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Pediatric reference intervals for clinical chemistry assays on Siemens ADVIA XPT/1800 and Dimension EXL in the CALIPER cohort of healthy children and adolescents

Houman Tahmasebi[a,b], Victoria Higgins[a,b], Amy Woroch[a], Shervin Asgari[a], Khosrow Adeli[a,b,*]

[a] CALIPER Program, Pediatric Laboratory Medicine, The Hospital for Sick Children, 555 University Avenue, Toronto, ON M5G 1X8, Canada
[b] Department of Laboratory Medicine & Pathobiology, Faculty of Medicine, University of Toronto, 1 King's College Cir, Toronto, ON M5S 1A8, Canada

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ABSTRACT

Introduction: Accurate reference intervals (RIs) are essential for clinical interpretation of laboratory test results; however, major gaps exist in pediatric RIs. The Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER) has established age- and sex-specific pediatric RIs on various analytical platforms. The current study expands the CALIPER database by establishing age- and sex-specific RIs for biochemical assays on Siemens ADVIA XPT/1800 and Dimension EXL Systems.

Methods: Serum samples from a total of 909 and 867 healthy children and adolescents (ages 0– < 19 y) were tested on ADVIA XPT/1800 and Dimension EXL systems, respectively. Age- and/or sex-specific RIs were calculated for a total of 54 biochemical assays.

Results: Serum concentrations of several biomarkers remained relatively constant across the pediatric age range and similar between sexes, including sodium and triglycerides. Other biomarkers, such as alkaline phosphatase and creatinine showed both age and sex differences. Furthermore, immunoglobulin A and iron showed only age differences.

Discussion: We established RIs for creatine kinase, random glucose, total iron binding capacity, and several electrolytes for the first time using the CALIPER cohort. Overall, pediatric RIs established in the current study will allow for more accurate laboratory test interpretation worldwide using Siemens chemistry assays.

1. Introduction

Clinical interpretation of laboratory test results requires appropriate population-based reference intervals (RIs), which most commonly represent the central 95% of the distribution of laboratory values gathered from a cohort of healthy reference individuals. The Clinical and Laboratory Standards Institute (CLSI) EP28-A3c guidelines has defined the process by which laboratory reference intervals should be established [1]. However, many gaps currently exist in the availability of accurate RIs, particularly for the pediatric population due to the difficulty in recruiting a large number of healthy reference individuals. Each RI partition requires a minimum sample size of 120 to use the non-parametric method, according to CLSI [1]. Thus, clinical laboratories often use adult RIs for interpretation of test results in children and adolescents, while others have calculated pediatric RIs based on insufficient sample size and/or employed inpatient or outpatient populations [2]. Additionally, many laboratories utilize RIs from manufacturer package inserts or other laboratories without initially verifying them for their local population. Some laboratories also adopt RIs calculated based on different analytical instruments and reagents than those used by their laboratory. The use of inaccurate RIs can introduce errors during clinical laboratory result interpretation, which can potentially compromise test interpretation and result in misdiagnosis or unnecessary further testing and/or anxiety for the patient.

Abbreviations: AHRIP, Australian Harmonized Reference Intervals for Paediatrics; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BCP, bromocresol purple; C3, complement component 3; C4, complement component 4; CALIPER, The Canadian Laboratory Initiative on Pediatric Reference Intervals; CHILDx, Children's Health Improvement through Laboratory Diagnostics; CHMS, Canadian Health Measures Survey; CLSI, Clinical and Laboratory Standards Institute; KiGGS, German Health Interview and Examination Survey for Children and Adolescents; L-P, L-lactate to pyruvate; NORIP, Nordic Reference Interval Project; P5P, pyridoxal-5-phosphate; Q-Q, quantile-quintile; SST, serum separator tubes.

* Corresponding author at: CALIPER Program, Pediatric Laboratory Medicine, The Hospital for Sick Children, 555 University Avenue, Toronto, ON M5G 1X8, Canada.
E-mail addresses: khosrow.adeli@sickkids.ca, k.adeli@utoronto.ca (K. Adeli).

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National RI initiatives have been established to fill the gap in pediatric reference intervals, including the Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER), the Children's Health Improvement through Laboratory Diagnostics (CHILDx), and the German Health Interview and Examination Survey for Children and Adolescents (KiGGS) [3]. CALIPER is a Canadian initiative that has established pediatric age- and sex-specific RIs for over 170 biomarkers on several analytical platforms using a large, healthy cohort of children and adolescents [4]. CALIPER RIs are available online (www.caliperdatabase.ca) and via a mobile application (CALIPER Reference App). To improve the utility of RIs for routine chemistry biomarkers, CALIPER has established reference intervals for immunoassays on several analytical systems or transferred pediatric RIs for biochemical markers originally established for Abbott assays to 4 other commonly used manufacturer instruments and reagents (i.e., Beckman Coulter, Ortho Clinical Diagnostics, Roche, and Siemens) [5–9]. Although there are similarities in lower and upper reference limits for many biomarkers, such as total protein, across analytical platforms [5–10], some show differences. Firstly, as demonstrated in CALIPER transference studies, several biomarkers lack strong correlations between measurements performed using different platforms. Some of the most notable examples include carbon dioxide (CO_2) [5–9], calcium [5–8], and magnesium [6,9]. Furthermore, some biomarkers, even with strong correlations, show a large bias in measurements between different manufacturer assays. For example, the upper reference limit for lipase was approximately 4-fold larger for the Ortho VITROS 5600 assay [5] and approximately 5-fold larger for the Siemens Vista 1500 assay [9] compared to the lipase assay on Abbott ARCHITECT c8000. These findings suggest that at least for some assays manufacturer-specific RIs may be required for appropriate test result interpretation.

CALIPER had previously transferred pediatric RIs from ARCHITECT to Vista 1500 for various biochemical markers, including common chemistry assays, enzymes, lipids and lipoproteins, as well as proteins [9]. In the current study, RIs were established for a combined total of 54 chemistry assays on ADVIA XPT/1800 and Dimension EXL with LM Integrated Chemistry Systems.

2. Materials and methods

2.1. Participant recruitment and sample acquisition

Information regarding participant recruitment and sample acquisition has been explained in detail elsewhere [10]. This study was approved by the Research Ethics Board at the Hospital for Sick Children (Toronto, ON). Healthy children and adolescents (ages 1– < 19 y) were recruited from the community (e.g., community centers, schools, summer camps, and daycares) in the Greater Toronto Area and Hamilton regions. None of the participants were required to fast prior to blood collection. Following the completion of written informed consent and a health questionnaire, blood was drawn by certified phlebotomists into serum separator tubes (SST™; Becton, Dickinson). These samples were centrifuged (3220 × *g*), separated, and aliquoted within 4 h of phlebotomy and stored at −80 °C until testing. Exclusion criteria included pregnancy, chronic illness, hormonal therapy, acute illness within the past week, and use of prescribed medication within the past 2 weeks. For neonates and infants (< 1 y), samples were collected from apparently healthy and metabolically stable subjects in outpatient clinics and maternity wards of collaborating hospitals.

