

ORIGINAL ARTICLE

Percentiles of fasting serum insulin, glucose, HbA1c and HOMA-IR in pre-pubertal normal weight European children from the IDEFICS cohort

J Peplies¹, D Jiménez-Pavón², SC Savva³, C Buck¹, K Günther¹, A Fraterman⁴, P Russo⁵, L Iacoviello⁶, T Veidebaum⁷, M Tornaritis³, S De Henauw⁸, S Mårild⁹, D Molnár¹⁰, LA Moreno² and W Ahrens^{1,11} on behalf of the IDEFICS consortium

OBJECTIVES: The aim of this study is to present age- and sex-specific reference values of insulin, glucose, glycosylated haemoglobin (HbA1c) and the homeostasis model assessment to quantify insulin resistance (HOMA-IR) for pre-pubertal children. **METHODS:** The reference population consists of 7074 normal weight 3- to 10.9-year-old pre-pubertal children from eight European countries who participated in at least one wave of the IDEFICS ('identification and prevention of dietary- and lifestyle-induced health effects in children and infants') surveys (2007–2010) and for whom standardised laboratory measurements were obtained. Percentile curves of insulin (measured by an electrochemiluminescence immunoassay), glucose, HbA1c and HOMA-IR were calculated as a function of age stratified by sex using the general additive model for location scale and shape (GAMLSS) method. **RESULTS:** Levels of insulin, fasting glucose and HOMA-IR continuously show an increasing trend with age, whereas HbA1c shows an upward trend only beyond the age of 8 years. Insulin and HOMA-IR values are higher in girls of all age groups, whereas glucose values are slightly higher in boys. Median serum levels of insulin range from 17.4 and 13.2 pmol l⁻¹ in 3- < 3.5-year-old girls and boys, respectively, to 53.5 and 43.0 pmol l⁻¹ in 10.5- < 11-year-old girls and boys. Median values of glucose are 4.3 and 4.5 mmol l⁻¹ in the youngest age group and 49.3 and 50.6 mmol l⁻¹ in the oldest girls and boys. For HOMA-IR, median values range from 0.5 and 0.4 in 3- < 3.5-year-old girls and boys to 1.7 and 1.4 in 10.5- < 11-year-old girls and boys, respectively. **CONCLUSIONS:** Our study provides the first standardised reference values for an international European children's population and provides the, up to now, largest data set of healthy pre-pubertal children to model reference percentiles for markers of insulin resistance. Our cohort shows higher values of HbA1c as compared with a single Swedish study while our percentiles for the other glucose metabolic markers are in good accordance with previous studies.

International Journal of Obesity (2014) **38**, S39–S47; doi:10.1038/ijo.2014.134

INTRODUCTION

Insulin resistance is one of the most common metabolic alterations related to obesity.^{1,2} It represents a key element of the metabolic syndrome and an important link between obesity and other metabolic as well as cardiovascular complications.^{3,4} Children with insulin resistance are also at risk for type 2 diabetes⁵ and a high proportion of cases apparently remain undiagnosed initially.⁶

Although reference data for body mass index (BMI) were published by organisations like International Obesity Taskforce, World Health Organization, Centers for Disease Control and Prevention and many others and these are widely used in paediatrics, there is still a lack of adequate reference data for markers of insulin resistance.⁷ Fasting insulin and homeostasis model assessment to quantify insulin resistance (HOMA-IR) have been suggested among others as surrogate markers for screening purposes in adults,⁸ as the gold standard method to measure insulin sensitivity (the hyperinsulinaemic euglycaemic clamp) is very labour- and time-intensive and thus not feasible in

epidemiological research. Oral glucose tolerance testing, a procedure widely used to measure insulin response in clinical practice, is also not feasible in a setting-based field study like ours. Matthews *et al.*⁹ showed that estimates of insulin resistance from HOMA-IR correlated well with estimates from the clamp-technique. Glycosylated haemoglobin (HbA1c) is a standard marker for glycaemic control in diabetic patients but has also been proposed as a predictive marker of insulin resistance.⁸

Population-based data on insulin resistance in children are rare, especially for pre-pubertal children and from large-scale epidemiological studies. Several authors have shown data on the distribution of insulin, glucose and HOMA-IR values in paediatric populations, and some also suggested cut-off values for insulin and HOMA-IR.^{10–17} However, all of these studies were limited to national study populations and sample sizes were mostly too small for statistical modelling of reference values. None of the studies included >1000 pre-pubertal children, except for a Mexican cross-section with about 2500 children in the age stratum of 6–10 years.¹³ All authors describe a pronounced age

¹Leibniz Institute for Prevention Research and Epidemiology—BIPS, Bremen, Germany; ²GENUD (Growth, Exercise, Nutrition and Development) Research Group, School of Health Sciences, University of Zaragoza, Zaragoza, Spain; ³Research & Education Institute of Child Health, Strovolos, Cyprus; ⁴Medizinisches Versorgungszentrum (MVZ), Dr Eberhard und Partner Dortmund, Dortmund, Germany; ⁵Epidemiology and Population Genetics, Institute of Food Science, CNR, Avellino, Italy; ⁶Centre for High Technology Research and Education in Biomedical Sciences, Università Cattolica del Sacro Cuore, Campobasso, Italy; ⁷National Institute for Health Development (NIHD), Tallinn, Estonia; ⁸Department of Public Health, Ghent University, Ghent, Belgium; ⁹Department of Paediatrics, The Queen Silvia Children's Hospital, Gothenburg, Sweden; ¹⁰Chronic Diseases Department, National Institute of Health Promotion, University of Pécs, Pécs, Hungary and ¹¹Institute of Statistics, Faculty of Mathematics and Computer Science, University Bremen, Bremen, Germany. Correspondence: Professor W Ahrens, Leibniz Institute for Prevention Research and Epidemiology—BIPS, Achterstrasse 30, 28359 Bremen, Germany. E-mail: ahrens@bips.uni-bremen.de

dependency of insulin and HOMA-IR, and to a lesser extent also of glucose values. Studies in adolescents show a peak of insulin and HOMA-IR values in puberty and again a slight decline towards adulthood.^{10,13,18} Comparability of results is limited due to the multitude of different laboratory procedures (for example, enzyme-linked immunosorbent assay, radioimmunoassay, immunoenzymometric assay, immunoelectrochemiluminometric assay) used for the determination of insulin. These different assays can show up to a twofold variation in insulin concentrations.¹⁹

The aim of this study is to present age- and sex-specific reference values for insulin, glucose and HOMA-IR based on a European population of normal weight pre-pubertal children from eight European countries who participated in at least one wave of the IDEFICS ('identification and prevention of dietary- and lifestyle-induced health effects in children and infants') surveys (2007–2010) and received standardised examinations and laboratory measurements.

MATERIALS AND METHODS

Study population

A population-based prospective cohort study was one of the key elements of the IDEFICS project. All children of the defined age group who lived in the selected study regions and attended one of the participating pre- or primary schools were eligible for participation. Children and parents were approached via schools and preschools to ensure inclusion of all social groups. Written consent of parents and verbal assent of children were given separately to the different modules of the examination, that is, participants were free to refrain from single components like blood drawing.

The baseline survey in the school year 2007/2008 included 16 228 preschool and primary school children aged 2–9 years from eight European countries (Belgium, Cyprus, Estonia, Germany, Hungary, Italy, Spain and Sweden). Of these, 11 292 were followed up again after 2 years. In the follow-up survey (2009–2010), 2517 new children were additionally included.

