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Lower Central Serotonergic Responsivity Is Associated With Preclinical Carotid Artery Atherosclerosis

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Background and Purpose—Central nervous system serotonergic neurotransmission appears to play a role in mood disorders, eating habits, and sleep, and also modulates blood pressure and metabolism. This investigation tested a hypothesized association between central serotonergic functioning and preclinical atherosclerosis.

Methods—Subjects were 244 adults 30 to 55 years of age and free of clinically evident vascular disease (52% men, 84% white). Central serotonergic responsivity was measured as the rise in serum prolactin concentration (area under the curve) over 2.5 hours, adjusted for baseline prolactin, after citalopram administered intravenously at 0.33 mg/kg lean body weight. Carotid artery morphology served as a marker of preclinical atherosclerosis, and carotid artery intima-media thickness and plaque occurrence were determined by B-mode ultrasonography.

Results—In linear regression models including age, gender, race, and citalopram concentration, a 1 SD lower prolactin response was associated with greater maximum intima-media thickness (+0.016 mm; $P=0.006$) and with greater mean intima-media thickness (+0.009 mm; $P=0.03$). The odds ratio for carotid artery plaque corresponding to a 1 SD decrease in prolactin response, adjusted for age, race, sex, and citalopram concentration, was 1.47 (95% CI, 0.98 to 2.19; $P=0.06$). The metabolic syndrome mediated ($P<0.01$), but did not fully account for, the association between lower prolactin response and greater maximum intima-media thickness.

Conclusions—In this young and relatively healthy sample, blunted prolactin response to citalopram was associated with carotid artery thickening, suggesting that individual differences in central serotonergic responsivity are inversely related to preclinical vascular disease. (*Stroke*. 2007;38:2228-2233.)

Key Words: atherosclerosis ■ central nervous system ■ serotonin

As currently understood, atherosclerosis is a degenerative and inflammatory disease process developing in the inner layers of large arteries over the course of several decades. In some individuals, substantial atherosclerotic lesions form before age 50 and lead to early morbidity and mortality, whereas other people reach advanced age with no measurable plaque. This variability appears to arise from individual differences in both biological (eg, blood pressure and serum lipid concentrations) and behavioral risk factors (eg, eating habits, physical activity, and depressed mood). It is now clear that risk factors for atherosclerotic cardiovascular disease (ASCVD) cluster within individuals, as in the metabolic syndrome, and such clustering suggests the existence of shared etiologic factor(s) that direct the coexpression of multiple biological and behavioral risk factors.

The central nervous system (CNS) receives afferent information from, and discharges efferent signals to, the cardiovascular, immune, gastrointestinal—metabolic, and endocrine systems. Given that the brain broadly regulates

the body's internal milieu and also accounts for cognition, affect, and behavior, CNS processes may give rise to the simultaneous expression of multiple risk factors for atherosclerosis. The brain's serotonergic (5-hydroxytryptamine [5-HT]) neural circuits, in particular, modulate many autonomic and neuroendocrine processes, emotions, and behaviors.¹

Prolactin release from the pituitary gland is regulated, in part, by 5-HT signals from the hypothalamus, and the measurement of prolactin responses to pharmacological challenge with direct or indirect 5-HT agonists provides a validated and minimally invasive in vivo index of central 5-HT function.^{2,3} Blunted prolactin response to serotonergic challenge by the 5-HT releasing agent, fenfluramine, has been associated with current and remitted major depression.^{4,5} Prolactin response to serotonergic challenge has also been found related inversely to elevated blood pressure, sedentary lifestyle, estimated insulin resistance, and the metabolic syndrome.^{6–8} Finally, several polymorphisms of

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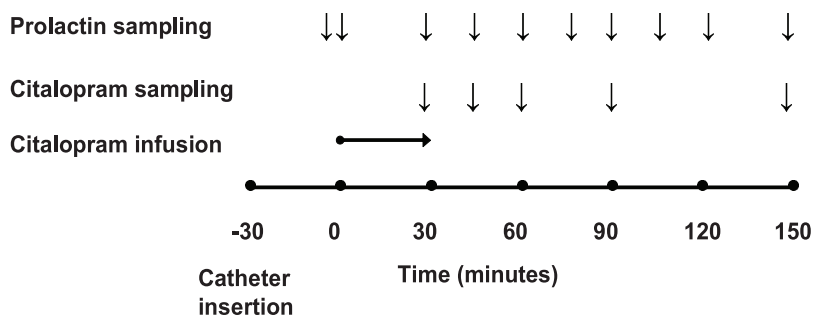


