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Evaluation of reference intervals of haematological and biochemical markers in an Austrian adolescent study cohort

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Abstract

Background: Reference intervals are a prerequisite for the interpretation of laboratory data related to diagnostic issues and treatment strategies. In adolescents, biomarker concentrations change with age, necessitating a continuous age-related definition of the reference intervals. The purpose of this pilot study was to evaluate the reference intervals for a healthy population of adolescents in Salzburg and compare these, when possible, with age- and gender-matched published data.

Methods: Anthropometrical parameters and blood samples were collected from adolescents (male and female; 14–17 years) in a school setting. Haematological samples were measured using Sysmex XS-1000i, lipid and carbohydrate metabolism markers as well as enzymes and hormones were determined by Cobas c311, Vitros ECiQ® or ELISA. The reference intervals were calculated according to the CLSI guidelines C28-A3c.

Results: Samples of 102 participants were included. Compared to age- and gender-matched reference intervals, the BMI levels were in the lower normal rage. Most haematological parameters and biomedical makers reveal similar ranges to values published in other studies.

Conclusions: This data analysis allowed for a partial comparison of reference values with published data and enabled a new determination of paediatric reference intervals for an Austrian cohort.

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Keywords: adolescents; laboratory parameters; paediatrics; post-analytics; reference intervals.

Introduction

Reference intervals of specific biomarkers are used to distinguish between healthy and diseased people. This enables an accurate interpretation of biomedical laboratory tests, which is a prerequisite for the proper treatment of patients. Reference intervals and target values are not only used for diagnosis, but also to monitor therapeutic treatments and to screen for risks of disease. Therefore, the continuous evaluation and fine-tuning of reference intervals, based on age-, gender- and ethnic-specific variations as well as on different methods, are essential. Physiological changes in biomedical parameters occur with age and are especially pronounced during childhood and adolescence due to constant growth, development as well as maturation, thereby causing alterations in metabolic processes [1–3]. Thus, the establishment and validation of paediatric reference values is of high relevance. Studies like the Children's Health Improvement through Laboratory Diagnostics program, the Lifestyle of Our Kids (LOOK) program, the German Health Interview and Examination Survey for Children and Adolescents (KiGGS), the Canadian Laboratory Initiative on Paediatric Reference Intervals (CALIPER) project (reviewed in [2]) as well as the Identification and prevention of Dietary- and lifestyle-induced health Effects in Children and infants (IDEFICs) project [4] contribute to meet this demand and close gaps in paediatric laboratory medicine. Although these studies all have a similar aim, the study protocols differ from each other. Nevertheless, the common denominator is the evaluation of reference intervals of different biomedical markers for age groups between 0 and 18 years. Recently, the MoYo (Motivating Young people to maintain a healthy life-style) project was performed. This was a school-based pilot study with an interventional design performed in Salzburg (Austria) from 2014 to 2015 [5]. It has provided baseline data from healthy adolescents at the age of

14–17 years and facilitated an "*a priori*" approach for the evaluation of reference values. The purpose of this paper is, to evaluate the reference intervals of haematological and biochemical laboratory parameters for local, Caucasian adolescents in Salzburg, and to compare these with available paediatric reference data.

Materials and methods

The anthropometric and biomedical data were obtained from the baseline data of the MoYo-pilot project, which was performed in schools and therefore in a non-clinical setting. In total, 110 per se healthy adolescents of Caucasian origin in the age group of 14–17 years participated. All but one indicated at the beginning of the study that their health status was good and that they did not have any known diseases. For 102 adolescents (63 males, 39 females) haematological parameters as well as carbohydrate and lipid metabolism markers, hormones, vitamins and micronutrients were determined. A full list with all the analysed parameters can be found in Table 1. All participants and their legal guardians gave informed consent to participate in this study. This informed consent could be withdrawn at any point of the study and beyond and included the deletion of all available data when required. The study design was approved by the local Ethics Committee (county of Salzburg, Austria) and meets the Declaration of Helsinki.

Venipuncture and anthropometric measurements were carried out between 07:30 and 08:30 AM. The participants were instructed not to eat or drink anything except for water at least 10 h before the day of the blood sample collection. Blood samples were drawn using a 21G Safety blood collection set (Ref. 450081, Greiner bio-one GmbH, Kremsmünster, Austria) in combination with Vacuette tubes for serum (Ref. 455092, Greiner bio-one GmbH, Kremsmünster, Austria), EDTA- (Ref. 454086, Greiner bio-one GmbH, Kremsmünster, Austria) and Li-Heparin-plasma (Ref. 454083, Greiner bio-one GmbH, Kremsmünster, Austria) and handled according to pre-analytical guidelines [6]. Serum samples were stored at room temperature in an upright position for 30 min to allow the sample to fully clot before further processing. Serum and plasma samples were centrifuged at $1500 \times g$ for 10 min at 22 °C.

Haematological parameters were measured using EDTA-samples on the XS-1000i instrument (Sysmex GmbH, Vienna, Austria). Clinical chemistry parameters, such as carbohydrate and lipid metabolism markers, hormones, vitamins and micronutrients were determined from serum using either the Cobas c311 (Roche Diagnostics, Switzerland), the Vitros ECiQ® (Assista, Vienna, Austria) or diagnostically approved ELISAs (IBL International GmbH [Hamburg, Germany] and Biovendor GesmbH [Vienna, Austria]). Assessment of the optical density of these assays was performed on the Infinite 200 pro plate reader (Tecan, Grödig, Austria) (Table 1).

Anthropometric measurements for evaluating body mass index (BMI), waist-to-hip ratio (WHR) and waist-to-height ratio (WHtR) were performed with a portable calibrated stadiometer, a measuring tape and a segmental body composition analyser (Tanita BC-418, Tanita Corporation of America, Inc., IL, USA) commonly used in clinical research.

