

## Original Article

# Peripheral artery tonometry demonstrates altered endothelial function in children with type 1 diabetes

Haller MJ, Stein J, Shuster J, Theriaque D, Silverstein J, Schatz DA, Earing MG, Lerman A, Mahmud FH. Peripheral artery tonometry demonstrates altered endothelial function in children with type 1 diabetes. *Pediatric Diabetes* 2007; 8: 193–198.

**Objectives:** To assess the ability of reactive hyperemia–peripheral artery tonometry (RH-PAT) to serve as a surrogate marker of endothelial dysfunction in children with type 1 diabetes (T1D).

**Research Design and Methods:** Forty-four children with T1D [age  $14.6 \pm 2.7$  yr; duration of diabetes  $6.01 \pm 4$  yr; range of diabetes duration 1–16 yr; and hemoglobin A1c (HbA1c)  $8.34 \pm 1.2\%$ ] and 20 children without diabetes (age  $14.1 \pm 1.5$  yr) underwent RH-PAT endothelial function testing after an overnight fast. Height, weight, body mass index (BMI), blood pressure (BP), fasting lipid profile, and glucose level were determined in each child. Children with T1D underwent a second RH-PAT study 4 wk after their initial study to determine the inpatient variability of the technique.

**Results:** Children with T1D had endothelial dysfunction as evidenced by lower mean RH-PAT scores ( $1.63 \pm 0.5$ ) when compared with children without diabetes (mean RH-PAT score  $1.95 \pm 0.3$ ) ( $p = 0.01$ ). Repeat RH-PAT scores were predicted by initial RH-PAT scores ( $p = 0.0025$ ). Mean inpatient standard deviation of RH-PAT score was 0.261 and mean coefficient of variation was 14.8. Variations in RH-PAT score were not explained by differences in glucose, HbA1c, BMI, systolic BP, diastolic BP, or lipids.

**Conclusions:** Although larger validation studies are required, RH-PAT is a promising non-invasive technique to assess endothelial function in children with T1D. Non-invasive measures of endothelial dysfunction may provide the additional risk stratification data needed to justify more aggressive primary prevention of cardiovascular disease in children with T1D.

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**Key words:** adolescents – cardiovascular endothelium – children/physiology – prevention – T1D

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Submitted 9 July 2006. Accepted for publication 16 November 2006

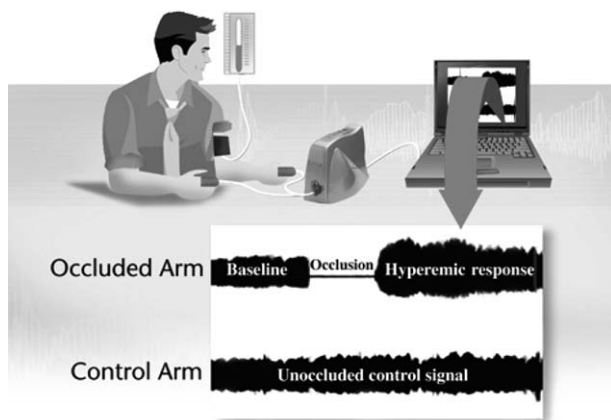
Type 1 diabetes (T1D) is a well-established risk factor for the development of premature cardiovascular disease (CVD) (1, 2). Despite advances in practice over the past 25 yr, the incidence of early CVD in the T1D population remains disproportionately high (3–6).

Although the epidemiologic associations between glycemic control and CVD risk remain weak (2), the Diabetes Control and Complications Trial (DCCT) and its longitudinal follow-up, the Epidemiology of Diabetes Interventions and Complications (EDIC)

study, have demonstrated that the risk of T1D-related microvascular and macrovascular complications is related to long-term glycemic control (7, 8).

However, even with intensive insulin therapy, the majority of children with T1D are unable to maintain near-normal glycemia. Children in the intensive arm of the DCCT were only able to achieve an average hemoglobin A1c (HbA1c) of 8.1% (9), but glycemic control is only one of the several important risk factors in defining CVD risk. Minimizing the long-term risks for CVD in patients with T1D may require early and aggressive management of other important CVD risk factors such as blood pressure (BP) and lipids (10, 11).

