Plasma non-esterified fatty acid levels in children and their relationship with sex steroids

Lorena Ortega[a], Alicia Garcia-Anguita[a], Pía Riestra[a], Henar Ortega[b], Leandro Soriano-Guillén[c], Miguel A. Lasunción[b], Manuel de Oya[a] and Carmen Garcés[a]

‡ Dedicated to the late Prof. Manuel de Oya, as the warmest homage to his memory.

[a]Lipid Metabolism Research Laboratory, IIS-Fundación Jiménez Díaz, Madrid.

[b]Servicio de Bioquímica-Investigación, Hospital Ramón y Cajal, Madrid.

[c]Department of Pediatrics, IIS-Fundación Jiménez Díaz, Madrid.

Corresponding author:

Carmen Garcés

Lipid Research Laboratory

IIS-Fundación Jiménez Díaz

Avda. Reyes Católicos, 2. 28040 Madrid, Spain

Telephone/fax: +34-91-5432880

cgarces@fjd.es

Abbreviations: BMI: body mass index; NEFA: non esterified fatty acids; SHBG: sex hormone binding protein.

Abstract

Objective: Puberty is associated with decreased insulin sensitivity. Sexual hormones have been related with the onset of insulin resistance, but their relationship with non-esterified fatty acids (NEFA) remains unexplored. The aim of this study was to evaluate circulating NEFA levels in population-based samples of prepubertal children and adolescents and to analyze the association of NEFA with obesity, insulin resistance, and sexual hormones in adolescents.

Experimental: The studied population included 854 randomly selected 6- to 8- year-old children and 822 children aged 12 to 16 years. NEFA levels were determined using a commercial kit. Testosterone and estradiol levels were determined by RIA, and insulin and sex hormone binding protein by IRMA. HOMA was calculated as an indicator of insulin resistance.

Results: NEFA levels were lower in adolescents than in 6- to 8-year-old children, and decreased progressively with age between 12-year-olds and 16-year-olds. No significant differences in NEFA levels were observed between obese and non-obese adolescents. NEFA were not correlated with insulin or HOMA in 12- to 16- year-old girls, and appear negatively correlated with these variables in boys. Insulin and HOMA were negatively correlated with SHBG levels in both sexes adjusting by age but NEFA levels were not.

Conclusions: NEFA levels decrease with age in adolescents and are not significantly increased in obese children, supporting the fact that the decreased insulin sensitivity at this age is not affecting NEFA metabolism. Although SHBG is related to insulin and HOMA independently of age in both sexes, SHBG levels are not associated with NEFA.

Keywords: children; hormone levels; insulin; non-esterified fatty acids; SHBG.

Introduction

It has been established that obesity is related to the onset of insulin resistance [1]. Among other reasons, obesity determines this onset of insulin resistance by impairing insulin signalling, interfering in glucose transport or decreasing insulin clearance related with elevated non-esterifed fatty acids (NEFA) [1]. Inhibition of NEFA release is the most sensitive action of insulin in normal conditions. In adults, it is believed that NEFA could be the main link between obesity and insulin resistance [2]. NEFA levels are increased in most obese adults due to an increase in the rate of lipolysis from the increased fat cell mass and elevated plasma NEFA concentrations trigger peripheral and hepatic insulin resistance [3]. In children and adolescents, on the other hand, although obesity has also been associated with impaired glucose tolerance and insulin resistance [4-6], the role of NEFA in the onset of insulin resistance remains unclear. In a previous study by our group, including 6- to 8-year-old obese children, NEFA levels were not different between obese and non-obese boys, and were significantly lower in obese girls [7].

Puberty is associated with decreased insulin sensitivity and increased insulin levels [8-12]. The role of sex hormones in insulin sensitivity has been studied, and a relationship between sex hormones and insulin resistance has been suggested in adults [13-15]. Sex hormone levels have been associated with insulin sensitivity in some studies in children [9, 16] but not in others [11, 17]. Besides, to our knowledge, the relationship between hormones and NEFA levels has not been investigated in a general population of adolescents. Thus, we aimed to determine whether NEFA levels are related to sexual hormones during sexual development.

