**DOI: 10.4274/jcrpe.galenos.2026.2025-12-20**

**Original Article**

# The Role of Sex Steroids, Sex Hormone-binding Globulin and Leptin in the Development of Pubertal Gynecomastia and its Persistence into Adulthood

**Petrova Todorova Z and Zdravkov BN. Hormonal Changes in Pubertal Gynecomastia**

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**Abstract**
**Objective:** Pubertal gynecomastia (PG) is a benign condition considered to be caused by imbalance between estrogen and testosterone, though this is not frequently evident from serum analysis. This study aimed to determine possible alteration in sex hormone levels, as well as to investigate the role of sex hormone-binding globulin (SHBG) and leptin in PG development and persistence.
**Methods:** A total of 95 boys with PG were included. They were divided into 3 groups considering stage. Anthropometric measurements, hormonal investigations, SHBG and leptin were collected and compared group of 64 controls without PG matched in pubertal status and anthropometrics.
**Results:** Investigations of patients with gonadal Tanner stage 2 proved statistically significant estradiol [120.0 pmol/l (73.4-240.0) vs.77.80 (23.2-120), p=0.023] and decrease in testosterone/estradiol ratio compared to control group. The same is not found in PG groups with Tanner stage 3, 4 5. Difference of the levels of testosterone and Free Androgen Index (FAI) were not found in Tanner stage 2, but significantly lower FAI was determined in mid-pubertal boys with gynecomastia compared to controls [36.0(6.8-197.0) vs.64.0(1.79-140.1), p=0.017]. Additionally, a lack of decrease of SHBG in the PG group with pubertal advancement was observed, which might explain the lower FAI in that cohort. No statistically significant difference leptin level was found.
**Conclusion:** The early rise of estradiol at the beginning of puberty might be responsible for PG, while lack of SHBG reduction with advancement of puberty and lower FAI might have an impact on its development and/or persistence in mid-pubertal boys.
**Keywords:** pubertal gynecomastia, estradiol, leptin, sex hormone-binding globulin, testosterone

**What is already known on this topic?**
Contradicting data on levels of sex hormones and gonadotropins in boys with pubertal gynecomastia exist. Some authors report elevated levels of estradiol and FSH while others not. Additionally, there is scarce data of the importance of SHBG and leptin on development and persistence of PG, found in single publications.

**What this study adds?**
We report that only boys with PG (Tanner stage 2) have significantly elevated Estradiol compared to controls and also that with advancement of puberty in boys with PG, SHBG does not decrease as much as in controls. This leads to lower FAI in gynecomastia group (Tanner 3-4), which impacts PG development/persistence.

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**Author contribution:** Both authors have contributed for the conceptualization, analysis, methodology, writing- draft and writing-review and editing

10.01.2026
17.08.2026

**Epub:** 31.08.2026

## Introduction

The benign growth of glandular tissue in the male breast is known as gynecomastia (GM). It is the most prevalent breast condition that affects men, and it can occur at any age. However, it is more common during the newborn period, puberty, and in older men, when it is thought to be physiologic [1,2]. It can be unilateral or bilateral, with the latter being more prevalent. Clinically it manifests as a palpable concentric firm or rubbery mass below the nipple-areola complex and should be distinguished from pseudogynecomastia, which is due to accumulation of adipose tissue. Breast discomfort and tenderness are frequently associated with GM development. The imbalance between estrogens and androgens that favors estrogen is the pathophysiological process that underlies the illness. [3] Similar to how they affect the female breast, estrogens induce ductal elongation and branching, epithelial hyperplasia, and periductal fibroblast proliferation, whereas testosterone (T) has a widespread inhibitory effect due to its antiestrogen action. [4,5] Growth hormone and insulin-like growth factor 1, which promote proliferation and growth through their respective receptors in breast tissue, are additional variables influencing breast development. [6]
GM can be divided into two primary categories: pathogenic and physiologic. Depending on the age of presentation, the development of breast in males outside the aforementioned periods is considered pathologic. Such an example is prepubertal GM. [7] Different endocrine and non-endocrine diseases may manifest with GM. The most common are primary and secondary hypogonadism, partial androgen insensitivity syndrome, Klinefelter syndrome, testicular or adrenal tumors, aromatase excess syndrome, hyperthyroidism, chronic liver and kidney disease,

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as well as some medications. [1,2,8] In each of these cases an obvious pathologic condition leading to either increased levels/action of estrogens or decreased testosterone secretion/action or both is established. Similar mechanism of transiently increased Estradiol (E2) to T ratio (E2/T ratio) in the absence of disease is considered a reason for development of physiologic GM [3,8].

