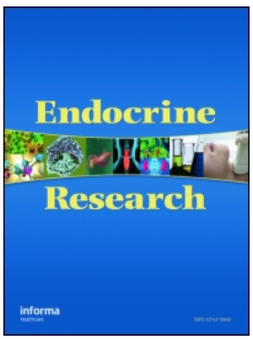

Endocrine Research

ISSN: 0743-5800 (Print) 1532-4206 (Online) Journal homepage: https://www.tandfonline.com/loi/ierc20

Obesity and Related Metabolic Biomarkers and Its Association with Serum Levels of Estrogen in Pre-pubertal Chilean Girls

Diana Carolina Mesa Valencia, Verónica Mericq, Camila Corvalán & Ana Pereira

To cite this article: Diana Carolina Mesa Valencia, Verónica Mericq, Camila Corvalán & Ana Pereira (2020) Obesity and Related Metabolic Biomarkers and Its Association with Serum Levels of Estrogen in Pre-pubertal Chilean Girls, Endocrine Research, 45:2, 102-110, DOI: 10.1080/07435800.2019.1681448

To link to this article: https://doi.org/10.1080/07435800.2019.1681448

Published online: 31 Oct 2019.

ENDOCRINE RESEARCH
2020, VOL. 45, NO. 2, 102–110
https://doi.org/10.1080/07435800.2019.1681448

Taylor & Francis
Taylor & Francis Group

Obesity and Related Metabolic Biomarkers and Its Association with Serum Levels of Estrogen in Pre-pubertal Chilean Girls

Diana Carolina Mesa Valencia[a], Verónica Mericq[b], Camila Corvalán[a], and Ana Pereira[a]

[a]Institute of Nutrition and Food Technology, University of Chile, Santiago, Chile; [b]Institute of Maternal and Child Research, Faculty of Medicine, University of Chile, Hospital San Borja Arriarán, Santiago, Chile

ABSTRACT

Purpose: Recent reports show that girls with higher body mass index (BMI) have an earlier puberty onset (thelarche). It has been suggested that earlier puberty is a consequence of higher levels of estrogen due to increased aromatization of androgens in adipose tissue. Thus, we aimed to assess the relation between serum levels of estrogen and excess weight (BMI ≥1SD) and central adiposity (>75th percentile for waist circumference) in prepubertal girls at age 7.

Materials and Methods: We conducted a cross-sectional study within the Growth and Obesity Cohort Study of 1190 low-middle income children from Santiago, Chile. We selected a random sample of 107 prepubertal girls at age 7. A trained dietitian measured weight, height and waist circumference. Additionally, a fasting blood sample was collected to measure serum levels of estradiol equivalents (via ultrasensitive recombinant cell bioassay), dehydroepiandrosterone sulfate (DHEAS), insulin, insulin-like growth factor 1 (IGF-1), and leptin.

Results: Excess weight was observed in 40% of our sample; 11.2% had high central adiposity, and the mean level of estradiol equivalents was 3.6 ± 2.3 pg/ml. In the univariate and multivariate analyzes, we did not observe an association between excess weight, central adiposity and estradiol equivalent levels; however, insulin was inversely associated with the serum level of estradiol equivalents.

Conclusions: Our participants had a mean level of estradiol equivalents of 3.6 pg/ml (±2.3 pg/ml) at the pre-pubertal stage. However, with the exception of insulin, we did not observe an association between estradiol equivalents and markers of adiposity and metabolic and hormonal factors.

ARTICLE HISTORY
Received January 18, 2019
Revised September 26, 2019
Accepted October 12, 2019

KEYWORDS
Obesity; childhood; puberty; estradiol; estrogen

Introduction

In the last 25 years, an earlier thelarche onset has been observed: from 11.7 years in a study published in 1969[1] by Marshall and Tanner to 9.7 years as reported in NHANES III (1988–1994).[2] On the other hand, the age of menarche onset has decreased only slightly (between 2.5 and 4 months)[3] since the early 1960s.

This earlier development of puberty in recent years coincides with the exponential increase in childhood obesity.[4] One underlying hypothesis is that greater estrogen availability by the aromatization of androgen precursors at the adipose tissue induces earlier breast development.[5] However, for this physiological process to occur, maturation of the adrenal reticularis must be achieved, leading to an increase in the production of weak androgens.[6] Indeed, obesity is a predisposing factor to premature adrenarche (i.e., increases in circulating levels of dehydroepiandrosterone sulfate (DHEAS)[7] and more potent androgens), and this process is partially reversed with weight loss.[8]

The peripheral conversion of androgens to estrogen in adipose tissue has been reported in adult women. In 1982, Kirschner noted that the aromatization of androstenedione to estrone in obese young women was 2.52% vs. 1.52% in non-obese young women.[9] However, in children, there are few studies describing the relationship between adiposity and levels of pre- and peripubertal estrogen, mainly due to the difficulty of measuring low levels of estrogen, such as those observed before puberty.