2.2. Sample analysis

A total of 909 and 867 serum samples were tested on ADVIA XPT/1800 (33 assays) and Dimension EXL with LM Integrated Chemistry (21 assays) Systems (Siemens Healthineers), respectively. These samples were tested in March and April of 2016 and October and November of 2017. ADVIA 1800 Chemistry and ADVIA Chemistry XPT analyzers were used to test samples for the same assays in 2016 and 2017, respectively. Due to the analytical equivalence of the 2 analyzers (i.e., use of identical reagents and equivalence of results), their results were combined. The following 21 assays were tested on both ADVIA and Dimension: alanine aminotransferase [ALT; with pyridoxal-5-phosphate (P5P) on Dimension only], albumin [bromocresol purple (BCP)], alkaline phosphatase (ALP), amylase, aspartate aminotransferase (AST; with P5P on both ADVIA and Dimension), direct bilirubin, total bilirubin, urea, calcium, chloride, total cholesterol, creatinine (enzymatic), glucose (random), lactate dehydrogenase (LDH), lipase, phosphate, potassium, sodium, total protein (TP), triglycerides (TG), and uric acid. An additional 12 assays were performed only on ADVIA: complement component 3 (C3), complement component 4 (C4), creatine kinase (CK), cystatin C, gamma-glutamyl transferase (GGT), high-sensitivity C-reactive protein (hsCRP), immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM), iron, TIBC, and transferrin. Analyzer diagnostic, calibration, and quality control for all assays passed Siemens specifications prior to sample testing. Analytical performance of assays on ADVIA and Dimension are summarized in Supplemental Tables 1–2.

2.3. Statistical analysis and calculation of RIs

Data were analyzed based on CLSI EP28-A3c guidelines [1]. Statistical analysis, adapted from a previous CALIPER publication [10], was performed using Microsoft Excel and R software. Biomarker concentrations were initially plotted against age and colour-coded by sex. They were visually inspected for age- and sex-based partitions, which were statistically confirmed using the Harris and Boyd statistical method [11]. Furthermore, normality of data for each partition was determined using quantile-quantile (Q–Q) plots. In the case that the distribution was skewed, effort was made to convert it to a parametric distribution by applying Box-Cox transformation. Outliers within each partition were identified and removed using Tukey or adjusted Tukey test twice for parametric and nonparametric data, respectively [12]. The lower and upper reference limits were calculated as the 2.5th and 97.5th percentiles for each age and sex partition using either the nonparametric rank method ($n \geq 120$) or the robust method of Horn and Pesce ($40 \leq n < 120$) [13]. This was followed by the calculation of 90% confidence intervals (CIs) around both upper and lower reference limits. The statistical procedure is summarized in Fig. 1.

3. Results

Age- and sex-specific pediatric RIs were calculated for 54 assays: 33 assays on ADVIA and 21 assays on Dimension, reported in SI units (Tables 1–2) and conventional units (Supplemental Tables 3 and 4). The age and sex distributions for each biomarker are shown in Figs. 2–3 and Supplemental Figs. 1–27. Several assays [i.e., 24 of the 54 assays (44%)] required both age and sex partitioning. These included eight assays (i.e., ALP, ALT, AST, creatinine, LDH, phosphate, urea, and uric acid) analyzed on both ADVIA and Dimension (i.e., a total of 16 assays) and an additional eight assays tested only on ADVIA (i.e., albumin, CK, cystatin C, direct bilirubin, GGT, potassium, total bilirubin, and total protein).

Furthermore, 16 out of the 54 assays (30%) demonstrated age differences, but not sex differences. Two of these assays, amylase and calcium, were tested on both ADVIA and Dimension (i.e., a total of 4 assays). Five of the 16 assays were tested on ADVIA (C3, IgA, IgG, IgM, and iron), while the remaining seven assays were tested on Dimension (albumin, chloride, direct bilirubin, potassium, total bilirubin, total protein, and transferrin).

Lastly, 14 out of the 54 assays (26%), did not show age or sex differences in their serum concentrations and, thus, were not partitioned. Five of these assays were tested on both ADVIA and Dimension (i.e., a total of 10 assays): total cholesterol, random glucose, lipase, sodium, and triglycerides. Additionally, 4 assays were only tested on ADVIA,

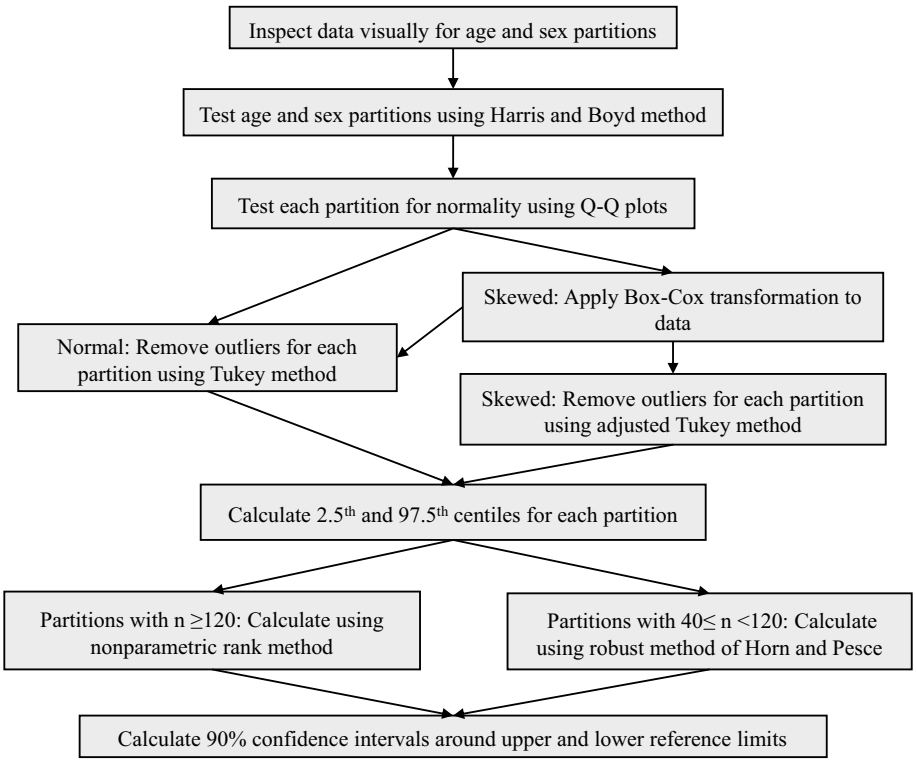

Fig. 1. Statistical approach to calculate age- and sex- reference intervals. Adapted from: Colantonio et al. [10]. n: sample size.

including C4, chloride, CRP, and TIBC.

While age and/or sex partitions were consistent between the 2 analyzers for some biomarkers (e.g., ALP, creatinine, and phosphate), they were different for others (e.g., albumin, calcium, and total bilirubin). These differences are possibly due to variation in sample size between ADVIA and Dimension used to calculate RIs. The findings for assays tested in the current study have been summarized below.

3.1. Anemia-related markers

Three markers of anemia were assessed in the current study: iron, TIBC, and transferrin. Iron and transferrin required partitioning by age in early childhood and early adolescence, but there were differences in the distribution of these 2 biomarkers with age. Iron showed a relatively slow, but consistent, increase throughout the pediatric age range. Transferrin concentrations, on the other hand, demonstrated a large increase in the first year of life, but remained stable for the remainder of the pediatric age range, with the exception of a slight increase at the age of 12 y. TIBC concentrations remained approximately constant for all participants < 19 y. Overall, although no sex differences were observed for anemia-related markers, age differences were present for iron and transferrin.

3.2. Electrolytes

The 5 electrolytes analyzed showed little change over the pediatric age range. Calcium and potassium concentrations decreased during early childhood, but their concentrations remained relatively constant throughout the rest of childhood and adolescence. Interestingly, potassium concentrations tested on ADVIA showed sex differences under the age of 1 y, but not on Dimension. Likewise, the upper reference limit for chloride decreased after early childhood and remained the same until adulthood. This reduction in the upper reference limit was much larger in data gathered from Dimension compared to ADVIA, requiring the need to partition by age for the RI established on the Dimension platform. Sodium concentrations were also consistent throughout the entire pediatric age range. Phosphate concentrations, however, varied in both children and adolescents, with concentrations decreasing from birth to 5 y, but remaining relatively stable until the age of 13 y. Sex differences in phosphate concentrations were also observed in adolescents, with generally higher values in males. With the exception of phosphate, the concentrations of electrolytes remained relatively stable after early childhood.

