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Current State of Pediatric Reference Intervals and the Importance of Correctly Describing the Biochemistry of Child Development A Review

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Abstract

IMPORTANCE—Appropriately established pediatric reference intervals are critical to the clinical decision-making process and should reflect the physiologic changes that occur during healthy child development. Reference intervals used in pediatric care today remain highly inconsistent across a broad range of common clinical biomarkers.

OBSERVATIONS—This narrative review assesses biomarker-specific pediatric reference intervals and their clinical utility with respect to the underlying biological changes occurring during development. Pediatric reference intervals from PubMed-indexed articles published from January 2015 to April 2021, commercial laboratory websites, study cohorts, and pediatric reference interval books were all examined. Although large numbers of pediatric reference intervals are published for some biomarkers, very few are used by clinical and commercial laboratories. The patterns, extent, and timing of biomarker changes are highly variable, particularly during developmental stages with rapid physiologic changes. However, many pediatric reference intervals do not capture these changes and thus do not accurately reflect the underlying biochemistry of development, resulting in significant inconsistencies between reference intervals.

CONCLUSIONS AND RELEVANCE—There is a need to correctly describe the biochemistry of child development as well as to identify strategies to develop accurate and consistent pediatric reference intervals for improved pediatric care.

Hypotehtical case scenario: a 1-week-old infant has an abnormal thyrotropin (previously, thyroid-stimulating hormone) laboratory result on the state newborn screening test. At the 10-day-old follow-up visit, the infant's primary care physician obtains a thyrotropin result of 9.4 mIU/L from the local laboratory, along with a pediatric reference interval (PRI) of 0.7 to 4.8 mIU/L for this age range. Based on these laboratory results, the physician tells the family that the infant has congenital hypothyroidism and begins treatment with levothyroxine. After performing additional literature reviews, the physician finds that PRIs published in the scientific literature and used in clinical laboratories vary greatly and that normal thyrotropin concentrations can measure as high as 27.2 mIU/L in the first month after birth. The physician realizes that he may have started the infant taking a medication that was not necessary.[1,2] The family is upset that they were given erroneous information and treatment. The infant is referred to a pediatric endocrinologist to discuss how and when to safely stop levothyroxine based on current clinical guidelines to avoid rebound hypothyroidism.[3]

PRIs are critical for correct clinical decision-making.[4–6] This case presents just 1 example of how inconsistent PRIs can impact clinical decision-making. Inconsistency between PRIs applies to a broad range of biomarkers. For example, a PRI study published for hematology biomarkers showed that 63% of the patient samples measured at the local hospital would

change classification from normal to disease depending on the reference interval used.[7] The physical development of children into adults requires appropriate growth and maturation of the body and its organ systems, which is accompanied by fluctuations in biomarker concentrations at various stages of healthy child development. In turn, PRIs must accurately reflect these dynamic biochemical and physiological changes in healthy children to ensure correct diagnosis and treatment of disease in children.

Despite notable efforts throughout the past 30 years, the PRIs currently used in pediatric care remain highly inconsistent.[7–9] To address these inconsistencies, reviews and guidelines about PRIs typically focus on the technical aspects of creating reference intervals, such as statistical methods, population characteristics, and analytical considerations.[4,9–11] In this review, we assess the consistency of PRIs reported on selected analytes in the context of the biological changes occurring during child development and identify strategies to develop accurate and consistent PRIs for pediatric care.

Methods

Literature Search Strategy

This review includes PRIs from PubMed-indexed articles published from January 2015 to April 2021. Transference studies and abstractswere not included. Literature search terms included, but were not limited to, *pediatric reference intervals* and/or *children* or *pediatric* in combination with the analytes listed below. After review, 18 articles were selected for inclusion. PRIs published by major commercial laboratories and study cohorts, such as the Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER), were collected from websites.[12] A member of the American Association for Clinical Chemistry (AACC) Pediatric Reference Interval Working Group (D.J.D) collected additional PRI data from 9 clinical practices and tertiary care, academic, and pediatric hospitals. References published prior to 2015 were included if referenced in selected PRI books or reviews.[2,13–15]

Analytes commonly measured in the pediatric clinical setting were used as examples for this review and included the thyroid axis hormones free thyroxine (FT_4) and thyrotropin, the kidney function biomarker cystatin C, insulin-like growth factor 1 (IGF-1), total testosterone, estradiol (E_2), and the iron-status indicators hemoglobin and ferritin. Each PRI source was examined for information on the laboratory method used, population surveyed, statistical approaches used, covariates, and published reference intervals.

Analysis of Analyte-Specific PRI

The use of PRIs for a specific biomarker in clinical practice is complicated by the availability of multiple PRIs from different sources. There is no common data source for deriving PRIs that uses a well-defined population representative of healthy US children. Many PRIs are from populations of other countries. PRIs reported by clinical laboratories often do not include information about the population from which they were derived.

Biomarker concentrations for most PRIs in this study differ across sources, in part, owing to variably calibrated analytical systems and differences in analytical system performance characteristics.[16] However, these kinds of differences among analytical systems should not

affect the patterns of biomarker changes at different developmental stages, such as the spike in thyrotropin in the first days of life. We observed inconsistent patterns for biomarker changes across PRIs (Figure 1 and Figure 2), partially attributable to the number of age groups used to establish PRIs. Age groups should reflect developmental stages during which biomarker concentrations are relatively constant. The number of age groups are highly variable across PRIs and range between 1 and 12 for hemoglobin and ferritin, for example. The number of individuals per age group is also highly variable and sometimes does not meet the minimum of 120 individuals per stratum to obtain robust estimates, as recommended by Clinical and Laboratory Standards Institute guideline document EP-28 (eTable 1 in the Supplement).[4]

Other factors that contribute to variability across PRIs for certain biomarkers are diurnal variability and menstrual cycle, inclusion and exclusion criteria used, and statistical procedures. Most studies do not provide details about these factors. Discussions of how these factors influence PRIs are addressed in detail by other reviews and are outside the scope of this review.[9,17–21] This review investigated how well dynamic changes in biomarkers during child development are reflected in recently published PRIs and PRIs currently used in pediatric care.

Results

Biomarkers of Thyroid Function: Free Thyroxine and Thyrotropin

Normal thyroid gland function in children is critical for early neurocognitive development and is important for growth and development from birth through adolescence, making early identification and treatment of thyroid diseases crucial. Hyperthyroidism accounts for 15% of pediatric thyroid disorders. Hypothyroidism affects 1 in 2000 to 4000 newborns, and screening is part of all newborn screening programs in the United States.[22,23] Physiologic thyrotropin and FT_4 serum concentrations surge in the first few days of life and normalize with the maturation of the thyrotropin/FT_4 axis throughout the next few months.[24,25]

For thyroid function analytes, a large number of inconsistent PRIs were found. We identified 21 PRIs for FT_4 and 21 PRIs for thyrotropin (Table 1).[26–29,31–34] Four PRIs for FT_4 and 5 for thyrotropin were derived from the same prospective study cohort (CALIPER) using the same statistical approaches but different assays. Information about the study populations for other PRIs was sometimes unavailable. PRIs stratified by sex were provided for 38% (8 of 21 studies) and 48% (10 of 21 studies) of the FT_4 and thyrotropin studies, respectively. Only the CHILDx study[30] described additional screening criteria used to ensure inclusion of healthy children with normal thyroid function, such as values for thyroid peroxidase autoantibodies.