For biometric data acquisition, Excel 2010 (Microsoft Corporation, WA, USA) was used. The BMI was calculated as the ratio of the body weight to the square of body height (kg/m^2). The WHR was calculated by dividing waist circumference by hip circumference and WHtR by dividing waist circumference by body height. Laboratory data were managed and validated by the laboratory software GLIMS (MIPS Diagnostics Intelligence Europe, Gent, Belgium).

For statistical analyses, the data were imported to IBM SPSS Statistics version 25 (IBM Corporation, NY, USA) and descriptive und inference statistical analyses were performed. The reference intervals were calculated according to the CLSI guidelines C28-A3c [7] after the data were normalized and outliers were identified and excluded using the Grubbs test. Before the 2.5 and the 97.5 percentiles were calculated, the data were weighted with respect to gender proportion (number of cases 120). In addition, the 90% confidence intervals (CIs) were computed for the upper and lower limit of the reference interval $\pm 1.64 \times SD/\sqrt{n}$. Differences between males and females were analysed conducting a multivariate analysis of variance.

Table 1: Measurement of laboratory parameters.

Measurement device	Reagents (Inc.)	Sample	Parameter
XS-1000i (Sysmex GmbH, Vienna, Austria)	Sysmex	EDTA	Erythrocytes, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, platelets, leucocytes, segmented neutrophils, lymphocytes, monocytes, eosinophils, basophils
Cobas c311 (Roche, Mannheim Germany)	Roche	Serum	Glucose, triglycerides, total cholesterol, LDL-cholesterol, HDL-cholesterol, apolipoprotein A1, apolipoprotein B, lipoprotein (a), hs-CRP, GOT (ASAT), GPT (ALAT), GGT, creatinine, cystatin-C, albumin, uric acid, urea, zinc, ferritin
	Roche	EDTA	HbA_{1c}
Vitros ECiQ® (Assista, Vienna, Austria)	Ortho Clinical Diagnostics	Serum	TSH, fT3, fT4, vitamin B12, folic acid, vitamin D, testosterone, oestrogene, FSH, LH, prolactin
Plate reader infinite 200-pro (Tecan, Grödig, Austria)	IBL	Serum	Insulin, proinsulin, C-peptide, SHBG
	Biovendor	Serum	Adiponectin

Standard equipment was used in combination with the corresponding test kits for clinical-chemical analysis.

Results

The analysis of the biometrical data showed that the BMI is distributed according to the age- and gender-matched reference range. In males, the median BMI was 20.37 (25 percentile 18.73 and 75 percentile 22.67), in females the median BMI was 20.52 (25 percentile 18.14 and 75 percentile 22.74). The WHR was normally distributed in males (mean WHR ± SD = 0.85 ± 0.05), but not in females (median WHR = 0.77 (25 percentile 0.74 and 75 percentile 0.80). The evaluated median WHtR for male was 0.42 (25 percentile 0.40 and 75 percentile 0.44) and for female 0.41 (25 percentile 0.39 and 75 percentile 0.44).

The calculated reference intervals, including the 90% CI of the lower and upper limit from investigated laboratory parameters, are listed in Table 2. According to the Grubbs outlier test, 17 individuals (11 boys and 6 girls) with a higher score than the critical Grubbs cut-off in at least one parameter were excluded. In addition, it should be mentioned that one male diabetes patient in the age of 15 years participated in the study, who was also excluded from the calculation of reference ranges. To compare these with reference intervals of similar study populations (age, gender and ethnicity), a survey for published reference intervals was performed. The results for the reference intervals for the Austrian adolescents as determined in the presented study and those published of matched age ranges [3, 8–12] are shown in Table 3.

Discussion

The MoYo-project was performed as a feasibility study for health screening in a non-clinical setting and health promotion in schools, including an interventional study design. The implementation of sampling for anthropometrical and biomedical measurements in the school setting was successfully established and samples of healthy adolescents were analysed for haematological and biochemical parameters (Table 1). Even though the number of cases was comparatively low for the analysis in terms of gender-matched groups, the data set provides a good basis for the calculation of paediatric reference intervals according to the CLSI guidelines [7].

Over the last 15 years, different studies assessing paediatric reference intervals have been published. These evolved from projects like KiGGS (Germany; 1–18 years), CALIPER (Canada; 0–18 years), NHANES (US; all age groups), LOOK (Australia; 8, 10 and 12 years), AACB (Australia and New Zealand; all age groups), COPENHAGEN (Denmark; 5–20 years) (reviewed in [2]) and IDEFICS (Italy, Spain, Germany, Belgium, Sweden, Hungary and Estonia; 2–11 years) [4].

The age- and gender-based evaluation of the BMI of the Austrian adolescents investigated shows that in comparison to German [13] and Greek [14] adolescents the Austrian cohort had a slightly lower BMI. This highlights that the study cohort is within the healthy range of BMI. The current WHO BMI tables are based on age- and gender-specific percentiles and z-scores (http://www.who.int/growthref/who2007_bmi_for_age/en/). These were derived by pooling the data sets of the Health Examination Survey, and the Health and Nutrition Examination Survey [15]. However, an age-, gender- and percentile-based BMI value calculation requires a different study set-up and was beyond the scope of the present study. The values for WHR and WHtR are provided as well, as these are described as discriminators for cardiovascular risk factors and metabolic syndrome diagnosis [16].

The present biomedical data analysis represents a synopsis of reference values for haematological and clinical biochemical parameters of adolescents in Europe and the Anglo-American region. Both the MoYo data set as well as the CALIPER data set [10–12] were derived from healthy and normal-weight adolescents and were analysed "*a priori*". As ethnical, environmental and lifestyle circumstances can influence the evaluation and establishment of reference intervals and the data of the CALIPER study are based on different ethnic groups [10], the MoYo data add value in regard of validation of reference intervals for Caucasian adolescents in the age of 14–17 years.