Because children rarely experience cardiovascular events, surrogate markers of CVD are needed to provide the additional risk stratification needed to justify and monitor the effects of more aggressive therapy (12). Reactive hyperemia–peripheral artery tonometry (RH-PAT) endothelial function testing employs the same endothelium-dependent mechanism used to induce reactive hyperemia in standard pediatric brachial artery reactivity measurements (13, 14). However, instead of measuring brachial artery diameter, RH-PAT testing uses fingertip plethysmography probes to measure the changes in pulse wave amplitude observed before and after the period of reactive hyperemia. RH-PAT score is then calculated to provide a measure of endothelial function (Fig. 1). In adults, the RH-PAT score has shown an excellent correlation with measures of coronary and peripheral endothelial dysfunction (15, 16). To date, correlations of RH-PAT score with invasive and non-invasive measures of endothelial function have not been performed in children. While differences in RH-PAT score indicate differences in endothelial function,



*Fig. 1.* Schematic of Endo-PAT in use. This figure demonstrates the set up and use of the Endo-PAT. The probes are placed on the index fingers of both arms. A reactive hyperemia procedure is performed by occluding the brachial artery of one arm for 5 min. Data are automatically analyzed by the software package and the reactive hyperemia peripheral artery tonometry score is provided (with permission from Itamar Medical Ltd.).

larger studies that provide receiver operator curves are needed to determine cutoff points for clinical application.

Only one previous study has used the RH-PAT score to demonstrate alterations in endothelial function in a small group of children with T1D ( $n = 20$ ) (12). We expanded the scope of the previous studies of RH-PAT score by measuring the reproducibility of RH-PAT score with serial testing in children with T1D. In addition, our study was designed to determine if RH-PAT score could differentiate endothelial function when comparing a larger group of children with T1D to controls and to determine if RH-PAT endothelial function testing could generate RH-PAT scores similar to those previously reported in a separate population of children with T1D. We hypothesized that children with T1D would have endothelial dysfunction (decreased RH-PAT score) when compared with healthy controls and that RH-PAT scores would have acceptable inpatient variability.

## Materials and methods

The study was approved by the University of Florida and Mayo Clinic Institutional Review Boards. Forty-four children (22 male and 22 female) with T1D for at least 1 yr were recruited from the University of Florida pediatric endocrinology clinic. Twenty control children (12 male and 8 female) were recruited from the Mayo Clinic. A comparison of the T1D and control subjects is shown in Table 1. Control subjects were non-smokers and community based and did not have any co-existing medical conditions or any family history of premature CVD or hyperlipidemia. All T1D and control patients who participated in this study were Caucasian. Following an overnight fast and using identical protocols, RH-PAT endothelial function was assessed in all children using the Endo-PAT device (Itamar Medical Ltd., Caesarea, Israel). The manufacturer of this device did not provide financial support for this study. Height, weight, and BP were recorded before RH-PAT testing. Following RH-PAT testing, blood was obtained for lipid profile and glucose determinations in all subjects and also for HbA1c determination in children with T1D. Four weeks after their initial test, T1D subjects had repeat RH-PAT testing to determine inpatient variability.

RH-PAT testing is a non-invasive technique that combines the traditional flow-mediated dilatation with pneumatic fingertip probes to measure arterial pulse wave amplitude and provide an objective measure of endothelial function. Briefly, the patient sits in a reclining chair with the hands at heart level and propped in a comfortable position such that the fingers are hanging freely. Fingertip probes are placed on both index fingers and pulse wave amplitudes are recorded for the duration of the study. After 5 min of