The total number of individuals and number of age groups differed across PRIs (eTable 1 in the Supplement). Many PRIs captured the neonatal surges in FT_4 and thyrotropin; however, others failed to do so. This is a result of the first age groups spanning anywhere from 0 to 3 days to 0 to 5 years for FT_4 and0 to3 days to0 to 11 years for thyrotropin (Figure 1 and eFigure in the Supplement). Nine published PRIs did not provide information about the number of individuals included. CALIPER studies reported instrument-specific PRIs for the

Cobas (Roche), Architect (Abbott), Vitros (Ortho), and DxL (Beckman) systems, illustrating how a lack of assay standardization can result in instrument-specific PRIs. Despite use of the same cohort and statistics, CALIPER PRIs still varied in the number of age groups, individuals per age group, and extent of changes between age groups (Table 1).

Of the 9 clinical laboratories that were surveyed (D.J.D.), 7 of 9 reported thyrotropin intervals indicative of the neonatal surge (eTable 2 in the Supplement). Only 3 laboratories use PRIs published in the literature, while 6 laboratories do not, suggesting that these laboratories did not adopt published PRIs and established PRIs internally. As observed with published PRIs, heterogeneity in patterns across clinical laboratory PRIs appear to stem from differences in age grouping, some of which inadequately reflect the dynamic changes that occur in childhood (eTable 2 in the Supplement). When comparing PRIs for thyrotropin and the corresponding FT_4 PRIs from the same laboratory, age grouping differed for 7 of 9 laboratories. Considering that FT_4 and thyrotropin are highly regulated through feedback loops, this observation suggests that FT_4 and thyrotropin PRIs used by some laboratories do not appropriately reflect the underlying physiologic pathway (eTable 2 in the Supplement).

Sex Hormone Biomarkers: E_2 and Testosterone

E_2 and testosterone are required for normal pubertal development and sexual maturation. The secondary sex characteristics that develop during puberty are characterized according to Tanner stage.[35] Clinically, E_2 and total testosterone concentrations are assessed in the evaluation of pituitary diseases, irregular menses, suspected polycystic ovary syndrome, hypogonadism, and other diseases related to sexual development.[36]

E_2 concentrations in female individuals are typically high at birth and decline rapidly throughout the first few weeks of life.[37] They decrease further in the first year and remain low until puberty, which typically occurs after age 8 years. Concentrations rise progressively throughout puberty and may demonstrate cyclic variation even before menarche. After menarche, E_2 concentrations vary with the menstrual cycle.[37]

Serum total testosterone concentrations are high in male individuals at birth, decline rapidly in the first week of life, and then increase again, peaking at about age 2 to 3 months, a phenomenon termed *the mini-puberty of infancy*.[38] By approximately age 6 months, total testosterone concentrations decline and remain low throughout the prepubertal years. In male individuals, puberty typically begins after age 9 years when total testosterone concentrations start gradually rising and reach adult levels by the later stages of puberty, typically in the midteenage years. In female individuals, total testosterone concentrations are low throughout infancy and childhood and increase modestly during puberty but remain much lower than in male individuals. Importantly, total testosterone concentrations exhibit diurnal patterns, being highest in the morning, and these diurnal variations are most pronounced in pubertal male individuals.[36]

For E_2 and total testosterone, we identified 15 PRIs and 12 PRIs, respectively (Table 2[39-48]). About half of these PRIs (6 for E_2 and 7 for total testosterone) were derived from the CALIPER study using the same statistical approaches. All studies reported sex-specific PRIs. Seven PRIs for E_2 and 4 for total testosterone provided data by Tanner stage. None

of the E_2 PRIs provided information about the time point in the menstrual cycle when the sample was collected, nor was time of day information provided to account for total testosterone diurnal variations.

The use of Tanner stages instead of age groups may not always be appropriate to characterize E_2 and total testosterone levels, especially in prepubertal children. For example, total testosterone concentrations for Tanner stage 1 are often slightly higher than those reported for male individuals who are about 5 years old because of inclusion of late prepubertal boys who may have early gonadarche or adrenarche. If a 5-year-old male individual presented in the clinic with early pubertal changes and total testosterone concentrations of 25 ng/dL (to convert to nanomoles per liter, multiply by 0.0347), which are high for his age and may indicate pathology, his total testosterone values would be flagged as high based on age-group PRIs but would fit into the normal range of a Tanner stage 1 group, causing the clinician to possibly miss the pathology.

PRIs for E_2 and total testosterone do not consistently reflect the dynamic changes that occur early in life. Similar to observations for thyrotropin and FT_4, the number of age groups for E_2 and total testosterone PRIs are highly variable (eTable 1 in the Supplement). For total testosterone, the first age group ranged from 0 to 10 days to 0 to 1.5 years for male and female individuals, while the first age group for E_2 ranged from 1 to 7 days to 0 to 19 years. The PRIs identified were generated with 13 different assays. Two PRIs for total testosterone are available for Cobas systems (Roche) using the CALIPER study, and each wasestablished using different age groups and numbers of individuals per age group (Table 2). Most commercial laboratories use mass spectrometry assays and have not adopted published PRIs. While mass spectrometry–based PRIs reflect low physiologic total testosterone concentrations expected in female individuals after birth, PRIs established by some clinical analyzers report female total testosterone concentrations approximately 8 times higher, suggesting that the use of accurate methodologies is central to establishing appropriate PRIs.

Growth Factor Biomarkers: IGF-1

The measurement of serum IGF-1 is often used in the diagnosis of disorders of growth hormone secretion, in the management of disorders that lead to nutritional insufficiency or catabolism, and for monitoring both growth hormone and IGF-1 replacement therapy.[49] IGF-1 is used to assess growth hormone secretion and activity because IGF-1 is a major downstream effector of growth hormone.[50,51] IGF-1 concentrations are highly dynamic from birth to adulthood, with large dynamic increases that occur with puberty before reaching a plateau around 15 years of age (Figure 2).[52]

We identified 8 PRIs for IGF-1 (eTable 3 in the Supplement). The PRI patterns differed considerably because of the number of age groups used to generate them (6–19; Figure 2 and eTable 1 in the Supplement). Three references in eTable 3 in the Supplement provided PRI data stratified by Tanner stage, and 6 PRIs provided data for each year of age. All PRIs were substratified by sex. Only 1 commercial laboratory used PRIs described in the literature.

Iron Status Indicators: Ferritin and Hemoglobin

Iron deficiency and iron deficiency anemia can have long-term effects on neurodevelopment; therefore, early diagnosis and treatment are critical.[53] The American Academy of Pediatrics recommends universal screening for anemia at age 1 year by determination of hemoglobin concentrations. If hemoglobin concentrations are less than 11.0 g/dL (to convert to grams per liter, multiply by 10) at age 1 year, then further evaluation for iron deficiency anemia is required using tests such as serum ferritin.[53] Serum ferritin concentrations decline in the first months and are lowest between age 15 and 24 months, after which they slightly increase.[54] Hemoglobin concentrations do not change considerably between 12 and 18 months of age and increase slightly with age thereafter.[55,56]

For hemoglobin and ferritin, 14 and 19 PRIs were identified (eTable 5 in the Supplement), respectively. The number of individuals and age groups per PRI are reported in supplemental eTable 1 in the Supplement. Eleven hemoglobin and 16 ferritin PRIs were stratified by sex. Two hemoglobin PRIs from CALIPER used the same age groups but different numbers of individuals per group, and 5 ferritin PRIs from CALIPER used different age groups and numbers of individuals per group. The hemoglobin PRIs reported by commercial laboratories do not seem to be derived from PRIs published in the literature. Only 1 commercial laboratory for each analyte used PRIs published in the literature. While PRI patterns for hemoglobin are somewhat consistent, with values increasing with age, the PRI patterns for ferritin are highly inconsistent and do not seem to reflect the normal biological changes described in the literature (Figure 2).

