

Pediatric reference intervals for testosterone, dihydrotestosterone, and T/DHT ratio by MS: need for harmonization

Critically Appraised Topic

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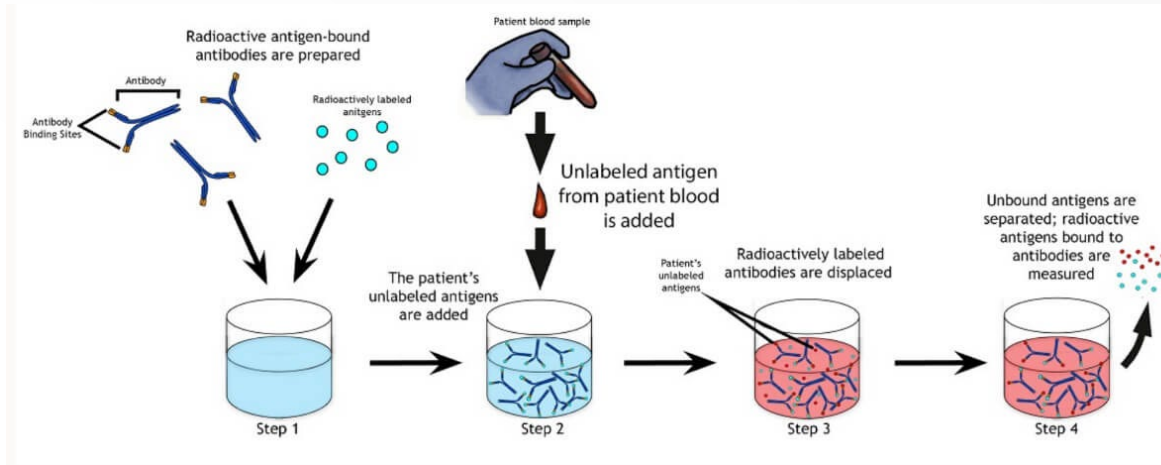
Introduction

T/DHT analysis – Pediatric Reference Intervals

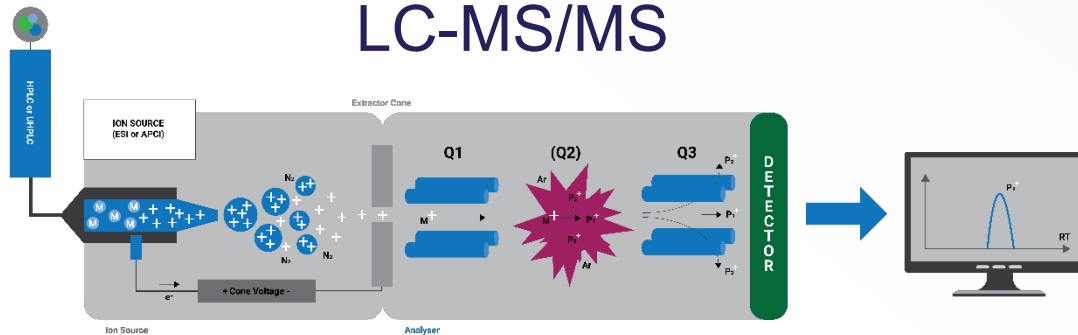


Analysis of Testosterone and Dihydrotestosterone

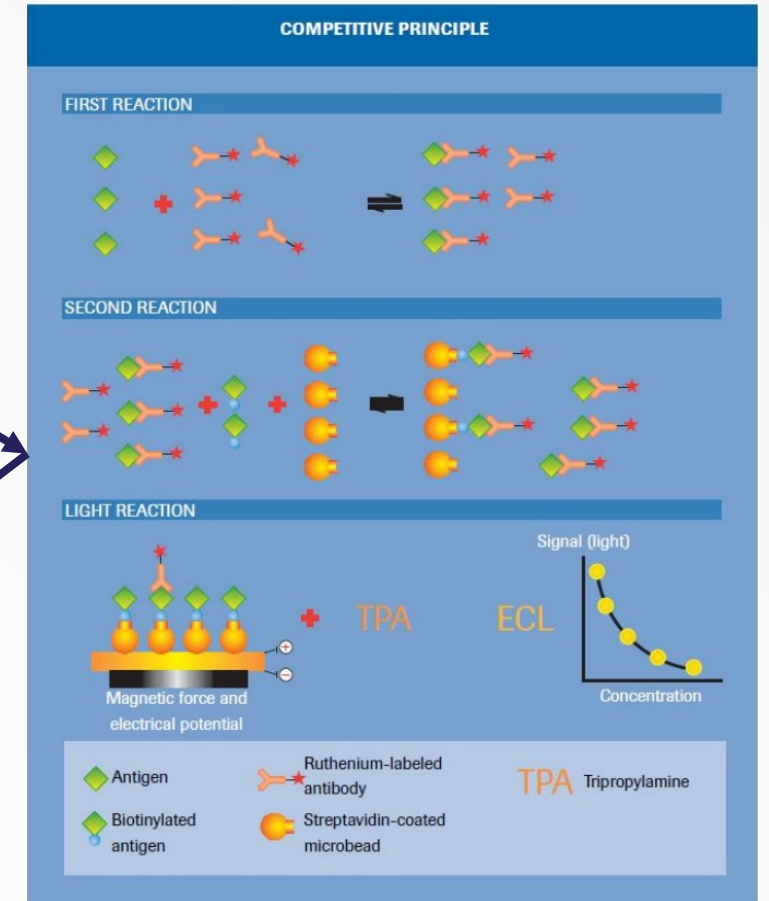
RIA



LC-MS/MS



ECLIA



Analysis of Testosterone and Dihydrotestosterone

RIA

- + Good analytical performance
 - Time-consuming
 - Radio-activity

ECLIA

- + Practical
- + High sample throughput
 - Insufficient specificity/sensitivity in female/pediatric population
- Interassay differences

LC-MS/MS

- + Superior sensitivity/accuracy/precision
- + Potential for standardization (traceability to international standard: NIST971)
 - Not always available
- Recent advancements: more easily accessible



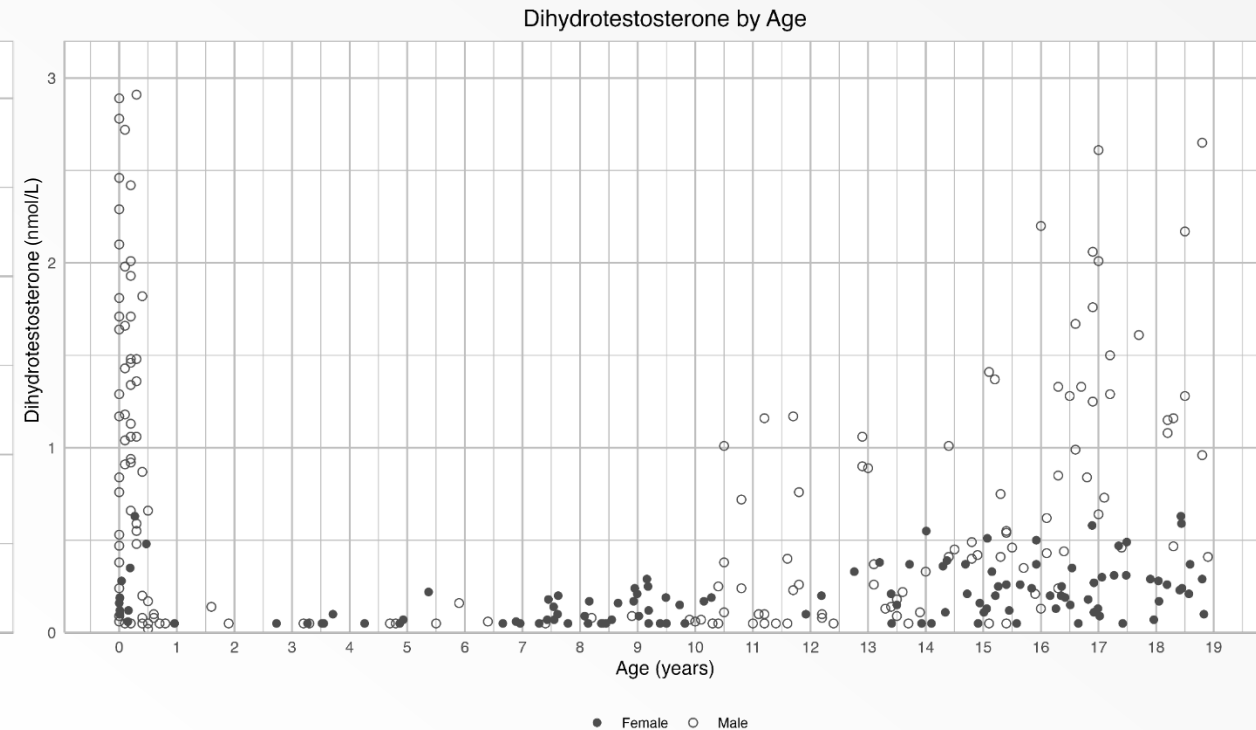
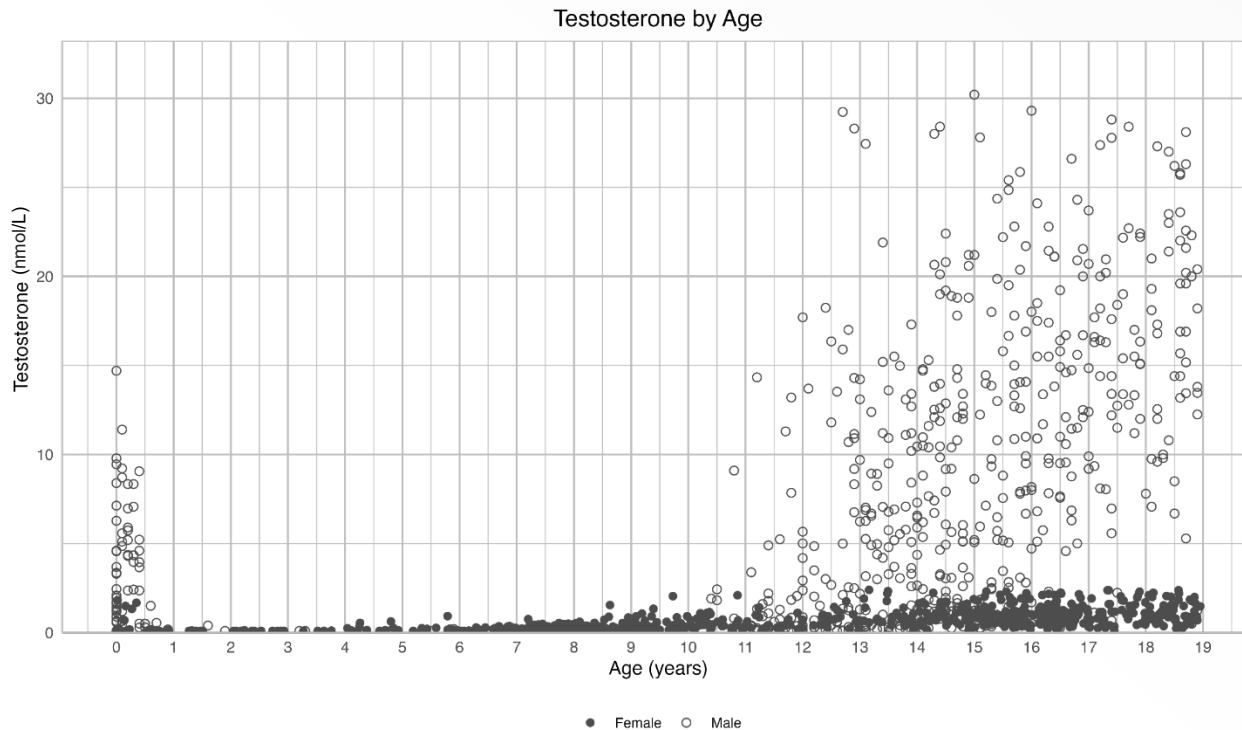
Analysis of T and DHT by MS-based assay