In our study we have analyzed NEFA levels in two cohorts of 6- to 8-year-old and 12- to 16-year-old children, respectively, evaluating their association with obesity, insulin, HOMA, testosterone, estradiol, and SHBG in our population-based sample of healthy 12- to-

16-year-old boys and girls, in order to assess the possible association between hormones and NEFA levels in adolescents.

Experimental

Study subjects: The studied population included two population-based samples comprising 854 (416 boys and 438 girls) 6- to 8-year-olds, and 822 (393 boys and 429 girls) 12- to 16-year-olds. Children were participants of a cross-sectional study designed to analyze cardiovascular risk factors in Spanish schoolchildren, and were selected by means of random cluster sampling in schools. Parents were required to provide written consent for their children to participate in the study. All children reported by their parents to be suffering from chronic diseases were excluded. 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: At each school, data were collected by a field team, comprised of a physician, several nurses, and a group of persons purpose-trained for taking anthropometric measurements.

Anthropometric variables: Measurements were taken with the children lightly dressed and barefoot. Height and weight were recorded, and body mass index (BMI) was computed based on these measurements. Children were categorized as obese according to the age- and sex-specific BMI cut-off points proposed by Cole et al [18].

Biochemical variables: Fasting (12-hour) venous blood samples were obtained by venipuncture into Vacutainer tubes. Plasma glucose was measured by the glucose oxidase method. NEFA levels were measured by using the Wako NEFA-C kit (Wako Industries, Osaka, Japan). Testosterone and estradiol were determined by coated tube radioimmunoassy (RIA), using commercial kits (DSL-4000 Active® Testosterone and DSL-43100 Active® Estradiol, respectively; Diagnostic Systems Laboratories, Inc., Webster, Texas, USA). Sex hormone binding globulin (SHBG) was measured using an Immunoradiometric Assay (IRMA) kit (DSL-7400 Active® SHBG; DSL, Inc., Webster, Texas, USA). Serum insulin concentration was also measured by IRMA 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 [microU/ml] x fasting glucose [mmol/L]/22·5).

Statistical analysis: All statistical analyses were conducted using the statistical package SPSS v9.0 (SPSS Inc, Chicago, IL). Differences in NEFA levels by age were evaluated by ANOVA and the appropriate post-hoc test. Differences in the studied variables between sexes and between non-obese and obese children were analyzed by t-test or Mann-Whitney test depending on the distribution of the variable. Partial correlation coefficients were estimated after adjusting by age to evaluate the relationships between insulin-resistance variables and hormone levels.

Results

Our study sample comprises of 76 obese children (35 boys and 41 girls) aged 6-8 years old, and 50 obese children (26 boys and 24 girls) aged 12-16 years old.

NEFA levels in 6- to 8-year-old and 12- to 16-year-old boys and girls are shown by age in table 1. No significant differences in NEFA levels between boys and girls were observed in adolescents, but NEFA levels were higher in girls than in boys at the age of 6 and 7. Mean NEFA levels were lower in 12- to 16-year-olds than in 6- to 8-year-old children. In addition, NEFA levels decreased gradually with age in 12- to 16-year-old boys and girls (table 1). Furthermore, NEFA levels were negatively correlated with days of life in both boys (-.302, $p<0.001$) and girls (-.282, $p<0.001$). BMI, insulin, and HOMA were significantly correlated with days of life in boys but not in girls. Days of life correlated positively with testosterone in boys and estradiol in girls, and negatively with SHBG in both sexes.

Insulin levels and HOMA were significantly higher in obese than in non-obese 12- to-16-year-old boys and girls (table 2). However, no significant differences in plasma glucose and NEFA levels were found between obese and non-obese 12- to- 16-year-old children, although obese girls showed slightly higher NEFA levels than non-obese girls (table 2).