Kidney Function Biomarker: Cystatin C

Cystatin C is a biomarker increasingly used for assessing kidney function. It is formed at a constant rate, freely filtered by the glomerulus, and reabsorbed and catabolized in the proximal tubules. Serum concentrations are highly dependent on glomerular function and increased concentrations indicate glomerular damage, whereas increased urinary concentrations indicate possible tubular injury.[57] Cystatin C concentrations remain constant between 28 and 37 weeks of gestation and decline throughout the first 2 years of life in healthy children.[58] When used in estimation equations, cystatin C shows a tighter correlation with criterion standard glomerular filtration rate (GFR) measures than creatinine, causing numerous organizations to recommend cystatin C use in routine clinical practice.[59] In children, cystatin C–based estimated GFR equations better predict endstage kidney disease, cardiovascular morbidities, hospitalization, and death than creatinine-based equations because cystatin C blood concentrations are less dependent on muscle mass, nutritional status, sex, age, and developmental stage than creatinine, which is also influenced by mild kidney impairment.[59–61] In pediatric patients, the creatinine-based Bedside Schwartz equation has predominantly been replaced with the creatinine–cystatin C–based Chronic Kidney Disease in Children Study equation, which has less bias and higher accuracy compared with a criterion standard method.[62]

Despite the increased use of cystatin C in clinical settings, very few PRIs exist for this biomarker. Six PRIs were identified (eTable 4 in the Supplement) and the number of individuals and age groups per PRI varied (eTable 1 in the Supplement). One study

developed continuous PRIs, providing a comprehensive picture of cystatin C changes by age and sex during development.[63] One additional PRI reflected the dynamic concentration changes occurring in early life. Four of the 6 PRIs were stratified by sex. Ziegelasch et al.[64] reported significant differences between older pediatric male and female individuals and recommended using age- and sex-specific PRIs for cystatin C. Two commercial laboratories reported a single range for cystatin C and provided no information about the ages to which the reference interval applied. Additional studies are needed to establish the clinical utility of and PRIs for cystatin C outside of estimated GFR calculations.

Discussion

PRIs should accurately reflect the underlying biological and biochemical changes that occur over the course of healthy child development. We found many PRIs do not adequately reflect patterns of biomarker changes during child development. Some PRIs appear inappropriate for assessing a child's health or monitoring treatment efficacy. PRI patterns and the extent and timing of changes should be similar across studies, even if absolute values vary across instruments. For IGF-1 and hemoglobin, PRIs were reasonably similar; however, a large degree of dissimilarity was observed for thyrotropin, ferritin, E_2, total testosterone, and cystatin C. Inconsistencies were most notable in developmental stages during which biomarker concentrations change rapidly, as observed in the first year of life for thyroid function tests or during puberty for steroid hormones. These inconsistencies are, in part, attributable to high variability in the number of age groups used to represent certain developmental stages. Several reference intervals were reported from the CALIPER study and used the same study population and statistics but different analytical systems. Despite these commonalities, the number of age groups and PRI patterns are still different. Some age groups cover long periods and miss short periods of time with highly dynamic changes, such as those in the first year in life. One promising solution to overcome the challenges with age grouping is to transition to continuous reference intervals, as demonstrated in reference interval studies in children and adults and in an American Academy of Pediatrics–developed IGF-1 study.[65–68]

Biomarkers, such as thyrotropin and FT_4 or hemoglobin and ferritin, are often used in combination to diagnose certain diseases. Therefore, PRI patterns should be consistent not only for individual biomarkers but also for the corresponding biomarkers within a physiologic pathway that are used to make clinical decisions. This is especially true for the tightly controlled thyrotropin and FT_4 axis. Consistent relationships between thyrotropin and FT_4, as well as hemoglobin and ferritin, were not reflected in the PRIs reviewed, including PRIs used in clinical laboratories. These findings also indicate that many PRIs do not reflect the underlying biochemistry of development, especially in very young children. When possible, PRI studies should measure all analytes used in the diagnostic workup in the same individual to better define their relationship in healthy children.

It is notable that despite the large number of PRIs published in the literature, very few are used by clinical laboratories. Only 4 of the 91 PubMed-derived and reference book–derived PRIs are used by the 13 commercial and clinical laboratories included in this study. Despite the large number of PRIs, it remains difficult for laboratories to identify the PRI that best

reflects their patient population. This is complicated by the fact that some PRIs are based on data generated with analytical systems no longer available and that only limited information is available about the comparability of different PRIs and their transferability to different analytical systems.[69,70] Additional well-designed studies from the US pediatric population may aid in determining PRIs for the national pediatric patient population and assessing comparability of PRIs from different regions. At the same time, facilitating the adoption of these PRIs by clinical laboratories could be achieved by using some of the approaches used in laboratory standardization and harmonization efforts.[71] Continued communication and collaboration between clinicians and their laboratory colleagues ensures appropriate clinical test interpretation and patient assessment and remains essential to effective implementation of common PRIs.

Limitations

Published laboratory PRIs were obtained and used where possible; however, observations and assessments made about PRI implementation are, in part, anecdotal in nature because they are based on limited survey results. For this review, 9 clinical laboratories measuring pediatric patients samples were surveyed about PRIs used in practice; thus, points raised about how laboratories practice the adoption and application of PRIs may not be representative of all clinical laboratories.

Conclusions

Development of PRIs requires overcoming many technical and logistical challenges and is often hampered by the lack of availability of sufficiently large study populations that are representative of healthy children. Furthermore, the lack of assay standardization results in instrument-specific reference intervals that cannot easily be transferred across laboratories. As a result, researchers and laboratories continue to develop new PRIs in addition to the many PRIs already available. On the other hand, increasingly used biomarkers such as cystatin C still lack PRIs derived from appropriate and well-designed study cohorts. The US Centers for Disease Control and Prevention (CDC) has partnered with the American Association for Clinical Chemistry and other key stakeholders to improve pediatric patient care. The goals of this joint effort include (1) using data acquired from the National Health and Nutrition Examination Survey to generate continuous PRIs, (2) providing these PRIs to laboratories, clinicians, and researchers, and (3) facilitating their adoption by clinical laboratories through assay standardization. The National Health and Nutrition Examination Survey, conducted by CDC's National Center for Health Statistics, is a well-established, continuous survey that collects nationally representative health data and information from adults and children across the United States' population through physical examinations, extensive health questionnaires, and laboratory biomarker measurements.[72] The CDC, in collaboration with clinical partners, has developed reference intervals in adults that can easily be adopted by clinical laboratories and is pursuing similar approaches through this partnership.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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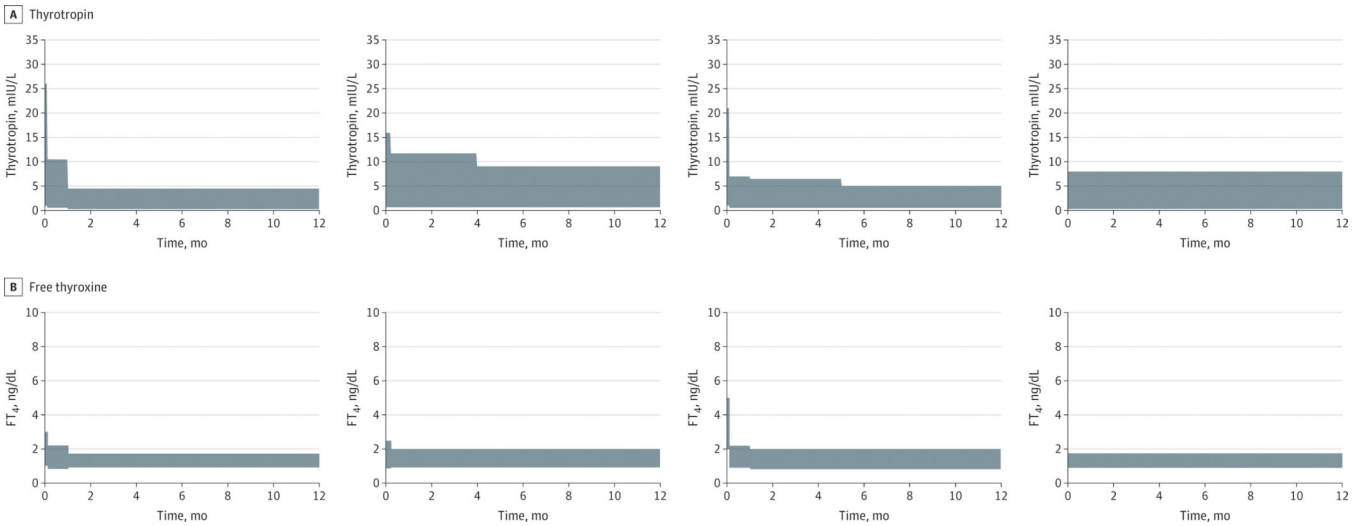

