Relation Between Adolescent Cardiovascular Risk Factors and Carotid Intima-Media Echogenicity in Healthy Young Adults: The Atherosclerosis Risk in Young Adults (ARYA) Study

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Background—Echogenicity is an ultrasound measure that reflects arterial wall composition. In adult populations, lower carotid intima-media echogenicity relates to an unfavorable cardiovascular risk burden yet appears to reflect a different aspect of arterial wall remodeling than carotid intima-media thickness (CIMT). Since studies on carotid intima-media echogenicity earlier in life are lacking, we investigated associations between adolescent cardiovascular risk factors and young adulthood carotid intima-media echogenicity and compared this to CIMT.

Methods and Results—In 736 participants of the Atherosclerosis Risk in Young Adults study, information on adolescent anthropometrics, puberty stage, and systolic blood pressure (SBP) was available. In young adulthood, demographics, anthropometrics, and fasting plasma samples were collected. Common CIMT and echogenicity, quantified as gray-scale median (GSM), were evaluated using B-mode ultrasonography. Lower and higher GSM values, respectively, represented lower and higher echogenicity. Associations of adolescent body mass index and SBP with young adulthood GSM and CIMT were evaluated using linear regression analysis. Mean age was 13.5 years in adolescence and 28.4 years in young adulthood (difference: 14.9 years). After full adjustment, adolescent body mass index related to GSM (β=−1.62/SD; 95% CI: −2.79, −0.46; P=0.006), independent of CIMT. Adolescent SBP did not relate to GSM. Moreover, adolescent body mass index (β=8.06 μm/SD [95% CI: 4.12, 11.99], P<0.001) and SBP (β=4.69 μm/SD [95% CI: 0.84, 8.54], P=0.02) related to CIMT.

Conclusions—Adolescent body mass index related to GSM and CIMT in young adulthood; SBP only related to CIMT. Hence, carotid intima-media echogenicity appears to be involved in arterial wall remodeling, yet may mimic a different facet of this process than CIMT. (*J Am Heart Assoc*. 2016;5:e002941 doi: 10.1161/JAHA.115.002941)

Key Words: adolescence • cardiovascular disease risk factors • risk factor • vascular biology • vascular remodeling • young adult

Cardiovascular events and their sequelae remain a major threat to global public health.[1] Atherosclerosis, the main cause of cardiovascular disease, is a chronic inflammatory condition that prompts adverse remodeling of various arterial walls. Atherosclerosis begins in early childhood, yet progresses silently for decades before evolving into clinically apparent cardiovascular disease.[2]

Noninvasive imaging modalities allow for the evaluation of arterial wall remodeling. For example, carotid intima-media thickness (CIMT), measured using B-mode ultrasound, is a widely used marker for subclinical atherosclerosis.[3] Yet there has been increasing interest in measuring arterial wall echogenicity.[4–7] Echogenicity is an ultrasound characteristic of tissue that reflects arterial wall composition instead of its thickness due to tissue's ability to reflect ultrasound waves. Tissue with lower echogenicity (echolucent tissue) reflects few ultrasound waves and therefore appears dark on the ultrasound image. Contrarily, tissue with higher echogenicity (echodense tissue) reflects many ultrasound waves and therefore appears light on the ultrasound image.

Studies have indeed demonstrated that echogenicity of atherosclerotic plaques is related to their composition.

Echolucent plaques mostly enclose lipid pools and hemorrhagic characteristics.[8] As such, they appear to be more vulnerable than echodense plaques that primarily enclose calcifications and fibrous tissue.[9] In fact, several studies observed that echolucent plaques related to unfavorable levels of cardiovascular risk factors and cardiovascular events, whereas echodense plaques did not.[8,10] Also, it was demonstrated that echogenicity of plaques was highly correlated with echogenicity of the intima-media complex of the carotid arterial wall.[11] A more echolucent carotid arterial wall is—similarly to echolucent plaques—thought to encompass a relatively high lipid content and inflammatory characteristics.[5,12] Studies performed in middle- to older-aged populations indeed showed that—similarly to echolucent plaques—an echolucent carotid intima-media was related, independent of CIMT, to unfavorable levels of cardiovascular risk factors.[5,6,12]

In view of the above, it has been postulated that carotid intima-media echogenicity may be involved in atherosclerosis, yet mirrors a pathophysiological aspect of the atherogenic process that is, at least partially, distinct from CIMT.[4,5] However, data on this matter in early stages of atherosclerosis are lacking. Therefore, we studied the relation of cardiovascular risk factors measured in adolescence with carotid intima-media echogenicity at 30 years of age. Additionally, we compared this with the association between the same adolescent cardiovascular risk factors and CIMT.

