Insulin and HOMA in Spanish prepubertal children. Relationship with lipid profile.

Running title: Insulin and HOMA in prepubertal children

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ABSTRACT

Objective: The effects of insulin or insulin resistance on lipid profile seem to change with age. The aim of this study was to analyze insulin levels and an insulin resistance index and to investigate the relationship between these and lipid profile in a population-based sample of Spanish prepubertal children. **Methods:** 1048 (524 boys and 524 girls) randomly selected prepubertal children were studied. Children were 6 to 8 with a mean age of 6.7. Plasma lipid, FFA, and insulin levels were measured. The homeostatic model assessment (HOMA) was calculated as an indicator of insulin resistance. **Results:** When analyzing percentile values of insulin, HOMA and FFA by sex, we observed that girls had significantly higher insulin concentrations than boys (except at the 10^{th} percentile) and significantly higher FFA (except at the 90^{th} percentile) with no significant differences between sexes for HOMA. Multivariate regression analyses showed that insulin was positively associated with glucose, triglycerides and apo B in boys but not in girls, and negatively associated with FFA in both genders. **Conclusions:** We report here data about the distribution of insulin in the Spanish prepubertal population. The higher levels of insulin in prepubertal girls could indicate that girls start to be more insulin resistant than boys at this age, although other manifestations of insulin resistance are not yet detectable.

Keywords: Insulin; HOMA; Free fatty acids; lipid profile, children.

INTRODUCTION

It is accepted that pathological processes related to the development of arteriosclerosis begin in childhood and seem to be related to the presence of cardiovascular risk factors at this age. Using intravascular ultrasound, atherosclerotic lesions have been detected in the coronary arteries of 17% of those individuals under 20 studied[1], indicating the start of the disease at early ages. High plasma lipid levels early in life could be contributing to the incidence of CHD in adults[2 3].

In adults, insulin and insulin resistance status are positively associated with metabolic syndrome and several associated disorders, such as altered lipid levels[4 5 6].

This relationship between insulin and insulin resistance and lipid profiles, clearly demonstrated in adults, seems to depend on the age of the population studied. Thus, the aim of this study has been to describe insulin and HOMA in Spanish prepubertal children and to analyze the relationship between these values and lipid, apolipoprotein and free fatty acid (FFA) levels in a representative sample of these children.

MATERIAL AND METHODS

Subjects and study design: The population included 1048 healthy school children (524 males and 524 females) 6 to 8 years old (mean age of 6.7 years) who participated in a voluntary survey of cardiovascular risk factors in Spain over the period 1998-2000. More detailed information about the design of the study is available in previous publications[7] [8].

Children were selected by means of random cluster-sampling in schools, and stratified by sex and type of school (i.e., public versus private). Sampling was carried out in two stages. In the first stage, schools were selected from lists supplied by the regional educational authorities. In the second stage, classrooms and scholars were selected. The response rate was 85%, with little variations among localizations. The study protocol complied with Helsinki Declaration guidelines and Spanish legal provisions governing clinical research on humans, and was approved by the Clinical Research Ethics Committee of the Fundación Jiménez Díaz in Madrid.

Data collection and study variables: The study was orally presented to the School Board of each of the schools. Following this, a letter was circulated to the parents of all children invited to participate in the study, outlining the study goals and procedures. Parents were required to sign a written consent for participation of their children in the study. All children reported by parents to be suffering from metabolic, endocrine, liver or kidney disorders were excluded to rule out any possible alteration in the values of the variables of interest.

Anthropometric variables: Measurements were taken with the children lightly dressed and barefoot. Height was measured to the last millimeter using a portable stadiometer, and weight was recorded to the nearest 0.1 kg using a standardized electronic digital

scale. From these measurements, body mass index (BMI)(weight in kilograms divided by the square of the height in meters: kg/m^2) was then computed. A questionnaire was provided along with consent forms and parents were asked to provide information regarding the child's general health.

Biochemical data: Fasting (12-hours) venous blood samples were obtained by venipuncture into Vacutainer tubes. Samples were kept on ice and sent to the study's central laboratory for analysis. Once centrifuged, the fractions were separated and frozen at –70ºC. Plasma glucose was measured by the glucose oxidase method and cholesterol and triglycerides (TG) were measured enzymatically (Menarini Diagnostics, Italy) with an RA-1000 Autoanalyzer. The coefficients of variation of the methods were 2,06% for cholesterol determinations and 3,42% for triglycerides determinations. HDL-cholesterol (HDL-C) was also measured in the RA-1000 after precipitation of apo B-containing lipoproteins with phosphotungstic acid and Mg (Roche Diagnostics, Spain). LDL-cholesterol (LDL-C) was calculated according to Friedewald´s formula. Plasma apo A-I and apo B concentrations were quantified by immunonephelometry (Dade Berhing, Germany).