Figure 1. Thyrotropin and Free Thyroxine (FT4) Pediatric Reference Interval (PRI) Patterns

PRI patterns are heterogeneous and illustrate dynamic changes at different ages. A, Thyrotropin PRIs from birth to age 12 months from 4 PRI sources. B, FT_4 PRIs from birth to age 12 months corresponding with the same 4 PRI sources as thyrotropin panel A.

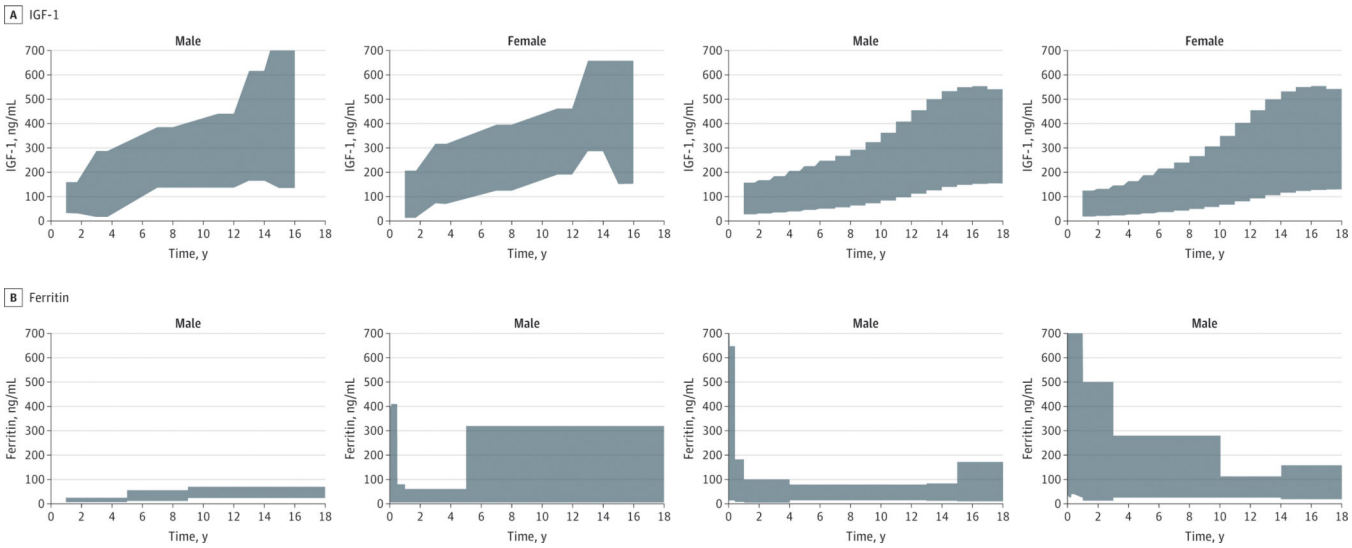

Figure 2. Insulin-like Growth Factor 1 (IGF-1) and Ferritin Pediatric Reference Interval (PRI) Patterns

PRI patterns are heterogeneous and illustrate dynamic changes at different ages. A, IGF-1 PRIs from birth to age 18 individuals from 4 PRI sources. years for males and female individuals from 2 PRI sources. B, Ferritin PRIs from birth to age 18 years for male

Table 1.

Pediatric Reference Intervals for Male and Female Individuals From the Literature and Major US Commercial Clinical Laboratories: FT_4 and Thyrotropin

Source	Age range	Sample size	Reference ranges	Analytical equipment
FT_4, ng/dL				
Higgins et al.,[26] 2018; CALIPER database[12]	0–<2 wk	60	0.97–5.63	ITROS 5600 (Ortho)
	2 wk-<5 y	136	0.89–2.27	
	5–19 y	434	0.76–1.33	
Soldin et al.,[15] 2011	0–3 d	N = 119	2.0–5.0	VITROS ECi (Ortho)
	3–30 d		0.9–2.2	
	31 d-18 y		0.8–2.0	
Karbasy et al.,[27] 2016; CALIPER database[12]	0-<20 d	40	1.35–4.48	DxI and Access2 (Beckman Coulter)
	20 d-<3 y	215	0.74–1.38	
	3–<19 y	455	0.61–1.06	
Bohn et al.,[1] 2016; CALIPER database[12]	0–<1 mo	46	1.24–3.89	Cobas 8000 e602 (Roche)
	1-<12 mo	79	1.09–1.71	
	1-<19 y	491	1.01–1.63	
Lim,[28] 2017	N = 1978			Cobas 6000 (Roche)
	10–12 y	Male: 298; female: 298	Male: 1.04–1.69; female: 0.98–1.64	
	13–15 y	Male: 386; female: 294	Male: 0.97–1.68; female: 0.95–1.53	
	16–18 y	Male: 360; female: 342	Male: 1.04–1.72; female: 0.95–1.62	
Gunapalasingham et al.,[29] 2019	N = 2407			Cobas 6000 (Roche)
	6.0–9.9 y	Male: 395; female: 471	Male: 0.99–1.18; female: 1.01–1.19	
	10.0–14.9 y	Male: 416; female: 605	Male: 0.95–1.17; female: 0.93–1.14	
	15.0–18.9 y	Male: 165; female: 359	Male: 0.94–1.20; female: 0.92–1.15	
Wong et al.,[2] 2020	0-≤5 y	Male: 208; female: 208	Male: 0.85–2.82; female: 0.85–2.82	Cobas 6000 (Roche)

Source	Age range	Sample size	Reference ranges	Analytical equipment
	5-≤15 y	Male: 238; female: 238	Male: 0.81–2.10; female: 0.81–2.15	
	15-≤20 y	Male: 28; female: 36	Male: 0.77–2.82; female: 0.78–1.46	
Wong et al.,[2] 2020; CALIPER database[12]	5-<15 d	66	1.05–3.21	Architect i2000 (Abbott)
	15-<30 d	55	0.68–2.53	
	30 d-<1 y	270	0.89–1.7	
	1-<19 y	952	0.89–1.37	
Soldin et al.,[15] 2011	1–3 d	Male: 24; female: 38	Male: 0.80–2.78; female: 0.88–1.93	IMx Analyzer (Abbott)
	4–30 d	Male: 73; female: 62	Male: 0.48–2.32; female: 0.61–1.93	
	1–12 mo	Male: 52; female: 54	Male: 0.76–2.0; female: 0.88–1.84	
	1–5 y	Male: 100; female: 117	Male: 0.90–1.59; female: 1.02–1.72	
	6–10 y	Male: 104; female: 101	Male: 0.81–1.68; female: 0.82–1.58	
	11–15 y	Male: 101; female: 100	Male: 0.92–1.57; female: 0.79–1.49	
	16–18 y	Male: 110; female: 101	Male: 0.92–1.53; female: 0.83–1.44	
Soldin et al.,[15] 2011	N = 6023			Architect ci8200 (Abbott)
	<2 mo	Male: 189; female: 160	Male: 0.78–1.83; female: 0.69–1.85	
	2-<12 mo	Male: 125; female: 138	Male: 0.71–1.56; female: 0.69–1.49	
	12–24 mo	Male: 190; female: 202	Male: 0.82–1.32; female: 0.73–1.41	
	2-<5 y	Male: 286; female: 253	Male: 0.80–1.32; female: 0.79–1.38	
	5-<10y	Male: 516; female: 655	Male: 0.78–1.29; female: 0.77–1.32	

