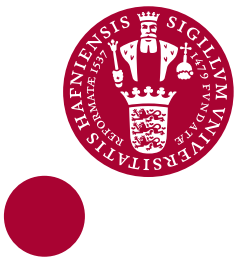

Glucose metabolism in children and adolescents

Population-based reference values and comparisons to children and adolescents enrolled in obesity treatment

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Keywords: Child, Glucose, Insulin, Obesity, Reference Values

Abbreviations:

BMI: Body mass index

CV: Coefficient of variation

DL: Detection limits

HOMA-IR: Homeostasis model assessment: insulin resistance

SDS: standard deviation score

Abstract

Background: Alterations in glucose metabolism that lead to the development of metabolic and cardiovascular disease may begin already in childhood.

Objective: This study aims to generate pediatric age and sex-specific reference values for fasting concentrations of glucose, HbA1c, insulin, C-peptide, and HOMA-IR in Danish/North-European white children and adolescents from a population-based cohort and to compare values from children and adolescents with overweight/obesity with this reference.

Methods: The population- and obesity clinic-based cohorts consisted of 2,451 and 1,935 children and adolescents between 6-18 years of age. Anthropometric measurements and blood samples were obtained and percentile curves were calculated.

Results: In the population based-cohort, glucose, insulin and HOMA-IR values increased before the expected onset of puberty ($P<0.05$). Thereafter, all variables decreased in girls ($P<0.05$) and HbA1c decreased in boys ($P<0.05$). Concentrations of all measured markers of glucose metabolism were higher in the obesity clinic-based cohort than the population-based cohort (both sexes $P<0.001$).

Specifically, insulin and HOMA-IR continued to increase to 18 years in the clinic-based cohort, particularly among boys.

Conclusions: Fasting glucose, insulin, and HOMA-IR change during childhood, making pediatric reference values essential for timely identification of derangements in glucose metabolism. Children and adolescents with obesity exhibit increased concentrations of these biomarkers.

Main text

Introduction

Alterations in glucose metabolism are important in the development of metabolic and cardiovascular disease [1–3] and may occur already in childhood. With the presence of childhood obesity [4], these alterations become increasingly common [5,6]. Though the prevalence of type 2 diabetes in children and adolescents is less than 1%, the prevalence is estimated to quadruple by 2050, mainly driven by the rising obesity rates [7,8] while prediabetes is already common [6,9].

Prediabetes is broadly defined as an altered glucose metabolism that is still below the diabetes threshold [2,10]. In adults with prediabetes, 30-40% will progress to manifest type 2 diabetes within a few years without intervention [11] and this transition has been reported to be even more rapid in children and adolescents [12].

Among children and adolescents with type 2 diabetes, the prevalence of obesity is much higher than in adults with type 2 diabetes [13], and there is a strong inverse association between BMI and age at onset of type 2 diabetes [14]. Therefore, focused efforts should be made in children and adolescents

with overweight/obesity to detect early alterations in glucose metabolism in order to begin timely interventions and furthermore to make preventive efforts.

Cardiovascular and metabolic risk factors vary with age, sex, ethnicity, and puberty stage [15]. This includes biochemical markers of glucose metabolism, emphasizing the importance of appropriate pediatric reference values for the physician. Different pediatric reference values for fasting concentrations of glucose, HbA1c, insulin, and HOMA-IR have been suggested in the literature [16–19] and especially fasting glucose has been shown to vary across populations [18]. Furthermore, concentrations of HbA1c were higher in a pre-pubertal combined European population compared to a Scandinavian population [16] supporting the need for establishing references values in regional and homogenous populations.

Our study contributes with age- and sex-specific reference values for glucose, HbA1c, insulin, C-peptide, and HOMA-IR in a homogenous population of Danish/North-European white children and adolescents. In addition, we present differences in these markers of glucose metabolism between the population-based cohort and children and adolescents with overweight/obesity.

Methods

Study populations

Two cohorts of children and adolescents were included in the study: 1) A population-based cohort and 2) a cohort with overweight/obesity.