Both waves of surveys comprised anthropometrical measurements and examinations of children as well as parental self-completion questionnaires on lifestyle habits and dietary intakes of children. The physical examination programme during the IDEFICS surveys covered standard anthropometric measures, that is, height, weight and circumferences of waist, hip, upper arm and neck, as well as skinfold thicknesses, and the measurement of blood pressure and pulse rate. BMI was calculated as weight (in kg) divided by height squared (in m) and classified according to the International Obesity Taskforce criteria.²⁰

Biomarkers were analysed in blood, urine and saliva samples. Standardised procedures were used by all survey centres and a quality management system was established.²¹ Venous blood was collected after an overnight fast from 9185 of the baseline and 962 of the children newly recruited at follow-up. In addition, 1011 children who did not provide blood at baseline survey, provided blood at follow-up. The present analyses included children of both waves of surveys with available data for height, weight and the laboratory analyses of interest. If serum measurements of a child were available for both survey waves, preference was given to the baseline survey. Girls and boys below 3 and above 10.9 years of age were excluded from the study sample due to the small number of children in these age strata ($n < 100$) to avoid instability of the statistical model. Children were also excluded if they had a diagnosis of diabetes or if they reported to be non-fasting at the time of blood withdrawal. The present analysis is thus based on 7074 children, as depicted in Figure 1. The background of the IDEFICS study, its research goals and instruments have been described elsewhere in detail.²²

Laboratory analyses

Children participating in the IDEFICS baseline survey were asked to provide fasting venous blood, morning urine and saliva samples. If consent was not given for venous blood withdrawal, capillary blood was taken with the consent of the parents and the children. Blood glucose was assessed on site at each study centre by point-of-care analysis using the Cholestech LDX analyser (Cholestech, Cholestech Corp., Hayward, CA, USA) either in venous or capillary blood. Precision and accuracy of this analyser were comparable to clinical diagnostic laboratory methods²³ with a slight

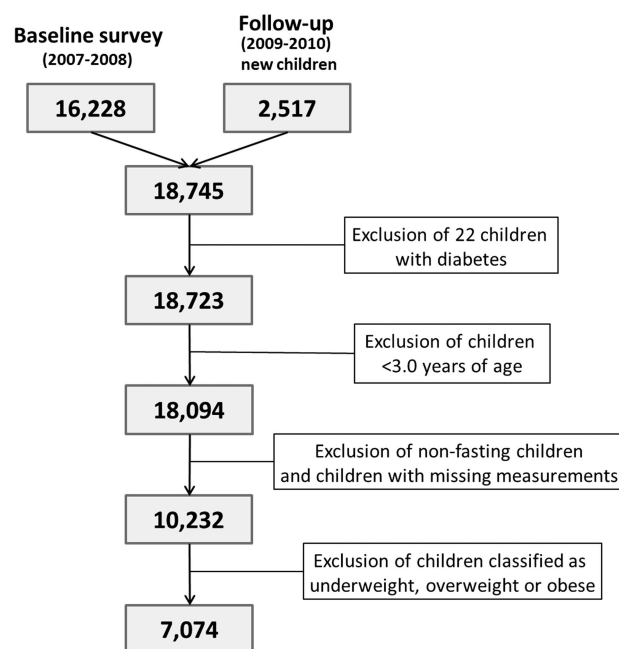


Figure 1. Flow chart of children included in the analysis.

positive bias of glucose measurement.²⁴ Pre-analytical sample processing of blood samples was done at the local survey centres or at local laboratories. Samples were then frozen (at -80°C) and shipped to a central laboratory, certified by ISO 15189:2007, for later analysis of insulin, HbA1c and other biological markers. Insulin was analysed by an electrochemiluminescence immunoassay (Roche Modular System, Mannheim, Germany), HbA1c was analysed by high-performance liquid chromatography (AUTO-GA variant). Details on the biological sampling procedures can be obtained from an earlier publication.²⁵ HOMA-IR was calculated as fasting insulin ($\mu\text{IU ml}^{-1}$) \times fasting glucose (mmol l^{-1})/22.5.

Statistical analyses

Percentile curves of insulin, glucose and HOMA-IR were calculated as a function of the covariate age stratified by sex using general additive model for location scale and shape (GAMLSS) method as an extension of the LMS method.²⁶ The LMS method models three parameters: the skewness L , the median M and the coefficient of variation S . The skewness accounts for the deviation from a normal distribution using a Box-Cox transformation, the median of the outcome variable is modelled depending on one explanatory variable and the coefficient of variation accounts for the variation of data points around the mean and adjusts for non-uniform dispersion. The GAMLSS method is able to model more than one covariate and also other distributions that particularly include the kurtosis. We used the gamlss package (version 4.2–6) of the statistical software R (version 3.0.1).²⁷ Different distributions were fitted to the observed distribution of insulin, glucose and HOMA-IR. Moreover, the influence of age on parameters of the considered distributions were modelled either as a constant, as a linear function, or as a cubic spline of the covariates. Goodness-of-fit was assessed by the Bayesian information criterion and by Q-Q plots to select the final model including the fitted distribution of insulin, glucose and HOMA-IR and the influence of covariates on distribution parameters. Worm plots were used as a diagnostic tool to assess whether adjustment for kurtosis was required.²⁸ Finally, percentile curves for the 5th, 10th, 25th, 50th, 75th, 90th and 95th were calculated based on the model that showed the best fit.^{27,29}

The final models for insulin and HOMA-IR for boys and girls considered a Box-Cox t (BCT) distribution modelling μ as a cubic spline depending on age, $\log(\sigma)$ as a linear function of age, and ν and τ as constants. With regard to glucose, a lognormal distribution was used for boys and girls considering μ as a linear function of age and $\log(\sigma)$ as a constant.

RESULTS

The reference population is composed of 3434 girls and 3640 boys. One-year age groups (3.0–10.9 years of age) include from about 250 up to 1500 children. Characteristics of this population are presented as mean values (\pm s.d.) or median (25th, 75th percentile) by age group in Table 1 for the study population before and after restriction to normal weight children. Anthropometrical measures and glucose concentrations show only little differences between boys and girls, but insulin concentrations are higher in girls than in boys through all age groups, which is also reflected in slightly higher HOMA-IR values.

Percentiles of insulin, glucose and HOMA-IR are presented in half-year age groups in Table 2 and Figures 2–4, respectively. Concentrations of all three biomarkers clearly show a positive trend with age, which is also observed for the variance of insulin and HOMA-IR, whereas for glucose the variance is similar in all age groups. Insulin and HOMA-IR values are higher in girls than in boys for all age groups, whereas glucose values are slightly higher in boys.

For insulin, 5th and 95th percentiles range from 4.2–49.3 and 3.5–41.0 pmol l⁻¹ in 3–<3.5-year-old girls and boys, respectively, to 25.7–100.7 and 19.4–88.2 pmol l⁻¹ in 10.5–<11-year-old girls and boys. For glucose, 5th and 95th percentiles were 3.6–5.2 and 3.7–5.3 mmol l⁻¹ in 3–<3.5-year-old girls and boys up to 4.2–5.7 and 4.3–5.9 mmol l⁻¹ in 10.5–<11-year-old girls and boys. For HOMA-IR, 5th and 95th percentiles ranged from 0.1–1.5 and 0.1–1.3 in 3–<3.5-year-old girls and boys to 0.8–3.4 and 0.6–3.0 in 10.5–<11-year-old girls and boys.

A table showing percentiles for children of all weight groups is provided in Supplementary Table A. Percentile values for glucose show only negligible differences as compared with the values for normal weight children. Inclusion of overweight and obese children has little impact on lower percentiles of insulin and HOMA-IR but has a very pronounced effect on the upper percentiles, especially in the older age groups. The 95th percentile of insulin in 10.5–<11-year-old girls for instance, is 125.7 compared with 100.7 pmol l⁻¹ in normal weight girls of the same age. A sensitivity analysis was conducted to illustrate the influence of BMI on age-specific values of insulin, glucose and HOMA-IR. A strong dependency on weight status was observed for insulin and HOMA-IR but not for glucose. Insulin percentile curves for children of all weight groups versus only normal weight girls and boys are depicted in Figure 5. Results of the sensitivity analyses for glucose and HOMA-IR are provided in Supplementary Figures A and B. Three different reference populations for BMI were used to test the robustness of the model.^{20,30–32} Preference was given to the classification by Cole *et al.*,²⁰ as differences between the three reference systems were not substantial and this classification led to the smallest number of excluded children.

Percentile curves for HbA1c are provided in Supplementary Figure C. Serum concentrations show nearly no variation for younger children (median of 26.8 for girls and 27.9 mmol mol⁻¹ for boys at 3 years of age to 29.0 mmol mol⁻¹ for both sexes at 8 years of age) and a slight increase for the 8–<11-year-old children (median of 34.4 mmol mol⁻¹ for girls and 33.3 mmol mol⁻¹ for boys at 10.9 years of age).

DISCUSSION

This study presents age- and sex-specific reference percentiles of insulin, glucose and HOMA-IR for pre-pubertal children on the basis of the IDEFICS cohort of children from eight European countries. This is the first time, reference percentiles of these biomarkers are based on a multinational children's cohort. At the same time, with over 7000 subjects, it is by far the biggest cohort ever used to generate such reference data. Nevertheless, previous studies of local populations have shown similar distributions of

insulin, glucose and HOMA-IR values in children. As the age group of 9-year-olds was considered most frequently, this age group will be used for comparison in the following.