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Source	Age range	Sample size	Reference ranges	Analytical equipment
	10-<15y	Male: 722; female: 1003	Male: 0.69–1.23; female: 0.66–1.22	
	15-<20y	Male: 507; female: 802	Male: 0.67–1.22; female: 0.67–1.22	
Soldin et al.,[15] 2011	N = 1459			Architect ci8200 (Abbott)
	0–12 mo	Male: 36; female: 43	Male: 0.92–1.83; female: 0.85–1.59	
	1–5 y	Male: 101; female: 93	Male: 0.86–1.62; female: 0.91–1.44	
	6–10 y	139	0.84–1.47	
	11–14y	161	0.78–1.31	
	15–20 y	163	0.79–1.34	
La'ulu et al.,[30] 2016	6 mo-6 y	840	1.40–2.70	Equilibrium dialysis-liquid chromatography-tandem mass spectrometry
	7–17 y	1373	1.10–2.00	
Soldin et al.,[15] 2011	1–3d	Male: 24; female: 38	Male: 1.16–2.95; female: 1.09–2.09	Immuno I (Bayer)
	4–30 d	Male: 73; female: 62	Male: 0.78–2.25; female: 0.85–2.09	
	1–12 mo	Male: 52; female: 54	Male: 1.00–2.17; female: 1.09–2.02	
	12 mo-5 y	Male: 100; female: 117	Male: 1.16–1.71; female: 1.24–1.86	
	6–10 y	Male: 104; female: 101	Male: 1.09–1.86; female: 1.0–1.71	
	11–15 y	Male: 101; female: 100	Male: 1.16–1.71; female: 1.0–1.63	
	16–18 y	Male: 110; female: 101	Male: 1.16–1.71; female: 1.09–1.63	

Source	Age range	Sample size	Reference ranges	Analytical equipment
Soldin et al.,[15] 2011	1 mo-18 y	1000	0.8–2.1	Isotope dilution tandem mass spectrometry in negative mode with ultracentrifugation
	1–3 d	ND	Male: 0.97–1.87; female: 0.93–1.44	Dimension RxLAnalyzer (Siemens)
	4–30 d		Male: 0.78–1.52; female: 0.81–1.44	
	1–12 mo		Male: 0.89–1.48; female: 0.93–1.40	
	1–5 y		Male: 0.97–1.25; female: 1.01–1.32	
	6–10 y		Male: 0.93–1.32; female: 0.93–1.25	
	11–15 y		Male: 0.97–1.25; female: 0.89–1.20	
	16–18 y		Male: 0.97–1.25; female: 0.93–1.25	
Wong et al.,[2] 2020	1–30 d	47	2.00–2.13	ADVIA Centaur (Siemens)
	31–60 d	58	0.96–1.69	
	61 d-1 y	317	0.84–1.52	
	1–5 y	2722	0.91–1.48	
	6–10 y	3452	0.88–1.45	
	11–14 y	3429	0.82–1.39	
	15–18 y	1019	0.81–1.40	
Soghier et al.,[13] 2019	5-<15 d	ND	1.05–3.21	DELFIA immunofluorometric system (PerkinElmer)
	15-<30 d		0.68–2.53	
	30 d-<1 y		0.89–1.70	
	1–19 y		0.89–1.37	
ARUP Laboratories[31]	25–30 Weeks' gestation	ND	0.5–3.3	Quantitative equilibrium dialysis/HPLC-MS/MS
	31–36 Weeks' gestation		1.3–4.7	

Source	Age range	Sample size	Reference ranges	Analytical equipment
	Birth-1 wk		2.2–5.3	
	2–3 wk		0.9–4.0	
	1–5 mo		1.1–2.2	
	6 mo-6 y		1.4–2.7	
	7–17 y		1.1–2.0	
	≥18 y		1.1–2.4	
LabCorp[32]	0–3 d	Varies	0.66–2.71	Electrochemiluminescence immunoassay
	4–30 d		0.83–3.09	
	31 d-12 mo		0.48–2.34	
	13 mo-5 y		0.85–1.75	
	6–10 y		0.90–1.67	
	11–19 y		0.93–1.60	
	>19 y		0.82–1.77	
Mayo Clinic Laboratories[33]	0–5 d	>120 Per age range per sex	0.9–2.5	Electrochemiluminescence Immunoassay
	6 d-2 mo		0.9–2.2	
	3–11 mo		0.9–2.0	
	1–5 y		1.0–1.8	
	6–10 y		1.0–1.7	
	11–19 y		1.0–1.6	
Quest Diagnostics[34]	<1 mo	ND	Not established	Immunoassay
	1–23 mo		0.9–1.4	
	2–12 y		0.9–1.4	
	13–20 y		0.8–1.4	
	>20 y		0.8–1.8	
Thyrotropin, mIU/L				
Bohn et al.,[1] 2019; CALIPER database[12]	0-<1 mo	50	1.23–27.2	Cobas 8000 e602 (Roche)
	1-<12 mo	86	1.03–6.80	
	1-<15 y	339	1.12–5.01	
	15-<19 y	148	0.68–4.09	

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Source	Age range	Sample size	Reference ranges	Analytical equipment
Wong et al.,[2] 2020; CALIPER database[12]	N = 600			Cobas 6000 (Roche)
	0-≤5 y	Male: 189; female: 189	Male: 0.84–6.22; female: 0.84–6.22	
	5-≤10 y	Male: 47; female: 61	Male: 1.18–5.33; female: 0.48–4.81	
	10-≤15 y	Male: 118; female: 118	Male: 0.76–4.20; female: 0.76–4.20	
	15-≤20 y	Male: 26; female: 35	Male: 0.64–5.37; female: 0.38–2.82	
Gunapalasingham et al.,[29] 2019	N = 2409			Cobas 6000 (Roche)
	6.0–9.9 y	Male: 395; female: 471	Male: 0.18–0.30; female: 0.16–0.30	
	10.0–14.9 y	Male: 416; female: 605	Male: 0.17–0.30; female: 0.17–0.31	
	15.0–18.9 y	Male: 165; female: 359	Male: 0.17–0.29; female: 0.16–0.30	
Lim,[28] 2017	N = 1044			Cobas 8000 e602 (Roche)
	10–12 y	Male: 298; female: 298	Male: 1.09–8.50; female: 0.61–7.84	
	13–15 y	Male: 386; female: 294	Male: 0.49–5.90; female: 0.63–7.39	
	16–18 y	Male: 360; female: 342	Male: 0.63–6.31; female: 0.55–6.49	
Higgins et al.,[26] 2018; CALIPER database[12]	0-<1 wk	51	0.71–31.5	VITROS 5600 (Ortho)
	1 wk-<19 y	600	0.86–5.7	
Soldin et al.,[15] 2011	0–3 d	119	1.0–20.0	VITROS ECi, chemilluminescent immunoassay (Ortho)
	3–30 d		0.5–6.5	
	1–5 mo		0.5–6.0	
	6 mo-18 y		0.5–4.5	
Karbasy et al.,[27] 2016; CALIPER database[12]	0-<12 y	423	0.79–5.85	DxI and Access2 (Beckman Coulter)
	12-<19 y	182	0.68–3.35	
Wong et al.,[2] 2020; CALIPER database[12]	4 d-<6 mo	139	0.73–4.77	Architect i2000 (Abbott)
	6 mo-<14 y	640	0.7–4.17	
	14-<19 y	259	0.47–3.41	

