

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

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Background: Plasma fasting insulin and the homeostasis model assessment of insulin resistance (HOMA-IR) are markers of IR, which, at least in part, mediates the relation of obesity to increased cardiovascular risk. Increased free fatty acids (FFAs) may be involved in the pathogenesis of IR. Our objectives were to describe the distributions of fasting plasma insulin, glucose, and FFAs and HOMA-IR in youth and to assess the relationship between FFAs and markers of IR.

Methods: Fasting plasma insulin, glucose, and FFAs were measured in a representative sample of Quebec youth comprising 2244 individuals 9, 13, and 16 years of age.

Results: In all age and sex groups, glucose exhibited remarkably tight distributions (median CV, 7.1%) in contrast to insulin, HOMA-IR, and FFAs (median CVs, 52%, 54% and 45%, respectively). For every percentile examined, 9-year-olds had lower insulin concentrations and HOMA-IR values than 13- and 16-year-olds. We observed strong correlations between insulin concentrations and HOMA-IR values, as well as close similarity in their rankings of individuals. The mean concentrations of glucose were higher in our population than in

other Caucasian pediatric populations. No positive correlations were detected between FFAs and markers of IR.

Conclusions: We report some of the first data on the distributions of fasting plasma insulin, HOMA-IR, and FFAs from a representative sample of youth. HOMA-IR does not appear more informative than fasting insulin as a marker of IR. Our findings on higher mean glucose concentrations in this population require confirmation in other representative samples of youth to assess whether the North American distribution of glucose concentrations is shifting positively.

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The insulin resistance syndrome (IRS),⁶ also known as the metabolic syndrome, is characterized by the clustering of some or all of the following anomalies: hyperinsulinemia, impaired glucose metabolism, dyslipidemia, abnormalities in fibrinolysis and coagulation, overweight, and hypertension (1). Numerous large prospective studies have shown that individuals with IRS are at increased risk for type 2 diabetes and cardiovascular diseases (1–3). It is therefore important to identify these individuals and to offer them proper preventive intervention (3).

Recently, there has been increased recognition of the need for prevention of cardiovascular disease risk factors in children and adolescents. Indeed, atherosclerosis begins early in life, and behaviors associated with increased

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⁶ Nonstandard abbreviations: IRS, insulin resistance syndrome; HOMA-IR, homeostasis model assessment of insulin resistance; FFA, free fatty acid; CEQAL, Canadian External Quality Assurance Laboratory; and CI, confidence interval.

risk of atherosclerotic disorders are often well established by the end of adolescence (4). Moreover, the noticeable increase in the prevalence of childhood obesity over the last two decades (5, 6), with the concomitant increase in the incidence of type 2 diabetes diagnosed during adolescence (7), underscores the need for substantial preventive efforts targeted toward childhood IRS and obesity.

IR, an important feature of IRS, can be measured with the euglycemic insulin glucose clamp. Because this method is laborious and not applicable to clinical practice or epidemiologic studies, fasting plasma insulin concentrations and the homeostasis model assessment (HOMA) are often used as markers of IR (8, 9). In adults, doubt has been expressed as to the validity of the HOMA as a better surrogate estimate of IR than fasting insulin (10). Surprisingly, there are few published data on the distributions of these important metabolic indices in pediatric populations (11–13) and, to the best of our knowledge, none in representative samples. Therefore, our first objective was to describe the distributions of fasting plasma insulin and glucose concentrations and of HOMA-IR values in a representative sample of Quebec children and adolescents. We also wished to test in a pediatric population whether an index of IR calculated from fasting glucose and insulin, such as HOMA, provided an evaluation of IR different from that obtained from the fasting insulin concentration alone.

Free fatty acids (FFAs) are an important energy source for most body tissues, particularly during periods of fasting. They also serve a broader function in whole body fuel homeostasis by virtue of their ability to act as potent signaling entities in various cellular processes. Strong evidence suggests that increases in FFAs may play a key role in the pathogenesis of IR (14). One may therefore wonder whether FFA concentrations can be used as an early marker of IR. Thus, our second objective was to examine the distribution of FFAs in our sample of children and adolescents and to assess the relationship between fasting plasma concentrations of FFA and markers of IR.