The population-based cohort (N= 2,898) is based on children and adolescents recruited from October 2010 to February 2015 from schools across 11 municipalities in the region of Zealand and the Capital region in Denmark. Additionally, pre-school children were recruited from March 2015 to March 2016 from the public dentistry and health care nurses in one municipality in the region of Zealand. This cohort has previously been described [20,21].

The exclusion criteria for the population-based cohort were: 1) age younger than 6.0 or older than 18.9 years (N=124); 2) no available blood sample data (N=3); 3) more than 30 days between the clinical examination and the blood sample (N=95); 4) ethnicity other than Danish/North European white (N=220); 5) diagnosed type 1 diabetes (N=4); 5) diagnosed type 2 diabetes (N=0); 7) intake of insulin, Liraglutid, or metformin (N=0), and 8) meeting type 2 diabetes criteria on the blood sample (fasting plasma glucose>7.0 mmol/l and/or HbA1c > 49 mmol/mol) (N=1).

The cohort of children and adolescents with overweight/obesity (N=2,873) was recruited from January 2009 to June 2018 from The Danish Childhood Obesity Biobank, prior to the patients' commencement of a multidisciplinary outpatient treatment program [22,23]. Applying identical exclusion criteria as for the population-based cohort, the following exclusions were made: 1) age (N=148); 2) no blood sample data (N=6); 3) more than 30 days between the clinical examination and the blood sample (N=464); 4) ethnicity (N=305); 5) diagnosed type 1 diabetes (N=7); 6) diagnosed type 2 diabetes (N=2); 7) medication (N=3), and 8) meeting type 2 diabetes criteria on the blood sample (N=3).

Outcome measures

Phenotyping was performed by trained medical staff and involved an extensive systemized questionnaire, a clinical examination and a fasting venous blood sample. For the population-based cohort, the questionnaire was completed at home before the examination. For the cohort with overweight/obesity, the questionnaire was completed by interview with a nurse or a pediatrician. Fasting samples of plasma glucose, whole blood HbA1c, serum insulin, and serum C-peptide were obtained. HOMA-IR was used in this study as a surrogate marker for insulin sensitivity and was calculated as insulin (mIU/L) x glucose (mmol/L)/22.5 [24] with a conversion factor of 6.00 between insulin in μU/L and mmol/L [25].

Anthropometric measurements

Anthropometric measurements were performed while wearing light indoor clothes and no shoes. Height was measured by stadiometer to the nearest 1 mm and weight was measured to the nearest 0.1 kg on a Tanita® BC418 Scale (Tanita Corp., Japan) in the population-based cohort, and on a Tanita® Digital Medical Scale, WB-110 MA (Tanita Corp., Japan) in the cohort with overweight/obesity. BMI SDS was calculated using the LMS method [26] according to Danish BMI charts [27].

Puberty stage

The puberty stage was assessed according to the Tanner classification [28,29]. In the population-based cohort, puberty was self-evaluated using a questionnaire with picture pattern recognition. In the cohort with overweight/obesity, a pediatrician evaluated the puberty stage. Self-evaluated puberty staging has been shown adequate for discriminating between pre-pubertal and pubertal stages [30]. Consequently, puberty stage in both the population-based cohort and the cohort with

overweight/obesity was defined as pre-pubertal (Tanner 1) or pubertal (Tanner 2-5) in the present study.

Blood samples

The blood samples were obtained from venipuncture of the antecubital vein between 7 and 9 AM after an overnight fast. If requested, the venipuncture was performed after application of a local anesthetic (lidocaine/prilocaine mixture, EMLA®, AstraZeneca, Sweden).

Plasma glucose concentrations (intra- and inter-assay coefficients of variation (CV): 2.3% and the detection limits (DL): 0.06 mmol/L) were determined on a Dimension Vista® 1500 Analyser (Siemens, Germany). Serum insulin concentrations (CV: 2.0% and DL: 1.4 pmol/L) and C-peptide concentrations (CV: 3.4% and DL: 0.003 nmol/L) were analyzed on a Cobas® 6000 Analyser (Roche Diagnostics, Germany). Whole-blood HbA1c (CV: 1.9% and DL: 24.6 mmol/mol) was analyzed on a Tosoh high-performance liquid chromatography G8 analyser (Tosoh Corporation, Japan).

