Endothelial Function as Measured by Peripheral Arterial Tonometry Increases during Pubertal Advancement

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Key Words

Peripheral arterial tonometry • Puberty • Endothelial function • Estradiol • DHEAS • Nitric oxide

Abstract

Background/Aims: Sex steroids, such as estrogens, are known to influence endothelial function by their vasodilator action. The aim of this study was to study the relation of puberty and sex steroids with endothelial function using peripheral arterial tonometry (PAT). **Methods:** In 89 healthy school boys and girls, we determined height, weight, waist circumference, percent body fat, BMI, BMI z-score, blood pressure (BP), BP percentiles, lipid profile, insulin, and glucose levels after overnight fast. Estrone (E₁), estradiol (E₂), DHEAS and E₁-sulfate were measured using ultrasensitive assays. Participants were divided into 3 pubertal groups on the basis of their estrogen levels: group 1 (Tanner stage I), group 2 (Tanner stages II–III), and group 3 (Tanner stages IV–V). Endothelial function was measured by Endo-PAT 2000® and expressed as PAT index. A higher PAT index represents a higher reactive hyperemia response. **Results:** The PAT index was lowest at 1.42 ± 0.44 (mean $\pm$ SD) in group 1 and significantly increased in group 2 at 1.71 ± 0.35 ($p = 0.02$) and group 3 at 1.92 ± 0.38 ($p < 0.001$). The PAT index correlated

positively with E₂, DHEAS and age. **Conclusion:** Enhancement of the PAT index was associated with an increment in Tanner stages. The changes in E₂ and DHEAS levels may contribute to increasing endothelial response to shear stress or arterial blood flow.

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Introduction

Peripheral arterial tonometry (PAT) is a reliable, non-invasive technology used to study endothelial function [1–3]. The PAT technology was initially designed to detect asphyxia during obstructive sleep apnea [4]; later it was applied to the field of cardiovascular medicine [1]. The Endo PAT 2000® device measures the changes in the amplitudes of digital artery pulse wave forms before and after an occlusion of the brachial artery [1, 2, 5–7]. The computerized algorithm generates a PAT index. The PAT index was also found to correlate with the conventional brachial artery ultrasound, which is a standard noninvasive testing of endothelial function in adults [1].

The PAT index represents the endothelial-derived nitric oxide (NO)-mediated vasodilation. The greater the vasodilation the higher the value of the PAT index [2].

The PAT index decreased significantly after a NO synthesis inhibitor, L-NAME (N(G)-nitro-L-arginine methyl ester) infusion into the brachial artery [6].

Estrogens enhance the endothelial-dependent flow-mediated vasodilation via the release of NO but have no effect on the endothelial-independent vasodilation [8–10]. Estrogens exert their effects by binding to the estrogen receptor (ER) located on the vascular endothelial and smooth muscle cells of the blood vessels [11, 12]; and this effect is demonstrated in both males and females [13–15]. The immediate NO production is mediated by estrogens via its action on endothelial nitric oxide synthetase enzyme (eNOS) [16, 17]. Another steroidal hormone DHEAS (dehydroepiandrosterone sulfate) is also associated with a cardioprotective role in older men [18, 19]. DHEA has been shown to have its own genomic and nongenomic effects [20]. Recently, the DHEA receptor has been identified on the endothelial cell plasma membrane, which binds with G-protein receptors to stimulate eNOS and mediate endothelial NO production [21]. However, there are no studies which have studied the relationship of estrogens and DHEAS to endothelial function in children. We hypothesized that the PAT index would increase as puberty progresses with increased effects of estrogens and DHEAS on the endothelium.

Participants and Methods

Participants

This study was a part of the POPS (Power of Prevention Study) project looking at the pathogenesis and prevention of type 2 diabetes in junior high school children through school-based lifestyle assessments and interventions. In the current substudy, 89 healthy participants were enrolled from Brooklyn, New York. The study protocol was approved by the New York City school board, the New York City Board of Health and the Institutional Review Board of Maimonides Medical Center. Informed consent was obtained from the parents of all of the participants and from the participants themselves if they were eight years of age or older. There was no history of diabetes, depression, anxiety, chronic disease or acute illness in the participants.

