Evaluation of Glucose Metabolism and Cardiovascular Risk Factors in Prepubertal Girls with Premature Pubarche

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What is already known on this topic?
Isolated premature pubarche (PP) is regarded as a warning sign of intrauterine-programmed metabolic syndrome.

What this study adds?
PP is not a risk factor alone for impaired glucose metabolism and insulin resistance in non-obese girls with normal birth weight before puberty.

Abstract
Objective: Premature pubarche (PP) is a risk factor for metabolic syndrome (MS). The aim was to evaluate if glucose-insulin metabolism, cardiovascular risk factors, familial cardiovascular risk factors (FCVRF) created a risk for insulin resistance (IR) and if PP was a risk factor alone for MS in normal weight prepubertal girls with PP.

Methods: Thirty-five prepubertal, non-obese girls with PP with normal birth weight and 35 age-matched control girls were evaluated for FCVRF, anthropometric measurements, blood pressure, lipid profile, fasting blood glucose-insulin, hemoglobin A1c (HbA1c), sex hormone binding globulin (SHBG), leptin, adiponectin, tumor necrosis factor-alpha (TNF-α), androgen levels, and bone age. Oral glucose tolerance test was performed in PP participants. Homeostasis model of assessment of IR (HOMA-IR), fasting glucose/insulin ratio, atherogenic index (AI), and free androgen index (FAI) were calculated. PP participants were further stratified by FCVRF.

Results: HbA1c, lipid profile, testosterone, leptin, adiponectin, TNF-α, HOMA-IR, glucose/insulin ratio, AI, and fasting glucose-insulin levels were similar. In the PP group FAI was significantly higher \((p=0.001)\), whereas SHBG was significantly lower \((p=0.010)\) than the control group. Leptin levels of FCVRF- and FCVRF+ subgroups were 15.2 ± 9.1 and 9.7 ± 7.2 ng/mL, respectively, and the difference was significant \((p=0.016)\).

Conclusion: As PP does not appear to be a risk factor alone for impaired glucose metabolism and IR in prepubertal non-obese girls with normal birth weight, it is our opinion that it is unnecessary to examine in detail such cases before puberty. Low SHBG levels in the PP group and high leptin levels in FCVRF+ subgroup might suggest that these may be predictive for MS in the future.

Keywords: Premature pubarche, prepubertal, leptin, glucose metabolism, adipocytokines, cardiovascular risk

Introduction
Puberty as a result of adrenal gland activity, known as adrenarche, is characterized by an increase in circulating androgen precursors, dehydroepiandrosterone (DHEA) and its sulfate ester (DHEA-S). Adrenarche is usually accompanied by pubarche; genital and/or axillary hair. If this situation occurs before the age of eight years in girls and before the age of nine years in boys, it is called, “premature pubarche” (PP’) (1). The incidence of PP, depending on societal and racial factors, varies between 5-8.6% (2). In a study conducted in Turkey, the incidence of PP was reported to be 4.6% (3). The female/male ratio in PP is approximately
10/1 (4). Prematurity, intrauterine growth retardation, being overweight or obese predispose to PP (1,2,3,4,5,6).

Even though isolated (normal variant) PP is a benign condition, PP is currently accepted as a warning sign for intrauterine-programmed metabolic syndrome (MS), especially in obese children with a diagnosis of PP. Early diagnosis of MS and its components is important for detection, prevention, and early treatment because patients with MS are at risk of type 2 diabetes mellitus and cardiovascular diseases (5,7,8,9).

It has been shown that isolated PP predisposes to hyperinsulinemia and insulin resistance (IR), not only in obese children, but also in non-obese children. In these cases, low sex hormone binding globulin (SHBG) levels have been shown to be a useful marker for detecting IR (5). It has also been reported that the risk of MS is increased within this group and increased tumor necrosis factor-α (TNF-α) levels may be a predictive marker for MS (10). When compared to healthy controls, SHBG and insulin-like growth factor binding protein-1 have been found to be lower, whereas insulin-like growth factor-1 (IGF-1), TNF-α, adiponectin and insulin levels have been found to be higher (10,11). It is speculated that in these cases, hyperinsulinemia also causes dyslipidemia, and as a result, at an early age, cardiovascular risk begins to increase (5). Family history of type 2 diabetes mellitus and cardiovascular disease has been reported to be more frequent in girls with PP (12). In the light of this evidence, the aim of this study was to evaluate if glucose and insulin metabolism, cardiovascular risk factors, and familial cardiovascular risk factors (FCVRF) are associated with the risk of IR in normal-weight, prepubertal PP girls.