Table 1. Laboratory and PAT score characteristics of controls and T1D subjects

Characteristic	Diabetes (n = 44)	Controls (n = 20)	p Value
PAT score	1.63 ± 0.5	1.95 ± 0.3	0.01
Mean intrasubject SD	0.261	—	—
Mean intrasubject CV	14.84	—	—
Age (years)	14.6 ± 1.5	14.1 ± 1.5	0.41
BMI (kg/m <sup>2</sup> )	22.6 ± 2.7	21.5 ± 2.6	0.34
Systolic BP (mmHg)	109.5 ± 10	103.9 ± 6.3	0.02
Diastolic BP (mmHg)	69.6 ± 9.6	69.3 ± 4.9	0.87
Glucose (mg/dL)	200.1 ± 89.7 (11.1 ± 4.9 mmol/L)	86.4 ± 11.5 (4.8 ± 0.64 mmol/L)	0.0001
HbA1c (%)	8.34 ± 1.2	—	—
Total cholesterol (mg/dL)	166.4 ± 35 (4.3 ± 0.9 mmol/L)	147.7 ± 20 (3.82 ± 0.52 mmol/L)	0.03
LDL (mg/dL)	87.6 ± 26 (2.26 ± 0.67 mmol/L)	78.1 ± 21 (2.02 ± 0.54 mmol/L)	0.16
HDL (mg/dL)	61.7 ± 12.9 (1.6 ± 0.33 mmol/L)	38.9 ± 11.1 (1.0 ± 0.28 mmol/L)	0.0001
Triglycerides (mg/dL)	85.5 ± 58.7 (0.96 ± 0.66 mmol/L)	68.6 ± 25.8 (0.77 ± 0.28 mmol/L)	0.22

BMI, body mass index; BP, blood pressure; HbA1c, hemoglobin A1c; LDL, low-density lipoprotein; HDL, high-density lipoprotein; T1D, type 1 diabetes. All values reported as mean ± SD except mean intrasubject SD and CV, coefficient of variation.

baseline measurement, arterial flow to the non-dominant arm is occluded for 5 min using a BP cuff inflated to 40 mmHg above systolic pressure. After the 5-min occlusion, the cuff is rapidly deflated to allow for reactive or flow-mediated hyperemia. Pulse wave amplitudes are recorded for at least 5 min after the cuff is deflated. An integrated software program compares the ratio of arterial pressure in the two fingers before and after the occlusion to calculate the RH-PAT score. The RH-PAT score is calculated as the ratio of the average pulse wave amplitude measured over 60 s starting 1 min after cuff deflation to the average pulse wave amplitude measured at baseline. This ratio is normalized to the concurrent signal from the contralateral finger to correct for changes in systemic vascular tone.

### Statistical methods

The primary end-point around which this study was designed was the RH-PAT score. Other variables were analyzed as secondary end-points. The two groups of patients (T1D and control) were compared using a two-sided two-sample t-test. Inpatient standard deviations for RH-PAT score were estimated from the repeated measures and averaged over the patients within each subgroup. With a sample of 44 T1D patients and 20 controls, a two-sided, two-sample t-test had 80% power at  $p = 0.05$  to detect a difference of 0.78 standard deviations between the two groups. Assuming a standard deviation of 0.4, this corresponds to sensitivity to an RH-PAT score difference of 0.32 units. Pearson correlation coefficients were used to evaluate the relationship between initial RH-PAT score and week 4 RH-PAT score as well as to evaluate the correlation between week 4 RH-PAT score, glucose, and HbA1c. A forward stepwise regression model was used to investigate associations between week 4 RH-PAT score and body mass index

(BMI), systolic BP, diastolic BP, glucose, HbA1c, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglycerides.

### Results

The summary statistics for the comparisons are presented in Table 1. Children with T1D ( $n = 44$ ) had endothelial dysfunction as evidenced by lower mean RH-PAT scores ( $1.63 \pm 0.5$ ) when compared with control children ( $n = 20$ ) (mean RH-PAT score  $1.95 \pm 0.3$ ,  $p = 0.01$ ). In children with T1D, the initial RH-PAT score was predictive of the week 4 RH-PAT score ( $p = 0.0025$ ). However, the range of RH-PAT scores was wide. Children with T1D had a range of

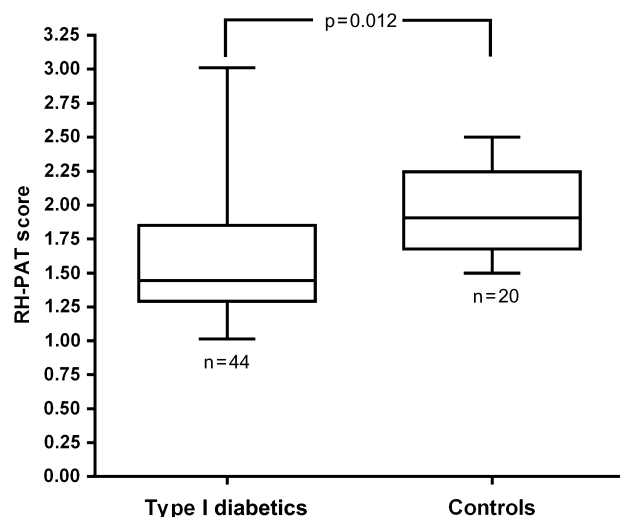


Fig. 2. Box plot of reactive hyperemia-peripheral artery tonometry (RH-PAT) score in controls and subjects with type 1 diabetes (T1D). The box plot demonstrates a significantly lower RH-PAT score in the 44 children with T1D when compared with the 20 control children. Although there was a wide range of RH-PAT scores, the lower RH-PAT score is indicative of relative endothelial dysfunction in the cohort with T1D.