Data Protection Agency. The study was carried out in accordance with the Helsinki Declaration of 1975 as revised in 2013.

Statistics

Statistical analyses were performed in R statistical software (v.3.5.1) [32]. Age- and sex-specific percentiles and percentile curves were calculated using the Generalized Additive Models for Location Scale and Shape (GAMLSS) package [33], using the Box–Cox transformation distribution family. To investigate effects of age within each sex, the participants were allocated into three groups: 6.0-9.9, 10.0-14.9, and 15.0-18.9 years of age. These age intervals were chosen to approximate periods of pre-puberty, puberty, and post-puberty respectively [34], as puberty stage was only available in a subset of children and adolescents. Each sex was analyzed separately and normality of data was evaluated using histograms and qq plots. Differences between cohorts were examined using Student's t-test or Wilcoxon rank sum test. Differences in concentrations of biomarkers and HOMA-IR between age groups were examined using Kruskal-Wallis test and pairwise Wilcoxon rank sum tests. Multiple comparisons were controlled for by the Holm's method. A generalized linear regression model was used to investigate effects of puberty for each sex adjusted for age and BMI SDS. Potential interactions between pubertal stage and biomarkers and HOMA-IR were investigated. A $P<0.05$ was considered statistically significant.

Results

From the population-based cohort, 2,451 (1,452 girls) children and adolescents with a median age of 11.5 years were included in the study. Of these, 5.9% (N=144) exhibited underweight (BMI<10th percentile) and 17.7% (N=435) exhibited overweight/obesity (BMI>90th percentile) according to Danish reference values [27]. From the cohort with overweight/obesity (all: BMI>90th percentile), 1,935 (1,050 girls) children and adolescents with a median age of 11.8 years were included in the study. The descriptive data for the two cohorts are shown in Table 1. The age distribution by sex for each 1-year age stratum for both cohorts is shown in Supplementary Table S1.

Relation to age and sex in the population-based cohort

Age- and sex-specific reference values for glucose, HbA1c, insulin, and HOMA-IR from the population-based cohort are presented as percentile curves in Figure 1 and percentile reference tables in Supplementary Table S2. Changes in C-peptide mirror the changes in insulin and will not be presented further in results. Reference curves for C-peptide are presented in Supplementary Figure S1.

Table 2 shows the population-based cohort divided into age groups. From the youngest (6.0-9.9 years) to the intermediate age group (10.0-14.9 years) glucose, insulin, and HOMA-IR increased in both girls and boys, while HbA1c increased in only boys (all P<0.001). From the intermediate to the oldest age group (15.0-18.9 years) the sexes differed: in girls, glucose, HbA1c, insulin, and HOMA-IR all decreased (P<0.01); in boys, only HbA1c decreased (P<0.001). Figure 1 similarly illustrates how glucose, insulin, and HOMA-IR peak around expected pubertal maturation [34], corresponding to age ~13 years for girls and ~15 years for boys.

Characteristics of participants in the highest percentiles from the population-based cohort

Compared to the rest of the population-based cohort, participants with glucose values above the 90th percentile exhibited a 0.4 points higher BMI SDS (P<0.001). Participants with insulin and HOMA-IR above the 90th percentile both exhibited a 1.0 points higher BMI SDS (both P<0.001), while no difference was found for participants with HbA1c above the 90th percentile, compared to the rest of the population. Overall 24.6% of the population had values of one or more of these biomarkers above the 90th percentile, while only 2.6% had values above the 90th percentile for both glucose and insulin.

Comparison between the population-based cohort and the cohort with overweight/obesity

Reference values for glucose, HbA1c, insulin, and HOMA-IR from the cohort with overweight/obesity are presented as percentile curves in Figure 2 and percentile reference tables in Supplementary Table S3.

