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ORIGINAL ARTICLE

Recommended Nordic paediatric reference intervals for 21 common biochemical properties

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Abstract

Paediatric reference intervals based on samples from healthy children are difficult to establish and consequently data are often from hospitalized children. Furthermore, biases may present in published data due to differences in the analytical methods employed. Blood samples from 1429 healthy Danish children were collected for establishing reference intervals for 21 common biochemical properties (Alanine transaminase, Albumin, Alkaline phosphatase, Aspartate transaminase, Bilirubin, Calcium, Cholesterol, Creatinine, Creatine kinase, HDL-Cholesterol, Iron, Lactate dehydrogenase, LDL-Cholesterol, Magnesium, Phosphate, Potassium, Protein, Sodium, Transferrin, Triglycerides and Urate). Samples were analyzed on a Roche-Modular-P/ISE-system. The NORIP reference material (NFKK Reference Serum X) was included in all the analytical runs. Reference values were recalculated according to the target values of X for the properties and statistical calculations carried out as performed in the NORIP study. Thus commutable (regarding analytical method) reference intervals for 20 properties were established and for LDL-Cholesterol reference intervals were reported for the specific analytical method employed. The data were compared to previous studies and to those obtained from the youngest age group in the NORIP study. Marked age differences were observed for most of the properties. Several properties also showed gender-related differences, mainly at the onset of puberty. Data are presented as suggested intervals for combined age groups, but can be accessed via the NORIP home page if more detailed division according to age or gender is desired.

Key Words: *Reference material, reference limit, reference individual, Nordic Reference Interval Project 2000, Paediatrics*

Introduction

Increasing electronic communication within healthcare services necessitates harmonization of laboratory measurements and reference intervals in clinical biochemistry. Analytical measurements are becoming more harmonized due to internationally established reference systems and external quality assessment results show that for many biochemical properties the results are of acceptable analytical quality. Reference intervals should be harmonized if possible to facilitate the interpretation of laboratory results and to reduce misinterpretations, but population differences as well as method differences can be a challenge in this process. Previously, laboratories have often used reference intervals from the literature, from the provider of the reagents employed or adjusted from old methods, since the establishment of own reference intervals can be cumbersome. For paediatric reference intervals the challenges are even bigger since samples from reference individuals are difficult to obtain. Data from the literature are frequently used, but are often insufficiently characterized regarding commutability. Biases may be introduced by collecting data from hospitalized children, using the Hoffmann approach as recommended in Soldin's textbook on paediatric reference intervals [1,2], or due to differences in analytical methods employed. Such biases may render the intervals invalid for their intended use.

The Nordic Reference Interval Project 2000 (NORIP) provided a reference material with values traceable to reference methods and reference intervals

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for 25 common biochemical properties in adults (≥18 years) [3].

In the present study we obtained blood samples from1429 Danish school children, analyzed the samples for 21 biochemical properties and included measurement of the NORIP reference material (NFKK Reference Serum X). This reference material has traceable values for 20 of the properties and in the present study commutable (regarding analytical method) paediatric reference intervals for these properties were established. For LDL-Cholesterol the reference intervals should be applied only for the analytical method and platform we employed.

Materials and methods

Study subjects

All children ($N = 6203$, boys $n = 3101$) in nine randomly selected schools in the Copenhagen area in Denmark were invited to participate in The COPENHAGEN Puberty Study (ClinicalTrials.gov ID: NCT01411527) conducted in 2006–2008 [4,5]. All participants filled out a questionnaire regarding current and previous diseases, medication, and ethnicity. A total of 4339 children (boys $n = 2334$) declined to participate resulting in 1097 girls and 767 boys who were clinically examined with an overall participation rate of 24.7% and 35.0% of boys and girls, respectively. Out of these, 168 were excluded from the final analysis because of chronic disease ($n = 5$), or one or both parents or one or more grandparents originating from a non-European country ($n = 160$), or age above 20 years ($n = 3$). Blood sampling for the present study was not performed in 275 of the children (venipuncture not accepted or difficult to perform), leaving a total of 596 boys aged 5.8–20.0 years and 825 girls aged 5.6–20.0 years for this study [4,5]. The age distribution of these participants is shown in Table I.