- Improved comparability
→ Need for harmonized age-related reference intervals
- Current RI (mostly immunoassay): differences due to method/assay, sex, developmental stage
- Role in diagnostic work-up of pediatric endocrine disorders:
 - e.g. hypogonadism, premature puberty, PCOS...
- Child development: significant variation in T and DHT



Analysis of T and DHT by MS-based assay

- Child development: significant variation in T and DHT
 - Mini-puberty
 - Adrenarche
 - Gonadarche



Research Questions

1. *What reference intervals for testosterone, dihydrotestosterone, and T/DHT ratio, analyzed using mass-spectrometry, are currently available in the literature?*
2. *What are the limitations of the currently published reference intervals?*
3. *Do published reference intervals still show heterogeneity and variability when using standardized MS-based assays?*



Methods

Search Strategy – Data Extraction – Real World Data Comparison



Literature review

- 1980-2024
- Testosterone;
Dihydrotestosterone;
Mass Spectrometry;
Reference Intervals

Data Extraction:

- Reference Interval
- Partitions
- Sample Size

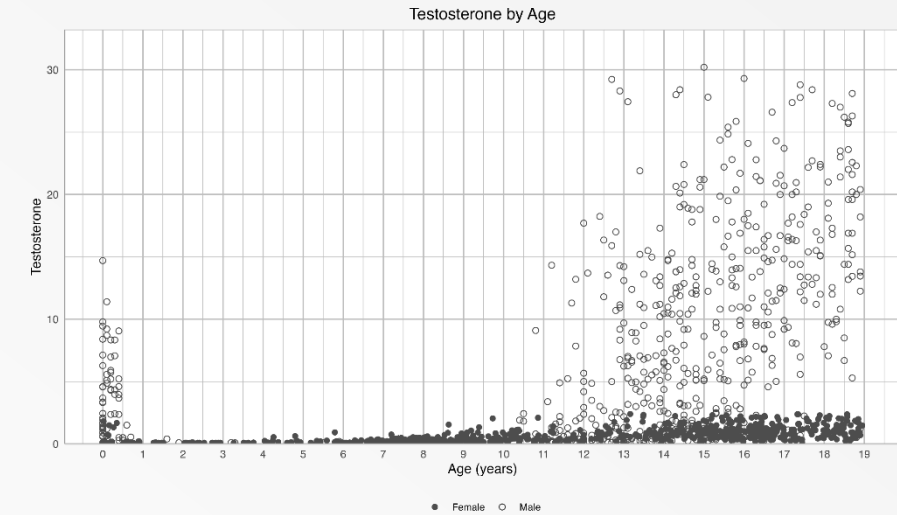
Inclusion Criteria	Exclusion Criteria
Reporting of Reference Intervals	Immunoassay
MS-based assay	Incorrect sample type (e.g. urine, amniotic fluid, saliva, DBS)
T; DHT; T/DHT Ratio	Only adult population
Plasma and/or Serum	Specific subgroup

Analytical Characteristics	Cohort Characteristics	Methodological Characteristics
- Method of analysis - Manufacturer - Calibrator traceability	- Age/sex composition - In- and exclusion criteria - Ethnicity	- Partitioning criteria - Statistical considerations



Real World Data Comparison

- 10-year retrospective data analysis (0-18y old)
 - Age, sex, T and/or DHT result
- Data cleaning:
 - Only 'solo' samples
 - Tukey's far-outlier detection method
- Application of published reference intervals
 - Evaluation of abnormal flagging rate

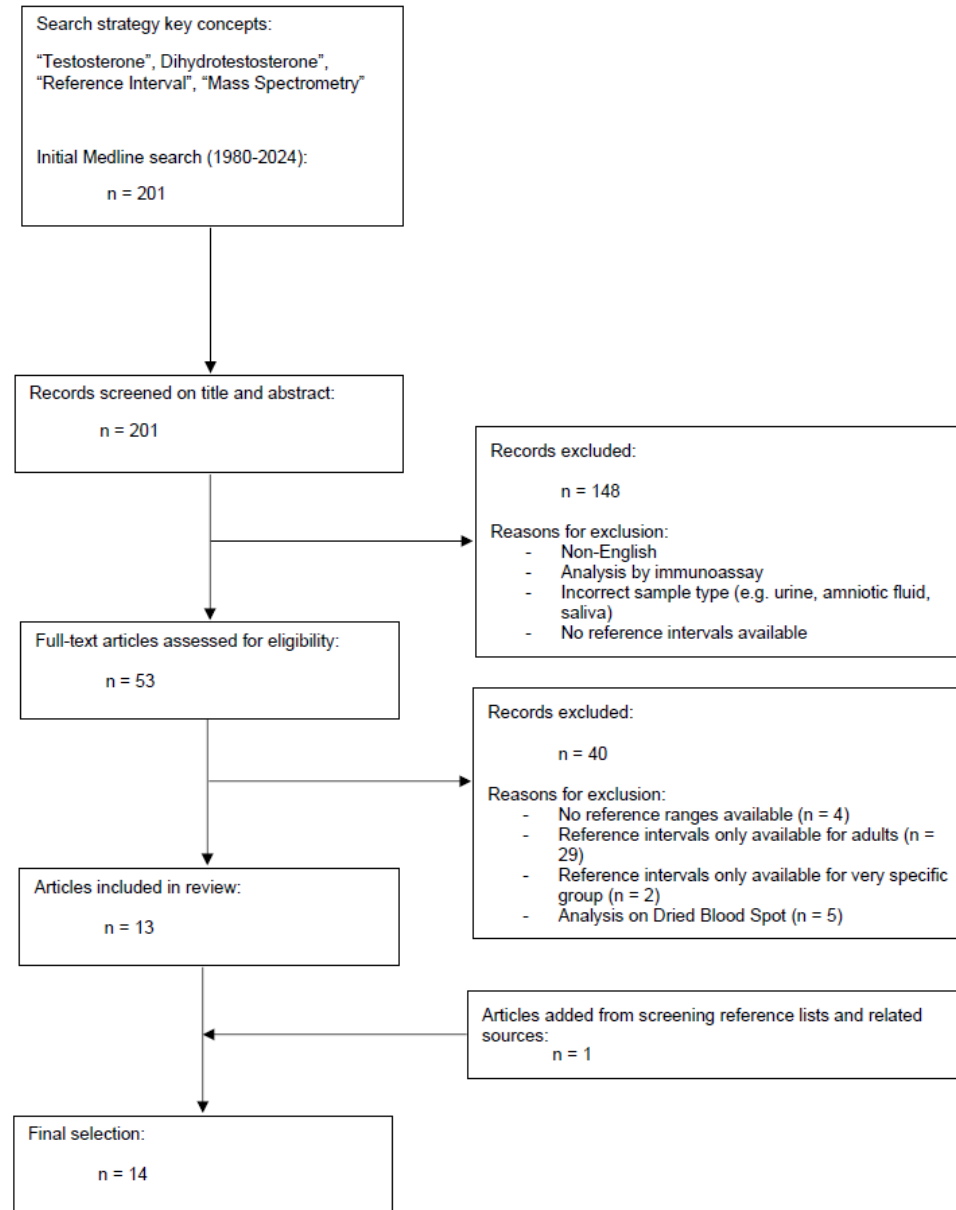


Results

Study Selection – Study Characteristics – Reference Intervals – Application of Reference Intervals on Real World Data



