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# Sex hormones and metabolic syndrome in children and adolescents

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**Abstract**

Cardiovascular risk starts early in life, yet the patterns of changes in metabolic syndrome (MS) during puberty and normal development have not been completely defined. Sex hormones are shown to play a pivotal role in the modulation of insulin resistance and MS. Our aim is to clarify the relation between sex hormones and MS in normal children and adolescents. This is a cross-sectional study of 365 (8-12 and 14-18 years old) school students. We analyzed the associations of sex hormones (testosterone, free androgen index, estradiol, free estradiol index [FEI], and sex hormone–binding globulin [SHBG]) with cardiovascular risk factors and MS. Prevalence of MS varied depending on the definition, and 33 (9%) students had MS based on at least 1 definition of MS. Frequency of MS doubled among 14- to 18-year–old adolescents compared with 8- to 12-year–old children (12.4% vs 5.6%, $P = .02$). Adolescent boys and girls with MS had significantly lower SHBG levels compared with controls. Adolescent boys with MS also had significantly higher FEI levels compared with controls. Logistic regression analysis was performed to find the predictors of MS. Among covariates of age, estradiol, testosterone, free androgen index, and FEI, SHBG was the only significant predictor of MS ($B = -0.3$, odds ratio = 0.8, 95% confidence interval for odds ratio are 0.64 and 0.92, $P = .005$, Nagelkarke $R^2 = 0.48$) in adolescent boys. In conclusion, sex hormone levels and androgen/estrogen balance may play an important role in determining MS and future cardiovascular risk among children and adolescents.


## 1. Introduction

Insulin resistance is associated with obesity, glucose intolerance or diabetes, hypertension, dyslipidemia, and cardiovascular disease. The constellation of these risk factors is called *metabolic syndrome* (MS) [1-3]. The National Cholesterol Education Program (NCEP) Adult Treatment Panel III [1], the World Health Organization (WHO) [2], and the International Diabetes Federation (IDF) [3] define MS in adults. These definitions carry certain differences; the NCEP definition is based on a number of risk factors, among which are abdominal obesity, elevated triglycerides (TG) (>150 mg/dL), low high-density lipoprotein cholesterol (HDL-C) (<40 mg/dL in men and <50 mg/dL in women), elevated blood pressure (>130/85 mm Hg), and elevated fasting glucose (>110 mg/dL) [1]. On the other hand, insulin resistance, hyperglycemia, and known diabetes are central components of the WHO definition [2]. The IDF was the first major organization to present a definition for children and adolescents [3,4]. The IDF definition considers central obesity as the prerequisite; and it has broader criteria for waist circumference, HDL-C, and fasting plasma glucose [3]. Increased waist circumference (>90th percentile) is a prerequisite for the IDF definition [4].

There is increasing evidence that MS starts early in life [5], yet the patterns of changes in insulin resistance and cardiovascular risk during puberty and normal development have not been completely defined. A rising level of estrogen for girls and a sharp increase in androgen for boys set the puberty process in motion [6]. Whereas prepubertal children are sensitive to insulin, adolescents become insulin resistant compared with prepubertal children [7]. In men, low testosterone and sex hormone–binding globulin (SHBG) levels were strongly associated with MS and its components [8]. On the other hand, in postmenopausal women not taking hormone replacement therapy, high levels of free androgen index (FAI) were associated with increased risk of MS [9]. High levels of FAI were also observed in premenopausal women with MS [10]. On the other hand, limited data exist in

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children and adolescents. Similar to pre- and postmenopausal women, testosterone increases the risk of MS in adolescent girls [11]. The aim of this study is to test the hypothesis that alterations in the androgen/estrogen balance might associate with MS among children and adolescents.

