Normative data for adiponectin, resistin, interleukin 6, and leptin/receptor ratio in a healthy Spanish pediatric population: relationship with sex steroids

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Abstract
Objectives: To investigate the circulating levels of adiponectin, resistin, interleukin 6 (IL-6), and leptin/receptor ratio in healthy Spanish children throughout the different stages of pubertal development. To analyze the relationship between adipokines and sex steroid level changes during puberty.

Study design: Serum adiponectin, resistin, IL-6 levels, and leptin/receptor ratio were studied in 160 healthy Spanish children grouped according to their pubertal stage (Tanner I, 23 girls and 22 boys; Tanner II, 19 girls and 16 boys; Tanners III and IV, 21 girls and 20 boys; and Tanner V, 20 girls and 19 boys). In addition, circulating levels of sex hormone-binding globulin (SHBG) were determined in every subject, and testosterone and estradiol levels in boys and girls respectively.

Results: Adiponectin levels decreased in boys from mid puberty ($P < 0.05$) to become significantly lower than in girls ($P < 0.001$), whereas IL-6 decreased in both sexes ($P < 0.05$). Resistin levels and leptin/receptor ratio showed no differences between sexes or according to pubertal stage, except in adult females, who had the highest levels of both parameters ($P < 0.001$). Serum IL-6 levels correlated significantly ($P < 0.05$) with testosterone and estradiol levels ($r = -0.37$ and $-0.42$ respectively), whereas estradiol, but not testosterone, correlated with leptin/receptor ratio ($r = 0.59; P < 0.001$). Furthermore, a positive relationship was found between SHBG and adiponectin and IL-6 ($P < 0.001$ and $P < 0.05$ respectively). In addition, a direct correlation between leptin/receptor and body mass index was found in both sexes ($P < 0.001$).

Conclusion: Variations in adipokine profiles throughout pubertal development appear to be related with progression of gonadal function.

Introduction
White adipose tissue (WAT) is an active player in energy balance and metabolic homeostasis expressing receptors for most hormonal and neurological signals, and also synthesizing a wide variety of peptides resulting in both paracrine and endocrine actions (1, 2). These peptides have numerous central and peripheral actions, some of which are not fully understood, and mediate the integration of WAT into body homeostasis control.

Among these peptides, leptin acts as a satiety signal and stimulates energy expenditure, thus regulating body weight (3). Its levels are related with body fat mass and distribution, changing in a sex-specific fashion as puberty progresses (4), as do levels of its specific soluble receptor (5), thus modulating leptin actions (6, 7). This peptide appears to be important for puberty onset and progression (8–10).

Adiponectin, specifically produced in adipocytes, is involved in glucose and lipid metabolism, directly influencing insulin sensitivity (11). Its circulating levels are negatively correlated with body mass index (BMI) and fat content (12) and, in association with visceral obesity, postulated to be a possible marker of metabolic syndrome risk (12). Adiponectin levels become significantly different between the sexes in early adulthood, suggesting that pubertal development influences adiponectin levels (13).

Circulating resistin, mainly produced by mononuclear cells in the adipose tissue matrix, has been positively associated with fat mass and insulin resistance (14,15), as well as interleukin 6 (IL-6) (16); however, its role in humans is controversial and sparse data regarding its levels during pubertal development are available to date.

Hence, situations modifying body fat content and distribution or specific metabolic requirements that...
involve adipose tissue could theoretically cause changes in adipokine profiles. During pubertal development, energy requirements necessary for the pubertal growth spurt, as well as the increase in fat mass deposits, increase (17). In addition, these fat mass deposits have a sex-specific distribution (17, 18), with increasing visceral fat accumulation in males, which could be involved in changes in adipokine secretion (19). These changes are influenced by increasing sex steroid and decreasing sex hormone-binding globulin (SHBG) levels, and thus increased bioavailability, as puberty progresses (19).

The parallel increase in fat mass and sex steroid levels during puberty could play an important role in the evolution of adipokine profiles throughout development in children and adolescents. Therefore, the aims of this study were to determine the normative data for adiponectin, resistin, and IL-6 levels and the leptin/receptor ratio in childhood and throughout the pubertal development, as well as to analyze the possible role of sex steroids in their changes.