Study Selection



Study Characteristics

Parameters	Method of analysis	Sample type	Sex
- Testosterone: 14 - DHT: 3 - T/DHT ratio: 0	- LC-MS/MS: 12 - GC-MS/MS: 2	- Serum: 13 - Plasma: 1	- M/F: 13 - M: 1

Partitioning criteria	Calibrator traceability	Ethnicity
- Age: 12 - Pubertal stage: 9 (Tanner PH, B, G; TV)	- NIST: 2 - Manufacturer: 12	- Reported: 4 - Conducted: North-America: 6 Europe: 8



Reference intervals

First author	Year	Reference Interval Definition	Subgroup	Dihydrotestosterone Reference Intervals (nmol/L)								
				Mixed Sex			Male			Female		
				n	LL	UL	n	LL	UL	n	LL	UL
Adriaansen	2024	2.5-97.5th	0-5y	62	<0,04	0,05						
			0-10y (M); 0-9y old (F)		<0,04	0,11	94			39		
		Range	11-17y (M); 10-17y (F)				12	0,05	1,59	16	0,04	0,29
Kulle	2010	Range	<1w				8	<0,1	0,7	6	<0,1	0,1
			2w-2m				19	<0,1	2,6	23	<0,1	1
			3-5m				12	<0,1	0,8	10	<0,1	0,1
			6-11m				4	<0,1	0,4	10	<0,1	0,1
			1-3y				9	<0,1	1,3	9	<0,1	0,4
			4-6y				10	0,1	0,8	13	<0,1	0,4
			7-9y				10	0,1	0,6	14	<0,1	0,5
			10-12y				18	0,1	1,9	17	<0,1	0,8
			13-15y				26	0,1	3,1	19	0,1	1
			16-18y				21	0,1	1,9	10	0,1	1

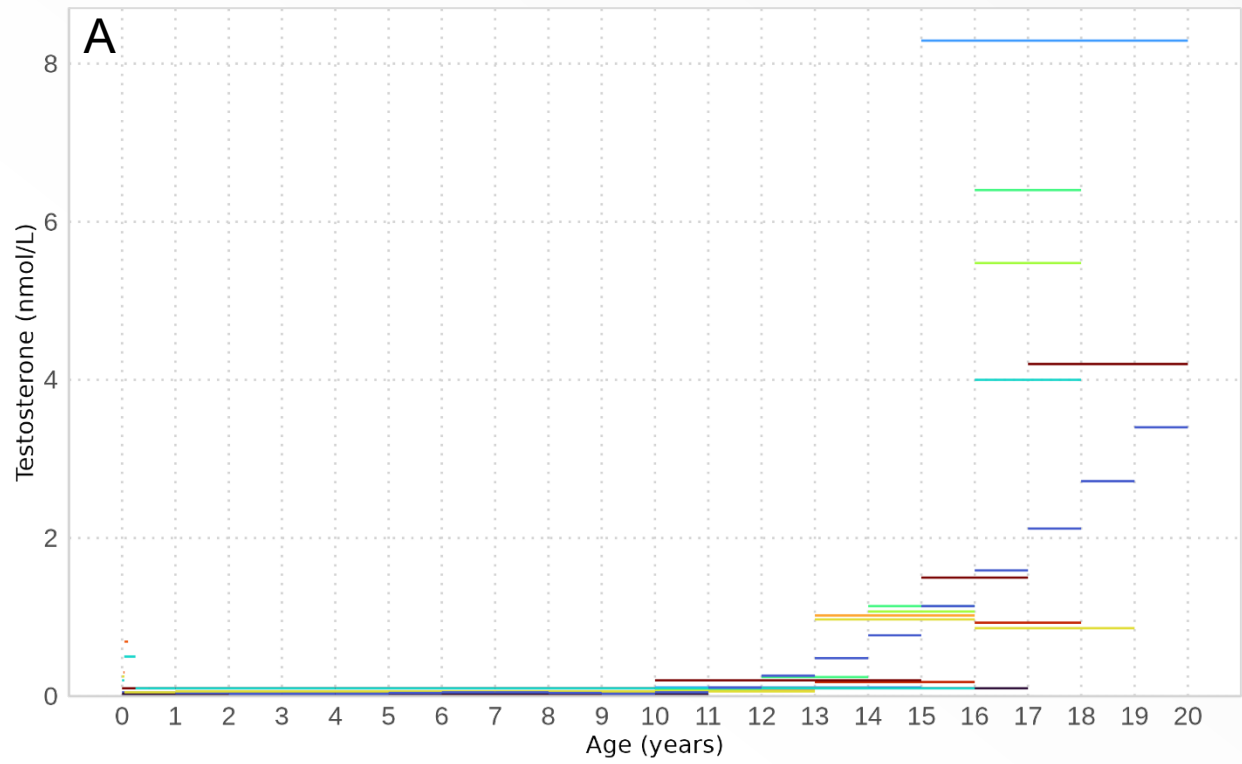
- Statistical inconsistency
- Heterogeneity in age partitions
- Heterogeneity in pubertal stage assessment
- Small sample sizes

First author	Year	Reference Interval Definition	Subgroup	Dihydrotestosterone Reference					
				Male			Female		
				n	LL	UL	n	LL	UL
Ankarberg-Lindgren	2018	5-95th	TV: 1-2 mL	9	<0,027	0,227			
			TV: 3-6 mL	9	0,08	0,31			
			TV: 8-12 mL	14	0,18	1,242			
			TV: 15-25 mL	9	0,65	1,363			
			Tanner B stage 1				16	<0,027	0,187
			Tanner B stage 2				15	0,028	0,337
			Tanner B stage 3				13	0,069	0,365
			Tanner B stage 4/5				16	0,027	0,528
Kulle	2010	Range	Tanner stage 1 (M: <6m; F: <8y)	41	0,1	3	71	0,1	1
			Tanner stage 1 (M: 6m-9y)	35	0,1	2,4			
			Tanner stage 1 (M: >9y; F: >8y)	13	0,1	1	14	0,1	0,5
			Tanner stage 2	11	0,1	1,8	10	0,1	0,5
			Tanner stage 3	12	0,1	1,9	10	0,1	0,8
			Tanner stage 4	10	0,1	1,8	11	0,1	1
			Tanner stage 5	18	0,2	3,2	12	0,1	0,7
			TV: 1-2 mL (<6m)	40	<0,1	3,4			
			TV: 1-2 mL (6m-9y)	26	<0,1	1,3			
			TV: 1-2 mL (>9y)	7	0,1	0,7			
			TV: 3-4mL	7	0,1	1,7			
			TV: 5-10mL	9	0,1	1,7			
			TV: 11-15mL	15	0,3	1,9			
			TV: >15mL	10	0,3	3,2			



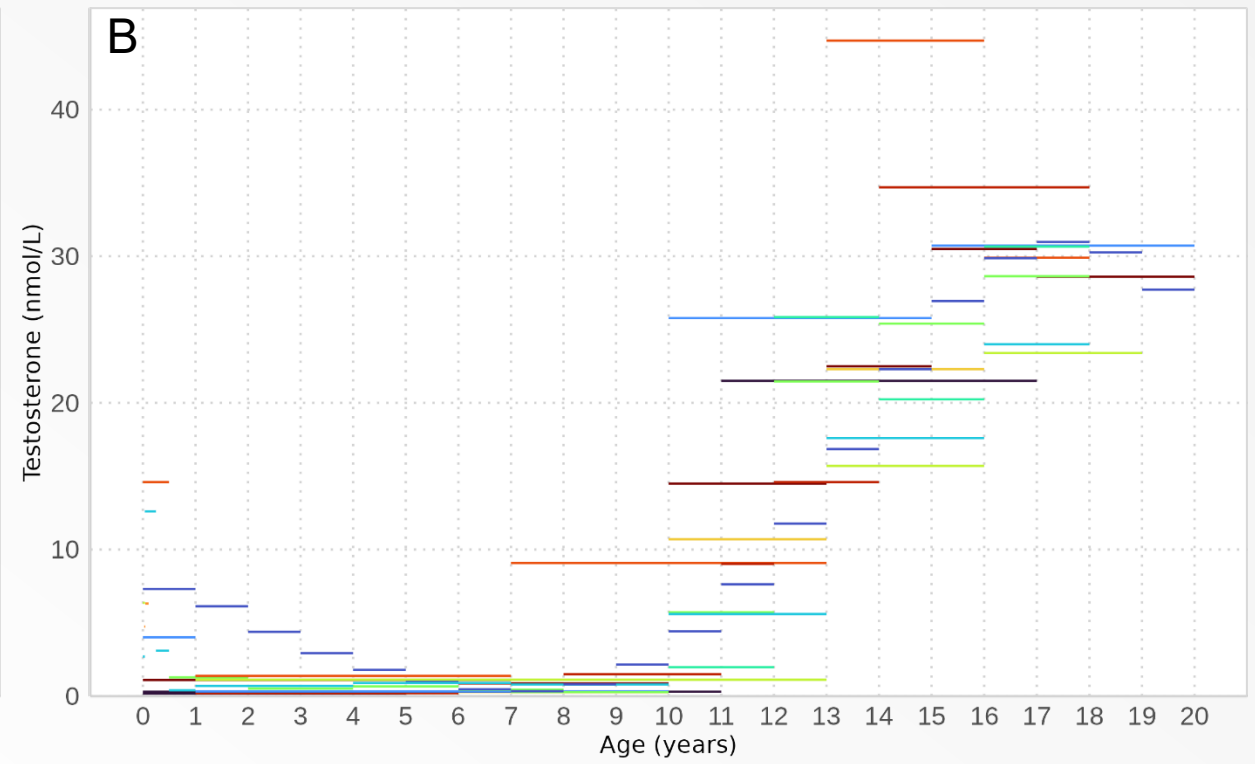


Lower Limit



- Adriaansen et al. 2024
- Kulle et al. 2010
- Kyriakopoulou et al. 2013
- Rauh et al. 2006
- Holmes et al. 2021
- Kushnir et al. 2006
- Madsen et al. 2020
- Soldin et al. 2009
- Ju Bae et al. 2019
- Kushnir et al. 2010
- Magnisali et al. 2008