In our reference population the 5th and 95th percentile values of insulin are 15.3–81.3 pmol l⁻¹ for 8.5–<9.5-year-old girls and 12.5–74.3 pmol l⁻¹ for 8.5–<9.5-year-old boys. Insulin values were also measured in a representative sample of 2244 school children from Quebec who were 9, 13, and 16 years old in 1999 (ref. 11) (insulin measured by the ultrasensitive insulin assay on the Access immunoassay system by Beckman Coulter, Mississauga, ON, Canada), in a representative sample of 1976 healthy French individuals aged 7–20 years in 2006–2008 (ref. 12) (insulin measured with a microparticle enzymeimmuno assay on an AxSYM analyser by Abbott, Abbott Park, IL, USA) and in a random sample of 1137 healthy 9- and 15-year-old school children examined in a cross-sectional study in Sweden in 1998/99 (ref. 17) (insulin assay not reported). In the pre-pubertal group of 9-year-olds, the 5th and 95th percentiles of insulin were 11.8–59.0,¹¹ 13.9–83.3 (ref. 12) and 11.1–78.5 pmol l⁻¹ (ref. 17) in girls and 10.4–54.2,¹¹ 10.4–69.5 (ref. 12) and 10.4–64.6 pmol l⁻¹ in boys. Overall, insulin concentrations were showing a positive trend with age until puberty and revealed pubertal peaks, which were sharper in females than in males. These results are in good accordance with the percentile values from our study. Studies in mixed non-Caucasian populations on 6132 school children aged 6–18 years in Mexico¹³ (insulin measured with a microparticle enzymeimmuno assay on an AxSYM analyser by Abbott) and 2153 normal weight children and adolescents in Chile¹⁶ (insulin measured by chemiluminescence on the ADVIA Centaur CP Immunoassay System, Bayer HealthCare AG, Leverkusen, Germany) also showed a gradual increase of insulin values between 6 and 13 years of life. Possibly due to the fact that older children were included, insulin concentrations were higher in the Chilean population than in our study cohort: the 5th and 95th percentiles were 29.9–150.0 pmol l⁻¹ for girls and 26.4–117.4 pmol l⁻¹ for boys in Tanner stages I and II.¹⁶

For glucose, the 5th and 95th reference percentiles in our population are 4.0–5.6 mmol l⁻¹ for 8.5–<9.5-year-old girls and 4.1–5.8 mmol l⁻¹ for 8.5–<9.5-year-old boys. Remarkably, glucose curves showed a linear trend in this pre-pubertal age group. To verify linearity of this functional relationship, we also applied a cubic model to the data. As both curves looked very similar, we preferred to use the simpler model. In addition, we know from literature¹² that glucose curves only start to flatten during puberty, which may lead to a linear functional relationship for the age range that we have considered here. Glucose values were very similar in the three above-mentioned studies from Canada,¹¹ France¹² and Sweden.¹⁷ For 9-year-olds, the 5th and 95th percentiles were 4.4–5.6,¹¹ 3.9–5.2 (ref. 12) and 4.3–5.4 mmol l⁻¹ (ref. 17) in girls and 4.7–5.7,¹¹ 4.0–5.2 (ref. 12) and 4.4–5.5 mmol l⁻¹ (ref. 17) in boys. Percentile values of glucose showed a positive trend with age, although the trend was less pronounced as compared with insulin. A distinct pubertal peak of serum glucose concentrations was only observed in females.

For HOMA-IR, the 5th and 95th percentiles in our reference population are 0.4–2.7 for 8.5–<9.5-year-old girls and 0.4–2.5 for boys of this age. Estimates from the 1999 Quebec sample¹¹ (insulin measured by the ultrasensitive insulin assay on the Access immunoassay system by Beckman Coulter) showed slightly lower HOMA-IR values (5th and 95th percentile) of 0.3–2.1 and 0.3–1.9 for 9-year-old girls and boys, respectively. A small Italian cross-section of healthy children and adolescents reported values of 0.6–2.2 for pre-pubertal girls and 0.4–2.2 for the respective boys¹⁵ (insulin measured by radioimmunoassay, Radim Kit, Rome, Italy). The above-mentioned studies in Mexico¹³ (insulin measured with a microparticle enzymeimmuno assay on an AxSYM analyser by Abbott) and Chile¹⁶ (insulin measured by chemiluminescence on the ADVIA Centaur CP Immunoassay System, Bayer

Table 1. Characteristics of study population by one-year age group, before and after restriction to normal weight children