Table 2 also shows the cohort with overweight/obesity divided into age groups. Glucose, insulin, and HOMA-IR increased from the youngest to the intermediate age group in both girls and boys (P<0.001). The changes from the intermediate to the oldest age group differed from the pattern observed in the population-based cohort: in girls, only HbA1c decreased (P=0.023), whereas glucose, insulin and HOMA-IR remained unchanged; in boys, glucose, insulin, and HOMA-IR all increased (P<0.005) compared with no change in the population-based cohort.

When comparing the two cohorts, the median age in the cohort with overweight/obesity was higher in the youngest age group and lower in the oldest age group than the population-based cohort (P<0.05). As expected, BMI SDS was higher in the cohort with overweight/obesity compared with the population-based cohort in all age groups (P<0.001).

Insulin and HOMA-IR were higher in the cohort with overweight/obesity compared with the population-based cohort in all age groups (P<0.001) (see Table 2). Glucose was higher in the youngest and oldest age group (P<0.005), but similar in the intermediate age group compared with the population-based cohort. HbA1C was higher only in the oldest age group of boys in the cohort with overweight/obesity compared with the population-based cohort (P=0.012).

Figure 2 compares percentile curves for the 5th, 50th, and 95th percentiles of the biomarkers between the two cohorts and illustrates marked differences. For insulin and HOMA-IR, the rise towards the described peak around pubertal maturation occurred at an earlier age in the cohort with overweight/obesity. For girls, these concentrations remained high thereafter, whereas for boys, they increased even further. Glucose and HbA1C showed a comparable, but less pronounced, pattern in the boys with overweight/obesity.

Relation to puberty stage

Data on puberty stage was available in 80.0% (N=1161) of girls and in 58.8% (N=587) of boys in the population-based cohort. When adjusting for age and BMI SDS, puberty compared with pre-puberty in girls was associated with 0.27 mmol/L higher concentrations of glucose ([95% CI:0.20;0.34], P<0.001); 0.49 mmol/mol higher concentrations of HbA1C ([95% CI:0.00;0.98], P=0.048); 21.7 pmol/L higher concentrations of insulin ([95% CI:16.4;26.9], P<0.001); and 0.94 mIU/L higher HOMA-IR ([95% CI:0.72;1.16], P<0.001). When adjusting for age and BMI SDS, puberty compared with pre-puberty in boys was associated with 0.09 mmol/L higher concentrations of glucose ([95% CI:0.01;0.18], P=0.024); and interactions were identified such that the association

between BMI SDS and insulin was 4.0 pmol/L higher per BMI SDS in pubertal than pre-pubertal boys ([95% CI:0.1;8.0], P=0.047).

Data on puberty stage was available in 85.0% (N=893) of girls and in 75.1% (N=665) of boys in the cohort with overweight/obesity. When adjusting for age and BMI SDS in girls in the cohort with overweight/obesity, puberty compared with pre-puberty was associated with 0.13 mmol/L higher concentrations of glucose ([95% CI:0.04;0.22], P=0.006). For the other biomarkers interactions were identified such that the association between BMI SDS and HbA1c was 1.44 mmol/mol higher per BMI SDS in pubertal than pre-pubertal girls ([95% CI:0.65;2.23], P<0.001); the association between BMI SDS and insulin was 32.8 pmol/L higher per BMI SDS ([95% CI:14.0;51.6], P<0.001); and the association between BMI SDS and HOMA-IR was 1.18 mIU/L higher per BMI SDS ([95% CI:0.60;1.77], P<0.001). In boys in the cohort with overweight/obesity, interactions were identified such that the association between BMI SDS and insulin was 31.8 pmol/L higher per BMI SDS in pubertal than pre-pubertal boys ([95% CI:4.7;58.8], P=0.022); and the association between BMI SDS and HOMA-IR was 1.46 mIU/L higher per BMI SDS ([95% CI:0.18;2.74], P=0.025).

These interactions were not present in girls in the population-based cohort, and while an interaction was present in boys in the population-based cohort for insulin, it showed an 8-fold lower effect than in the cohort with overweight/obesity (^{2} = 4.0 vs. ^{2} = 31.8).