Methods

Study Design and Population

Our study population consisted of individuals who participated in the Atherosclerosis Risk in Young Adults (ARYA) study. The ARYA study is a prospective, population-based, birth cohort study. A comprehensive rationale and description of the study has been published previously.[13] In short, individuals were suitable for participation in the ARYA study if they were born between 1970 and 1973, attended secondary school in the city of Utrecht, the Netherlands, and if original medical files from the Utrecht Municipal Health Service were obtainable. The medical records were required to include data on birth weight and at least 1 blood pressure measurement obtained in adolescence. Ultimately, the ARYA study population consisted of 750 subjects. From October 1, 1999 to December 31, 2000, these 750 subjects visited our outpatient clinic 2 times within 3 weeks. The Institutional Review Board of the University Medical Center Utrecht approved the ARYA study and written informed consent was collected from all subjects before enrollment.

Measurements

Adolescent measurements

In the Netherlands, the Municipal Health Service carries out regular physical examinations of all children throughout their primary and secondary school years. Data on adolescent anthropometric characteristics, pubertal stage (Tanner stadia), and blood pressure measurements were acquired from the original medical files. Blood pressure measurements were performed using a manual sphygmomanometer. Our analyses were performed on the initial adolescent examination (time point 1) where the average age was 13.5 years. Overweight and obesity in adolescence were assessed using the following method. First, the subjects were subdivided into 5 groups according to their age at the adolescent examination ($\leq$12, 12–13, 13–14, 14–15, and $\geq$15 years). Overweight was defined as having a body mass index (BMI; kg/m^2) between the age-group and sex-specific 85th and 95th percentile for the adolescent study population. Obesity was defined as having a BMI above the age- and sex-specific 95th percentile for the adolescent study population.[13,14]

Young adulthood measurements

With a standardized written questionnaire, information on cardiovascular risk factors was acquired. Additionally, anthropometric measurements were acquired during the first visit to the outpatient clinic. Weight and height measurements were performed with the study subjects wearing indoor clothes without shoes and standing with the feet slightly apart. BMI was calculated. Moreover, blood pressure measurements were acquired 2 times during the first and second visit to the outpatient clinic. Blood pressure was measured at the left brachial artery, with the subject in a sitting position and a time interval of 5 to 15 minutes between each measurement using an automated oscillometric device (Dynamap; Portanje, Schiedam, the Netherlands). Mean diastolic (DBP) and systolic (SBP) blood pressure were computed as the average of all 4 measurements. At the second visit, a fasting venous blood sample was obtained in which glucose, triglycerides, high-density lipoprotein, and total cholesterol levels were measured with a Vitros950 dry-chemistry analyzer (Johnson & Johnson, Rochester, NY). The Friedewald formula was used to compute the levels of low-density lipoprotein cholesterol.[15]

Arterial wall characteristics

Ultrasonography measurements of the right and left common carotid artery were carried out in all subjects with the use of a 7.5-MHz linear array transducer (Acuson Aspen, Mountain View, CA, USA).[13] On a 2-dimensional ultrasonography image of the common carotid artery in the longitudinal plane, the far and near walls of the common carotid artery were visualized as

2 illuminated white lines divided by a hypoechogenic area. The width between the primary edge of the first and second illuminated line of the far wall represented the common CIMT. When an ideal image was acquired, the image was frozen on the R-wave of the electrocardiogram and stored on videotape. This method was carried out at 4 prespecified angles for each side using the Meijer's arc. For the left and right common carotid artery, these angles were 180, 210, 240, and 270 and 180, 150, 120, and 90, respectively. Next, the image measurements were carried out offline. First, the videotaped images were digitized and saved. A region of interest (ROI) indicating the position of the intima-media complex in the far and near wall of the distal common carotid artery was constructed using dedicated software with automated edge detection. The ROI was saved.[16] The anatomical landmark that served as the starting point of the measurement was the beginning of the dilatation of the distal part of the common carotid artery. For each subject, the mean common CIMT of all available angles was calculated. The ultrasonographers and readers were, except for sex and presence of obesity, blinded for each subject's cardiovascular risk profile. Reproducibility of mean common CIMT measurement was high (intraclass correlation coefficient of 0.84 for both left and right carotid arteries).[13]

Echogenicity quantification

A post-hoc analysis of the ultrasound images that were collected in the ARYA study was performed to measure carotid intima-media echogenicity. For the evaluation of carotid intima-media echogenicity, we used an updated version of the originally used software (Artery Measurement System, software developed by Chalmers University of Technology in cooperation with The Wallenberg Laboratory for Cardiovascular Research, Goteborg University, Goteborg, Sweden) and the previously saved ROIs. The echogenicity of the arterial wall can be accurately quantified with the gray-scale median (GSM). Pixels that are white have a gray-value of 255; pixels that are black have a gray-value of 0. Accordingly, the GSM distribution goes from 0 (maximum echolucent) to 255 (maximum echogenic). An example of ultrasound images representing a higher and lower carotid intima-media echogenicity are displayed in Figure. For the current study, the GSM values were collected using the following method: In each ultrasound image, pixels within the ROI were examined and their gray-value was assessed. The median of the gray-values of all pixels within the ROI represented the GSM value for that particular image. The mean GSM value for each subject was acquired by adding up all obtainable GSM values (all 8 angles, both left and right carotid artery) from the far wall of the common carotid artery and dividing it by the number of obtained GSM values. In 14 subjects, GSM and CIMT measurements were missing. Therefore, complete case analysis was performed in 736 of the 750 (98%) subjects. Reproducibility for GSM measurements has previously been assessed using the same device and software program (Artery Measurement System) and was shown to be highly reproducible, as indicated by an intraclass correlation coefficient of 0.79.[7]