Anthropometric Measurements

Height was measured by using a wall mounted stadiometer to the nearest 0.1 cm (Holtain Ltd., Crymych, UK) and weight was measured nearest to 0.1 kg on a standing scale (Scale-Tronix, Wheaton, Ill., USA). We performed the measurement of body fat using the Omron Body Fat Analyzer HBF-300 (Omron Health Care, Inc., Vernon Hills, Ill., USA) in all participants. Waist circumference was measured to the nearest 0.1 cm using a nonelastic measuring tape along the horizontal line of the upper border of the iliac crests [22]. Blood pressure was measured with a standard sphygmomanometer in a quiet and comfortable environment af-

ter 3–5 min rest. Blood pressure percentiles were calculated according to the previously published methodology [23].

Frequently Sampled Intravenous Glucose Tolerance Test

We performed the frequent-sample intravenous glucose tolerance test (IVGTT) on all participants to assess the parameters of insulin sensitivity [24]. Participants and their parents were contacted the night before and reminded not to eat breakfast or consume any foods or beverages except for water on the morning of testing. Testing was performed between 08.00 and 12.00 h in the school by a physician. A plastic intravenous catheter was placed, one in each arm, and basal blood samples were collected at –1 min. Each subject received an intravenous bolus of glucose 0.5 mg/kg (50% dextrose; maximum, 25 g), over 3 min, and blood was drawn through the same catheter for measurement of serum insulin concentrations at 3 and 5 min after glucose administration.

Insulin resistance was calculated as $1/(\log_{10}[\text{fasting glucose}] + \log_{10}[\text{fasting insulin}])$ is defined as Quantitative Insulin Sensitivity Check Index (QUICKI), and insulin secretory capacity was defined as the acute insulin response (AIR) [24, 25]. QUICKI is well correlated and a reliable mean of following changes in insulin sensitivity over time [25, 26]. AIR (mean incremental rise in plasma insulin at 3 and 5 min after an intravenous glucose load) correlates well with first phase insulin release measured by IV glucose tolerance testing; Glucose Disposal Index (GDI) was calculated as: $\log_{10}(\text{AIR} \times \text{fasting glucose concentration}/\text{fasting insulin concentration})$ [27].

Biochemical Analysis

Venous blood was obtained before the IVGTT testing for serum levels of triglycerides (TG), low-density lipoprotein (LDL), total cholesterol (TC), and high-density lipoproteins (HDL). Glucose was measured by the hexokinase method (Glucose/HK; Roche Molecular Biochemicals, Werk Penzberg, Germany). Plasma insulin was measured by solid phase ^{125}I -RIA (Coata-count; DPC, Los Angeles, Calif., USA). Lipid profile was measured calorimetrically using an automated Hitachi 704 spectrometer. Steroids were analyzed by radioimmunoassay (RIA) after selective solvent extraction, column chromatography, hydrolysis and HPLC. Estradiol (E_2), estrone (E_1), estrone sulfate and DHEAS assays were measured by an ultra-sensitive liquid chromatography (HPLC) with triple quadrupole mass spectrometer detection (Esoterix, Calabasas Hill, Calif., USA).

Peripheral Arterial Tonometry

The endothelial function of all 89 participants was evaluated using the Endo PAT 2000 (Itamar Medical Ltd., Caesarea, Israel). Participants wear pneumatic finger-tip probes on the first finger of both hands. The participants lay on a comfortable bed with their hands at heart level in a comfortable position and their fingers resting freely on an armrest. The temperature of the room was maintained at 23°C; lighting and noise in the room were kept to the bare minimum. The testing was performed in the morning in a fasting state. The fingertip probes recorded the pulse wave continuously throughout the study. Baseline measurements were collected for 5 min. Then, the arterial blood flow to one arm was occluded for 5 min using a blood pressure cuff inflated to 40 mm Hg above systolic pressure. After the occlusion, the cuff was rapidly deflated, stimulating increased blood flow which would lead to endothelial-dependent, flow-mediated vasodilation (reactive hyperemia), as described earlier. Pulse wave amplitudes were recorded for at least

Table 1. Anthropometric data, hormonal profile and parameters of metabolic profile of participants in the three groups