## 2. Methods

### 2.1. Study population and data collection

The study sample is derived from a cross-sectional survey on the prevalence of cardiovascular risk factors in a representative sample of school children in Istanbul, Turkey. Five different state elementary and secondary schools were selected. Students who did not want to participate in the survey were excluded (around 10% for elementary school children and 20% for secondary school students). The study has been conducted in 2007. Subjects were instructed to fast for at least 12 hours before the screening. Their compliance was ascertained by an interview on the day of examination. The fasting status was based on self-report. Blood samples were drawn at 9:00 AM, and screening took place at schools during normal school hours. Participating students were asked about second-hand smoke exposure and the presence cardiovascular disease in the family.

The Institutional Review Board and the Educational Board approved the study protocol. Informed consent was obtained from parents or guardians. Subjects were given case numbers, and identities were kept confidential. At least 1 parent signed an informed consent for participation after having read an explanatory note.

### 2.2. Anthropometric measurements

History and physical examination were performed including anthropometric measurements of weight, height, blood pressure, skin fold thickness, waist and hip circumference, and arm span. Standardized protocols were used by the trained examiners. The weight of children wearing minimal clothing was measured to the nearest 0.1 kg with a portable electronic scale. Each time it was moved, the scale was recalibrated and standardized. Height was measured with a fiberglass tape. Body mass index (BMI) was calculated as weight (in kilograms)/height (in meters)$^2$. Body circumferences were measured with subjects in the standing position. Hip and waist (just above the iliac crest) circumferences and arm span were measured to the nearest 0.1 cm. Using the tables provided by the waist circumference percentiles in a nationally representative sample, we determined subjects with increased waist circumference (>90th percentile) [12]. Biceps skin fold thickness was measured to the nearest 1.0 mm with a Holtain caliper. Body proportions normally change during pubertal development and may vary among persons of different race and ethnic groups. Age- and sex-specific cutoff points of BMI were used to assess the overweight and obesity status. These cutoff points of BMI were developed and published from the centile curves of an international reference population [13].

### 2.3. Blood pressure

Blood pressure was measured by automatic blood pressure monitor (Omron, Bannockburn, IL). Blood pressure was measured 3 times while the subjects were seated, and the last 2 measurements were averaged for analysis. Small and medium cuffs were used for arm circumferences of less than 22 and 22 to 32 cm, respectively. To find the age-specific height percentile level for each case, we used the growth curves drawn for healthy Turkish children [14]. Using the tables provided by the Task Force Report on High Blood Pressure in Children and Adolescents, we determined children and adolescents with elevated blood pressure (≥95th percentile) [15].

### 2.4. Definitions of MS in children and adolescents

In our study, the MS criteria in children and adolescents were modified from those of the NCEP Adult Treatment Panel [1]. As age- and sex-specific lipid percentiles are not available in Turkish children, we used the National Heart Lung and Blood Institute Growth and Health Study (NGHS) as the reference population [16]. A TG level of at least the 90th percentile or an HDL-C level not exceeding the 10th percentile was considered as a risk determinant of MS.

Subjects with 3 or more of the following 5 criteria were considered to have MS:

1. Elevated systolic and/or diastolic blood pressure (>95th percentile) [15]
2. BMI level indicating overweight or obesity [13]
3. Elevated TG (>90th percentile level) [16]
4. Low HDL-C (<10th percentile level) [16]
5. Impaired fasting glucose (>100 mg/dL) [17]

We also examined the prevalence of MS based on the adapted WHO and IDF definitions for children and adolescents [2-4]. Impaired fasting glucose or insulin resistance (assessed by homeostasis model assessment of insulin resistance [HOMA-IR]) was a prerequisite for WHO definition. *Impaired fasting glucose* is defined as greater than 100 mg/dL [17]. *Hyperinsulinemia* was arbitrarily defined as fasting value greater than 18 $\mu$U/mL, a value considered indicative of insulin resistance in normoglycemic subjects [18]. Homeostasis model assessment–estimated insulin resistance is calculated using the formula glucose (in millimoles per liter) × fasting insulin (in microunits per milliliter)/22.5. The HOMA-IR has been validated as a measure of insulin resistance in nondiabetic children [19]. *Insulin resistance* is defined based on a threshold of greater than 3.16 [20]. In addition, 2 of the following 4 criteria were required in WHO definition: raised TG level, reduced HDL-C, raised blood pressure, and BMI corresponding to overweight or obesity [2].