In terms of haematological data, Zierk et al. [8, 17] used a two-fold different approach: first, Zierk et al. used an indirect approach by retrospectively evaluating haematological parameters from the laboratory database of the University Children's Hospital Erlangen containing healthy and pathologic samples for data analysis; and secondly, they calculated continuous reference intervals to meet the demands of variations in age and sex. In the first study [8], they compared the reference intervals for erythrocytes, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and platelets to the 2.5th and 97.5th percentile (95% CI) of the data retrieved from the KiGGS study, which investigated, among others, haematological parameters in a cohort representative for healthy 1–18 year old German children "*a priori*" [18] (Table 3). The analysis of the haematological parameters in the different studies was performed either using a Sysmex haematological analyser [5, 8] or a Coulter STKS haematological flow cytometer [9], and the results are in the same range. However, although the continuous

Table 2: Calculation of reference intervals (2.5–97.5 percentile [$P_{2.5\%}$–$P_{97.5\%}$]) from the baseline data of the MoYo-study based on the CLSI guidelines C28-A3c.

Parameter	All						Male						Female					
	$P_{2.5\%}$	CI lower $P_{2.5\%}$	CI upper $P_{2.5\%}$	$P_{97.5\%}$	CI lower $P_{97.5\%}$	CI upper $P_{97.5\%}$	$P_{2.5\%}$	CI lower $P_{2.5\%}$	CI upper $P_{2.5\%}$	$P_{97.5\%}$	CI lower $P_{97.5\%}$	CI upper $P_{97.5\%}$	$P_{2.5\%}$	CI lower $P_{2.5\%}$	CI upper $P_{2.5\%}$	$P_{97.5\%}$	CI lower $P_{97.5\%}$	CI upper $P_{97.5\%}$
Erythrocytes, $\times10^6/\mu L$*	3.99	3.93	4.05	5.97	5.91	6.03	4.62	4.57	4.66	6.04	5.99	6.08	3.83	3.78	3.88	5.14	5.09	5.19
Haemoglobin, g/dL*	11.74	11.54	11.94	16.48	16.28	16.68	13.22	13.08	13.36	16.70	16.56	16.84	9.00	8.84	9.16	14.60	14.44	14.76
HCT, %*	36.08	35.60	36.56	48.06	47.58	48.54	39.04	38.66	39.43	48.88	48.49	49.26	29.20	28.81	29.59	42.70	42.31	43.09
MCV, fL*	76.18	75.53	76.84	93.42	92.76	94.07	76.39	75.89	76.89	88.92	88.42	89.42	66.70	65.90	67.50	95.30	94.50	96.10
MCH, pg*	25.44	25.20	25.68	31.18	30.94	31.42	25.90	25.72	26.08	31.26	31.07	31.44	20.50	20.20	20.80	31.10	30.80	31.40
MCHC, g/dL*	31.64	31.50	31.78	35.76	35.62	35.90	33.22	33.11	33.32	35.83	35.72	35.93	30.80	30.65	30.95	35.40	35.25	35.55
Platelets, $\times10^3/\mu L$	157.21	150.69	163.73	354.40	347.87	360.92	152.04	145.34	158.75	352.85	346.14	359.55	175.00	168.89	181.11	366.00	359.89	372.11
Leucocytes, $\times10^3/\mu L$	3.52	3.25	3.78	11.44	11.17	11.70	3.90	3.63	4.16	11.24	10.98	11.51	3.48	3.22	3.74	12.00	11.74	12.26
Segmented neutrophils, %	37.24	35.83	38.66	76.30	74.88	77.72	38.81	37.42	40.20	75.18	73.79	76.57	36.60	35.24	37.96	76.80	75.44	78.16
Segmented neutrophils, $\times10^3/\mu L$*	1.70	1.46	1.94	8.26	8.02	8.49	1.79	1.56	2.03	8.03	7.80	8.27	1.67	1.44	1.91	9.22	8.98	9.45
Lymphocytes, %	16.04	14.84	17.24	51.00	49.80	52.20	17.56	16.37	18.76	50.60	49.40	51.79	15.90	14.74	17.06	51.20	50.04	52.36
Lymphocytes, $\times10^3/\mu L$	1.27	1.20	1.34	3.15	3.08	3.22	1.38	1.32	1.45	3.26	3.19	3.33	0.94	0.87	1.01	2.94	2.87	3.01
Monocytes, %	4.32	4.13	4.51	9.28	9.09	9.47	4.50	4.31	4.69	9.64	9.45	9.83	4.10	3.91	4.29	9.10	8.91	9.29
Monocytes, $\times10^3/\mu L$	0.22	0.20	0.24	0.83	0.81	0.85	0.23	0.21	0.26	0.86	0.84	0.88	0.22	0.20	0.24	0.65	0.64	0.67
Eosinophils, %*	0.70	0.23	1.17	14.28	13.81	14.75	0.76	0.24	1.28	14.41	13.89	14.93	0.70	0.36	1.04	9.40	9.06	9.74
Eosinophils, $\times10^3/\mu L$*	0.04	0.01	0.07	1.04	1.01	1.07	0.07	0.03	0.10	1.10	1.07	1.14	0.03	0.01	0.05	0.59	0.57	0.61
Basophils, %	0.10	0.05	0.15	1.38	1.33	1.43	0.17	0.12	0.22	1.36	1.31	1.41	0.10	0.06	0.14	1.40	1.36	1.44
Basophils ($\times10^3/\mu L$)*	0.009	0.006	0.011	0.088	0.085	0.090	0.010	0.007	0.013	0.088	0.085	0.091	0.008	0.006	0.011	0.062	0.060	0.065
Glucose, mg/dL*	68.20	67.30	69.10	93.80	92.90	94.70	68.00	67.07	68.93	95.13	94.20	96.07	69.00	68.26	69.74	88.00	87.26	88.74
HbA_{1c}, %	4.70	4.67	4.73	5.60	5.57	5.63	4.87	4.84	4.90	5.60	5.57	5.63	4.60	4.57	4.63	5.60	5.57	5.63
Insulin, µIU/mL	6.96	6.39	7.53	22.45	21.87	23.02	7.24	6.68	7.80	21.47	20.92	22.03	6.93	6.33	7.53	24.87	24.27	25.47
Proinsulin, pmol/L	0.62	0.37	0.86	8.34	8.09	8.58	0.55	0.30	0.80	8.89	8.64	9.13	0.84	0.61	1.07	7.80	7.57	8.03
Triglycerides, mg/dL	28.81	24.15	33.47	150.80	146.14	155.46	30.87	26.15	35.59	140.96	136.24	145.68	26.00	21.50	30.50	158.00	153.50	162.50
Total cholesterol, mg/dL	110.01	105.88	114.15	232.98	228.84	237.11	120.00	116.79	123.21	214.28	211.07	217.50	104.00	98.73	109.27	255.00	249.73	260.27
LDL-cholesterol, mg/dL	51.62	47.76	55.48	156.21	152.36	160.07	56.69	53.47	59.91	146.94	143.72	150.16	44.00	39.33	48.67	170.20	165.53	174.87
HDL-cholesterol, mg/dL*	35.20	33.45	36.95	82.19	80.44	83.95	34.43	32.72	36.15	81.26	79.55	82.98	36.00	34.26	37.74	83.00	81.26	84.74
Lipoprotein (a), mg/dL	0.00	−6.42	6.42	171.39	164.97	177.81	0.00	−5.89	5.89	151.37	145.48	157.26	0.00	−7.13	7.13	181.00	173.87	188.13
ApoA1, mg/dL*	115.65	112.36	118.94	208.34	205.05	211.63	121.55	118.70	124.39	207.67	204.82	210.51	114.00	110.27	117.73	222.00	218.27	225.73
ApoB, mg/dL	42.80	40.24	45.37	115.84	113.27	118.41	47.55	45.25	49.84	115.43	113.13	117.73	41.40	38.46	44.34	125.40	122.46	128.34
Adiponectin, µg/mL	5.34	4.83	5.85	18.80	18.29	19.31	5.36	4.87	5.84	17.46	16.98	17.95	4.87	4.34	5.40	19.09	18.56	19.62
hs-CRP, mg/L	0.01	−0.15	0.17	4.32	4.16	4.48	0.00	−0.12	0.12	3.23	3.11	3.35	0.06	−0.14	0.26	6.79	6.59	6.99
TSH, mIU/L	0.89	0.77	1.02	4.13	4.00	4.25	1.02	0.89	1.14	4.51	4.39	4.64	0.51	0.39	0.63	4.15	4.03	4.27
FT3, pg/mL*	3.07	2.98	3.16	5.72	5.62	5.81	3.76	3.69	3.84	5.95	5.87	6.02	2.87	2.78	2.96	5.30	5.21	5.39
FT4, ng/dL	0.70	0.68	0.73	1.38	1.35	1.41	0.71	0.68	0.73	1.36	1.34	1.39	0.70	0.67	0.73	1.40	1.37	1.43