Statistical Analysis

Whole cohort and sex-stratified demographic characteristics of the study subjects in adolescence and young adulthood were expressed as numbers and percentages for categorical
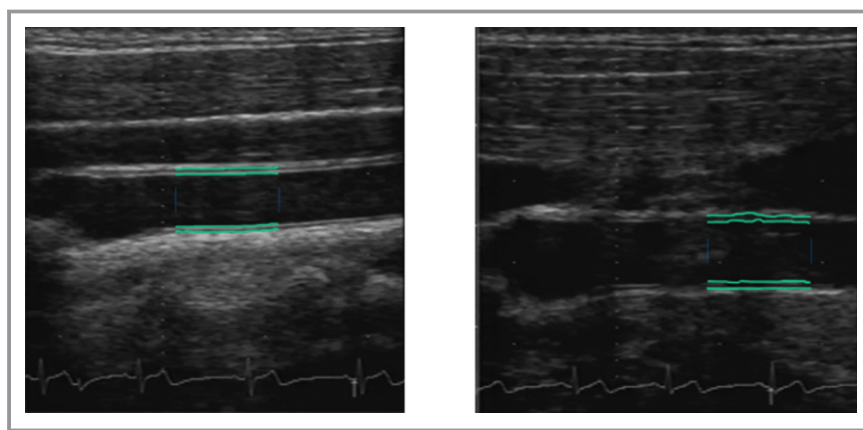
Figure. Example of higher and lower carotid intima-media echogenicity. Example of ultrasound images obtained in the ARYA study illustrating the differences in carotid intima-media echogenicity using identical gain-settings (60 dB). The left image shows a higher carotid intima-media echogenicity that, accordingly, has a higher GSM value and appears light on the ultrasound image. Contrarily, the right image displays a lower carotid intima-media echogenicity (more echolucent carotid intima-media) that, accordingly, has a low GSM value and appears dark on the ultrasound image. The blue lines represent the regions of interest of both images. ARYA indicates Atherosclerosis Risk in Young Adults Study; GSM, gray-scale median.

variables and as medians with interquartile ranges or means with SD or for continuous (non)-normal distributed variables, respectively.

We assessed associations of adolescent BMI and SBP with young adulthood GSM using linear regression analyses in which GSM was the dependent and BMI and SBP were separate independent variables. For this analysis, 2 linear regression models were constructed. First, we constructed a univariable model (Model 1) in which we evaluated the unadjusted association of BMI and SBP with GSM. Then, we constructed a multivariable model (Model 2) in which we adjusted the association of BMI and SBP with GSM for the following possible confounders: adolescent age (years), sex, SBP (mm Hg), BMI (kg/m^2) and adult SBP (mm Hg), adult BMI (kg/m^2), adult total–cholesterol level (mmol/L), and adult mean common CIMT (μm). Since we assumed that adult BMI and adult SBP are intermediates in the causal pathway between the adolescent cardiovascular risk factors and GSM, estimates for adolescent BMI and SBP were not adjusted for adult BMI and SBP. Associations of adolescent BMI and SBP with GSM were also evaluated for quartiles of adolescent BMI and SBP and for BMI subdivided into lean, overweight, and obese adolescents.

Second, we assessed the associations of adolescent BMI and SBP with young adulthood CIMT using the same method as above. CIMT served as dependent variable and adolescent BMI and SBP served as separate independent variables. Furthermore, since puberty stage and adult age may relate to GSM, CIMT, the adult cardiovascular risk factors, and adolescent BMI and SBP, we performed additional analyses in which we further adjusted our multivariable models for puberty stage and adult age.

Finally, we performed 2 subanalyses. First, we assessed differences in the adolescent cardiovascular risk factor levels as well as differences in adolescent age and sex (dependent variables) between 2 "extreme" groups (independent variable). The first group comprised a subset of participants with a high GSM (≥75th percentile) and low CIMT (≤25th percentile). The second group comprised a subset of participants with a low GSM (≤25th percentile) and high CIMT (≥75th percentile) (independent variable; reference category: High GSM/low CIMT group). Second, we assessed the differences in the abovementioned variables between a subset of participants with a high GSM (≥75th percentile) and high CIMT (≥75th percentile) and a subset of participants with a low GSM (≤25th percentile) and low CIMT (≤25th percentile) (independent variable; reference category: High GSM/high CIMT group). Since age and sex are considered key drivers of cardiovascular risk, we adjusted the difference between the 2 groups for adolescent age and sex. For both subanalyses, we used linear regression models for the dependent variables adolescent age, adolescent SBP, and

adolescent BMI and a logistic regression model for the dependent variable sex.