Physiologic pubertal gynecomastia (PG) typically manifests between the ages of 13 and 14 during the Tanner stage of genital development 3–4 [3,9-12]. This is actually intermediate or advanced pubertal development and T level has already appropriately increased at this stage.

There are, however, boys who develop PG as early as the age of 10 at the very beginning of puberty which is Tanner stage 2. To our knowledge there are no investigations of hormonal levels and their impact which differentiate boys with PG by Tanner staging. So maybe this is the reason why sex steroid imbalances can rarely be detected in serum samples from boys with PG and data in the literature is controversial. [12-19]

Pubertal gynecomastia evolution is generally benign as in majority of the cases it regresses within 6 months to 2 years. [3, 12, 20] A recent study found that persistence of PG in adulthood ranges from 10% of males [21] to 16.4%. [22] The mechanisms that can lead to its persistence in maturity are not well understood. Kilic et al. [15] found that sex hormone-binding globulin (SHBG) do not decline in the same manner in the group of boys with PG with advancement of pubertal development when compared to control group without PG and respectively has an impact on the Free androgen index (FAI) in PG group. So they concluded that lack of significant decrease in SHBG and as a consequence of this no significant increase in FAI during the stages of pubertal development can play a key role in the hormonal etiology of PG [15] or even further explain its persistence. Furthermore, there is a substantial association between GM, including pubertal, and obesity, with a reported incidence of up to 80% [23–25]. Due to their increased adipose tissue both generally and in the breast area, obese people are more likely to acquire GM. The aromatase enzyme complex, which helps convert T to E2, is known to be present in adipose tissue. [27] Additionally, a number of pathways link circulating leptin to the formation of GM in obese adolescents. [10] Given the conflicting data on sex hormone and gonadotropin levels, as well as the limited evidence regarding the role of SHBG and leptin in the development and persistence of pubertal gynecomastia (PG), this study aimed to evaluate serum estradiol (E2), testosterone (T), the T/E2 ratio, SHBG, and leptin levels in adolescents with PG compared with age- and pubertal stage–matched controls without PG. We also aimed to assess whether gonadal stage and the duration of GM influence these parameters.

## Materials and Methods

### Study subjects

This study began as retrospective cross-sectional analysis of boys referred with GM to a pediatric endocrinology tertiary clinic at a specialized pediatric hospital between 2009 and 2016. It continued with a prospective evaluation through 2019. Within this period 157 with GM were identified. Twelve boys were with prepubertal GM and another 35 were diagnosed with pathologic GM (previously published data). [28] The remaining 110 teenagers were classified as having PG since no pathologic cause for breast growth was found. Fifteen cases from the retrospective group were excluded due to incomplete data (from history or some investigation) so at the end 95 patients were included in the study. Patients were divided into three groups with respect to gonadal stage – group 1 –boys with gonadal development stage 2 (corresponding testicular volume 4-8 ml) according to Marshal-Tanner (beginning of puberty), group 2 – middle pubertal development –3-rd and 4-th stage (testicular volume 9 to 20 ml) and group 3- completed pubertal development (Tanner 5 or testicular volume more than 20 ml) [29]. For complete evaluation the same cohort was divided by another characteristic into 2 groups – recently developed PG with duration less than 6 months and long-lasting PG (more than 12 months).[21] A group of 64 adolescents without GM at the same age, with matching anthropometric measurements and gonadal staging was investigated as a reference cohort. Most of them were referred for obesity and were selected as controls due to absence of true or pseudogynecomastia. Leftover samples for blood checks for exclusion of endocrine or other disease of otherwise healthy boys were also used.