In recent decades, the prevalence of obesity in Chile among 6-year-old children has almost

CONTACT Ana Pereira apereira@inta.uchile.cl Institute of Nutrition and Food Technology, University of Chile, Av. El Líbano 5524, Macul, Santiago, Chile

tripled, rising from 7% in 1987 to 19.4% in. 2006[10] Since 2006, we have followed approximately 1100 middle- and low-income Chilean children (50% girls) in whom obesity increased from 10.4% at three years to 12.6% at five years.[11] In these children, we have shown that greater adiposity is associated with the presence of higher concentrations of DHEAS at 7 years.[7] Thus, the current study aimed to assess whether total and central adiposity, as well as their metabolic biomarkers, were related to estrogen levels measured by an ultrasensitive technique in prepubertal girls at age seven.

Materials and Methods

Study Design and Population

The Growth and Obesity Chilean Cohort Study (GOCS) started in 2006 by enrolling 1190 children (50% female) born in 2002 and 2003 from the National Child Daycare Centres (JUNJI) from the South-East area of Santiago de Chile. The participants are representative of middle- and lower-income Chilean children. The participants were from single births, had a birth weight between 2500 and 4500 g, and did not have any physical or mental illness. Further details of the cohort have been published previously.[11] In this study, we carried out a cross-sectional study of 107 randomly selected girls who were assessed in 2009 (mean age = 6.7, SD = 0.4) and were prepubertal (breast Tanner stage = 1). The sample size was calculated to detect a mean difference of estradiol equivalents of 0.27 pg/ml between girls with obesity ($\mu = 0.76$ pg/ml) and normal weight girls ($\mu = 0.44$ pg/ml) with a standard deviation of 0.54 pg/ml in each group, assuming a 30% prevalence of obesity, 80% power and 5% error.

Anthropometry

Anthropometric measurements were performed by trained dietitians using standardized protocols (intraclass correlation ≥ 0.75).[12] All children were barefoot and measured in light clothes. Weight was measured with a portable electronic scale (Seca 770, 0.1 kg), height was measured with a portable stadiometer (Harpenden 603, nearest 0.1 cm), body mass index (BMI) was calculated as weight (kg)/height $(m)^2$, and BMI z-score was determined based on the World Health Organization 2007 growth curves.[13] Waist circumference was measured at the midpoint between the last rib and the right iliac crest with a LUFKIN W606PM tape that has 0.1 cm accuracy. The Fernandez 2004 reference for waist circumference in a Mexican American population was used for the classification of high central obesity (NHANES III).[14] The fat mass percentage (% fat mass) was estimated by bioimpedance using a TANITA 418 BC. Age, sex, and height were needed for calculation of the % fat mass.

Sexual Maturation

The assessment of sexual maturity (breast development) was performed by visual inspection and palpation of the breast using the Tanner scale.[1] A single trained dietitian conducted the evaluation (Kappa > 0.8)[15], and a pediatric endocrinologist evaluated doubtful cases.

Metabolic – Hormonal Measurements

A fasting blood sample was taken (minimum 8 hours, maximum 12 hours) for hormonal and metabolic measurements. Samples were centrifuged for 10 minutes at 3000 RPM. The extracted serum was maintained at −20°C.

Measurement of ultrasensitive estrogens was performed by an ultrasensitive recombinant cell bioassay (RCBA). This technique uses a strain of *Saccharomy cescerevisiae*, which is transformed with two plasmids. One plasmid is the DNA of the complementary human estrogen receptor, and the other plasmid includes an estrogen response element upstream of the beta-galactosidase structural gene. Yeast was incubated for 8 hours with either extracts of 0.8 ml of standard serum or estradiol. Beta-galactosidase activity was measured with ortho-nitrophenol galactopyranoside as a substrate. A standard linear interpolation curve was used to estimate the estradiol equivalent units.[16] The sensitivity of the bioassay was 0.02–0.2 pg/ml (0.07 to 0.7 pmol/l). The intra- and interassay coefficients of variation of 0.2 pg/ml (0.7 pmol/l) were 10–50%.[17]

Other metabolic compounds (hormones) were measured, such as DHEAS, which was determined

by ELISA using a commercial kit (Diagnostic Inc. product, TX, USA) with a 10 pg/ml sensitivity and intra- and interassay coefficients of variation (CVs) of 4% and 8%, respectively. Insulin-like growth factor 1 (IGF-1) was measured by radioimmunoassay (RIA) with 5 ng/ml sensitivity and intra- and interassay CVs of 8.6% and 10.2%, respectively. Insulin was measured by RIA (sensitivity of 0.5 IU/ml, intra- and interassay CVs of 8.6% and 10.2%, respectively).

Other Variables

Maternal data were collected through a survey to the mothers, in which women reported their age at first menstrual cycle (menarche) and their total years of formal education.

Analysis Plan

A descriptive analysis of the dependent and independent variables was performed. Means with their corresponding standard deviations were calculated. Serum estradiol equivalent results below the level of detection (LOD) were imputed as $\frac{LOD}{\sqrt{2}}$ [18] Levels of serum estradiol equivalents were normally distributed, based on skewness and kurtosis tests for normality. We categorized overweight as a BMI z-score ≥ 1 SD and < 2 SD, and obesity as ≥ 2 SD High central obesity was defined as a waist circumference ≥ 90th percentile.[14] Fat mass percentage was categorized in tertiles (low < 25%; medium 25% to 29%; high greater than 29%).

Bivariate analysis was performed to evaluate the association between the levels of serum estradiol equivalents, excess weight and central adiposity using Student's t-test. Pearson correlations between estradiol equivalents and metabolic hormone levels (DHEAS, insulin, IGF-1, leptin) were estimated for continuous variables and Spearman correlation was used to assess quartiles of estradiol equivalents and metabolic hormone variables.