Source	Age range	Sample size	Reference ranges	Analytical equipment
Soldin et al.,[15] 2011	N = 1459			Architect ci8200 (Abbott)
	0–12 mo	Male: 71	Male: 0.9–5.4; female: 0.9–5.4	
	1–5 y	Male: 152; female: 155	Male: 0.7–4.5; female: 0.7–4.8	
	11–14 y	Male: 93; female: 201	Male: 0.6–3.6; female: 0.5–4.1	
Soldin et al.,[15] 2011	N = 6023			Architect ci8200 (Abbott)
	<2 mo	Male: 212; female: 171	Male: 1.1–6.3; female: 1.1–5.5	
	2-<12 mo	Male: 138; female: 152	Male: 1.0–4.9; female: 1.1–4.5	
	12–24 mo	Male: 197; female: 203	Male: 0.9–4.8; female: 1.0–4.4	
	2-<5 y	Male: 297; female: 264	Male: 0.8–4.4; female: 0.9–4.1	
	5-<10 y	Male: 537; female: 697	Male: 0.8–4.1; female: 0.9–4.1	
	10-<15 y	Male: 746; female: 1063	Male: 0.8–4.0; female: 0.7–3.7	
	15-<20 y	Male: 547; female: 877	Male: 0.6–3.6; female: 0.5–3.6	
Soldin et al.,[15] 2011	1–30 d	Male: 63; female: 89	Male: 0.5–16.0; female: 0.7–13.1	IMx analyzer (Abbott)
	1 mo-5 y	Male: 95; female: 95	Male: 0.6–7.1; female: 0.5–8.1	
	6–18 y	Male: 95; female: 96	Male: 0.4–6.0; female: 0.4–5.8	
Soghier et al.,[13] 2019	4 d-<1 y	ND	0.73–4.77	House RIA; TSH immunoradiometric assay (Dynotest)
	6 mo-<14 y		0.7–4.17	
	14-<19 y		0.47–3.41	
Soldin et al.,[15] 2011	0–1 mo	Male: 84; female: 62	Male: <6.5; female: <6.0	Immunofluorescent TSH Kit (Delphia)
	1–12 mo	Male: 114; female: 103	Male: <4.1; female: <4.0	
	1–3 y	Male: 128; female: 126	Male: <3.0; female: <3.3	
	4–6 y	Male: 109; female: 82	Male: <3.0; female: <2.8	

Source	Age range	Sample size	Reference ranges	Analytical equipment
	7–12 y	Male: 112; female: 107	Male: <3.1; female: <2.9	
	13–18 y	Male: 106; female: 106	Male: <3.1; female: <3.0	
Soldin et al.,[15] 2011	1–30 d	Male: 63; female: 89	Male: 0.7–16.1; female: 1.0–13.7	Immuno I
	1 mo-5 y	Male: 95; female: 95	Male: 0.8–7.5; female: 0.7–8.6	
	6–18 y	Male: 95; female: 96	Male: 0.6–6.4; female: 0.6–6.2	
Soldin et al.,[15] 2011	N = 1459			ACS 180 using Chiron Diagnostic TSH-3 kit
	0-<2 y	36	0.7–4.5	
	2-<7 y	149	0.4–3.2	
	7-<13 y	128	0.3–2.7	
	13-<18 y	123	0.4–1.9	
Soldin et al.,[15] 2011	1–30 d	ND	Male: 0.6–12.8; female: 0.8–10.8	Dimension RxL Analyzer (Siemens)
	1 mo-5 y		Male: 0.7–6.0; female: 0.6–6.8	
	6–18 y		Male: 0.45–5.1; female: 0.5–4.9	
Wong et al.,[2] 2020	1–30 d	48	1.08–11.8[a]	ADVIA Centaur (Siemens)
	31–60 d	56	0.68–12.56[a]	
	61 d-1 y	321	0.62–7.3[a]	
	1–5 y	2782	0.75–6.57[a]	
	6–10 y	3531	0.79–6.0[a]	
	11–14 y	3487	0.72–5.77[a]	
	15–18 y	1086	0.63–6.28[a]	
ARUP Laboratories[31]	Cord blood	ND	2.000–40.000	Quantitative Electro-chemiluminescent Immunoassay, 3rd Generation
	0–3 d		5.170–14.600	
	4–30 d		0.430–16.100	
	1–24 mo		0.620–8.050	

Source	Age range	Sample size	Reference ranges	Analytical equipment
	2–6 y		0.540–4.530	
	7–11 y		0.660–4.140	
	12–19 y		0.530–3.590	
LabCorp[32]	0–6 d	ND	0.0000007–0.0000152	Electrochemiluminescence immunoassay
	7 d-3 mo		0.00000072–0.000011	
	>3–12 mo		0.00000073–0.00000835	
	1–5 y		0.0000007–0.00000597	
	6–10 y		0.0000006–0.00000484	
	>10 y		0.000000450–0.0000045	
Mayo Clinic Laboratories[33]	0–5 d	>120 Per age range per sex	0.7–15.2	E immunoassay TSH method (Cobas)
	6 d-2 mo		0.7–11.0	
	3–11 mo		0.7–8.4	
	1–5 y		0.7–6.0	
	6–10 y		0.6–4.8	
	11–19 y		0.5–4.3	
Quest Diagnostics[34]	Premature infants	ND	0.20–27.90	Ultrasensitive 3rd generation
	1–2 d		3.20–34.60	
	3–4 d		0.70–15.40	
	1–11 mo		0.80–8.20	
	1–19 y		0.50–4.30	

Abbreviations: FT_4, free thyroxine; HPLC-MS/MS, high-performance liquid chromatography-tandem mass spectrometry; ND, not disclosed; RIA, radioimmunoassays; TSH, thyroid-stimulating hormone.

SI conversion factors: To convert FT_4 to picomoles per liter, multiply by 12.871; thyrotropin to milli-international units per liter, multiply by 1.

[a]Values are reported as milligrams per liter.

Table 2.