Discussion

We generated age- and sex-specific reference values for fasting blood concentrations of glucose, HbA1c, insulin, C-peptide, and the HOMA-IR index from a large population-based cohort of

Danish/Northern European white children and adolescents aged 6.0-18.9 years and demonstrated that these markers of glucose metabolism vary significantly during childhood and adolescence. We further examined how the concentrations of these markers in a cohort of children and adolescents with overweight/obesity compared with the established reference.

Our presented references values are in accordance with the existing literature [16,17,19,35]. The IDEFICS study by Peplies *et al*. presented reference values for 7,074 pre-pubertal children with normal weight and ages of 3.0–10.9 years from eight European countries and showed that fasting glucose, insulin, and HOMA-IR increase with age [16]. The HELENA study by Koester-Weber *et al* calculated reference values for fasting glucose and insulin in 927 adolescents aged 12.5–17.4 years from nine European countries and showed stable glucose values and deceasing insulin values with age [17]. Most adolescents in the HELENA study were classified as Tanner 4 or 5. Our study included both pre-pubertal, pubertal, and post-pubertal children and adolescents, and we observed both a significant increase in these biomarkers with age before expected onset of puberty, similar to the IDEFICS study, and a significant decrease (mainly in girls) or a plateau in these biomarkers with age after expected puberty, similar to the HELENA study [17]. We observed a 45–50% increase in median HOMA-IR from age 10 to 14 years in both girls and boys. This phenomenon of relative pubertal insulin resistance is well known, and an approximate 30% reduction in estimates of insulin sensitivity during mid-puberty has previously been reported [36,37]. Interestingly, while insulin levels rose sharply during puberty, fasting glucose was only minimally affected in the present study, probably due to an adequate ability to increase insulin production.

When comparing the two cohorts, we found that the cohort with overweight/obesity was affected in their markers of glucose metabolism: glucose was higher in the youngest and oldest age group, whereas insulin and C-peptide concentrations as well as HOMA-IR were higher in all age groups. This is similar to the findings in the IDEFICS study, where a strong dependency on weight status was observed for insulin and HOMA-IR but not glucose [16]. The HELENA study and Koester-Weber *et al*. also showed a positive association between BMI and insulin and they did not detect an association with glucose [17]. However in contrast to the present study, these two studies did not include entire cohorts with overweight/obesity, as they considered solely the participants from their population-based samples with overweight/obesity and as such might be underpowered to detect the associations for fasting glucose.

In a sub analysis of the population-based cohort with values above the 90th percentile for each biomarker, we found that these participants had a significantly higher BMI SDS, especially for insulin and HOMA-IR. We further found that only a very small proportion of the population had high values of both glucose and insulin. While normal variations in markers of glucose metabolism are complex, these results indicate that also in a population-based pediatric sample, increased weight affects glucose metabolism mainly through increased insulin resistance matched by an increased insulin production.

In our population-based cohort, puberty compared with pre-puberty was associated with increased values of both glucose and insulin in girls, but only glucose in boys. Interestingly, in the cohort with overweight/obesity the associations between BMI SDS and both insulin and HOMA-IR were much higher per BMI SDS in pubertal than pre-pubertal girls and boys. These results suggest a greater

and more harmful effect of overweight and obesity on glucose metabolism after the onset of puberty. This is in line with the findings of a recent registry study of 62,565 Danish men, examining associations between weight status from childhood through early adulthood and risk of adult type 2 diabetes [38]. Bjerregaard et al. [38] found that childhood overweight at 7 years of age that remitted to normal weight at 13 years of age was associated with no increased risk of adult type 2 diabetes, whereas childhood overweight that continued into adolescence and early adulthood was associated with a fourfold increase in risk.