Crude and adjusted linear regression models were calculated to assess the association between independent variables: (i) nutritional status, (ii) high central obesity, (iii) tertile of % fat mass and serum levels of estradiol equivalents. Adjusted analyses included participant age, mother's age at menarche and maternal education. Additionally, the association between estradiol equivalents and hormonal/metabolic variables (insulin, IGF-1, leptin, and DHEAS) was evaluated. We tested potential interactions between hormonal/metabolic variables and adiposity markers. Identified interactions were stratified by the median levels of the hormone/metabolic variables, and linear regression models were carried out between adiposity and estradiol equivalents within each stratum. For all statistical analyses, a confidence level of 95% was defined, and values of $p < .05$ were considered statistically significant. The STATA statistical package version 13.0 was used.

Ethics

The Ethical Committee Board of the Institute of Nutrition and Food Technology approved the research protocol. All procedures performed that involved human participants were conducted in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study. Parents or guardians of the adolescents provided written informed consent, and adolescents provided written assent.

Results

We randomly selected 107 girls who, in 2009, had a mean age of 6.7 ± 0.4 years. Of these participants, 16% were obese, and 11.2% were categorized as having high central obesity. The mean age of maternal menarche was 12.7 ± 1.6 years. This sample was not different from the remaining girls of the larger cohort with regard to anthropometric measures, maternal age of menarche and metabolic and hormonal levels (insulin, leptin, IGF-1, and DHEAS) (data not shown). The mean level of estradiol equivalents in the subsample was 3.6 pg/ml ± 2.4 pg/ml (18% of the girls had levels under the detection limit).

The estradiol equivalents levels were not statistically associated with BMI z-score and high waist circumference (> 90th percentile). Only % fat mass as a categorical variable was inversely correlated

with estradiol equivalents, $p < .05$, (Figure 1). Similar results were found for the metabolic variables (Figure 2). Insulin was inversely related to estradiol equivalents when the estradiol equivalents variable was left in its continuous data form (r = −0.25, $p < .05$, data not shown); however, the quartile of estradiol equivalents was not significantly associated with insulin. We did not observe an association between estradiol equivalents and leptin, IGF-1 and DHEAS. We observed a positive trend between leptin, DHEAS, and IGF-1 with BMI z-score and % fat mass, but no association was found with insulin (Figure 3). However when BMI z-score was assessed as a continuous variable we observed a positive association (r = 0.25, p-value = 0.009; data not shown in tables).

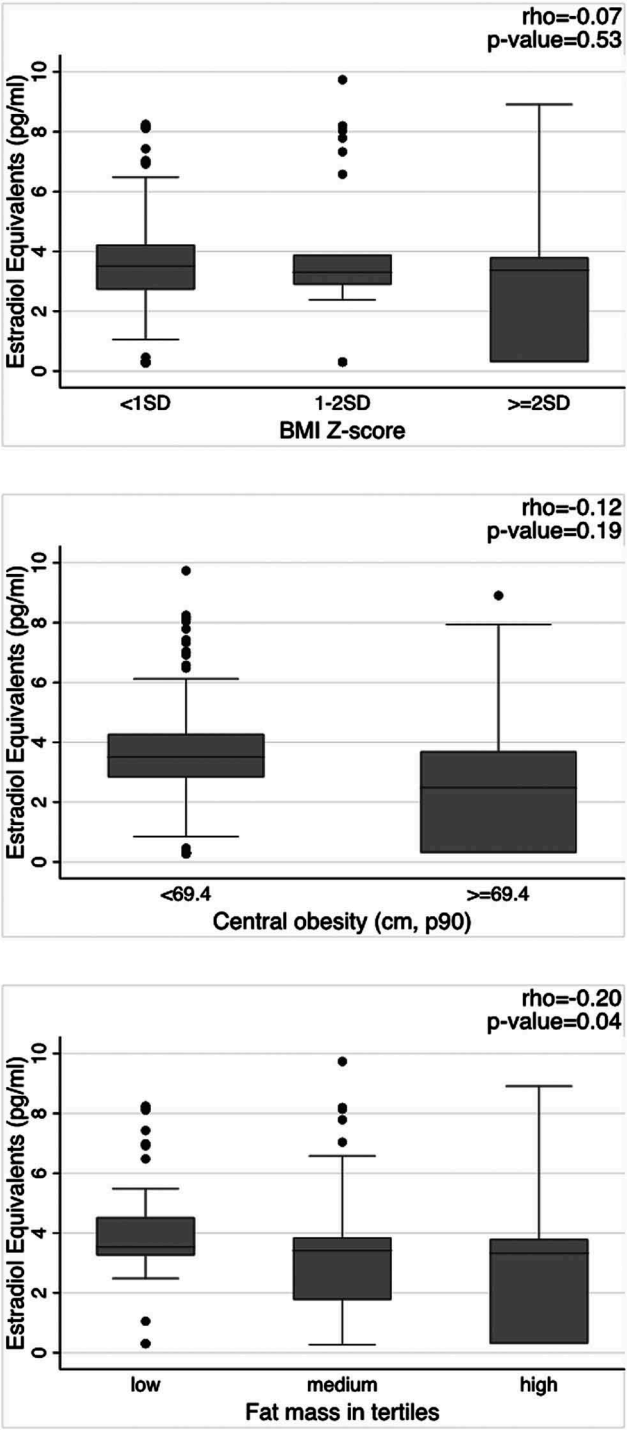

Figure 1. Correlation between total and central adiposity markers and estradiol equivalent in pre-pubertal girls.