Methods

The patient group consisted of non-obese girls having no breast development and birth weights appropriate for gestational age (AGA), who were in follow-up with the diagnosis of PP from the outpatient clinic of Trakya University Medical Faculty Hospital, Department of Pediatric Endocrinology. Patients with breast development, chronic illnesses, any kind of drug use, congenital adrenal hyperplasia or virilizing tumor were excluded. An equal number of girls, born AGA, and who were non-obese and prepubertal were included as the control group. The patients and the families of the participants were informed about the study and signed informed consent forms were obtained from the mother or father of the participant and the child before they were recruited to the study. The study was conducted after approval was given by the Ethics Committee of Trakya University Faculty of Medicine (TÜBF-TÜBAPK date: 11.06.2013, approval no: 121).

Participants’ data, including birth date, gestational age, birth weight, current height and weight measurements, waist-hip circumference measurements, and blood pressure (BP) measurements, and pubertal examination based on Tanner staging, were obtained and recorded. The presence or absence of cardiovascular risk factors, including type 2 diabetes mellitus, polycystic ovary syndrome, MS, dyslipidemia and familial cardiovascular disease (premature atherothrombotic cardiovascular disease, occurring in a first-degree male relative before 55 years of age or in a first-degree female relative before 65 years of age) were recorded (13). Height, weight measurements, and the body mass index (BMI) values and their standard deviation (SD) scores (SDS) were calculated using reference values for Turkish children (14).

Two mL of blood were drawn from all participants at 08.30 in the morning after 10 hours of fasting, and DHEA-S, androstenedione (AS), 17-hydroxyprogesterone (17-OHP), total testosterone (T), lipid profile [high density lipoprotein (HDL), low density lipoprotein (LDL), total cholesterol, triglyceride (TG)], hemoglobin A1c (HbA1c), SHBG, TNF-α, leptin and adiponectin levels were measured. Free androgen index (FAI), defined as total T/SHBG, and atherogenic index (AI) defined as TG/HDL, were calculated. Oral glucose tolerance test (OGTT) (with 1.75 g/kg glucose, maximum of 75 g glucose) was performed in participants with PP, again at 8.30 in the morning after 10 hours of fasting and blood glucose and insulin values were obtained at baseline (0 minutes) and two hours (120 minutes). From the OGTT results, patients with hyperinsulinemia (those with fasting insulin >15 mIU/mL and/or 120th minute insulin level >75 mIU/mL) and IR (homeostasis model of assessment (HOMA-IR) >2.5) were identified. HOMA-IR was calculated using the standard formula: fasting blood glucose (mmol/L) x 18 x insulin (mIU/L)/22.5 and/or fasting glucose/insulin ratio <7 (15). For the determination of bone age (BA), a radiographic examination of the left wrist was completed for each participant, and BA was assessed by comparison with the Greulich-Pyle radiological atlas. BA SDS were calculated using the programme BoneXpert V3.1 (16). The study group was divided into two subgroups based on the presence (FCVRF+) or absence (FCVRF-) of FCVRF.

Blood glucose, LDL, HDL, TG, and total cholesterol levels were measured by spectrophotometry on an Advia 1800 device; insulin, DHEA-S, and T levels were measured by microparticle chemical immunoassay on an Abbott Architect i2000SR and leptin, adiponectin, and TNF-α levels were measured by immunoassay, also on the Architect i2000SR. SHBG and
AS levels were measured by immunoassay on the Siemens Immulite 2000 (Siemens Healthcare Diagnostics Inc. Flanders, HbA1c level was determined by high performance ion-exchange liquid chromatography using Adams HA-8180V analyzer (Arkay Factory Inc., Shiga, Japan) and 17-
OHP levels were measured by radioimmunoassay on the gamma counter DPC Gambyt CR (DPC Diagnostic Products Corporation, Los Angeles, CA, USA) at the department of nuclear medicine. Left hand wrist radiographs were taken with the Philips Optimus Bucky Diagnost TS device.