RH-PAT scores of 1.01–3.01, while control children had a range of 1.5–2.5 (Fig. 2). Mean inpatient standard deviation of RH-PAT score in the T1D patients was 0.261 and the mean coefficient of variation for RH-PAT score was 14.84%.

Children with T1D had higher mean systolic BP ( $p = 0.02$ ), mean total cholesterol ( $p = 0.03$ ), and mean HDL ( $p = 0.0001$ ) than control children. No significant differences in age, BMI, diastolic BP, LDL, or triglycerides were observed between the children with T1D and the control children. Pearson correlation coefficients comparing week 4 RH-PAT score with glucose and HbA1c were not significant ( $p = 0.67$  and  $p = 0.56$ , respectively). Use of forward stepwise regression modeling of week 4 RH-PAT score with BMI, systolic and diastolic BP, glucose, HbA1c, total cholesterol, LDL, HDL, and triglycerides was not significant ( $p = 0.16$ ).

## Discussion

This study demonstrates that RH-PAT score can detect group differences in endothelial function between children with T1D and controls. Overall, children with T1D have lower RH-PAT scores than controls. In addition, this study provides the first known measures of the coefficient of variation for RH-PAT endothelial function testing in children.

Several characteristics of our study population warrant further comment. The two study populations compared were of similar age and gender, racially homogeneous, and tested using an identical protocol and technique despite the fact that they derived from different locations in North America (Florida and Minnesota). Our T1D population had higher systolic BP, HDL, and total cholesterol than the control group. While higher systolic BP and total cholesterol are risk factors for CVD, higher HDL is typically associated with lower CVD risk. Nevertheless, HDL cholesterol levels are typically higher in T1D patients than in matched controls (17); yet, CVD risk remains elevated in the T1D population. In addition to these differences in cholesterol metabolism, multiple additional risk factors associated with both increased and decreased CVD risk may explain some of the difference in RH-PAT score between the two groups. We must accept, therefore, that the difference in endothelial function seen in the two groups may be partially explained by selection bias and not by CVD risk associated solely with the disease state of T1D.

In addition, regression modeling of HbA1c, fasting glucose, systolic and diastolic BP, cholesterol, and BMI failed to explain a significant portion of the variability in RH-PAT score. Similar findings have been observed with other surrogate markers of CVD in children with T1D (11). Perhaps, the larger variations in RH-PAT score seen among T1D subjects can

be explained by the acute effects of rapid blood glucose changes experienced by nearly all subjects with T1D as opposed to single measures of blood glucose or HbA1c. As such, additional studies, including measures of change in recent glycemic control, will likely be needed to determine the relative importance of traditional and non-traditional CVD risk factors on RH-PAT score. Finally, while similar measures of endothelial function do predict cardiovascular events, RH-PAT score has not yet been used to evaluate the risk of future cardiovascular events in either adult or pediatric populations.

Despite the limitations of this particular study, RH-PAT score has several potential advantages over traditional reactive hyperemia measurements. Specifically, RH-PAT testing is affordable, reproducible, and, being operator independent, not subject to subjective interpretations of blood vessel diameter associated with brachial artery ultrasounds. While larger studies are needed to confirm the association between low RH-PAT score and traditional measures of endothelial dysfunction, RH-PAT score may be useful as both a research and a clinical tool in assessing endothelial dysfunction in populations at high risk for developing premature CVD. Nevertheless, further validation studies are needed to confirm the relevance of low RH-PAT scores in detecting CVD and predicting future CVD.