To enable effect size comparisons, the means of adolescent BMI and SBP were divided by their SDs to create a standardized scale. The described regression coefficient reflects the change in GSM, adult cardiovascular risk factor level, or CIMT related to 1 SD increase in adolescent BMI or SBP. Also, assumptions for linear regression models were assessed; there were no violations. We did not report sex-stratified estimates since the interactions between sex and adolescent BMI and adolescent SBP were not significant (*P*=0.27 and 0.41, respectively).

Conclusions were based on *P*-values and (standardized) linear and logistic regression coefficients (βs and odds ratios, respectively) with 95% CIs. Statistical significance was defined as $P_{2\text{ sided}} < 0.05$. Data analyses were performed using the statistical environment R (R-studio version 3.1.2, R-studio, Boston, MA, USA).

Results

Table 1 summarizes the characteristics of the study population. The 736 subjects under study had a mean age of 13.5 years in adolescence and 28.4 years in young adulthood; 47% were male. Median adolescent BMI was 18.2 kg/m^2 (16.7, 20.0); mean SBP was 110.0 mm Hg (±12.0). Moreover, 70 (9.5%) adolescents were overweight and 43 (5.8%) were obese. Young adulthood mean common CIMT and mean far-wall GSM were 487.1 μm (±50.2) and 78.5 (±14.8), respectively.

Tables 2 and 3 and Table S1 summarize the results of the associations of adolescent BMI and SBP with young adulthood GSM. The multivariable model (Model 2) demonstrated an inverse association of adolescent BMI with young adulthood GSM: 1 SD higher adolescent BMI related to a 1.62 ([95% CI: −2.79, −0.46], *P*=0.006) lower young adulthood GSM. SBP did not relate to GSM (Table 2). There was no difference in GSM between obese and overweight adolescents as compared to their lean counterparts (Table 3). Yet each higher fourth of adolescent BMI (reference category: first fourth) related to a 0.51 ([95% CI: −3.53, 2.50], *P*=0.73), 2.68 ([95% CI: −5.75, 0.39], *P*=0.09), and 3.40 ([95% CI: −6.65, −0.16], *P*=0.04) lower GSM, respectively. Of note, fourths of SBP did not relate to GSM (Table S1).

Tables 2 and 3 and Table S2 summarize the results of the associations of adolescent BMI and SBP with young adulthood CIMT. The multivariable model showed a positive association of adolescent BMI and SBP with CIMT. One SD increase in adolescent BMI and SBP related to an 8.06 μm ([95% CI: 4.12, 11.99], *P*<0.001) and to a 4.69 μm ([95% CI: 0.84, 8.54], *P*=0.02) increase in CIMT, respectively (Table 2). Furthermore, obese adolescents had a 24.55 μm higher CIMT as compared to their lean counterparts ([95% CI: 9.07, 40.02], *P*=0.002)

Table 1. Baseline Characteristics

	All (n=736)	Men (n=344)	Women (n=392)
Adolescence			
Age (y), mean (±SD)	13.5 (1.1)	13.5 (1.1)	13.4 (1.2)
SBP (mm Hg), mean (±SD)	110.0 (12.0)	112.0 (12.0)	109.0 (11.0)
DBP (mm Hg), mean (±SD)	67.0 (10.0)	67.0 (10.0)	66.0 (10.0)
BMI (kg/m^2), median (IQR)	18.2 (16.7, 20.0)	17.9 (16.7, 19.5)	18.5 (16.8, 20.6)
Breast development stage (0–5), mean (±SD)	—	—	3.6 (1.2)
Genital development stage (1–6), mean (±SD)	—	2.9 (1.2)	—
Pubic hair development stage (0–6), mean (±SD)	3.3 (1.4)	2.9 (1.3)	3.7 (1.4)
Had menarche, n (%)	—	—	235 (59.9)
Time between measurements (yrs), median (IQR)	15.0 (13.9, 15.9)	14.9 (13.9, 15.8)	15.0 (13.9, 15.9)
Young adulthood			
Age (yr), mean (±SD)	28.4 (0.9)	28.4 (0.9)	28.4 (0.9)
BMI (kg/m^2), median (IQR)	23.91 (21.7, 26.7)	24.3 (22.0, 26.6)	23.4 (21.1, 26.8)
SBP (mm Hg), mean (±SD)	125.0 (13.0)	131.0 (12.0)	121.0 (12.0)
DBP (mm Hg), mean (±SD)	72.0 (8.0)	73.0 (8.0)	71.0 (8.0)
Cigarette smoking status, yes (%)	221 (30.0)	121 (35.3)	100 (25.5)
Total cholesterol (mmol/L), mean (±SD)	4.8 (0.9)	4.8 (1.0)	4.8 (0.8)
HDL cholesterol (mmol/L), mean (±SD)	1.44 (0.4)	1.3 (0.3)	1.6 (0.4)
LDL cholesterol (mmol/L), mean (±SD)	2.8 (0.9)	2.9 (0.9)	2.7 (0.8)
Triglycerides (mmol/L), median (IQR)	1.1 (0.8, 1.6)	1.1 (0.8, 1.7)	1.1 (0.8, 1.5)
Glucose (mmol/L), median (IQR)	5.0 (4.7, 5.2)	5.0 (4.7, 5.2)	5.0 (4.7, 5.2)