Statistical Analysis

Normality of the distribution of the parameters was analyzed with Shapiro-Wilk test. Logarithmic or square root transformations were applied to non-normally distributed parameters to obtain normal distribution. Independent samples t-test was used for two group comparisons. All the results except for BA SDS were expressed as mean ± SD also for the data before the transformation was applied for easy understanding clinically. BA SDS data was expressed as median (minimum-maximum) because of normal distribution could not be obtained. Mann-Whitney U test was used for the comparison of BA SDS between two groups. All statistical analysis were performed by SPSS 20 statistical software (IBM Corp., Armonk, NY, USA). The value p < 0.05 was accepted as statistically significant.

Results

There were 35 patients in the PP group and 35 healthy girls in the control group. The mean ages of the PP and control groups at the time of the study were 8.3 ± 1.1 and 8.1 ± 1 years, respectively. Birth weights, gestational weeks, presence of FCVRFs, weight SDSs, height SDSs, waist/hip ratios, BA SDSs and BP values of the groups were similar. Although, the mean BMI SDS values were within normal ranges in both groups, they were significantly higher in the PP group than in the control group (Table 1).

There was no difference between the groups in terms of HbA1c level, lipid profiles, HOMA-IR scores, glucose/insulin ratios and AIs. In the PP group, serum DHEA-S, AS, and 17-
OHP levels were significantly higher, whereas T levels were similar. Serum leptin, adiponectin, and TNF-α levels were similar in both groups. Fasting and 120th minute glucose and insulin levels of the PP participants and the fasting glucose and insulin levels of the control group participants were within normal limits, and there was no significant difference between the groups in terms of fasting glucose and insulin levels. FAI was significantly higher and SHBG was significantly lower in the PP group. Laboratory findings of the groups are shown in Table 2.

When the PP group was divided by the presence of FCVRF, the clinical and laboratory findings, except for leptin levels, were similar. The FCVRF+ PP group had significantly lower leptin levels than in FCVRF- PP group (p = 0.016). Comparisons of the clinical and laboratory findings of the PP sub-groups, stratified by the presence of FCVRF, are shown in Tables 3 and 4.

Discussion

Regardless of age and developmental stage, nutritional status is a physiological regulator of adrenarche. Starting from the age of five, an increase in fat tissue, insulin and IGF-1 levels associated with an increase in BMI enhances the expression of steroidogenic enzymes and the production of androgens in adrenocortical cells (17). Although, weight SDS, height SDS, and BMI SDS were within normal limits in our study, it was within normal limits for age and pubertal stage. As suggested by Sopher et al. (22), in our study when BMI was within normal limits, PP alone does not appear to be a factor that increased BA, despite hyperandrogenemia. Systolic and diastolic BP values are components of the MS criteria and are significant in cases with PP. In some studies, in which PP and healthy control participants were compared (10,18,19,23,24), blood pressure values were found to be within normal limits, similar to our study. Based on this evidence, it has been suggested that PP alone does not affect BP, if there is no increase in fat tissue, resulting in obesity or overweight in prepubertal period.

In the PP group, androgen precursors other than for T were significantly higher than in the control group. However, TNF-α, leptin, and adiponectin levels, all adipocytokines, were found to be similar in both groups. When glucose metabolism, another component of the MS, was evaluated
Table 1. Clinical findings of PP and control cases