Derangements in glucose metabolism often precede the development of type 2 diabetes. Although overt type 2 diabetes in childhood is relatively rare, prediabetes is common [6,8]. Prediabetes constitutes a heterogeneous group of early alterations in glucose metabolism [5] and for this reason, there are several different criteria for the diagnosis. Diagnosing alternations in glucose metabolism in children and adolescents is further complicated by marked variations in normal values during growth and development. When obesity is also present, this natural variation is even more complex [5,37]. Our study demonstrated that pubertal insulin resistance occurs at an earlier age and either persists in girls or increases further in boys with overweight and obesity. The natural alterations in glucose metabolism, as well as the exaggerated alterations during childhood obesity, emphasize the importance of appropriate reference values.

Established reference values are a useful tool for clinical assessment of children having increased risk of metabolic derangements and subsequent morbidity. Given the relative overlap between the presented cohorts and taking into consideration the complexity and relative heterogeneity of childhood obesity, no definitive single threshold can be given based on this data and accordingly,

individualized risk stratification should be done by taking the individual patients' phenotype and biochemical marker into consideration.

Strengths and limitations

Insight into insulin resistance can be achieved in many ways. The gold standard measure of insulin resistance is obtained from clamp-studies [39], but this method is time consuming and not easily applicable in daily clinical practice. Therefore in the present study, fasting measures of plasma glucose and serum insulin were used, as well as HOMA-IR calculated from fasting values of glucose and insulin, as these measures have been shown to correlate with insulin sensitivity values obtained in clamp studies [24,40].

Strengths of the present study include the large homogenous group of children and adolescents included, making the population-based cohort and the cohort with overweight/obesity comparable. Moreover, the biochemical methods were meticulous, as all samples were collected in a narrow time interval in the morning after an overnight fast. Additionally, all samples were analyzed at the same time and in the same batch using identical laboratory procedures for all participants. This is important as it has been shown that different assays for insulin measurement can produce important variations in concentrations [41].

A limitation to the study is that only one blood sample was collected on each of the participants. Preferably, insulin would have been measured from blood samples taken at three consecutive time points to correct for the pulsatile insulin secretion. Likewise, with only a single measurement of glucose we were unable to adjust for natural day-to-day variation. Although the thorough

biochemical method would minimize this variation, and the fact that sampling and time of analysis was identical for all study participants allows for comparison.

In conclusion, our study provides age- and sex-specific reference values for fasting blood concentrations of glucose, HbA1c, insulin, C-peptide, and the HOMA-IR index from a large population-based cohort of Danish/Northern European white children and adolescents. Markers of glucose metabolism vary during childhood and adolescence, increase transiently around puberty, and are dependent on age and sex. Children and adolescents with obesity exhibit increased concentrations of these biomarkers, especially after onset of puberty, which should raise awareness for both prevention and treatment of childhood obesity.

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Tables

Table 1. Descriptive information on the population-based cohort and the cohort with overweight/obesity

Characteristic		Population-based cohort		Cohort with overweight/obesity	
		n	Median (interquartile range)	*n*	Median (interquartile range)
Age (years)					
	f	1452	11.9 [9.3-15.0]	1050	11.7 [9.3, 14.4]
	m	999	11.0 [8.5-13.9]	885	11.9** [9.9, 13.8]
Height (cm)					
	f	1452	154.0 [137.0-165.5]	1050	154.2 [141.5, 164.5]
	m	999	148.5 [135.0-169.0]	885	155.0** [144.5, 167.0]
Weight (kg)					
	f	1452	43.5 [30.6-55.7]	1050	62.7** [47.3, 81.8]
	m	999	38.5 [29.3-56.2]	885	63.4** [50.0, 82.2]
BMI SDS					
	f	1452	0.2 [-0.5-1.0]	1050	2.7** [2.3, 3.0]
	m	999	0.3 [-0.4-1.0]	885	3.1** [2.6, 3.6]
Puberty: pre-pubertal/ pubertal (%)	f	1161	32/68	893	34/66
	m	587	38/62	665	48/52
Glucose (mmol/L)					
	f	1413	4.9 [4.7-5.2]	1018	5.0** [4.7, 5.3]
	m	971	5.0 [4.8-5.2]	861	5.1** [4.9, 5.4]
HbA1c (mmol/mol)					
	f	1428	34.0 [32.0-35.0]	1043	34.0* [32.0, 36.0]
	m	974	34.0 [32.0-35.0]	877	34.0* [32.0, 36.0]
Insulin (pmol/L)					
	f	1440	61.6 [45.0-84.6]	1014	101.7** [70.1, 143.6]
	m	984	51.4 [34.1-68.8]	863	93.4** [63.2, 136.8]
C-peptide (nmol/L)					
	f	1421	0.6 [0.4-0.7]	976	0.8** [0.6, 1.1]
	m	969	0.5 [0.4-0.6]	840	0.8** [0.6, 1.0]
HOMA-IR (mIU/L)					
	f	1404	2.3 [1.6-3.2]	983	3.8** [2.5, 5.5]
	m	960	1.9 [1.3-2.6]	840	3.6** [2.4, 5.3]