3.3. General chemistry markers

Five general chemistry markers were assessed and, similar to electrolytes, many of them required either few or no age partitions. Glucose and lipase activities did not vary by age or sex across the pediatric age range. Furthermore, amylase activities increased during the first year of life, but, similar to glucose and lipase, levels remained stable thereafter. Conversely, CK activities were stable throughout childhood, but in adolescence, male activities increased and female activities decreased with respect to childhood activities. While the other 4 general chemistry markers showed stable concentrations at some point during childhood, total protein constantly increased in children. This increase was relatively sharp in infants, but was more gradual between 1 and 9 y. Total protein concentrations, thereafter, remained stable until adulthood. Sex differences in total protein concentrations were also observed in infants. In summary, RIs for most general chemistry markers required minimal age and sex partitioning.

3.4. Hepatic markers

Five of the 8 hepatic markers studied were enzymes (i.e., ALT, ALP, AST, GGT, and LDH), all of which varied by age and sex. ALT and GGT activities decreased in early childhood, and remained constant until adolescence, where sex differences were observed, with higher upper and lower reference limits in males. Moreover, LDH activities were stable throughout childhood and showed sex differences mainly during

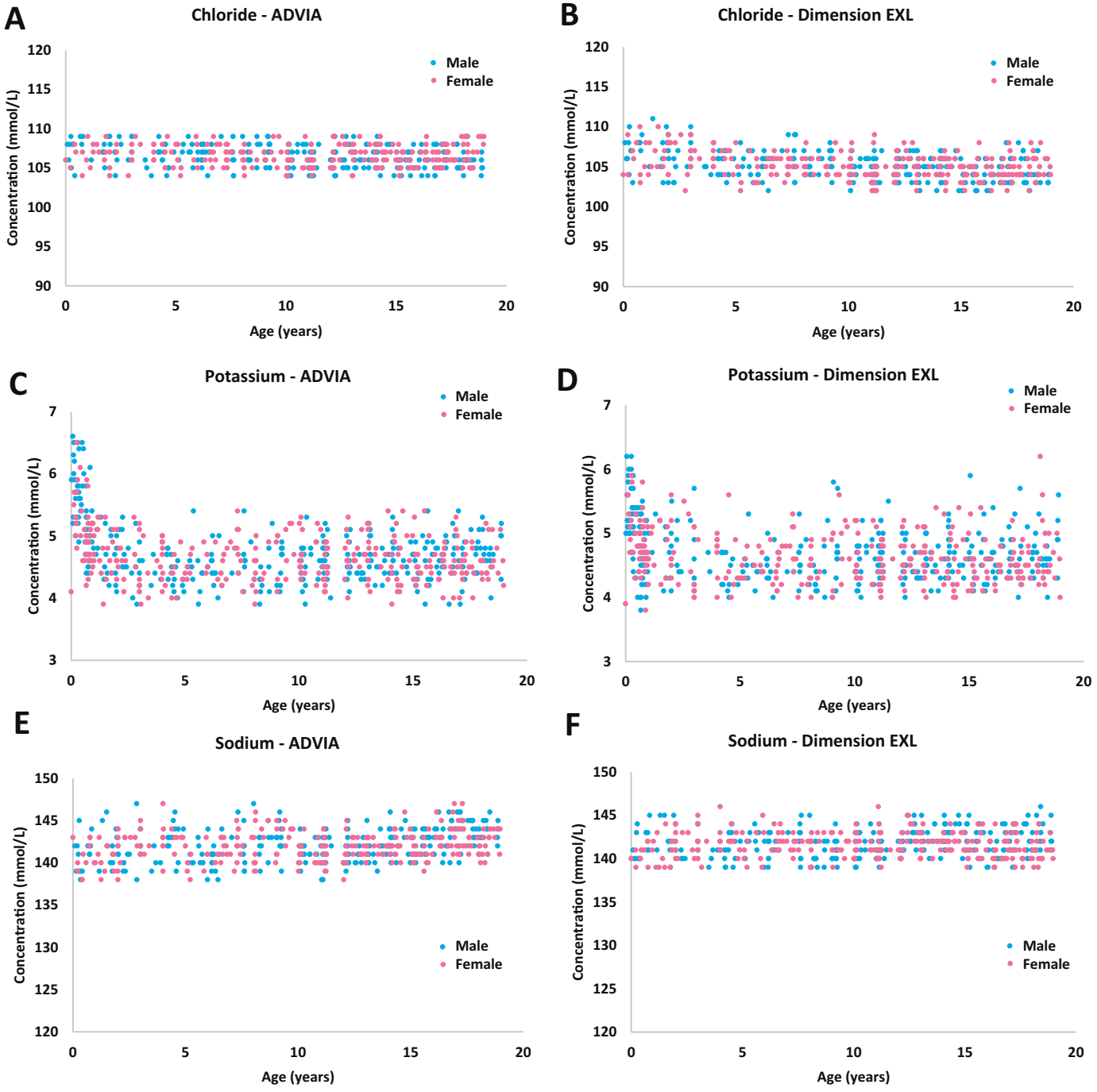

Fig. 2. Scatterplots demonstrating age-specific (0– < 19 y) population reference values for chloride (A–B), potassium (C–D), and sodium (E–F) measured on Siemens ADVIA Chemistry XPT/1800 and Dimension EXL with LM Integrated Chemistry Systems. Note: scatterplots demonstrate data after removing outliers.

adolescence, with higher concentrations in males than females. Additionally, AST activities persistently decreased throughout childhood, but remained constant with age and showed sex differences in adolescence. Lastly, ALP activities demonstrated both increases and decreases with age. Although ALP activities were relatively constant in early childhood, its activites increased prior to and decreased during adolescence. In addition to requiring 5 age partitions, ALP RIs were also partitioned by sex. Similar to the other hepatic enzymes, ALP and AST activities were generally higher in males than females. Overall, hepatic enzymes required relatively complex age and sex partitioning.

Three non-enzymatic hepatic markers were also tested: albumin, direct bilirubin, and total bilirubin. These biomarkers showed differences in partitioning between ADVIA and Dimension results. As an example, albumin RIs on ADVIA not only consisted of a larger number of age partitions compared to those on Dimension, but were also partitioned by sex, unlike the Dimension albumin RIs. Furthermore, even though ADVIA and Dimension direct bilirubin RIs consisted of the same age partitions, ADVIA RIs demonstrated sex differences. This resulted in the creation of narrower male RIs compared to female RIs on ADVIA. Lastly, total bilirubin upper reference limits for ADVIA and Dimension showed a large age-dependent increase. Additionally, there were sex differences in total bilirubin concentrations for ADVIA, while this difference was absent for the Dimension assay. In brief, differences in concentrations and/or distribution of data were observed between