Pubertal stages were examined clinically in all the participating boys and girls [4,5]. There was no significant difference in height, weight or pubertal stage between the children who had a blood sample taken and those who did not.

Table I. Age distribution of subjects included in the study.

Age (years)	Female (*n*)	Male (*n*)
5	7	1
6	41	35
7	59	37
8	76	56
9	89	84
10	115	74
11	108	60
12	78	75
13	41	29
14	55	34
15	32	31
16	19	18
17	51	31
18	30	18
19	24	13

Blood sampling

The study subjects were not requested to fast on the day of blood sampling. The subjects were situated in the sitting position for approximately 10 min before blood sampling. During school hours between 08:30 and 13:00 blood samples were drawn from an antecubital vein, using a vacuum collecting system (Vacuette®, Greiner Bio-One, Belgium) in Li-Heparin-tubes with gel separator. The samples were left at room temperature for less than 2 h, centrifuged at 2000 *g* and plasma was separated, frozen and stored at −20°C for < 6 months until analysis.

Laboratory analysis

Reagents, calibrators and instruments: P-Alanine transaminase, P-Albumin, P-Alkaline phosphatase, P-Aspartate transaminase, P-Bilirubin, P-Calcium, P-Cholesterol, P-Creatinine, P-Creatine kinase, P-HDL-Cholesterol, P-Iron, P-Lactate dehydrogenase, P-LDL-Cholesterol, P-Magnesium, P-Phosphate, P-Potassium, P-Protein, P-Sodium, P-Transferrin, P-Triglycerides and P-Urate were all analyzed in heparin-plasma on the Modular® ANALYTICS P/ISE-system (ROCHE Diagnostics, Mannheim, GmbH), using the CFAS-calibrator and the Roche Modular® reagents for all assays (Table II).

Reference material: The commutable NFKK Reference Serum X (The Nordic Society of Clinical Chemistry (NFKK) has certified values traceable to SI-units, or to an international measurement standard through reference measurement procedures and international reference materials, for the components: Albumin, Calcium, Cholesterol, Creatinine, Iron, Potassium, Magnesium, Sodium, Triglycerides and Urate. Reference Serum X also has indicative values for the components: Alkaline phosphatase, Alanine transaminase, Aspartate transaminase, Bilirubin, Creatine kinase, HDL-cholesterol, Lactate dehydrogenase, Phosphate, Protein, and Transferrin, as obtained in the frame of the Nordic Reference Interval Project 2000 [6].

Protocol for analysis

Frozen NFKK Reference Serum X, CFAS, internal control samples and study samples were placed in the dark at room temperature for 1 h on the day of analysis, mixed gently for 10 min and analyzed on Roche Modular® P/ISE-system. Each series consisted of 80 study samples and 10 aliquots of reference serum X distributed throughout the series. Samples

Table II. Components and methods.