Age	Girls							Boys						
	N	Height (cm) ^a	Weight (kg) ^a	BMI (kg m ⁻²) ^a	Insulin (pmol l ⁻¹) ^b	Glucose (mmol l ⁻¹) ^a	HOMA-IR ^b	N	Height (cm) ^a	Weight (kg) ^a	BMI (kg m ⁻²) ^a	Insulin (pmol l ⁻¹) ^b	Glucose (mmol l ⁻¹) ^a	HOMA-IR ^b
<i>All children (n = 10232)</i>														
3-<4	415	100.5 (±4.9)	16.0 (±2.4)	15.8 (±1.4)	18.1 (11.1, 27.8)	4.4 (±0.5)	0.5 (0.3, 0.8)	457	101.4 (±5.1)	16.3 (±2.7)	15.8 (±1.4)	15.3 (7.6, 24.3)	4.5 (±0.5)	0.4 (0.2, 0.7)
4-<5	611	106.7 (±5.0)	17.8 (±2.7)	15.6 (±1.8)	20.8 (13.9, 31.9)	4.5 (±0.5)	0.6 (0.4, 1.0)	667	108.1 (±4.8)	18.5 (±2.8)	15.8 (±1.7)	18.8 (11.8, 28.5)	4.6 (±0.5)	0.6 (0.3, 0.9)
5-<6	606	113.9 (±5.4)	20.6 (±3.6)	15.8 (±1.9)	25.7 (16.7, 37.5)	4.6 (±0.5)	0.7 (0.5, 1.1)	673	114.8 (±5.2)	21.0 (±3.7)	15.9 (±2.0)	22.9 (13.9, 32.6)	4.7 (±0.5)	0.7 (0.4, 1.0)
6-<7	801	120.4 (±5.4)	23.5 (±4.5)	16.1 (±2.3)	27.8 (18.1, 41.0)	4.7 (±0.5)	0.8 (0.5, 1.2)	809	121.9 (±5.4)	24.3 (±5.1)	16.3 (±2.5)	25.7 (17.4, 37.5)	4.8 (±0.5)	0.8 (0.5, 1.1)
7-<8	1089	126.5 (±5.7)	26.9 (±5.6)	16.7 (±2.6)	31.9 (22.2, 45.1)	4.7 (±0.5)	1.0 (0.7, 1.4)	1110	127.8 (±6.1)	27.4 (±5.8)	16.7 (±2.7)	28.5 (19.4, 41.0)	4.8 (±0.5)	0.9 (0.6, 1.3)
8-<9	873	131.6 (±5.9)	29.9 (±6.4)	17.2 (±2.9)	37.5 (26.4, 52.8)	4.8 (±0.4)	1.1 (0.8, 1.7)	887	132.7 (±6.1)	30.4 (±6.6)	17.2 (±3.0)	31.9 (20.8, 47.2)	4.9 (±0.5)	1.0 (0.6, 1.5)
9-<10	422	137.8 (±7.0)	33.9 (±7.9)	17.7 (±3.2)	38.2 (32.6, 65.3)	4.9 (±0.4)	1.4 (1.0, 2.2)	416	138.7 (±6.2)	35.1 (±8.2)	18.1 (±3.4)	41.7 (29.9, 58.3)	5.0 (±0.5)	1.3 (0.9, 1.9)
10-<11	207	143.0 (±7.0)	37.8 (±8.8)	18.3 (±3.4)	54.2 (41.0, 79.9)	5.0 (±0.5)	1.7 (1.3, 2.6)	189	142.2 (±6.4)	38.1 (±9.2)	18.7 (±3.5)	44.4 (32.6, 70.1)	5.0 (±0.5)	1.5 (1.0, 2.2)
All	5024	122.0 (±12.9)	25.1 (±7.9)	16.5 (±2.6)	30.6 (19.4, 45.1)	4.7 (±0.5)	0.9 (0.6, 1.4)	5208	122.6 (±12.8)	25.5 (±8.1)	16.6 (±2.7)	23.4 (16.7, 40.3)	4.8 (±0.5)	0.8 (0.5, 1.3)
Age	Girls							Boys						
	N	Height (cm) ^a	Weight (kg) ^a	BMI (kg m ⁻²) ^a	Insulin (pmol l ⁻¹) ^{b,c}	Glucose (mmol l ⁻¹) ^a	HOMA-IR ^b	N	Height (cm) ^a	Weight (kg) ^a	BMI (kg m ⁻²) ^a	Insulin (pmol l ⁻¹) ^b	Glucose (mmol l ⁻¹) ^a	HOMA-IR ^b
<i>Normal weight children according to Cole et al.²⁰ (n = 7074)</i>														
3-<4	318	100.2 (±4.9)	15.9 (±1.8)	15.8 (±0.9)	18.8 (11.1, 27.8)	4.4 (±0.6)	0.5 (0.3, 0.8)	329	101.3 (±4.7)	16.4 (±1.8)	15.9 (±0.8)	13.9 (7.6, 25.0)	4.5 (±0.5)	0.4 (0.2, 0.7)
4-<5	433	106.4 (±4.8)	17.6 (±2.0)	15.5 (±0.8)	20.1 (13.9, 29.2)	4.5 (±0.4)	0.6 (0.4, 0.9)	497	107.9 (±4.8)	18.3 (±2.0)	15.7 (±0.8)	18.8 (11.8, 27.1)	4.6 (±0.5)	0.5 (0.3, 0.8)
5-<6	422	113.4 (±5.3)	19.8 (±2.2)	15.3 (±0.9)	24.3 (16.0, 35.4)	4.6 (±0.5)	0.7 (0.4, 1.1)	491	114.5 (±5.0)	20.5 (±2.2)	15.6 (±0.8)	20.8 (13.2, 30.6)	4.7 (±0.5)	0.6 (0.4, 0.9)
6-<7	558	120.1 (±5.3)	22.3 (±2.5)	15.4 (±1.0)	24.3 (17.4, 36.1)	4.6 (±0.5)	0.7 (0.5, 1.1)	561	121.3 (±5.1)	23.0 (±2.5)	15.6 (±1.0)	23.6 (16.7, 34.7)	4.8 (±0.5)	0.7 (0.5, 1.1)
7-<8	738	126.1 (±5.6)	25.2 (±3.1)	15.8 (±1.1)	29.2 (20.8, 39.6)	4.7 (±0.5)	0.9 (0.6, 1.2)	763	127.3 (±5.7)	25.7 (±3.1)	15.8 (±1.0)	26.4 (18.1, 37.5)	4.8 (±0.5)	0.8 (0.5, 1.2)
8-<9	558	131.1 (±5.8)	27.8 (±3.5)	16.2 (±1.3)	34.7 (25.0, 45.8)	4.7 (±0.4)	1.1 (0.7, 1.5)	619	132.2 (±6.1)	28.3 (±3.5)	16.2 (±1.2)	29.2 (19.4, 40.3)	4.9 (±0.5)	0.9 (0.6, 1.3)
9-<10	278	137.1 (±6.9)	31.1 (±4.2)	16.5 (±1.3)	41.0 (30.6, 55.6)	4.9 (±0.4)	1.3 (0.9, 1.8)	255	137.6 (±6.0)	31.5 (±4.2)	16.6 (±1.3)	36.8 (28.5, 47.2)	5.0 (±0.5)	1.2 (0.9, 1.6)
10-<11	129	142.7 (±6.7)	35.3 (±5.3)	17.2 (±1.5)	50.7 (39.6, 65.3)	5.0 (±0.5)	1.6 (1.3, 2.1)	125	140.5 (±6.1)	33.8 (±5.3)	17.1 (±1.4)	40.3 (29.2, 53.5)	5.0 (±0.5)	1.3 (0.9, 1.7)
All	3434	121.0 (±12.9)	23.5 (±5.8)	15.8 (±1.2)	27.8 (18.8, 40.3)	4.6 (±0.5)	0.8 (0.5, 1.3)	3640	121.7 (±12.5)	23.9 (±5.8)	15.9 (±1.1)	24.3 (15.3, 36.1)	4.8 (±0.5)	0.8 (0.5, 1.1)

Abbreviations: BMI, body mass index; HOMA-IR, homeostasis model assessment to quantify insulin resistance. ^aData of parameters with normal distribution are presented as mean values (±s.d.). ^bData of parameters with skewed distributions are presented as median (25th, 75th percentile). ^cMeasured by an electrochemoluminescence immunoassay.

Table 2. Percentiles of insulin ($\mu\text{mol l}^{-1}$), glucose (mmol l^{-1}) and HOMA-IR in normal weight children calculated with GAMLSS