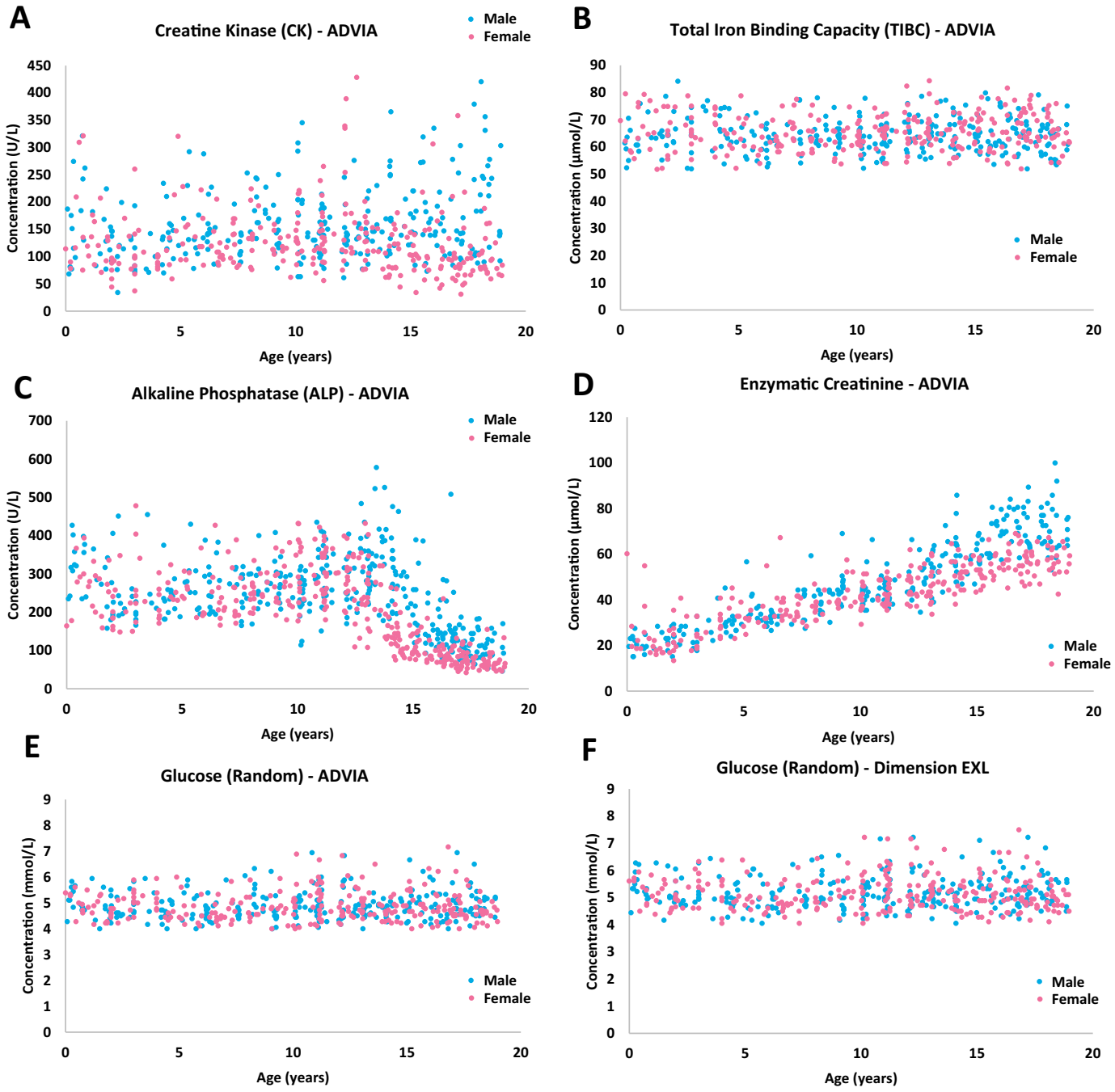

Fig. 3. Scatterplots demonstrating age-specific (0– < 19 y) population reference values for creatine kinase (A), total iron binding capacity (B), alkaline phosphatase (C), and creatinine (D) measured on Siemens ADVIA XPT/1800, as well as glucose (E–F) measured on Siemens ADVIA XPT/1800 and Dimension EXL with LM Integrated Chemistry Systems. Note: scatterplots demonstrate data after removing outliers.

ADVIA and Dimension assays for non-enzymatic hepatic markers.

3.5. Inflammatory markers

Six inflammatory and immunological markers were studied. C4 and CRP did not vary with age or sex. C3 and IgM required 2 age partitions, without the need for sex partitioning. Their concentrations increased in early childhood and remained relatively constant until adulthood. IgA and IgG, on the other hand, required multiple age partitions due to consistent increases throughout childhood. IgA concentrations greatly increased after the second year of life and remained stable between the ages of 2 and 10 y, after which the upper reference limit increased. This resulted in the widening of its RI for adolescents. IgG concentrations increased sharply during the first year of life and again around 4 and 10 y. Even though there were age differences in the concentrations of some inflammatory markers, no sex differences were observed.

3.6. Lipid markers

Cholesterol and triglycerides, the only 2 lipid markers assessed, remained relatively stable from birth until the age of 19 y, with no sex differences. Thus, no sex or age RI partitioning was required for the lipid markers.

Table 1
Age- and sex-specific pediatric reference intervals for chemistry assays on Siemens ADVIA Chemistry XPT and ADVIA 1800 Chemistry analyzers in SI units.