In crude and adjusted linear regression models (Table 1), there was no association between adiposity, serum metabolic markers, serum hormonal markers and serum estradiol equivalents in prepubertal girls. An inverse association between insulin and estradiol equivalents was observed in the crude model; however, this association lost significance after adjusting for participant age, maternal age at menarche and maternal education.

We observed an interaction between the median serum levels of leptin and DHEAS with different adiposity markers (Table 2). Among girls with serum leptin levels over the median (> 4.7 µg/ml), we observed an inverse association of obesity, central obesity and the highest tertile of % fat mass with levels of estradiol equivalents. Similar results were observed in the group of girls with DHEAS levels over 304 ng/ml. Girls that were classified in the medium or highest tertile of % fat mass had significantly lower levels of estradiol equivalents compared to girls in the lowest % of fat mass tertile. In contrast, in girls with lower levels of DHEAS (≤ 304 ng/ml), nutritional status (BMI z-score) was positively associated with estradiol equivalents, but the association was only significant in overweight girls (ß = 2.2; 95% CI = 0.5; 3.9).

Discussion

In a random sample of prepubertal girls from the GOCS study (mean age = 6.7 ± 0.4 y), we did not observe an association between serum estradiol equivalents and different markers of adiposity (nutritional status measured by BMI z-score, central obesity measured by waist circumference and % fat mass). We observed an inverse relationship between estradiol equivalents and all adiposity markers in girls who had leptin levels

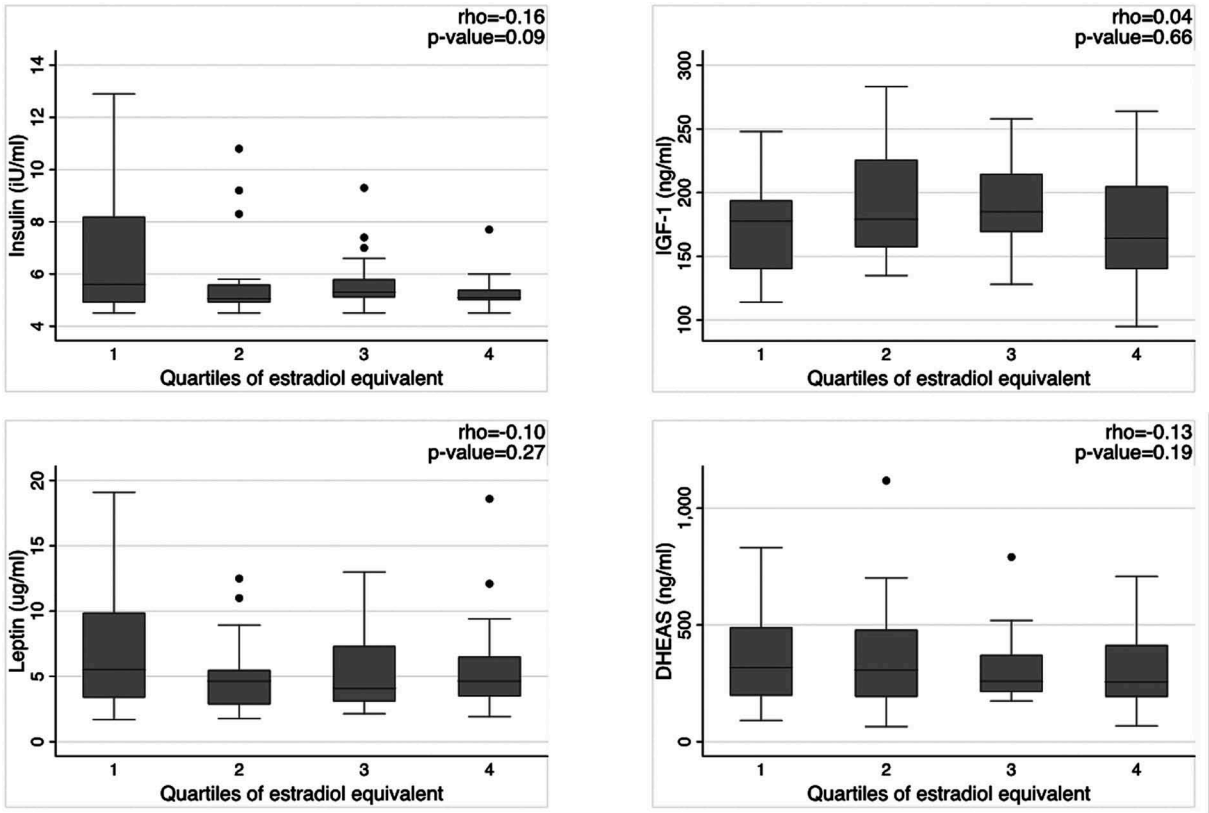

Figure 2. Correlation between metabolic and hormonal markers and estradiol equivalent in pre-pubertal girls.

above the median (> 4.7 μg/ml) and with % fat mass in girls with DHEAS > 304 ng/ml.