The IDF definition of MS was also adapted for children and adolescents, where increased waist circumference (>90th percentile based on nationally representative sample) is the prerequisite [4]. In addition, 2 of the following 4 criteria were required in the IDF definition of MS in children and adolescents: raised TG level (≥150 mg/dL), reduced HDL-C (≤40 mg/dL), raised blood pressure (systolic blood pressure ≥130 mm Hg or diastolic blood pressure ≥85 mm Hg), and raised fasting blood glucose (≥100 mg/dL) [4].

### 2.5. Sex hormone levels

To estimate free (non–protein bound) testosterone, we calculated the FAI, the molar ratio of total testosterone to SHBG [21]. To convert testosterone to nanomolar, the nanogram per deciliter value is multiplied by 0.0347. Likewise, to estimate the free estradiol concentration, the free estradiol index (FEI), the molar ratio of estradiol to SHBG was calculated. To convert estradiol to picomolar, the picogram per milliliter value is multiplied by 3.67.

### 2.6. Biochemical analysis

Triglyceride, HDL-C, and glucose were measured by enzymatic colorimetric assay method using Cobas Integra 800 kit (Roche Diagnostic, Indianapolis, IN). Insulin, testosterone, estradiol, and SHBG levels were measured by chemiluminescence immunoassay method using Modular E170 kit (Roche Diagnostic). Analyses were performed in an accredited laboratory (Centro Laboratories, which are based in Istanbul, Turkey). The laboratory is accredited by DAR (*Deutscher Akkreditierungs Rat*) according to ISO 15189 by clinical laboratory accreditation committee. Measurement uncertainty of each method in use for this laboratory is 6.01% for LDL-C, 3.0% for total cholesterol, 10.5% for TG, 6.5% for HDL-C, 2.9% for glucose, 5.07% for insulin, 3.37% for testosterone, 3.88% for estradiol, and 5.78% for SHBG.

### 2.7. Statistical analyses

Descriptive parameters are shown as means ± SD and in percentages for variables with normal distribution. Comparisons were made to detect significance between groups of means; independent-samples *t* test was used to analyze the differences in means for variables with normal distribution. Nonparametric test (Mann-Whitney) was used to compare the groups for variables with skewed distribution (insulin, HOMA-IR, FEI, SHBG, FAI, and FAI/FEI); mean (range = maximum-minimum) is shown for variables with skewed distribution in Tables 2 and 3. The $\chi^2$ test was used to compare categorical variables between the groups (ie, MS vs others). Logistic regression analysis was performed to find the predictors of MS. A value of *P* less than .05 on the 2-sided test was considered statistically significant. Statistical analyses were performed using SPSS-13 for Windows (SPSS, Chicago, IL, No. 9026510).

## 3. Results

A total of 365 children and adolescents were enrolled in the study (175 boys; mean age, 12.8 ± 3.4 and 190 girls; mean age, 13.2 ± 3.3). Parental smoking was common (59.2%), and family history of cardiovascular disease was reported in 31% of the cases.

### 3.1. Prevalence of MS risk criteria in children and adolescents

We observed a trend of increasing frequency of obesity and insulin resistance in addition to a decreasing level of HDL-C