| Clinical findings       | PP (n = 35) | Control (n = 35) | p     |
|-------------------------|-------------|------------------|-------|
| Mean ± SD               | Mean ± SD   |                  |       |
| Birth weight (g)        | 3230±405.9  | 3290.2±371.7     | NS    |
| Gestation week (week)   | 39.4±0.9    | 39.4±0.8         | NS    |
| Family                  | n (%)       | n (%)            |       |
| FCVRF+                  | 16 (45.7)   | 14 (40)          | NS    |
| FCVRF-                  | 19 (54.3)   | 21 (60)          | NS    |
| At the time of study    | Mean ± SD   | Mean ± SD        |       |
| Age (year)              | 8.3 ± 1.1   | 8.1 ± 1          | NS    |
| Weight SDS              | 0.56 ± 0.97 | 0.15 ± 0.95      | NS    |
| Height SDS              | 0.92 ± 1.1  | 0.65 ± 1.23      | NS    |
| BMI SDS                 | 0.34 ± 0.83 | -0.04 ± 0.78     | 0.026 |
| Waist/Hip ratio         | 0.86 ± 0.04 | 0.85 ± 0.04      | NS    |
| Systolic BP (mmHg)      | 93 ± 3.47   | 93.14 ± 3.44     | NS    |
| Diastolic BP (mmHg)     | 63.29 ± 4.36| 63.71 ± 3.5      | NS    |
| BA SDS                  | -0.40 (-2.5-2.1) | -0.90 (-3.8-2.3) | NS    |

PP: premature pubarche, SDS: standard deviation score, FCVRF: familial cardiovascular risk factor, BMI: body mass index, BA: bone age, BP: blood pressure, NS: not significant

Table 2. Laboratory findings of PP and control cases

| Laboratory findings                         | PP (n = 35) | Control (n = 35) | p     |
|---------------------------------------------|-------------|------------------|-------|
| Mean ± SD                                   | Mean ± SD   |                  |       |
| Adipocytokine and adipokines                |             |                  |       |
| TNF-α (pg/mL)                              | 1.95±0.84   | 1.81±0.93        | NS    |
| Leptin (ng/mL)                             | 12.27±8.53  | 13.33±13.03      | NS    |
| Adiponectin (µg/mL)                        | 14.06±5.42  | 15.81±11.69      | NS    |
| Fasting lipid profile (mg/dL)               |             |                  |       |
| Cholesterol                                | 152.3±26.6  | 155.4±29.4       | NS    |
| Triglyceride                               | 65.8±31     | 67.7±27.6        | NS    |
| LDL                                        | 90.2±25.8   | 93.14±3.44       | NS    |
| HDL                                        | 58±12.4     | 57.9±11.3        | NS    |
| OGGTT                                      |             |                  |       |
| Glucose 0.min                               | 79.7±8.2    | 80.6±7.6         | NS    |
| 120.min                                    | 97±15       | -                | -     |
| Insulin 0.min                               | 6.6±2.9     | 7.3±4.1          | NS    |
| 120.min                                    | 31.6±18     | -                | -     |
| HbA1c (%)                                  | 5.1±0.3     | 5.1±0.2          | NS    |
| HOMA-IR                                    | 1.2±0.6     | 1.4±0.8          | NS    |
| Fasting glucose/insulin ratio               | 15.2±8.6    | 14.7±8.8         | NS    |
| SHBG (nmol/L)                              | 52.9±24.2   | 72.9±38.2        | 0.010 |
| AI                                         | 1.2±0.69    | 1.22±0.62        | NS    |
| FAI                                        | 1.15±0.62   | 0.85±0.85        | 0.001 |

PP: premature pubarche, SDS: standard deviation score, TNF-α: tumor necrotizing factor-α, LDL: low density lipoprotein, HDL: high density lipoprotein, OGGTT: oral glucose tolerance test, HbA1c: hemoglobin A1c, HOMA-IR: homeostasis model of assessment-insulin resistance, SHBG: sex hormone binding globulin, AI: atherogenic indeks, FAI: free androgen index, NS: not significant
in PP participants, in many studies fasting glucose values (24,25,26,27,28), fasting insulin values, glucose/insulin ratio, HOMA-IR, postprandial glucose-insulin values (12,25,29,30) and HbA1c values (19) were within normal limits, and we obtained similar results. Liimatta et al. (31) evaluated patients diagnosed with PP twice, both at an average age of seven years and at an average age of 18 years. These authors showed that the factor affecting glucose metabolism was not the risk factors present in the prepubertal period, but the adipose tissue present in the pubertal stage. However, in some studies, fasting insulin levels and HOMA-IR were reported to be high and glucose/insulin ratio was reported to