Recent EDIC study data demonstrate that intensive diabetes management significantly decreases cardiovascular events among patients with T1D (8). However, the overall risk of CVD in T1D patients remains disproportionately high. Because T1D is still associated with a two- to threefold increased risk of premature CVD, further efforts are needed to accurately identify and aggressively treat those patients at high risk for CVD. CVD is a process rooted in early childhood. A multitude of studies now demonstrate that risk factors present in childhood predict cardiovascular event rates in adulthood (18, 19). Furthermore, autopsy studies have shown that permanent plaques associated with CVD appear in the arteries of children as young as 8 yr of age (20). Still, outside of striving for tight glycemic control in children with T1D, many pediatric endocrinologists struggle with measures to appropriately identify and manage comorbidities associated with CVD (21).

The average HbA1c in our T1D study population (8.34%) approached the HbA1c of the adolescents in the intensive therapy arm of the DCCT and yet differences in endothelial function were identified in our T1D study population compared with our control subjects. Thus, until technological advances provide for even greater improvements in glycemic control for all children with T1D, we are likely to continue to observe average HbA1c levels well above those that eliminate risk of complications even in relatively compliant patients.

Similarly, while slightly higher than those seen in the control population, the average LDL cholesterol (LDL) in our T1D population (87.6 mg/dL, 2.26 mmol/L) and systolic BP (109 mmHg) easily met American Diabetes Association (22) and American Heart Association (22, 23) goals. Recent data have demonstrated better endothelial function in adults with CVD who have LDL levels <80 mg/dL when compared with those with LDL between 80 and 100 mg/dL (24). Thus, adequate primary prevention of CVD in patients with T1D may require both tight glycemic control and aggressive (i.e. pharmacologic) management of lipids and BP to levels lower than those currently labeled as 'normal'. Furthermore, to truly minimize risk of CVD in patients with T1D, initiation of low-dose statins and angiotensin-converting enzyme inhibitors may need to be initiated shortly after diagnosis regardless of cholesterol and BP values in select, high-risk patients.

While prospective studies are needed to determine if more aggressive management of lipids and BP can decrease the CVD risk associated with T1D, non-invasive measures of vascular function such as RH-PAT may provide the additional level of risk stratification needed to justify early initiation of pharmacologic lipid- and BP-lowering therapy in children with T1D.

## Acknowledgements

This study was supported by the Diabetes Action Research and Education Foundation and the Children's Miracle Network with additional funding provided by NIH grants 42288-05 and 39250-06 and GCRC grant MO1-RR00082. Funding was also provided by the Mayo Clinic Clinical Research Fund.