System–Component	Reagent	Calibrator	Instrument	Unit
P–Alanine transaminase	ALT IFCC (Roche)	C.f.a.s. (Roche)	Roche Modular® P	U/L
P–Albumin	Albumin Plus BCG (Roche)	C.f.a.s. (Roche)	Roche Modular® P	g/L
P–Alkaline phosphatase	ALP IFCC (Roche)	C.f.a.s. (Roche)	Roche Modular® P	U/L
P–Aspartate transaminase	AST IFCC (Roche)	C.f.a.s. (Roche)	Roche Modular® P	U/L
P–Bilirubin	BIL-T DPD (Roche)	C.f.a.s. (Roche)	Roche Modular® P	µmol/L
P–Calcium	Calcium (Roche)	C.f.a.s. (Roche)	Roche Modular® P	mmol/L
P–Cholesterol	CHOD-PAP (Roche)	C.f.a.s. (Roche)	Roche Modular® P	mmol/L
P–Creatine kinase	CK IFCC (Roche)	C.f.a.s. (Roche)	Roche Modular® P	U/L
P–Creatinine	CREA plus	C.f.a.s. (Roche)	Roche Modular® P	µmol/L
P–HDL-Cholesterol	HDL-C plus (Roche)	C.f.a.s. (Roche)	Roche Modular® P	mmol/L
P–Iron	Fe (Roche)	C.f.a.s. (Roche)	Roche Modular® P	µmol/L
P–Lactate dehydrogenase	LDH IFCC (Roche)	C.f.a.s. (Roche)	Roche Modular® P	U/L
P–LDL-Cholesterol	LDL-C plus (Roche)	C.f.a.s. (Roche)	Roche Modular® P	mmol/L
P–Magnesium	Mg (Roche)	C.f.a.s. (Roche)	Roche Modular® P	mmol/L
P–Phosphate	Phos (Roche)	C.f.a.s. (Roche)	Roche Modular® P	mmol/L
P–Potassium	ISE/Na^+, K^+, Cl^- (Roche)	ISE Internal Standard (Roche)	Roche Modular® ISE	mmol/L
P–Protein	TP (Roche)	C.f.a.s. (Roche)	Roche Modular® P	g/L
P–Sodium	ISE/Na^+, K^+, Cl^- (Roche)	ISE Internal Standard (Roche)	Roche Modular® ISE	mmol/L
P–Transferrin	Tina-quant Transferrin (Roche)	C.f.a.s. (Roche)	Roche Modular® P	µmol/L
P–Triglycerides	TG GPO-PAP	C.f.a.s. (Roche)	Roche Modular® P	mmol/L
P–Urate	UA plus (Roche)	C.f.a.s. (Roche)	Roche Modular® P	µmol/L

were all analyzed in singlicate within 3 h after thawing. Internal control samples were analyzed in the beginning, middle and at the end of the series. Analyses of samples were carried out according to normal procedures including acceptance of control values and rerun of samples if error flags were displayed. The study samples were analyzed in a total of 18 series over a period of 1 year.

Data analysis

Recalculation, using the mean of intra-serial determination of NFKK Reference Serum X

All reference values in the series were recalculated according to the formula $Rc = R \bullet T/Mr$ where Rc is the corrected reference value and R is the measured reference value, T is the certified or assigned value for NFKK Reference Serum X and Mr is the mean of the 10 replicate measurements of NFKK Reference Serum X in the series.

The statistical analysis was performed as previously published for the Nordic Reference Interval Project 2000 [3]. A simple non-parametric method was used to calculate low and high reference limits according to the 2.5 and 97.5 percentiles of the distribution of reference values. Calculations were done using either MS Excel® (Table III) or the computer program RefVal 4.0 [7] based on the IFCC recommendations (Table IV). Automatic outlier detection as incorporated in RefVal 4.0, using Dixon's range test, was used. The number of outliers detected is given in Table IV.

Partitioning of distribution was performed according to Lahti [8]. The criterion for no partitioning is that each sub-distribution should have a number of reference values between 0.9% and 4.1% outside the reference limit (2.5 or 97.5 percentile) of the common distribution.

Gender partitioning was mostly decided by use of this criterion. Reasonable age limits were estimated by 'qualified guessing' prior to exposure to the partitioning criteria. The age trend of reference intervals was evaluated by splitting the reference individuals into six age groups for each gender: 5–6 years, 7–8 years, 9–10 years, 11–13 years, 14–16 years and 17–19 years, and calculate and plot the reference intervals for each group using MS Excel®.

Ethical aspects

All children and parents received written information, and they were invited to an information meeting. All participants and their parents gave their informed consent. The study was approved by the regional ethics committees (KF 01 282214 and V200.1996/90) and the Danish Data Protection Agency (2010-41-5042).

Results

The low and high reference limits for each gender in the six age groups are shown in Table III and the suggested reference limits are shown in Table IV. No age- or gender-related differences were observed for

Table III. Reference limits (RL) in the 6 age groups.