### Table 3. Comparison of clinical findings of the PP cases regarding the presence of FCVRF

| Clinical findings       | FCVRF+ (n = 16) Mean ± SD | FCVRF- (n = 19) Mean ± SD | p     |
|-------------------------|---------------------------|---------------------------|-------|
| Birth weight (g)        | 3300 ± 445.5              | 3171 ± 371.1              | 0.357 |
| Gestation week (week)   | 39.5 ± 0.8                | 39.3 ± 1                  | 0.541 |
| Diagnosis age (year)    | 7.2 ± 0.7                 | 7.1 ± 0.9                 | 0.817 |
| **At the time of the study** |                           |                           |       |
| Age (year)              | 8.3 ± 1.1                 | 8.3 ± 1.1                 | 0.960 |
| Weight SDS              | 0.9 ± 0.81                | 0.28 ± 1.02               | 0.069 |
| Height SDS              | 1.3 ± 1.22                | 0.59 ± 0.92               | 0.085 |
| BMI SDS                 | 0.61 ± 0.5                | 0.12 ± 0.99               | 0.159 |
| BA SDS                  | 0.15 ± 1.24               | -0.52 ± 1.33              | 0.123 |
| Waist/hip ratio         | 0.87 ± 0.48               | 0.86 ± 0.03               | 0.228 |
| Systolic BP (mmHg)      | 92.8 ± 3.6                | 93.1 ± 3.4                | 0.774 |
| Diastolic BP (mmHg)     | 62.5 ± 4.4                | 63.9 ± 4.2                | 0.336 |

**SDS**: standard deviation score, **FCVRF**: familial cardiovascular risk factor, **BMI**: body mass index, **BA**: bone age, **BP**: blood pressure, **PP**: premature pubarche

### Table 4. Comparison of laboratory findings of the PP cases regarding the presence of FCVRF

| Laboratory findings        | FCVRF+ (n = 16) Mean ± SD | FCVRF- (n = 19) Mean ± SD | p       |
|----------------------------|---------------------------|---------------------------|---------|
| DHEA-S (µg/dL)             | 124.9 ± 54.2              | 109.6 ± 48.4              | 0.329   |
| 17-OHP (ng/mL)             | 0.81 ± 0.3                | 0.96 ± 0.28               | 0.120   |
| AS (ng/mL)                 | 0.75 ± 0.37               | 0.7 ± 0.41                | 0.733   |
| T (ng/dL)                  | 16.3 ± 8.5                | 14.2 ± 3.5                | 0.341   |
| SHBG (nmol/L)              | 47.7 ± 16.6               | 57.4 ± 28.8               | 0.512   |
| TNF-α (pg/mL)              | 2.07 ± 0.78               | 1.84 ± 0.9                | 0.415   |
| Leptin (ng/mL)             | 15.2 ± 9.1                | 9.7 ± 7.2                 | 0.016   |
| Adiponectin (µg/mL)        | 13.4 ± 5                  | 14.5 ± 5.7                | 0.596   |
| Cholesterol (mg/dL)        | 154.7 ± 26.8              | 150.3 ± 26.9              | 0.635   |
| Triglyceride (mg/dL)       | 71.8 ± 35                 | 60.6 ± 27.2               | 0.267   |
| LDL (mg/dL)                | 97.7 ± 27.1               | 83.9 ± 23.6               | 0.117   |
| HDL (mg/dL)                | 54.8 ± 11.5               | 60.6 ± 12.9               | 0.174   |
| Fasting glucose (mg/dL)    | 79.6 ± 9.5                | 79.8 ± 7.3                | 0.765   |
| 120. min blood glucose (mg/dL) | 96.5 ± 14.4            | 97.4 ± 15.9               | 0.778   |
| Fasting insulin (µU/mL)    | 7.1 ± 3.3                 | 6.1 ± 2.6                 | 0.312   |
| 120. min blood insulin (µU/mL) | 32.4 ± 14.8         | 30.9 ± 20.8               | 0.371   |
| HbA1c (%)                  | 5 ± 0.2                   | 5.1 ± 0.3                 | 0.435   |
| HOMA-IR                    | 1.39 ± 0.72               | 1.21 ± 0.58               | 0.508   |
| Fasting glucose/insulin ratio | 14.7 ± 9.9                | 15.7 ± 7.5                | 0.312   |
| AI                         | 1.58 ± 0.82               | 1.05 ± 0.53               | 0.208   |
| FAI                        | 1.54 ± 0.77               | 0.99 ± 0.41               | 0.085   |