Materials and Methods

STUDY POPULATION

The study population comprised a probability sample of Quebec youth 9, 13, and 16 years of age who participated in the Quebec Child and Adolescent Health and Social Survey, which was conducted in schools between January and May 1999. Three (one per age) independent, stratified, cluster random samples were drawn from the Quebec Ministry of Education list of all children attending public or private schools. Schools in the most remote regions of Quebec, federal government and aboriginal schools, those in which >50% of students had severe handicaps, and those with <12 eligible students were excluded. This sampling frame represented 97% of youth of the targeted ages. Among eligible children, 51.5% (783 of 1520), 54.6% (818 of 1498), and 58.5% (874 of 1495) of 9-,

13-, and 16-year-olds, respectively, agreed to blood collection. These low response proportions raised the possibility of selection bias. However, age-specific comparisons of youth who did and did not participate in the blood collection process revealed no significant differences for parental income and education, for family history of cardiovascular disease, for urban or rural residence, and for weight category of youth. French Canadians comprised 79.6% of the sample. Of 2475 blood samples available, 7 were excluded for blood glucose analysis because the individuals reported they were diabetic; an additional 224 specimens were excluded for measurement of insulin and FFAs because (a) 107 parents refused to provide consent for further analyses and (b) 117 samples arrived thawed at the laboratory or were of insufficient amount. This study was approved by the Ethics Review Boards of Sainte-Justine Hospital and Institut de la statistique du Québec. Informed consent was obtained from parents or guardians.

BIOCHEMICAL ANALYSES

Blood was obtained by venipuncture between 0800 and 1000 in the morning, after an overnight fast, in a EDTA (1g/L) collection tube. Samples were placed on ice, centrifuged on site within 45 min, frozen on dry ice, and sent within 24 h to the laboratory, where they were stored at -80°C . Adequacy of the fasting period was checked by nurses before blood was collected.

Plasma glucose concentrations were measured on the Beckman Coulter CX⁷ analyzer using the glucose oxidase method. The interassay CVs for controls at 2.17, 6.38, and 13.69 mmol/L (Triad[®] Link levels 1, 2 and 3; Beckman Coulter, Inc.) were 3.8%, 1.3%, and 1.4%, respectively ($n = 24$ for each of the three concentrations). To assess agreement between measurements done in our laboratory and those obtained with the hexokinase/glucose 6-phosphate dehydrogenase reference method (15), 50 samples randomly selected from among all the samples were analyzed by the Canadian External Quality Assurance Laboratory, Ltd (CEQAL; Vancouver, BC). Over a range of 4.0–6.1 mmol/L, the mean difference between our measurements and those of CEQAL (our laboratory glucose value minus the CEQAL glucose value) was 0.15 mmol/L [95% confidence interval (CI), 0.10–0.20] (16).

Plasma insulin concentrations were measured with the Ultrasensitive Insulin assay on the Access[®] immunoassay system (Beckman Coulter). This is a double sandwich immunoassay with no cross-reactivity with proinsulin or C-peptide. The interassay CVs for controls at 92 and 285 pmol/L (Lyphochek[®] Immunoassay Plus Control, levels 1 and 2; Bio-Rad) were 4.1% and 5.0%, respectively ($n = 24$ at level 1 and $n = 23$ at level 2). Duplicate measurements of a systematic random sample of 1 in 20 specimens, analyzed 1 week apart, showed a median CV across specimens of 3.1% (5th and 95th percentiles, 0.3% and 13.5%; $n = 116$).

Plasma FFA concentrations were measured manually

Table 1. Distribution of plasma insulin concentrations.