Figure 1. Intravenous citalopram challenge test protocol. Each test was scheduled between 1:00 and 3:00 pm after a 2-hour fast. Catheter insertion was followed by a 30-minute adaptation period. Citalopram was dosed at 0.33 mg/kg lean body mass and was administered by a controlled infusion pump over 30 minutes. Both prolactin and drug concentrations were monitored over 2.5 hours.

genes potentially affecting serotonergic neurotransmission have been associated with ASCVD.^{9,10}

In view of the evidence linking variation in 5-HT function with several biological and behavioral risk factors for cardiovascular disease, the current investigation tested the hypothesis that individual differences in central serotonergic activity are likewise related to preclinical vascular disease. Central serotonergic function was indexed as the prolactin response to a selective serotonergic probe, citalopram, and vascular disease was assessed with sonographic imaging of the carotid arteries. We further tested whether any such association between central serotonergic responsivity and preclinical vascular disease is mediated by the metabolic syndrome or its component ASCVD risk factors.

Patients and Methods

Subjects were participants in the University of Pittsburgh’s Adult and Human Behavior Project and were recruited from Allegheny County, Pennsylvania, from February 2002 to August 2004 using mailed brochures. All were non-Hispanic white and black community volunteers 30 to 55 years of age. Exclusion criteria included clinical history of atherosclerotic disease (eg, stroke, myocardial infarction, angioplasty, or bypass surgery), cancer diagnosis, or treatment within the past year, chronic liver or kidney disease, as well as use of cardiovascular, lipid-lowering, diabetic, glucocorticoid, weight-loss, or psychotropic medications. Women were excluded if they were not using reliable birth control, or were pregnant, lactating, or currently experiencing age-related menstrual period irregularities.

A total of 259 subjects completed the protocol and 244 were included in analyses. Eight subjects were excluded because they experienced adverse reactions during the citalopram challenge that confound interpretation of hormonal responses (vomiting, vasovagal syncope, or both), 3 because baseline prolactin levels were >40 ng/mL, 2 because measurements of plasma citalopram concentration were missing, 1 because the left carotid artery could not be visualized adequately, and 1 outlier whose prolactin response exceeded that sample mean by >10 SDs.

Risk Factor Assessments

Subjects arrived at the University of Pittsburgh’s Behavioral Physiology Laboratory between 7:30 and 10:30 AM after a 12-hour overnight fast. After they rested in the seated position for at least 10 minutes, a trained staff member obtained 2 blood pressure measurements from the right arm using a mercury sphygmomanometer and a regular, large, or extra large adult cuff, according to the subject’s arm circumference. The 2 readings were averaged. Then, phlebotomy was performed along with measurement of height, weight, waist circumference at the umbilicus, and lean body mass (estimated using a bioelectrical impedance body composition analyzer; Tanita Corporation of America, Inc, Arlington Heights, Ill).

Determinations of standard serum lipids, glucose, and insulin were performed by the Heinz Nutrition Laboratory, Department of Epidemiology, University of Pittsburgh Graduate School of Public

Health, according to previously described methods.¹¹ The metabolic syndrome was defined by the criteria of the National Cholesterol Education Program.¹² An estimate of insulin resistance based on the Homeostasis Model Assessment was calculated as follows: $HOMA-IR = \text{serum insulin } (\mu\text{IU/mL}) \times \text{fasting blood glucose (mmol/L)} / 22.5$.¹³

Citalopram Challenge Test

Central serotonergic responsivity was measured as the change in serum prolactin concentration after administration of citalopram, a selective 5-HT reuptake inhibitor (SSRI). Stimulation of hypothalamic 5-HT receptors promotes the pituitary release of prolactin, and citalopram increases serotonergic neurotransmission by highly selective inhibition of 5-HT reuptake.¹⁴ The increase in circulating prolactin concentration induced by citalopram is dose-dependent¹⁵ and provides an index of net serotonergic responsivity in the hypothalamic–pituitary axis.³ Individual differences in prolactin response to serotonergic challenge are reasonably reproducible over 6 months ($r=0.59$ when menstrual cycling is controlled¹⁶) and correlate across methods of assessment.¹⁷

Participants reported to the University of Pittsburgh’s General Clinical Research Center between 1:00 and 3:00 PM after a 2-hour fast. Testing was conducted in the afternoon to minimize the influence of circadian variation on prolactin levels. Premenopausal women were scheduled during the early follicular phase (ie, between 3 and 9 days after onset of menses).