There are few epidemiological studies in children assessing the relationship between adiposity and levels of pre- and peri-pubertal estrogen, mainly due to the difficulty of measuring low concentrations. Three studies used the same RCBA assay for this purpose; all were cross-sectional with sample sizes lower than 30 girls.[19–21] The first study was conducted in prepubertal US children with BMI values less than 2 SD in 1994 by Klein et al[21] (23 males, 21 females). These authors found that girls had an eight-fold higher level of estradiol equivalents compared to boys (0.6 ± 0.6 pg/ml and 0.08 ± 0.2 pg/ml, respectively). Subsequently, the same author studied obese children (6 males, 12 females) at a mean age of 8.9 ± 1.8 y and 30 nonobese children (19 males, 11 female) at a mean age of 10.0 ± 1. 9 y, in the prepubertal or early pubertal stage (girls were all Tanner stage 2). Mean estradiol levels were not different between obese and nonobese children (0.45 ± 0.40 pg/ml versus 0.75 ± 0.90 pg/ml) and they did not correlate with any measure of body fat.[19] Later, in 2002, Larmore and colleagues compared the relationship of leptin and estradiol in 5 groups of girls with different combinations of pubertal status and weight. In the prepubertal group, obese girls (n = 12) had slightly higher levels of estradiol than nonobese girls (n = 12) (0.59 ± 1.27 pg/ml vs 0.25 ± 0.40 pg/ml, respectively). In contrast, normal weight pubertal girls had levels of estradiol that were 3.5 times higher than the levels observed in obese pubertal girls (7.7 ± 11.1 pg/ml vs 2 ± 1.8 pg/ml).[20] Thus, despite a large number of younger age girls assessed in this report and the higher concentrations of estradiol equivalents that were detected, our findings indicate that estradiol was not higher in higher adiposity conditions. In a recent case-control study, we found that girls with high levels of prepubertal estradiol equivalents had a higher risk of presenting earlier thelarche, even after adjusting for BMI, insulin and IGF-1 at age. 7[22] Thus, whole-body adiposity and increased adrenal activity did not explain the observed prepubertal estrogen activity. In contrast, in 2015, Mauras and colleagues, using a different high resolution technique (liquid chromatography-mass spectrometry

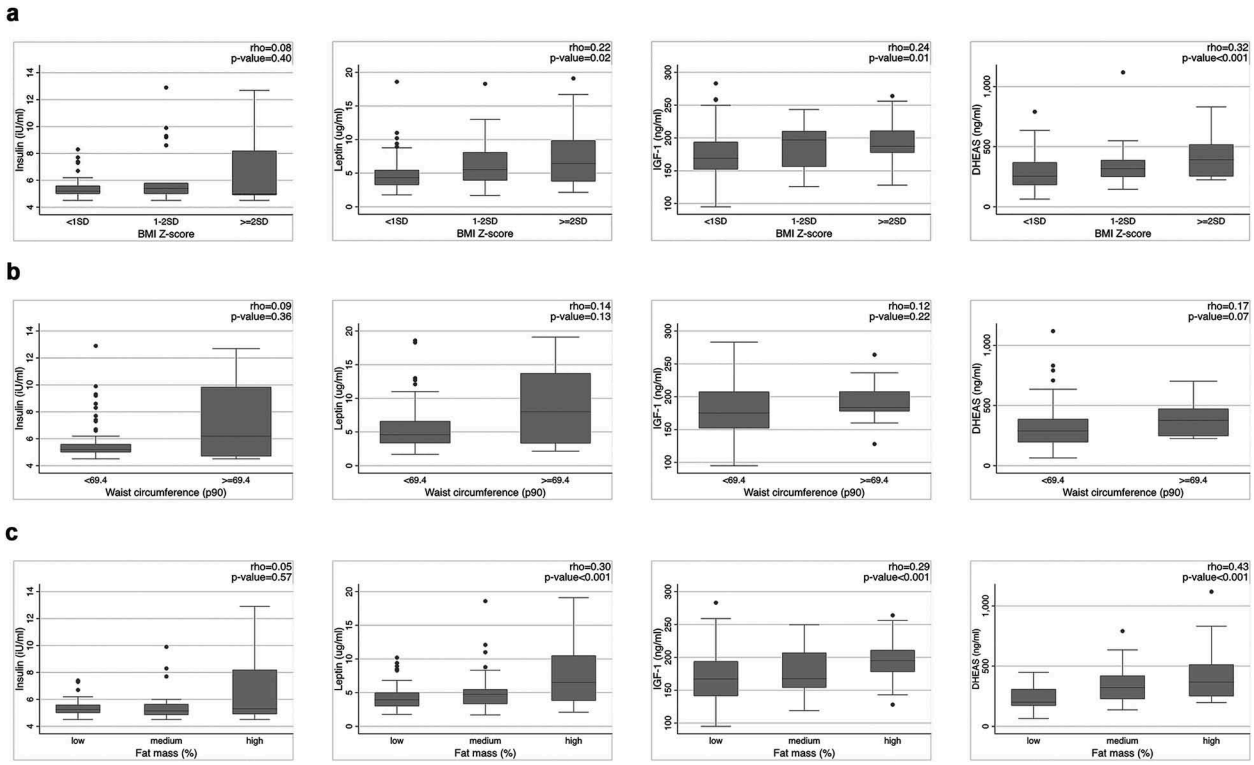

Figure 3. Correlation between metabolic and hormonal markers and different methods for assessing adiposity (a) BMI z-score (b) central adiposity (c) % fat mass in pre- pubertal girls.