	Group 1 (Tanner I)	Group 2 (Tanner II–III)	Group 3 (Tanner IV–V)	p value		
				groups 1 and 2	groups 1 and 3	groups 2 and 3
Number of participants	21	35	33			
Male/Female	19/2	14/21	11/22			
Age, years	12.06 ± 0.6	12.65 ± 0.7	12.96 ± 0.7	0.002	<0.001	n.s.
Weight, kg	42.7 ± 9.8	52.8 ± 12.4	57.5 ± 14.5	0.002	<0.001	n.s.
BMI	18.9 ± 3.1	21.9 ± 4.5	23.1 ± 4.9	0.006	<0.001	n.s.
BMI z-score	0.18 ± 1.1	0.68 ± 1.13	0.86 ± 1.1	n.s.	0.04	n.s.
Ht z-score	−0.13 ± 1.2	0.06 ± 0.88	0.02 ± 1.0	n.s.	n.s.	n.s.
WC, cm	66.6 ± 12.4	76.2 ± 11.3	76.7 ± 12.3	0.006	0.006	n.s.
WC z-score	0.23 ± 0.97	0.91 ± 1.17	0.85 ± 1.32	0.02	0.05	n.s.
Body fat, %	24.0 ± 6.1	25.2 ± 7.3	26.1 ± 8.2	n.s.	n.s.	n.s.
SBP percentile	40.4 ± 25.7	35.4 ± 25.2	45.9 ± 27.4	n.s.	n.s.	n.s.
DBP percentile	47.9 ± 21.3	48.7 ± 20.7	35.6 ± 20.4	n.s.	0.04	0.01
Insulin, IU/ml*	7.3 ± 2.8	8.8 ± 4.8	8.7 ± 4.1	n.s.	n.s.	n.s.
Glucose, mg/dl*	94.2 ± 6.8	91 ± 6.3	91.6 ± 7.3	n.s.	n.s.	n.s.
Cholesterol, mg/dl*	170.3 ± 29.6	157.9 ± 28	168.7 ± 33.3	n.s.	n.s.	n.s.
LDL, mg/dl*	99.3 ± 21.4	85.6 ± 23.5	100.4 ± 25.7	0.03	n.s.	0.02
Triglyceride, mg/dl*	69.3 ± 28.5	87.5 ± 27.3	88 ± 43.7	0.03	n.s.	n.s.
HDL, mg/dl*	57.4 ± 17.2	54.3 ± 14.5	54 ± 10.9	n.s.	n.s.	n.s.
Triglyceride/HDL ratio	1.37 ± 0.9	1.79 ± 0.9	1.78 ± 1.3	n.s.	n.s.	n.s.
QUICKI	0.36 ± 0.02	0.35 ± 0.02	0.39 ± 0.24	n.s.	n.s.	n.s.
GDI	2.77 ± 0.49	3.12 ± 0.28	3.06 ± 0.27	0.04	0.08	n.s.
AIR	70.2 ± 56.9	152.7 ± 151.3	123 ± 64.7	0.02	0.02	n.s.
Estrone, pg/ml	0.69 ± 0.29	2.29 ± 1.12	4.73 ± 2.82	<0.001	<0.001	<0.001
Estradiol, pg/ml	0.23 ± 0.24	2.68 ± 1.46	8.99 ± 8.27	<0.001	<0.001	<0.001
DHEAS, µg/dl	60.0 ± 35.1	72.8 ± 38.8	113.6 ± 73.9	n.s.	<0.001	0.007
Estrone sulfate, µg/dl	21.9 ± 12.3	67.1 ± 41.9	157.6 ± 123.2	<0.001	<0.001	<0.001
PAT index	1.46 ± 0.44	1.71 ± 0.35	1.92 ± 0.38	0.02	<0.001	0.037

p values between the three groups as shown in the table. Data expressed as mean ± SD.

* Fasting values. n.s. = Nonsignificant; BMI = body mass index; Ht = height; WC = waist circumference; SBP = systolic blood pressure; DBP = diastolic blood pressure; DHEAS = dehydroepiandrosterone sulfate.