### History and clinical examination

All patients' age at PG development and duration, presence of pain/tenderness and family history for PG, were collected and analyzed. Height was measured to nearest 0.1 cm using a standardized stadiometer. Children were weighed barefoot with light clothing by a scale with precision to 0.1 kg. Body mass index (BMI) was calculated using the standard formula of weight (kg) divided by height (m) squared. Height, weight and BMI standard deviation scores (SDS) were calculated using the standard formula and national standards. A BMI SDS over (+2.0) was classified as obesity, between (+2) and (+1) as overweight. [30] Pubic hair and genital development were assessed by clinical examination according to Marshall and Tanner and testicular volume (ml) was measured by palpation using Prader orchidometer to the nearest bud. Gynecomastia was evaluated by inspection and palpation as described by Braunstein. [8] In some disputable cases (difficult to differentiate between true gynecomastia and pseudogynecomastia), breast ultrasonography was performed by an experienced specialist so as to prove the presence of glandular tissue.

### Serum hormones analysis

Blood samples were drawn between 7.30 and 9.00 and were clotted, centrifuged and analyzed the same day with exclusion of leptin which was analyzed from frozen at -200 C serum not later than 1 year. Serum concentrations of follicle stimulating hormone (FSH), luteinizing hormone (LH), Testosterone (T), Estradiol (E2), sex hormone-binding globulin (SHBG) were analyzed by chemiluminescent immunoassay method (IMMULITE 2000, Siemens Healthcare Diagnostics). Intra- and inter-assay coefficient of variation of all investigations was less than 5%.

To evaluate the relationship between T and E2 we calculated T to E2 ratio. Based on the serum concentrations of T and SHBG we calculated FAI by the formula total T divided to SHBG, the result multiplied by 100 [FAI = testosterone (nmol/L)/SHBG (nmol/L) × 100]. Free testosterone (freeT) was calculated based on total testosterone, SHBG and albumin using Vermeulen formula.[31] Serum albumin was analyzed by Cobas integra 400 plus (Roche Diagnostics). Leptin levels in serum samples of 50 boys with PG (referred for evaluation within 2016-2019) and all controls were analyzed by enzyme-linked immunosorbent assay (ELISA) (DIAsource ImmunoAssays Human leptin ELISA kit, Belgium).

### Ethics

All procedures were conducted in accordance with the national and institutional ethical standards and with the Helsinki Declaration. In the retrospective part no formal approval or informed consent was required. All participants and responsible caregivers in the prospective part gave general informed consent as patients of the hospital for investigation and the study of leptin only, received a formal approval by institutional bioethics committee (1327-28-03-2017).

### Statistical analysis

Statistical analysis was performed using Statistical Package for Social Sciences (version 22). A p value of $<0.05$ was considered significant. Descriptive statistics for continuous variables are expressed as mean, median, standard deviation (SD), minimum and maximum. Whether quantitative data are distributed normally was tested by Kolmogorov-Smirnov test. To compare variables Independent –Samples t-test and nonparametric Mann-Whitney test were used as appropriate. For correlation analyses of continuous variables that are parametric was used Persons correlation and for nonparametric–Spearman coefficient.

## Results

A total of 95 boys with PG were included in the study. When analyzed by anthropometric measurements 72 (75.79 %) adolescents were with obesity and 4 were overweight (4.21%) or only 20 % were with BMI < 1 SD. Five of the boys with PG had unilateral and 90 (94.74 %)

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had bilateral breast. Mean age of development was 11.85 years (9.33-16.92). Additional analysis was performed with regard to overweight and obesity as the excessive adipose tissue has impact on the beginning of pubertal development. The boys with normal weight developed PG at a mean age of 13.13 years and those with overweight/obesity at 11.69 ($p<0.001$). Figure 1 presents the detailed age distribution of PG development in the groups according to BMI.

Prior to medical reference, the average length of PG was 1.66 years (0.04-5). Within the first year of PG development, 46 boys (48.42%) sought medical attention, the remaining 49 boys with longer presence. Twenty-four boys, or nearly a quarter, had GM for more than three years, which strongly suggests that they will have persitent GM as adults. Forty teenagers (42.1%) responded favorably when asked about pain and/or soreness, all of them within the first year of breast development. Twenty-three boys (24.21 %) had a positive family history for PG, 56.84 % (54 boys) did not have and in the rest 18 data was missing. According to the GM staging – 7 were stage 1 (5 normal weight) – 7.37 %, stage 2a – 32 boys (12 –normal weight) – 33.69 %, stage 2b-28 patients (25 obese)- 29.47%, and stage 3- 28 patients- all obese (29.47%) according to Simon classification. [32] The cohort of adolescents with PG was divided into 3 groups according to stage of genital development as described above. As it is well established that adipose tissue has an impact on sex hormone levels and particularly influences leptin, boys with PG in each of the three Tanner stage groups were matched with their corresponding control group for all anthropometric indices and their SDS values. (Table 1) Data presented in table 1 emphasizes that although majority of the patients were also diagnosed with obesity the reference cohort was selected so as to match by anthropometrics. Serum levels of FSH, LH, T, E2, SHBG and Leptin were investigated in adolescents with PG and compared with control groups. T/E2 ratio and free T were calculated and also compared. (Table 2).

