Original Article

Noninvasive Evaluation of Endothelial Function and Arterial Mechanics in Overweight Adolescents

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Objective: *To evaluate endothelial function and arterial mechanics in apparently healthy overweight adolescents.* ***Design:*** *Analytical observational study.* ***Setting:*** *Tertiary hospital.* ***Methods:*** *40 asymptomatic, normotensive and non-smoking adolescents (11 to 18 years old) were evaluated. Of these 20 were overweight or obese as per International Obesity Task Force criteria while 20 were controls. High resolution ultrasonography was performed to measure flow mediated and Glyceryltrinitrate induced dilation in brachial artery, and arterial mechanics in common carotid artery.* ***Results:*** *Overweight adolescents had significantly lower ratio of flow mediated dilation to Glyceryltrinitrate mediated dilation (0.40 ± 0.41 versus 0.61 ± 0.17; P = 0.039). On age and sex adjusted multiple regression analysis, the ratio of flow mediated to Glyceryltrinitrate mediated dilation had a significant negative association with body mass index (P = 0.012) and mean skin fold thickness (P = 0.011). However, for mean skin fold thickness, flow mediated dilation also had a significant negative association (P = 0.027). None of the measures of arterial mechanics were significantly different amongst overweights and controls, or significantly associated with either body mass index or mean skin fold thickness.* ***Conclusions:*** *Endothelial function can be mildly impaired in apparently healthy adolescents who are overweight (assessed by body mass index) or adipose (assessed by skin fold thickness). The use of overweight for screening adolescents likely to develop coronary artery disease is therefore justified. Skin fold thickness is a better indicator than Body Mass Index for predicting endothelial function.*

Key words: *Arterial wall mechanics, Body mass index, Coronary artery disease, Endothelial function, Skin fold thickness.*

CORONARY artery disease (CAD) is a leading cause of mortality and morbidity among adults. The prevalence of the disease is rising in India and is assuming epidemic proportions(1). It is well established that risk factors for CAD in adults are obesity, hypertension, dyslipidemia, diabetes mellitus, and smoking. Efforts are now being made to detect risk factors at the earliest in order to prevent CAD. Thus the preventive focus is shifting to older children and adolescents as the available data suggests an increased prevalence of adiposity and clustering of CAD risk factors even in this age group(2,3). The use of overweight has been proposed as a screening tool for risk factor clustering for early identification of persons likely to develop CAD(4).

Endothelial dysfunction is an early physiological event in atherogenesis(5).

Studies *in vitro* have shown that endothelium is abnormal in the earliest stages before plaques exist and certainly before clinical detection of disease(6). One of the accepted non-invasive techniques of quantifying endothelial function is flow mediated dilation of brachial or femoral arteries(7,8). Atherosclerosis is also preceded by a phase of changes in arterial wall mechanics that could have functional consequences even before the appearance of atheromatous changes. These preclinical alterations are generally present at several sites in arterial tree (including common carotid arteries) and seem indicative of overall tendency to develop atherosclerosis(9). These novel echotracking techniques can now be used to investigate the mechanical properties of the common carotid artery and the endothelial function of the brachial artery in children(7); changes in these functions are established markers for coronary artery atherosclerosis.

A recent report(7) from a developed country has documented presence of increased stiffness of the common carotid artery and endothelial dysfunction in severely obese children. However, there is a paucity of similar reports from developing countries and it is still not clear if similar abnormalities are present in less obese or overweight children and adolescents. The current study was therefore designed to provide information on these aspects.

Subjects and Methods

A total of 40 school going adolescents (20 overweight or obese and 20 controls) aged between 11 to 18 years were evaluated at a tertiary hospital after obtaining an informed consent from the parents and children. The appropriate institutional review committee had approved the study. Only asymptomatic, normotensive and non-smoking adolescents were evaluated in the study. The measurements recorded in each child included weight to the nearest 100 g by electronic weighing machine, height to the nearest 1mm by fibre glass tape, skin fold thicknesses (triceps, biceps, subscapular and suprailiac) to the nearest 0.2 mm by Holtain's caliper (mean of three readings at each site) and blood pressure in left brachial artery using appropriate size blood pressure cuffs (mean of three readings). Overweight and obesity were defined by International Obesity Task Force (IOTF) age and sex specific cut off points based on body mass index (BMI)(10).