Pediatric Reference Intervals for Male and Female Individuals From the Literature and Major US Commercial Clinical Laboratories: E_2 and Total Testosterone

Source	Age range and Tanner stage	Sample size	Reference ranges	Analytical equipment
E_2pg/ml				
Wong et al.,[2] 2020; CALIPER database[12]	Male: 15 d-<1 y; female: 15 d-<1 y	Male: 197;female: 197	<25	Architect Í2000SR (Abbott)
	Male: 1-<11 y; female: 1-<9 [y]	Male: 331; female: 261	Male: <13; female: <10	
	Male: 11-<13 y; female: 9-<11 y	Male: 81; female: 81	Male: <26; female: <48	
	Male: 13-<15 y; female: 11-<12 y	Male: 80; female: 87	Male: <28; female: <94	
	Male: 15-<19 y; female: 12-<14 y	Male: 37; female: 83	Male: <38; female: 11–172	
	Female: 14-<19 y	Female: 175	Female: <255	
	T1	Male: 27; female: 22	Male: <19; female: <20	
	T2	Male: 38; female: 23	Male: <18; female: <26	
	T3	Male, 23; female: 26	Male: <21; female: <86	
	T4	Male: 47; female: 57	Male: <35; female: 13–141	
	T5	Male: 16; female: 30	Male: 17–34; female: 19–208	
Higgins et al.,[26] 2018; CALIPER database[12]	0-<1 mo	Male: 68; female: 68	44.2–376	VITROS 5600 (Ortho)
	1 mo-<6 y	Male: 196; female: 196	<6.36–48.2	
	6-<11y	Male: 144; female: 144	<6.36–18.5	
	11-<15 y	Male: 105; female: 86	Male: 6.92–39.6; female: 18.6–93.6	
	15-<19 y	Male: 88; female: 85	Male: 13.2–39.2; female: 12.2–181	
Bogner 2019[39]	14–17y	Male: 63; female: 39	Male: 6.929–38.507; female: 6.780–106.897	VITROS ECiQ (Ortho)
Karbasy et al.,[27] 2016; CALIPER database[12]	0-<1 y	Male: 174; female: 174	<20–52.51	DxI (for age >1 y) and Access2 (for age <1 y) (Beckman Coulter)
	1-<12 y	Male: 321; female: 321	<20-<20	
	12-<19 y	Male: 95; female: 96	Male: <20–42.83; female: <20–198.2	
Bohn et al.,[1] 2019; CALIPER database[12]	0-<1 mo	Male: 50; female: 50	2.54–96	Cobas 8000 e602 (Roche)

Source	Age range and Tanner stage	Sample size	Reference ranges	Analytical equipment
	1 mo-<10 y	Male: 267; female: 267	<4.90	
	Male: 10-<19 y; female: 10-<14 y	Male: 157; female: 76	Male: <4.90–36.5; female: <4.90–68.1	
	Female: 14-<19 y	Female: 86	Female: 14.6–248	
Bae et al.,[40] 2019	<1 y	Male: 124; female: 156	Male: <20.484; female: <22.064	LC-MS/MS
	1–5 y	Male: 206; female: 47	Male: <19.449; female: <17.243	
	5–10 y	Male: 649; female: 557	Male: <10.078; female: <11.849	
	10–15 y	Male: 731; female: 629	Male: <28.330; female: <228.084	
	T1	Male: 266; female: 224	<10.078	
	T2	Male: 75; female: 99	Male: <11.005; female: <83.410	
	T3	Male: 63; female: 74	Male: <32.743; female: <227.894	
	T4	Male: 73; female: 68	Male: <35.412; female: <271.697	
	T5	Male: 114; female: 90	Male: <52.764; female: <269.027	
Wong et al.,[2] 2020	7-<10 y	Male: 94; female: 65	Male: <6; female: <35	LC-MS/MS
	10-<13 y	Male: 95; female: 120	Male: <10; female: <87	
	13-<16 y	Male: 81; female: 127	Male: 1–36; female: 9–248	
	16-<18 y	Male: 49; female: 129	Male: 3–34; female:	
			2–266	
	T1	Male: 134; female: 158	Male: <8; female: <55	
	T2	Male: 60; female: 75	Male: <9; female: 2–133	
	T3	Male: 53; female: 100	Male: 1–35; female: 12–277	
	T4, T5	Male: 74; female: 108	Male: 3–35; female: 2–259	
Ankarberg-Lindren et al.,[41] 2018	ND	Male (TV, mL): 1–2: 9; female (TBS): 1: 15	Male: <0.54–3.81; female: <0.54–1.91	GC-MS/MS
		Male (TV, mL): 3–6: 9; female (TBS): 2: 15	Male: <0.54–5.99; female: 1.63–12.26	
		Male (TV, mL): 8–12: 14; female (TBS): 3: 13	Male: 0.82–19.34; female: 10.08–160.45	
		Male (TV, mL): 15–25: 9; female (TBS): 4–5: 16	Male: 6.81–25.88; female: 24.24–211.93	
Soldin et al.,[15] 2011	Male: 0-<19 y; female: 0-<6 y	Male: 132; female: 50	Male: <20–40; female: <20–53	DPC Immulite 1000 (Siemens)
	Female: 6-<11 y	Female: 103	Female: <20–59	

Source	Age range and Tanner stage	Sample size	Reference ranges	Analytical equipment
	Female: 11-<15 y	Female: 61	Female: <20–87	
	Female: 15-<19 y	Female: 55	Female: <20–111	
Wu,[14] 2006	30–60d	ND	Male: 10–32; female: 5–50	RIA
	6 mo-10 y		<15	
	T1		Male: 3–15; female: 5–10	
	T2		Male: 3–10; female: 5–115	
	T3		Male: 5–15; female: 5–180	
	T4		Male: 3–40; female: 25–345	
	T5		Male: 15–45; female: 25–410	
Mayo Clinic[42]	T1 (>14 d-prepubertal)	ND	Male: LOD-13; female: L0D-20	LC-MS/MS
	T2		Male: LOD-16; female: LOD-24	
	T3		Male: LOD-26; female: L0D-60	
	T4		Male: LOD-38; female: 15–85	
	T5		Male: 10–40; female: 15–350	
ARUP Laboratories[31]	7–9 y	ND	Male: <7.0; female: <36.0	LC-MS/MS
	10–12y		Male: <11.0; female: 1.0–87.0	
	13–15 y		Male: 1.0–36.0; female: 9.0–249.0	
	16–17 y		Male: 3.0–34.0; female: 2.0–266.0	
	T1 (>14 d-prepubertal)		Male: <8.0; female: <56.0	
	T2		Male: <10.0; female: 2.0–133.0	
	T3		Male: 1.0–35.0; female: 12.0–277.0	
	T4, T5		Male: 3.0–35.0; female: T5: 2.0–259	
LabCorp[43]	Male: 0.5–10 y; female: 1–9 y	ND	<15.0	LC-MS/MS
	T1: male: <9.8 y; female: <9.2y		T1: male: 5.0–11.0; female: 5.0–20.0	
	T2: male: 9.8–14.5 y; female: 9.2–13.7 y		T2: male: 5.0–16.0; female: 10.0–24.0	
	T3: male: 10.7–15.4y; female: 10.0–14.4 y		T3: male:5.0–25.0; female: 7.0–60.0	
	T4: male: 11.8–16.2 y; female: 10.7–15.6 y		T4: male:10.0–36.0; female: 21.0–85.0	

Source	Age range and Tanner stage	Sample size	Reference ranges	Analytical equipment
	T5: male: 12.8–17.3 y; female: 11.8–18.6 y		T5: male:10.0–36.0; female: 34.0–170.0	
LabCorp[44]	1–10 y	ND	Male: 0–20.0; female: 6.0–27.0	Electrochemiluminescence immunoassay analyzer method (Roche)
Quest Diagnostics[45]	1–9 y		Male: <4; female: <16	LC-MS/MS
	10–11y		Male: <12; female: <65	
	12–14 y		Male: <24; female: <142	
	15–17 y		Male: <31; female: <283	
Total testosterone, ng/dL				
Wong et al.,[2] 2020; CALIPER database[12]	Male: 4 d-<6 mo; female: 4 d-<9 y	Male: 72; female: 125	Male: 8.6–299; female: 1.15–62.0	Architect Í2000SR (Abbott)
	Male: 6 mo-<9 y; female: 9-<13 y	Male: 61; female: 102	Male: <35.7; female: <28.2	
	Male: 9-<11 y; female: 13-<15 y	Male: 45; female: 79	Male: <23.3; female: 10.4–44.4	
	Male: 11-<14y; female: 15-<19 y	Male: 89; female: 110	Male: <444; female: 14.1–45.0	
	Male: 14-<16y	Male: 78	Male: 36.0–632	
	Male: 16-<19y	Male: 94	Male: 148–794	
	T1	Male: 20; female: 21	Male: <17.9; female: <19.3	
	T2	Male: 32; female: 21	Male: <24.5; female: <19.9	
	T3	Male: 22; female: 26	Male: <543; female: <41.8	
	T4	Male: 52; female: 62	Male: 8.65–636; female: 8.93–41.5	
	T5	Male: 17; female: 34	Male: 99.7–760; female: 3.75–49.6	
Bohnetal,[1] 2019; CALIPER database[12]	0-<6 mo	Male: 47; female: 49	Male: 5.8–548; female: <2.9–346	Cobas 8000 e602 (Roche)
	6 mo-<11 y	Male: 244; female: 244	<2.9	
	Male: 11-<15 y; female: 11-<19 y	Male: 76; female: 150	Male: <2.9–563; female: <2.9–52	
	Male: 15-<19y	Male: 75	Male: 49–769	
Soldinet al.,[15] 2011; CALIPER database[12]	0-<1 y	Male: 18; female: 29	Male: 2–10; female: 2–20	Cobas 6000 (Roche)
	>1-<5 y	Male: 97; female: 97	2.0–11.4	
	>5-<10 y	Male: 96; female: 96	2.0–22.2	