Table 2 (continued)

Parameter	All						Male						Female					
	$P_{2.5\%}$	CI lower $P_{2.5\%}$	CI upper $P_{2.5\%}$	$P_{97.5\%}$	CI lower $P_{97.5\%}$	CI upper $P_{97.5\%}$	$P_{2.5\%}$	CI lower $P_{2.5\%}$	CI upper $P_{2.5\%}$	$P_{97.5\%}$	CI lower $P_{97.5\%}$	CI upper $P_{97.5\%}$	$P_{2.5\%}$	CI lower $P_{2.5\%}$	CI upper $P_{2.5\%}$	$P_{97.5\%}$	CI lower $P_{97.5\%}$	CI upper $P_{97.5\%}$
Albumin, g/L*	42.32	41.79	42.85	57.98	57.45	58.51	44.20	43.70	44.70	58.68	58.18	59.18	40.30	39.77	40.83	54.80	54.27	55.33
Creatinine, mg/dL*	0.58	0.57	0.60	1.02	1.00	1.03	0.61	0.59	0.62	1.04	1.02	1.05	0.58	0.57	0.59	0.99	0.98	1.00
Urea (BUN), mg/dL*	12.54	11.76	13.32	34.18	33.40	34.96	16.87	16.15	17.60	34.30	33.57	35.03	12.40	11.67	13.13	30.30	29.57	31.03
Cystatin C, mg/L*	0.70	0.68	0.72	1.08	1.06	1.10	0.70	0.69	0.71	1.10	1.09	1.11	0.70	0.69	0.71	1.00	0.99	1.01
Uric acid, mg/dL*	3.22	3.04	3.40	7.94	7.76	8.12	4.04	3.90	4.19	8.00	7.85	8.15	2.80	2.69	2.91	5.80	5.69	5.91
AST, IU/L*	15.00	14.04	15.96	44.60	43.63	45.56	17.72	16.73	18.71	45.57	44.57	46.56	14.00	13.23	14.77	37.00	36.23	37.77
ALT, IU/L*	10.00	9.12	10.88	35.80	34.92	36.68	9.72	8.79	10.64	36.85	35.92	37.77	10.00	9.34	10.66	32.00	31.34	32.66
GGT, IU/L*	5.20	4.49	5.91	26.80	26.09	27.51	5.72	5.03	6.40	26.57	25.88	27.25	5.00	4.43	5.57	27.00	26.43	27.57
Vitamin B12, pmol/L	228.53	209.49	247.57	704.23	685.19	723.27	224.21	205.35	243.08	724.18	705.31	743.04	263.00	244.00	282.00	707.00	688.00	726.00
Folic acid, nmol/L	2.66	2.32	3.01	11.47	11.12	11.82	3.01	2.68	3.34	11.32	10.99	11.65	2.44	2.07	2.81	14.60	14.23	14.97
Vitamin D, ng/mL	17.12	15.95	18.29	43.88	42.70	45.05	17.35	16.17	18.52	44.20	43.02	45.38	17.00	15.87	18.13	44.40	43.27	45.53
Zinc, µg/dL*	9.20	4.38	14.03	117.59	112.76	122.42	8.59	3.72	13.45	119.00	114.13	123.87	9.00	4.52	13.48	101.00	96.52	105.48
Ferritin, ng/mL*	5.50	3.22	7.77	69.80	67.52	72.07	11.60	9.43	13.77	73.06	70.89	75.22	3.28	1.19	5.37	51.70	49.61	53.79
Testosterone, nmol/L*	0.45	−0.78	1.68	28.30	27.07	29.53	0.82	−0.21	1.86	29.74	28.71	30.78	0.44	0.36	0.52	2.81	2.73	2.89
Oestradiol, pmol/L*	25.20	13.34	37.07	380.28	368.42	392.15	25.44	21.14	29.74	141.36	137.07	145.66	24.89	10.06	39.72	392.42	377.59	407.25
FSH, IU/L*	1.06	0.71	1.41	9.61	9.26	9.95	1.23	0.89	1.57	9.83	9.50	10.17	0.66	0.35	0.97	9.11	8.80	9.42
LH, IU/L*	0.41	−0.13	0.95	15.86	15.32	16.39	0.59	0.39	0.79	5.83	5.63	6.03	0.22	−0.49	0.93	21.50	20.79	22.21
Prolactin, ng/mL*	147.26	133.86	160.65	561.57	548.18	574.96	143.57	130.40	156.73	517.29	504.12	530.45	165.60	152.63	178.57	578.60	565.63	591.57
SHBG, nmol/L*	12.11	6.36	17.86	149.09	143.33	154.84	10.78	7.35	14.21	116.87	113.44	120.30	20.29	13.05	27.53	271.31	264.07	278.55