Partial correlations, after adjusting by age, between BMI, plasma sex hormones, and insulin resistance-related parameters were evaluated in our 12- to- 16-year-old children (table 3). BMI correlated positively with insulin and HOMA in both genders, but not with NEFA. In boys, NEFA levels correlated negatively with insulin levels and HOMA, although this correlation was not present in girls (table 3). SHBG levels were significantly and negatively related to insulin and HOMA in boys and girls, after adjusting by age, but the correlations were more substantial in girls. No significant correlations were observed between testosterone or estradiol and the studied variables. No correlation was found when analyzing partial correlation coefficients between NEFA and hormone levels after adjusting by age (table 3).

Discussion

In our study, we report data on the distribution of NEFA levels by age in Spanish children, describing that NEFA concentrations are lower in 12- to- 16-year-old children than in prepubertal children. In addition, we show a progressive decrease of NEFA levels with age in the age interval studied, between 12 and 16 years of age. Lower NEFA levels in pubertal than in prepubertal children have also been described in other pediatric populations [12, 19] even though, as discussed previously, a decrease in insulin sensitivity associated with the onset of puberty is accepted [8-12]. It has been reported that pubertal insulin resistance is associated with decreased insulin stimulated glucose and decreased insulin suppression of NEFA oxidation [20]. In our study, no positive correlations have been found between insulin and HOMA and NEFA; in fact, in boys, significant negative correlations were detected. A negative correlation between insulin and NEFA was also found for 13-year-old girls and 16-years-olds of both sexes in the study of Allard et al [19]. No relationships or negative associations between insulin and NEFA have been found in studies in prepubertal children [21, 22].

Although obese boys and girls in our study had higher plasma insulin levels and HOMA values than their non-obese peers, no significant differences were found in NEFA levels between them. BMI is positively correlated with insulin and HOMA but not with NEFA. Even though some studies have reported higher NEFA levels in obese children [23, 24], others have described lower NEFA levels in obese versus normal-weight children [7, 21, 25] or no differences between them [7, 26]. In general, in adults, the onset of insulin resistance is accompanied by glucose intolerance and an increase in NEFA concentrations [3]. One of the more important primary actions of insulin is the inhibition of lipolysis in fat cells. In our study, while other consequences of high BMI are similar to those associated with insulin resistance in adults (higher fasting insulin levels and higher HOMA), NEFA levels are not increased in obese adolescents, suggesting that adipose tissue in these children remains

sensitive to the action of insulin. It seems that the sequence of the metabolic changes associated with insulin resistance in adult obesity (high levels of insulin, glucose intolerance, and, finally, NEFA increase) is not yet complete in our 12- to 16-year-old population.

However, we observed some differences between boys and girls regarding the relationship between insulin/HOMA and NEFA and regarding NEFA levels in the obese individuals that suggest slightly differences in insulin sensitivity between sexes. It has been described that females throughout life are more insulin resistant than males [27]. Studies in different populations have observed differences in insulin sensitivity between boys and girls depending on age [19, 28], particularly at the pubertal age [10], possibly owing to sexual development. The girls in our study could have characteristics regarding impaired fatty acid metabolism and insulin sensitivity that are closer to those of adults than those seen in boys.

When analyzing the association of insulin-related variables with hormone levels in these 12- to- 16-year-old boys and girls, we observed that, even though a negative association of insulin and HOMA with SHBG levels is present, no significant association has been found between NEFA concentrations and hormone levels at this age. SHBG levels have been previously related to lower insulin levels and insulin resistance in adults [13, 29] as well as in obese children [30, 31], and has being suggested as playing an important role in the changes in glucose metabolism and body composition occurring during the pubescent transition [32]. In children and adolescents, SHBG has been related to diabetes [33] and presence of metabolic syndrome [34]. However, the association with NEFA has not been analyzed in any of these studies.