Serum levels of FSH, LH, testosterone, free T, and leptin did not differ between the PG group and the control at any pubertal stage. In group 1, boys at the beginning of puberty have significantly higher E2, respectively lower T/E2 ratio. On the contrary, a statistically significant decreased FAI was determined for males in group 2 who were in the middle of pubertal development. The values of SHBG merit a special consideration. The PG group and the control cohort of boys in the second, third, and fourth stages of puberty differ significantly. While SHBG is significantly lower in PG group compared to controls at the beginning of puberty, it does not decline with advancement of sexual maturation as much as SHBG in boys without breast development. In boys without PG there is a statistically significant difference in SHBG value measured at the beginning of puberty and those in the middle [27.0 (25.4-40.7) vs. 14.4(7.27-76.4), $p=0.02$] and with completed puberty [27.0 (25.4-40.7) vs. 14.6(6.10-23.5), $p<0.001$]. No such a decrease was found in PG group: stage 2 vs stage 3-4 [21.75(7.12-69.4) vs.18.9 (9.19-47.4), $p=0.433$] and stage 2 compared with stage 5 [21.75(7.12-69.4) vs.17.2 (8.83-30.0), $p=0.340$]. Figure 2.

This may be one of the factors contributing to lower FAI in PG group in 3-rd and 4-th pubertalsstage. We did not manage to prove any hormonal imbalance in boys with completed puberty and PG compared to controls.

Since most of the boys were also with obesity we tried to find a correlation between the adiposity reflected by weight and BMI SD scores and the serum levels of sex hormones, their ratio, SHBG, free T and FAI in cohort with PG. In pubertal stage 2 a moderate positive correlation was found between weight SDS and T ($r=0.338$, $p=0.041$), free T ($r=0.346$, $p=0.039$), FAI ($r=0.349$, $p=0.046$) and T/E2 ratio ($r=0.319$, $p=0.047$) and stronger correlation between BMI SDS and T ($r=0.537$ $p<0.008$), free T ($r=0.534$, $p<0.007$), FAI ($r=0.507$, $p<0.008$) and T/E2 ratio ($r=0.478$, $p<0.007$). With advancement of puberty (stage 3 and 4) the positive correlation is substituted by a negative one between weight SDS and T ($r=-0.314$, $p=0.0367$) and T/E2 ratio ($r=-0.351$, $p=0.048$). In boys with 5-th stage of gonadal development a strong negative correlation was found between weight SDS score and free T ($r=-0.592$, $p<0.034$) and FAI ($r=-0.593$, $p<0.03$) Although expected we could not prove a correlation between the extent of adiposity and E2.

The same patient cohort was split into two groups based on how long the PG had been present: Group A consisted of boys who had developed PG within the last six months, while group B consisted of PG that had been present for more than a year. Age, age of PG development, mean length of PG before to investigations, anthropometric characteristics, and hormone levels were compared between the two groups (Table3). As there is a significant difference in age (more than 2 years) it is anticipated that there is a corresponding significant difference in height and levels of T, free T and FAI as well as gonadotropins (not shown). However, at an average age of 12.13 ±1.34 and 14.75 ±1.54, there is no difference in E2 levels and SHBG in boys with PG, which supports earlier findings about their involvement in the onset and/or persistence of PG.