Biomarker	Units	Male Reference Intervals						Female Reference Intervals					
		Age (y)	Lower Limit	Upper Limit	n	Lower 90% CI	Upper 90% CI	Age (y)	Lower Limit	Upper Limit	n	Lower 90% CI	Upper 90% CI
Albumin BCP	g/l	0– < 5	35	49	115	(33,36)	(48,49)	0– < 5	35	49	115	(33,36)	(48,49)
		5– < 15	41	47	285	(40,41)	(47,47)	5– < 15	41	47	285	(40,41)	(47,47)
		15– < 19	41	53	79	(40,42)	(52,53)	15– < 19	40	50	80	(40,41)	(50,51)
ALP	U/l	0– < 10	163	427	251	(154,171)	(398,451)	0– < 10	163	427	251	(154,171)	(398,451)
		10– < 13	132	432	150	(109,177)	(412,484)	10– < 13	132	432	150	(109,177)	(412,484)
		13– < 15	176	515	58	(160,191)	(477,560)	13– < 15	70	370	57	(62,78)	(332,418)
		15– < 17	86	390	56	(79,93)	(333,446)	15– < 17	52	182	51	(47,56)	(157,206)
		17– < 19	53	191	50	(47,62)	(176,209)	17– < 19	43	132	51	(40,47)	(119,147)
ALT (without P5P)	U/l	0– < 3	13	45	66	(11,15)	(42,49)	0– < 3	13	45	66	(11,15)	(42,49)
		3– < 13	15	35	273	(15,15)	(32,41)	3– < 13	15	35	273	(15,15)	(32,41)
		13– < 19	15	47	112	(14,15)	(42,53)	13– < 19	12	26	114	(11,12)	(25,28)
Amylase	U/l	0– < 1	6	79	119	(5,7)	(71,87)	0– < 1	6	79	119	(5,7)	(71,87)
		1– < 19	35	115	535	(33,37)	(110,129)	1– < 19	35	115	535	(33,37)	(110,129)
AST (with P5P)	U/l	0– < 6	28	57	145	(27,30)	(53,60)	0– < 6	28	57	145	(27,30)	(53,60)
		6– < 12	26	44	165	(24,27)	(42,44)	6– < 12	26	44	165	(24,27)	(42,44)
		12– < 19	22	52	135	(21,24)	(45,53)	12– < 19	19	42	136	(19,21)	(36,46)
Bilirubin (Direct)	µmol/l	0– < 9	1.7	6.8	211	(1.7,1.7)	(5.1,10.3)	0– < 9	1.7	6.8	211	(1.7,1.7)	(5.1,10.3)
		9– < 13	1.7	3.4	123	(1.7,1.7)	(3.4,3.4)	9– < 13	1.7	3.4	123	(1.7,1.7)	(3.4,3.4)
		13– < 19[a]	3.4	5.1	84	N/A	N/A	13– < 19[b]	1.7	8.6	119	(1.7,1.7)	(6.8,12.0)
Bilirubin (Total)	µmol/l	0– < 10	5.1	12.0	188	(5.1,5.1)	(10.3,13.7)	0– < 10	5.1	12.0	188	(5.1,5.1)	(10.3,13.7)
		10– < 14[c]	3.4	13.7	193	(3.4,3.4)	(12.0,13.7)	10– < 14[c]	3.4	13.7	193	(3.4,3.4)	(12.0,13.7)
		14– < 19[c]	6.2	19.1	115	(5.9,6.7)	(18.0,20.4)	14– < 19[c]	3.4	15.4	127	(3.4,5.1)	(13.7,15.4)
Complement C3	g/l	0– < 1	0.60	1.39	123	(0.55,0.64)	(1.34,1.54)	0– < 1	0.60	1.39	123	(0.55,0.64)	(1.34,1.54)
		1– < 19	0.89	1.62	555	(0.86,0.92)	(1.56,1.68)	1– < 19	0.89	1.62	555	(0.86,0.92)	(1.56,1.68)
Complement C4	g/l	0– < 19	0.12	0.36	580	(0.10,0.12)	(0.34,0.38)	0– < 19	0.12	0.36	580	(0.10,0.12)	(0.34,0.38)
Calcium	mmol/l	0– < 2	2.38	2.87	44	(2.31,2.44)	(2.82,2.92)	0– < 2	2.38	2.87	44	(2.31,2.44)	(2.82,2.92)
		2– < 5	2.37	2.69	65	(2.35,2.40)	(2.67,2.72)	2– < 5	2.37	2.69	65	(2.35,2.40)	(2.67,2.72)
		5– < 19	2.28	2.55	409	(2.28,2.30)	(2.53,2.55)	5– < 19	2.28	2.55	409	(2.28,2.30)	(2.53,2.55)
Chloride	mmol/l	0– < 19	104	109	497	(104,104)	(109,109)	0– < 19	104	109	497	(104,104)	(109,109)
Total Cholesterol	mmol/l	0– < 19	3.08	5.49	575	(3.00,3.19)	(5.26,5.65)	0– < 19	3.08	5.49	575	(3.00,3.19)	(5.26,5.65)
Creatine Kinase	U/l	0– < 13[d]	68	293	347	(61,75)	(262,321)	0– < 13[d]	68	293	347	(61,75)	(262,321)
		13– < 19[d]	80	354	108	(73,86)	(327,382)	13– < 19[d]	48	200	112	(44,53)	(186,215)
Creatinine (Enzymatic)	µmol/l	0– < 2	15	46	44	(14,15)	(39,55)	0– < 2	15	46	44	(14,15)	(39,55)
		2– < 5	16	43	70	(15,17)	(40,46)	2– < 5	16	43	70	(15,17)	(40,46)
		5– < 9	27	54	94	(26,28)	(50,57)	5– < 9	27	54	94	(26,28)	(50,57)
		9– < 12	36	60	52	(34,37)	(56,65)	9– < 12	32	56	50	(31,34)	(54,59)
		12– < 15	38	75	58	(35,40)	(71,80)	12– < 15	35	64	58	(33,37)	(61,66)
		15– < 19	54	90	75	(52,56)	(87,94)	15– < 19	44	68	74	(42,46)	(66,71)
Cystatin C	mg/l	0– < 1	0.69	1.62	116	(0.66,0.73)	(1.54,1.71)	0– < 1	0.69	1.62	116	(0.66,0.73)	(1.54,1.71)
		1– < 13	0.59	0.94	325	(0.58,0.61)	(0.89,0.97)	1– < 13	0.59	0.94	325	(0.58,0.61)	(0.89,0.97)
		13– < 19	0.66	1.02	106	(0.64,0.68)	(0.99,1.05)	13– < 19	0.58	0.83	116	(0.56,0.59)	(0.81,0.85)
GGT	U/l	0– < 4	7	54	81	(6,7)	(41,67)	0– < 4	7	54	81	(6,7)	(41,67)
		4– < 13	9	18	231	(9,9)	(17,19)	4– < 13	9	18	231	(9)	(17,19)
		13– < 19	11	29	104	(11,11)	(27,31)	13– < 19	6	15	90	(5,7)	(14,15)
Glucose (Random)	mmol/l	0– < 19	4.05	6.22	542	(4.00,4.11)	(5.99,6.66)	0– < 19	4.05	6.22	542	(4.00,4.11)	(5.99,6.66)
hsCRP	mg/l	0– < 19[e]	0.0	1.6	512	(0.0,0.0)	(1.5,1.8)	0– < 19[e]	0.0	1.6	512	(0.0,0.0)	(1.5,1.8)
IgA	g/l	0– < 2	0.2	2.2	44	(0.2,0.2)	(1.7,2.9)	0– < 2	0.2	2.2	44	(0.2,0.2)	(1.7,2.9)
		2– < 10	0.6	2.1	180	(0.2,0.7)	(2.0,2.3)	2– < 10	0.6	2.1	180	(0.2,0.7)	(2.0,2.3)
		10– < 19	1.0	4.0	335	(0.9,1.0)	(3.6,4.7)	10– < 19	1.0	4.0	335	(0.9,1.0)	(3.6,4.7)
IgG	g/l	0– < 1[f]	1.2	10.6	112	(1.0,1.4)	(9.7,11.8)	0– < 1[f]	1.2	10.6	112	(1.0,1.4)	(9.7,11.8)
		1– < 4	5.2	16.0	54	(4.9,5.6)	(14.3,18.1)	1– < 4	5.2	16.0	54	(4.9,5.6)	(14.3,18.1)
		4– < 10	6.2	12.9	141	(5.1,6.8)	(12.1,13.9)	4– < 10	6.2	12.9	141	(5.1,6.8)	(12.1,13.9)
		10– < 19	8.0	15.6	343	(7.3,8.4)	(15.0,16.4)	10– < 19	8.0	15.6	343	(7.3,8.4)	(15.0,16.4)
IgM	g/l	0– < 3[f]	0.2	2.0	68	(0.1,0.2)	(1.8,2.2)	0– < 3[f]	0.2	2.0	68	(0.1,0.2)	(1.8,2.2)
		3– < 19	0.5	2.4	517	(0.4,0.5)	(2.2,2.6)	3– < 19	0.5	2.4	517	(0.4,0.5)	(2.2,2.6)
Iron	µmol/l	0– < 2	2.2	22.6	44	(1.4,3.3)	(18.8,26.6)	0– < 2	2.2	22.6	44	(1.4,3.3)	(18.8,26.6)
		2– < 14	2.5	26.9	346	(1.8,3.2)	(25.1,30.4)	2– < 14	2.5	26.9	346	(1.8,3.2)	(25.1,30.4)
		14– < 19	4.1	30.4	198	(2.3,5.5)	(27.4,32.0)	14– < 19	4.1	30.4	198	(2.3,5.5)	(27.4,32.0)
LDH	U/l	0– < 12	195	349	306	(179,207)	(337,363)	0– < 12	195	349	306	(179,207)	(337,363)
		12– < 16	177	358	78	(171,184)	(327,396)	12– < 16	146	297	77	(138,154)	(278,315)
		16– < 19	134	314	59	(126,142)	(295,334)	16– < 19	120	250	60	(109,130)	(238,262)
Potassium	mmol/l	0– < 1	4.3	6.7	55	(4.1,4.5)	(6.5,7.0)	0– < 1	4.2	6.2	45	(4.0,4.3)	(5.9,6.5)
		1– < 19	4.0	5.3	562	(3.9,4.0)	(5.3,5.4)	1– < 19	4.0	5.3	562	(3.9,4.0)	(5.3,5.4)
Sodium	mmol/l	0– < 19[g]	139	146	561	(138,139)	(145,146)	0– < 19[g]	139	146	561	(138,139)	(145,146)
Lipase	U/l	0– < 19	22	48	545	(21,22)	(44,52)	0– < 19	22	48	545	(21,22)	(44,52)
Phosphate (inorganic)	mmol/l	0– < 1	1.36	2.49	125	(1.29,1.49)	(2.23,2.52)	0– < 1	1.36	2.49	125	(1.29,1.49)	(2.23,2.52)
		1– < 5	1.42	1.99	86	(1.38,1.47)	(1.95,2.03)	1– < 5	1.42	1.99	86	(1.38,1.47)	(1.95,2.03)
		5– < 13	1.29	1.84	233	(1.23,1.32)	(1.81,1.94)	5– < 13	1.29	1.84	233	(1.23,1.32)	(1.81,1.94)
		13– < 16	1.05	1.82	57	(0.95,1.13)	(1.77,1.89)	13– < 16	1.05	1.68	56	(1.00,1.10)	(1.62,1.75)
		16– < 19	0.87	1.57	118	(0.82,0.92)	(1.53,1.61)	16– < 19	0.87	1.57	118	(0.82,0.92)	(1.53,1.61)
TIBC	µmol/l	0– < 19	53.7	78.6	573	(52.1,54.1)	(77.3,79.3)	0– < 19	53.7	78.6	573	(52.1,54.1)	(77.3,79.3)