**SD**: standard deviation, **FCVRF**: familial cardiovascular risk factor, **DHEA-S**: dehydroepiandrosterone-sulphate, **17-OHP**: 17-hydroxyprogesterone, **AS**: androstenodione, **T**: total testosterone, **SHBG**: sex hormone binding globulin, **TNF-α**: tumor necrotizing factor-α, **LDL**: low density lipoprotein, **HDL**: high density lipoprotein, **HbA1c**: hemoglobin A1c, **HOMA-IR**: homeostasis model of assessment-insulin resistance, **AI**: atherogenic indeks, **FAI**: free androgen index, **PP**: premature pubarche
be low in children with PP (26,32). The reason for impaired glucose metabolism and IR in these studies might possibly be related to the pubertal status of the cases. In our study, it was found that glucose, insulin, and HOMA-IR levels were similar between the PP and the control groups, and there was no impairment in glucose metabolism in the PP group, which could be due to the participants not being overweight or obese, participants being prepubertal and thus not yet experiencing any increase in adiposity. Thus we suggest that isolated PP in the prepubertal period might not pose a risk in terms of MS.

Dyslipidemia is an important biochemical disorder that causes cardiovascular risk to begin at an early age. In PP cases, some studies reported deterioration in lipid profile including an increase in total cholesterol, TG, LDL and decrease in HDL (18,25), whereas other studies reported the absence of dyslipidemia and the lipid profile to be within normal limits (19,29,32). In our study, lipid profile and AI were within normal limits in both groups. In studies in which dyslipidemia was detected, PP subjects were pubertal, and who were also obese and had hyperinsulinemia (18,25). It is possible that the reason lipid profiles were within normal limits, both in the PP group and in the control group in our study, was that the PP subjects were both prepubertal and of normal weight. We therefore speculate that isolated PP in the prepubertal period does not pose a strong risk for the development of dyslipidemia.

In our study, SHBG levels in the PP group were significantly lower than in the control group, and this finding was similar to other published studies (18,19,26,32). FAI was significantly higher in the PP group when compared to the control group. Although, this finding was similar to some studies (18,26,32), some of these studies included pubertal participants (26,32), while some of the others included obese PP participants, who were prepubertal. Ibáñez et al. (18) suggested that elevated FAI in prepubertal participants was a result of hyperinsulinemia. In our study, even though the participants in the PP group were prepubertal, non-obese and had normal birth weight, FAI was significantly elevated. This suggests that some mechanism(s) other than puberty, an increase in adipose tissue and hyperinsulinemia might be responsible for the occurrence of low SHBG and elevated FAI. One of these mechanisms may be intrauterine programming or genetic polymorphisms and other genetic variations that could affect enzyme expression, which plays a role in SHBG production in the liver. In order to test this hypothesis, studies with large numbers of participants, genetic studies and long-term follow-up are needed.