HCT, haematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; HbA_{1c}, haemoglobin A_{1c} (glycated haemoglobin); LDL, low density lipoprotein; HDL, high density lipoprotein; Apo, apolipoprotein; hs-CRP, high sensitive C-reactive protein; TSH, thyroid-stimulating hormone; FT, free thyroxine; BUN, blood urea nitrogen; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, γ-glutamyltransferase; vitamin D (total 25-OH vitamin D [D3+D2]); FSH, follicle stimulating hormone; LH, luteinising hormone; SHBG, sex hormone binding globulin. The age range is 14–17 years. Significant gender differences for the calculated reference intervals are indicated with asterisk (*) in the first column.

Table 3: Reference intervals derived from the MoYo-study compared to published, age- and gender-matched paediatric reference values.

Analyte	Male						
	Bogner et al.[a]		Soldin et al. [3]		CALIPER or other studies		SI units
	Reference values	Age, years	Reference values	Age, years	Reference values	Age, years	(CF US to SI)
Erythrocytes, $\times 10^6/\mu L$*	4.62–6.04	14–17	3.74–4.93	12 to <18	4.3–5.8 [8]	16	$\times 10^{12}/L$ ($\times 1$)
Haemoglobin, g/dL*	13.22–16.70	14–17	11.0–14.3	12 to <18	12.3–17.0 [8]	16	g/L ($\times 10$)
HCT, %*	39.04–48.88	14–17	31.4–41.0	12 to <18	36.3–50.2 [8]	16	Portion of 1 ($\times 0.01$)
MCV, fL*	76.39–88.92	14–17	80.8–86.56	12 to <18	76–95 [8]	16	%
MCH, pg*	25.90–31.26	14–17	28.2–30.5	12 to <18	26–31 [8]	16	fL
MCHC, g/dL*	33.22–35.83	14–17	34.2–35.6	12 to <18	31–36 [8]	16	g/L ($\times 10$)
Platelets, $\times 10^3/\mu L$	157.21–354.40	14–17	180–299	12 to <18	153–344.7 [8]	16	$\times 10^9/L$ ($\times 1$)
Leucocytes, $\times 10^3/\mu L$	3.52–11.44	14–17	5.24–9.74	12 to <18	4.2–12.2 [9]	14–19	$\times 10^9/L$ ($\times 0.001$)
Segmented neutrophils, %	37.24–76.30	14–17	43.2–76.7	12 to <18	n.a.	adol.	Portion of 1 ($\times 0.01$)
Segmented neutrophils, $\times 10^3/\mu L$*	1.79–8.03	14–17	2.73–6.68	12 to <18	1.6–6.1 [9]	14–19	$\times 10^9/L$ ($\times 0.001$)
Lymphocytes, %	16.04–51.00	14–17	8–41	12 to <18	n.a.	adol.	Portion of 1 ($\times 0.01$)
Lymphocytes, $\times 10^3/\mu L$	1.27–3.15	14–17	1.03–2.18	12 to <18	1.4–3.3 [9]	14–19	$\times 10^9/L$ ($\times 0.001$)
Monocytes, %	4.32–9.28	14–17	4–8	12 to <18	n.a.	adol.	Portion of 1 ($\times 0.01$)
Monocytes, $\times 10^3/\mu L$	0.22–0.83	14–17	0.18–0.78	12 to <18	0.27–0.87 [9]	14–19	$\times 10^9/L$ ($\times 0.001$)
Eosinophils, %*	0.76–14.41	14–17	2–4	12 to <18	n.a.	adol.	Portion of 1 ($\times 0.01$)
Eosinophils, $\times 10^3/\mu L$*	0.07–1.10	14–17	0.04–0.20	12 to <18	0.05–0.61 [9]	14–19	$\times 10^9/L$ ($\times 0.001$)
Basophils, %	0.10–1.38	14–17	0–1	12 to <18	n.a.	adol.	Portion of 1 ($\times 0.01$)
Basophils, $\times 10^3/\mu L$*	0.010–0.088	14–17	0.01–0.05	12 to <18	0.01–0.18 [9]	14–19	$\times 10^9/L$ ($\times 0.001$)
Glucose, mg/dL*	68.00–95.13	14–17	54–117	>7 days	n.a.[b]	adol.	mmol/L ($\times 0.0555$)