Table 1
Prevalence of MS and risk determinants

| Risk determinant | Boys, n (%) | | | Girls, n (%) | | |
|---|---|---|---|---|---|---|
| | 8-12 y old (n = 92) | 14-18 y old (n = 83) | *P* | 8-12 y old (n = 88) | 14-18 y old (n = 102) | *P* |
| Age | 9.7 ± 1.0 | 16.1 ± 1.0 | <.001 | 9.7 ± 0.9 | 16.2 ± 0.6 | <.001 |
| Overweight or obesity | 7 (7.6) | 16 (19.3) | .02 | 7 (8.0) | 13 (12.7) | .28 |
| Waist circumference >90th percentile | 5 (5.4) | 9 (10.8) | .15 | 8 (9.1) | 14 (13.7) | .32 |
| Waist to hip ratio (>0.9 for boys, >0.85 for girls) | 5 (5.4) | 1 (1.2) | .13 | 8 (9.1) | 4 (3.9) | .12 |
| Diastolic BP >95th percentile | 5 (5.4) | 0 (0) | .03 | 7 (8.0) | 6 (5.9) | .57 |
| Systolic BP >95th percentile | 26 (28.3) | 9 (10.8) | .003 | 29 (33) | 12 (11.8) | <.001 |
| Systolic or diastolic BP >95th percentile | 26 (28.3) | 9 (10.8) | .003 | 30 (34.1) | 14 (13.7) | .001 |
| HDL-C ≤10th percentile of NGHS population | 4 (4.3) | 23 (27.7) | <.001 | 9 (10.2) | 9 (8.8) | .74 |
| TG ≥90th percentile level | 19 (20.7) | 18 (21.7) | .51 | 4 (4.5) | 7 (6.9) | .36 |
| Fasting glucose >100 mg/dL | 0 (0) | 1 (1.2) | .47 | 0 (0) | 1 (1) | .54 |
| Hyperinsulinemia (fasting value >18 μU/mL) | 2 (2.2) | 18 (21.7) | <.001 | 3 (3.4) | 9 (8.8) | .11 |
| Insulin resistance (HOMA-IR >3.16) | 2 (2.2) | 23 (27.7) | <.001 | 3 (3.4) | 12 (11.8) | .03 |
| MS by adapted NCEP definition | 1 (1.1) | 6 (7.2) | .04 | 1 (1.1) | 4 (3.9) | .23 |
| MS by adapted WHO definition | 0 (0) | 9 (10.8) | .001 | 2 (2.3) | 4 (3.9) | .41 |
| MS by adapted IDF definition | 3 (3.3) | 3 (3.6) | .61 | 3 (3.4) | 0 (0) | .10 |
| MS by at least 1 definition | 4 (4.3) | 15 (18.1) | .003 | 6 (6.8) | 8 (7.8) | .51 |

BP indicates blood pressure.