Data are medians and interquartile ranges. All biomarkers are fasting blood concentrations. BMI SDS: Body mass index standard deviation score; HbA1c: Hemoglobin A1c; HOMA-IR: Homeostasis model assessment: insulin resistance. * P<0.05 and ** P<0.001 compared with population-based cohort.

Table 2. Descriptive information by age group on the population-based cohort and the cohort with overweight/obesity

Characteristic		6.0-9.9 years		10.0-14.9 years		15.0-18.9 years	
		Population-based cohort	**Cohort with overweight/obesity**	**Population-based cohort**	**Cohort with overweight/obesity**	**Population-based cohort**	**Cohort with overweight/obesity**
No. of Subjects							
	f	481	326	610	523	361	201
	m	396	238	435	510	168	137
Age (years)							
	f	8.1 [7.1-9.2]	8.3* [7.5, 9.2]	12.3 [11.2-13.5]	12.3 [11.1, 13.6]	16.9 [15.9-17.9]	16.2** [15.5, 17.2]
	m	8.0 [7.1-9.0]	8.6** [7.6, 9.3]	12.3 [11.0-13.5]	12.2 [11.3, 13.3]	16.8 [15.9-17.6]	16.0** [15.5, 16.8]
Height (cm)							
	f	131.0 [124.5-138.4]	136.2** [130.7, 141.7]	157.5 [150.0-164.0]	158.1 [150.9, 164.6]	168.0 [164.0-172.0]	166.5* [163.5, 170.6]
	m	132.0 [125.5-138.0]	139.0** [132.0, 144.5]	156.0 [147.2-166.3]	157.3 [150.8, 164.5]	179.9 [175.0-185.1]	179.4 [173.5, 184.3]
Weight (kg)							
	f	27.7 [24.0-32.6]	41.8** [37.0, 47.7]	45.4 [38.2-53.4]	68.7** [56.8, 81.0]	59.5 [54.4-65.5]	89.3** [80.9, 99.5]
	m	27.9 [24.7-31.7]	45.5** [39.1, 52.8]	44.4 [37.2-53.8]	65.8** [57.8, 76.2]	68.7 [62.4-76.8]	108.3** [93.3, 122.1]
BMI SDS							
	f	0.2 [-0.5-1.0]	2.7** [2.3, 3.0]	0.2 [-0.5-0.9]	2.7** [2.3, 3.0]	0.4 [-0.3-0.9]	2.8** [2.4, 3.2]
	m	0.2 [-0.5-1.0]	3.4** [2.8, 4.0]	0.3 [-0.4-1.1]	2.9** [2.5, 3.4]	0.5 [-0.0-1.1]	3.4** [2.9, 3.7]
Glucose (mmol/L)							
	f	4.8 [4.6-5.0]	4.9** [4.7, 5.2]	5.1 [4.9-5.3]	5.0 [4.8, 5.4]	4.9 [4.6-5.1]	5.0* [4.7, 5.3]
	m	4.9 [4.7-5.1]	5.0** [4.7, 5.2]	5.1 [4.9-5.3]	5.1 [4.9, 5.4]	5.1 [4.9-5.3]	5.2** [5.0, 5.5]
HbA1c (mmol/mol)							
	f	34.0 [32.0-35.0]	34.0 [32.0, 36.0]	34.0 [32.0-36.0]	34.0 [32.0, 36.0]	33.0 [31.8-35.0]	33.0 [31.0, 36.0]
	m	34.0 [32.0-35.0]	34.0 [32.0, 36.0]	34.0 [32.0-36.0]	34.0 [32.0, 36.0]	33.0 [31.0-35.0]	34.0* [32.0, 36.0]
Insulin (pmol/L)							
	f	43.2 [30.8-56.2]	71.7** [52.1, 97.4]	73.8 [55.4-97.0]	116.2** [84.4, 158.4]	67.8 [53.9-89.4]	113.8** [82.2, 160.5]
	m	34.5 [24.7-49.7]	68.7** [48.6, 99.1]	60.3 [44.7-79.0]	95.3** [68.8, 130.1]	60.0 [47.5-79.1]	159.5** [114.6, 217.1]
C-peptide (nmol/L)							
	f	0.4 [0.3-0.5]	0.6** [0.5, 0.8]	0.6 [0.5-0.8]	0.9** [0.7, 1.2]	0.7 [0.6-0.8]	1.0** [0.8, 1.2]

