

# Endothelial function evaluation in patients with anorexia nervosa

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## Abstract

**Introduction:** This study evaluated endothelial function in patients with anorexia nervosa (AN) using Endothelial Pulse Amplitude Testing (Endo-PAT) and correlated findings with the patients' history and biochemical data.

**Method:** Twenty-one patients age 13-21 years diagnosed with AN by the Division of Adolescent Medicine at Cohen Children's Medical Center of New York between 6/1/2012 and 5/31/2013 were studied along with 19 healthy controls similar in age and gender distribution. Digital pulse amplitude was examined using Endo-PAT. Raw data were automatically transferred into a reactive hyperemia index (RHI) and the natural log transformation of RHI (LnRHI). Subjects' and controls' electrocardiograms and biochemical markers were obtained.

**Results:** AN and controls had similar RHI ( $P=0.7542$ ) and LnRHI ( $P=0.9497$ ). AN had lower mean weight ( $P<0.0001$ ), height ( $P=0.0207$ ), BMI ( $P<0.0001$ ), resting HR ( $P<0.0001$ ), systolic ( $P<0.0001$ ) and diastolic BP ( $P=0.0141$ ). AN also had lower mean HR during EndoPAT testing ( $P<0.0001$ ), triiodothyronine (T3) ( $P<0.0001$ ), luteinizing hormone (LH) ( $P=0.0055$ ) and estradiol ( $E_2$ ) ( $P=0.0052$ ). Total cholesterol (Chol) ( $P=0.0004$ ) was higher in AN subjects. No correlation was observed between RHI and other parameters.

**Conclusion:** No significant differences in RHI or LnRHI were found between the two groups. There were significantly higher Chol and lower HR, T3, LH and  $E_2$  levels in the AN group compared to controls. There were no correlations of these parameters to RHI.

**Abbreviations:** AN: anorexia nervosa; BMI: body mass index; Chol: total cholesterol; DBP: diastolic blood pressure;  $E_2$ : estradiol; ECG: electrocardiogram; Endo-PAT: Endothelial Pulse Amplitude Testing; FSH: follicle-stimulating hormone; Hcy: homocysteine; HDL: high-density lipoprotein; LDL: low-density lipoprotein; LH: luteinizing hormone; LnRHI: natural logarithm transformation of Reactive Hyperemia Index;  $\ln(Lp(a))$ : natural logarithm transformation of lipoprotein A; Lp(a): lipoprotein A; PAT: Peripheral Arterial Tone; PRL: prolactin; RHI: Reactive Hyperemia Index; SBP: systemic blood pressure; T3: triiodothyronine; TG: triglyceride; TSH: thyroid stimulating hormone

## Background

The cardiovascular complications in patients with eating disorders, especially anorexia nervosa (AN), have been well described, including sinus bradycardia, a prolonged QT interval, decreased left ventricular wall thickness, alterations of left ventricular morphology and function, and silent pericardial effusion [1,2]. There is evidence that these cardiovascular complications are reversible after patients with AN achieve a normal body mass index (BMI) [2,3].

Endothelial dysfunction contributes to atherogenesis and the development of clinical cardiovascular disease [4]. This can occur even

in young children with certain medical conditions [5]. Endothelial pulse amplitude tonometry (Endo-PAT) is a new non-invasive method used to assess peripheral vascular function. It is based on noninvasive Peripheral Arterial Tone (PAT) signal technology that measures endothelium-mediated changes in vascular tone using unique biosensors placed on the fingertips. The changes in arterial tone are elicited by creating a down-stream hyperemic response induced by a standard 5-minute occlusion of the brachial artery. Measurements from the contralateral arm are used to control for concurrent non-endothelial dependent changes in vascular tone. The results are automatically calculated as an index of endothelial function. Endo-PAT has been validated in multiple studies, including in adolescent populations [6-8]. Digital vascular dysfunction assessed by Endo-PAT

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has a strong correlation with multiple cardiovascular risk factors [9].

The association between endothelial dysfunction and an unfavorable lipid profile has also been described in athletic females who have amenorrhea, eating disorders, and osteoporosis, also known as the female athlete triad. The mechanism for endothelial dysfunction and an impaired lipid profile in this group of patients is believed to be related to hypoestrogenism. However, unlike the direct cardiovascular complications, whether the endothelial dysfunction and impaired lipid profile found in young athletic women with amenorrhea is reversible after resumption of menses is still not known [10].

The purpose of this study was to evaluate endothelial function in patients with AN using Endo-PAT and to correlate findings with the patients' histories, and hormonal levels.