SBP indicates systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; IQR, interquartile range; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

(Table 3). Additionally, each higher fourth of adolescent BMI (reference category: first fourth) related to an 11.92 μm ([95% CI: 1.62, 22.23], *P*=0.02), 13.73 μm ([95% CI: 3.26, 24.20], *P*=0.01), and 20.26 μm ([95% CI: 9.25, 31.28], *P*<0.001) higher CIMT. Fourths of SBP did not relate to CIMT (Table S2).

Tables 4 and 5 summarize the results of the age- and sex-adjusted differences in the studied adolescent cardiovascular risk factors, adolescent age and sex between the above-described groups. Regarding the comparison between the high GSM/low CIMT and low GSM/high CIMT group, only adolescent BMI was significantly different between the 2 groups. The age- and sex-adjusted model showed that adolescent BMI was 1.97 kg/m^2 higher in the low GSM/high CIMT group as compared to the high GSM/low CIMT group (95% CI: 0.95, 2.99, *P*<0.001) (Table 4). Moreover, regarding the comparison between the high GSM/high CIMT and low GSM/low CIMT group, no significant age- and sex-adjusted difference in adolescent cardiovascular risk factor levels of adolescent age and sex was observed (Table 5).

Additional analyses with puberty stage and adult age as variables in our model did not affect our results, suggesting that the relation of adolescent BMI and SBP with echogenicity of the carotid intima-media later in life was independent of these factors.

Discussion

The present study showed that a higher adolescent BMI related to a lower common carotid intima-media echogenicity in young adulthood, independent from CIMT, suggesting that carotid intima-media echogenicity indeed mirrors an aspect of arterial wall remodeling that is, at least partially, distinct from CIMT. Although this relation was not primarily determined by the overweight and obese adolescents, the highest quartile of adolescent BMI did exert the strongest effect on GSM. In addition, although adolescent SBP did not relate to GSM, a higher adolescent BMI and adolescent SBP both related to a higher CIMT. For BMI, this relation was primarily induced by the obese adolescents. Hence, mainly metabolic factors, and not muscular hypertrophy and hemodynamic remodeling, appear to be important determinants of arterial wall echogenicity. However, the subanalyses demonstrated that adolescent BMI was significantly higher in the low GSM/high

Table 2. Relation of Adolescent BMI and SBP With Young Adulthood GSM and CIMT (n=736)

	Young Adulthood GSM*	Young Adulthood CIMT (μm)*
Adolescent BMI per SD[†‡]		
Model 1	−2.19 (−3.25, −1.14)	9.02 (5.47, 12.57)
P-value[§]	<0.001	<0.001
Model 2	−1.62 (−2.79, −0.46)	8.06 (4.12, 11.99)
P-value[§]	0.006	<0.001
Adolescent SBP per SD[†‖]		
Model 1	−0.69 (−1.77, 0.38)	8.22 (4.66, 11.77)
P-value[§]	0.21	<0.001
Model 2	−0.15 (−1.28, 0.98)	4.69 (0.84, 8.54)
P-value[§]	0.79	0.02

BMI indicates body mass index; CIMT, carotid intima-media thickness; GSM, gray-scale median; SBP, systolic blood pressure.
*Values are standardized betas (per 1 SD increase in adolescent BMI or SBP) with 95% CIs.
[†]Model 1: univariable. Model 2: adjusted for adolescent age, sex, BMI, and SBP and adult total cholesterol level, SBP, BMI, and CIMT.
[‡]Model 2 not adjusted for adult BMI.
[§]P-value of model.
[‖]Model 2 not adjusted for adult SBP.