High resolution ultrasonography by Agilent Sonos 4500 machine was used to evaluate endothelial function and arterial mechanics. The diameter of right brachial artery was measured from two-dimensional ultrasound images with a 10 MHz linear array transducer. The brachial artery was scanned in longitudinal section 2-15 cm above the elbow. The transmit (focus) zone was set to the depth of the near wall, in view of the greater difficulty of evaluating the near than the far wall "m" line (the interface between media and adventitia)(11). Depth and gain settings were set to optimize images of the lumen-arterial wall interface. In each study, scans were taken at rest, during reactive hyperemia, again at rest, and after sublingual Glyceryl-trinitrate (GTN). The subject lay at rest for at least 15 min before a first resting scan was recorded (*Fig.1*). Reactive hyperemia was induced by inflating blood pressure cuff to a pressure of 300 mm Hg for four minutes and then deflating it. A second scan was taken after 45-60 sec of cuff deflation. After 10 minutes, again a resting scan was recorded. Then GTN (400 microgram) aerosol was given sublingually, and the artery was scanned 3 min later. All diameters were measured from the anterior to posterior "m"

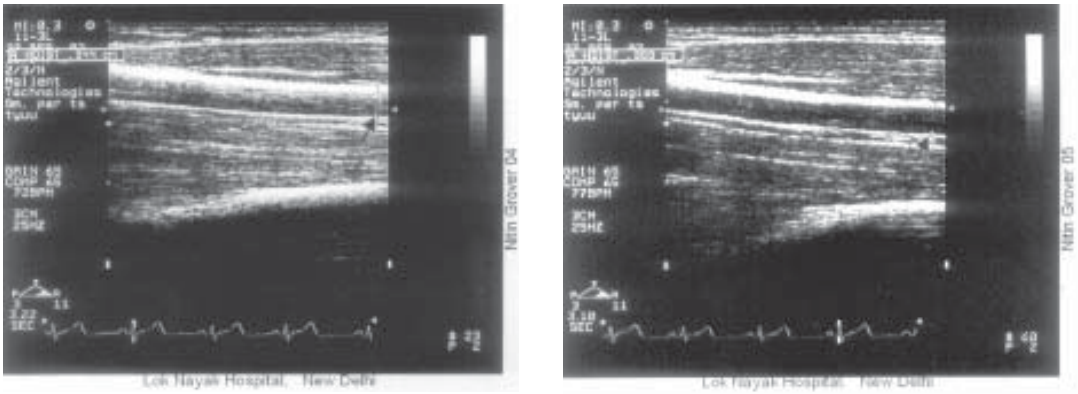

Fig. 1. Brachial artery diameters recorded by electronic callipers at baseline (left) and after reactive hyperemia (right). Black arrows depict the measurement place.

line at end diastole incident with the R-wave on the electrocardiogram (ECG). Four cardiac cycles were analyzed for each scan and measurements averaged. We measured flow mediated dilation, defined as percentage changes in arterial diameter in response to reactive hyperemia (increased flow producing endothelium-dependent vasodilation), and glyceryl-trinitrate-mediated dilation, defined as percentage changes in arterial diameter in response to the endothelium-independent vasodilator glyceryl trinitrate. A lower than expected flow mediated dilation is indicative of endothelial dysfunction and is considered to be an early marker for coronary-artery atherosclerosis.