Age ^a	Percentiles for girls										Percentiles for boys											
	1	3	5	10	25	50	75	90	95	97	99	1	3	5	10	25	50	75	90	95	97	99
Insulin ($\mu\text{mol l}^{-1}$)^b																						
3-<3.5	1.74	3.34	4.41	6.40	10.69	17.23	26.59	39.05	49.52	58.12	80.10	1.30	2.46	3.25	4.73	8.03	13.28	21.07	31.78	40.99	48.66	68.60
3.5-<4	2.09	3.87	5.05	7.20	11.76	18.62	28.31	41.07	51.70	60.41	82.53	1.63	2.99	3.91	5.61	9.35	15.19	23.75	35.40	45.34	53.59	74.90
4-<4.5	2.48	4.44	5.72	8.02	12.85	19.99	29.95	42.93	53.68	62.43	84.57	2.00	3.56	4.60	6.51	10.63	16.99	26.20	38.61	49.12	57.79	80.09
4.5-<5	2.91	5.07	6.45	9.15	14.00	21.42	31.64	44.84	55.68	64.48	86.61	2.37	4.12	5.27	7.36	11.81	18.57	28.26	41.17	52.02	60.94	83.76
5-<5.5	3.40	5.75	7.23	9.85	15.17	22.85	33.29	46.65	57.56	66.36	88.41	2.78	4.71	5.96	8.21	12.96	20.07	30.14	43.43	54.52	63.60	86.70
5.5-<6	3.90	6.41	7.98	10.72	16.24	24.07	34.62	47.99	58.83	67.54	89.25	3.22	5.33	6.68	9.09	14.10	21.53	31.92	45.50	56.76	65.94	89.16
6-<6.5	4.41	7.08	8.73	11.57	17.22	25.16	35.73	48.99	59.68	68.25	89.47	3.68	5.96	7.40	9.95	15.20	22.87	33.49	47.24	58.57	67.76	90.90
6.5-<7	4.99	7.83	9.56	12.52	18.34	26.41	37.04	50.28	60.88	69.33	90.19	4.16	6.61	8.14	10.82	16.27	24.15	34.94	48.79	60.13	69.29	92.24
7-<7.5	5.70	8.76	10.59	13.71	19.77	28.34	38.94	52.33	62.98	71.44	92.24	4.69	7.31	8.92	11.73	17.38	25.47	36.42	50.35	61.68	70.80	93.55
7.5-<8	6.60	9.92	11.90	15.23	21.64	30.34	41.58	55.34	66.23	74.84	95.90	5.29	8.08	9.79	12.75	18.62	26.93	38.08	52.15	63.52	72.64	95.26
8-<8.5	7.69	11.34	13.50	17.10	23.95	33.15	44.94	59.25	70.50	79.38	100.98	6.00	9.02	10.85	13.97	20.14	28.77	40.24	54.59	66.12	75.33	98.07
8.5-<9	8.99	13.02	15.38	19.28	26.65	36.45	48.88	63.86	75.57	84.78	107.09	6.90	10.20	12.17	15.94	22.40	31.20	43.17	58.04	69.91	79.36	102.59
9-<9.5	10.53	14.99	17.57	21.83	29.78	40.25	53.43	69.19	81.44	91.04	114.20	7.97	11.59	13.74	17.38	24.42	34.07	46.66	62.18	74.50	84.26	108.18
9.5-<10	12.30	17.24	20.06	24.69	33.28	44.48	58.46	75.05	87.88	97.90	121.98	9.15	13.11	15.44	19.36	26.87	37.08	50.29	66.43	79.18	89.25	113.80
10-<10.5	14.26	19.68	22.77	27.77	36.99	48.90	63.66	81.04	94.42	104.83	129.75	10.41	14.71	17.22	21.40	29.37	40.09	53.85	70.55	83.66	93.98	119.04
10.5-<11	16.37	22.27	25.59	30.97	40.78	53.37	68.82	86.91	100.77	111.51	137.13	11.77	16.39	19.07	23.52	31.92	43.12	57.38	74.56	87.98	98.51	123.97
Glucose (mmol l^{-1})																						
3-<3.5	3.19	3.48	3.62	3.82	4.11	4.39	4.68	4.97	5.16	5.30	5.59	3.22	3.53	3.68	3.89	4.19	4.49	4.79	5.10	5.30	5.45	5.76
3.5-<4	3.23	3.52	3.66	3.85	4.14	4.43	4.72	5.00	5.20	5.34	5.63	3.26	3.57	3.72	3.92	4.23	4.53	4.83	5.14	5.34	5.49	5.80
4-<4.5	3.26	3.56	3.69	3.89	4.18	4.46	4.75	5.04	5.23	5.37	5.67	3.30	3.61	3.76	3.96	4.27	4.57	4.87	5.17	5.38	5.53	5.84
4.5-<5	3.30	3.59	3.73	3.93	4.21	4.50	4.79	5.07	5.27	5.41	5.70	3.34	3.65	3.79	4.00	4.30	4.61	4.91	5.21	5.42	5.56	5.87
5-<5.5	3.33	3.63	3.77	3.96	4.25	4.54	4.82	5.11	5.31	5.45	5.74	3.38	3.69	3.83	4.04	4.34	4.64	4.95	5.25	5.46	5.60	5.91
5.5-<6	3.37	3.66	3.80	4.00	4.28	4.57	4.86	5.15	5.34	5.48	5.77	3.41	3.72	3.87	4.08	4.38	4.68	4.99	5.29	5.49	5.64	5.95
6-<6.5	3.41	3.70	3.84	4.03	4.32	4.61	4.90	5.18	5.38	5.52	5.81	3.45	3.76	3.91	4.11	4.42	4.72	5.02	5.33	5.53	5.68	5.99
6.5-<7	3.44	3.73	3.87	4.07	4.36	4.64	4.93	5.22	5.41	5.55	5.85	3.49	3.80	3.95	4.15	4.46	4.76	5.06	5.36	5.57	5.72	6.03
7-<7.5	3.48	3.77	3.91	4.11	4.39	4.68	4.97	5.25	5.45	5.59	5.88	3.53	3.84	3.98	4.19	4.49	4.80	5.10	5.40	5.61	5.76	6.06
7.5-<8	3.51	3.81	3.95	4.14	4.43	4.72	5.00	5.29	5.49	5.62	5.92	3.57	3.88	4.02	4.23	4.53	4.83	5.14	5.44	5.65	5.79	6.10
8-<8.5	3.55	3.84	3.98	4.18	4.46	4.75	5.04	5.33	5.52	5.66	5.95	3.61	3.91	4.06	4.27	4.57	4.87	5.18	5.48	5.69	5.83	6.14
8.5-<9	3.59	3.88	4.02	4.21	4.50	4.79	5.07	5.36	5.56	5.70	5.99	3.64	3.95	4.10	4.31	4.61	4.91	5.21	5.52	5.72	5.87	6.18
9-<9.5	3.62	3.91	4.05	4.25	4.54	4.82	5.11	5.40	5.59	5.73	6.03	3.68	3.99	4.14	4.34	4.65	4.95	5.25	5.56	5.76	5.91	6.22
9.5-<10	3.66	3.95	4.09	4.28	4.57	4.86	5.15	5.43	5.63	5.77	6.06	3.72	4.03	4.18	4.38	4.68	4.99	5.29	5.59	5.80	5.95	6.25
10-<10.5	3.69	3.99	4.13	4.32	4.61	4.90	5.18	5.47	5.67	5.80	6.10	3.76	4.07	4.21	4.42	4.72	5.03	5.33	5.63	5.84	5.98	6.29
10.5-<11	3.73	4.02	4.16	4.36	4.64	4.93	5.22	5.51	5.70	5.84	6.13	3.80	4.10	4.25	4.46	4.76	5.06	5.37	5.67	5.88	6.02	6.33
HOMA-IR^c																						
3-<3.5	0.04	0.08	0.11	0.16	0.29	0.48	0.78	1.17	1.51	1.79	2.51	0.03	0.06	0.08	0.12	0.22	0.38	0.62	0.97	1.27	1.52	2.18
3.5-<4	0.05	0.10	0.13	0.19	0.32	0.53	0.83	1.24	1.59	1.88	2.61	0.04	0.07	0.10	0.15	0.26	0.44	0.71	1.10	1.43	1.71	2.42
4-<4.5	0.06	0.11	0.15	0.21	0.35	0.57	0.89	1.31	1.66	1.95	2.69	0.05	0.09	0.12	0.17	0.30	0.50	0.80	1.21	1.57	1.86	2.61
4.5-<5	0.07	0.13	0.17	0.24	0.39	0.62	0.95	1.38	1.74	2.03	2.77	0.06	0.11	0.14	0.20	0.33	0.55	0.87	1.30	1.67	1.97	2.75
5-<5.5	0.08	0.15	0.19	0.27	0.43	0.67	1.00	1.44	1.81	2.10	2.85	0.07	0.12	0.16	0.23	0.37	0.60	0.93	1.38	1.76	2.07	2.86
5.5-<6	0.10	0.17	0.21	0.29	0.46	0.71	1.05	1.49	1.86	2.15	2.89	0.08	0.14	0.18	0.25	0.41	0.65	0.99	1.46	1.84	2.16	2.95
6-<6.5	0.11	0.19	0.23	0.32	0.49	0.74	1.09	1.53	1.89	2.18	2.90	0.10	0.16	0.20	0.28	0.44	0.69	1.05	1.52	1.91	2.22	3.02
6.5-<7	0.13	0.21	0.26	0.35	0.53	0.78	1.13	1.57	1.93	2.22	2.93	0.11	0.18	0.23	0.31	0.48	0.74	1.10	1.57	1.96	2.28	3.07
7-<7.5	0.15	0.24	0.29	0.38	0.57	0.84	1.19	1.64	2.00	2.29	3.00	0.11	0.18	0.25	0.34	0.52	0.78	1.15	1.63	2.02	2.34	3.13
7.5-<8	0.17	0.27	0.33	0.43	0.63	0.91	1.28	1.75	2.12	2.41	3.14	0.14	0.23	0.28	0.37	0.56	0.83	1.21	1.70	2.09	2.41	3.20
8-<8.5	0.21	0.31	0.38	0.49	0.71	1.01	1.40	1.89	2.27	2.58	3.33	0.17	0.26	0.31	0.41	0.61	0.90	1.29	1.79	2.19	2.51	3.31
8.5-<9	0.24	0.36	0.44	0.56	0.79	1.12	1.54	2.05	2.46	2.78	3.56	0.19	0.29	0.35	0.46	0.67	0.98	1.39	1.92	2.33	2.66	3.48
9-<9.5	0.29	0.43	0.51	0.64	0.90	1.25	1.70	2.24	2.67	3.01	3.83	0.23	0.34	0.40	0.52	0.75	1.08	1.52	2.06	2.50	2.84	3.68
9.5-<10	0.34	0.50	0.59	0.73	1.02	1.39	1.87	2.46	2.91	3.26	4.12	0.26	0.39	0.46	0.59	0.83	1.18	1.64	2.21	2.66	3.02	3.89
10-<10.5	0.41	0.57	0.67	0.83	1.14	1.55	2.06	2.67	3.15	3.52	4.41	0.30	0.44	0.52	0.65	0.92	1.28	1.77	2.36	2.82	3.19	4.07
10.5-<11	0.47	0.66	0.76	0.94	1.27	1.70	2.24	2.88	3.38	3.77	4.69	0.35	0.49	0.58	0.72	1.00	1.39	1.89	2.50	2.97	3.35	4.25

Abbreviations: GAMLSS, general additive model for location scale and shape; HOMA-IR, homeostasis model assessment to quantify insulin resistance. ^aPercentile values were modelled for midpoints of age groups. ^bMeasured by an electrochemoluminescence immunoassay. ^cHOMA-IR was calculated as fasting insulin ($\mu\text{U ml}^{-1}$) x fasting glucose (mmol l^{-1})/22.5.