5 min after the cuff was deflated. The PAT index is provided automatically by the system's software and represents the ratio between the post- to pre-occlusion average signal size in both the fingers, corrected for systemic vascular changes and baseline level [2].

Participants were characterized and divided into three groups defined by Tanner-stage on the basis of their ultrasensitive serum E₂ and E₁ levels. The norms of the steroids were provided by the Esoterix laboratories and were standardized for the ultrasensitive assay used in the study [28]. In group 1 both E₂ and E₁ levels corresponded to Tanner stage I (prepubertal); in group 2 E₂ and/or E₁ levels were in the range defined for Tanner stage II–III (initiation or early puberty). Group 3 had E₂ and/or E₁ levels in the range of Tanner stages IV–V (mature puberty) (table 1) [28]. Participants with severe obesity (BMI z-score ≥ +2.0 SDS) were excluded from the groups for statistical analysis.

Statistics and Calculations

The data was analyzed by Statsoft® software and the data are presented as the mean ± SDS.

Results

Participants

The anthropometric data, hormonal and metabolic profile of the three groups are given in table 1. Age, weight and waist circumference significantly increased from group 1 to group 2 and again from group 1 and group 3. The difference was not significant between groups 2 and 3. Percent body fat also increased from groups 1 through 3; however, there was no statistical difference among the groups with respect to this parameter. The diastolic blood pressure percentile decreased in group 3 as compared to group 1 and 2. BMI and BMI z-score also followed the trend to significantly increase from groups 1 and 3. No significant difference was observed when group 2 was compared to group 3 (table 1).

Fig. 1. Increment of average PAT index and E₂ levels with advancement of puberty from group 1 to group 3. * p value significant when Tanner I was compared with groups 2 and 3. ± denotes p value significant when group 2 was compared with group 3.

Fig. 2. Increment of average PAT index, age and DHEAS levels with advancement of puberty from group 1 to group 3. * p value significant when Tanner I was compared with groups 2 and 3. ± denotes p value significant when group 2 was compared with group 3.

Height z-score, fasting glucose and insulin levels were similar in all three groups. QUICKI, AIR and GDI indices were calculated from the glucose and insulin values from the IVGTT. QUICKI was similar amongst the three groups (table 1). AIR and GDI were the highest in group 2 and significantly different when compared to groups 1 and 3; there was no significant difference between groups 1 and 3 (table 1). The LDL levels were significantly lower in group 2 as compared to group 1 and group 3. The triglycerides levels were lowest in group 1 and significantly different from group 3. The total cholesterol, HDL and triglyceride/HDL ratio was similar in all the three groups (table 1).

Table 2. a Anthropometric data, hormonal profile and parameters of metabolic profile of female participants in the two groups

	Group 2 (Tanner II–III)	Group 3 (Tanner IV–V)	p value
Number of participants	21	22	
Age, years	12.47 ± 0.62	12.78 ± 0.72	n.s.
Weight, kg	51.1 ± 12.9	56.1 ± 14.1	n.s.
BMI	21.3 ± 4.6	23.3 ± 4.8	n.s.
BMI z-score	0.48 ± 1.09	0.86 ± 1.08	n.s.
Ht z-score	0.20 ± 0.72	−0.23 ± 1.03	n.s.
WC, cm	75.1 ± 10.2	77.2 ± 12.3	n.s.
WC z-score	0.91 ± 1.11	0.79 ± 1.23	n.s.
Body fat, %	26.5 ± 6.5	28.5 ± 6.4	n.s.
SBP percentile	31.7 ± 23.7	52.3 ± 21.2	n.s.
DBP percentile	41.2 ± 24.4	34.3 ± 18.6	0.008
Insulin, IU/ml*	8.6 ± 5.7	8.4 ± 3.5	n.s.
Glucose, mg/dl*	89.2 ± 4.8	90.1 ± 7.4	n.s.
Cholesterol, mg/dl*	162.1 ± 31.8	168.1 ± 40.7	n.s.
LDL, mg/dl*	86.9 ± 26.8	102.7 ± 29.1	0.09
Triglyceride, mg/dl*	86.8 ± 31.2	83.2 ± 46.7	n.s.
HDL, mg/dl*	57.9 ± 17.3	53.8 ± 11.5	n.s.
Triglyceride/HDL ratio	1.78 ± 1.12	1.47 ± 0.66	n.s.
QUICKI	0.35 ± 0.02	0.35 ± 0.02	n.s.
AIR	111.4 ± 57.8	138.2 ± 67.5	n.s.
GDI	3.10 ± 0.23	3.12 ± 0.28	n.s.
Estrone, pg/ml	2.64 ± 0.73	5.95 ± 2.93	<0.001
Estradiol, pg/ml	3.31 ± 1.12	12.85 ± 8.52	<0.001
DHEAS, µg/dl	70.4 ± 39.4	101.7 ± 74.4	n.s.
Estrone sulfate, µg/dl	75.1 ± 39.1	193.3 ± 125.5	0.001
PAT index	1.66 ± 0.38	1.91 ± 0.29	0.023