LC-MS) in 35 prepubertal girls (~ 9 y), observed 7-fold higher levels of estradiol in obese versus lean girls (3.45 pg/ml (IQR: 0.5, 4.65) versus 0.5 pg/ml (IQR: 0.5, 2.37)) and 16 alpha-OH estrone (an estradiol metabolite considered genotoxic) was 14 times higher in obese prepubertal girls.[23] We observed no statistical association between estradiol and fat mass or BMI z-scores. These differences in findings should be addressed since the recombinant cell assay is not specific to the different types of

Table 1. Crude and adjusted linear regression between adiposity, hormonal and metabolic markers and estradiol equivalent in pre-pubertal girls.

	β^1 (95% CI)	β^2 (95% CI)
Nutritional Status		
Normal weight (−1 SD; 1 SD)	Reference	Reference
Overweight (1 SD; 2 SD)	0.33 (−0.77; 1.44)	0.78 (−0.45; 2.01)
Obese (≥ 2 SD)	−0.50 (−1.78, 0.77)	−0.37 (−1.78; 1.03)
Central Adiposity		
No Central Adiposity (< p90)	Reference	Reference
Central Adiposity (> p90)	−0.82 (−2.24; 0.61)	−0.80 (−2.39; 0.78)
% Fat Mass (tertiles)		
Low (< 25%)	Reference	Reference
Medium (25%–29.0%)	−0.57 (−1.67; 0.53)	−0.70 (−1.94; 0.54)
High (≥ 29.0%)	−1.01 (−2.12; 0.10)	−0.89 (−2.13; 0.34)
Metabolic – Hormonal markers		
Insulin (IU/ml)	−0.35 (−0.62, −0.09)	0.32 (−0.65; 0.01)
Leptin (ug/ml)	−0.10 (−0.22; 0.02)	−0.05 (−0.20; 0.10)
IGF-1 (ng/ml)	0.002 (−0.01; 0.01)	0.003 (−0.01; 0.02)
DHEAS (ng/mL)	−0.001 (−0.004, 0.001)	−0.002 (−0.005, 0.001)

IGF-1: insulin-like growth factor 1, DHEAS: Dehydroepiandrosterone sulfate.
β^1: Crude model. β^2: Adjusted by: girl's age, maternal age at menarche, maternal education.

Table 2. Adjusted linear regression models between adiposity and estradiol equivalent in pre-pubertal girls, stratified by median levels of serum leptin and DHEAS.

	Leptin (p50)		DHEAS (p50)	
	≤ 4.7 ug/ml	> 4.7 ug/ml	≤ 304 ng/ml	> 304 ng/ml
BMI z-score				
BMI z-score < 1 SD	Reference	Reference	Reference	Reference
BMI z-score 1–2 SD	1.17 (−0.07; 3.06)	0.42 (−1.29; 2.14)	2.21 (0.49; 3.93)	−0.58 (−2.52; 1.36)
BMI z-score > 2 SD	1.78 (−0.24; 3.80)	−2.29 (−4.50; −0.08)	1.60 (−0.90; 4.09)	−1.38 (−3.32; 0.55)
Central Adiposity				
Central Adiposity < p90	Reference	Reference	Reference	Reference
Central Adiposity > p90	2.06 (−0.34; 4.47)	−3.07 (−5.38; −0.77)	0.98 (−2.08; 4.04)	−1.50 (−3.52; 0.51)
% Fat Mass				
Low (< 25%)	Reference	Reference	Reference	Reference
Medium (25%–29.0%)	−0.05 (−1.72; 1.62)	−1.65 (−3.55; 0.25)	0.29 (−1.35; 1.93)	−2.66 (−4.67; −0.65)
High (> 29.0%)	0.89 (−0.94; 2.71)	−2.55 (−4.40; −0.7)	0.83 (−1.14; 2.79)	−2.85 (−4.70; −1.00)

DHEAS: Dehydroepiandrosterone sulfate.

estrogen, and therefore, determines the total pool of circulating estrogens, whereas LC-MS identifies different types.

We found higher concentrations of estradiol equivalents compared to the previous studies[17,20,21], suggesting that levels of estradiol equivalents may not be due to an obesogenic environment but may be influenced by other variables. Among the possible explanations for the higher levels includes the variability of the estradiol assay. It is important to note that these samples were processed in the same laboratory as the previous studies, so another possibility is that these higher levels may be related to endocrine disruptor chemicals, which are present in our daily lives via different products (personal care, plastics, food and food containers, etc.).[3,24] Some of these products mimic estrogen activity; thus, they could be detected by the technique used in this protocol. There is evidence that similar bioassays (yeast-based in vitro assay) detect exogenous estrogen activity.[25,26]