Quantity	Age (years) Gender	5–6 RL	7–8 RL	9–10 RL	11–13 RL	14–16 RL	17–19 RL
Alanine transaminase U/L	All	10	9	8	8	7	7
		24	28	35	32	31	43
	Female	10	7	8	8	7	6
		24	35	32	33	32	31
	Male	7	9	10	8	10	12
		24	28	37	37	31	64
Albumin g/L	All	36.1	39.5	40.1	39.4	35.8	34.8
		45.6	47.0	47.4	47.5	48.4	49.9
	Female	37.6	39.7	40.4	39.0	34.8	32.4
		46.2	47.2	47.9	47.6	47.9	49.2
	Male	35.1	39.1	39.2	39.8	38.8	40.3
		45.3	47.1	47.1	47.5	49.5	50.9
Alkaline phosphatase U/L	All	144	154	163	121	61	41
		367	358	404	455	376	176
	Female	149	157	166	119	49	39
		363	365	423	402	291	106
	Male	127	149	159	150	100	55
		370	345	365	492	402	242
Aspartate transaminase U/L	All	26	25	22	19	15	14
		47	46	46	47	41	49
	Female	27	25	22	18	13	14
		47	46	43	45	34	35
	Male	22	25	22	20	17	14
		47	46	50	51	46	83
Bilirubin µmol/L	All	2	3	3	4	4	4
		13	17	20	20	28	30
	Female	2	3	3	3	4	3
		14	17	20	19	22	15
	Male	2	3	4	4	5	5
		9	22	21	21	35	47
Calcium mmol/L	All	2.17	2.28	2.27	2.26	2.08	1.94
		2.55	2.59	2.59	2.57	2.58	2.57
	Female	2.21	2.26	2.28	2.26	2.08	1.79
		2.57	2.60	2.60	2.55	2.57	2.59
	Male	2.12	2.28	2.25	2.26	2.09	2.14
		2.55	2.54	2.56	2.59	2.60	2.60
Cholesterol mmol/L	All	2.69	2.74	2.66	2.69	2.29	2.56
		5.37	5.36	5.41	5.52	5.54	5.58
	Female	3.04	2.74	2.66	2.63	2.29	2.56
		5.37	5.43	5.95	5.51	5.36	5.65
	Male	2.37	2.82	2.65	2.84	2.28	2.50
		5.82	5.24	5.31	5.60	5.67	5.33
Creatine kinase U/L	All	83	65	70	57	50	46
		230	277	277	303	724	668
	Female	78	63	73	57	46	38
		207	252	253	296	337	601
	Male	86	65	62	54	66	81
		291	321	356	332	786	1363
Creatinine µmol/L	All	25	28	31	36	42	44
		43	50	58	64	81	91
	Female	26	28	33	35	41	40
		43	51	58	62	77	81
	Male	23	28	31	39	47	57
		45	50	59	67	82	95
HDL-Cholesterol mmol/L	All	0.9	1.0	0.9	1.0	0.8	0.9
		2.4	2.3	2.4	2.3	2.2	2.3
	Female	1.0	1.0	0.9	1.1	0.9	1.0
		2.5	2.3	2.3	2.3	2.3	2.4
	Male	0.8	0.9	0.9	1.0	0.8	0.9
		2.4	2.3	2.5	2.4	2.0	2.1
Iron µmol/L	All	5.2	7.2	7.8	6.9	7.8	5.5
		29.6	30.6	28.2	31.9	35.4	32.8
	Female	7.9	6.1	7.8	5.6	7.3	4.2
		31.1	31.5	28.2	33.7	35.4	34.2
	Male	4.3	6.9	7.8	8.1	8.7	7.7
		28.1	30.9	28.7	30.7	38.4	36.9

(Continued)

Table III. *(Continued)*.