Source	Age range and Tanner stage	Sample size	Reference ranges	Analytical equipment
	Male: >10-<15 y; female: >10-<20 y	Male: 49; female: 80	Male: 2–830; female: 2–78	
	Male: >15-<20 y	Male: 25	Male: 103–1011	
Higgins et al.,[26] 2018; CALIPER database[12]	0-<6 mo	Male: 68; female: 55	Male: 8.05–642; female: 5.54–401	VITROS 5600 (Ortho)
	6 mo-<12 y	Male: 388; female: 388	Male: <4.90–46.1; female: <4.90–46.1	
	Male: 12-<15 y; female: 12–19 y	Male: 86; female: 153	Male: 10.4–717; female: 15.3–63.7	
	Male: 15-<19y	Male: 83	Male: 117–782	
Bogner 2019[39]	14–17y	Male: 63; female: 39	Male: 23.63–857.06; female: 12.68–80.98	VITROS EciQ (Ortho)
Karbasy et al.,[27] 2016; CALIPER database[12]	0-<1.5 y	Male: 46; female: 48	Male: 0–284; female: 0–63.1	DxI and Access2 (Beckman Coulter)
	1.5-<7 y	Male: 169; female: 169	0–10.1	
	7–9 y	Male: 58; female: 58	0–17.9	
	9-<12 y	Male: 79; female: 79	0–47	
	Male: 12–19 y; female: 12-<15 y	Male: 94; female: 43	Male: 11–566; female: 10.1–65.1	
	Female: 15-<19 y	Female: 59	Female: 17.9–85.9	
Wong et al.,[2] 2020	0-<15 d	Male: 18; female: 13	Male: 7.2–183; female: 1.7–13.2	LC-MS/MS
	15 d-<1 y	Male: 34; female: 28	Male: 1.4–257; female: 0.6–6.3	
	1-<13 y	Male: 138; female: 138	Male: 1.7–32.3; female: 1.7–32.3	
	13-<16 y	Male: 18; female: 17	Male: 28–453; female: 14.4–39.2	
	16-<19 y	Male: 30; female: 31	Male: 27.7–674; female: 3.2–66.6	
Soldin et al.,[15] 2011	Male: 0–6 y; female: 0–5 y	Male: 158; female: 127	Male: 4–31; female: 2–10	LC-MS/MS
	Male: 7–9 y; female: 6–9 y	Male: 148; female: 171	Male: 4–25; female: 5–13	
	Male: 10–12 y; female: 10–14y	Male: 188; female: 190	Male: 5–418; female: 14–50	
	Male: 13–14y; female: 15–16 y	Male: 245; female: 175	Male: 6–647; female: 12–53	
	Male: 15–16 y; female: 17–18 y	Male: 209; female: 123	Male: 42–880; female: 16–50	
	Male: 17–19 y	Male: 123	Male: 121–823	
Mayo Clinic[46]	0–5 mo	ND	Male: 75–400; female: 20–80	LC-MS/MS

Source	Age range and Tanner stage	Sample size	Reference ranges	Analytical equipment
	6 mo-9 y		Male: <7–20; female: <7–20	
	10–11y		Male: <7–130; female: <7–44	
	Male: 12–13 y; female: 12–16 y		Male: <7–800; female: <7–75	
	Male: 14 y		Male: <7–1200	
	Male: 15–16 y		Male: 100–1200	
	17–18 y		Male: 300–1200; female: 20–75	
	T1		Male: <7–20; female: <7–20	
	T2		Male: 8–66; female: <7–47	
	T3		Male: 26–800; female: 17–75	
	T4		Male: 85–1200; female: 20–75	
	T5		Male: 300–950; female: 12–60	
LabCorp[47]	Newborn	ND	Male: 75.0–400.0; female: 20.0–64.0	LC-MS/MS
	1–7 d		Male: 20.0–50.0; female:<10.0	
	20–60d		Male: 60.0–400.0	
	61 d-7 mo		Male: <2.5–10.0	
	Male: 1–10 y; female: 1–9 y		<2.5–10.0	
	>18 y		Male: 264.0–916.0; female: 10.0–55.0	
	T1		Male: <2.5–10.0; female: <2.5–10.0	
	T2		Male: 18.0–150.0; female: 7.0–28.0	
	T3		Male: 100.0–320.0; female: 15.0–35.0	
	T4		Male: 200.0–620.0; female: 13.0–32.0	
	T5		Male: 350.0–970.0; female: 20.0–38.0	
ARUP Laboratories[31]	Newborn	ND	Male: 75–400; female: 20–64	LC-MS/MS
	1–5 mo		Male: 14–363; female: <20	
	6–24 mo		Male: <37; female: <9	
	2–3 y		Male: <15; female: <20	

Source	Age range and Tanner stage	Sample size	Reference ranges	Analytical equipment
	4–5 y		Male: <19; female: <30	
	6–7 y		Male: <13; female: <7	
	8–9 y		Male: 2–8; female: 1–11	
	10–11y		Male: 2–165; female: 3–32	
	12–13 y		Male: 3–619; female: 6–50	
	14–15y		Male: 31–733; female: 6–52	
	16–17 y		Male: 158–826; female: 9–58	
	T1		Male: 2–15; female: 2–17	
	T2		Male: 3–303; female: 5–40	
	T3		Male: 10–851; female: 10–63	
	T4, T5		Male: 162–847; female: 11–62	
Quest Diagnostics[48]	1–10 d	ND	Male: <187; female: <24	Extraction RIA
	11–30 d		Not established	
	1–2 mo		Male: 72–344; female:<17	
	3–4 mo		Male: <201; female: <12	
	5–6 mo		Male: <59; female: <13	
	7–11 mo		Male: <16; female: <11	
	1–5 y		Male: <5; female: <8	
	2–7 y		Male: <25; female: <20	
	8–10 y		Male: <42; female: <35	
	11 y		Male: <260; female: <40	
	12–13 y		Male: <420; female: <40	
	14–17.9 y		Male: <1000; female: <40	

Abbreviations: E_2, estradiol; GC-MS/MS; gas chromatography–tandem mass spectrometry; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LOD, limit of detection; ND, not disclosed; RIA, radioimmunoassays; T, Tanner stage; TBS, Tanner breast stage; TV, testicular volume.

SI conversion factors: To convert E_2 to picomoles per liter, multiply by 3.671; testosterone to nanomoles per liter, multiply by 0.0347.

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