HbA_{1c}, %	4.70–5.60	14–17	4–7	All	n.a.[b]	adol.	Proportion of total haemoglobin ($\times 0.01$)
Insulin, µIU/mL	6.96–22.45	14–17	7.1–66.1	15–18	2.2–49.6[b]	6 to <19	pmol/L ($\times 6.945$)
Proinsulin, pmol/L	0.62–8.34	14–17	n.a.	adol.	n.a.[b]	adol.	pmol/L
Triglycerides, mg/dL	28.81–150.80	14–17	38–243	≥1 to ≤20	44–197 [10]	1 to <19	mmol/L ($\times 0.0113$)
Total cholesterol, mg/dL	110.01–232.98	14–17	95–208	>15 to ≤20	112–208 [10]	1 to <19	mmol/L ($\times 0.0259$)
LDL-cholesterol, mg/dL	51.62–156.21	14–17	50–154	≥1 to ≤20	n.a.[b]	adol.	mmol/L ($\times 0.0259$)
HDL-cholesterol, mg/dL*	34.43–81.26	14–17	25–68	>15 to ≤20	32–6 [10]	13 to <19	mmol/L ($\times 0.0259$)
Lipoprotein (a), mg/dL	<171.39	14–17	n.a.	adol.	n.a.[b]	adol.	µmol/L ($\times 0.0357$)
ApoA1, mg/dL*	121.55–207.67	14–17	88–164	>15 to ≤20	72–154 [10]	14 to <19	g/L ($\times 0.01$)
ApoB, mg/dL	42.80–115.84	14–17	38–105	≥1 to ≤20	31–84 [10]	6 to <19	g/L ($\times 0.01$)
Adiponectin, µg/mL	5.34–18.80	14–17	n.a.	adol.	n.a.[b]	adol.	µg/mL
hs-CRP, mg/L	0.01–4.32	14–17	0.4–7.9	15–18	0.1–1.7 [10]	15 to <19	nmol/L ($\times 9.524$)
TSH, mIU/L	0.89–4.13	14–17	0.5–4.5	0.5–18	0.47–3.41 [11]	14 to <19	mIU/L
FT3, pg/mL*	3.76–5.95	14–17	n.a.	adol.	2.25–3.85 [11]	15 to <19	pmol/L ($\times 1.54$)
FT4, ng/dL	0.70–1.38	14–17	0.8–2.0	31 days–18	0.89–1.37 [11]	1 to <19	pmol/L ($\times 12.87$)
Albumin, g/L*	44.20–58.68	14–17	37–56	10–19	38–50 [10]	15 to <19	g/L
Creatinine, mg/dL*	0.61–1.04	14–17	0.6–1.0	15–20	0.45–1.08 [10]	12 to <19	µmol/L ($\times 88.4$)
Urea (BUN), mg/dL*	16.87–34.30	14–17	n.a.	adol.	7.3–21 [10]	10 to <19	mmol/L ($\times 0.3571$)

Table 3 (continued)

Cystatin C, mg/L*	0.70–1.10	14–17	0.5–1.3	>3–16	2.6–7.6 [10]	12 to <19	mg/L
Uric acid, mg/dL*	4.04–8.00	14–17	2.4–8.6	14–19	2.6–7.6 [10]	12 to <19	µmol/L (×59.485)
AST, IU/L*	17.72–45.57	14–17	<39	13–18	14–35 [10]	12 to <19	µkat/L (×0.0167)
ALT, IU/L*	9.72–36.85	14–17	5–30	10–18	9–24 [10]	13 to <19	µkat/L (×0.0167)
GGT, IU/L*	5.72–26.57	14–17	2–42	13–18	7–21 [10]	11 to <19	µkat/L (×0.0167)
Vitamin B12, pmol/L	228.53–704.23	14–17	158–638	13–18	180–655 [11]	14 to <17	pmol/L
Folic acid, nmol/L	2.66–11.47	14–17	2.7–19.9	13–18	n.a.[b]	adol.	nmol/L
Vitamin D, ng/mL	17.12–43.88	14–17	6–39	13–18	4.8–42.32 [11]	14 to <19	nmol/L (×2.496)
Zinc, µg/dL*	8.59–119.00	14–17	46–130	14 to <18	n.a.[b]	adol.	µmol/L (×0.153)
Ferritin, ng/mL*	11.60–73.06	14–17	10–300	10–18	11.1–171.9 [11]	16 to <19	pmol/L (×2.247)
Testosterone, nmol/L*	0.82–29.74	14–17	<0.069–35.075	>10 to ≤20	1.25–27.55 [12]	14 to <19	nmol/L
Oestradiol, pmol/L*	25.44–141.36	14–17	37–184	13–17	≤141 [12]	15 to <19	pmol/L
FSH, IU/L*	1.23–9.83	14–17	0.0–10.0	13–18	0.78–5.1 [12]	13 to <19	IU/L
LH, IU/L*	0.59–5.83	14–17	1.3–5.7	13–17	0.79–4.76 [12]	15 to <17	IU/L
Prolactin, ng/mL*	143.57–517.29	14–17	1.8–13.3	11–20	4.2–23.04 [12]	1 to <19	pmol/L (×43.478)
SHBG, nmol/L*	10.78–116.87	14–17	10.6–62.6	13–18	9.7–49.6 [12]	15 to <19	nmol/L (×8.896)