m	0.4 [0.3-0.5]	0.6** [0.5, 0.7]	0.5 [0.4-0.7]	0.8** [0.6, 1.0]	0.6 [0.5-0.7]	1.1** [0.9, 1.4]
HOMA-IR (mIU/L)						
f	1.6 [1.1-2.1]	2.6** [1.8, 3.7]	2.8 [2.1-3.7]	4.5** [3.1, 6.2]	2.5 [2.0-3.2]	4.3** [2.9, 6.2]
m	1.3 [0.9-1.8]	2.6** [1.8, 3.6]	2.3 [1.7-3.1]	3.7** [2.6, 5.0]	2.2 [1.8-3.1]	6.0** [4.2, 8.7]

Data are medians and interquartile ranges. All biomarkers are fasting blood concentrations. BMI SDS: Body mass index standard deviation score; HbA1c: Hemoglobin A1c; HOMA-IR: Homeostasis model assessment: insulin resistance. * P<0.05 and ** P<0.001 compared with same age group in the population-based cohort.

Legends to figures

Figure 1. Percentile curves for fasting blood concentrations of glucose, Hemoglobin A1c (HbA1c), insulin, and Homeostasis model assessment: insulin resistance (HOMA-IR). Smoothed 5th, 50th, and 95th percentile curves for girls (first column, red) and boys (second column, blue) from the population-based cohort. Individual values represented by light grey dots.

Figure 2. Percentile curves for fasting blood concentrations of glucose, Hemoglobin A1c (HbA1c), insulin, and Homeostasis model assessment: insulin resistance (HOMA-IR). Smoothed 5th, 50th, and 95th percentile curves for girls (first column, red) and boys (second column, blue). Full lines and light grey dots represent the population-based cohort, and dotted lines and dark grey dots represent the cohort with overweight/obesity. (Fourteen very high values from the cohort with overweight/obesity were outside depicted range: Girls, insulin: 694 pmol/L (10 years), 710 (11y), 751 (12y), 941 (10y); Girls, HOMA-IR: 27 mIU/L (12y), 34 (10y), 35 (11y); Boys, insulin: 680 (15y), 811 (12y), 1401 (16y), 2098 (14y); Boys, HOMA-IR: 28 (12y), 68 (16y), 101 (14y).)

Supplementary Figure S1. Percentile curves for fasting blood concentrations of C-peptide. Smoothed 5th, 50th, and 95th percentile curves for girls (first column, red) and boys (second column, blue) from the population-based cohort (first row) and with the cohort with overweight/obesity superimposed (second row). Full lines and light grey dots represent the population-based cohort, and dotted lines and dark grey dots represent the cohort with overweight/obesity. (Three very high values from the cohort with overweight/obesity were outside depicted range: Girls: 3.3 nmol/L (12 years); Boys: 3.9 (16y), 5.1 (14y).)