		Insulin, pmol/L					
		Percentile (95% CI)					
Age, years (n)	Mean (95% CI)	5th	25th	50th	75th	95th	
Boys	9 (342)	29.4 (27.0–31.8)	10.7 (9.5–11.6)	17.9 (16.8–19.1)	25.6 (23.9–27.1)	35.0 (32.6–37.8)	56.3 (52.0–77.8)
	13 (370)	50.0 (45.3–54.6)	19.2 (16.0–20.6)	28.8 (27.7–30.1)	41.9 (39.0–45.2)	60.0 (56.0–63.7)	98.5 (88.7–119.4)
	16 (375)	46.9 (43.1–50.6)	19.6 (17.6–21.7)	29.5 (28.0–31.0)	38.7 (37.5–40.4)	50.7 (46.9–56.3)	97.6 (86.5–121.9)
Girls	9 (369)	35.4 (30.2–40.7)	12.0 (9.9–13.5)	20.0 (19.1–22.1)	29.0 (27.6–30.1)	40.6 (37.3–43.2)	61.1 (58.1–78.7)
	13 (352)	58.4 (53.8–63.1)	25.4 (22.8–27.1)	38.7 (36.3–41.0)	51.1 (48.0–53.5)	69.9 (65.3–74.0)	114.4 (101.1–126.1)
	16 (436)	51.0 (48.5–53.6)	22.4 (19.8–24.6)	34.2 (32.5–36.4)	46.4 (43.5–48.4)	62.8 (59.0–68.4)	95.1 (89.0–104.3)

by means of an enzymatic, colorimetric method (Wako Chemicals). The interassay CV, assessed with two pools of plasma, was 4.0% and 3.0% at 0.25 and 0.42 mmol/L, respectively ($n = 43$ for each concentration). Duplicate measurements of a systematic random sample of 1 in 20 specimens, analyzed 1 week apart, showed a median CV across specimens of 2.8% (5th and 95th percentiles, 0.0% and 11.3%; $n = 111$).

HOMA-IR was calculated as proposed by Matthews et al. (9): insulin (mIU/L) \times glucose (mmol/L)/22.5.

STATISTICAL ANALYSES

Wilcoxon rank-sum tests were used to assess differences by ethnic origin (French Canadian vs other) in plasma concentrations of insulin, glucose, and FFAs. Statistically significant ($P < 0.05$) differences were observed only in 13-year-old boys for FFAs and in 16-year-old boys for insulin; therefore, ethnic origin was not taken into consideration in subsequent analyses. We used the sample quantiles to estimate the corresponding population percentiles. Nonparametric CIs for the quantiles of interest were constructed by approximating the distribution of the linear interpolation estimator of the quantile function with the distribution of the fractional order statistic, using the algorithm described by Hutson (17). When comparing percentile values between sexes or across ages, we concluded that they were significantly different if their respective 95% CIs were nonoverlapping. For data that were not normally distributed (insulin, FFAs, and HOMA-IR), CVs were computed as the square root of the variance of the natural logarithm (\log_e)-transformed data (18). For normally distributed data, CVs were computed as usual

as the SD divided by the mean. To take the complex sampling design into account, sample weights and clustering effects were estimated and incorporated into our calculation of quantiles, CIs and CVs. Pearson correlation coefficients were computed without use of sample weights and clustering effects. Statistical analyses were performed with SAS statistical software (SAS Institute, Inc) and SUDAAN (Research Triangle Institute).

Results

The means and selected percentile values of plasma insulin concentrations by age and sex are shown in Table 1. Distributions of plasma insulin concentrations were positively skewed. For all ages, the 25th percentile and median insulin values were higher in girls than in boys, whereas no significant differences between sexes were detected at the 5th and 95th percentiles (except for the 5th percentile in 13-year-olds). For every percentile examined, in both sexes, 9-year-olds had lower insulin concentrations than did 13- and 16-year-olds.