## Hypothesis

Patients with AN have abnormal endothelial function demonstrated by endothelial pulse amplitude tonometry (Endo-PAT).

## Methods

### Study population

All patients between the age of 13 and 21 years who were admitted to the adolescent unit at the Cohen Children's Medical Center of New York for eating disorder management, as well as all ambulatory patients seen in the Division of Adolescent Medicine clinic for eating disorder evaluation and treatment, between June 1, 2012 and May 31, 2013, were screened for eligibility. Those who met the diagnostic criteria for AN by DSM-IV [11] were approached for study participation. Healthy control subjects, similar in age and gender distribution, were screened from all patients who presented to the pediatric cardiology and adolescent medicine clinics during the same time period. Subjects with chronic underlying medical conditions (e.g., congenital or acquired heart disease, inflammatory bowel disease, diabetes mellitus, endocrine disease, renal disease), or current cigarette smoking, alcohol, or illegal drug use were excluded. All subjects aged 18 years or greater, or parents of those who were 17 years or younger, gave informed consent, and minors gave assent, before enrolling in the study.

### Demographic, anthropometric and blood pressure measurements

Baseline demographic data were obtained by history, or abstracted from the medical record, and included: race, ethnicity, height, weight, body mass index (BMI), BMI percentile, Tanner stage, heart rate, and systolic and diastolic blood pressures (SBP and DBP). For the subjects with AN, duration of weight loss, percentage of weight loss and last menstrual period were obtained from the subjects' medical records.

### Biochemical analyses

The following hormones were either measured at the time of the study or abstracted from the medical record if the results were within 3 months of the Endo-PAT procedure: total cholesterol (Chol), high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride (TG), lipoprotein A (Lp(a)), homocysteine (Hcy), thyroid stimulating hormone (TSH), triiodothyronine (T3), follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol ( $E_2$ ), and prolactin (PRL). All biochemical analyses were performed at the Northwell Health Core Laboratory.

## Electrocardiogram

Electrocardiograms (ECGs) performed within one week prior to enrollment to the study were obtained from the medical record. For subjects who did not have an ECG performed within that timeframe, an ECG was performed in the pediatric cardiology clinic. All ECGs were interpreted by a pediatric cardiologist.

## Peripheral arterial tonometry

Peripheral arterial tonometry testing was performed in a quiet, restful environment with a comfortable temperature (between 72° to 75°F). The subjects were asked to fast at least 4 hours prior to the procedure and to avoid caffeine consumption for 12 hours before testing. Patients who were on psychotropic medications were not excluded from the study. The testing protocol was performed using the Endo-PAT device as instructed by the manufacturer (Itamar Medical, Caesarea, Israel). Endo-PAT is a Federal Drug Administration (FDA)-approved commercially available tool introduced by the Itamar Corporation. This machine non-invasively measures peripheral arterial tone (PAT), which reflects changes in arterial pulsatile blood volume. The PAT amplitude is equivalent to the arterial pulse volume. The device comprises a pneumatic plethysograph that applies uniform pressure to the entire distal finger surface, allowing measurement of pulse volume changes in the finger. Non-invasive pneumatic probes were placed on the index fingers of both hands. The pulse wave amplitude was recorded continuously from both index fingers. A reactive hyperemia procedure was performed by occluding the brachial artery of the non-dominant arm with a blood pressure cuff at 250 mmHg for 5 minutes. The tracing in the non-occluded arm served as a control for changes in overall physiologic state. The Endo-PAT data were analyzed by a computerized, automated algorithm (Itamar Medical), without any input from the examiner. Endo-PAT provides an index of endothelial function in two forms: The Reactive Hyperemia Index (RHI) and the natural log transformation of RHI (LnRHI). The RHI was defined as the ratio of the average pulse amplitude during the 1 minute period beginning after exactly 90 seconds of reactive hyperemia compared with the average pulse amplitude during the pre-occlusion baseline period. The LnRHI is a similar index to the RHI after natural log transformation. The LnRHI provides a better double-sided distribution that is closer to normal distribution than RHI. It offers better separation between disease states.

## Statistical analysis

Demographic parameters were compared between groups using the two-sample t-test for continuous parameters and the chi-square test, or Fisher's Exact test, for categorical parameters.