p values between the two groups as shown in the table. Data expressed in mean ± SD. The prepubertal group is not included in the table as there were only 2 female participants.

* Fasting values. n.s. = Nonsignificant; BMI = body mass index; Ht = height; WC = waist circumference; SBP = systolic blood pressure; DBP = diastolic blood pressure; DHEAS = dehydroepiandrosterone sulfate.

The PAT index of 1.42 ± 0.44 in group 1 was significantly ($p < 0.001$) lower as compared to that in groups 2 (1.71 ± 0.35) and 3 (1.92 ± 0.38). A similar trend in PAT index was observed when the data was stratified by gender (table 2a, b). The E₂ levels were higher in groups 2 and 3 compared to group 1 (table 1; fig. 1). The E₁ and estrone sulfate levels, following the same pattern, were the lowest in group 1 and significantly increased in groups 2 and 3 (table 1). Estradiol, estrone and estrone sulfate levels were the highest in group 3 in both males and females and were significantly higher than in groups 2 and 1 (table 2b). Similarly, the DHEAS levels were highest in

Table 2. b Anthropometric data, hormonal profile and parameters of metabolic profile of male participants in the three groups

	Group 1 (Tanner I)	Group 2 (Tanner II–III)	Group 3 (Tanner IV–V)	p value		
				groups 1 and 2	groups 1 and 3	groups 2 and 3
Number of participants	19	14	11			
Age, years	12.06 ± 0.66	12.88 ± 0.78	13.28 ± 0.69	0.004	<0.001	n.s.
Weight, kg	43.1 ± 9.8	55.6 ± 11.5	61.4 ± 15.3	0.003	0.003	n.s.
BMI	18.9 ± 3.1	22.7 ± 4.2	22.8 ± 5.0	0.009	0.03	n.s.
BMI z-score	0.20 ± 1.10	1.01 ± 1.03	0.96 ± 1.20	0.04	n.s.	n.s.
Ht z-score	−0.01 ± 1.15	0.04 ± 1.03	0.36 ± 0.89	n.s.	n.s.	n.s.
WC, cm	66.4 ± 12.7	78.1 ± 12.6	76.5 ± 13.3	0.01	0.05	n.s.
WC z-score	0.22 ± 0.96	1.01 ± 1.28	0.71 ± 1.42	0.06	n.s.	n.s.
Body fat, %	24.7 ± 6.1	23.6 ± 7.3	21.9 ± 10.0	n.s.	n.s.	n.s.
SBP percentile	43.9 ± 24.4	42.8 ± 26.9	48.8 ± 31.8	n.s.	n.s.	n.s.
DBP percentile	46.8 ± 22.1	44.8 ± 21.3	28.3 ± 10.9	n.s.	0.006	0.02
Insulin, IU/ml	7.3 ± 2.8	8.6 ± 1.9	9.9 ± 5.3	n.s.	n.s.	n.s.
Glucose, mg/dl*	94.1 ± 6.8	92.5 ± 7.5	93.4 ± 6.4	n.s.	n.s.	n.s.
Cholesterol, mg/dl*	172.4 ± 28.8	148.3 ± 20.0	170.2 ± 16.5	0.01	n.s.	0.01
LDL, mg/dl*	100.5 ± 21.3	80.4 ± 17.0	94.9 ± 21.0	0.007	n.s.	n.s.
Triglyceride, mg/dl*	71.1 ± 28.2	84.6 ± 21.0	99.0 ± 41.2	n.s.	n.s.	n.s.
HDL, mg/dl*	57.9 ± 17.5	50.8 ± 10.4	55.6 ± 9.9	n.s.	n.s.	n.s.
Triglyceride/HDL ratio	1.41 ± 0.91	1.77 ± 0.70	1.93 ± 1.23	n.s.	n.s.	n.s.
QUICKI	0.36 ± 0.02	0.35 ± 0.01	0.49 ± 0.44	n.s.	n.s.	n.s.
GDI	2.82 ± 0.49	3.10 ± 0.38	2.93 ± 0.24	n.s.	n.s.	n.s.
AIR	75.8 ± 56.7	140.1 ± 106.6	101.9 ± 65.6	n.s.	n.s.	n.s.
Estrone, pg/ml	0.74 ± 0.27	1.34 ± 0.41	2.63 ± 1.07	0.001	<0.001	0.003
Estradiol, pg/ml	0.25 ± 0.25	1.27 ± 0.65	2.48 ± 1.68	<0.001	0.001	0.04
DHEAS, µg/dl	59.0 ± 35.9	77.3 ± 41.8	138.5 ± 77.0	n.s.	0.007	0.03
Estrone sulfate, µg/dl	22.3 ± 12.7	41.0 ± 16.2	72.8 ± 31.2	0.003	0.001	0.01
PAT index	1.41 ± 0.35	1.78 ± 0.30	1.93 ± 0.67	0.003	0.03	n.s.