Analyte	**Female**						
	Bogner et al.[a]		**Soldin et al. [3]**		**CALIPER or other studies**		**SI units**
	Reference values	**Age, years**	**Reference values**	**Age, years**	**Reference values**	**Age, years**	**(CF US to SI)**
HCT, %*	29.20–42.70	14–17	32.1–38.7	12 to <18	35.2–45.6 [8]	16	Portion of 1 (×0.01)
MCV, fL*	66.70–95.30	14–17	82.1–87.7	12 to <18	80–96 [8]	16	%
MCH, pg*	20.50–31.10	14–17	28.4–30.7	12 to <18	26–32 [8]	16	fL
MCHC, g/dL*	30.80–35.40	14–17	33.9–35.4	12 to <18	31–35 [8]	16	g/L (×10)
Platelets, $\times10^3$/µL	157.21–354.40	14–17	192–307	12 to <18	126–390 [8]	16	$\times10^9$/L (×1)
Leucocytes, $\times10^3$/µL	3.52–11.44	14–17	5.52–9.29	12 to <18	4.6–10.7 [9]	14–19	$\times10^9$/L (×0.001)
Segmented neutrophils, %	37.24–76.30	14–17	46.4–75.6	12 to <18	n.a.	adol.	Portion of 1 (×0.01)
Segmented neutrophils, $\times10^3$/µL*	1.67–9.22	14–17	3.04–6.06	12 to <18	2.1–7.2 [9]	14–19	$\times10^9$/L (×0.001)
Lymphocytes, %	16.04–51.00	14–17	8–39	12 to <18	n.a.	adol.	Portion of 1 (×0.01)
Lymphocytes, $\times10^3$/µL	1.27–3.15	14–17	1.17–2.30	12 to <18	1.4–3.3 [9]	14–19	$\times10^9$/L (×0.001)
Monocytes, %	4.32–9.28	14–17	4–7	12 to <18	n.a.	adol.	Portion of 1 (×0.01)
Monocytes, $\times10^3$/µL	0.22–0.83	14–17	0.19–0.72	12 to <18	0.37–1.06 [9]	14–19	$\times10^9$/L (×0.001)
Eosinophils, %*	0.70–9.40	14–17	1–3	12 to <18	n.a.	adol.	Portion of 1 (×0.01)
Eosinophils, $\times10^3$/µL*	0.03–0.59	14–17	0.05–0.17	12 to <18	0.05–0.61 [9]	14–19	$\times10^9$/L (×0.001)
Basophils, %	0.10–1.38	14–17	0–1	12 to <18	n.a.	adol.	Portion of 1 (×0.01)
Basophils, $\times10^3$/µL*	0.008–0.062	14–17	0.01–0.05	12 to <18	0.01–0.18 [9]	14–19	$\times10^9$/L (×0.001)
Glucose, mg/dL*	69.00–88.00	14–17	54–117	>7 days	n.a.[b]	adol.	mmol/L (×0.0555)
HbA_{1c}, %	4.70–5.60	14–17	4–7	All	n.a.[b]	adol.	Proportion of total haemoglobin (×0.01)
Insulin, µIU/mL	6.96–22.45	14–17	7.4–51.3	15–18	2.2–49.6[b]	6 to <19	pmol/L (×6.945)
Proinsulin, pmol/L	0.62–8.34	14–17	n.a.	adol.	n.a.[b]	adol.	pmol/L

Table 3 (continued)

Triglycerides, mg/dL	28.81–150.80	14–17	38–243	≥1 to ≤20	44–197 [10]	1 to <19	mmol/L (×0.0113)
Total cholesterol, mg/dL	110.01–232.98	14–17	93–233	>15 to ≤20	112–208 [10]	1 to <19	mmol/L (×0.0259)
LDL-cholesterol, mg/dL	51.62–156.21	14–17	50–154	≥1 to ≤20	n.a.[b]	adol.	mmol/L (×0.0259)
HDL-cholesterol, mg/dL*	36.00–83.00	14–17	25–89	>15 to ≤20	32–72 [10]	13 to <19	mmol/L (×0.0259)
Lipoprotein (a), mg/dL	<171.39	14–17	n.a.	adol.	n.a.[b]	adol.	µmol/L (×0.0357)
ApoA1, mg/dL*	114.00–222.00	14–17	89–203	>15 to ≤20	72–154 [10]	14 to <19	g/L (×0.01)
ApoB, mg/dL	42.80–115.84	14–17	38–105	≥1 to ≤20	31–84 [10]	6 to <19	g/L (×0.01)
Adiponectin, µg/mL	5.34–18.80	14–17	n.a.	adol.	n.a.[b]	adol.	µg/mL
hs-CRP, mg/L	0.01–4.32	14–17	0.6–7.9	15–18	0.1–1.7 [10]	15 to <19	nmol/L (×9.524)
TSH, mIU/L	0.89–4.13	14–17	0.5–4.5	0.5–18	0.47–3.41 [11]	14 to <19	mIU/L
FT3, pg/mL*	2.87–5.30	14–17	n.a.	adol.	2.31–3.71 [11]	15 to <19	pmol/L (×1.54)
FT4, ng/dL	0.70–1.38	14–17	0.8–2.0	31 days-18	0.89–1.37 [11]	1 to <19	pmol/L (×12.87)
Albumin, g/L*	40.30–54.80	14–17	37–56	10–19	35–49 [10]	15 to <19	g/L
Creatinine, mg/dL*	0.58–0.99	14–17	0.6–0.9	15–20	0.45–0.84 [10]	12 to <19	µmol/L (×88.4)
Urea (BUN), mg/dL*	12.40–30.30	14–17	n.a.	adol.	7.3–19 [10]	10 to <19	mmol/L (×0.3571)
Cystatin C, mg/L*	0.70–1.00	14–17	0.5–1.3	>3–16	2.6–5.9 [10]	12 to <19	mg/L
Uric acid, mg/dL*	2.80–5.80	14–17	3.0–5.9	14–19	2.6–5.9 [10]	12 to <19	µmol/L (×59.485)
AST, IU/L*	14.00–37.00	14–17	<32	13–18	13–26 [10]	12 to <19	µkat/L (×0.0167)
ALT, IU/L*	10.00–32.00	14–17	5–20	10–18	8–22 [10]	13 to <19	µkat/L (×0.0167)
GGT, IU/L*	5.00–27.00	14–17	4–24	13–18	6–21 [10]	11 to <19	µkat/L (×0.0167)
Vitamin B12, pmol/L	228.53–704.23	14–17	134–605	13–18	180–655 [11]	14 to <17	pmol/L
Folic acid, nmol/L	2.66–11.47	14–17	2.7–16.3	13–18	n.a.[b]	adol.	nmol/L
Vitamin D, ng/mL	17.12–43.88	14–17	6–28	13–18	4.8–42.32 [11]	14 to <19	nmol/L (×2.496)
Zinc, µg/dL*	9.00–101.00	14–17	46–130	14 to <18	n.a.[b]	adol.	µmol/L (×0.153)
Ferritin, ng/mL*	3.28–51.70	14–17	10–70	10–18	5.46–67.42 [11]	14 to <19	pmol/L (×2.247)
Testosterone, nmol/L*	0.44–2.81	14–17	<0.069–2.715	>10 to ≤20	0.36–1.7 [12]	13 to <19	nmol/L
Oestradiol, pmol/L*	24.89–392.42	14–17	37–1100	13–17	≤936 [12]	14 to <19	pmol/L
FSH, IU/L*	0.66–9.11	14–17	0.0–15.0	13–18	0.26–7.77 [12]	11 to <19	IU/L
LH, IU/L*	0.22–21.50	14–17	1.3–10.2	11–20	0.37–13.08 [12]	13 to <17	IU/L
Prolactin, ng/mL*	165.60–578.60	14–17	1.4–14.3	11–20	4.2–23.04 [12]	1 to <19	pmol/L (×43.478)
SHBG, nmol/L*	20.29–271.31	14–17	9.3–76.5	13–18	9.83–154.6 [12]	15 to <19	nmol/L (×8.896)