**Discussion**

Adolescent boys with pubertal gynecomastia experience psychological discomfort, worry, and low self-esteem. The diagnostic process and available treatment options become considerably more challenging when obesity is present, as is frequently observed This article examines the sex hormone levels and ratios, free fractions of androgens, and the effects of SHBG and leptin on the development and persistence of PG, primarily in boys with obesity, which reflects the current milieu. Studies have shown that prevalence of GM is higher in adolescents with obesity. [9,25,26] A database was searched for young male "breast" specimens in a study carried out between 1997 and 2008. Based on BMI, 51% of the 69 patients were categorized with obesity, 16% as overweight, and 33% as normal weight. [26] The majority of the adolescents in our sample assessed at a tertiary pediatric endocrine facility were diagnosed also with obesity, which may possibly be explained by the requirement for a more thorough endocrine examination when two problems coexist. According to reports, 50–60% of teenagers have bilateral PG [33], however 90% of our cohort has bilateral PG. Breast pain or tenderness was reported in higher number of our patients 42.1 % vs. 30.2 % [34] all of them with recently developed PG, which is consistent with literature. In most of the studies the average age of PG development is 13-14 years and Tanner staging 3-4 according to gonadal size or pubic hair. [3,12] The average age in our study is earlier - 11.85 years with an average difference of 1.44 years between obese and non-obese males. Excessive adipose tissue (measured by BMI) may influence the age at which puberty begins and, in turn, cause pubertal phenomena like PG to emerge earlier due to their positive link with androgen levels at the onset of puberty. Some studies report that a family history for PG is a predisposing factor [33] and such data are confirmed in 24.21 % of our patients which is more than other reports. [34] The imbalance between levels of estrogen and androgen is considered a major cause for the development of pathologic GM as well as in physiologic periods although data are controversial. [12-17, 19] In a longitudinal study an increase of E2 was reported before the beginning of PG development, whereas T levels did not differ. [18] According to our research, samples from boys in the second gonadal stage showed a large rise in E2, a drop in the T/E2 ratio, and no difference in T, free T, or FAI when compared to controls. This outcome is consistent with prior research and is not seen in more advanced pubertal development. [11,19, 35] The results of the comparison between group A and group B confirm the impact of early E2 rise and lowered T/E2 ratio on PG development. We found no difference in the E2 level between these groups, which is moderately elevated for both based on reference values. However, the first group has significantly lower levels of T and free T, while the second group has significantly higher levels of androgens respectively there is a significant difference between T/E2 ratio which is much higher in the second one. So even moderate increase of E2 at the beginning of puberty, when not opposed by androgens may stimulate breast development in a susceptible cohort.

The hormones of group 2 manifested a distinct pattern. Boys with PG in this gonadal stage had significantly lower FAI compared to controls. FAI is calculated according to a formula which considers the level of SHBG-a glycoprotein that binds sex hormones with much more affinity to T. Levels of SHBG determine to great extent the levels free T and FAI so the higher SHBG levels, the lower the biologically active testosterone

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fraction. As PG may develop due to decreased levels/action of androgens we may speculate that the development/persistence of PG in that pubertal group may be explained by higher SHBG respectively lower FAI. In their study of healthy boys Kim et al. [36] found a significant decrease in the serum level of SHBG with the beginning of puberty accompanied by a rise in T level, which allows calculation of FAI and its use as a marker of pubertal development. In our investigation, SHBG significantly decreased as puberty progressed in the control group, but this decline did not occur in the PG group, not even between Tanner stages 2 and 5.(Fig. 2). Similar results were reported by Kilic et al. [15] They proved lack of corresponding increase in FAI in PG group with progression of puberty compared to control group, found also in our study. Furthermore, in the comparison of SHBG between groups A and B we received the same result - no difference of the level of SHBG between 12.13 ±1.34 years old boys with florid PG and the group consisting of 14.75 years old boys ±1.54 within fibrous phase of PG.
Dundar et al. [10] discovered a substantially greater level of leptin in the PG group when they compared the serum leptin levels of boys with PG, normal weight, and pubertal development 2-3 stage to a control group that was matched by age, anthropometric indices, and pubertal stage. As far as we are aware, this is the sole study on leptin in males with PG. Although leptin levels were higher in all PG groups in our cohort, the differences between each PG group and its corresponding control group were not statistically significant. However, since leptin receptors are present in breast epithelial cells and its effects on their proliferation, local aromatase activity, and estrogen receptor activity are well documented, leptin may play a local role in PG formation.
The study has several limitations. First it is a single center cross sectional study which makes it susceptible to some confounders. Second we cannot exclude the possibility that other androgens and estrogens might influence the development of PG. Third most of the patients, respectively controls were obese and this may have an impact on results. However, the study has some strengths. To our knowledge it is the first study that differentiates boys with PG according to their pubertal status and duration of GM and contributes to elucidation of hormonal milieu while confirming data that were previously reported by single studies.