Table 3. Comparison of Lean, Overweight, and Obese Adolescents in Their Relation With Young Adulthood GSM and CIMT (n=736)

	Young Adulthood GSM*	Young Adulthood CIMT (μm)*
Overweight adolescent[†]		
Model 1 (reference: lean adolescent)	−2.02 (−5.66, 1.62)	9.86 (−2.37, 22.09)
P-value[‡]	0.28	0.11
Model 2 (reference: lean adolescent)	−0.83 (−4.43, 2.76)	5.21 (−7.08, 17.51)
P-value[‡]	0.65	0.41
Obese adolescent[†]		
Model 1 (reference: lean adolescent)*	−6.23 (−10.79, −1.67)	30.04 (14.74, 45.34)
P-value[‡]	0.007	<0.001
Model 2 (reference: lean adolescent)*	−3.00 (−7.55, 1.55)	24.55 (9.07, 40.02)
P-value[‡]	0.20	0.002

CIMT indicates carotid intima-media thickness; GSM, gray scale median.
*Values are betas with 95% CIs.
[†]Model 1: univariable. Model 2: adjusted for adolescent age, sex, and systolic blood pressure (SBP), and adult total cholesterol level, SBP and CIMT.
[‡]P-value of model.

CIMT group as compared to the high GSM/low CIMT group and that there was a trend toward a significantly higher adolescent BMI in the high GSM/high CIMT group as compared to the low GSM/low CIMT group. From these subanalyses it appears that although both GSM and CIMT contribute to cardiovascular risk, BMI does tend to relate more strongly to CIMT than to GSM. Yet these results should be interpreted with care since few study subjects were eligible for the subanalyses.

The pathophysiology of carotid intima-media echogenicity is not entirely known. Histological studies observed that carotid artery plaques that have an echolucent or echogenic appearance on ultrasound enclose different matter.[8,9] An echolucent plaque primarily comprises an aggregation of lipids, macrophages, a necrotic core, and a weak fibroid cap.[8,9] Contrarily, an echogenic plaque is calcified and comprises an accumulation of fibroid tissue.[8,9] Due to their difference in enclosed matter, it has been implied that, as compared to echogenic plaques, echolucent plaques are more vulnerable to rupture and thus may lead to unfavorable cardiovascular sequelae.[8,10] Research performed in subjects with cardiovascular disease indeed described that echolucent atherosclerotic plaques related to unfavorable levels of cardiovascular risk factors, events, and mortality whereas echogenic atherosclerotic plaques did not.[8,10] The same applies to common carotid intima-media echogenicity. Studies performed in middle- to older-aged healthy adults and in adults with an increased cardiovascular risk burden observed that unfavorable levels of the cardiovascular risk factors age,

sex, BMI, high-density lipoprotein cholesterol, and triglycerides related to a decreased GSM and thus to a lower carotid intima-media echogenicity, thus reflecting a more echolucent carotid intima-media.[5–7,11,12,17] Our results expand these observations to a younger, healthy population.

The possible significance of measuring arterial wall echogenicity is that it might reflect a pathophysiological aspect of arterial wall remodeling that is different from CIMT, thereby providing other information on factors associated with arterial remodeling (ie, cardiovascular risk factors) than CIMT. Prior studies demonstrated that GSM and CIMT only weakly correlated to each other and that GSM related to cardiovascular risk factors independent of CIMT.[4–6,12] The present study also observed a modest, inverse correlation between GSM and CIMT (Spearman's $r=-0.23$). Moreover, we showed that adolescent BMI related to CIMT and GSM independent of CIMT. Yet adolescent SBP only related to CIMT. It has been postulated that CIMT mainly reflects hemodynamic remodeling and smooth muscle cell hypertrophy, making blood pressure a key cardiovascular risk factor. However, echogenicity is primarily influenced by metabolic factors, making BMI a crucial cardiovascular risk factor.[4] In echogenicity, the presence and extent of lipoprotein content

Table 4. Age- and Sex-Adjusted Differences in Adolescent Cardiovascular Risk Factors Between Participants With a GSM ≥75th Percentile and CIMT ≤25th Percentile (High GSM/Low CIMT) and Participants With a GSM ≤25th Percentile and CIMT ≥75th Percentile (Low GSM/High CIMT)

	High GSM/Low CIMT (n=70)	Low GSM/High CIMT (n=65)	Age and Sex Adjusted Difference*[¶]	P Value[§]
Adolescent age (y), mean (±SD)[†‖]	13.4 (1.2)	13.5 (1.1)	0.07 (−0.33, 0.46)	0.74
Sex (men), n (%)[‡‖]	32 (45.7)	39 (60.0)	1.16 (0.93, 1.45)	0.47
Adolescent SBP (mm Hg), mean (±SD)[†]	108.1 (11.5)	112.7 (12.9)	3.89 (-1.00×10^{-3}, 7.79)	0.05
Adolescent BMI (kg/m^2), median (IQR)[†]	17.6 (16.1, 19.4)	19.5 (17.3, 21.0)	1.97 (0.95, 2.99)	<0.001

BMI indicates body mass index; CIMT, carotid intima-media thickness; GSM, gray-scale median; IQR, interquartile range; SBP, systolic blood pressure.