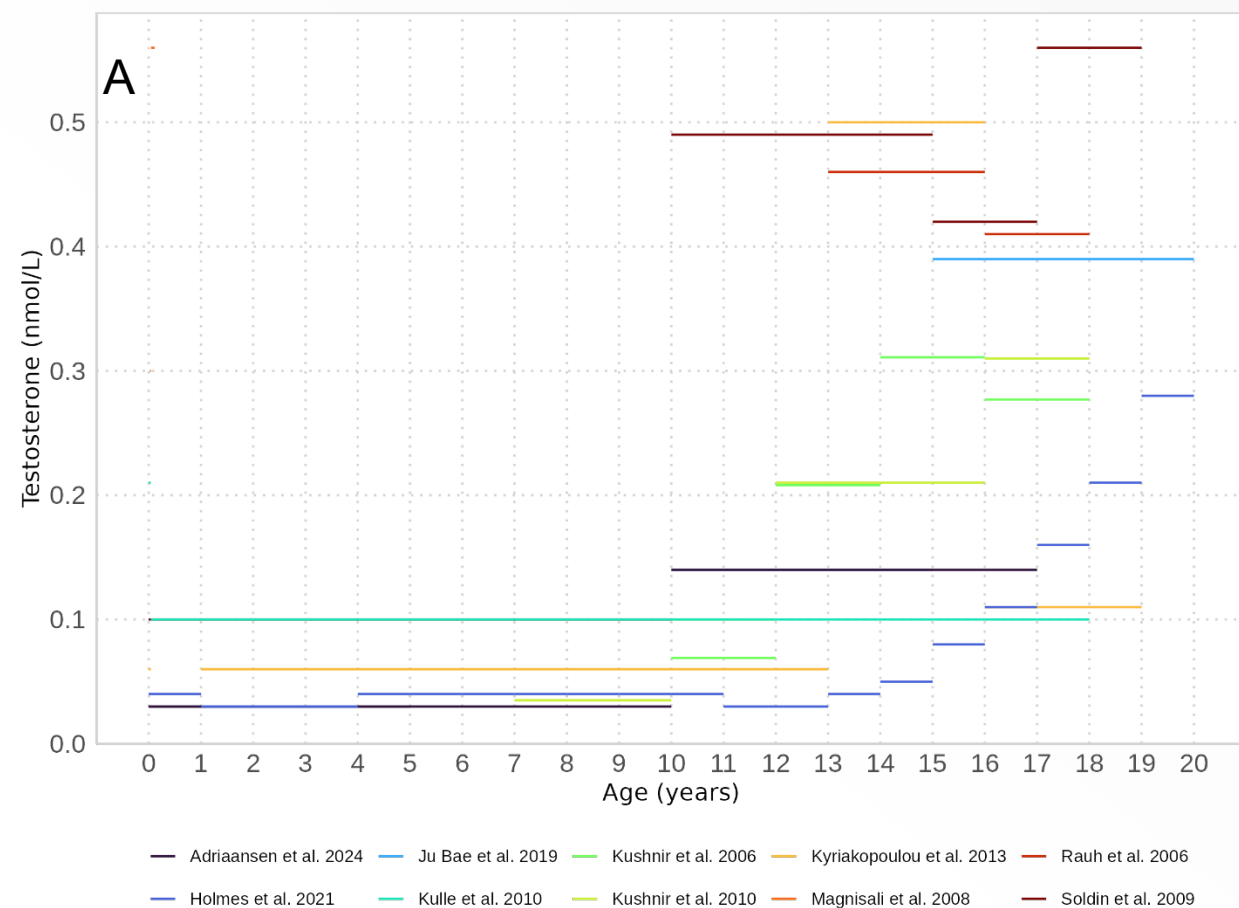
Upper Limit



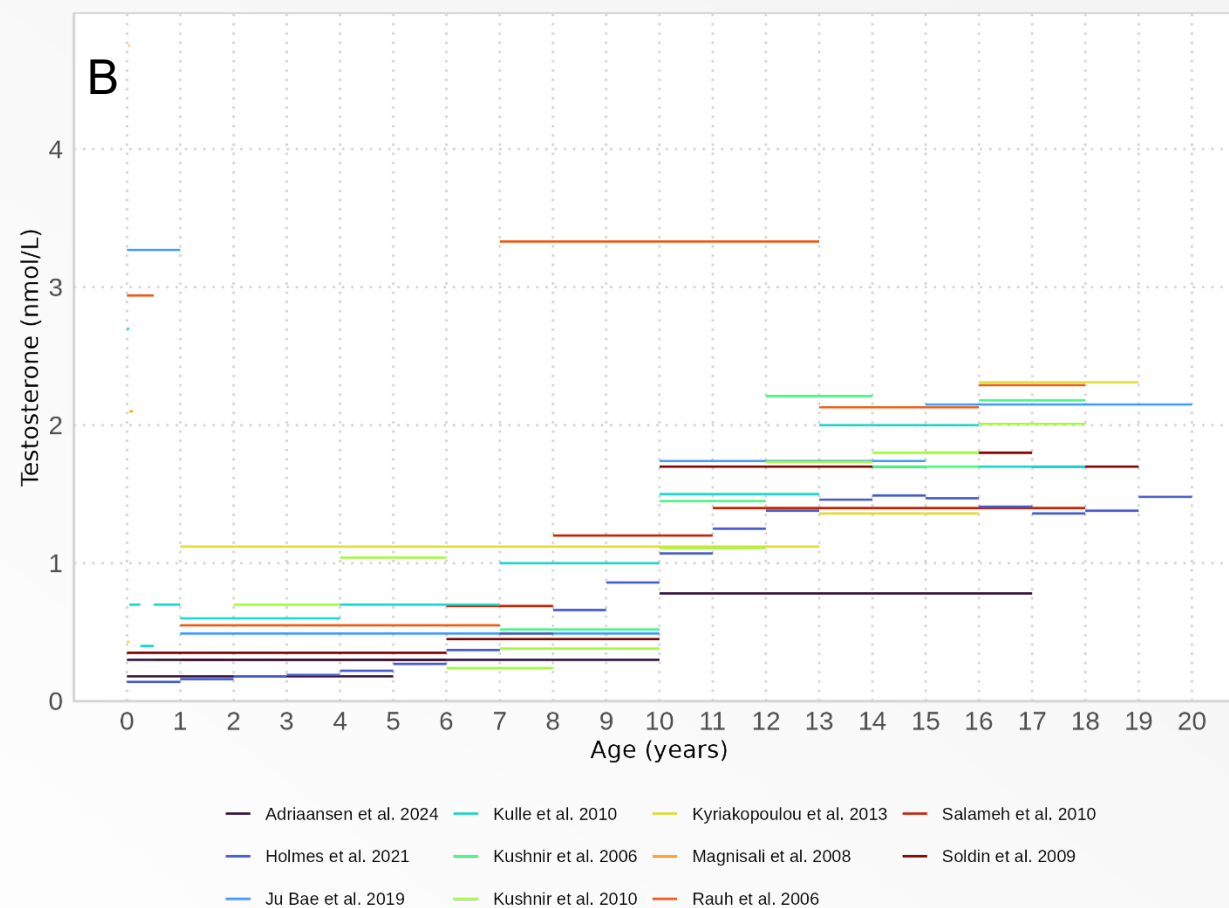
- Adriaansen et al. 2024
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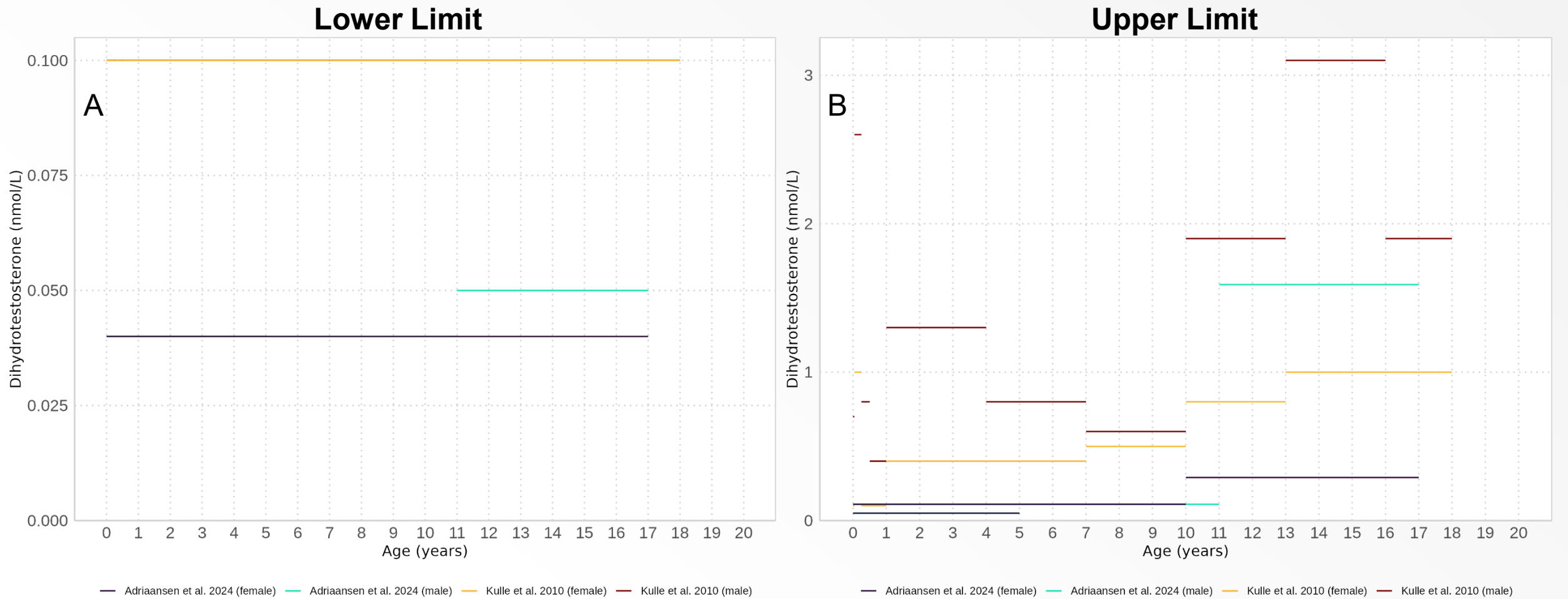