Quantity	Age (years) Gender	5–6 RL	7–8 RL	9–10 RL	11–13 RL	14–16 RL	17–19 RL
Lactate dehydrogenase U/L	All	187	188	170	149	124	123
		313	356	319	320	272	272
	Female	200	186	170	145	119	112
		310	356	318	293	259	267
	Male	186	185	164	158	143	140
		314	381	324	340	272	308
LDL-Cholesterol mmol/L	All	1.1	1.1	1.3	1.3	0.7	1.1
		3.3	3.4	3.6	3.5	3.4	3.3
	Female	1.1	1.0	1.3	1.1	0.7	1.0
		3.3	3.6	3.9	3.5	3.3	3.3
	Male	1.1	1.1	1.4	1.4	0.7	1.0
		3.7	3.2	3.4	3.6	3.5	3.6
Magnesium mmol/L	All	0.69	0.74	0.73	0.73	0.69	0.66
		0.90	0.94	0.94	0.94	0.93	0.92
	Female	0.71	0.75	0.74	0.73	0.66	0.65
		0.92	0.95	0.93	0.94	0.94	0.92
	Male	0.69	0.73	0.72	0.73	0.71	0.69
		0.89	0.93	0.95	0.95	0.93	0.92
Phosphate mmol/L	All	1.11	1.16	1.08	1.07	0.80	0.74
		1.71	1.74	1.71	1.73	1.63	1.52
	Female	1.19	1.10	1.10	1.06	0.72	0.72
		1.75	1.68	1.73	1.72	1.50	1.49
	Male	1.03	1.21	1.05	1.06	0.81	0.90
		1.64	1.78	1.69	1.76	1.70	1.65
Potassium mmol/L	All	3.38	3.35	3.33	3.29	3.27	3.27
		4.11	4.18	4.20	4.27	4.32	4.29
	Female	3.44	3.29	3.31	3.27	3.27	3.24
		4.12	4.17	4.20	4.22	4.27	4.26
	Male	3.38	3.36	3.34	3.30	3.28	3.28
		4.00	4.23	4.32	4.35	4.40	4.35
Protein g/L	All	58.0	64.2	64.4	65.0	61.7	58.0
		76.8	80.6	81.3	81.2	84.3	83.2
	Female	58.6	64.0	65.8	65.8	62.3	56.1
		77.5	81.2	82.0	81.2	83.7	83.6
	Male	57.8	64.0	63.3	63.4	60.4	63.0
		76.2	77.0	79.8	82.0	84.6	83.6
Sodium mmol/L	All	132.3	136.0	136.9	136.6	135.5	133.7
		144.8	145.6	147.3	147.5	146.6	147.6
	Female	133.2	135.7	136.3	136.4	135.5	131.8
		145.8	145.4	147.3	146.0	147.4	147.8
	Male	132.3	135.7	137.0	136.9	136.2	134.3
		144.4	145.9	147.8	148.9	145.6	146.1
Transferrin µmol/L	All	2.12	2.14	2.14	2.27	2.07	2.10
		3.05	3.28	3.24	3.37	3.64	3.96
	Female	2.00	2.13	2.02	2.27	2.07	2.04
		3.29	3.25	3.21	3.40	3.80	4.16
	Male	2.16	2.13	2.25	2.25	2.08	2.12
		2.93	3.28	3.26	3.32	3.36	3.32
Triglycerides mmol/L	All	0.34	0.29	0.34	0.34	0.36	0.43
		1.79	1.82	1.66	2.21	2.28	2.43
	Female	0.40	0.29	0.31	0.42	0.38	0.40
		1.79	1.82	1.71	1.93	2.16	2.50
	Male	0.32	0.29	0.35	0.31	0.32	0.41
		1.86	1.72	1.51	2.41	2.68	3.97
Urate µmol/L	All	132	141	132	158	171	176
		306	302	322	377	449	413
	Female	122	137	144	157	159	168
		308	318	325	338	406	358
	Male	143	140	129	154	215	251
		284	300	317	401	472	443

cholesterol, LDL-Cholesterol, Potassium, Sodium or Aspartate transaminase. For many of the remaining components, partitioning into two age-groups at 14 years (Albumin, Bilirubin, Calcium, HDL-Cholesterol, Lactate dehydrogenase, Magnesium, Phosphate, Protein, Transferrin) or at 11 years (Iron, Triglycerides) were suggested, whereas partitioning into three age groups was proposed for Alanine transaminase, Creatine kinase, and Urate. For Alkaline phosphatase and Creatinine, four age-related reference intervals were suggested.