(*continued on next page*)

Table 1 (*continued*)

Biomarker	Units	Male Reference Intervals						Female Reference Intervals					
		Age (y)	Lower Limit	Upper Limit	n	Lower 90% CI	Upper 90% CI	Age (y)	Lower Limit	Upper Limit	n	Lower 90% CI	Upper 90% CI
Total Protein	g/l	0– < 1	48	73	67	(46,49)	(71,76)	0– < 1	43	73	47	(41,44)	(69,77)
		1– < 6	61	74	105	(60,62)	(73,75)	1– < 6	61	74	105	(60,62)	(73,75)
		6– < 9	65	77	69	(65,66)	(75,78)	6– < 9	65	77	69	(65,66)	(75,78)
		9– < 19	68	80	355	(68,68)	(79,81)	9– < 19	68	80	355	(68,68)	(79,81)
Transferrin	g/l	0– < 1	1.68	3.42	124	(1.43,1.75)	(3.16,3.52)	0– < 1	1.68	3.42	124	(1.43,1.75)	(3.16,3.52)
		1– < 12	2.14	3.07	240	(2.05,2.25)	(3.02,3.09)	1– < 12	2.14	3.07	240	(2.05,2.25)	(3.02,3.09)
		12– < 19	2.35	3.63	273	(2.26,2.38)	(3.58,3.70)	12– < 19	2.35	3.63	273	(2.26,2.38)	(3.58,3.70)
Triglycerides	mmol/l	0– < 19	0.43	2.83	584	(0.40,0.46)	(2.66,2.98)	0– < 19	0.43	2.83	584	(0.40,0.46)	(2.66,2.98)
Urea	mmol/l	0– < 1	1.8	7.2	102	(1.7,1.9)	(6.7,7.8)	0– < 1	1.8	7.2	102	(1.7,1.9)	(6.7,7.8)
		1– < 10	3.2	7.9	202	(2.9,3.6)	(7.5,8.2)	1– < 10	3.2	7.9	202	(2.9,3.6)	(7.5,8.2)
		10– < 19	3.2	7.1	167	(2.5,3.6)	(6.8,7.1)	10– < 19	3.6	6.8	151	(3.6,3.6)	(6.4,7.5)
Uric Acid	μmol/l	0– < 12	137	327	307	(125,143)	(315,339)	0– < 12	137	327	307	(125,143)	(315,339)
		12– < 19	161	404	127	(119,178)	(393,404)	12– < 19	167	339	129	(149,184)	(321,357)

BCP: bromocresol purple; L-P: assay converting L-lactate to pyruvate; P5P: pyridoxal-5-phosphate; TIBC: total iron binding capacity.
Note: Sex differences are underlined. These reference intervals are applicable to both ADVIA Chemistry XPT and ADVIA 1800 Chemistry analyzers. Supplemental Table 3 includes ADVIA XPT/1800 reference intervals in conventional units.

[a] Due to the categorical nature of data, robust method of Horn and Pesce could not calculate the reference interval. Instead, the Harrell-Davis bootstrap method [25], another reference interval calculation method recommended by CLSI [1], was used to calculate the reference intervals and the confidence intervals. However, due to the categorical nature of data, confidence intervals could not be calculated.

[b] Due to the categorical nature of data, robust method of Horn and Pesce could not calculate the reference interval. Instead, the non-parametric rank method was used to calculate the reference interval, as well as the confidence intervals around the upper and lower limits.

[c] Total bilirubin values above 21 μmol/l were removed, based on the harmonized upper limit recommended by the Australian Association of Clinical Biochemists (AACB) [26].

[d] Creatine kinase values above 450 U/l were manually removed based on visual assessment of data (Fig. 3A) prior to analysis, as Tukey only removed a limited number of outliers. Additionally, Harris and Boyd did not indicate age differences, but sex difference was indicated between 13 and 19 year old males and females.

[e] Neither Tukey nor adjusted Tukey removed any outliers. Thus, CRP values above 2 mg/l were manually removed due to the visual assessment of the scatterplot (Supplemental Fig. 14).

[f] Harris and Boyd indicated sex difference, but sex-specific reference intervals were not calculated due to insufficient sample sizes for males and/or females.

[g] Due to the skewed nature of data, Box-Cox transformation could not be performed prior to applying adjusted Tukey. Thus, outliers were removed using adjusted Tukey without applying Box-Cox transformation. Additionally, Harris and Boyd test could not be performed, since the data did not meet the requirements for the test. However, visual examination of the scatterplot indicated no age or sex difference.

3.7. Renal markers

Lastly, 4 renal markers were studied. Uric acid concentrations remained approximately constant in childhood, but increased and deviated by sex during adolescence. Cystatin C concentrations decreased sharply during the first year of life, remained approximately constant for the remainder of childhood, and showed sex differences in adolescence, with higher concentrations in males compared to females. On the other hand, urea concentrations increased sharply from birth to 1 y, remained relatively stable during childhood, and decreased in adolescence, while also showing sex differences. Creatinine concentrations required sex and multiple age partitions. Its concentrations demonstrated constant increases throughout the pediatric age range, with sex differences starting at the age of 9 y. Overall, renal marker concentrations changed in childhood and adolescence and showed sex differences, mainly after puberty.

4. Discussion

Age- and sex-specific RIs were established for a combined total of 54 chemistry assays on ADVIA XPT/1800 and Dimension EXL systems. Furthermore, this is the first study to establish RIs for chloride, CK, glucose, potassium, sodium, and TIBC using the CALIPER cohort. Scatterplots and calculated RIs from the current study were compared to those from previous CALIPER studies: Colantonio et al. [10], Kelly et al. [14], and Estey et al. [9]. Colantonio et al. used data from a large number of participants (i.e., 2188) to establish RIs for 40 chemistry assays on the ARCHITECT system, many of which were also tested in the current study. Kelly et al. established RIs for specialized biomarkers for ARCHITECT assays, including cystatin C, based on data from a relatively large sample size (i.e., 1917 participants). Lastly, Estey et al. transferred ARCHITECT RIs to 4 other analytical platforms, including Vista. Generally, most assays in the current study followed a similar age- and sex-related trends to those observed in the 3 previous CALIPER studies mentioned above. Additionally, analyte concentration for the majority of the assays tested in the current study were either consistent with or had only minor differences in comparison to those found in the 3 other CALIPER studies. However, a limited number of the assays exhibited major differences, which are discussed below.

Out of the 3 anemia-related markers, RIs for iron and transferrin had previously been established by CALIPER on ARCHITECT [10] and Vista [9]. The concentrations of iron and transferrin from these 2 CALIPER studies are very similar to those observed in the current study. Additionally, the upper TIBC reference limit calculated in the current study was generally consistent with that of Soldin et al. calculated for those between the ages of 1 and 18 y [15], while the lower reference limit was higher in the current study. The difference in the findings may be due to the use of patient samples and the use of a different statistical approach (i.e., Hoffmann) by Soldin et al.