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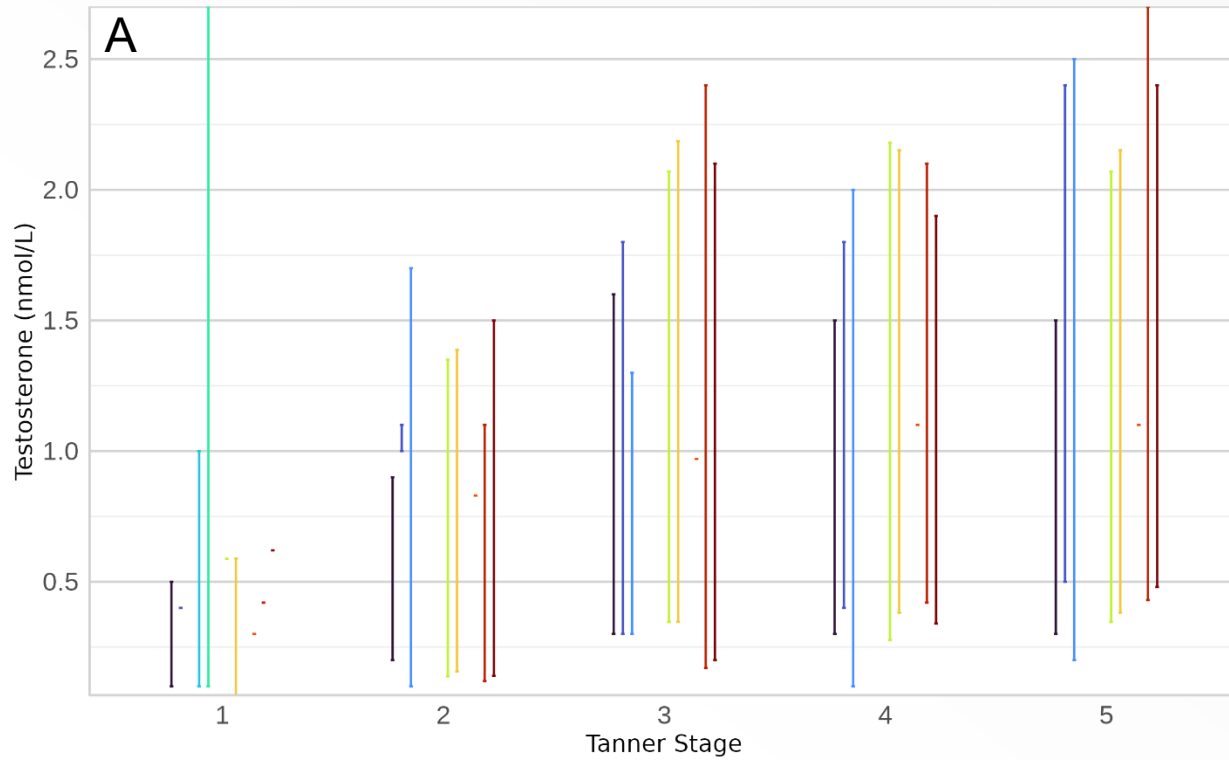


Upper Limit



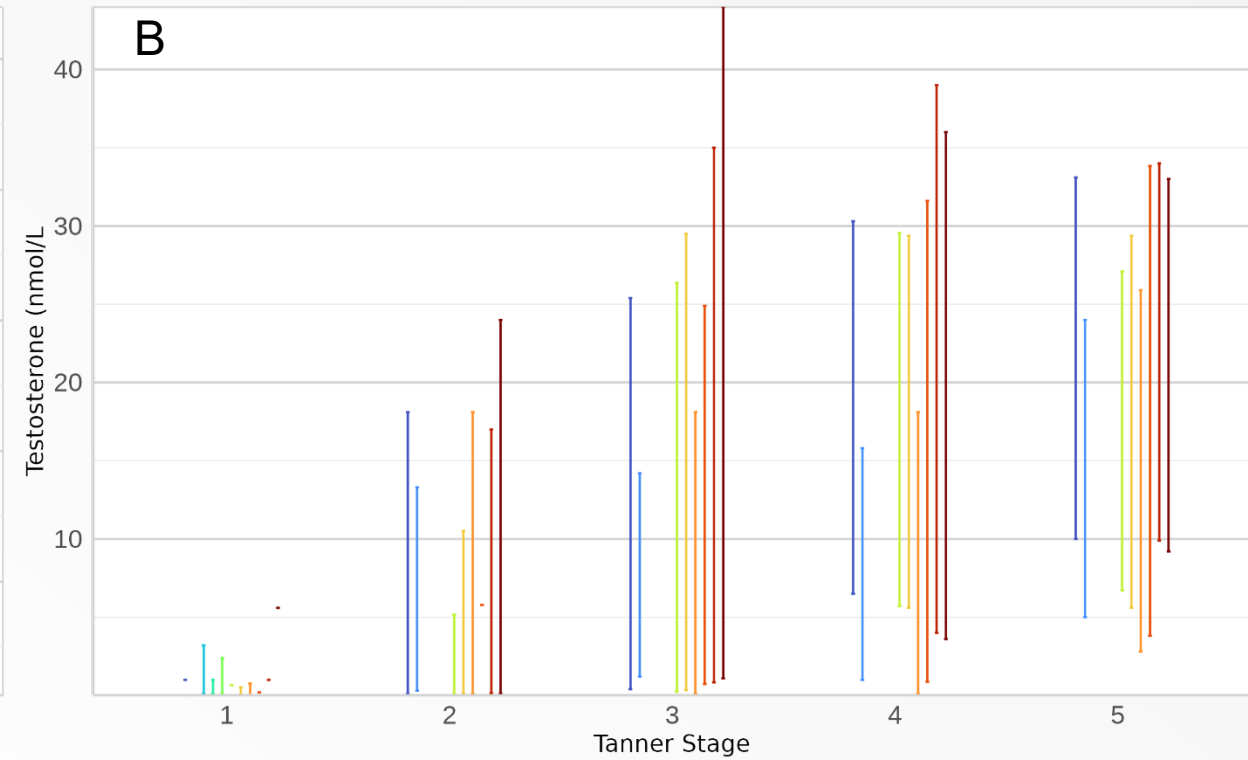


Female



Ankarberg-Lindgren et al. 2018 Kulle et al. 2010 ((M: <6m; F: <8y)) Kushnir et al. 2006 Salameh et al. 2010
 Bae et al. 2019 Kulle et al. 2010 (M: >9y; F: >8y) Kushnir et al. 2010 Søbørg et al. 2014 (G/B)
 Kulle et al. 2010 Kulle et al. 2010 (M: 6m - 9y) Madsen et al. 2020 Søbørg et al. 2014 (PH)