Gender differences were observed around the onset of puberty for Alkaline phosphatase, Bilirubin, Calcium, Creatinine, Creatine kinase, Iron, Phosphate, Triglycerides and Urate. High levels of Alanine transaminase and Aspartate transaminase was observed in the group of males aged 17–19 years using MS Excel® (Table III), but was not found in the oldest age group (14–18 years) using RefVal with an outlier test (Table IV).

Discussion

This study reports age- and gender-specific reference intervals, calculated using a non-parametric method, for 21 different components measured in plasma from 1421 healthy children and adolescents from the Copenhagen area. Establishing comprehensive paediatric reference intervals is a cumbersome task, in particular due to the difficulty in obtaining blood samples from healthy children. Therefore, laboratories frequently rely on published data, either from journals, textbooks or from manufacturers' kit inserts. Such reference intervals on healthy children are rarely complete, i.e. limited age intervals are examined, or both genders are not covered. A review of the literature in 2009, included in the CALIPER initiative, showed that comprehensive data only existed for a limited number of analytes [9]. Furthermore, data were often from older studies using equipment with lower precision than current technology provides, and applying other analytical methodologies, including different reagents and calibrators, all of which frequently hampers commutability of the reference intervals. This may lead to misinterpretation of results produced in a modern laboratory if such reference intervals are applied uncritically and may cause grave errors in diagnostics.

Subsequent studies in the CALIPER initiative have established paediatric reference intervals on the Abbott Architect ci8200 [10], the Vitros 5600 Integrated system [11] and the Cobas 6000 analyser [12], all using samples from children attending selected out-patient clinics, and deemed to lack metabolic diseases.

In contrast, our population was not associated to the medical health care-sector but consisted of healthy children and adolescents who were recruited in their schools.

The pre-analytical conditions chosen for the children in our study were comparable to those relevant for children attending outpatient clinics. Fasting was not required, physical exercise was not restricted except for resting for 10 min before blood sampling, and samples were drawn in a time slot of 5 h, beginning in the morning.

We were able to perform a large study since inclusion of far more than 120 subjects in all the suggested age groups (except for 5–6 years ($n = 84$) and 15–16 years ($n = 93$)) was achieved, as recommended in the CLSI guideline [13].

Furthermore, our study is unique in one particular aspect. The NORIP reference material (NFKK Reference Serum X) has certified values for 10 of the components examined (Albumin, Calcium, Cholesterol Creatinine, Iron, Potassium, Magnesium, Sodium, Triglycerides and Urate) and indicative values for another 10 components (Alkaline phosphatase, Alanine transaminase, Aspartate transaminase, Bilirubin, Creatine kinase, HDL-Cholesterol, Lactate dehydrogenase, Phosphate, Protein and Transferrin). By including NFKK Reference Serum X in all analytical runs, commutability of the reference intervals regarding the important variable factor, measurement method, was obtained. In the NORIP study this was achieved, as exemplified for the reference intervals for P-Albumin, analyzed on six different analytical platforms (http://pweb.furst.no//norip/). By comparing measured and assigned values on 'X', present biases could be detected and the measured values of the different quantities in the paediatric samples could be corrected accordingly before establishing the reference intervals.

The data were partitioned by age and gender by statistical analysis as previously published [8] and combined when possible into the suggested reference intervals. This partitioning by age was done somewhat arbitrarily and another approach could be to combine age groups less extensively and instead include all the reference intervals in the Laboratory Information System. Our data can be accessed online and recalculated and interpreted according to other statistical methods (http://www.furst.no/norip/ped).

The observed age- and gender-related differences for Creatinine and Alkaline phosphatase confirm textbook data [2] and the data for Uric acid and Creatine kinase is in agreement with previous studies (Roche Modular P and Abbott Ci8200 analyzers) [14,15]. For Bilirubin the high levels observed in boys older than 13 years were also found in the study by Chan [10].

