturbidimetric	<0.05	−3.2
Total protein	Vitros 5,1 FS	Copper tartrate	<0.05	−1.7
Transferrin	Cobas Integra	Immunoturbidimetric	0.5	
Triglyceride	Vitros 5,1 FS	Enzyme-coupled reaction	<0.05	+20.5
Urea	Vitros 5,1 FS	Dye complex	0.5	
Uric acid	Vitros 5,1 FS	Leuco dye	<0.05	+4.0