In summary, in our study we describe lower NEFA levels in Spanish adolescents than in prepubertal children, reporting a progressive decrease in NEFA concentration between 12-and 16-year-olds. Additionally, we describe that NEFA levels are not significantly elevated in obese children, suggesting that, in the obese adolescent, insulin retains its antilipolytic effect. Although the association between SHBG and insulin and HOMA suggests a relationship

between androgen bioavailability and insulin sensitivity, NEFA levels are not related to sexual hormones or SHBG.

Acknowledgements: This work was supported by a grant from the *Fondo de Investigación Sanitaria* (FIS 11/00344) and Biobank grant FEDER RD09/0076/00101. The contract of C Garcés is co-financed by the *Fondo de Investigación Sanitaria.* Alicia Garcia-Anguita and Lorena Ortega are fellows of the Conchita Rábago Foundation. We thank Oliver Shaw for his revision of our manuscript.

References

[1] Kahn BB, Flier JS. Obesity and insulin resistance. J Clin Invest 2000;106: 473-81.

[2] Boden G. Free fatty acids – the link between obesity and insulin resistance. Endocr Pract 2001; 7: 44-51.

[3] Boden G. Role of free fatty acids in the pathogenesis of insulin resistance and NIDDM. Diabetes 1997; 46: 3-10.

[4] Sinha R, Fisch G, Teague B, Tamborlane WV, Banyas B, Allen K, et al. Prevalence of impaired glucose tolerance among children and adolescents with marked obesity. N Engl J Med 2002; 346: 802-10.

[5] Freedman DS, Mei Z, Srinivasan SR, Berenson GS, Dietz WH. Cardiovascular risk factors and excess adiposity among overweight children and adolescents: the Bogalusa Heart Study. J Pediatr 2007;150: 12-7.

[6] Nathan BM, Moran A. Metabolic complications of obesity in childhood and adolescence: more than just diabetes. Curr Opin Endocrinol Diabetes Obes 2008; 15: 21-9.

[7] Garcés C, Gutiérrez-Guisado J, Benavente M, Cano B, Viurro E, Ortega H, et al. Obesity in Spanish schoolchildren: relationship with lipid profile and with insulin resistance. Obes Res 2005; 13: 959-63.

[8] Amiel SA, Sherwin RS, Simonson DC, Lauritano AA, Tamborlane WC. Impaired insulin action in puberty. A contributing factor to poor glycemic control in adolescents with diabetes. N Engl J Med 1986; 315: 215-9.

[9] Bloch CA, Clemons P, Sperling MA. Puberty decreases insulin sensitivity. J Pediatr 1987; 110: 481-7.

[10] Moran A, Jacobs DR Jr, Steinberger J, Hong CP, Prineas R, Luepker R, et al. Insulin resistance during puberty: results from clamp studies in 357 children. Diabetes 1999; 48: 2039-44.

[11] Goran MI, Gower BA. Longitudinal study on pubertal insulin resistance. Diabetes 2001; 50: 2444-50.

[12] Hannon TS, Janosky J, Arslanian SA. Longitudinal study of physiologic insulin resistance and metabolic changes of puberty. Pediatr Res 2006; 60: 759-63.

[13] Haffner SM. Sex hormones, obesity, fat distribution, type 2 diabetes and insulin resistance: epidemiological and clinical correlation. Int J Obes Relat Metab Disord 2000; 24: S56-8.

[14] Laaksonen DE, Niskanen L, Punnonen K, Nyyssönen K, Tuomainen TP, Salone R, et al. Sex hormones, inflammation and the metabolic syndrome: a population-based study. Eur J Endocrinol 2003;149: 601-8.

[15] Muller M, Grobbee DE, den Tonkelaar I, Lamberts SW, van der Schouw YT. Endogenous sex hormones and metabolic syndrome in aging men. J Clin Endocrinol Metab 2005; 90: 2618-23.