The common carotid artery (CCA) was scanned in a longitudinal section with 10 MHz vascular probe using a real time B-mode ultrasound imager. The intima-media thickness (IMT) and lumen diameter measurements were performed in all subjects 1 to 2 cm proximal to the carotid bifurcation (*Fig.2*). The sound beam was adjusted perpendicular to the far wall of the vessel, thereby obtaining two parallel echogenic lines corresponding to lumen intima-media and media-adventitia interfaces. These two parallel line echoes were separated by a small echo free space. The IMT was measured between these two leading edges corresponding to the far wall of the CCA. At each longitudinal projection, determinations of IMT were made at the point of greatest thickness and at two points 1.0 cm upstream and 1.0 cm downstream from the point of greatest thickness. The mean of six IMT measurements (3 from left and 3 from right CCA) was used as representative value for each subject(12,13). Ultrasound-assessed carotid IMT is extensively used as a marker of the atherosclerotic burden. The internal lumen diameters of the CCA were measured along the same distance as the intima-media thickness between the near and far wall lumen-intima interfaces in the right CCA(14). The diastolic diameter (Dd) was calculated as the mean of the minimum values of CCA diameter for five consecutive cardiac cycles, measured at R-wave on the ECG. Systolic diameter (Sd) was calculated as the mean value of the maximum CCA diameter during the same cardiac cycles at T-wave on the ECG. The following formulae based on IMT, Sd, Dd and pulse pressure (PP) were used to calculate the other dimensions of arterial mechanics(7): cross-sectional compliance

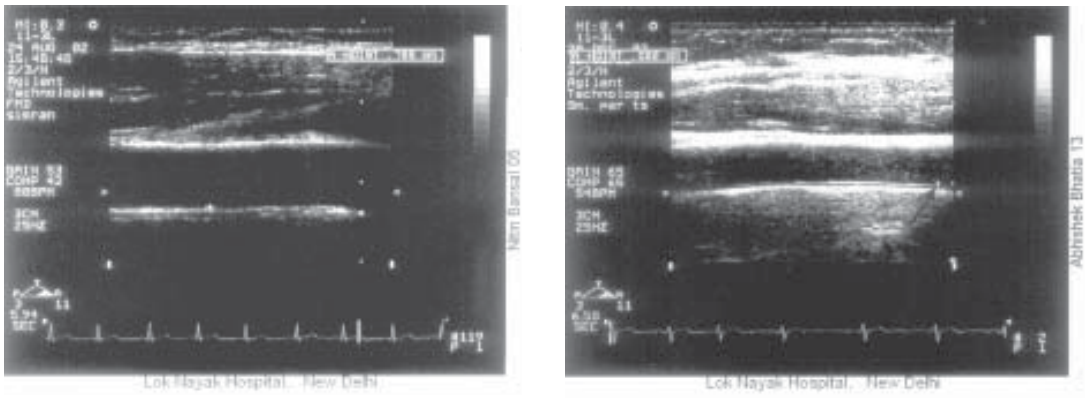

Fig. 2. Recording of common carotid artery diameter (left) and intima-media thickness (right. Intima-media thickness measured by electronic callipers in the posterior wall represents the black line in between the thin and thick white lines (see tip of the black arrow).

(mm^2.mm Hg^{-1}) = p (Sd^2 – Dd^2)/(4*PP; cross-sectional distensibility (mm $Hg^{-1}.10^{-2}$) = (Sd^2 – Dd^2)/(Dd^2*PP); diastolic wall stress (mm $Hg.10^2$) = MAP*Dd/2IMT; incremental elastic modulus (mm Hg. 10^3) = 3(1+ lumen cross-sectional area/wall cross-sectional area)/cross-sectional distensibility; mean arterial pressure (MAP) = 2/3 diastolic BP+1/3 systolic BP; lumen cross-sectional area = p*Dd^2/4; and wall cross-sectional area = $p(Dd/2+IMT)^2$ – $p(Dd/2)^2$. The various calculated dimensions of carotid artery mechanics therefore represent quantification of arterial stiffness. Whereas, compliance provides information on elasticity of the artery as a hollow structure, incremental elastic modulus provides information on the properties of the wall material independently from arterial geometry(7). Lower cross-sectional compliance, lower cross-sectional distensibility, higher wall stress and higher incremental elastic modulus, all indicate greater arterial stiffness. A stiffer artery is considered to be an early marker for cardiovascular disease including coronary artery atherosclerosis.