The two-sample t-test was used to compare outcomes of interest between the subjects with AN and the healthy, normal controls. Not all outcome variables met the standard assumptions of normality and equality of variance required to conduct a t-test. However, the data was also analyzed using the Mann-Whitney U test, a non-parametric counterpart to the t-test. Results between the t-test and the Mann-Whitney U test did not differ qualitatively. Therefore, for ease of interpretation and consistency, only results from the t-test are reported and presented here. If results between parametric and non-parametric tests differed qualitatively, then an appropriate transformation was applied, such as the natural logarithm, in order to meet the necessary assumptions of the t-test (this applied specifically to Lp(a)).

The correlation of demographic and laboratory results with the Endo-PAT findings was examined using the Spearman correlation coefficient.

All statistical analyses were conducted in SAS version 9.3 (SAS Institute, Cary, NC). Results were considered significant at a p-value of less than 0.05 for all primary aim which was to compare RHI and LnRHI between two groups. A Bonferroni-like adjustment, alpha level of 0.01, was applied to all secondary aims which were the comparison of other biochemical parameters and the correlation of demographic and laboratory results with EndoPAT results.

The study protocol was reviewed and approved by the Northwell Health Institutional Review Board.

## Results

During the study period, 254 subjects who came for eating disorder management, either as an in-patient admission or in the ambulatory clinic, were screened for eligibility. A total of 35 (13.78%) subjects were eligible, 27 (77.14%) of whom were consented and enrolled for the study. The Endo-PAT was performed on 21 (77.78%) subjects; 5 (18.52%) subjects did not come for the appointment and 1 (3.7%) subject withdrew from the study.

For the control group, 350 subjects were screened and 37 (10.57%) subjects were eligible for the study. Twenty-four (64.86%) subjects consented, 2 (8.33%) subjects did not come for the appointment, 1 (4.17%) subject withdrew from the study, and 2 (8.33%) subjects were excluded from the analysis because of lack of cooperation during the Endo-PAT study, which resulted in significant movement artifacts in one subject and a poor Endo-PAT signal in the other subject.

Of the total of 40 subjects who had the Endo-PAT performed (21 patients with AN (52.5%) and 19 normal, healthy controls (47.5%)), two subjects (5%) (1 patient with AN and 1 healthy control) were male. All of the patients with AN were non-Hispanic White. In the control group, 1 (5.26%) subject was Asian, 4 (21.05%) were African American, 1 (5.26%) was Hispanic White, and the remaining were non-Hispanic White (68.43%). A description of demographic parameters is shown in Table 1.

There were no significant differences between the RHI of  $1.84 \pm 0.51$  in the AN group and  $1.90 \pm 0.69$  in the control group ( $P=0.7542$ ) or LnRHI of  $0.58 \pm 0.29$  and  $0.58 \pm 0.35$  ( $P=0.9497$ ) respectively.

The patients with AN had a lower mean weight ( $P<0.0001$ ), height ( $P=0.0207$ ), BMI ( $P<0.0001$ ), resting HR ( $P<0.0001$ ), SBP ( $P<0.0001$ )

**Table 1.** Demographic information.

Variable	Anorexia Nervosa	Controls	P value
Total patients (N)	21	19	
Age (years)	16.44 ± 1.50	16.87 ± 2.13	0.4579
Height (cm)	160.11 ± 6.38	165.02 ± 6.48	0.0207
Weight (Kg)	41.14 ± 6.73	60.86 ± 9.62	< 0.0001
Body Mass Index (Kg/m <sup>2</sup> )	16.00 ± 2.12	22.29 ± 2.74	< 0.0001
Weight loss (%)	23.06 ± 8.04	n/a	
Weight loss Duration (months)	13.71 ± 12.10	n/a	
Resting heart rate (BPM)	52.57 ± 10.91	67.26 ± 7.38	< 0.0001
SBP (mmHg)	95.24 ± 8.83	111.79 ± 9.23	< 0.0001
DBP (mmHg)	58.00 ± 7.22	64.26 ± 8.18	0.0141
Tanner Stage			0.9608
• 3	5 (23.81)	5 (26.32)	
• 4	13 (61.90)	11 (57.89)	
• 5	3 (14.29)	3 (15.79)	

Value are expressed as mean ± SD or number of patients (percentage). BPM = beats per minute, SBP = systolic blood pressure, DBP = diastolic blood pressure. P-value of less than 0.05 is considered statistically significant.

and DBP ( $P=0.0141$ ) than the healthy normal subjects. No significant differences were observed in mean age ( $P=0.4579$ ) or Tanner stage ( $P=0.9608$ ) between the groups.