[16] de Sousa G, Brodoswki C, Kleber M, Wunsch R, Reinehr T. Association between androgens, intima-media thickness and the metabolic syndrome in obese adolescent girls. Clin Endocrinol 2010; 72 :770-4.

[17] Travers SH, Jeffers BW, Bloch CA, Hill JO, Eckel RH. Gender and Tanner stage differences in body composition and insulin sensitivity in early pubertal children. J Clin Endocrinol Metab 1995; 80: 172-8.

[18] Cole TJ, Bellizi CB, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. BMJ 2000; 320: 1240-3.

[19] Allard P, Delvin EE, Paradis G, Hanley JA, O`Loughlin J, Lavallée C, et al. Distribution of fasting plasma insulin, free fatty acids, and glucose concentrations and of homeostasis model assesment of insulin resistance in a representative sample of Quebec children and adolescents. Clin Chem 2003;49: 644-9.

[20] Arslanian S, Suprasongsin C. Glucose-fatty acid interactions in prepubertal and pubertal children: effects of lipid infusion. Am J Physiol 1997;272: E523-9.

[21] Valle M, Gascón F, Martos R, Ruz FJ, Bermudo F, Morales R, et al. Metabolic cardiovascular syndrome in obese prepubertal children: the role of high fasting insulin level. Metabolism 2002; 51: 423-8.

[22] Garcés C, Cano B, Granizo JJ, Benavente M, Viturro E, Gutiérrez-Guisado J, et al. Insulin and HOMA in Spanish prepubertal children. Relationship with lipid profile. Clin Biochem 2005; 38: 920-4.

[23] Reinehr T, Kiess W, Andler W. Insulin sensitivity indices of glucose and free fatty acid metabolism in obese children and adolescents in relation with serum lipids. Metabolism 2005; 54: 397-02.

[24] Sabin MA, De Hora M, Holly JM, Hunt LP, Ford AL, Williams SR, et al. Fasting nonesterified fatty acid profiles in childhood and their relationship with adiposity, insulin sensitivity, and lipid levels. Pediatrics 2007;120: e1426-33.

[25] Gil-Campos M, Ramírez Tortosa MC, Aguilera CM, Cañete R, Gil A. Fasting and postprandial adiponectin alterations anticipate NEFA and TNF-α changes in prepubertal obese children. Nutr Metab Cardiovasc Dis. 2011;21:62-68

[26] Mihalik SJ, Michaliszyn SF, de las Heras J, Bacha F, Lee S, Chace DH, et al. Metabolomic profiling of fatty acid and amino acid metabolism in youth with obesity and type 2 diabetes: evidence for enhanced mitochondrial oxidation. Diabetes Care 2012; 35: 605-11.

[27] Wilkin TJ, Voss LD, Metcalf BS, Mallam K, Jeffery AN, Alba S, et al. Metabolic risk in early childhood: the EarlyBird Study. Int J Obes Relat Metab Disord 2004; 28: S64-9.

[28] Guillaume M, Lapidus L, Beckers F, Lambert A, Bjorntorp P. Cardiovascular risk factors in children from the Belgian province of Luxembourg. The Belgian Luxembourg Child Study. Am J Epidemiol 1996;144: 867-80.

[29] Hajamor S, Despres JP, Couillard C, Lemieux S, Tremblay A, Prud`homme D, et al. Relationship between sex hormone-binding globulin levels and features of the metabolic syndrome. Metabolism 2003;52: 724-30.

[30] Gascon F, Valle M, Martos R, Ruz FJ, Ríos R, Montilla P, et al. Sex hormone-binding globulin as a marker for hyperinsulinemia and/or insulin resistance in obese children. Eur J Endocrinol 2000;14: 85-89.