We observed a positive association between adiposity (BMI z-score and % fat mass) and higher levels of leptin, IGF-1, and DHEAS but not higher levels of insulin; however, we did observe a positive association with the latter when the variables were analyzed continuously (r = 0.25, *p*-value = 0.009; data not shown in the tables). It has been frequently postulated that estrogen availability is increased by the aromatization of androgen precursors in adipose tissue. The reasoning behind this hypothesis has been supported by the fact that premature adrenarche in obese children increases the concentration of weak androgens. Hyperinsulinemia and hyperleptinemia increase the aromatization of weak androgens.[5,27,28] Additionally, hyperinsulinemia decreases the hepatic production of sex hormone-binding globulin (SHBG), which results in increased bioavailability and production of sex hormones.[5,29,30] Furthermore, growth hormone (GH) levels are lower in obese children[5], while basal levels of IGF-1 are regularly normal or elevated, which may potentially increase aromatase activity and, together with lower levels of SHBG, lead to higher levels of "free" sex steroids.[30] Moreover, girls with more adipose and higher levels of leptin or DHEAS had lower levels of estradiol equivalents. We lacked data on some parameters, such as SHBG and gonadotropins (luteinizing hormone (LH), follicle-stimulating hormone (FSH)), to establish whether changes in the concentrations of these steroids would facilitate greater estrogen bioavailability in obese girls or not, which may explain this association. Additionally, leptin is associated with subcutaneous fat and not with visceral fat, which is the type of fat that increases insulin[31]; thus, this could explain the lack of positive association between adiposity and estrogen in girls with higher levels of DHEAS and leptin.

Our study is not exempt from limitations. We did not evaluate other hormones, such as SHBG, FSH, and LH; therefore, we were not able to accurately elucidate the role of these hormones in our hypothesis and how they may be involved in the relationship between adiposity and prepubertal estrogen. Although we used an ultrasensitive

technique for the measurement of the estradiol equivalent levels, which allowed for the detection of low levels of estradiol equivalents, 18% of the sample had values below the detection limit. Additionally, this technique detects estrogenic activity, and it cannot differentiate endogenous estrogen from exogenous estrogen. In contrast, our primary strength is that even though the design was cross-sectional, the study was set up within a cohort study allowing us to validate our anthropometric and pubertal measurements and assess all girls at the same stage of sexual maturation (Tanner 1) at the time of blood collection. Finally, our sample size was larger than previously reported, and all participants were prepubertal girls at the same chronological age (7 y).

In conclusion, we used a precise and sensitive estrogen detection method to assess prepubertal levels of estradiol equivalents. We did not observe an association between estradiol level and adiposity markers; however, there was an inverse association among girls with higher levels of leptin and DHEAS. More studies are needed to elucidate the role of obesity in estrogen activity at early ages, which is essential to understand the recent early puberty onset trends observed among girls.

Acknowledgments

We thank GOCs participants for their constant contribution in the study and all our team that have worked in data collection and coordination of the project and Dr Karen Klein at University of California, San Diego for performing the ultrasensitive recombinant cell bioassay

Conflict of Interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This work was supported by the Program U-INICIA VID 2011, [Grant U-Inicia11/18], University of Chile. The Chilean Council for Science and Technology: FONDECYT 11170670 and 1140447 and the World Cancer Research Fund [grant 2010/245].

References

1. Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. *Arch Dis Child*. 1969;44 (235):291–303. doi:10.1136/adc.44.235.291.
2. Herman-Giddens ME, Slora EJ, Wasserman RC, et al. Secondary sexual characteristics and menses in young girls seen in office practice: a study from the pediatric research in office settings network. *Pediatrics*. 1997;99 (4):505–512. doi:10.1542/peds.99.4.505.
3. Sorensen K, Mouritsen A, Aksglaede L, Hagen CP, Mogensen SS, Juul A. Recent secular trends in pubertal timing: implications for evaluation and diagnosis of precocious puberty. *Horm Res Paediatr*. 2012;77 (3):137–145. doi:10.1159/000336325.
4. Jasik CB, Lustig RH. Adolescent obesity and puberty: the "perfect storm". *Ann N Y Acad Sci*. 2008;1135:265–279. doi:10.1196/nyas.2008.1135.issue-1.
5. Burt Solorzano CM, McCartney CR. Obesity and the pubertal transition in girls and boys. *Reproduction*. 2010;140(3):399–410. doi:10.1530/REP-10-0119.
6. Golub MS, Collman GW, Foster PM, et al. Public health implications of altered puberty timing. *Pediatrics*. 2008;121(Suppl 3):S218–230. doi:10.1542/peds.2007-1813G.
7. Corvalán C, Uauy R, Mericq V. Obesity is positively associated with dehydroepiandrosterone sulfate concentrations at 7 y in Chilean children of normal birth weight. *Am J Clin Nutr*. 2013;97(2):318–325. doi:10.3945/ajcn.112.037325.
8. Reinehr T, de Sousa G, Roth CL, Andler W. Androgens before and after weight loss in obese children. *J Clin Endocrinol Metab*. 2005;90(10):5588–5595. doi:10.1210/jc.2005-0438.
9. Kirschner MA, Schneider G, Ertel NH, Worton E. Obesity, androgens, estrogens, and cancer risk. *Cancer Res*. 1982;42:3281s–3285s.
10. Kain J, Lera L, Rojas J, Uauy R. Obesidad en preescolares de la Región Metropolitana de Chile. *Rev méd Chile*. 2007;135(1):63–70.doi:10.4067/S0034-98872007000100009.
11. Kain J, Corvalan C, Lera L, Galvan M, Uauy R. Accelerated growth in early life and obesity in preschool Chilean children. *Obesity (Silver Spring)*. 2009;17(8):1603–1608. doi:10.1038/oby.2009.37.
12. Corvalán C, Uauy R, Kain J, Martorell R. Obesity indicators and cardiometabolic status in 4-y-old children. *Am J Clin Nutr*. 2010;91(1):166–174. doi:10.3945/ajcn.2009.27547.
13. de Onis M, Onyango AW, Borghi E, Siyam A, Nishida C, Siekmann J. Development of a WHO growth reference for school-aged children and adolescents. *Bull World Health Organ*. 2007;85 (9):660–667. doi:10.2471/BLT.07.043497.
14. Fernandez JR, Redden DT, Pietrobelli A, Allison DB. Waist circumference percentiles in nationally representative samples of African-American, European-American, and