Serum insulin concentrations were measured by radioimmunoassay (RIA) using a commercial kit (BI-Insulin IRMA, Bio-Rad, France). Insulin resistance was estimated using the homeostasis model assessment for insulin resistance (HOMA; fasting insulin [μU/ml] x fasting glucose [mmol/l]/22.5) [9]. We measured FFA levels by using the Wako NEFA-C kit (Wako Pure Chemical Industries Ltd., Japan).

Statistical analysis: Differences in anthropometric and biochemical measurements between boys and girls were evaluated using a t-test. We used sample quantiles to estimate the corresponding population percentiles. When comparing percentile values

between sexes, we concluded that they were significantly different if their respective 95% CIs were non-overlapping. In separate multivariate linear regression models, we regressed the lipid profiles onto plasma insulin and HOMA before and after adjusting for BMI with gender-specification. All statistical analyses were conducted using the statistical package SPSS v9.0 (SPSS Inc, Chicago, IL).

RESULTS

Anthropometric and biochemical parameters in boys and girls in our study are shown in Table 1. The boys had significantly higher mean height ($p < 0.05$) than girls. Glucose and apoAI levels were significantly higher in boys than in girls. Apo B concentrations and FFA levels were significantly higher in girls than in boys ($p < 0.01$ and $p < 0.05$, respectively). Mean values of insulin and HOMA were not significantly different between boys and girls

The percentile values of plasma insulin levels, HOMA and FFA concentrations by sex are shown in Table 2. Girls had significantly higher insulin concentrations than boys for all the percentiles except at the 10^{th} percentile. FFA levels were significantly higher in girls than in boys for all the percentiles except the 90^{th} percentile. No significant differences between sexes were detected for any percentile of HOMA (Table 2).

Multivariate regression analyses of insulin and HOMA on lipid profiles among children by sex, after adjusting for BMI, are shown in Table 3. Insulin was positively associated with glucose in boys but not in girls. Insulin and HOMA were positively associated with TG and apo B in boys. However, in girls, only insulin was positively associated with triglycerides. Insulin and HOMA were negatively associated with FFA in both genders.

DISCUSSION

Insulin resistance (IR) can be measured with the euglycemic insulin glucose clamp, but because this method is not suitable for large epidemiological studies, fasting plasma insulin concentrations and the homeostasis model assessment (HOMA) are often used as markers of IR[9 10].

There is data on the distributions of these metabolic variables in some pediatric populations[11 12], but little on representative samples of prepubertal children in the Mediterranean area. This study analyzes percentile values within the distributions of fasting plasma insulin, HOMA and FFA in a population based sample of prepubertal Spanish children. Since there is no consensus on cutoffs to define hyperinsulinemia or increased IR as assessed by HOMA, the possibility of establishing percentile values may be useful to categorize the population.

For all percentiles examined, we found small but consistent differences between fasting plasma insulin concentrations and HOMA values in boys and girls, with girls having slightly higher concentrations. These differences between sexes were statistically significant when we looked at fasting insulin concentrations (except the 10th percentile), although differences in HOMA values were not. The lack of validity of the HOMA in detecting IR in our population is not surprising. Multiple studies have reported that subrogate indices of IR are not accurate predictors of IR in children[13]. Other studies have previously reported differences in insulin sensitivity between boys and girls depending on age and percentile analyzed[14 15], with significant differences in insulin resistance between boys and girls at the puberal age[16]. The insulin concentrations in our study are lower than those reported in older children[14 17]. This seems to be related to the lowering in insulin sensitivity associated with the onset of

puberty[18]. We think that at the prepubertal age that we are studying the insulin resistance may be starting.

The important increase in the prevalence of childhood obesity over the last two decades, accompanied by an increase in the incidence of type 2 diabetes early in life justifies the need to understand the relationship between IR, anthropometric variables, and altered lipid levels in children. We have found an important association of insulin and HOMA with BMI in both genders. The association between BMI and blood lipid and lipoprotein profiles among children has been reported[19]. In our study, we found that insulin was positively associated with glucose, TG, and apo B in boys. However, in girls, insulin is positively associated with TG but not with glucose or apoB after adjusting for BMI. Apparently, more insulin is needed to modify glycemia in girls than in boys; as has been described in the literature, females throughout life are more insulin resistant than males[20]. The association between insulin and TG levels has been consistently described in both genders in children of different ages[21 22]. The association between insulin and apoB is present in both sexes only in older children[21]. We did not find any association between insulin and HOMA with HDL-C in either gender, other studies in older children have reported a negative association with HDL-C [21 22].

Strong evidence suggests that increases in FFAs may play a key role in the pathogenesis of insulin resistance[23]. Thus, we examined the relationship between fasting plasma concentrations of FFA and markers of insulin resistance. We observed a negative association between FFA and both insulin concentrations and HOMA in our children. This negative correlation between FFA and insulin in younger children has been previously reported[15] although it has not been found in older children[16]. This seems to mean that, with regards to lipid metabolism, the insulin resistance is not yet manifest.

The different association of insulin and HOMA and lipid profiles between boys and girls depending on age might be due to hormonal influences on insulin and HOMA levels. During puberty, higher levels of sex hormones, which were associated with a more apparent insulin resistance status have been reported[24]. At the prepubertal age no sexual hormones are present but we have found significantly different dehydroepiandrosterone-sulfate (DHEA-S) levels in boys and girls[25].