The various evaluated outcome measures were compared in the overweight subjects and control adolescents by Chi-square or Fisher's exact test (wherever applicable) for proportions and by Student 't' test. The mean skin fold thickness (SFT) was computed as the mean of skin fold thicknesses at four places (biceps, triceps, subscapular and suprailiac). Simple and multiple linear regression analyses were also conducted with various outcome measures as dependent variables.

Results

Among the 'overweight subjects', 15 adolescents (75%) were overweight whereas 5 (25%) were obese as per IOTF classification(10) (*Table I*). The 'overweight subjects' were comparable to controls with respect to age, sex distribution, height and blood pressure. However, they had significantly greater weight, BMI and skin fold thickness.

The flow mediated dilation (%) was lower in overweight subjects but the difference was not statistically significant (P = 0.230). However, the ratio of flow mediated dilation to GTN induced dilation (FMD/GTN) was significantly (P = 0.039) lower in overweight

TABLE I– *Comparison of baseline descriptive characteristics, endothelial function and arterial mechanics in overweight (based on BMI) and control adolescents.*

Variable	Controls (n =20)	Overweight (n = 20)	P value
Males (No.)	18	18	0.698
Age (yr)	14.9 ± 2.3 (11.5 – 18.0)	13.8 ± 2.0 (11.0 –17.0)	0.146
Weight (kg)	44.8 ± 8.4 (31.0 – 63.2)	63.4 ± 12.7 (43.2 – 84.3)	<0.001
Height (m)	1.560 ± 0.093 (1.388 – 1.712)	1.559 ± 0.116 (1.353 – 1.765)	0.975
BMI (kg/m^2))	18.33 ± 2.33 (15.09 – 23.52)	25.83 ± 2.18 (22.66-30.71)	<0.001
Mean skin fold thickness (mm)*	9.0 ± 2.0 (5.6 –13.8)	16.2 ± 3.4 (10.9 – 21.8)	<0.001
Systolic blood pressure (mm Hg)	117 ± 6 (106 –128)	120 ± 7 (108–134)	0.226
Diastolic blood pressure (mm Hg)	75 ± 5 (64 – 86)	74 ± 6 (68 – 86)	0.618
Pulse pressure (mm Hg)	42 ± 4 (33 – 52)	46 ± 4 (38 – 54)	0.013
Brachial measures			
Baseline diameter (mm)	3.39 ± 0.30 (2.87–3.93)	3.47 ± 0.55 (2.79 – 4.34)	0.585
Flow mediated dilation (FMD %)	9.5 ± 2.7 (7.1 –16.2)	7.6 ± 6.5 (–8.8 –15.8)	0.230
Glyceryltrinitrate induced dilation (GTN %)	16.2 ± 4.5 (10.0 – 24.4)	19.4 ± 8.7 (9.6 – 43.4)	0.156
Ratio of flow mediated dilation to Glyceryltrinitrate induced dilation	0.61 ± 0.17 (0.35 – 1.00)	0.40 ± 0.41 (–0.69 – 0.81)	0.039
Common carotid measures			
Systolic diameter (mm)	6.67 ± 0.62 (5.49 – 8.03)	6.96 ± 0.42 (6.23 – 7.78)	0.101
Diastolic diameter (mm)	6.11 ± 0.55 (5.00 – 7.45)	6.37 ± 0.37 (5.73 – 7.01)	0.085
Intima-media thickness (mm)	0.65 ± 0.07 (0.57 – 0.75)	0.68 ± 0.09 (0.56 – 0.89)	0.203
Cross-sectional compliance (mm^2. mm Hg^{-1})	0.14 ± 0.05 (0.07 – 0.26)	0.14 ± 0.04 (0.06 – 0.21)	0.865
Cross-sectional distensibility (mm Hg^{-1}. 10^{-2})	0.29 ± 0.10 (0.10 – 0.50)	0.27 ± 0.08 (0.10 – 0.50)	0.611
Diastolic wall stress (mm Hg . 10^2)	4.24 ± 0.54 (3.40 – 5.30)	4.28 ± 0.76 (2.96 – 5.89)	0.842
Incremental elastic modulus (mm Hg . 10^3)	3.71 ± 1.42 (2.01 – 6.97)	3.82 ± 1.25 (1.76 – 6.96)	0.790