*Values are betas (†) and odds ratios (‡) reflecting the adolescent age and sex-adjusted difference in adolescent cardiovascular risk factor levels between both groups with 95% CIs.

[§]P-value of model.

[‖]The regression coefficients for adolescent age and sex are only adjusted for sex and adolescent age, respectively.

[¶]Reference category: High GSM/low CIMT group.

in the arterial intima appears to play a vital part since lipid accumulation appears echolucent on ultrasound, yet does not necessarily imply intimal thickening. However, CIMT appears to mainly mirror arterial wall remodeling due to changes in blood flow.[4,8,10] This hypothesis is supported by our study and other studies that observed that GSM mainly related to levels of metabolic cardiovascular risk factors, whereas CIMT had a more robust association with the cardiovascular risk factors age and blood pressure.[4–7,11]

Nevertheless, as opposed to our results, some studies did report a relation between SBP and GSM.[4,18] This may be due to the cross-sectional nature of these studies; cross-sectional relations are usually stronger than relations with a time interval between determinant and outcome. Hence, the GSM value in adulthood is possibly primarily determined by the acquired adult SBP. Another explanation may be that, contrary to prior echogenicity studies, our study population is young and healthy. Therefore, lipid accumulation into the arterial intima, an early phase of atherosclerosis, may already have occurred whereas hemodynamic remodeling, which is mirrored in CIMT and takes a longer time to develop, may

not.[4,8,10] These reasons might also clarify why in the present study adolescent BMI exerted a stronger effect on CIMT than adolescent SBP and a stronger effect on CIMT than on GSM.

Showing the association between adolescent BMI and echogenicity in young adulthood is a step in the process of determining whether echogenicity reliably detects adverse arterial wall remodeling already from a young age onward and is distinct from CIMT. The possible use of this proxy for cardiovascular research and cardiovascular risk assessment purposes in young individuals warrants further exploration. Hence, various aspects should be elucidated. The relation of GSM with cardiovascular risk factors and cardiovascular disease morbidity and mortality and the role of GSM in monitoring the effect of anti-atherosclerotic therapy require further evaluation in larger observational cohorts and clinical trials. Only 1 clinical trial has been carried out in low-risk, middle-aged subjects demonstrating that, in general, rosuvastatin therapy did not modify arterial wall echogenicity.[7] Yet the researchers suggested that in subjects with a low echogenic vascular wall at baseline, a possible effect of rosuvastatin was highly conceivable.[7] Next to studies

Table 5. Age- and Sex-Adjusted Differences in Adolescent Cardiovascular Risk Factors Between Participants With a GSM ≥75th Percentile and CIMT ≥75th Percentile (High GSM/High CIMT) and Participants With a GSM ≤25th Percentile and CIMT ≤25th Percentile (Low GSM/Low CIMT)

	High GSM/High CIMT* (n=30)	Low GSM/Low CIMT* (n=24)	Age and Sex Adjusted Difference*[¶]	P Value[§]
Adolescent age (y), mean (±SD)[†‖]	13.6 (0.9)	13.0 (1.1)	−0.50 (−1.07, 0.07)	0.09
Sex (men), n (%)[‡‖]	16 (53.3)	6 (25.0)	0.77 (0.59, 1.01)	0.06
Adolescent SBP (mm Hg), mean (±SD)[†]	108.2 (12.4)	104.8 (11.2)	−2.36 (−9.29, 4.57)	0.50
Adolescent BMI (kg/m^2), median (IQR)[†]	18.2 (17.3, 19.9)	17.0 (16.1, 18.5)	−0.93 (−1.97, 0.10)	0.08

BMI indicates body mass index; CIMT, carotid intima-media thickness; GSM, gray-scale median; IQR, interquartile range; SBP, systolic blood pressure.

*Values are betas (†) and odd's ratio's (‡) reflecting the adolescent age- and sex-adjusted difference in adolescent cardiovascular risk factor levels between both groups with 95% CIs.

[§]P-value of model.

[‖]The regression coefficients for adolescent age and sex are only adjusted for sex and adolescent age, respectively.

[¶]Reference category: High GSM/high CIMT group.

performed in living individuals, autopsy studies are a necessity to allow for an improved understanding of the biological trail underlying carotid intima-media echogenicity. Not only may this further unravel the pathophysiology of echogenicity, but also it possibly allows for the preclinical identification of high-risk individuals at a young age.