CF, conversion factor; HCT, haematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; HbA_{1c}, haemoglobin A_{1c} (glycated haemoglobin); LDL, low density lipoprotein; HDL, high density lipoprotein; Apo, apolipoprotein; hs-CRP, high sensitive C-reactive protein; TSH, thyroid-stimulating hormone; FT, free thyroxine; BUN, blood urea nitrogen; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, γ-glutamyltransferase; vitamin D (total 25-OH vitamin D [D3 + D2]); FSH, follicle stimulating hormone; LH, luteinising hormone; SHBG, sex hormone binding globulin. [a]This study, n.a., not available; adol., adolescents; [b]CALIPER database (https://app3.ccb.sickkids.ca/caliper/caliperlogin). Significant gender differences (indicated with asterisk [*] in the first column) of reference intervals of the MoYo-study were calculated by multivariate analysis of variance. For parameters that did not show significant gender differences, the $P_{2.5\%}$ and $P_{97.5\%}$ are deviated from the mixed cohort.

reference interval approach is appropriate for specific parameters that are changing continuously with age (e.g. creatinine or uric acid [10, 19]), currently, the prevalent application for reference intervals is still the age-grouped reference range. One reason for that is that currently most laboratory IT systems are not designated for data interpretation using continuous reference intervals.

The CALIPER database (https://app3.ccb.sickkids.ca/caliper/caliperlogin) comprises data of healthy children between 0 and 18 years derived from a nation-wide, multi-centre study performed in hospitals across Canada. It contains validated reference intervals for over 100 biomedical blood tests for common biochemical, endocrine, metabolic disease and tumour biomarkers as well as vitamins [2, 10–12, 20–22]. The correlation with selected biomedical parameters also determined in the MoYo-study reveals a high correlation of the values for both genders, even though the laboratory analysis was performed with different devices (Cobas c311, Vitros ECiQ® and diagnostically approved ELISAs in the MoYo-study vs. Abbott Architect ci4100, c8000 or i2000 in the CALIPER project) and the study population was different in terms of regional aspects. Ideally, measurement values and therefore reference ranges should not differ depending on the technique or equipment used. However, it is known from the literature as well as from reference guides that there are significant alterations of the reference values depending on the used methodology [3, 7, 23]. Therefore, every clinical laboratory needs to validate the reference intervals for the parameters that are measured and released for diagnostic and therapeutic use [7], and it is of main importance to clearly indicate the methods and equipment used for clinical parameters tested. The availability of carefully established and validated databases for laboratory reference intervals and medical decision levels can serve not only as general reference work, but also as a standard for other laboratories.

The parameters proinsulin, lipoprotein (a) and adiponectin were neither analysed by Soldin et al. [3] nor by the CALIPER study. The cut-off for lipoprotein (a) is reported to be 30 mg/dL for 9.1–18-year-old persons [24]. The upper limit of the MoYo-study population was much higher (171.39 mg/dL). The adiponectin concentration in the serum varies significantly with age, but generally the range of the adiponectin values of the Austrian cohort match with the year-of-age specified reference intervals of Lausten-Thomsen et al. [25]. To the best of our knowledge, currently no reference intervals for proinsulin at fasting for 14–17-year-old patients exist. The analysis of the MoYo cohort revealed no significant gender differences and indicates a range of 0.62–8.34 pmol/L.

As already described, the MoYo study was performed as a pilot study and it should be mentioned that the small sample size is a limitation of this work. However, it is challenging to gain data of a per se healthy study cohort of people within this age range and the analysis of the data set in terms of reference limits gives valuable insights in both non-gender-matched and gender-matched reference intervals. Therefore, the data set provides a good basis for the calculation of paediatric reference intervals according to the CLSI recommendations [7].

To conclude, the results of this "*a priori*" study add value to the evaluation of paediatric reference intervals of Caucasian adolescents in terms of an increase of the spectrum and a broader validation of the available biomarkers.

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