The patients with AN also had a lower mean HR during Endo-PAT testing ( $P<0.0001$ ), T3 ( $P<0.0001$ ), LH ( $P=0.0055$ ) and E<sub>2</sub> ( $P=0.0052$ ) as compared to the healthy, normal controls (Table 2). The Chol level was higher in the AN subjects compared to the healthy controls ( $P=0.0004$ ). There were no significant differences between the groups for HDL ( $P=0.0546$ ), LDL ( $P=0.0108$ ), TG ( $P=0.3182$ ), ln(Lp(a)) ( $P=0.0447$ ), Hcy ( $P=0.6833$ ), TSH ( $P=0.4421$ ), FSH ( $P=0.6182$ ) or PRL ( $P=0.1052$ ) at a significance level of 0.01.

We further examined the correlation of demographic and laboratory values with Endo-PAT finding using the Spearman correlation coefficient (Table 3). No correlations were observed between BMI, HR during EndoPAT, weight loss, duration of amenorrhea, Chol,

**Table 2.** Comparison of heart rate and biochemical analysis data.

Variable	Anorexia Nervosa		Controls		P value
	N		N		
HR during EndoPAT testing (BPM)	21	53.57 ± 7.88	19	67.79 ± 8.89	< 0.0001
Chol (mg/dL)	13	181.77 ± 22.55	6	138.83 ± 10.46	0.0004
HDL (mg/dL)	13	71.23 ± 15.36	6	57.00 ± 9.86	0.0546
LDL (mg/dL)	13	95.62 ± 19.81	6	69.67 ± 14.31	0.0108
Triglyceride (mg/dL)	13	78.08 ± 28.19	6	61.33 ± 42.36	0.3182
ln(Lipoprotein A)	11	3.46 ± 0.85	5	4.37 ± 0.51	0.0447
Homocysteine (μmol/L)	11	10.43 ± 2.58	6	9.90 ± 2.33	0.6833
Triiodothyronine (ng/dL)	13	67.46 ± 19.68	6	110.33 ± 10.52	< 0.0001
TSH (IU/mL)	21	2.59 ± 1.93	6	1.94 ± 1.27	0.4421
FSH (IU/mL)	20	4.20 ± 3.62	5	5.06 ± 2.30	0.6182
LH (IU/mL)	20	1.30 ± 2.00	5	4.80 ± 3.32	0.0055
Estradiol (pg/mL)	18	16.56 ± 10.07	5	140.60 ± 178.99	0.0052
Prolactin (ng/mL)	19	8.45 ± 5.02	5	13.34 ± 8.31	0.1052

Value are expressed as mean ± SD. HR = heart rate, BPM = beats per minute, Chol = total cholesterol, HDL = high-density lipoprotein, LDL = low-density lipoprotein, TSH = thyroid stimulating hormone, FSH = follicle-stimulating hormone, LH = luteinizing hormone. P-value of less than 0.01 is considered statistically significant.

**Table 3.** Correlation between demographic and biochemical parameters with EndoPAT results.

Parameter	N	Correlation coefficient (ρ)	P-value
Body Mass Index	40	0.2196	0.1734
HR during EndoPAT testing	40	-0.1688	0.2978
Weight Loss <sup>1</sup>	21	-0.4139	0.0621
Amenorrhea duration <sup>1,2</sup>	18	-0.2345	0.3489
Total cholesterol	19	0.0649	0.7918
HDL	19	-0.3423	0.1515
LDL	19	0.1845	0.4497
Triglyceride	19	0.5266	0.0206
ln(Lipoprotein A)	16	-0.0412	0.8797
Homocysteine	17	0.5841	0.0138
Triiodothyronine	19	-0.1984	0.4155
TSH	27	-0.1112	0.5810
FSH	25	0.1444	0.4910
LH	25	-0.0995	0.6361
Estradiol	23	-0.1950	0.3726
Prolactin	24	-0.3480	0.0957

N = total patients, HR = heart rate, HDL = high-density lipoprotein, LDL = low-density lipoprotein, TSH = thyroid stimulating hormone, FSH = follicle-stimulating hormone, LH = luteinizing hormone. P-value of less than 0.01 is considered statistically significant.

<sup>1</sup>Only applies to anorexic subjects.

<sup>2</sup>Two subjects were primary amenorrhea and one subject was male.

HDL, LDL, TG, In(Lp(a)), Hcy, TSH, T<sub>3</sub>, FSH, LH, E<sub>2</sub>, PRL and RHI at a significance level of 0.01. No significant abnormalities were found on ECG in the patients with AN except for various degrees of sinus bradycardia.