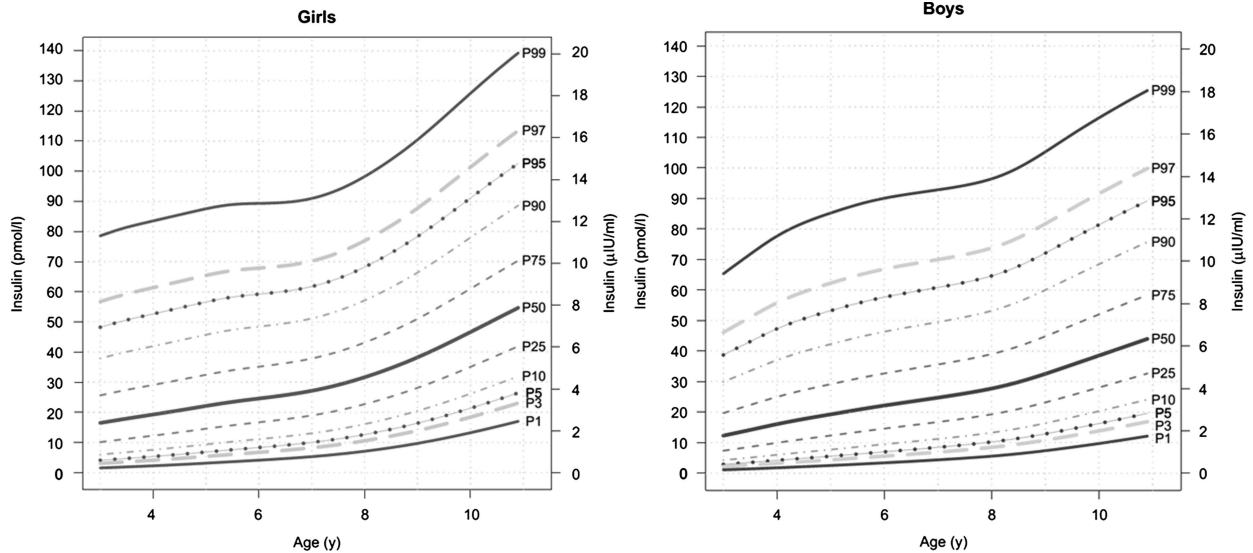


Figure 2. Percentiles of fasting serum insulin (measured by an electrochemiluminescence immunoassay) from normal weight children of the IDEFICS cohort (2007–2010).

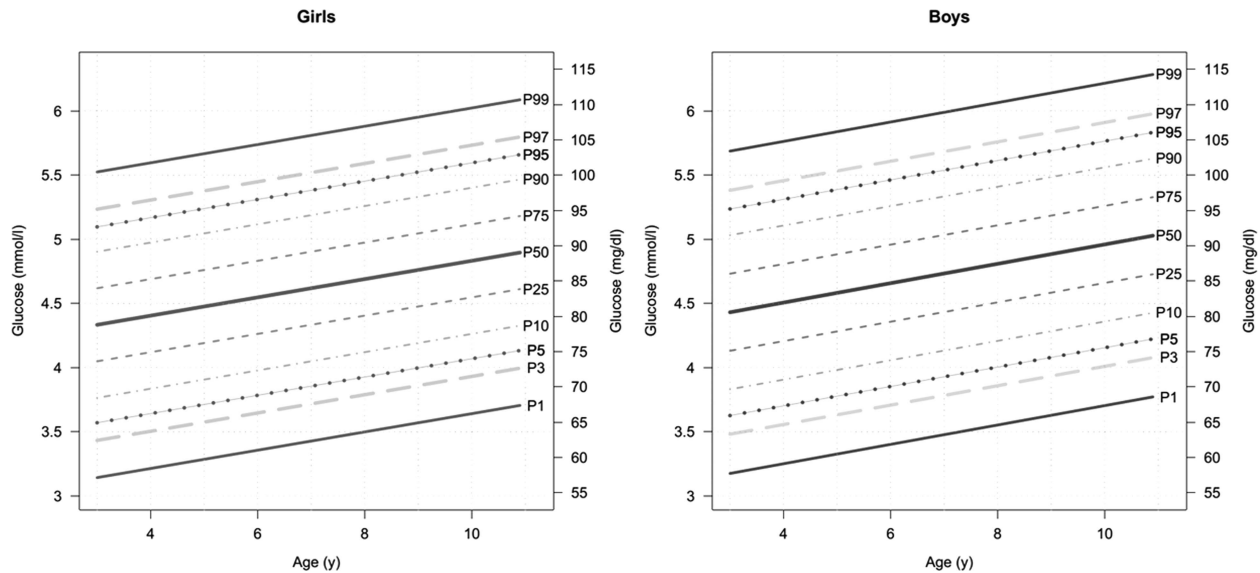


Figure 3. Percentiles of fasting blood glucose (measured by point-of-care analysis) from normal weight children of the IDEFICS cohort (2007–2010).

HealthCare AG) also showed an increase of HOMA-IR from the age of 6 to the age 13 years. Similar to insulin, percentile values were obviously higher for the combined group of Tanner stages I and II, as compared with our study population, that is, HOMA-IR values were 0.9–4.9 for girls and 0.8–3.9 for boys. A HOMA-IR value of 2.0 was suggested as a cut-off point for pre-pubertal children in a population-based small Italian study¹⁴ (insulin measured by an immunoenzymometric assay, AIA-Pack IRI, Tosoh, Tokyo, Japan). Cut-off values were also derived from oral glucose tolerance testing in studies with obese children. Two Turkish studies on 148 and 82 obese paediatric patients, proposed cut-off points of HOMA-IR ≥ 2.7 for both sexes³³ (insulin was measured using the IMMULITE immunoassay, IMMULITE Diagnostic Products Corporation, Los Angeles, CA, USA) or HOMA-IR ≥ 2.2 for girls and ≥ 2.7 for boys³⁴ (insulin measured by an immunoradiometric assay kit, INS-Irma Biosource, Nivelles, Belgium).

HbA1c as a standard marker for glycaemic control in diabetes has rather little relevance in healthy children. However, as it is

frequently measured in population-based studies, we included it in our analysis and we present the results in Supplementary Figure C. To our knowledge, there is only one recent study that aimed to define paediatric reference values for HbA1c.³⁵ This Swedish cohort investigated healthy children from 6 months to 18 years of age and suggested cut-off values of 16.9–27.9 mmol mol⁻¹ (2.5th and 97.5th percentile), which are lower than the 5th and 95th percentiles observed in our study population, that is, 15.8–34.4 and 16.9–34.4 mmol mol⁻¹ for 3-year-old girls and boys, and up to 26.8–38.8 and 15.8–39.9 mmol mol⁻¹ for 10.9-year-old girls and boys.

Overall, the distributions of insulin, glucose and HOMA-IR shown in previous studies are in good accordance with the reference percentiles in the present analysis. For the subgroup of 9-year-old girls and boys, the 5th and 95th percentile values are very similar to cohorts from European countries or Canada. Concentrations were generally higher in countries with mixed populations including people with indigenous ancestry or when

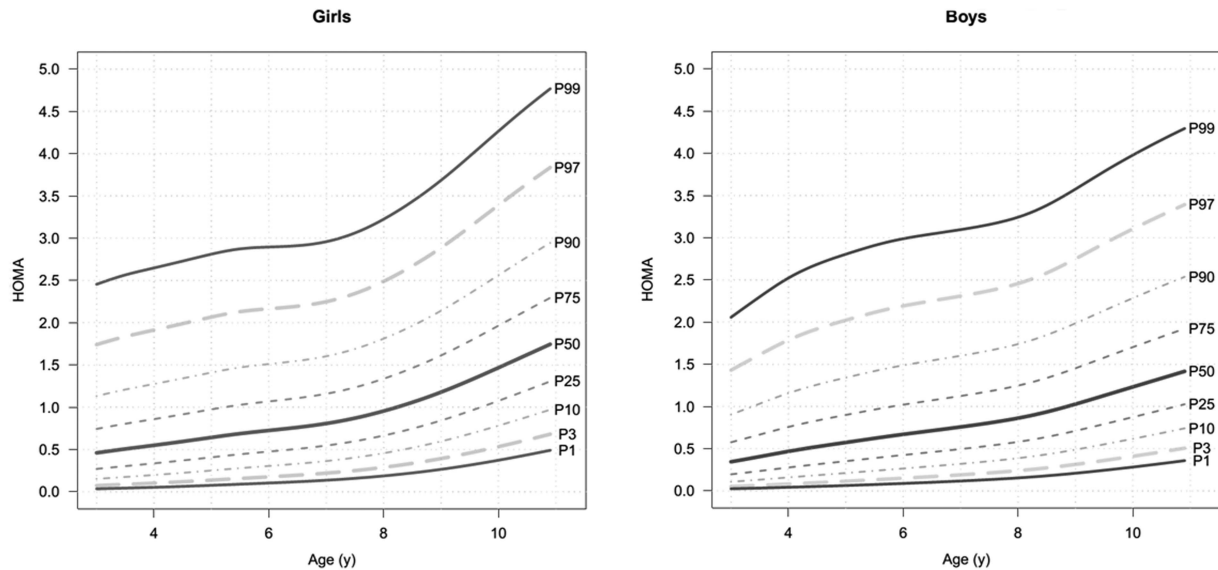


Figure 4. Percentiles of HOMA-IR from normal weight children of the IDEFICS cohort (2007–2010). Insulin was measured by an electrochemiluminescence immunoassay, glucose was measured by point-of-care analysis.