p values between the three groups as shown in the table. Data expressed in mean ± SD.

* Fasting values. n.s. = Nonsignificant; BMI = body mass index; Ht = height; WC = waist circumference; SBP = systolic blood pressure; DBP = diastolic blood pressure; DHEAS = dehydroepiandrosterone sulfate.

group 3 and were significantly lower in groups 2 and 1; the DHEAS levels between groups 1 and 2 were not significantly different (table 1; fig. 2). In males, the DHEAS levels increased with the increase in pubertal advancement and were statistically significant between groups 2 and 3 and groups 1 and 3 (table 2b). While in females an increase in DHEAS was also observed, it was not significant; however, the number of females in the Tanner I group was small for analysis.

Univariate correlation analysis was performed to study the relation of PAT index and various other factors. PAT index was found to be positively correlated with E_2 levels ($r^2 = 0.10$; $p < 0.05$), DHEAS levels ($r^2 = 0.12$; $p < 0.05$), and age ($r^2 = 0.04$; $p < 0.05$) levels. There was a negative correlation with total cholesterol ($r^2 = 0.03$; $p < 0.05$) and in males with diastolic BP percentiles ($r^2 = 0.12$;

$p < 0.05$). No correlation was observed between the PAT index and height, weight, BMI, BMI z-score, percent body fat, waist circumference, fasting insulin, glucose, LDL, HDL, triglyceride levels, triglyceride/HDL ratio, GDI, QUICKI, AIR, estrone or estrone sulfate levels.

Discussion

The major findings of this study are: (1) The PAT index, a marker of endothelial function, increases during puberty. (2) PAT index is significantly correlated with E_2 , DHEAS levels and age. (3) PAT index is negatively correlated with higher diastolic BP percentiles and higher total cholesterol levels.

Endothelial dysfunction is an early event in the cascade of processes that culminate in atherosclerosis. In adolescents and young adults, atherosclerosis develops in association with high-risk factors like metabolic syndrome and diabetes [29, 30]. Endothelial function has been shown to alter in presence of metabolic syndrome, menopause and polycystic ovarian syndrome (PCOS) [31–33]. During puberty, the increase in concentrations of estrogens and DHEAS can potentially affect the endothelium-dependent vasodilation. In various in vitro animal models, it was shown that endothelial NO (nitric oxide) production and vasodilation increases during puberty [34, 35]. However, the role of estrogens and DHEAS during puberty in the development of endothelial function has not been studied in humans. Pediatric obesity and dyslipidemia, which are increasing in prevalence, are other factors that can contribute to an increasing prevalence of endothelial dysfunction [36]. The complex hormonal and metabolic changes during puberty make it important to establish norms for endothelial function, possibly enabling detection of cardiovascular compromise at this young age.