As expected, plasma glucose distributions were symmetric. In 9-, 13-, and 16-year-olds, boys tended to have higher glucose concentrations than girls except at the 95th percentile, where they were similar (Table 2). For every percentile estimated, 9-year-old boys tended to have lower glucose concentrations than 13- and 16-year-old boys, whereas for girls, 9- and 16-year-olds showed similar values that were slightly lower than those of 13-year-olds for the 25th, 50th, and 75th percentiles.

Similar to insulin, HOMA-IR distributions were positively skewed. Percentile values for HOMA-IR were similar in boys and girls of the same age except for the 5th,

Table 2. Distribution of plasma glucose concentrations.

		Glucose, mmol/L					
		Percentile (95% CI)					
Age, years (n)	Mean (95% CI)	5th	25th	50th	75th	95th	
Boys	9 (382)	5.2 (5.1–5.2)	4.7 (4.5–4.7)	5.0 (4.9–5.0)	5.2 (5.1–5.2)	5.4 (5.3–5.4)	5.7 (5.6–5.7)
	13 (416)	5.3 (5.3–5.3)	4.8 (4.7–4.9)	5.1 (5.0–5.1)	5.3 (5.3–5.3)	5.5 (5.4–5.5)	5.9 (5.7–6.0)
	16 (406)	5.3 (5.3–5.3)	4.7 (4.7–4.8)	5.1 (5.0–5.1)	5.3 (5.2–5.3)	5.5 (5.5–5.6)	6.0 (5.8–6.3)
Girls	9 (400)	5.0 (5.0–5.0)	4.4 (4.3–4.5)	4.8 (4.7–4.8)	5.0 (5.0–5.0)	5.2 (5.2–5.3)	5.6 (5.5–5.7)
	13 (400)	5.2 (5.1–5.2)	4.6 (4.5–4.7)	4.9 (4.9–5.0)	5.2 (5.1–5.2)	5.4 (5.4–5.5)	5.8 (5.7–5.8)
	16 (464)	5.0 (5.0–5.1)	4.5 (4.4–4.6)	4.8 (4.7–4.8)	5.0 (4.9–5.0)	5.2 (5.2–5.3)	5.7 (5.6–5.9)

Table 3. Distribution of HOMA-IR values.

		HOMA-IR, mmol/L					
		Percentile (95% CI)					
Age, years (n)	Mean (95% CI)	5th	25th	50th	75th	95th	
Boys	9 (342)	0.95 (0.87–1.03)	0.33 (0.29–0.36)	0.55 (0.52–0.61)	0.83 (0.76–0.86)	1.15 (1.05–1.22)	1.88 (1.71–2.49)
	13 (370)	1.66 (1.50–1.82)	0.58 (0.48–0.65)	0.91 (0.87–0.99)	1.40 (1.28–1.52)	2.04 (1.83–2.16)	3.28 (2.95–3.80)
	16 (375)	1.55 (1.43–1.67)	0.61 (0.56–0.70)	0.94 (0.90–0.99)	1.28 (1.21–1.36)	1.69 (1.57–1.85)	3.31 (2.96–4.01)
Girls	9 (369)	1.13 (0.94–1.32)	0.34 (0.28–0.39)	0.63 (0.56–0.68)	0.90 (0.84–0.96)	1.29 (1.18–1.36)	2.07 (1.87–2.39)
	13 (352)	1.90 (1.73–2.06)	0.79 (0.68–0.83)	1.25 (1.14–1.31)	1.62 (1.52–1.75)	2.27 (2.10–2.41)	3.86 (3.34–4.42)
	16 (436)	1.60 (1.52–1.68)	0.68 (0.62–0.75)	1.03 (0.98–1.12)	1.42 (1.35–1.53)	2.01 (1.82–2.14)	3.10 (2.80–3.38)

25th, and 50th percentiles values of 13-year-olds (Table 3). For all percentiles examined, in both sexes, 9-year-olds had lower HOMA-IR values than did 13- and 16-year-olds.