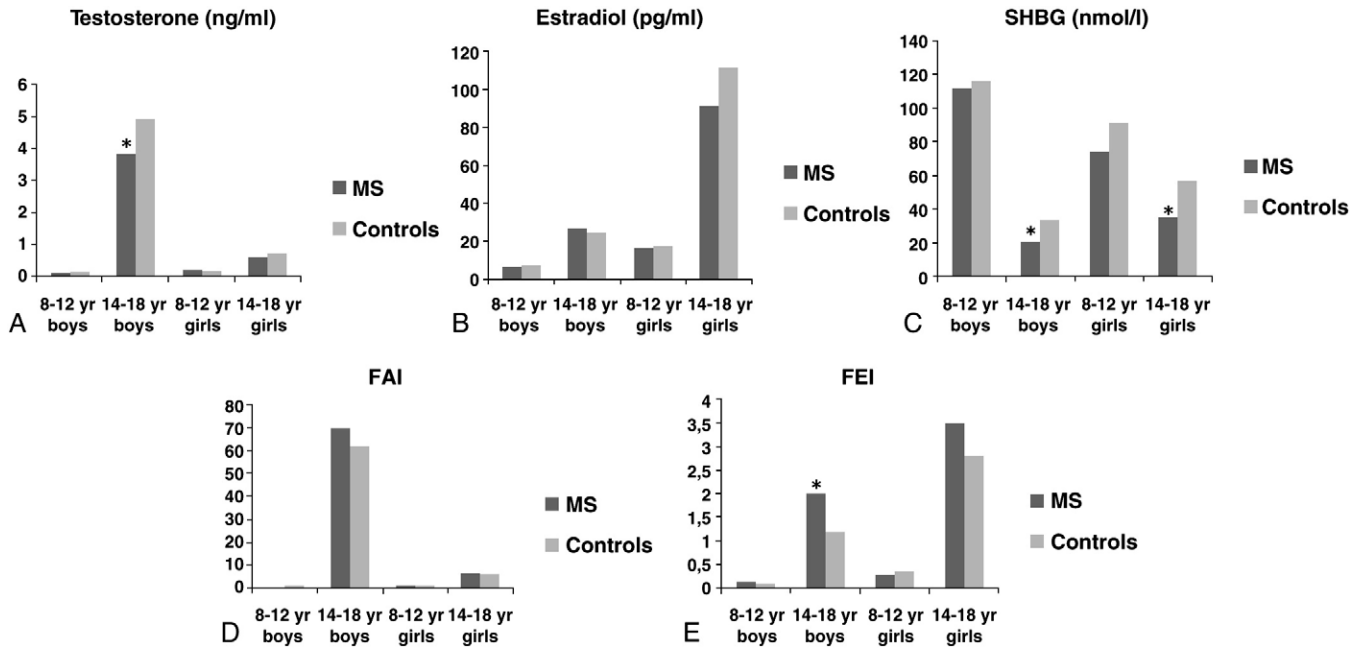


Fig. 1. Testosterone (A), estradiol (B), SHBG (C), FAI (D), and FEI (E) levels in students with MS vs controls. *$P$ <.05.

among 14- to 18-year–old adolescents compared with 8- to 12-year–old children (Table 1). These changes likely reflect physical and biochemical changes around the ages of puberty. The frequency of overweight status and obesity (based on the international criteria) doubled among adolescents compared with children. Most significant change in the lipid levels was observed in HDL-C levels of boys (Table 1). As a result, nearly one fourth of the adolescent boys had HDL-C levels less than the 10th percentile of NGHS population. Similarly, an increase was noted in the prevalence of insulin resistance among adolescent girls and boys. Prevalence of low HDL-C among girls did not show significant change (Table 1).

Blood pressure, on the other hand, displayed a different trend in this age range; high blood pressure (based on the age-, sex-, and height-specific percentile) was more common among children compared with 14- to 18-year–old adolescents (Table 1).

### 3.2. Metabolic syndrome

Prevalence of MS varied depending on the definition; 3.3%, 4.1%, and 2.5% of students had MS based on the adapted NCEP, WHO, and IDF definitions, respectively. The overlap among the definitions of the MS was small. For

Table 2
Univariate comparison of boys with and without MS

| | 8- to 12-y–old boys | | | 14- to 18-y–old boys | | |
|---|---|---|---|---|---|---|
| | MS (n = 4) | Controls (n = 88) | $P$ | MS (n = 15) | Controls (n = 68) | $P$ |
| Age | 9.5 ± 1 | 9.7 ± 1 | .68 | 16.2 ± 1 | 15.8 ± 1 | .20 |
| Total cholesterol | 168 ± 25 | 151 ± 27 | .22 | 153 ± 32 | 136 ± 25 | .03 |
| BMI (kg/m$^2$) | 22 ± 3 | 16 ± 2 | <.01 | 26 ± 5 | 21 ± 2 | <.01 |
| HDL-C (mg/dL) | 54 ± 9 | 63 ± 16 | .13 | 39 ± 8 | 49 ± 11 | <.01 |
| LDL-C (mg/dL) | 93 ± 23 | 75 ± 20 | .09 | 79 ± 25 | 72 ± 22 | .30 |
| Fasting glucose (mg/dL) | 82 ± 8 | 81 ± 9 | .88 | 80 ± 9 | 76 ± 11 | .22 |
| TG (mg/dL) | 89 (88) | 63 (161) | .13 | 117 (224) | 70 (222) | <.01 |
| Insulin level ($\mu$U/mL) | 8.4 (9.5) | 5.1 (20.7) | .04 | 26.8 (80.9) | 11.0 (55.7) | <.01 |
| HOMA-IR | 1.7 (1.9) | 1.0 (4.7) | .05 | 5.5 (17.9) | 2.1 (11.0) | <.01 |
| Estradiol (pg/mL) | 6.4 (2.4) | 6.9 (10.2) | .86 | 26.5 (39.4) | 24.1 (105.0) | .25 |
| FEI | 0.13 (0.29) | 0.09 (0.31) | .93 | 2.0 (2.9) | 1.2 (5.8) | <.01 |
| Testosterone (ng/mL) | 0.08 (0.21) | 0.13 (1.2) | .35 | 3.8 (6.0) | 4.9 (8.8) | .04 |
| FAI | 0.21 (0.36) | 0.41 (3.4) | .23 | 70 (96) | 62 (186) | .31 |
| SHBG (nmol/L) | 112 (167) | 116 (169) | .85 | 21 (27) | 34 (94) | <.01 |