LDL-Cholesterol levels and Magnesium were slightly lower than reported by Chan et al. [10]. For the lipids HDL-Cholesterol was higher, LDL-Cholesterol was lower, Cholesterol was similar and Triglycerides were lower in our study than in the study by Kulasingam et al. [12] – which may reflect differences in the population (geography, ethnicity, obesity, food intake and other cultural habits) in the

Table IV. Suggested reference limits. NORIP reference limits are shown for comparison.

Quantity	Gender	Age (years)	Estimated reference limits for children from this investigation								Adults NORIP reference limits		
			Low	CiI	CiH	n/ outliers	High	CiI	CiH	n/ outliers	Age (years)	Low	High
Alanine transaminase U/L	Female	5–18	8	8	8	1419/1	32	29	35	823	≥18	10	45
	Male	5–8					27	25	35	129			70
		9–13					37	29	44	322			
		14–18					47	33	69	145/1			
Albumin g/i	Female	5–13	39	38	40	610/1	47	47	48	1268	18–39	36	48
		14–18	35	31	35	207							
	Male	5–13	39	38	39	595							
		14–18					50	49	53	144			
Alkaline phosphatase U/L	Female	5–13	143	120	155	613	396	381	434	–	≥18	35	105
		14–16	42	38	43	211	288	–	–	106			
		17–18					102	–	–	105			
	Male	5–8	151	141	157	450	351	331	398	129			
		9–13					457	415	515	321			
		14–16	96	–	–	83	412	–	–	–			
		17–18	56			62	238	–	–	–			
Aspartate transaminase U/L	Female	5–18	17	16	18	1420/1	46	44	47	–	≥18	15	35
	Male	5–18											45
Bilirubin μmol/L	Female	5–18	3	3	3	1415/1	18	17	20	822/1	≥18	5	25
	Male	5–13					20	16	24	447			
		14–18					40	29	55	145			
Calcium mmol/L	Female	5–13	2.26	2.23	2.28	611	2.58	2.57	2.59	1412	≥18	2.15	2.51
		14–18	1.95	1.72	2.03	210							
	Male	5–13	2.22	2.18	2.26	447							
		14–18	2.10	2.06	2.23	144							
Cholesterol mmol/L	Both	5–18	2.7	2.6	2.7	1418	5.5	5.3	5.6	–	18–29	2.9	6.1
Creatine kinase U/L	Female	5–13	56	52	59	1415	254	237	318	611	≥18	35	210
		14–16					334	–	–	106			
		17–18					600	–	–	105			
	Male	5–13					310	282	386	449	18–49	50	400
		14–18					945	702	1446	144			
Creatinine μmol/L	Female	5–8	28	24	29	182	50	48	64	–	≥18	45	90
		9–10	32	28	34	202	58	56	63	–			
		11–13	34	33	36	226	62	59	65	–			
		14–18	41	37	46	156	80	78	86	–			
	Male	5–8	26	22	29	128	49	47	51	–		60	105
		9–10	31	26	35	157	59	56	67	–			
		11–13	39	37	39	164	68	64	75	–			
		14–18	52	–	–	111	93	–	–	–			
HDL–Cholesterol mmol/L	Female	5–18	1.0	0.9	1.0	1273	2.3	2.3	2.4	–	≥18	1.0	2.7
	Male	5–13											
		14–18	0.8	0.5	0.9	145	2.0	1.9	2.1	–			
Iron μmol/L	Female	5–10	7.7	5.5	8.8	385	29.3	27.8	31.9	–	≥18	9	34
		11–18	6.3	4.0	7.1	437	33.4	30.2	36.1	–			
	Male	5–10	6.18	4.36	8.19	284	29.79	27.48	32.3	–			
		11–18	8.34	7.86	8.85	309	32.39	31.14	36.35	–			
Lactate dehydrogenase U/L	Both	5–13	157	149	167	1062	327	316	351	–	18–69	105	205
		14–18	121	118	130	356	271	263	294	–			
LDL–Cholesterol mmol/L	Both	5–18	1.1	1.1	1.2	1420	3.4	3.4	3.6	–	18–29	1.2	4.3
Magnesium mmol/L	Female	5–13	0.73	0.72	0.74	611	0.93	0.93	0.94	1415	≥18	0.71	0.94
		14–18	0.65	0.57	0.68	211							
	Male	5–18	0.71	0.70	0.73	593							

(Continued)

Table IV. *(Continued)*.