RIs calculated for calcium and phosphate in the current study were generally consistent with other studies [9,10]. Additionally, sodium, potassium, and chloride RIs were generally comparable to those observed in other pediatric RI studies: the CALIPER Canadian Health Measures Survey (CHMS) study [16], Australian Harmonized Reference Intervals for Paediatrics (AHRIP) study [17], and the Nordic Reference Interval Project (NORIP) [18]. The 2 exceptions included the NORIP potassium upper and lower reference limits and the AHRIP chloride lower reference limit, compared to both the ADVIA and Dimension RIs. Such differences between studies for potassium may be due to pre-analytical issues such as hemolysis. Overall, similar to anemia-related markers, most differences in electrolytes RIs were minor and the findings were relatively consistent with those of other studies.

Table 2
Age- and sex-specific pediatric reference intervals for chemistry assays on Siemens Dimension EXL analyzer in SI units.

Biomarker	Units	Male Reference Intervals						Female Reference Intervals					
		Age (y)	Lower Limit	Upper Limit	n	Lower 90% CI	Upper 90% CI	Age (y)	Lower Limit	Upper Limit	n	Lower 90% CI	Upper 90% CI
Albumin BCP	g/l	0– < 5	34	48	111	(33,36)	(48,49)	0– < 5	34	48	111	(33,36)	(48,49)
		5– < 19	40	47	403	(39,40)	(47,47)	5– < 19	40	47	403	(39,40)	(47,47)
ALP	U/l	0– < 10	163	417	220	(112,178)	(396,430)	0– < 10	163	417	220	(112,178)	(396,430)
		10– < 13	138	436	126	(111,183)	(421,449)	10– < 13	138	436	126	(111,183)	(421,449)
		13– < 15	176	540	52	(159,194)	(485,597)	13– < 15	70	370	54	(60,78)	(331,408)
		15– < 17	97	402	51	(91,103)	(341,471)	15– < 17	51	159	46	(44,57)	(145,173)
		17– < 19	55	204	46	(48,65)	(188,223)	17– < 19	46	138	48	(43,49)	(125,153)
ALT (with P5P)	U/l	0– < 1	16	53	128	(9,18)	(48,55)	0– < 1	16	53	128	(9,18)	(48,55)
		1– < 13	20	38	266	(19,21)	(37,39)	1– < 13	20	38	266	(19,21)	(37,39)
		13– < 19	22	60	106	(21,22)	(54,67)	13– < 19	18	38	102	(18,19)	(36,40)
Amylase	U/l	0– < 1	5	80	137	(4,6)	(61,96)	0– < 1	5	80	137	(4,6)	(61,96)
		1– < 19	36	100	444	(35,37)	(95,112)	1– < 19	36	100	444	(35,37)	(95,112)
AST (with P5P)	U/l	0– < 10	31	66	188	(31,32)	(58,99)	0– < 10	31	66	188	(31,32)	(58,99)
		10– < 12	23	44	64	(21,24)	(42,46)	10– < 12	23	44	64	(21,24)	(42,46)
		12– < 19	20	54	126	(18,21)	(47,61)	12– < 19	16	40	124	(15,17)	(36,47)
Bilirubin (Direct)	μmol/l	0– < 9	0.9	1.7	185	(0.9,0.9)	(1.7,1.7)	0– < 9	0.9	1.7	185	(0.9,0.9)	(1.7,1.7)
		9– < 13[a]	0.9	3.4	125	(0.9,0.9)	(1.7,3.4)	9– < 13[a]	0.9	3.4	125	(0.9,0.9)	(1.7,3.4)
		13– < 19[a]	0.9	5.1	217	(0.9,0.9)	(3.4,5.1)	13– < 19[a]	0.9	5.1	217	(0.9,0.9)	(3.4,5.1)
Bilirubin (Total)	μmol/l	0– < 12	1.7	10.3	272	(1.7,1.7)	(8.6,12.0)	0– < 12	1.7	10.3	272	(1.7,1.7)	(8.6,12.0)
		12– < 19[b]	4.6	18.4	109	(4.3,5.0)	(17.0,19.9)	12– < 19[b]	4.6	18.4	109	(4.3,5.0)	(17.0,19.9)
Calcium	mmol/l	0– < 2	2.18	2.63	42	(2.09,2.24)	(2.59,2.67)	0– < 2	2.18	2.63	42	(2.09,2.24)	(2.59,2.67)
		2– < 19	2.13	2.43	494	(2.13,2.15)	(2.40,2.43)	2– < 19	2.13	2.43	494	(2.13,2.15)	(2.40,2.43)
Chloride	mmol/l	0– < 4	102	111	81	(101,103)	(110,112)	0– < 4	102	111	81	(101,103)	(110,112)
		4– < 19	102	108	477	(102,102)	(108,108)	4– < 19	102	108	477	(102,102)	(108,108)
Total Cholesterol	mmol/l	0– < 19	2.85	5.36	569	(2.77,3.00)	(5.23,5.54)	0– < 19	2.85	5.36	569	(2.77,3.00)	(5.23,5.54)
Creatinine (Enzymatic)	μmol/l	0– < 2[c]	17	50	42	(16,17)	(43,60)	0– < 2[c]	17	50	42	(16,17)	(43,60)
		2– < 5	18	43	65	(16,20)	(41,45)	2– < 5	18	43	65	(16,20)	(41,45)
		5– < 9	31	58	94	(30,32)	(54,63)	5– < 9	31	58	94	(30,32)	(54,63)
		9– < 12	40	59	48	(38,41)	(57,62)	9– < 12	39	59	48	(38,40)	(57,62)
		12– < 15	41	82	57	(38,44)	(78,86)	12– < 15	40	69	58	(38,43)	(66,72)
		15– < 19	58	98	75	(56,61)	(94,103)	15– < 19	49	77	73	(47,50)	(75,80)
Glucose (Random)	mmol/l	0– < 19	4.22	6.66	543	(4.16,4.27)	(6.38,7.10)	0– < 19	4.22	6.66	543	(4.16,4.27)	(6.38,7.10)
LDH	U/l	0– < 10	226	373	192	(217,230)	(345,386)	0– < 10	226	373	192	(217,230)	(345,386)
		10– < 15	188	320	81	(183,194)	(309,333)	10– < 15	148	325	80	(139,157)	(309,340)
		15– < 19	130	302	68	(121,138)	(283,321)	15– < 19	123	233	68	(112,133)	(225,242)
Potassium	mmol/l	0– < 1	3.9	6.0	131	(3.8,4.0)	(5.9,6.2)	0– < 1	3.9	6.0	131	(3.8,4.0)	(5.9,6.2)
		1– < 19	4.0	5.5	533	(4.0,4.0)	(5.3,5.6)	1– < 19	4.0	5.5	533	(4.0,4.0)	(5.3,5.6)
Sodium	mmol/l	0– < 19	139	145	556	(139,139)	(145,145)	0– < 19	139	145	556	(139,139)	(145,145)
Lipase	U/l	0– < 19	69	212	495	(65,72)	(196,225)	0– < 19	69	212	495	(65,72)	(196,225)
Phosphate	mmol/l	0– < 1	1.39	2.36	135	(1.39,1.55)	(2.23,2.36)	0– < 1	1.39	2.36	135	(1.39,1.55)	(2.23,2.36)
		1– < 5	1.57	2.09	82	(1.53,1.60)	(2.05,2.14)	1– < 5	1.57	2.09	82	(1.53,1.60)	(2.05,2.14)
		5– < 13	1.49	2.00	202	(1.49,1.49)	(1.91,2.07)	5– < 13	1.49	2.00	202	(1.49,1.49)	(1.91,2.07)
		13– < 16	1.08	1.86	55	(0.94,1.19)	(1.80,1.92)	13– < 16	1.14	1.77	56	(1.10,1.18)	(1.69,1.84)
		16– < 19	0.93	1.64	116	(0.88,0.97)	(1.59,1.68)	16– < 19	0.93	1.64	116	(0.88,0.97)	(1.59,1.68)
Total Protein	g/l	0– < 1	51	80	141	(50,53)	(78,83)	0– < 1	51	80	141	(50,53)	(78,83)
		1– < 6	68	81	83	(67,69)	(79,82)	1– < 6	68	81	83	(67,69)	(79,82)
		6– < 9[c]	70	82	60	(69,71)	(80,83)	6– < 9[c]	70	82	60	(69,71)	(80,83)
		9– < 19	72	83	295	(72,72)	(82,83)	9– < 19	72	83	295	(72,72)	(82,83)
Triglycerides	mmol/l	0– < 19	0.34	2.78	574	(0.28,0.36)	(2.60,2.97)	0– < 19	0.34	2.78	574	(0.28,0.36)	(2.60,2.97)
Urea	mmol/l	0– < 1	1.4	7.1	128	(1.4,1.8)	(5.7,7.1)	0– < 1	1.4	7.1	128	(1.4,1.8)	(5.7,7.1)
		1– < 10	2.5	8.2	203	(2.5,2.9)	(7.5,8.6)	1– < 10	2.5	8.2	203	(2.5,2.9)	(7.5,8.6)
		10– < 19	3.2	7.5	167	(2.9,3.2)	(6.8,7.9)	10– < 19	2.9	6.4	174	(2.1,2.9)	(6.1,7.1)
Uric Acid	μmol/l	0– < 12	125	321	257	(119,131)	(303,327)	0– < 12	125	321	257	(119,131)	(303,327)
		12– < 19	170	418	116	(148,190)	(402,434)	12– < 19	155	326	115	(143,165)	(313,336)