In summary, from this school children study, we found that even though girls had higher insulin levels than boys, HOMA values were not statistically significant. However, while insulin is clearly associated with blood glucose and triglycerides in boys, in girls only TG correlate with insulin and glucose does not. In our population, girls may be starting to be more insulin resistant at this age, without all of the characteristics of IR found in adults yet. Further studies are needed to evaluate the possible mechanisms of this different insulin resistance by gender.

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Table 1. Anthropometric and biochemical characteristics (mean±SD) among Spanish school children.

	BOYS N=524	**GIRLS N=524**	**P**
Age (years)	6.7 ± 0.7	6.7 ± 0.7	0.80
Weight (Kg)	26.8 ± 5.3	26.6 ± 5.5	0.60
Height (m)	1.26 ± 0.06	1.25 ± 0.07	0.02
BMI (Kg/m^2)	16.9 ± 2.4	17. 0 ± 2.6	0.51
Glucose (mmol/l)	5.12 ± 0.46	4.96 ± 0.67	P<0.001
Total Cholesterol (mmol/l)	4.74 ± 0.73	4.79 ± 0.70	0.27
Triglycerides (mmol/l)	0.80 ± 0.29	0.83 ± 0.27	0.20
HDL-C (mmol/l)	1.54 ± 0.34	1.52 ± 0.33	0.20
LDL-C (mmol/l)	2.83 ± 0.72	2.90 ± 0.66	0.12
Apo AI (g/L)	1.39 ± 0.19	1.37 ± 0.18	0.05
Apo B (g/L)	0.69 ± 0.14	0.72 ± 0.14	0.005
Insulin (pmol/L)	24.61 ± 17.72	26.69 ± 21.45	0.08
HOMA	0.79 ± 0.61	0.82 ± 0.71	0.40
FFA (mEq/L)	0.68 ± 0.27	0.72 ± 0.30	0.03

Table 2. Distribution of plasma insulin concentrations, HOMA values and FFA concentrations in boys and girls.

	Percentile (95%CI)				
	P_{10}	P_{25}	P_{50}	P_{75}	P_{90}
Insulin (pmol/L)					
Boys	4.73 (4.16-5.31)	7.96 (7.39-8.53)	16.50 (16.14-16.93)	25.18 (24.61-25.69)	39.68 (38.67-40.61)
Girls	5.81 (5.23-6.38)	9.25 (8.68-9.83)	17.86 (17.51-18.30)	26.55 (25.97-27.12)	41.83 (40.97-42.69)
HOMA					
Boys	0.13 (0.12-0.15)	0.24 (0.22-0.26)	0.52 (0.50-0.53)	0.80 (0.78-0.81)	1.28 (1.24-1.31)
Girls	0.16 (0.14-0.17)	0.27 (0.25-0.28)	0.54 (0.53-0.56)	0.82 (0.80-0.83)	1.30 (1.28-1.33)
FFA (mEq/L)					
Boys	0.30 (0.28-0.31)	0.38 (0.36-0.39)	0.55 (0.55-0.56)	0.72 (0.71-0.74)	0.97 (0.95-0.99)
Girls	0.33 (0.32-0.34)	0.41 (0.40-0.42)	0.58 (0.57-0.59)	0.76 (0.75-0.77)	1.00 (0.98-1.02)

Table 3. Multivariate regression analysis of insulin and HOMA on lipid profiles among prepubertal children by sex, adjusting for BMI.

	Boys		**Girls**	
	INSULIN B (95%CI)	HOMA B (95%CI)	INSULIN B (95%CI)	HOMA B (95%CI)
Glucose	0.067 *** (0.042-0.091)	-	0.009 (-0.013-0.032)	-
Triglycerides	0.022 *** (0.012-0.031)	0.005 *** (0.003-0.007)	0.017 ** (0.004-0.029)	0.003 (0.000-0.006)
HDL-C	-0.010 (-0.033-0.013)	-0.002 (-0.008-0.003)	0.015 (-0.017-0.047)	0.003 (-0.004-0.010)
LDL-C	-0.017 (-0.029-(-0.004))	-0.004 (-0.007-(-0.001))	-0.013 (-0.030-0.004)	-0.003 (-0.006-0.001)
Apo AI	0.000 (-0.015-0.014)	0.000 (-0.003-0.003)	-0.027 (-0.048-(-0.006))	-0.006 (-0.010-(-0.001))
Apo B	0.035 ** (0.010-0.059)	0.008 ** (0.002-0.013)	0.024 (-0.005-0.053)	0.006 (0.000-0.012)
FFA	-1.463 *** (-2.233-(-0.693))	-0.309 ** (-0.485-(-0.133))	-1.678 ** (-2.644-(-0.711))	-0.400 *** (-0.611-(-0.188))
R^2	**0.295**	**0.356**	**0.171**	**0.186**

**p<0.01 ***p<0.001

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