This study demonstrates that endothelial function changes in children during puberty. Children in the advanced stages of pubertal development had a higher peripheral vasodilatory response as measured by PAT. These changes were consistent in both sexes and among the three groups of increasing pubertal development, suggesting a potential role for estrogens and DHEAS in the development of vascular function during that period.

In adults, a lower PAT index means worsened endothelial function. A cutoff value of PAT index of 1.35 or less has been reported to have 80% sensitivity and 85% specificity to detect coronary endothelial function [2]. This cutoff value of PAT was studied and established in an older population. It was striking that the PAT index in our healthy pre-pubertal children was comparable to the PAT index reported in adults with cardiovascular disease [2, 37]. Therefore, the standards established for the adult population cannot be extrapolated in the pediatric age groups.

The PAT indices was lowest at 1.42 ± 0.44 (mean $\pm$ SD) in Tanner I participants and higher in Tanner II–III participants at 1.71 ± 0.35 ($p = 0.02$) and Tanner IV–V participants at 1.92 ± 0.38 ($p < 0.001$). The PAT index was also higher in the Tanner IV–V as compared to the Tanner II–III group (0.04). The trend in the increase in the PAT index was similar in both sexes when the data were stratified. The mean PAT index in females increased from 1.66 in the Tanner II–III group to 1.91 in the Tanner

IV–V group (table 2a). In males, the increase in PAT index was noted from 1.41 in Tanner I to 1.78 and 1.93 in Tanner II–III and Tanner IV–V, respectively (table 2b). A lower PAT index in childhood may not be indicative of a disease process but should be assessed in relation to the pubertal status of the child.

The data from this study showed that the PAT index significantly increases with the progression of puberty (Tanner I to Tanner IV–V) and correlates with increasing estradiol ($r^2 = 0.10$) and DHEAS levels ($r^2 = 0.12$). The increase in the PAT index is also correlated with a significant increase in estradiol, estrone, estrone sulfate and DHEAS levels through the various stages of puberty (fig. 1, 2; table 1). A positive correlation of PAT with estradiol and DHEAS and a negative correlation with increased cholesterol and diastolic BP percentile underlines the fact that sex steroids and other factors may play an important role in the development of vascular reactivity and endothelial function. However, there was no correlation between estrone and estrone sulfate. Other factors studied, including height, weight, BMI, insulin, glucose, LDL, and HDL levels, GDI, QUICKI and AIR, failed to show any correlation with PAT index.

During puberty, the exact mechanism of the complex interplay of metabolic parameters and hormonal factors is unknown at this time, but this study gives an outline of the changes in the endothelial-dependent vascular function during puberty. Independently from other changes in puberty such as the increase in estrogens, androgens, DHEA/DHEAS levels, height, accrual of bone mass and changes in behavior, changes in vascular function with increased vasodilation may be of physiological significance. However, more studies will be needed to elucidate the mechanism of endothelial development in humans.

The data do not explain all the correlations of PAT index, suggesting that there might be other unknown factors which may play an important role in the development of endothelial function during puberty. Another limitation of the study is the unequal distribution of males and females in the three groups. This is because very few girls were prepubertal at 12 years of age; on the other hand, few boys were in late puberty at 12 years of age. The analysis of the study was conducted based upon the ultrasensitive estrogen assay irrespective of gender. According to the study protocol, the investigators were not authorized to examine the participants. So, pubertal staging of children was established by ultrasensitive estrogen assay and not by physical examination.

In summary, we report the values for PAT index in healthy children during different stages of puberty. This

underlines the fact that when endothelial function is assessed in children, their pubertal status should be taken into consideration. We have established a relationship between estradiol and DHEAS levels and the PAT index in children during puberty. We have also shown that PAT index increases with advancement in the pubertal stage. The study emphasizes the fact that endogenously produced estrogens and DHEAS can have an effect in maturation of endothelial function. The data from this study can be used as a reference when endothelial function is assessed in various estrogen-deficient conditions like hypogonadism and Turner syndrome.

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