Insulin, HOMA-IR, FEI, SHBG, FAI, and FAI/FEI displayed skewed distribution. Mean (range) levels were displayed at the table for these variables, and nonparametric test (Mann-Whitney) was used to compare the groups. Independent-samples $t$ test was used to compare groups for variables with normal distribution (HDL-C, LDL-C, total cholesterol). Mean ± SD are displayed for these variables.

Table 3
Univariate comparison of girls with and without MS

| | 8- to 12-y–old girls | | | 14- to 18-y–old girls | | |
|---|---|---|---|---|---|---|
| | MS (n = 6) | Controls (n = 82) | *P* | MS (n = 8) | Controls (n = 94) | *P* |
| Age | 9.7 ± 1 | 9.8 ± 1 | .82 | 16.0 ± 1 | 16.2 ± 1 | .44 |
| Total cholesterol | 165 ± 12 | 157 ± 23 | .18 | 164 ± 30 | 147 ± 22 | .05 |
| BMI (kg/m$^2$) | 19 ± 4 | 16 ± 2 | .02 | 25 ± 3 | 21 ± 3 | <.01 |
| HDL-C (mg/dL) | 59 ± 13 | 60 ± 15 | .93 | 54 ± 16 | 57 ± 10 | .66 |
| LDL-C (mg/dL) | 80 ± 25 | 82 ± 20 | .88 | 88 ± 27 | 76 ± 20 | .13 |
| Fasting glucose (mg/dL) | 75 ± 9 | 76 ± 8 | .77 | 74 ± 13 | 74 ± 8 | .91 |
| TG (mg/dL) | 128 (273) | 75 (182) | .14 | 110 (185) | 69 (119) | .15 |
| Insulin level ($\mu$U/mL) | 13.5 (23) | 5.9 (22.7) | <.01 | 13.2 (20.7) | 8.6 (28) | .02 |
| HOMA-IR | 2.6 (4.7) | 1.1 (5.6) | .01 | 2.5 (4.5) | 1.6 (5.0) | .07 |
| Estradiol (pg/mL) | 15.9 (46.8) | 16.9 (346) | 1.0 | 91.2 (251) | 111 (542) | .42 |
| FEI | 0.29 (0.8) | 0.34 (8.9) | .44 | 3.5 (8.4) | 2.8 (9.1) | .46 |
| Testosterone (ng/mL) | 0.17 (0.15) | 0.16 (0.41) | .72 | 0.6 (0.6) | 0.7 (6.6) | .87 |
| FAI | 0.94 (1.9) | 0.77 (2.8) | .40 | 6.5 (8.5) | 5.7 (103) | .06 |
| SHBG (nmol/L) | 74 (76) | 91 (158) | .21 | 35 (29) | 57 (130) | <.01 |

Insulin, HOMA-IR, FEI, SHBG, FAI, and FAI/FEI displayed skewed distribution. Mean (range) levels were displayed at the table for these variables, and nonparametric test (Mann-Whitney) was used to compare the groups. Independent-samples *t* test was used to compare groups for variables with normal distribution (HDL-C, LDL-C, total cholesterol). Mean ± SD are displayed for these variables.