Subjects and methods

Subjects

One hundred and sixty healthy children and adolescents (83 girls (F) and 77 boys (M)) were included. They were divided into four groups according to the degree of their pubertal development: prepubertal (Tanner I) 23 F and 22 M; puberty onset (Tanner II) 19 F and 16 M; mid puberty (Tanners III and IV) 21 F and 20 M and early adults (Tanner V) 20 F and 19 M.

These children were referred to our department for suspected endocrine abnormalities and were found to be normal, with no auxological, clinical or analytical abnormalities. Their height, weight, and BMI were between ±1 SDS according to Spanish standards (20). Their mean BMI was 0.09 ±0.08 SDS, mean height −0.21 ± 0.11 SDS and mean weight −0.15 ± 0.09 SDS. Their mean age was 8.16 ± 0.45 years for prepubertal children; 10.65 ± 0.31 and 12.22 ± 0.39 at puberty onset for F and M respectively; 11.99 ± 0.35 (F) and 13.86 ± 0.39 (M) at mid puberty and 17.22 ± 0.41 (F) and 17.67 ± 0.52 (M) for young adults. All blood samples were obtained after overnight fasting, centrifuged, and the serum stored at −80 °C until assayed.

All patients and their parents or guardians were informed about the purpose of the study and gave informed consent as required by the local ethics committee, which had previously approved the study.

Biochemical measurements

Serum adiponectin and testosterone were measured by commercial RIA (LINCO Research, Inc., St Charles, MO, USA and DSL, Webster, TX, USA respectively). Leptin was measured by RIA as previously reported (4). Soluble leptin receptor and resistin levels were determined by commercial ELISA assays (BioVendor Laboratory Medicine, Inc., Brno, Czech Republic and LINCO Research, Inc. respectively). Ultrsensitive ELISA assays were used to quantify circulating IL-6 (Quantikine HS, R&D Systems, Inc., Minneapolis, MN, USA) and estradiol levels (DSL). IRMA was used to determine SHBG levels (DSL). Intra- and inter-assay coefficients of variation were below 10% for all assays.

Statistical analysis

All data are reported as mean ± S.D. Analysis was performed by one-way ANOVA for comparisons between different pubertal stages and a two-tailed Student’s t-test was used for comparisons between sexes. The relationship between different quantitative variables was determined by linear regression analysis. A value of $P < 0.05$ was chosen as the level of significance. Statistical analyses were performed using SPSS 12.0 software for Windows (MapInfo Corporation, Troy, NY, USA).

Results

Adiponectin, resistin, IL-6, and leptin/receptor ratio

Serum adiponectin levels showed no differences between the sexes in prepubertal children. Values did not change during puberty in girls, whereas in boys there was a significant decrease at mid puberty ($P < 0.05$), becoming significantly lower compared with girls ($P < 0.001$; Fig. 1A).

Resistin levels and leptin/receptor ratio remained unchanged during development and showed no differences between the sexes until after puberty was completed. Both parameters were higher in Tanner V girls compared with previous stages ($P < 0.001$) and with Tanner V boys ($P < 0.001$; Fig. 1B and C respectively). IL-6 levels decreased in both sexes from mid puberty ($P < 0.05$), without any significant differences between the sexes at any pubertal stage (Fig. 1D).

Correlations between adipokines and BMI

A significant correlation was found between BMI and leptin/receptor ratio in both sexes ($r$ = 0.57, $P < 0.001$ for girls and $r$ = 0.60, $P < 0.001$ for boys). No significant correlation between BMI and the other adipokines studied was found.

Adipokines correlations

A significant correlation was found between resistin levels and leptin/receptor ratio when the whole cohort

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was analyzed together \((r=0.44, P<0.001)\). When this relationship was studied in males and females separately, the correlation remained in girls \((r=0.54, P<0.001)\), while it disappeared in boys. No other significant correlations between adipokines were found.

**Correlations of sex steroids and SHBG with adipokines**

Significant correlations \((P<0.05)\) were found between estradiol and leptin/receptor ratio \((r=0.59)\) in girls, as well as between IL-6 and both testosterone and estradiol \((r=-0.37\) and \(-0.42\) respectively; Fig. 2A and B). SHBG levels were positively correlated with IL-6 \((r=0.3, P<0.05); Fig. 2C\), as well as with adiponectin \((r=0.41, P<0.001); Fig. 3\).