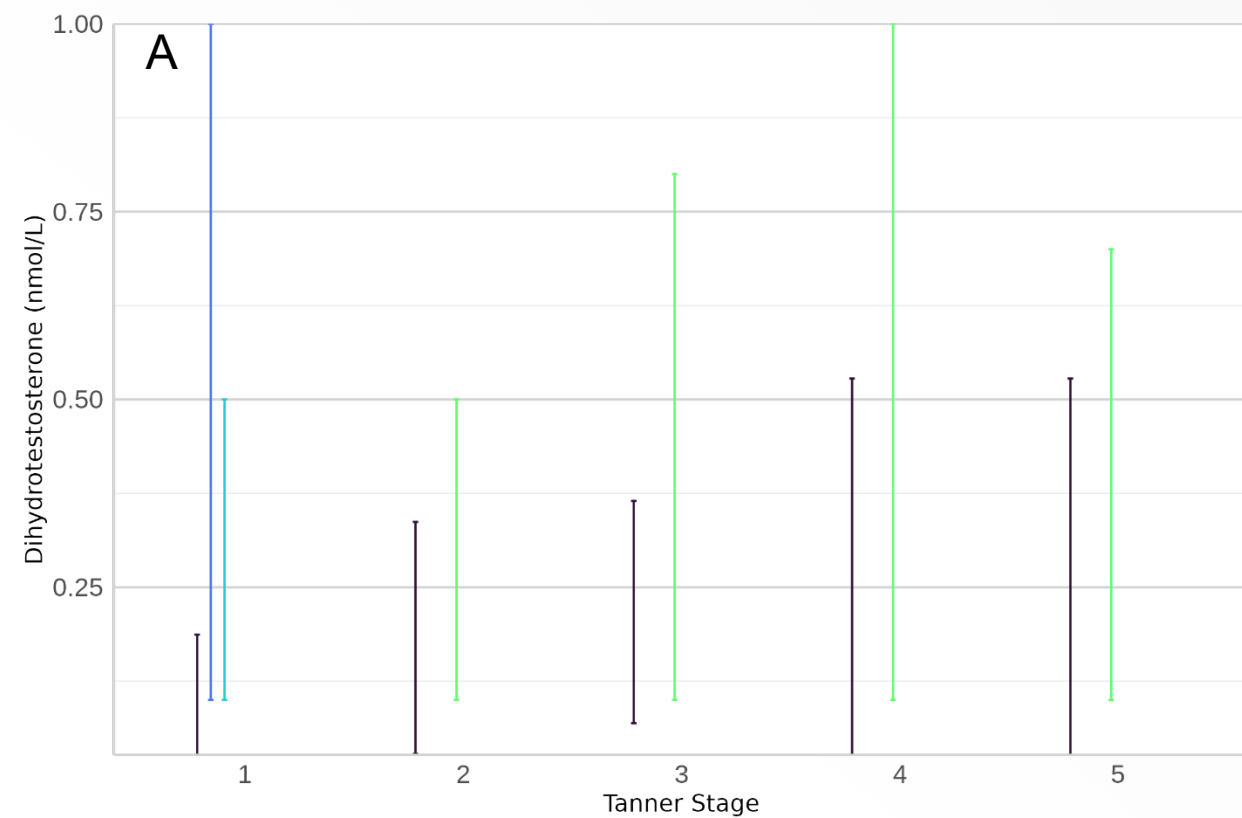
Male



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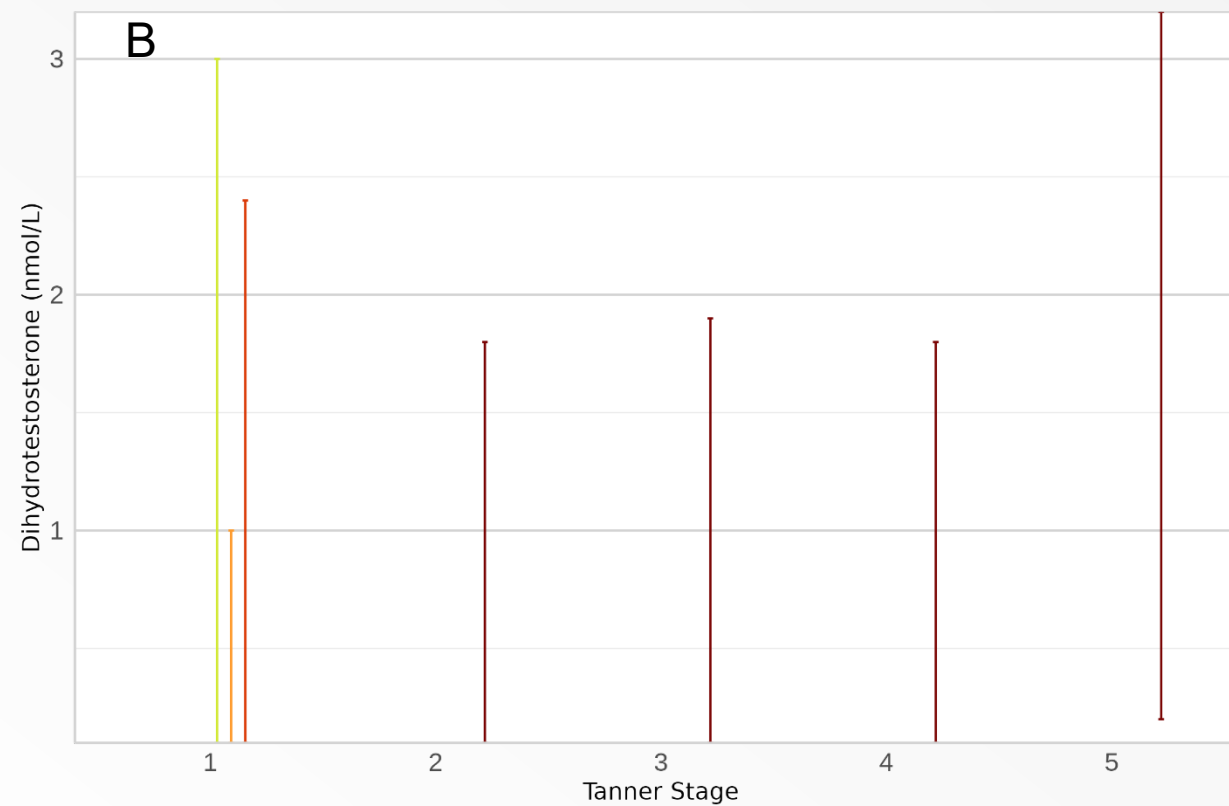


Female



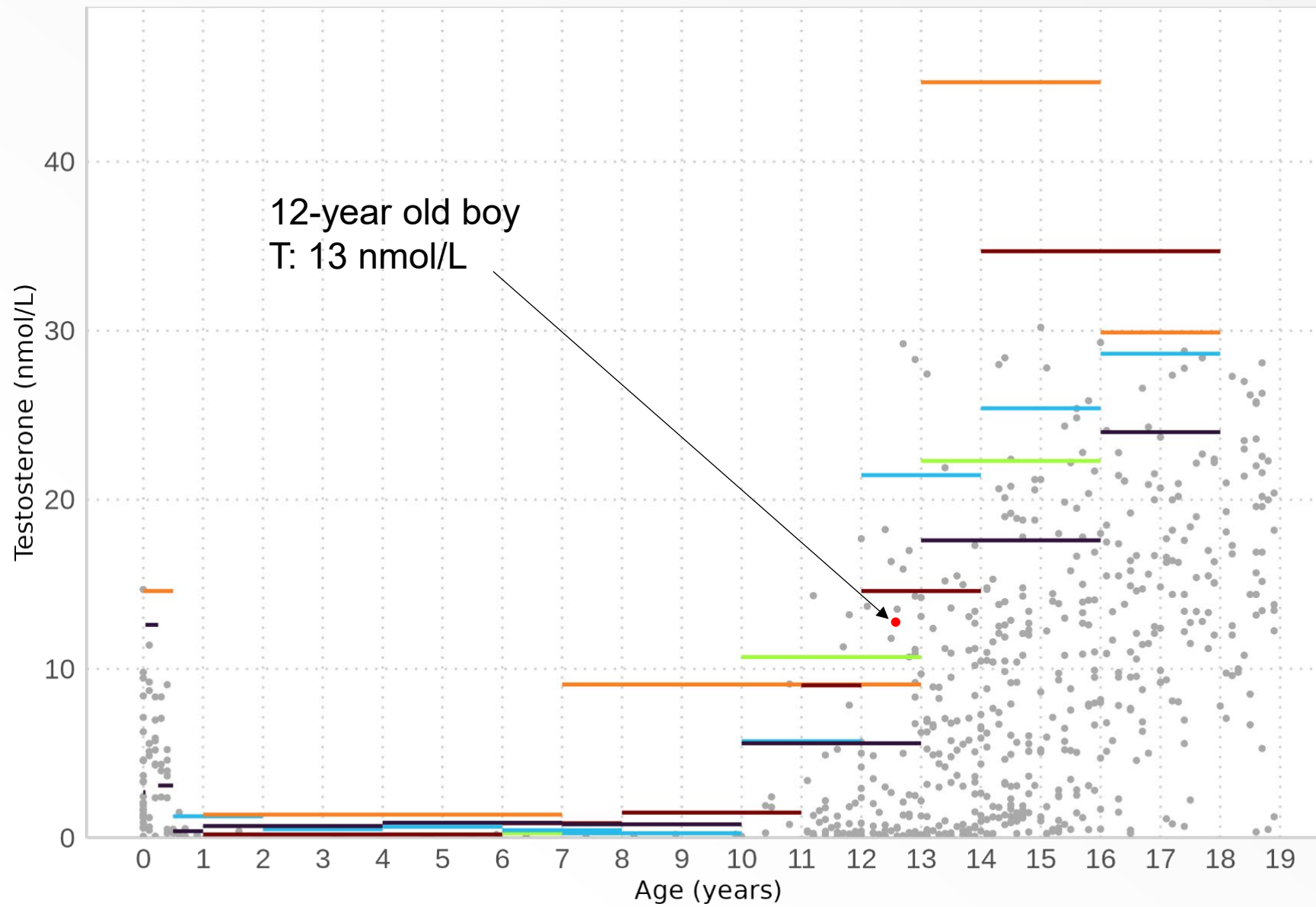
— Ankarberg-Lindgren et al. 2018 (F) — Kulle et al. 2010 (F: <8y) — Kulle et al. 2010 (F) — Kulle et al. 2010 (M: <6m) — Kulle et al. 2010 (M: 6m - 9y)
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Male