[31] Galloway PJ, Donaldson MDC, Wallace AM. Sex hormone binding globulin concentration as a prepuberal marker for hyperinsulinaemia in obesity. Arch Dis Child 2001; 85:489-91.

[32] Sorensen K, Aksglaede L, Munch-Andersen T, Aachmann-Andersen NJ, Petersen JH, Hilsted L, et al. Sex hormone-binding globulin levels predict insulin sensitivity, disposition index and cardiovascular risk during puberty. Diabetes Care 2009; 32: 909-14.

[33] Danielson KK, Drum ML, Lipton RB. Sex hormone-binding globulin and testosterone in individuals with childhood diabetes. Diabetes Care 2008; 31: 1207-13.

[34] de Oya I, Schoppen S, Lasunción MA, López-Simón L, Riestra P, de Oya M, et al. Sex hormone-binding globulin levels and metabolic syndrome and its features in adolescents. Pediatr Diabetes 2010; 11: 188-94.

Table 1. Plasma NEFA levels (mEq/L) among 6- to- 8-year-old and 12- to- 16-year-old boys and girls by age.

Boys									
6y n=86	7y n=228	8y n=102	p	12y n=53	13y n=54	14y n=110	15y n=120	16y n=56	p
0.69±0.27	0.67±0.28	0.68±0.25	n.s.	0.54±0.22	0.50±0.17	0.42±0.22	0.43±0.19	0.33±0.14	12-14[a], 12-15[a], 12-16[c] 13-16[c], 14-16[a], 15-16[b]
Girls									
6y n=110	7y n=227	8y n=101	p	12y n=48	13y n=51	14y n=141	15y n=131	16y n=58	p
0.75±0.29	0.72±0.30	0.66±0.27	n.s.	0.58±0.22	0.49±0.21	0.46±0.19	0.40±0.18	0.41±0.22	12-14[a], 12-15[c], 12-16[b] 13-15[a], 14-15[b]

Data shown as mean±SD. NEFA: non-esterified fatty acids.

p: ANOVA, Tukey post hoc test; [a]$p \leq 0.05$ [b]$p \leq 0.01$ [c]$p \leq 0.001$

Table 2. Biochemical variables (mean±SD) in non-obese and obese 12- to- 16-year-old children.

	Boys			Girls		
	Non-obese (n=392)	Obese (n=26)	P	Non-obese (n=432)	Obese (n=24)	p
Age (years)	14.3±1.2	14.5±1.2	n.s.	14.4±1.1	14.2±1.5	n.s.
Glucose (mg/dl)	92.3±9.1	94.5±12.4	n.s.	88.8±8.7	91.8±5.0	n.s.
Insulin (μU/ml)	8.3±6.6	12.8±7.8	0.000	8.3±5.1	13.5±5.0	0.000
HOMA	1.89±1.53	3.00±1.89	0.000	1.83±1.23	3.04±1.19	0.000
NEFA (mEq/L)	0.43±0.20	0.39±0.19	n.s.	0.43±0.19	0.53±0.23	0.079

Table 3. Correlations (adjusted by age) between NEFA, glucose, insulin, HOMA, and BMI and hormone levels in 12- to- 16-year-old boys and girls.

Boys				
	NEFA	Glucose	Insulin	HOMA
NEFA	-	.030	-.132[b]	-.120[a]
BMI	.005	.092	.161[b]	.189[c]
Testosterone	.089	.057	.064	.056
SHBG	.001	-.046	-.142[b]	-.144[b]
Girls				
	NEFA	Glucose	Insulin	HOMA
NEFA	-	.241[c]	-.045	.008
BMI	.024	.041	.208[c]	.225[c]
Estradiol	.026	-.000	.036	.031
SHBG	-.025	-.082	-.264[c]	-.282[c]

BMI: body mass index; SHBG: sex hormone binding protein.

[a]$p \leq 0.05$ [b]$p \leq 0.01$ [c]$p \leq 0.001$