Values depict mean ±SD (range).
*Mean of skin fold thicknesses at biceps, triceps, subscapular and suprailiac points.

subjects. None of the carotid vascular outcome measures (IMT, cross-sectional compliance, cross-sectional distensibility, diastolic wall stress and incremental elastic modulus) were significantly different in the two groups.

BMI was a significant (P <0.01) predictor of the mean SFT; however, it cannot be considered a perfect indicator of the fat content of an individual. The comparative analysis was therefore repeated with two new groups based on mean SFT (11.49 mm cut off), a better parameter of adiposity. The ratio of flow mediated dilation to Glyceryltrinitrate induced dilation was significantly (P = 0.024) lower in adipose subjects (*Table II*). Paradoxical vasoconstriction was documented in two obese subjects instead of flow mediated vasodilation.

Age and sex adjusted multiple regression analyses were also used to evaluate the relationship between the various vascular outcome measures and BMI or mean SFT (*Table III*). The ratio of flow mediated dilation to GTN induced dilation had a significant negative association with BMI (P = 0.012) and mean SFT (P = 0.011). However, for mean SFT, flow mediated dilation also had a significant negative association (P = 0.027). Thus SFT had a better predictive value for endothelial dysfunction. None of the carotid outcome measures could be significantly related to either BMI or mean SFT.

Discussion

The results of this study indicate mildly impaired endothelial function in apparently healthy overweight (assessed by BMI) or adipose (assessed by mean SFT) adolescents. However, there was no simultaneous evidence of altered carotid arterial mechanics. Skin fold thickness proved to be a better indicator than BMI for predicting endothelial dysfunction of brachial vascular tree.

The current study represents a preliminary observational effort on a subject for which information, particularly from a developing country setting, is scanty. However, the endothelial function and arterial mechanics were not related to serum biochemistry (lipids, glucose or insulin). Also an intervention-based design would have provided a firmer evidence for inferring causality.

In a recent study conducted in severely obese (≥3SD BMI), normotensive children in France(7), both flow mediated dilation and GTN mediated dilation were significantly (P <0.001) impaired. A strong positive correlation was documented between flow mediated dilation and apolipoprotein A-I concentration and a negative correlation between flow mediated dilation and fasting insulin concentration. The greater endothelial dysfunction in this study could be related to the severity of obesity and biochemical aberrations in the evaluated subjects. Consistent with this possibility, our study on multiple regression analyses provided evidence that increasing childhood adiposity is associated with greater endothelial dysfunction. Further, the magnitude of endothelial dysfunction in obese adults has been related to the duration and severity of adiposity(15,16). In the French study, the arterial mechanics were also altered in the severely obese but normotensive children. Diastolic diameter, lumen cross-sectional area, diastolic wall stress, and incremental elastic modulus were all significantly higher while cross sectional compliance and distensibility were significantly lower in the obese group. However, the intimal medial thickness was not increased in obese children. In contrast, in our study, we did not document any alteration in arterial mechanics, a difference probably related to the severity of obesity and biochemical aberrations. Another

TABLE II- *Comparison of baseline descriptive characteristics, endothelial function and arterial mechanics in adipose (based on mean SFT) and control adolescents.*