The citalopram challenge test protocol is illustrated in Figure 1. On arrival on the General Clinical Research Center (GCRC), nursing staff inserted an intravenous catheter into each forearm, one for blood sampling and one for drug infusion. After a 30-minute adaptation period, blood samples for baseline prolactin were drawn at 5 minutes and 1 minute before citalopram infusion. Subjects then received citalopram by controlled infusion over 30 minutes at a dose of 0.33 mg/kg lean body mass. Eight subsequent timed blood samples for prolactin concentration and 4 timed samples for citalopram concentration were obtained. After allowing serum samples to clot, all samples were centrifuged, separated, and stored at -70°C until analysis. Methods for determining serum prolactin and plasma citalopram concentrations have been described previously.¹⁵ Prolactin concentrations from the two baseline prolactin samples were averaged.

Carotid Artery Ultrasound

Images were taken from 4 locations in both the right and left carotid arteries: the near and far walls of the distal common carotid artery (1 cm proximal to the carotid bulb), and the far walls of the bulb and first centimeter of the internal carotid. Intima-media thickness (IMT) was measured by electronically tracing the lumen–intima interface and the media–adventitia interface across a 1-cm segment at each location (with the exception of the bulb which was measured in its entirety). Measures were obtained using software that uses an edge detection algorithm and generates one measurement for each pixel, yielding ≈ 140 measures for each segment.¹⁸ Computerized readings were over-read by certified sonographers who made adjustments to the computer readings as necessary. Mean IMT was calculated as the average of the mean IMT measured at each location. Maximum IMT was calculated as the average of the maximum IMT from each

location. Plaque was defined as any focal area in which the IMT was at least 50% thicker than adjacent regions.

Statistical Analysis

The citalopram-induced prolactin response area under the curve (AUC) in nanograms/milliliter per hour was calculated by trapezoidal integration using prolactin concentrations measured from 0 to 150 minutes after infusion. Time-integrated citalopram exposure (citalopram AUC) was calculated in an analogous fashion from plasma citalopram concentrations. Prolactin AUC was adjusted for covariation with baseline serum prolactin to control for the influence of central dopaminergic tone and lactotroph function on prolactin release. Specifically, prolactin response (AUC) and baseline prolactin levels were first log-transformed (base 10) to ensure normality and then log prolactin AUC was regressed on log baseline prolactin. The prolactin AUC residuals, also log-transformed, were used in analyses. HOMA-IR, triglycerides, and insulin also required log transformation.

Mean and maximum carotid IMT were averaged across the eight measured sites and then subjected to inverse transformation to ensure normality. Plaque was categorized as the presence or absence of any observable plaque. Bivariate associations between transformed IMT measures and risk factors were evaluated using Pearson correlations but are reported with the sign reversed for ease of interpretation. Linear regression was used to evaluate the association between the prolactin AUC and mean and maximum IMT. Logistic regression was used in analyses of carotid artery plaque. Covariates in all regression analyses of carotid artery morphology were age, sex, race, and citalopram AUC (the latter to avoid potential confounding by variation citalopram exposure). Potential mediation of any revealed associations by blood pressure and other metabolic syndrome risk factors was evaluated in subsequent linear and logistic regression models, using the difference in coefficients test.¹⁹ Effect size estimates were calculated based on a 1 SD decrease in prolactin response (AUC). Potential sex and race interactions were evaluated in all models.

Results

Characteristics of the study participants are summarized in Table 1. This generally healthy sample tended to be somewhat overweight but had acceptable average blood pressure, lipid, and glucose concentrations. Thirteen percent had detectable carotid artery plaque. The citalopram challenge protocol adjusted the administered dose for participant body size to standardize citalopram exposure. This dose adjustment was successful insofar as the plasma citalopram AUC was unrelated to BMI ($r=0.02$, $P=0.74$). As expected, mean and maximum IMT correlated positively with age, waist circumference, systolic blood pressure, diastolic blood pressure, triglycerides, glucose, insulin, and insulin resistance estimated by HOMA-IR ($r=0.16$ to 0.40), and negatively with HDL cholesterol ($r=-0.23$) (all $P<.05$). Similarly, carotid artery plaque was positively associated with age, systolic blood pressure, diastolic blood pressure, triglycerides, insulin, and HOMA-IR (Spearman $r=0.13$ to 0.24 , all $P<.05$).