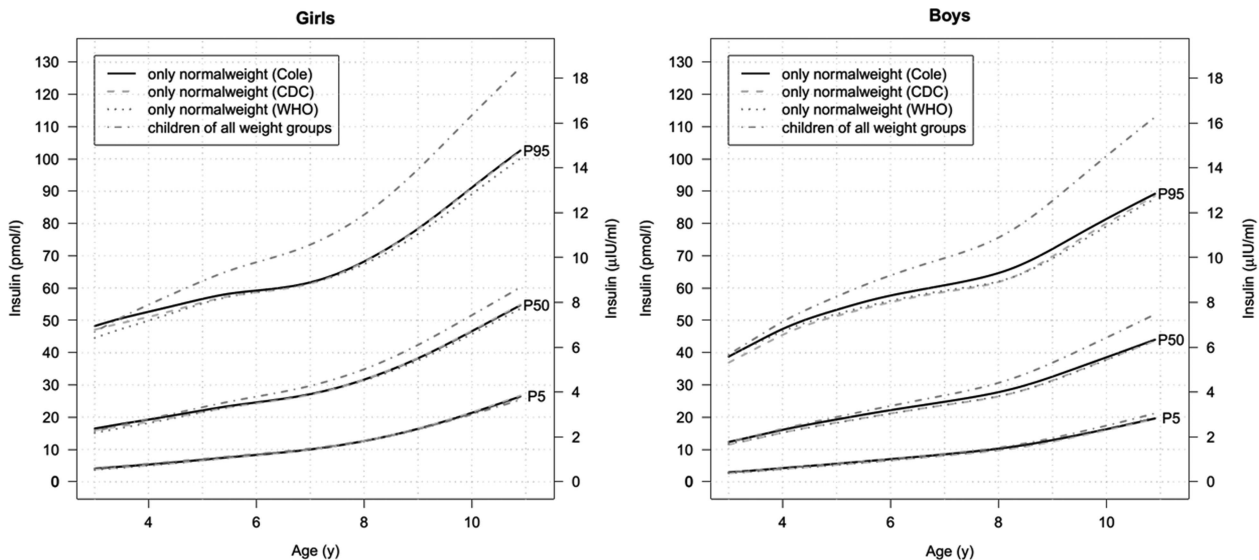


Figure 5. Sensitivity analysis showing percentiles of fasting serum insulin according to different definitions of normal weight and for the whole study group including children of all weight groups (classification of normal weight according to Cole *et al.*/International Obesity Taskforce,²⁰ Centers for Disease Control and Prevention³⁰ and World Health Organization^{31,32}).

adolescents were included (for example, combined analysis for Tanner stages I and II), which corresponds well with the pubertal peak described for all of the considered markers. Differences in insulin and HOMA-IR levels could also be due to the variety of different insulin assays used in the referenced studies.

In our study, only normal weight children were included into the modelling of percentile curves as the insulin level is known to be strongly influenced by BMI.³⁶ Despite the strong influence of BMI on markers of insulin resistance, the relationship between BMI and HOMA-IR has been shown to be much weaker in children than in adults.³⁷ Within the growing group of children with overweight and obesity, paediatricians are faced with the challenge of identifying individuals at the greatest risk of comorbidity. It has been pointed out by other authors that interventions to halt weight gain and promote weight loss in children are of limited success and demand significant resources and continuous follow-up and monitoring.³⁸ This puts the caregiver in the dilemma of

where to allocate the limited available resources and who among the obese children will benefit most from an intervention.¹ The provision of paediatric reference curves for insulin resistance is thus long overdue.

The main strengths of the present study are the large sample size and the standardised assessment of anthropometrical and laboratory measurements. The statistical modelling was done according to the most advanced methodology, which has also been used by the most recent studies in this field.^{29,39,40} Some limitations of the study should also be accounted for: even though the study was designed to reach all eligible children in the selected study regions via their settings, the overall participation rate was just above 50%,²² and a non-response bias, for example, towards higher social status may well be present. Nevertheless, the influence of social status on insulin resistance is likely to be mediated by weight status, which is one of the reasons why we restricted our analyses to normal weight children. Another

problem might be caused by the fact that pubertal stages were not assessed in the IDEFICS surveys. Even though children of our age range are considered to be pre-pubertal, we cannot exclude that a few of them might already have developed signs of beginning puberty that might better be excluded from the reference population.

In summary, the reference percentiles presented here are to our knowledge the first to be based on a multinational children's population that is also the largest cohort that was up to now used to model reference percentiles for markers of insulin resistance (insulin measured by electrochemiluminescence immunoassay). These reference values may help to identify children with insulin resistance who have an elevated risk for cardiovascular disease and type 2 diabetes mellitus. These reference values will thus allow for a more focused and earlier behavioural or therapeutic intervention. As insulin resistance has been shown to be reversible in most cases by healthy eating and physical activity,^{41,42} these reference values may hopefully contribute to reduce the burden of disease in the long run.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This work was done as part of the IDEFICS study (www.idefics.eu). We gratefully acknowledge the financial support of the European Community within the Sixth RTD Framework Programme Contract No. 016181 (FOOD). We express our sincerest gratitude to Timm Intemann for his enduring help with the GAMLSS modelling and to Ina Alvarez for technical support. We also wish to thank the IDEFICS children and their parents for their participation. The authors are also grateful to the Volkswagen Foundation that financially supported the production of this supplement.

STATEMENT OF ETHICS

We certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during this research. Approval by the appropriate Ethical Committees was obtained by each of the eight centres involved in the field work. Study subjects were not submitted to any study procedure before both the children and their parents had given their consent to examinations, collection of samples, subsequent analyses and storage of personal data and collected samples. Study subjects and their parents could consent to single components of the study while refraining from others. For ethical reasons the amount of blood drawn varied according to age, weight and height of the children and did not exceed 1% of the estimated blood volume of the child. Consequently, a volume of 10–25 ml (depending on the weight of the child) was not exceeded during blood withdrawal from the 2–11-year-old children.

DISCLAIMER

The information in this document reflects the author's view and is provided as is.