**Discussion**

Our results indicate that levels of circulating adipokines change during normal pubertal development in a sex-dependent fashion. Adult females have higher leptin/receptor ratio and resistin levels compared with previous pubertal stages and adult males. There is a progressive decrease in IL-6 levels in both sexes and in adiponectin levels in boys as puberty progresses. We found testosterone and estradiol levels to be inversely correlated with IL-6 levels, whereas estradiol was directly correlated with leptin/receptor ratio. In contrast, SHBG levels were directly correlated with IL-6, as well as with adiponectin levels.

We have found, consistent with our previous data (4), that leptin levels significantly increase throughout the pubertal development in females and decrease at the final stage of male puberty. It was demonstrated that leptin soluble receptor, which modulates serum leptin levels (21), decreases in both sexes after puberty onset (5), with free leptin suggested to be the active form (22). Thus, evaluation of the leptin/receptor ratio offers more valuable information than leptin levels alone. The increase in leptin bioavailability during pubertal development could be a signal to the central nervous system that metabolic conditions are adequate to undergo pubertal development (22).

The highest leptin/receptor ratio in adult females is most likely the result of changes in fat mass and sex steroid levels, as BMI and percentage and distribution of body fat seem to be the most important predictors of the leptin/receptor ratio (23). This is consistent with the correlation found between BMI and leptin/receptor ratio in our cohort overall and in both sexes when analyzed separately. Sex steroids affect this ratio through regulating the amount and distribution of fat mass and directly modulating leptin transcriptional control (24).

Adipose tissue has receptors for sex steroids (24) and testosterone exerts a negative effect on leptin transcription and secretion (25, 26), while estrogens are stimulatory (26, 27). The positive correlation found between estradiol and leptin/receptor ratio in females supports this hypothesis, whereas the lower increase of leptin/receptor ratio throughout the pubertal
The lack of sex differences prior to mid puberty and the decrease in adiponectin levels found in males after mid puberty suggest that androgens could decrease adiponectin levels. Moreover, higher testosterone bioavailability in males due to the progressive decrease in SHBG throughout puberty may be involved as suggested by the direct correlation between adiponectin and SHBG levels. Indeed, plasma adiponectin levels decrease after androgen replacement in hypogonadal males (28) and experimental models (29). Moreover, adiponectin levels are also decreased in females with polycystic ovarian syndrome, who have increased testosterone levels and bioavailability, as SHBG levels are reduced (30). However, adiponectin production is also negatively correlated with visceral fat accumulation (19, 31, 32), thus the decrease in circulating adiponectin in males could also be influenced by an androgen mediated increase in visceral fat as puberty progresses (17).

Among proinflammatory cytokines, the tumoral necrosis factor α acts mainly as a local factor, whereas adipose-derived IL-6 is secreted to the bloodstream and constitutes up to one-third of circulating levels (1). Although circulating IL-6 levels in healthy infants have been reported (33), to our knowledge no information regarding IL-6 levels during pubertal development is available. Both testosterone (34) and estradiol (35) have a direct inhibitory effect on IL-6 production in humans, which is consistent with the decrease in IL-6 levels observed in both sexes after puberty onset. Likewise, IL-6 levels correlated negatively with both sex steroids and positively with SHBG levels.

We found serum resistin levels to be higher in young adult females than in males, as previously reported (36, 37). However, in agreement with most (38, 39), but not all (37) reports, we found no correlation of resistin with sex steroids or BMI. This lack of correlation is consistent with results from animal studies where estradiol did not modify plasma resistin levels (40). However, the fact that resistin levels are their highest in adult females and correlate with the leptin/receptor ratio, which does not occur in males, suggests a possible link between resistin levels and female body fat content changes. Taken together, these results might indicate a sex-specific role for adipokines in metabolic changes throughout development. Hence, the higher insulin requirements
observed in late puberty could be mainly influenced by adiponectin decrease in males and resistin increase in females.

In conclusion, our findings indicate that changes in adipokine levels throughout the pubertal development are sexually dimorphic, suggesting that gonadal function progression and increasing circulating sex steroids may play an important role in adipokine changes.

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