Variable	Controls (mean SFT≥11.49mm) (n = 20)	Adipose (mean SFT>11.49mm) (n = 20)	P value
Males (No.)	18	18	0.698
Age (yr)	14.9 ± 2.34 (11.5 – 18.0)	13.8 ± 2.0 (11.0 – 17.5)	0.127
Weight (kg)	47.4 ± 13.5 (31.0 – 82.3)	60.8 ± 11.7 (43.2 – 84.3)	0.002
Height (m)	1.570 ± 0.104 (1.388 – 1.730)	1.548 ± 0.105 (1.353 – 1.765)	0.490
BMI (kg/m^2)	18.96 ± 3.56 (15.09 – 28.01)	25.21 ± 2.58 (20.71 – 30.71)	<0.001
Systolic blood pressure (mm Hg)	118 ± 6 (106 – 128)	119 ± 8 (108 – 134)	0.689
Diastolic blood pressure (mm Hg)	75 ± 5 (64 – 86)	74 ± 6 (68 – 86)	0.542
Pulse pressure (mm Hg)	43 ± 5 (33 – 54)	45 ± 4 (38 – 50)	0.224
Brachial measures			
Baseline diameter (mm)	3.46 ± 0.35 (2.87 – 4.26)	3.40 ± 0.52 (2.79 – 4.34)	0.650
Flow mediated dilation (FMD %)	9.7 ± 2.6 (7.1 – 15.4)	7.4 ± 6.5 (–8.8 – 16.2)	0.135
Glyceryltrinitrate induced dilation (GTN %)	16.5 ± 5.0 (10.0 – 28.7)	19.1 ± 8.5 (9.6 – 43.4)	0.236
Ratio of flow mediated dilation to Glyceryltrinitrate induced dilation	0.62 ± 0.17 (0.35 – 1.00)	0.39 ± 0.41 (–0.69 – 0.81)	0.024
Common carotid measures			
Systolic diameter (mm)	6.69 ± 0.64 (5.49 – 8.03)	6.94 ± 0.42 (6.23 – 7.78)	0.145
Diastolic diameter (mm)	6.12 ± 0.57 (5.00 – 7.25)	6.35 ± 0.37 (5.73 – 7.01)	0.139
Intima-media thickness (mm)	0.65 ± 0.07 (0.56 – 0.75)	0.68 ± 0.09 (0.56 – 0.89)	0.189
Cross-sectional compliance ($mm^2 . mm\ Hg^{-1}$)	0.14 ± 0.05 (0.08 – 0.23)	0.14 ± 0.05 (0.06 – 0.26)	0.824
Cross-sectional distensibility ($mm\ Hg^{-1} . 10^{-2}$)	0.28 ± 0.08 (0.20 – 0.40)	0.28 ± 0.10 (0.10 – 0.50)	0.980
Diastolic wall stress ($mm\ Hg . 10^2$)	4.27 ± 0.59 (3.40 – 5.51)	4.24 ± 0.73 (2.96 – 5.89)	0.867
Incremental elastic modulus ($mm\ Hg.10^3$)	3.70 ± 1.28 (2.10 – 6.97)	3.82 ± 1.40 (1.76 – 6.96)	0.784

Values depict numbers as mean ±SD (range).

TABLE III–*Age and sex adjusted multiple regression analysis for brachial and common carotid vascular measures based on BMI and mean SFT.*