REFERENCES

- Weiss R, Kaufman FR. Metabolic complications of childhood obesity: identifying and mitigating the risk. *Diabetes Care* 2008; **31**(Suppl 2): S310–S316.
- International Obesity Task Force. *Obesity in Europe, the Case for Action*. International Obesity Task Force: London, UK, 2002.
- Martin BC, Warram JH, Krolewski AS, Bergman RN, Soeldner JS, Kahn CR. Role of glucose and insulin resistance in development of type 2 diabetes mellitus: results of a 25-year follow-up study. *Lancet* 1992; **340**: 925–929.
- Berenson GS, Sririvasan SR, Bao W, Newman WP III, Tracy RE, Wattigney WA. Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. The Bogalusa Heart Study. *N Engl J Med* 1998; **338**: 1650–1656.
- International Diabetes Foundation. *Global IDF/ISPAD Guideline for Diabetes in Childhood and Adolescence*. International Diabetes Foundation: Brussels, Belgium, 2011.
- Demmer RT, Zuk AM, Rosenbaum M, Desvarieux M. Prevalence of diagnosed and undiagnosed type 2 diabetes mellitus among US adolescents: results from the continuous NHANES, 1999–2010. *Am J Epidemiol* 2013; **178**: 1106–1113.
- Mansoub S, Chan MK, Adeli K. Gap analysis of pediatric reference intervals for risk biomarkers of cardiovascular disease and the metabolic syndrome. *Clin Biochem* 2006; **39**: 569–587.
- Singh B, Saxena A. Surrogate markers of insulin resistance: a review. *World J Diabetes* 2010; **1**: 36–47.
- Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; **28**: 412–419.
- Grant DB. Fasting serum insulin levels in childhood. *Arch Dis Child* 1967; **42**: 375–378.
- Allard P, Delvin EE, Paradis G, Hanley JA, O'Loughlin J, Lavallee C et al. Distribution of fasting plasma insulin, free fatty acids, and glucose concentrations and of homeostasis model assessment of insulin resistance in a representative sample of Quebec children and adolescents. *Clin Chem* 2003; **49**: 644–649.
- Mellerio H, Alberti C, Druet C, Capelier F, Mercat I, Josserand E et al. Novel modeling of reference values of cardiovascular risk factors in children aged 7 to 20 years. *Pediatrics* 2012; **129**: e1020–e1029.
- Aradillas-Garcia C, Rodriguez-Moran M, Garay-Sevilla ME, Malacara JM, Rascon-Pacheco RA, Guerrero-Romero F. Distribution of the homeostasis model assessment of insulin resistance in Mexican children and adolescents. *Eur J Endocrinol* 2012; **166**: 301–306.
- Masuccio FG, Lattanzio FM, Matera S, Giannini C, Chiarelli F, Mohn A. Insulin sensitivity in prepubertal Caucasian normal weight children. *J Pediatr Endocrinol Metab* 2009; **22**: 695–702.
- d'Annunzio G, Vanelli M, Pistorio A, Minuto N, Bergamino L, Lufasco D et al. Insulin resistance and secretion indexes in healthy Italian children and adolescents: a multicentre study. *Acta Biomed* 2009; **80**: 21–28.
- Barja S, Arnaiz P, Dominguez A, Villarroel L, Casis B, Castillo O et al. [Normal plasma insulin and HOMA values among Chilean children and adolescents]. *Rev Med Chil* 2011; **139**: 1435–1443.
- Wennlof AH, Yngve A, Nilsson TK, Sjoström M. Serum lipids, glucose and insulin levels in healthy schoolchildren aged 9 and 15 years from Central Sweden: reference values in relation to biological, social and lifestyle factors. *Scand J Clin Lab Invest* 2005; **65**: 65–76.
- Steene-Johannessen J, Kolle E, Anderssen SA, Andersen LB. Cardiovascular disease risk factors in a population-based sample of Norwegian children and adolescents. *Scand J Clin Lab Invest* 2009; **69**: 380–386.
- Manley SE, Stratton IM, Clark PM, Luzio SD. Comparison of 11 human insulin assays: implications for clinical investigation and research. *Clin Chem* 2007; **53**: 922–932.
- Cole TJ, Lobstein T. Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. *Pediatr Obes* 2012; **7**: 284–294.
- Peplies J, Fraterman A, Scott R, Russo P, Bammann K. Quality management for the collection of biological samples in multicentre studies. *Eur J Epidemiol* 2010; **25**: 607–617.
- Ahrens W, Bammann K, Siani A, Buchecker K, De HS, Iacoviello L et al. The IDEFICS cohort: design, characteristics and participation in the baseline survey. *Int J Obes (Lond)* 2011; **35**(Suppl 1): S3–15.
- Panz VR, Raal FJ, Paiker J, Immelman R, Miles H. Performance of the CardioChek PA and Cholestech LDX point-of-care analysers compared to clinical diagnostic laboratory methods for the measurement of lipids. *Cardiovasc J S Afr* 2005; **16**: 112–117.
- Whitehead SJ, Ford C, Gama R. A combined laboratory and field evaluation of the Cholestech LDX and CardioChek PA point-of-care testing lipid and glucose analysers. *Ann Clin Biochem* 2013; **51**: 54–67.
- Peplies J, Gunther K, Bammann K, Fraterman A, Russo P, Veidebaum T et al. Influence of sample collection and preanalytical sample processing on the analyses of biological markers in the European multicentre study IDEFICS. *Int J Obes (Lond)* 2011; **35**(Suppl 1): S104–S112.
- Cole TJ, Green PJ. Smoothing reference centile curves: the LMS method and penalized likelihood. *Stat Med* 1992; **11**: 1305–1319.
- Stasinopoulos DM, Rigby RA. Generalized additive models for location scale and shape (GAMLSS) in R. *J Stat Softw* 2007; **23**: 1–46.
- van Buuren S, Fredriks M. Worm plot: a simple diagnostic device for modelling growth reference curves. *Stat Med* 2001; **20**: 1259–1277.
- Cole TJ, Stanojevic S, Stocks J, Coates AL, Hankinson JL, Wade AM. Age- and size-related reference ranges: a case study of spirometry through childhood and adulthood. *Stat Med* 2009; **28**: 880–898.
- Kuczmarki RJ, Ogden CL, Guo SS, Grummer-Strawn LM, Flegal KM, Mei Z et al. 2000 CDC Growth Charts for the United States: Methods and Development:

- Vital Health Statistics Series 11*. Department of Health and Human Services, CDC: Hyattsville, MD, USA, 2000. vol 246, pp 1–190.
- 31 de Onis M, Lobstein T. Defining obesity risk status in the general childhood population: which cut-offs should we use? *Int J Pediatr Obes* 2010; **5**: 458–460.
- 32 de Onis M, Onyango AW, Borghi E, Siyam A, Nishida C, Siekmann J. Development of a WHO growth reference for school-aged children and adolescents. *Bull World Health Organ* 2007; **85**: 660–667.
- 33 Atabek ME, Pirgon O. Assessment of insulin sensitivity from measurements in fasting state and during an oral glucose tolerance test in obese children. *J Pediatr Endocrinol Metab* 2007; **20**: 187–195.
- 34 Kurtoglu S, Hatipoglu N, Mazicioglu M, Kendirici M, Keskin M, Kondolot M. Insulin resistance in obese children and adolescents: HOMA-IR cut-off levels in the prepubertal and pubertal periods. *J Clin Res Pediatr Endocrinol* 2010; **2**: 100–106.
- 35 Rodoo P, Ridefelt P, Aldrimer M, Niklasson F, Gustafsson J, Hellberg D. Population-based pediatric reference intervals for HbA1c, bilirubin, albumin, CRP, myoglobin and serum enzymes. *Scand J Clin Lab Invest* 2013; **73**: 361–367.
- 36 Whitrow MJ, Davies MJ, Giles LC, De Stavola BL, Owens JA, Maftei O *et al*. Effects of birth size, post-natal growth and current size on insulin resistance in 9-year-old children: a prospective cohort study. *Eur J Pediatr* 2013; **172**: 1207–1214.
- 37 Voss LD, Metcalf BS, Jeffery AN, Wilkin TJ. IOTF thresholds for overweight and obesity and their relation to metabolic risk in children (EarlyBird 20). *Int J Obes (Lond)* 2006; **30**: 606–609.
- 38 Summerbell CD, Waters E, Edmunds LD, Kelly S, Brown T, Campbell KJ. Interventions for preventing obesity in children. *Cochrane Database Syst Rev* 2005; **3**: CD001871.
- 39 Neuhauser HK, Thamm M, Ellert U, Hense HW, Rosario AS. Blood pressure percentiles by age and height from nonoverweight children and adolescents in Germany. *Pediatrics* 2011; **127**: e978–e988.
- 40 Rosario AS, Kurth BM, Stolzenberg H, Ellert U, Neuhauser H. Body mass index percentiles for children and adolescents in Germany based on a nationally representative sample (KiGGS 2003–2006). *Eur J Clin Nutr* 2010; **64**: 341–349.
- 41 Thomas DE, Elliott EJ, Naughton GA. Exercise for type 2 diabetes mellitus. *Cochrane Database Syst Rev* 2006; **3**: CD002968.
- 42 Waters E, de Silva-Sanigorski A, Hall BJ, Brown T, Campbell KJ, Gao Y *et al*. Interventions for preventing obesity in children. *Cochrane Database Syst Rev* 2011; **12**: CD001871.



This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International License. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>

Supplementary Information accompanies this paper on International Journal of Obesity website (<http://www.nature.com/ijo>)

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.