BCP: bromocresol purple; P5P: pyridoxal-5-phosphate.

Note: Sex differences have been underlined. Supplemental Table 4 includes Siemens Dimension EXL reference intervals in conventional units.

[a] Since the outlier test identified a large proportion of values as outliers in the corresponding age partitions, reference intervals were calculated without removing outliers in these age partitions.

[b] Total bilirubin values above 21 μmol/l were removed, based on the harmonized upper reference limit recommended by the Australian Association of Clinical Biochemists (AACB) [26].

[c] Harris and Boyd indicated sex difference, but sex-specific reference intervals were not calculated due to insufficient sample sizes for males and/or females.

General chemistry markers showed marked differences between ADVIA and Dimension measurements and compared to other studies [9,10]. As demonstrated in Tables 1–2, lipase lower and upper reference limits calculated for Dimension were approximately 3 and 4.5 times higher than those calculated for ADVIA, respectively. ADVIA RIs for lipase were more similar to CALIPER ARCHITECT RIs [10], while the CALIPER Vista RIs had a much higher upper reference limit (9). Interestingly, the Dimension upper reference limit for lipase was more comparable to the upper reference limit calculated for Vista, whereas Dimension upper and lower reference limits were approximately 17 and 5.5 times higher than those calculated for the ARCHITECT assay, respectively. The analytical differences in lipase assay between ADVIA and Dimension may be explained by differences in calibration, assay reagents, and analytical measurement ranges (i.e., higher in Dimension)

between the 2 instruments.

RIs for 2 general chemistry markers, glucose and CK, had not previously been established based on the CALIPER cohort. Thus, their concentrations were compared with studies from other research studies. Compared to CHMS [16], the lower reference limits for glucose observed in the present study are similar, whereas the upper reference limits (i.e., ADVIA and Dimension) are higher. Furthermore, CK values above 450 U/l were observed in some teens but were removed as outliers based on visual assessment of the scatterplot (Fig. 3A), as these extreme values may have resulted from intense physical activity. On the other hand, CK upper reference limits were substantially lower than those proposed by other pediatric studies [18,19]. Such a major difference in upper CK reference limits may be due to differences in environmental factors (e.g., physical activity) influencing the study cohorts.

Comparison of scatterplots for the hepatic markers between the current study and Colantonio et al. study (i.e., ARCHITECT) [10] yielded similar results for numerous assays, while some differences were observed. For instance, the observed values for the Abbott assay for ALT were lower than ADVIA and Dimension assays and narrower than ADVIA activities in the current study. Moreover, Vista assay RIs established by CALIPER [9] were consistently lower than the RIs calculated in the current study for the majority of assays, including ALT (Dimension). In addition to the relatively consistent observed differences for hepatic markers between the current and the other CALIPER studies, there were differences in the activities of ALT and direct bilirubin between the ADVIA and Dimension systems. As demonstrated in Tables 1–2, compared to ADVIA, ALT activities were higher and direct bilirubin concentrations were lower for Dimension. Overall, these findings suggest analytical differences between ADVIA, Dimension, Vista, and ARCHITECT assays for some hepatic markers.

All 6 inflammatory and immunological markers in the current study were only measured on ADVIA. The majority of RIs in the current study were consistent with those calculated by Estey et al. (i.e., Vista) [9] and Colantonio et al. (i.e., ARCHITECT) [10], but with minor differences. For instance, IgA RIs were found to be slightly higher and wider in the current study compared to those established for Vista and ARCHITECT assays by Estey et al. and Colantonio et al., respectively. Overall, the minor differences between the current study and the previous CALIPER studies may be due to the differences in assay calibration.

Of the 2 lipid biomarkers studied, cholesterol RIs were similar to those reported in previous CALIPER studies (i.e., ARCHITECT and Vista) [9,10]. Triglyceride RIs for ADVIA and Dimension assays in the current study were slightly wider than those previously reported by CALIPER (i.e., ARCHITECT and Vista) [9,10]. These minor differences are likely due to differences in lifestyle and dietary factors of the 2 study cohorts and/or assay calibration.

Lastly, RIs for the majority of renal makers were similar to those previously reported by CALIPER [9,10,14], although there were slight differences. For example, creatinine (Dimension) RIs were slightly narrower and higher than those reported using the ARCHITECT [10] and Vista assays [9]. Similar to many other biomarkers, only minor differences exist in renal markers between the current study and other CALIPER studies.

In addition to the discussed differences, a common variation between the findings in the current study and those in other CALIPER studies [9,10,14] were in the concentrations of biomarkers in neonatal and infantile age ranges. One example is the relatively large AST upper reference limit (i.e., 186 U/l) for the age range of 0– < 14 days reported in the Colantonio et al. study [10] compared to the upper reference limits calculated on either ADVIA and Dimension platforms. Such differences are likely the result of using serum samples from a relatively small number of neonates and infants in the current study. A second limitation of the current study is that participants were not fasting, since the concentrations of certain biomarkers, such as iron, may be variable depending on the time of last meal. However, it is important to note that children are routinely tested under non-fasting conditions, and therefore non-fasting reference concentrations for iron may better reflect routine clinical practice. Another limitation of the current study is the lack of ethnic partitioning for some of the biomarkers (e.g., amylase, CK, IgG, and IgM) that were shown by other studies to demonstrate ethnic differences [10,20–24]. This limitation is due to the insufficient sample size from certain ethnicities, such as Black and East Asian, to allow statistical comparisons. Regardless of these limitations, the majority of our findings are either relatively consistent with or show minor deviations from those published in other studies, suggesting the potential for harmonization of reference intervals for many biomarkers across different analytical platforms.

5. Conclusions

Overall, pediatric reference intervals reported in the present study will allow for more accurate laboratory assessment of pediatric patients tested using biochemical assays on commonly used Siemens platforms in clinics and hospitals around the world. It is however recommended that these reference values be verified by each laboratory using local pediatric blood samples, based on CLSI guidelines, before clinical implementation.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2018.12.011.

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