## Discussion

To our knowledge, this is the first report that examines endothelial function in adolescent patients with AN by studying the relationship of baseline digital pulse amplitude and digital pulse amplitude hyperemic response, a non-invasive measure of vascular endothelial function. We observed no significant differences in both RHI and LnRHI between the AN subjects and the control group. In addition, we found no significant correlations between RHI and BMI, HR, weight loss, duration of amenorrhea, Chol, HDL, LDL, TG, In(Lp(a)), Hcy, TSH, T<sub>3</sub>, FSH, LH, E<sub>2</sub>, and PRL. We found significantly lower HR, T<sub>3</sub>, LH and E<sub>2</sub> level in the AN group compared to controls. We also found that total cholesterol was significantly higher in the AN group. These findings confirm previous published studies [10,12-16].

There is strong evidence to support that T<sub>3</sub> and E<sub>2</sub> can potentially affect endothelium-dependent vasodilatation [17-22]. In various studies, it has been shown that endothelial function changes during puberty in both human [23,24] and animal models [25,26]. Adolescents in the advanced stages of pubertal development have a higher peripheral vasodilatory response, as measured by Endo-PAT, compared to the initial stages of pubertal development [23,24]. Bhangoo *et al.*, [23] studied 89 children and adolescents at different stages of puberty, which was based on ultrasensitive estrogen assays, and found significant positive correlations between the RHI and steroid hormone levels. These findings are similar to data in the adult population which showed that estrogens enhance the endothelial-dependent flow-mediated vasodilatation [18-20] Estrogens exert their effects by binding to the estrogen receptor (ER) located on the vascular endothelial and smooth muscle cells of the blood vessels [21,22].

Napoli *et al.* [17] conducted a double blind, placebo-controlled trial to study the effect of acute changes in serum T<sub>3</sub> concentration on endothelial function in 10 healthy subjects aged 24 ± 1 year. They found that T<sub>3</sub> exerts direct and significant acute effects on blood vessel resistance by enhancing endothelial dilatation, with a significant increase in forearm blood flow in the extremity receiving a continuous T<sub>3</sub> infusion when compared to the placebo.

Another interesting finding in our study was a significant higher Chol and the tendency of higher LDL levels in patients with AN compared to controls (P=0.0108). An increase in the plasma levels of Chol, especially LDL, is a well-known risk factor for atherosclerotic disease [27]. Previous studies have demonstrated that decreased levels of endogenous estrogen unfavorably modify the lipid profile and vascular function in postmenopausal women [28-30]. Elevated lipid levels, especially LDL, have also been reported in premenopausal women with hypoestrogenic conditions due to caloric deficiency such as in AN or athletic amenorrhea [10,31,32]. There are several reasons for this abnormal lipid profile, which include changes in thyroid hormones, increased lipolysis and decreased endogenous cholesterol synthesis with a resulting decrease in LDL removal [33].

In contrast to previously published studies, we failed to show any correlations between E<sub>2</sub>, T<sub>3</sub>, Chol and LDL with RHI. In addition to a small sample size, this may be because AN patients, despite having well established laboratory risk factors for arteriosclerosis, have normal endothelial function. We suggest that future studies be performed to

investigate whether our findings in patients with AN are reproducible *in vitro*, such as by biochemical markers of endothelial function, to confirm our results. Furthermore, prospective, longitudinal studies to determine whether patients with AN have a higher incidence of arteriosclerosis or acute coronary syndrome in adulthood, as long term consequences of these abnormal biomarkers at a very young age, should be considered.

## Limitations

One limitation of this study is that there were some subjects who had missing laboratory data, which could result in limiting our ability to show significant correlations between the RHI and other variables. The norm for RHI and LnRHI values in pediatric populations have not been determined, which also limited our ability to evaluate whether some of the patients with AN have abnormal endothelial function and to understand the factors that might have caused the abnormality. A further study with a larger cohort, including age-, gender- and Tanner stage-matched controls, is warranted.

## Conclusion

In our cohort, there were no significant differences in RHI or LnRHI between a group of patients with AN and controls. There was a significantly increased mean Chol and decreased HR, T<sub>3</sub>, LH and E<sub>2</sub> levels in the patients with AN compared to controls, which confirms other published data. No correlations were observed between RHI and BMI, HR, weight loss, duration of amenorrhea, Chol, HDL, LDL, TG, In(Lp(a)), Hcy, TSH, T<sub>3</sub>, FSH, LH, E<sub>2</sub>, and PRL.

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The authors declare no financial relationships relevant to this article to disclose.

## Conflict of interest

The authors declare no conflicts of interest relevant to this article to disclose.

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