instance, among 12 subjects who qualified for MS based on the adapted NCEP definition, only 1 (8.3%) fulfilled the criteria for MS based on the adapted WHO or IDF definitions. Similarly, among 15 subjects of MS based on the adapted WHO definition, only 1 (6.7%) and 2 (13.3%) qualified for MS based on the adapted NCEP or IDF definitions, respectively. As a result, 33 (9%) students had MS based on at least 1 definition. Among subjects with MS, male sex was more common (58% vs 47%). More than half of the subjects (57.6% vs 7.6%) with MS were overweight or obese. Students with MS frequently had high blood pressure (51.5%), elevated TG (60.6%), low HDL-C levels (45.5%), and insulin resistance (42.4%). Sex hormone levels displayed significant differences among adolescent subjects with and without MS (Fig. 1, Tables 2 and 3). The frequency of MS increased among 14- to 18-year–old boys compared with 8- to 12-year–old boys (Table 1). This trend was more evident for boys (Table 1). Adolescent boys with MS had significantly higher FEI and lower SHBG and testosterone levels compared with controls (Fig. 1, Table 2). Adolescent girls with MS had significantly lower SHBG levels compared with controls (Fig. 1, Table 3).

Table 4
Logistic regression results (predictors of MS) in adolescent boys

| | $\beta$ Coefficient | *P* | OR | 95% CI for OR | |
|---|---|---|---|---|---|
| | | | | Lower | Upper |
| Age | −0.48 | .28 | 0.6 | 0.26 | 1.48 |
| Estradiol | −0.02 | .75 | 1.0 | 0.86 | 1.11 |
| FEI | 2.5 | .15 | 12.1 | 0.41 | 353.2 |
| Testosterone | 1.0 | .16 | 2.6 | 0.67 | 10.32 |
| FAI | −0.1 | .06 | 0.9 | 0.79 | 1.00 |
| SHBG | −0.3 | .005 | 0.8 | 0.64 | 0.92 |
| Constant | 12.9 | .06 | 400266 | | |

Dependent: MS, $R^2$ = 0.48. CI indicates confidence interval.

Logistic regression was performed separately to find the predictors of MS in all 4 age and sex groups (8- to 12- vs 14- to 18-year–old boys and girls). Sex hormone–binding globulin remained as the significant predictor of MS in adolescent boys (Table 4).

Logistic regression analysis was also performed in all participants. Among covariates of sex, age, estradiol, testosterone, FAI, FEI, and FAI/FEI ratio, SHBG was the only significant predictor of MS ($B$ = −0.22, odds ratio [OR] = 0.98, 95% confidence interval for OR are 0.960 and 0.996, $P$ = .02, Nagelkarke $R^2$ = 0.19).

## 4. Discussion

In this cross-sectional study of school children, we observed significant increases in the MS risk parameters such as insulin resistance, obesity, and low HDL-C among 14- to 18-year–old adolescents compared with 8- to 12-year–old children. Adolescent boys and girls with MS had significantly lower SHBG levels compared with controls. Adolescent boys with MS also had significantly lower testosterone and higher FEI levels compared with controls.

Puberty-related changes in the components of MS contributed to this observation. Interestingly, low SHBG level was the strongest predictor of the constellation of MS components. Pubertal insulin resistance occurs during a time of profound change in body composition and sex hormone levels. Free androgen index and FEI levels increase and SHBG levels decrease with puberty [6]. As we observed, significant sex-related differences can occur in the developmental changes during the transition from late childhood to adolescence. Lean body mass and fat mass increase in both sexes. Increased body fat and BMI can cause

insulin resistance and mediate the pubertal changes that we observed [22].