Variable	P for whole equation	R square	BMI/ mean SFT beta (S.E.)		P	P for age	P for sex
BMI							
Flow mediated dilation	0.131	0.143	–0.287	(0.178)	0.115	0.981	0.055
Glyceryltrinitrate induced dilation	0.471	0.067	–0.379	(0.260)	0.154	0.959	0.642
Ratio of flow mediated dilation to Glyceryltrinitrate induced dilation	0.029	0.219	–0.030	(0.011)	0.012	0.682	0.077
Intima-media thickness	0.699	0.038	0.0027	(0.003)	0.357	0.710	0.616
Cross-sectional compliance	0.584	0.052	0.0014	(0.002)	0.437	0.945	0.305
Cross-sectional distensibility	0.854	0.021	0.0001	(0.000)	0.765	0.958	0.450
Diastolic wall stress	0.455	0.069	2.359	(2.416)	0.335	0.670	0.269
Incremental elastic modulus	0.988	0.003	0.793	(5.067)	0.876	0.897	0.788
Mean SFT							
Flow mediated dilation	0.043	0.200	–0.386	(0.167)	0.027	0.883	0.056
Glyceryltrinitrate induced dilation	0.611	0.049	–0.301	(0.255)	0.246	0.939	0.554
Ratio of flow mediated dilation to Glyceryltrinitrate induced dilation	0.027	0.223	–0.029	(0.011)	0.011	0.777	0.112
Intima-media thickness	0.798	0.027	0.0019	(0.003)	0.505	0.710	0.555
Cross-sectional compliance	0.581	0.052	0.0014	(0.002)	0.432	0.975	0.274
Cross-sectional distensibility	0.762	0.031	0.0002	(0.000)	0.497	0.902	0.443
Diastolic wall stress	0.631	0.046	0.6100	(2.38)	0.799	0.631	0.224
Incremental elastic modulus	0.984	0.004	–1.18	(4.92)	0.812	0.856	0.814

recent study has confirmed that endothelial dysfunction can be detected before adulthood; endothelial function was found to be impaired in children with diabetes mellitus within the first decade of its onset and preceded an increase in carotid IMT(17).

Paradoxical vasoconstriction was documented in two obese subjects instead of flow mediated vasodilation. Although surprising, a similar observation has been documented earlier(14).

Our data suggests that the ratio of flow mediated dilation (acting via nitric oxide release) to Glyceryltrinitrate induced dilation (direct vascular smooth muscle action) may be a more sensitive marker of endothelial function rather than either of these indicators taken individually. This may be related to the fact that the brachial artery has the potential to dilate more when stimulated by Glyceryltrinitrate acting independently of the endothelium than following reactive hyperemia(18); as the later dilation is endothelium dependent.

This study also highlights the subtle but important differences in utilizing BMI and

Key Messages

- Endothelial dysfunction and changes in arterial wall mechanics are considered to be early (preclinical) markers for development of coronary artery disease.
- Endothelial function was mildly impaired in apparently healthy adolescents who were overweight (assessed by BMI) or adipose (assessed by mean skin fold thickness).
- Arterial wall mechanics were not altered in these subjects.
- Skin fold thickness was a better indicator than BMI for predicting endothelial dysfunction.

SFT as indicators of adiposity in adolescents. Mean SFT was a better predictor of endothelial dysfunction in comparison to BMI. This has practical implications for screening for fat associated morbidity. Recent studies using sophisticated techniques of fat assessment indicate that for a comparable body mass index, Indian adults have more body fat and lower muscle volumes than other ethnic groups(19,20). Similar differences have been documented even in the newborn(21).

The documentation of endothelial dysfunction in apparently healthy adolescents who were overweight adds strength to the philosophy of using this indicator as a screening tool for early identification of individuals likely to develop CAD. A confirmation of these findings in other settings and an improvement with intervention measures would establish mild obesity and overweight as premorbid entities.

In conclusion, endothelial function is mildly impaired in apparently healthy adolescents who are overweight or adipose and the use of overweight for screening adolescents likely to develop coronary artery disease is therefore justified.

Contributors: VK was responsible for data collection and preliminary drafting of the manuscript. AK supervised the ultrasonographic studies. HPSS conceived the idea, did the statistical analysis and finalized the drafted manuscript. HPSS and VK will act as the guarantors for the study.

