Interpretation of Cortisol Concentrations and Reference Intervals from the CALIPER Database

To the Editor:

Measuring chronic stress and its effects is particularly challenging in the pediatric population. Infants and children are more vulnerable to the effects of chronic stress because the brain structures associated with stress regulation (hypothalamic–pituitary–adrenal axis, autonomic nervous system, limbic system) are still developing and thus susceptible to allostatic load. In early childhood, these effects are often unmasked when diurnal cortisol patterns are measured over successive days, or when baseline cortisol concentrations are compared with those obtained after exposure to psychological stressors, physiological tests, or pharmacologic interventions (the insulin tolerance test, the adrenocorticotropic hormone stimulation test, the dexamethasone suppression test, and others).

The investigators reporting from the CALIPER (Canadian Laboratory Initiative on Pediatric Reference Intervals) program database (1–3) are laudably addressing the need for normative levels and reference intervals (RIs) for serum biomarkers in healthy children and adolescents. Nonetheless, there appear to be discrepancies in the age-associated concentrations of cortisol reported for the sampling of a single population for the 2 platforms of analysis: a chemiluminescence assay with the Abbott ARCHITECT i2000 platform (1) and liquid chromatography–tandem mass spectrometry assay (3). It is unclear whether the selection of “age-specific” categories for these 2 studies is due to technological aspects of these platforms or whether other factors can explain the differences between these 2 reports in cortisol RIs. Certain points of interpretation of the cortisol RIs are also inconsistent with the published literature, as we discuss below.

First, cortisol samples are reported for age by sex categories, unadjusted for ethnicity [Table 1 in (1), 1381 samples; and Table 6 in (3), 328 samples]. Table 2 in (2) summarizes the census data for the province of Ontario, and the authors of this report state that “Caucasians, East Asians, and South Asian participants were evaluated.” Table 3 in the online Data Supplement of (1) does not describe the effect of ethnicity on cortisol, although the authors report linear modeling results for the concentrations of other analytes. Other groups have reported racial and ethnic differences for cortisol (4), and race and ethnicity are widely accepted as robust sources of variation. It remains unclear whether the observed “high variance” (1) for cortisol reported by the authors can be attributed to an “ethnically diverse” population.

Second, the authors acknowledge some of the limitations in their finding of minimal (10%) differences between morning and evening cortisol values; however, they still conclude that “. . . when applying RIs for cortisol to a pediatric population, daytime sampling time does not appear to importantly affect interpretation” (1). Without performing sequential sampling for cortisol concentrations at multiple times per day for each individual (and repeated for 3–5 days), any estimate of diurnal variation becomes meaningless. Moreover, the authors’ statement that infants lack a circadian cortisol rhythm is questionable.

Fetuses, newborns, and infants are known to express adrenocortical circadian cortisol rhythms, which have been measured across varying times of day within a 24-h cycle. Whereas the peak cortisol concentration in newborns occurs in the late afternoon, an adultlike pattern of cortisol rhythms may be observed in infancy by 3 months of age.

Third, identical RIs by age and sex [cortisol values reported in micrograms per deciliter in (1) vs. nanomoles per liter in (3)] are suspect because the age categories in these 2 reports are different. Moreover, the authors claim no divergence in sex-based RIs at any age (1) and do not acknowledge that this finding might be unique to the cohort sampled. These results are in contrast to a bulk of emerging literature that supports the divergence of cortisol concentrations by sex, particularly during puberty and adolescence. An ample literature indicates that dysregulation of the hypothalamic–pituitary–adrenal axis (as measured by cortisol, although the authors report sex, race, and age specific (4, 5). Recurrent exposure to stressors early in life alters physiological responses to subsequent stress. This allostatic load may lead to the manifestation of psychological, emotional, behavioral, immune, or metabolic disorders that is often associated with a flattening of the diurnal cortisol curve and a lack of postpubertal sex differences. Arguably, RIs for cortisol should be reported from selected populations with normative hypothalamic–pituitary–adrenal axis responses, thus minimizing the effects of allostatic load from early-life stressors.

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1 Nonstandard abbreviations: CALIPER, Canadian Laboratory Initiative on Pediatric Reference Intervals; RI, reference interval.
In Reply

We appreciate the interest in our recently published CALIPER (Canadian Laboratory Initiative for Pediatric Reference Intervals) manuscripts and are delighted to see that the publications are stimulating further discussion in the field. Overall, the authors of these letters have raised several important questions, but they appear to have overlooked some important points when reviewing the published studies. They also fail to acknowledge the numerous limitations inherent in interpretation of cortisol as addressed in our published articles: namely, that circadian rhythms in infants differ from those of adults, that the stress of phlebotomy can alter cortisol concentrations, and that we examined a single time point in each individual within a large population of children rather than multiple time points in a small number of individuals. That being said, in our population, the reference intervals are not altered by points raised in these letters. We agree that performing sequential sampling for cortisol measurement at multiple time points over 3–5 days would be ideal to assess the diurnal variation for cortisol. However, such a determination was well beyond the scope of the recently published CALIPER studies, which established reference value distributions in a large, healthy population of children.

We would like to respond first to the question raised by Rovnaghi et al. (1) regarding the differences in age partitions between the CALIPER immunoassay study published in Clinical Chemistry (2) and the HPLC-MS/MS study published in Clinical Biochemistry (3). Although the concentration units used in these studies are different, a review of the cortisol scatterplots, Fig. 4B in Bailey et al. (2) and Fig. 3A in Kyriakopoulou et al. (3), shows that the patterns observed were very similar despite the sample size differences between the 2 studies. The partitions for these studies were chosen to ensure that each study had a homogeneous population (i.e., the same mean and variance in all age groups). Owing to the smaller sample size in the study by Kyriakopoulou et al., we were not able to observe differences in distributions that were more noticeable in the Bailey et al. study; with a larger number of data points, different partitions were found, resulting in different reference intervals.

We examined the data for differences in cortisol concentrations between ethnic groups, but found no statistically significant differences among the 3 ethnic groups examined (Caucasians, South Asians, and East Asians). However, this may have resulted from the fact that only 3 ethnicities were tested. In the paper referenced by Martin...