This study has some limitations. First, the relation between adolescent BMI and echogenicity lost its statistical significance when we additionally adjusted for adult BMI. It appears that adolescent BMI does not exert an independent effect on GSM and that GSM is primarily determined by the acquired adult BMI. Yet this is logical given the strong correlation between adolescent and adult BMI (Spearman's r=0.62), the cross-sectional nature of the relation between adult BMI and GSM, and the notion that adult BMI is probably an intermediate in the causal pathway between adolescent BMI and GSM. Therefore, we feel that it is justified that we did not adjust our multivariable model for adult BMI. Second, since we performed a post hoc analysis of the ultrasound images to measure GSM, procedures for image collection and processing were not specially designed for GSM measurements. GSM measurements can be affected by gain-settings and study subject features may in turn affect the gain settings. This may have generated an increase in measurement error. Yet it is unlikely that this has affected our results. If anything, an underestimation may have occurred. An analysis of a random selection of saved images demonstrated that there was no large variation in gain-settings. Moreover, it has been demonstrated that variation in gain-settings leaves the strength and direction of the studied relations unchanged.[19] Third, we were unable to assess the effect of other potential adolescent cardiovascular risk factors on young adulthood GSM since information on these factors was lacking. Therefore, our conclusion is restricted to the cardiovascular risk factors available at baseline. Finally, GSM measurements were not performed in adolescence. Hence, it is unknown whether participants with lower echogenicity in young adulthood may already have had a lower echogenicity in adolescence.

In conclusion, a higher adolescent BMI related to a lower carotid intima-media echogenicity and to a higher CIMT in young adulthood, whereas a higher adolescent SBP solely related to a higher CIMT. These results suggest that carotid intima-media echogenicity may indeed reflect arterial wall remodeling from adolescence onward, yet may mirror a pathophysiological pathway that is, at least partially, distinct from CIMT.

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Disclosures

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Table S1. Relation between quartiles of adolescent BMI and SBP and GSM in young adulthood (*n*=736)

	Q1*†	Q2*†	Q3*†	Q4*†
Adolescent BMI*‡§				
Range	13.51 - 16.73	16.73 - 18.19	18.19 - 19.97	19.97 - 33.71
Model 1 (reference: Q1)	-	-0.90 (-3.91, 2.11)	-3.20 (-6.21, -0.20)	-4.68 (-7.69, -1.67)
P value‖	-	0.56	0.04	0.002
Model 2 (reference: Q1)	-	-0.51 (-3.53, 2.50)	-2.68 (-5.75, 0.39)	-3.40 (-6.65, -0.16)
P value‖	-	0.73	0.09	0.04
Adolescent SBP*†**				
Range	80.00 - 100.00	100.00 - 110.00	110.00 - 120.00	120.00 - 165.00
Model 1 (reference: Q1)	-	-1.24 (-3.97, 1.49)	-1.24 (-4.28, 1.80)	-1.40 (-5.23, 2.43)
P value‖	-	0.37	0.42	0.47
Model 2 (reference: Q1)	-	-1.39 (-4.10, 1.31)	-0.66 (-3.74, 2.43)	0.69 (-3.23, 47.61)
P value‖	-	0.31	0.68	0.73

*Q: quartile, BMI: body mass index, SBP: systolic blood pressure

†Values are standardized beta's (per quartile increase in adolescent BMI or SBP) with 95% confidence intervals

‡Model 1: univariable. Model 2: adjusted for adolescent age, sex, body mass index and systolic blood pressure and adult total cholesterol level,

body mass index, systolic blood pressure and carotid intima-media thickness

§Model 2 not adjusted for adult BMI

||P value

**Model 2 not adjusted for adult SBP

Table S2. Relation between quartiles of adolescent BMI and SBP and CIMT in young adulthood (*n*=736)

	Q1*†	Q2*†	Q3*†	Q4*†
Adolescent BMI*‡§				
Range	13.51 - 16.73	16.73 - 18.19	18.19 - 19.97	19.97 - 33.71
Model 1 (reference: Q1)	-	14.41 (4.31, 24.50)	15.25 (5.16, 25.34)	22.98 (12.89, 33.08)
P value||	-	0.005	0.003	<0.001
Model 2 (reference: Q1)	-	11.92 (1.62, 22.23)	13.73 (3.26, 24.20)	20.26 (9.25, 31.28)
P value||	-	0.02	0.01	<0.001
Adolescent SBP*†**				
Range	80.00 - 100.00	100.00 - 110.00	110.00 - 120.00	120.00 - 165.00
Model 1 (reference: Q1)*†§	-	4.64 (-4.49, 13.77)	12.69 (2.51, 22.87)	22.30 (9.47, 35.12)
P value||	-	0.32	0.01	<0.001
Model 2 (reference: Q1)*†§	-	2.62 (-6.68, 11.92)	6.47 (-4.14, 17.07)	10.36 (-3.10, 23.83)
P value||	-	0.58	0.23	0.13

*Q: quartile, BMI: body mass index, SBP: systolic blood pressure

†Values are standardized beta's (per quartile increase in adolescent BMI or SBP) with 95% confidence intervals

‡Model 1: univariable. Model 2: adjusted for adolescent age, sex, body mass index and systolic blood pressure and adult total cholesterol level,

body mass index, systolic blood pressure and carotid intima-media thickness

§Model 2 not adjusted for adult BMI

||P value

**Model 2 not adjusted for adult SBP