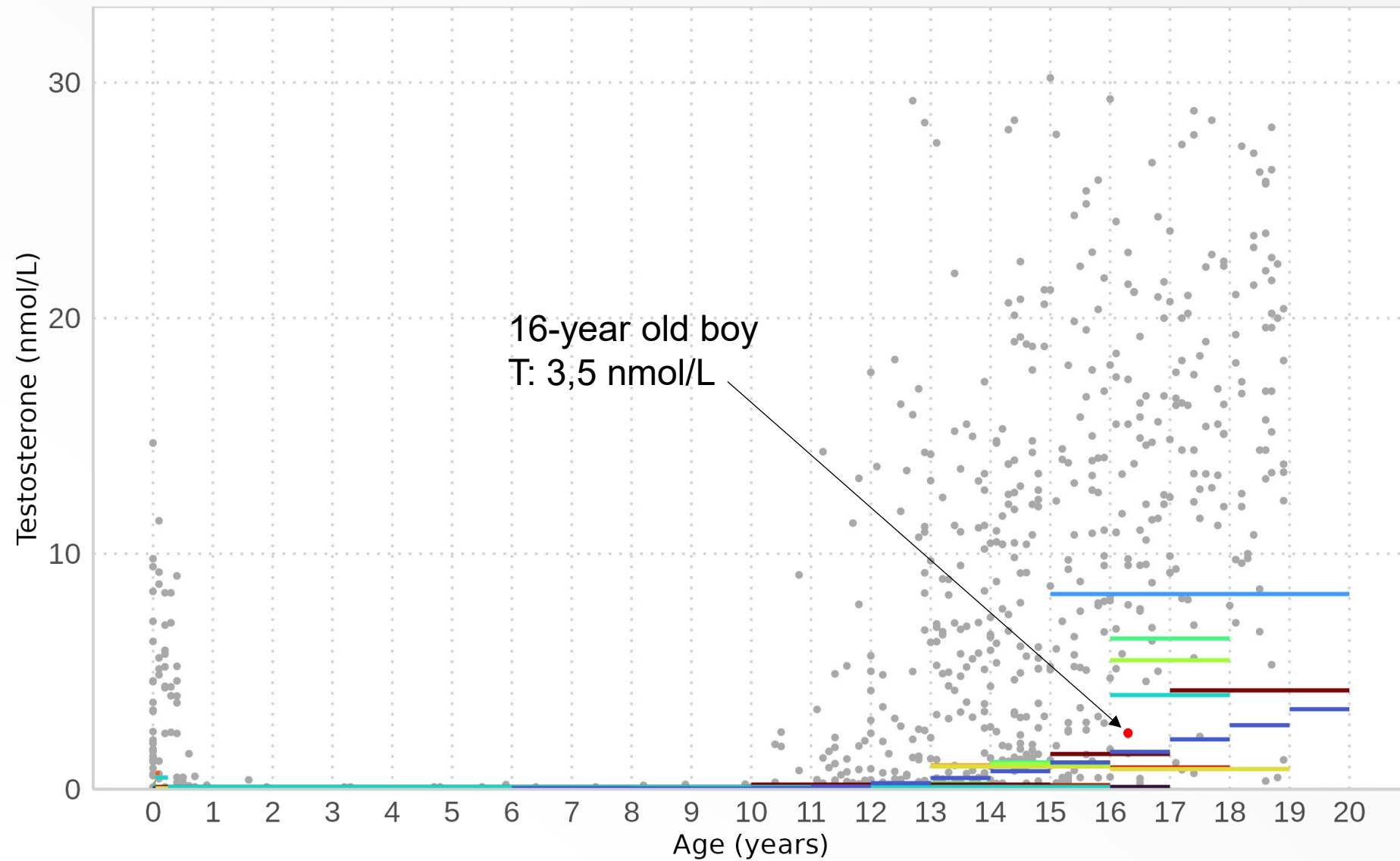
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 — Kulle et al. 2010 (M: >9y) — Kulle et al. 2010 (M)





Abnormal flagging rate: 33%





Abnormal flagging rate: 44%





Abnormal flagging rate: 60%



Discussion

Age Partitions and Physiological Variability – Challenges in Sample Size and Data Representation – Inaccuracies due to Opportunistic Sampling – Variability due to Ethnic differences



Published Pediatric Reference Intervals

- Testosterone:
 - Variability in LL/UL
 - Inconsistent age-partitions
- Dihydrotestosterone
 - Limited availability
 - Variability in LL/UL
 - Inconsistent age-partitions
- T/DHT ratio
 - No RIs available

→ Consequence of several limitations in RI studies



Age Partitions and Physiological Variability

Age Partitions

Rationale:

- Not mentioned (7/12)
- Statistical (3/12)
- Literature (1/12)
- 'Standard Partitions' (1/12)

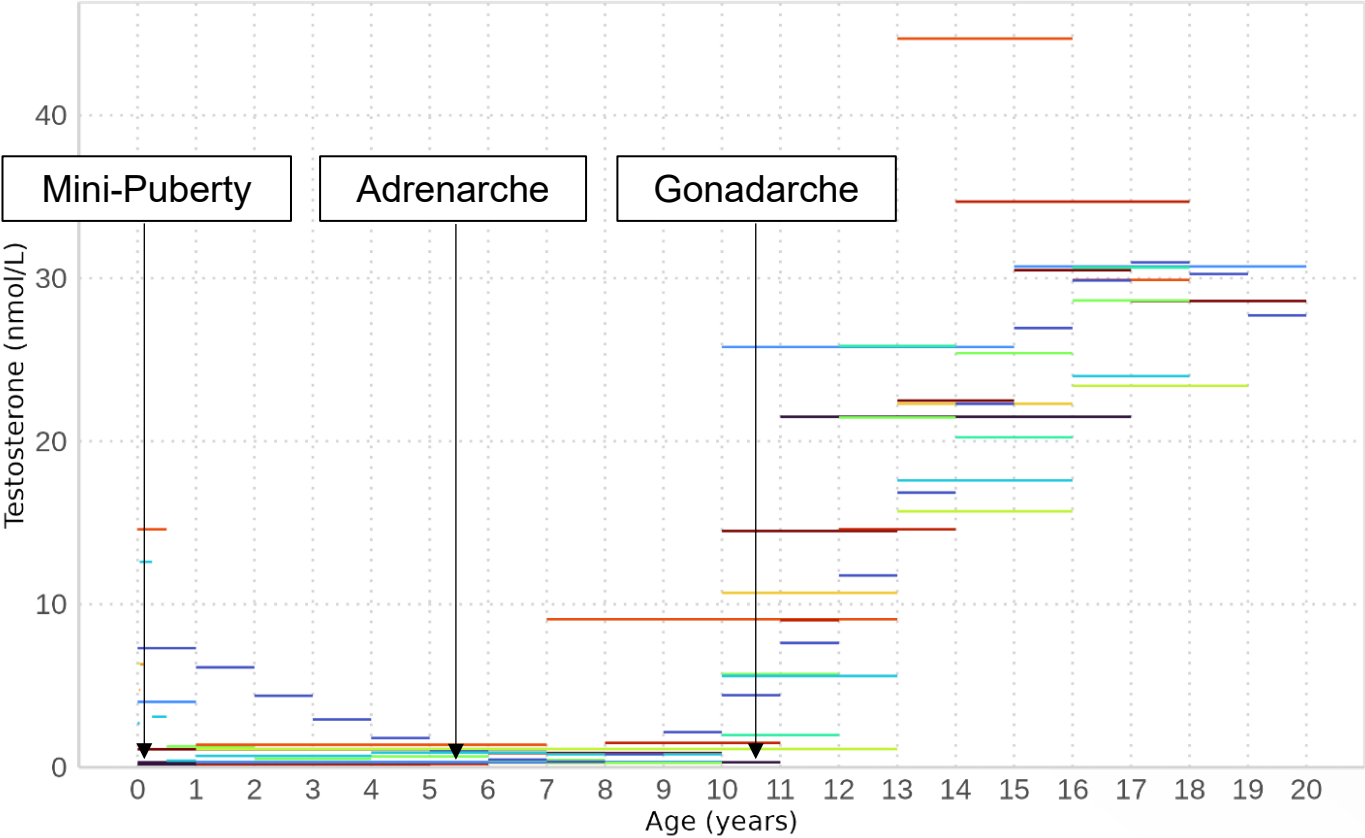
Partitions by Pubertal Stage

- Subjective evaluation
- Heterogeneity in assessment:
 - Orchidometer vs US
 - Tanner G vs Tanner PH
 - Self-reporting vs physician



Age Partitions and Physiological Variability

→ Inconsistent age-partitions: different developmental stage within same subgroup