Quantity	Gender	Age (years)	Estimated reference limits for children from this investigation								Adults NORIP reference limits		
			Low	Cil	Cih	n/ outliers	High	Cil	Cih	n/ outliers	Age (years)	Low	High
Phosphate mmol/L	Female	5–13	1.09	1.06	1.13	612/1	1.72	1.70	1.74	–	≥18	0.85	1.5
		14–18	0.72	0.69	0.79	209	1.49	1.45	1.52	–			
	Male	5–13	1.07	1.03	1.10	449	1.74	1.71	1.78	594	18–49	0.75	1.65
		14–18	0.85	0.79	0.96	145							
Potassium mmol/L	Both	5–18	3.26	3.20	3.29	1419	4.29	4.26	4.41	–	≥18	3.6	4.6
Protein g/L	Both	5–13	63	63	64	1062	81	80	82	–	≥18	62	78
		14–18	60	56	62	356	84	83	85	–			
Sodium mmol/L	Both	5–18	135	135	136	1375	147	146	148	–	≥18	137	145
Transferrin μmol/L	Female	5–13	2.13	2.11	2.17	1420	3.31	3.27	3.33	1209	≥18	1.95	3.31
		14–18					3.93	3.74	4.34	211			
	Male	5–18					3.31	3.27	3.33	1209			
Triglycerides mmol/L	Female	5–18	0.34	0.32	0.36	1419	1.95	1.78	2.17	823	≥18	0.45	2.60
	Male	5–10					1.58	1.50	1.86	287/1			
		11–18					2.54	2.18	3.08	309			
Urate μmol/L	Female	5–13	140	132	150	613	328	318	337	–	18–49	155	350
		14–18	163	142	176	210	376	348	417	–			
	Male	5–10	133	122	144	451	311	289	345	287	≥18	230	480
		11–13					401	379	452	164			
		14–18	223	173	248	145	462	427	487	–			

Cil: Confidence interval low. Cih: Confidence interval high.

two studies [12]. Traditional (2.5–97.5 percentiles) reference intervals are, however, less clinically relevant for lipids and lower percentiles are often presented [16].

Comparison of reference intervals in the youngest age group from the NORIP study with those of the oldest age group in the present study (Table IV) shows similar values for Albumin, Calcium, Iron, Magnesium, Phosphate, Protein, Sodium, Transferrin, Triglycerides and Urate. For Alkaline phosphatase and Lactate dehydrogenase the levels in our group were higher than those of the NORIP group – in agreement with the observed decrease during adolescence. The opposite pattern was observed for Creatinine in agreement with Creatinine levels increasing with muscle mass. For Cholesterol, LDL-Cholesterol and HDL-Cholesterol the upper limits were lower in our study compared to the NORIP groups. Whether these differences in reference limits could reflect differences in the two populations is unresolved. For LDL-Cholesterol the discrepancy could also be due to method differences since Friedewald's formula for calculation of LDL-Cholesterol was used in the NORIP study, whereas we used direct measurement. For Bilirubin and Aspartate transaminase the upper limits for males ($n = 144$) in our study was slightly higher than in the NORIP group and for Creatine kinase much higher – for both genders. The data for Creatine kinase in our oldest age groups contrast with those presented in Soldin's textbook [2], but are in agreement with those reported by Clifford et al. [14]. Differences in physical activity prior to blood sampling may explain some of the differences observed in Creatine kinase concentrations.

In conclusion the reference intervals reported in the present study could be applied not only for laboratories using the same equipment and reagents as we have used, but could be used, irrespective of instrumentation, for the 10 components with certified values in the NFKK Reference Serum X. However, reference intervals should in all cases be appropriately validated in the laboratory since commutability may be hampered by population differences as well as undetected measurement errors. This is also the case for the reference intervals obtained for the components where NFKK Reference Serum X only has indicative values – and for LDL-cholesterol where reference material was not co-measured with the subject samples.

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