## References

- LAING SP, SWERDLOW AJ, SLATER SD et al. The British Diabetic Association Cohort Study, I: all-cause mortality in patients with insulin-treated diabetes mellitus. *Diabet Med* 1999; 16: 459–465.
- ORCHARD TJ, COSTACOU T, KRETOWSKI A, NESTO RW. Type 1 diabetes and coronary artery disease. *Diabetes Care* 2006; 29: 2528–2538.
- PAMBIANCO G, COSTACOU T, ELLIS D, BECKER DJ, KLEIN R, ORCHARD TJ. The 30-year natural history of type 1 diabetes complications: the Pittsburgh Epidemiology of Diabetes Complications Study experience. *Diabetes* 2006; 55: 1463–1469.
- ORCHARD TJ, FORREST KY, KULLER LH, BECKER DJ. Lipid and blood pressure treatment goals for type 1 diabetes: 10-year incidence data from the Pittsburgh Epidemiology of Diabetes Complications Study. *Diabetes Care* 2001; 24: 1053–1059.
- The absence of a glycemic threshold for the development of long-term complications: the perspective of the Diabetes Control and Complications Trial. *Diabetes* 1996; 45: 1289–1298.
- SOEDAMAH-MUTHU SS, FULLER JH, MULNIER HE, RALEIGH VS, LAWRENSEN RA, COLHOUN HM. High risk of cardiovascular disease in patients with type 1 diabetes in the U.K.: a cohort study using the general practice research database. *Diabetes Care* 2006; 29: 798–804.
- WRITING TEAM FOR THE DIABETES CONTROL AND COMPLICATIONS TRIAL/EPIDEMIOLOGY OF DIABETES INTERVENTIONS AND COMPLICATIONS RESEARCH GROUP. Effect of intensive therapy on the microvascular complications of type 1 diabetes mellitus. *JAMA* 2002; 287: 2563–2569.
- NATHAN DM, CLEARY PA, BACKLUND JY et al. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. *N Engl J Med* 2005; 353: 2643–2653.
- DIABETES CONTROL AND COMPLICATIONS TRIAL RESEARCH GROUP. Effect of intensive diabetes treatment on the development and progression of long-term complications in adolescents with insulin-dependent diabetes mellitus: diabetes control and complications Trial. *J Pediatr* 1994; 125: 177–188.
- SOEDAMAH-MUTHU SS, STEHOUWER CD. Cardiovascular disease morbidity and mortality in patients with type 1 diabetes mellitus: management strategies. *Treat Endocrinol* 2005; 4: 75–86.
- HALLER MJ, SAMYN M, NICHOLS WW et al. Radial artery tonometry demonstrates arterial stiffness in children with type 1 diabetes. *Diabetes Care* 2004; 27: 2911–2917.
- MAHMUD F, EARING MG, LEE RA, LTEIF AN, DRISCOLL DJ, LERMAN A. Altered endothelial function in asymptomatic male adolescents with type 1 diabetes. *Congenit Heart Dis* 2006; 1: 98–103.
- ROSENTHAL DN, CHIN C. Brachial artery reactivity: a modified technique with applicability to children. *J Am Soc Echocardiogr* 1999; 12: 850–852.
- SINGH TP, GROEHN H, KAZMERS A. Vascular function and carotid intimal-medial thickness in children with insulin-dependent diabetes mellitus. *J Am Coll Cardiol* 2003; 41: 661–665.
- KUVIN JT, PATEL AR, SLINNEY KA et al. Assessment of peripheral vascular endothelial function with finger arterial pulse wave amplitude. *Am Heart J* 2003; 146: 168–174.
- BONETTI PO, PUMPER GM, HIGANO ST, HOLMES DR Jr, KUVIN JT, LERMAN A. Noninvasive identification of patients with early coronary atherosclerosis by assessment of digital reactive hyperemia. *J Am Coll Cardiol* 2004; 44: 2137–2141.
- VALABHUI J, DONOVAN J, MCCOLL AJ, SCHACHTER M, RICHMOND W, ELKELES RS. Rates of cholesterol esterification and esterified cholesterol net mass transfer between high-density lipoproteins and apolipoprotein B-containing lipoproteins in type 1 diabetes. *Diabet Med* 2002; 19: 424–428.
- BERENSON GS, SRINIVASAN SR, BAO W, NEWMAN WP 3rd, TRACY RE, WATTIGNEY WA. Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. The Bogalusa Heart Study. *N Engl J Med* 1998; 338: 1650–1656.
- DAVIS PH, DAWSON JD, RILEY WA, LAUER RM. Carotid intimal-medial thickness is related to cardiovascular risk factors measured from childhood through middle age: the Muscatine Study. *Circulation* 2001; 104: 2815–2819.
- LI S, CHEN W, SRINIVASAN SR et al. Childhood cardiovascular risk factors and carotid vascular changes in adulthood: the Bogalusa Heart Study. *JAMA* 2003; 290: 2271–2276.
- SCHWAB KO, DOERFER J, HECKER W et al. Spectrum and prevalence of atherogenic risk factors in 27,358 children, adolescents, and young adults with type 1 diabetes: cross-sectional data from the German diabetes

- documentation and quality management system (DPV). *Diabetes Care* 2006; 29: 218–225.
22. SILVERSTEIN J, KLINGENSMITH G, COPELAND K et al. Care of children and adolescents with type 1 diabetes: a statement of the American Diabetes Association. *Diabetes Care* 2005; 28: 186–212.
  23. WILLIAMS CL, HAYMAN LL, DANIELS SR et al. Cardiovascular Health in Childhood: A Statement for Health Professionals From the Committee on Atherosclerosis, Hypertension, and Obesity in the Young (AHOY) of the Council on Cardiovascular Disease in the Young, American Heart Association. *Circulation* 2002; 106: 143–160.
  24. KUVIN JT, PATEL AR, SLINNEY KA, PANDIAN NG, KARAS RH. Comparison of flow-mediated dilatation of the brachial artery in coronary patients with low-density lipoprotein cholesterol levels <80 mg/dl versus patients with levels 80 to 100 mg/dl. *Am J Cardiol* 2005; 95: 93–95.