It has been shown that TNF-α, an adipocytokine, is a predictive marker for type 2 diabetes mellitus and coronary heart disease (33). Mathew et al. (10) reported that BP and TNF-α levels were high in both normal weight and overweight PP cases, and that this adipocytokine might be an important factor in the development of hypertension, a component of MS. In another study (32), it was shown that TNF-α levels in PP participants were similar to the control group. In our study, TNF-α level was found to be similar in both the PP group and the control group, and this similarity was primarily attributed to the presence of similar weight SDS and similar insulin levels in both groups. Leptin, also an adipokine, reflects the nutritional status of the body. Teixeira et al. (29) found that serum leptin levels were normal in PP cases with normal BMI and elevated in overweight cases. This finding led them to suggest that an increase in leptin level was not associated with PP but was directly related to increased BMI. Corvalán et al. (34) showed that obesity in 7-year-old children with normal birth weight was directly related to DHEA-S levels, whereas insulin, IGF-1 and leptin levels were only weakly associated with high DHEA-S, but strongly associated with obesity. In our study, serum leptin levels in PP participants were similar to the control group and this finding was attributed to the normal BMI SDS values of PP participants. Once again, we suggest that this is evidence that isolated PP does not lead directly to an elevation in serum leptin levels. It has been shown that adiponectin, another adipokine, increases insulin sensitivity and has a lower level in babies born small for gestational age (SGA) (8,35). Unlike in some studies (28,36), in our study serum adiponectin levels were normal in PP participants. Larqué et al. (28) found that postprandial adiponectin levels were lower in cases with PP when compared to the control group, yet they did not present birth weight data for their cases. Nieuwenhuis et al. (36) reported that adiponectin triggers puberty earlier in obese children. The serum adiponectin levels of the PP participants being similar to the control group in our study might be due to the fact that the study participants had normal birth weight, were non-obese and prepubertal without hyperinsulinemia.

A family history of cardiovascular disease is important for the development of MS, and it has been reported that it is more common in patients with PP than in healthy children (12). There are studies reporting the incidence of FCVRF in PP cases varies greatly, from 31.8 % (23) to as high as 92.5% (25). In our study, there was no significant difference in the proportion reporting FCVRF between the PP group (45.7 %) and the healthy control group (40 %). When the PP group was divided into two subgroups, based on the presence or absence of FCVRF, it was found that mean leptin levels in the FCVRF+ subgroup were significantly higher than in the FCVRF- subgroup. The present study is the first published to divide and compare PP cases according to FCVRF history in
terms of clinical, biochemical, hormonal and adipocytokine statuses. The high serum leptin levels in the FCVRF+ PP group suggested that high serum leptin levels might be a predictive criterion for the development of obesity, MS or cardiovascular disease in the future. Once again, to test this hypothesis, studies with large numbers of participants and long-term follow-up are needed.

This study was conducted only in non-obese and prepubertal girls with normal birth weight, excluding SGA birth, puberty and obesity, which pose risks for the development of MS, independent of PP. The results appear to show that the prepubertal period in these PP patients does not pose a risk in terms of IR and cardiovascular risk.

Study Limitations
This study had several limitations. The sample size was small. OGTT was not performed in the control group and glucose metabolism was evaluated only with fasting glucose and insulin. Low SHBG levels and high leptin levels were found to be predictive factors but to support these findings long term follow up would be needed.

Conclusion
In conclusion, PP alone does not appear to be a risk factor for impaired glucose metabolism and IR in non-obese girls with PP with normal birth weight and before puberty. Additionally, low SHBG levels can be a predictive marker of hyperandrogenism in prepubertal girls with PP and high leptin levels in the FCVR+ subgroup may be a marker of future obesity risk but long term follow up studies are needed to test these hypotheses.

Ethics
Ethics Committee Approval: The ethics committee approval was obtained from Ethics Committee of Trakya University Faculty of Medicine (TÜF-TÜBAPK date: 11.06.2013, approval no: 121).

Informed Consent: Informed consent was obtained from all parents/guardians and children included in this study.

Peer-review: Externally peer-reviewed.

Authorship Contributions
Concept: Diğdem Bezen, Filiz Tütüncü Kökenli, Design: Diğdem Bezen, Filiz Tütüncü Kökenli, Data Collection or Processing: Diğdem Bezen, Analysis or Interpretation: Diğdem Bezen, Filiz Tütüncü Kökenli, Emine Dilek, Didem Ağ Seleci, Hakan Erbaş, Literature Search: Diğdem Bezen, Filiz Tütüncü Kökenli, Writing: Diğdem Bezen.

Financial Disclosure: This study was supported by a project grant from the Trakya University Scientific Research Project Institution (TÜBAP date: 11.06.2013, approval no: 121).

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