**Conclusion**

In conclusion, our study shows that early rise of E2 and an altered T/E2 ratio at the beginning of puberty might be responsible for PG development. Additionally, lack of SHBG reduction with advancement of puberty and lower FAI might have an impact of PG development and/or persistence in mid-pubertal boys.

**Acknowledgement and funding sources:** A part of the research (investigation of serum leptin level) was funded by Medical University-Sofia by a Grant project № 8426/2016

**Conflicts of interest:** Both authors declare no conflicts of interest.

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Table 1. Anthropometric measurements of boys with PG and controls, divided by pubertal stage

| Group 1 | | | | Group 2 | | | Group3 | | |
|---|---|---|---|---|---|---|---|---|---|
| PG, N 39 | | **Control group, N 21** | **p-value** | **PG, N43** | **Control group, N 21** | **p-value** | **PG, N 13** | **Control group, N 23** | **p-value** |
| Height, cm | 156.52±6.27 | 152.78±7.8 | 0.062 | 171.0(157.7-189.0) | 172(144-185.9) | 0.227 | 183.5(160.0-194.0) | 177.34(164.7-189.0) | 0.046 |
| Height SDS | 1.05±0.98 | 0.68±1.44 | 0.273 | 1.34(1.27-3.1) | 0.83 (1.8-3.3 2) | 0.105 | 1.81(0-1.13-3.54) | 1.04(0.52-3.8) | 0.120 |
| Weight, Kg | 72.00±10.12 | 65.2±17.39 | 0.081 | 88.5(46.5-139.0) | 92.0 (32-125.8) | 0.180 | 101.5 (75.5-132.9) | 91.0(46.6-128.7) | 0.091 |
| Weight SDS | 3.55±1.4 | 2.66±2.06 | 0.069 | 3.90 (1.34-8.68) | 3.52 (2.01-6.86) | 0.132 | 4.45(1.3-11.1) | 3.40(1.33-9.33) | 0.082 |
| BMI, kg/m$^2$ | 29.27±3.79 | 27.72±5.68 | 0.242 | 31.16(17.28-48.55) | 30.62 (15.43-41.26) | 0.249 | 30.81(20.70-46.6) | 29.21 (17.20-41.87) | 0.200 |
| BMI SDS | 3.78±1.47 | 3.08±1.94 | 0.145 | 4.17(-1.42-9.68) | 3.93(1.6-8.04) | 0.358 | 4.21 (0.52-10.7) | 3.3(1.45-8.68) | 0.142 |

*PG- pubertal gynecomastia, ‡ SDS- standard deviation score, § BMI – Body mass index; Parametric values are presented as Mean± standard deviation, non-parametric values are presented as Median (minimal-maximal) values; Independent –Samples t-test and nonparametric Mann-Whitney test were used as appropriate.
P-values represent comparisons between boys with pubertal gynecomastia and their corresponding control group within each pubertal stage.

Table 2. Comparison between serum hormone values in PG boys and controls according to pubertal stage