Mexican-American children and adolescents. *J Pediatr*. 2004;145(4):439–444. doi:10.1016/j.jpeds.2004.06.044.

15. Pereira A, Garmendia ML, Gonzalez D, et al. Breast bud detection: a validation study in the Chilean growth obesity cohort study. *BMC Womens Health*. 2014;14:96. doi:10.1186/1472-6874-14-96.
16. Klein KO, Baron J, Barnes KM, Pescovitz OH, Cutler GB Jr. Use of an ultrasensitive recombinant cell bioassay to determine estrogen levels in girls with precocious puberty treated with a luteinizing hormone-releasing hormone agonist. *J Clin Endocrinol Metab*. 1998;83(7):2387–2389. doi:10.1210/jcem.83.7.4929.
17. Janfaza M, Sherman TI, Larmore KA, Brown-Dawson J, Klein KO. Estradiol levels and secretory dynamics in normal girls and boys as determined by an ultrasensitive bioassay: a 10 year experience. *J Pediatr Endocrinol Metab*. 2006;19(7):901–909. doi:10.1515/JPEM.2006.19.7.901.
18. Succop PA, Clark S, Chen M, Galke W. Imputation of data values that are less than a detection limit. *J Occup Environ Hyg*. 2004;1(7):436–441. doi:10.1080/15459620490462797.
19. Klein KO, Larmore KA, de Lancey E, Brown JM, Considine RV, Hassink SG. Effect of obesity on estradiol level, and its relationship to leptin, bone maturation, and bone mineral density in children. *J Clin Endocrinol Metab*. 1998;83(10):3469–3475. doi:10.1210/jcem.83.10.5204.
20. Larmore KA, O'Connor D, Sherman TI, Funanage VL, Hassink SG, Klein KO. Leptin and estradiol as related to change in pubertal status and body weight. *Med Sci Monit*. 2002;8:Cr206–210.
21. Klein KO, Baron J, Colli MJ, McDonnell DP, Cutler GB. Estrogen levels in childhood determined by an ultrasensitive recombinant cell bioassay. *J Clin Invest*. 1994;94(6):2475–2480. doi:10.1172/JCI117616.
22. Pereira A, Corvalan C, Uauy R, Klein KO, Mericq V. Ultrasensitive estrogen levels at 7 years of age predict earlier thelarche: evidence from girls of the growth and obesity Chilean cohort. *Eur J Endocrinol*. 2015;173(6):835–842. doi:10.1530/EJE-15-0327.
23. Mauras N, Santen RJ, Colon-Otero G, et al. Estrogens and their genotoxic metabolites are increased in obese prepubertal girls. *J Clin Endocrinol Metab*. 2015;100(6):2322–2328. doi:10.1210/jc.2015-1495.
24. Rosen D. physiologic growth and development during adolescence. *pediatr rev*. 2004;25(6):194–200. doi:10.1542/pir.25-6-194.
25. Wagner M, Oehlmann J. Endocrine disruptors in bottled mineral water: total estrogenic burden and migration from plastic bottles. *Environ Sci Pollut Res Int*. 2009;16(3):278–286. doi:10.1007/s11356-009-0107-7.
26. Wagner M, Oehlmann J. Endocrine disruptors in bottled mineral water: estrogenic activity in the E-screen. *J Steroid Biochem Mol Biol*. 2011;127(1–-2):128–135. doi:10.1016/j.jsbmb.2010.10.007.
27. Shalitin S, Phillip M. Role of obesity and leptin in the pubertal process and pubertal growth–a review. *Int J Obes Relat Metab Disord*. 2003;27(8):869–874. doi:10.1038/sj.ijo.0802328.
28. Kaplowitz PB. Link between body fat and the timing of puberty. *Pediatrics*. 2008;121(Suppl 3):S208–217. doi:10.1542/peds.2007-1813F.
29. Garnett SP, Hogler W, Blades B, et al. Relation between hormones and body composition, including bone, in prepubertal children. *Am J Clin Nutr*. 2004;80(4):966–972. doi:10.1093/ajcn/80.4.966.
30. Dunger DB, Ahmed ML, Ong KK. Early and late weight gain and the timing of puberty. *Mol Cell Endocrinol*. 2006;254–255:140–145. doi:10.1016/j.mce.2006.04.003.
31. Van Harmelen V, Reynisdottir S, Eriksson P, et al. Leptin secretion from subcutaneous and visceral adipose tissue in women. *Diabetes*. 1998;47(6):913–917. doi:10.2337/diabetes.47.6.913.