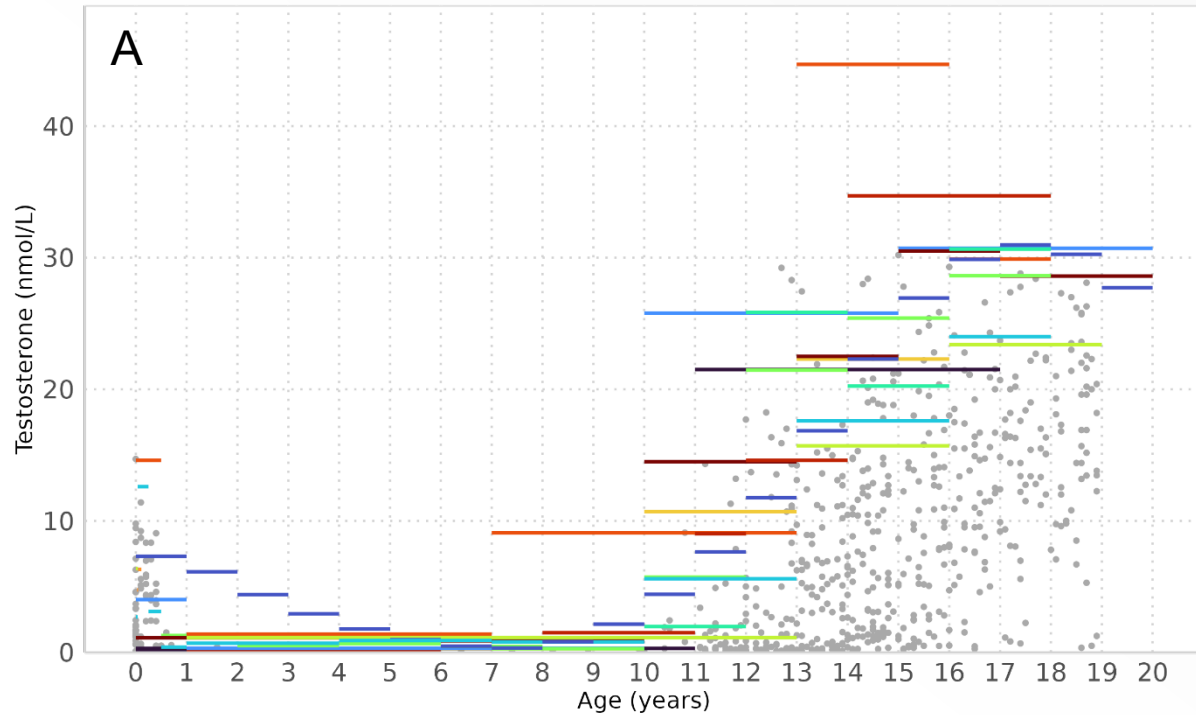


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		Male		
		n	LL	UL
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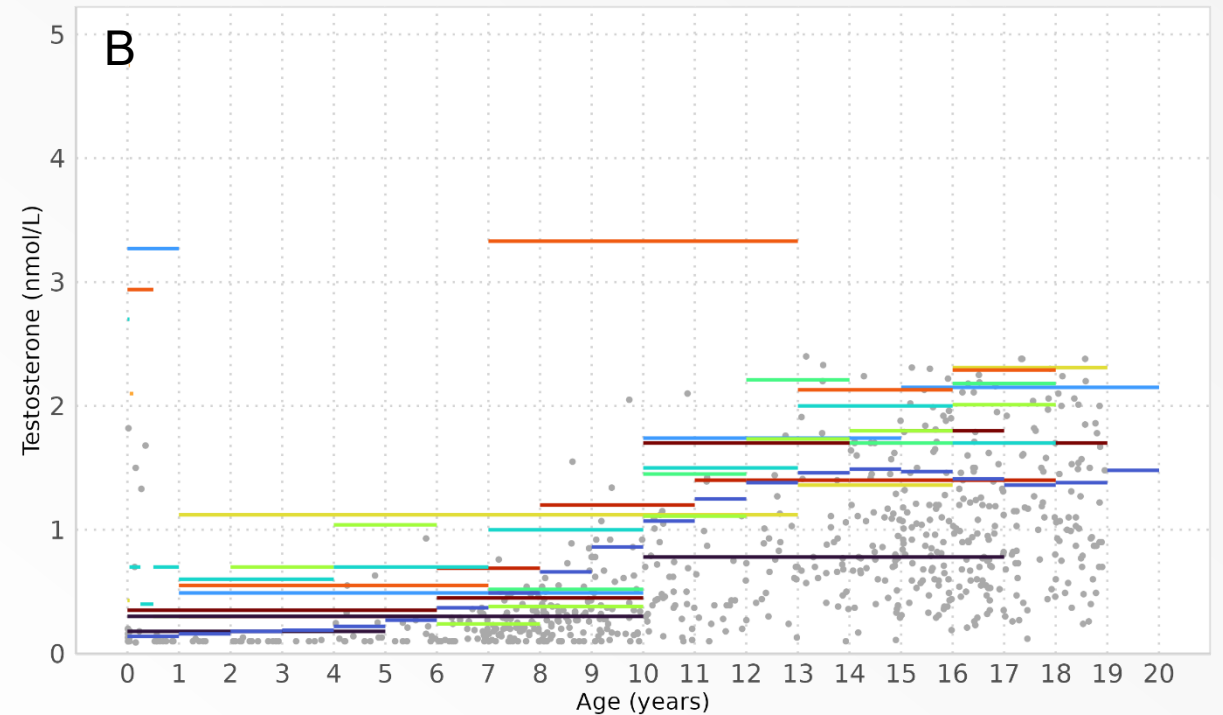
First Author	Subgroup	Testosterone Reference Intervals (nmol/L)		
		Mixed Sex		
		n	LL	UL
Adriaansen	0-5y	62	<0,03	0.18
	0-10y (M); 0-9y old (F)	133	<0,03	0.3

Age Partitions and Physiological Variability

Male



Female



Challenges in Sample Size and Data Representation

CLSI EP28-A3C: ≥ 120 samples (in each homogenous subgroup)

- Testosterone: 68/189 subgroups
- DHT: 1/52 subgroups
- Age <6 months: all subgroups <25 samples

Blood samples from healthy children: invasive procedure

Rapid changing physiology: numerous subgroups

→ Small sample sizes: limited generalizability



Inaccuracies due to Opportunistic Sampling

Difficulties obtaining samples → opportunistic sampling:

- Hospitalized children, elective surgery, leftover specimens
- Unrelated medical reasons: no impact on selected parameter
 - Samples for steroid analysis, exclusion for endocrine diseases

→ Pre-analytical conditions: not standardized

- E.g. children awaiting surgery: surge in adrenal androgens

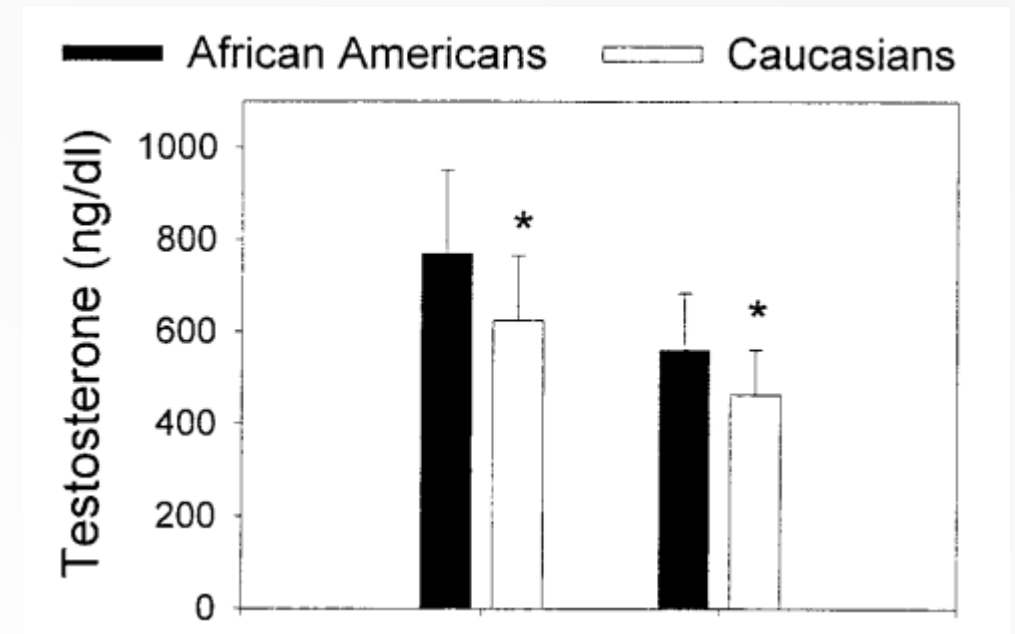


Variability due to Ethnic Differences

- African population: higher T levels
- ↔ Other studies: lower T levels in black population

- Selected RI studies:

- North-America: 6
- Europe: 8



Conclusion



Conclusion

- Limited RIs for DHT, no RI for T/DHT ratio
- Variability and inconsistency for T and DHT

Consequence of:

- Partitions not reflecting underlying physiology
- Insufficient sample size
- Opportunistic sampling

→ RIs not reflecting dynamic endocrine changes of the developing child

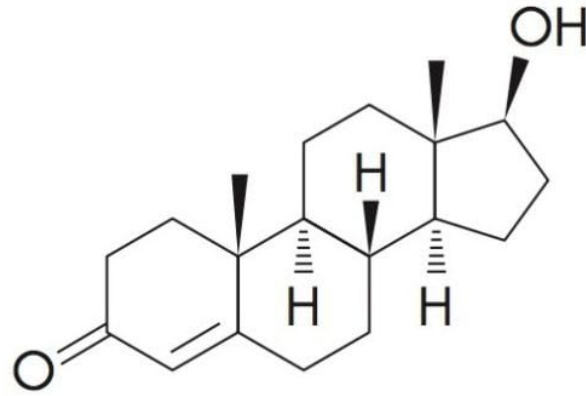


Conclusion

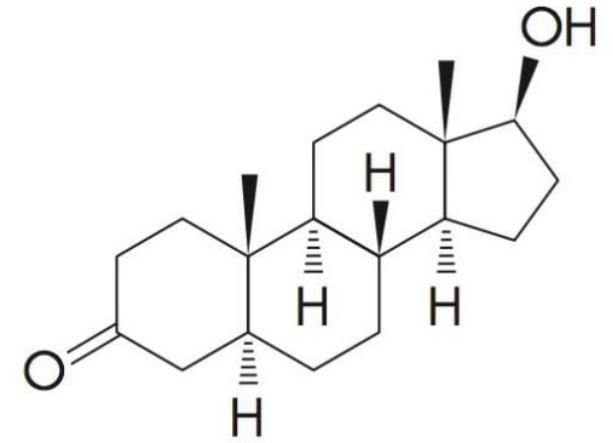
- More studies: DHT, T/DHT
- Better studies: Testosterone
 - Sufficiently large cohort
 - Statistical evaluation of partitions to reflect physiological changes
- Alternative: indirect RI studies
 - MS: standardization → multicenter approach
 - Accessible, less-invasive, cost-effective
 - Robust age-partitioned RI reflecting physiological changes
 - Cave: more pathological results in pediatric population

To do: data collection





Testosterone



Dihydrotestosterone

Questions?