| | **Group 1** | | | **Group 2** | | | **Group 3** | | |
|---|---|---|---|---|---|---|---|---|---|
| | **PG, N 39** | **Control group, N21** | **p-value** | **PG, N43** | **Control group, N21** | **p-value** | **PG, N 13** | **Control group, N 22** | p-value |
| **FSH,mIU/ml** | 1.96(0.19-9.24) | 1.28(0.83-5.0) | 0.229 | 3.59 (1.37-9.79) | 3.26(0.65-9.23) | 0.667 | 4.80(2.02-9) | 3.89(2.2-7.58) | 0.173 |
| **LH, mIU/ml** | 1.2 (0.1-7.6) | 1.45(0.13-3.93 | 0.969 | 3.68 (0.88-7.6) C | 1.97 (2.4-9.3) | 0.710 | 4.67(1.69-8.80) | 3.80(2.15-8.05) | 0.754 |
| **T, nmol/l** | 0.89 (0.69-5.86) | 2.13(0.69-6.21) | 0.085 | 6.89(2.74-22.30) C | 8.81(1.69-20.8) | 0.214 | 11.20 (4.96-28.9) | 11.20(5.97-22.70) | 1.000 |
| **$E_2$, pmol/l** | **120.0(73.4-240.0)** | **77.80 (23.2-120)** | **0.023** | 119.5 (73.4-247) | 109.0 (73.4-269) | 0.324 | 165.0 (80.0-359.0) | 153.5 (73.4-245.0) | 0.442 |
| **T/$E_2$** | **7.69 (3.12-37.80)** | **38.15(5.78-91.81)** | **0.016** | 64.25(15.96-331.06) | 84.51(6.13-283.38) | 0.233 | 64.0(34.8-206.41) | 79.11(29.65-243.87) | 0.130 |
| **SHBG, nmol/l** | **22.6(7.39-69.4)** | **27.0(25.4-40.4)** | **0.028** | **18.9(9.19-47.40)** | **14.40(7.27-76.4)** | **0.039** | 17.20(8.83-30.0) | 14.6(6.10-23.5) | 0.142 |
| **FreeT, pmol/l** | 17.10(11.2-142.0) | 43.0 (12.1-132.0) | 0.182 | 175(36.0-680.0) | 252.0(11.2-640.0) | 0.060 | 289.0(122-704) | 305.0 (172-731) | 0.593 |
| **FAI** | 3.40 (1.8-23.6) | 7.5(2.04-23.3) | 0.354 | **36.0(6.8-197.0)** | **64.0(1.79-140.1)** | **0.017** | 68.5 (21.0-140.0) | 76.5(36.0-267.0) | 0.218 |
| **Leptin, ng/ml** | PG (N=23) 22.58(9.31-35.65) | 20.21(2.04-35.69) | 0.272 | PG (N=18) 14.61(4.34-43.0) | 14.74(0.77-41.54) | 0.367 | PG(N=9) 17.22(0.60-28.51) | 10.23(1.36-24.82) | 0.415 |

*PG- pubertal gynecomastia, † C- controls, ‡ FSH –Follicle stimulating hormone, § LH –Luteinizing hormone; § T-Testosterone, || $E_2$ -Estradiol, ¶ SHBG –Sex hormone-binding globuline, ** FAI- Free androgen index; Non-parametric values are presented as Median (minimal-maximal) values; Mann-Whitney test was used.
P-values represent comparisons between boys with pubertal gynecomastia and their corresponding control group within each pubertal stage.

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| Table 3. Comparison between boys with recently developed PG (Group A) and boys with long –standing PG (Group B) | | | |
|---|---|---|---|
| | Group A, N=21 | Group B, N=57 | P |
| Age, y | 12.13 ±1.34 | 14.75±1.54 | **<0.001** |
| Age at PG development, y | 11.81±1.30 | 12.02±0.97 | 0.417 |
| Duration of PG, y | 0.31 ±0.15 | 2.66±1.07 | **<0.001** |
| Height, cm | 157.97 ±8.6 | 172.29 ±9.67 | **< 0.001** |
| Height SDS | 1.33±1.06 | 1.24±1.18 | 0.783 |
| Weight, kg | 100.9±12.56 | 106.81±14.75 | 0.108 |
| BMI kg/m$^2$ | 31.30±5.46 | 32.68±6.76 | 0.406 |
| BMI SDS | 4.69±2.35 | 4.53±2.57 | 0.796 |
| T, nmol/l | 1.82±2.80 | 8.05±2.16 | **<0.001** |
| $E_2$, pmol/l | 120 (73.40-470) | 132 (73.40-359.0) | 0.993 |
| T/$E_2$ | 7.29 (1.47-152.5) | 51.87(3.77-290.19) | **0.001** |
| SHBG, nmol/l | 22.6(7.12-69.40) | 17.55(7.39-47.40) | 0.165 |
| Free T, pmol/l | 17.10 (13.10-339.0) | 155.5(11.20-704) | **<0.001** |
| FAI | 3.85(2.32-87.0) | 37.0(1.80-197) | **<0.001** |
| Leptin, ng/ml | 26.87(4.34-43.0) | 17.79 (0.06-43) | 0.092 |
| *PG- pubertal gynecomastia, † C- controls, ‡ SDS- standard deviation score, § BMI – Body mass index; , ‡‡ FSH –Follicle stimulating hormone, ††LH –Luteinizing hormone; # T-Testosterone, \|\| $E_2$ -Estradiol, ¶ SHBG –Sex hormone-binding globuline, ** FAI- Free androgen index; Parametric values are presented as Mean± standard deviation, non-parametric values are presented as Median (minimal-maximal) values; Independent –Samples t-test and nonparametric Mann-Whitney test were used as appropriate | | | |