Age- and sex-specific distributions of FFA concentrations were also positively skewed. Except for the central portion of the distribution in 16-year-olds, boys and girls of the same age exhibited similar FFA concentrations (Table 4). In boys, we observed a progressive decrease in FFA concentrations from 9 to 16 years of age. In girls, we observed a reduction between ages 9 and 13, whereas FFA concentrations were similar among 13- and 16-year-olds.

In all age and sex groups, glucose exhibited remarkably tight distributions (median for total CV, 7.1%; range for total CV across age and sex groups, 6.1–7.5%) in contrast to insulin (median CV, 52%; range, 47–56%), HOMA-IR (median CV, 54%; range, 49–59%), and FFAs (median CV, 45%; range, 39–48%).

We observed strong correlations between \log_e insulin concentrations and \log_e HOMA-IR values (range, 0.991–0.995 across age and sex groups). To further evaluate the agreement between these two surrogate measures of IR, we computed differences between age- and sex-specific Z-scores for \log_e insulin and the corresponding Z-scores for \log_e HOMA-IR (16). Differences had a SD of only 0.12 Z-scores, supporting close agreement between these two measures of IR. We observed weak but significant ($P < 0.05$) negative correlations between \log_e FFAs and markers of IR in 9-year-old boys (\log_e FFAs and \log_e insulin, $r = -0.34$; \log_e FFAs and \log_e HOMA-IR, $r = -0.33$), in 9-year-old girls (\log_e FFAs and \log_e insulin, $r = -0.19$; \log_e FFAs and \log_e HOMA-IR, $r = -0.18$), and in

13-year-old boys (\log_e FFAs and \log_e insulin, $r = -0.19$; \log_e FFAs and \log_e HOMA-IR, $r = -0.17$). We detected no other relationship. Indeed, for 13-year-old girls and 16-year-olds of both sexes, Pearson correlation coefficients between \log_e FFAs and \log_e insulin and between \log_e FFAs and \log_e HOMA-IR ranged from -0.05 to 0.03 .

Discussion

This study is the first to report selected percentile values within the distributions of fasting plasma insulin, glucose, FFAs, and HOMA-IR in a representative sample of youth. In children and adults, there is no widely agreed consensus on cutoffs to define hyperinsulinemia or increased IR as assessed by HOMA. Because numerous cardiovascular disease risk factors exert their adverse consequences on a continuum often encompassing reference intervals, rather than in a dichotomous manner, the availability of selected percentile values may be useful to clinicians by allowing a finer categorization of their patients.

The marked increase in insulin concentrations and in HOMA-IR values observed in 13- and 16-year-olds compared with 9-year-olds is most likely explained by the lowering in insulin sensitivity associated with the onset of puberty (19, 20). Our data agree with the previously reported differences in insulin sensitivity between boys and girls (20–22). However, these differences between sexes were statistically significant only when we used fasting insulin concentrations as surrogate measures of insulin sensitivity. As expected, they reflected a central tendency more than an effect of sex at the extremes. This is worth noting because most often it is the values of the extremes that are used to categorize individuals.

Table 4. Distribution of plasma FFA concentrations.

		FFAs, mmol/L					
		Percentile (95% CI)					
Age, years (n)	Mean (95% CI)	5th	25th	50th	75th	95th	
Boys	9 (342)	0.49 (0.47–0.51)	0.20 (0.18–0.22)	0.32 (0.30–0.35)	0.47 (0.44–0.50)	0.60 (0.58–0.63)	1.01 (0.82–1.07)
	13 (370)	0.41 (0.38–0.43)	0.18 (0.16–0.20)	0.28 (0.26–0.29)	0.37 (0.35–0.38)	0.49 (0.46–0.52)	0.76 (0.69–0.99)
	16 (375)	0.33 (0.31–0.35)	0.14 (0.12–0.15)	0.20 (0.20–0.22)	0.30 (0.28–0.31)	0.40 (0.38–0.44)	0.61 (0.57–0.74)
Girls	9 (369)	0.54 (0.52–0.57)	0.24 (0.21–0.27)	0.39 (0.36–0.41)	0.51 (0.49–0.54)	0.65 (0.62–0.68)	1.01 (0.82–1.11)
	13 (352)	0.42 (0.40–0.44)	0.21 (0.18–0.23)	0.30 (0.28–0.32)	0.39 (0.38–0.42)	0.50 (0.47–0.52)	0.70 (0.66–0.78)
	16 (436)	0.43 (0.41–0.45)	0.17 (0.15–0.19)	0.29 (0.28–0.31)	0.39 (0.37–0.42)	0.52 (0.50–0.55)	0.77 (0.72–1.00)