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REFERENCES

1. Krishnaswami S. Prevalence of coronary artery disease in India. Indian Heart J 2002; 54: 103-107.
2. Freedman DS, Dietz WH, Srinivasan SR, Berenson GS. The relation of overweight to cardiovascular risk factors among children and adolescents: The Bogalusa Heart Study. Pediatrics 1999; 103: 1175-1182.
3. Chu NF, Rimm EB, Wang DJ, Liou HS, Shieh SM. Clustering of cardiovascular disease risk factors among obese school children: The Taipei Children's Heart Study. Am J Clin Nutr 1998; 67: 1141-1146.
4. Berenson GS, Srinivasan SR, Bao W, William PN, Tracy RE, Wendy AW. Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. N Engl J Med 1998; 338: 1650-1656.
5. Healy B. Endothelial cell dysfunction: an emerging endocrinopathy linked to coronary disease. J Am Coll Cardiol 1990; 16: 357-358.
6. Drexler H. Endothelial dysfunction: Clinical Implications. Prog Cardiovas Dis 1997; 39: 287-324.
7. Tounian P, Aggoun Y, Dubern B, Varille V, Grand BG, Sidi D, et al. Presence of increased stiffness of the common carotid artery and endothelial dysfunction in severely obese children: a prospective study. Lancet 2001; 358: 1400-1404.
8. Celermajer DS, Sorensen KE, Gooch VM,

Spiegelhalter DJ, Miller OI, Lloyd JK, *et al.* Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. Lancet 1992; 340:1111-1115.

9. Simionescu N, Mora R, Vasile E, Lupu DA, Simionescu M. Prelesional modifications of the vessel wall in hyperlipidemic atherogenesis: extracellular accumulation of modified and reassembled lipoproteins. Ann NY Acad Sci 1990; 598:1-16.

10. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. BMJ 2000; 320:1240-1243.

11. Wendelhag I, Gustavsson T, Suurkula M, Berglund G, Wikstrand U. Ultrasound measurement of wall thickness in the carotid artery: fundamental principles and description of computerized analyzing system. Clin Physiol 1991; 11: 565-577.

12. Mohan V, Ravikumar R, Rani SS, Deep R. Intimal medial thickness of the carotid artery in South Indian diabetic and non-diabetic subjects: the Chennai Urban Population Study (CUPS). Diabetologia 2000; 43: 494-499.

13. Geroulakos G, Ramaswami G, Veller MG, Fisher GM, Renton S, Nicolaides A, *et al.* Arterial wall changes in type 2 diabetic subjects. Diabet Med 1994; 11: 692-695.

14. Aggoun Y, Bonnet D, Sidi D, Girardet JP, Brucker E, Polak M, *et al*. Arterial mechanical changes in children with familial hypercholesterolemia. Arterioscler Thromb Vasc Biol 2000; 20: 2070-2075.

15. Steinberg HO, Chaker H, Leaming R, Johnson A, Brechtel G, Baron AD. Obesity/insulin resistance is associated with endothelial dysfunction. J Clin Invest 1996; 97: 2601-2610.

16. Arcaro G, Zamboni M, Rossi L, Govi G, Turcato E, Armellini F, *et al*. Body fat distribution predicts the degree of endothelial dysfunction in uncomplicated obesity. International J Obes 1999; 23: 936-942.

17. 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.

18. Leeson P, Thorne S, Donald A, Mullen M, Clarkson P, Deanfield J. Non-invasive measurement of endothelial function: effect on brachial artery dilation of graded endothelial dependent and independent stimuli. Heart 1997; 78: 22-27.

19. Chowdhary B, Lantz H, Sjostrom L. Computed tomography determined body composition in relation to cardiovascular risk factors in Indian and matched Swedish males. Metabolism 1996; 45: 634-644.

20. Banerji MA, Faridi N, Atluri R, Chaiken RL, Labovitz HE. Body composition, visceral fat, leptin and insulin resistance in Asian Indian men. J Clin Endocrinol Metab 1999; 84: 137-144.

21. Yajnik CS, Lubreb HG, Rege SS, Naik SS, Deshpande JA, Deshpande SS, *et al*. Adiposity and hyperinsulinemia in Indians are present at birth. J Clin Endocrinol Metab 2002; 87: 5575-5580.