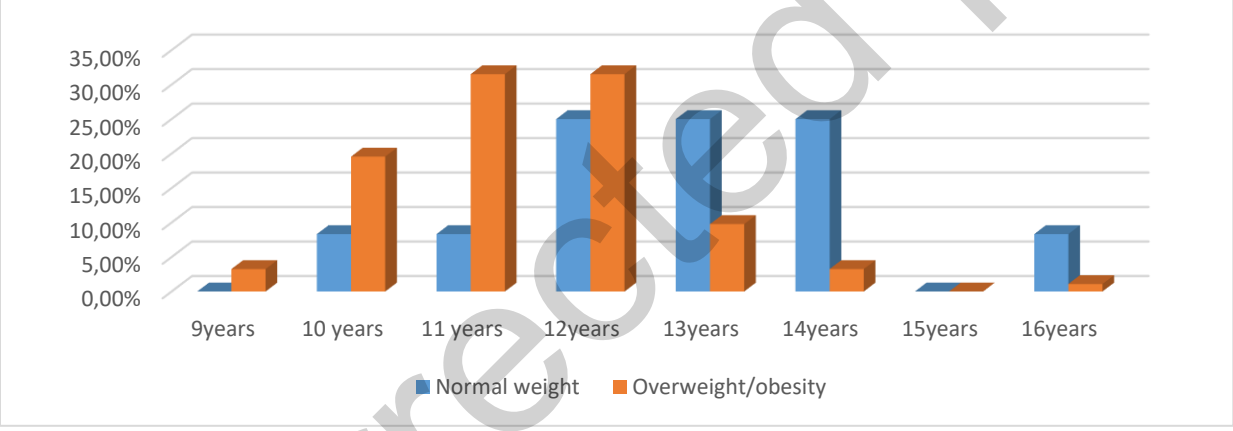


Figure 1. Age distribution of PG development in the groups with normal weight and overweight/obesity

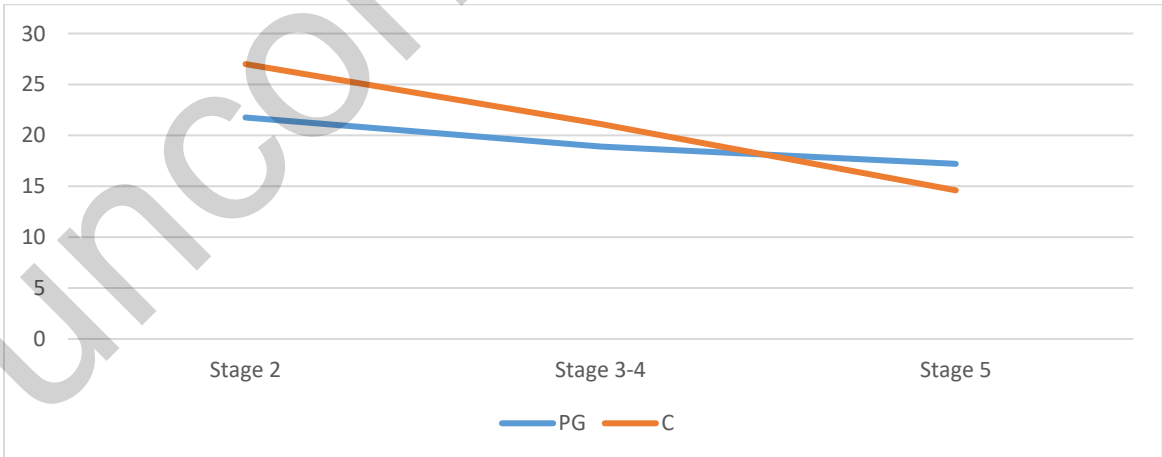


Figure 2. SHBG level change according to Tanner-Marshal stage of pubertal development in boys with PG and without.

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