We observed strong correlations between insulin concentrations and HOMA-IR values, as well as close similarity in their rankings of individuals. Because HOMA-IR values agreed closely with fasting plasma insulin concentrations, at least in our cross-sectional data, they might not be more informative. This finding was not unexpected because HOMA-IR values are derived from insulin and glucose concentrations. Moreover, similar observations have been made in adults (10).

At all ages and for all percentiles examined (except the 95th percentile), we found small but consistent differences between fasting plasma glucose concentrations in boys and girls, girls having slightly lower concentrations. Although some reports do not concur (23, 24), most large pediatric studies have shown differences between sexes for mean fasting glucose concentrations (12, 21, 22). In all age and sex groups, the mean concentrations of fasting glucose were higher in our population than those observed in other Caucasian pediatric populations. Indeed, in four large studies conducted between 1986 and 1996, mean glucose concentrations ranged from 4.43 to 4.88 mmol/L in children 8–10 years of age and from 4.40 to 4.66 mmol/L in adolescents 12–17 years of age (21, 22, 25, 26). To explain this discrepancy between our results and those of others, we considered four possibilities: differences in measurement methods, in the nature of the specimens used and in the timing of the specimen collection, and the presence of a bias in our results. Concerning differences in measurement methods, although the different studies used different analytical procedures, glucose measurements do not vary significantly according to method used (27). Concerning the nature of the specimens, in our population as well as in two previous reports (22, 26), glucose was measured in plasma, which has lower glucose concentrations than the serum used in the two other studies (21, 25). Thus, in our population, higher glucose concentrations cannot be explained by differences in composition of plasma and serum. With regard to the timing of specimen collection, there is diurnal variation in fasting plasma glucose concentrations, with the highest values being observed early in the morning (28). However, it seems unlikely that blood collection in our study was performed systematically earlier than in the other four studies. Finally, with regard to possible bias, a mean difference of 0.15 mmol/L between our glucose measurements and those performed with the reference method shows that there was no large positive bias in our results. We therefore believe that the differences in mean glucose concentrations observed between our data and those in other Caucasian pediatric populations are not artifactual. Because earlier data are not available in our population, we are not able to assess secular trends. However, interpreted in the context of the increase in obesity in children (5, 6), our findings suggest that the North American distribution of glucose concentrations might be shifting to the right. Indeed, in our population we observed a positive correlation between

body mass index and fasting plasma glucose (Pearson correlation coefficient between Z-scores for log_e body mass index and Z-scores for glucose, 0.12; $P < 0.0001$). Confirmation of these results in other representative samples of youth is required.

Similar to others (29), we observed that fasting plasma FFA concentrations decreased with age, reflecting the greater dependency of younger children on fat mobilization and production of ketone bodies as alternative sources of energy during fasting. We observed a negative correlation between FFA and insulin concentrations in younger children that is consistent with their early mobilization of fat to sustain ketogenesis during fasting. There was no indication that plasma FFAs were positively associated with markers of IR. Similar observations were made in adults in the Paris Prospective Study and the Quebec Cardiovascular Study, in which no significant correlations were seen between fasting plasma FFA and fasting plasma insulin concentrations (30, 31). Therefore, although the biological imprecision of a single FFA measurement and the cross-sectional design of our study limit the interpretation of our results, it is doubtful that fasting plasma FFA concentrations can be used as an early marker of IR.

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