Several studies suggest that MS starts early in life [5], yet definitions of MS in children and adolescents still remain controversial. Most studies have been performed at middle and older ages. Information is lacking on the long-term, longitudinal, and progressive changes of the risk variables of the MS from childhood to younger adulthood. Ethnic differences exist in the criteria, definition, and prevalence of MS in adolescents between populations. As expected, most subjects with MS were either overweight or obese [23]. Compared with our previous reports [24], we observed a temporal trend of increasing obesity in Turkish children and adolescents. In contrast, we observed that high blood pressure was more common among children compared with adolescents. This supports our prior observations indicating that contrasting temporal trends exist for obesity and blood pressure among Turkish adolescents [25].

Little is known on the distribution of sex hormones in children and adolescents, particularly their relation to various cardiometabolic risk factors. We observed lower testosterone levels in adolescent boys with MS. Similarly, in adults, testosterone appears to relate negatively to MS in men [8] and positively in premenopausal [10] and postmenopausal women [9] and in adolescent girls [11]. Therefore, we analyzed 8- to 12- vs 14- to 18-year–old boys and girls separately. Although we had only 33 subjects with MS, we observed higher FEI levels in relation to MS in adolescent boys. This finding is also consistent with previous reports indicating a positive relationship of estradiol with cardiovascular risk factors [26] and MS [27] in men.

In logistic regression analysis, SHBG remained as the only significant predictor of MS. As with MS, ethnic and racial variations are known to occur in SHBG levels among children [28]. We observed differences in the prevalence of MS according to the selected definition in children and adolescents. We were able to identify more students at risk by combining all 3 available definitions. Such a strategy may enable us to identify and prevent MS at an earlier stage.

The study was a cross-sectional survey of school students and comes with several limitations. Healthy children may experience a decline in insulin sensitivity as a transition from prepuberty to puberty. Thus, to assume that this decline is a manifestation of MS may be unreasonable. However, we demonstrate that SHBG remained as the significant predictor of MS for adolescent boys when the groups were analyzed separately. Similarly, a dose-response relationship between sex hormone levels and odds of the MS in men has been reported that was consistent across race/ethnic groups [29].

The standard method of assessing pubertal stages is the Tanner Sexual Maturation Scale, yet this assessment is impractical for a school screening project because it may be uncomfortable and invasive to parents and students.

Obviously, larger prospective studies are needed to test the standard definition and ethnic variations of MS in children and adolescents. We compared lipid, insulin, and glucose levels with published ranges from different populations. Differences in methodologies and blood collection procedures may account for the different trends that we report. The methodologies may have limitations in the measurement of estradiol and testosterone for the values that are observed in children and adolescents [30-32]. Ultrasensitive recombinant cell bioassay or high-sensitivity liquid chromatography–tandem mass spectrometry may provide better sensitivity and specificity for analyzing estrogens in samples from children [30-31]. Estradiol had a diurnal variation in girls and boys, with the trough occurring from 8.00 AM to 8:00 PM in girls and from 12:00 PM to 8:00 PM in boys [31]. Blood samples were drawn at 9:00 AM, and screening took place at schools during normal school hours. Similarly, immunoassays for testosterone may give inaccurate results for samples from girls; liquid chromatography–tandem mass spectrometry may provide better sensitivity and specificity assay to measure testosterone in this age group [32].

Insulin sensitivity in humans is a spectrum with no universally accepted and clinically well defined cutoffs that will identify and diagnose those who are insulin sensitive and those who are insulin resistant. Therefore, taking an arbitrary cutoff with a value of insulin in children may be misleading. We compared the change from 8- to 12-year–old children to 14- to 18-year–old adolescents within the same population. We used standardized procedures in an internationally accredited laboratory. Therefore, we do not believe that methodological differences can account for all the variations that we observed. Furthermore, the analyses for the relationships between sex hormones and MS were evaluated separately for the children and adolescents.

In conclusion, low SHBG level proved to be an important element determining constellation of MS components. Similar observations were made in men [8] and postmenopausal women [9]. Thus, it is likely that the relationship of sex hormones and MS is bidirectional throughout the lifespan, with low SHBG levels being predictive of the development of MS in children and adolescents. Decline in sex hormone levels may contribute to the increasing cardiovascular risk and MS occurring in postmenopausal women and elderly men.

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