We evaluated the association between prolactin response (prolactin AUC) and carotid artery maximum and mean IMT using linear regression and the relationship between prolactin response and carotid artery plaque using logistic regression (Table 2). All regression models included age, gender, race, and citalopram AUC as covariates. Potential sex and race interactions were not significant for any of the models. Lower prolactin response was associated with greater maximum IMT ($+0.016$ mm per 1 SD lower prolactin AUC; $P=0.006$), and this relationship is illustrated in Figure 2. Lower prolactin

TABLE 1. Participant Characteristics

Characteristics	Mean±SD*
N	244
Age, y	44.4±6.7
Male, n (%)	126 (52)
White, n (%)	206 (84)
Current smokers, n (%)	40 (16.5)
Waist circumference (cm)	90.4±13.5 (35.6±5.3 in)
BMI, kg/m ²	26.5±4.4
Systolic blood pressure, mm Hg	115.0±11.6
Diastolic blood pressure, mm Hg	75.5±8.4
Total cholesterol, mmol/L	5.15±0.88 (197.8±34.0 mg/dL)
HDL cholesterol, mmol/L	1.38±0.37 (53.3±14.4 mg/dL)
Triglycerides,† mmol/L	1.05 (0.82) (93 [73] mg/dL)
LDL cholesterol, mmol/L	3.16±0.79 (121.9±30.4 mg/dL)
Glucose,† mmol/L	5.2 (0.67) (94 [12] mg/dL)
Average IMT,‡ mm	0.63 (0.10)
Maximum IMT,‡ mm	0.79 (0.14)
Carotid plaque present, n (%)	31 (13)
Citalopram exposure (AUC), ng/mL*hr	56.8±14.1
Baseline prolactin, ng/mL	10.4±5.2
Prolactin response‡ (AUC), ng/mL*hr	30.1±13.7

*Unless otherwise indicated.

†Values are median (interquartile range).

‡Values are unadjusted.

response was also associated with greater mean IMT ($+0.009$ mm greater IMT per 1 SD lower prolactin response; $P=0.03$) and, marginally, with the presence of carotid artery plaque (OR, 1.47 per 1 SD lower prolactin response; 95% CI, 0.98 to 2.19 $P=0.06$).

Blunted serotonergic responsivity has been associated with the metabolic syndrome in previous reports and, in this sample, prolactin response was correlated inversely with waist circumference, diastolic blood pressure, triglycerides, glucose, insulin, and HOMA-IR (Pearson $r=-0.13$ to -0.22), and positively correlated with HDL cholesterol ($r=0.16$) (all $P<.05$). Therefore, mediational analyses were conducted and demonstrated that the metabolic syndrome (yes/no by NCEP criteria) was a significant mediator in the relationship between lower prolactin response and thicker maximum IMT ($P<.01$ according to Freedman-Schatzkin's difference in coefficients test).¹⁹ However, neither the metabolic syndrome nor its components (eg, waist circumference, systolic blood pressure, and fasting serum glucose, triglycerides, and HDL cholesterol) fully explained the association between lower prolactin response and thicker maximum carotid IMT, as seen by the relatively small change in the adjusted parameter estimates, which remained significant (Table 2). The association between blunted prolactin response and thicker maximum IMT remained significant after the final model in Table 2 was additionally adjusted for pack-years of smoking and BMI ($P=0.04$; data not shown).

Table 2 also provides results from additional linear regression analyses of prolactin response and mean IMT. As noted,

TABLE 2. Regression Model Findings Relating Low Central Serotonergic Responsivity (Prolactin AUC) to Carotid Artery Morphology

Change Associated With a 1 SD (0.048) Decrease in Prolactin AUC*	Increase in Maximum IMT†, mm	P	Increase in Mean IMT†, mm	P	Odds of Carotid Plaque (any vs none)	P
Model 1 covariates: age, race, sex, citalopram drug levels	0.016	0.006	0.009	0.03	1.47 (0.98, 2.19)	0.06
Model 1 covariates+metabolic syndrome by IDF criteria	0.012	0.03	0.006	0.08	1.39 (0.92, 2.10)	0.11
Model 1 covariates+metabolic syndrome by NCEP criteria	0.014	0.01	0.007	0.045	1.45 (0.97, 2.18)	0.07
Model 1 covariates+waist., systolic BP, glucose, triglycerides, and HDL cholesterol	0.011	0.04	0.006	0.11	1.47 (0.97, 2.22)	0.07
Model 1+waist., systolic BP, glucose, triglycerides, HDL cholesterol, and HOMA-IR	0.011	0.04	0.005	0.11	1.45 (0.95, 2.22)	0.08

*Prolactin AUC was adjusted for baseline prolactin and log-transformed.

†Maximum and mean IMT data were normalized by reciprocal (1/x) transformation transformed for hypothesis testing. To improve interpretability, nontransformed variables are presented to estimate the amount of change in millimeters.

blunted prolactin response was similarly associated with mean carotid artery IMT. The addition of covariates related to the metabolic syndrome diminished the association between prolactin response and mean IMT, in several instances causing it to lose statistical significance. Thus, the metabolic syndrome again appears to mediate, at least in part, the relationship between serotonergic responsivity and carotid IMT.

Discussion

The role of CNS processes in atherosclerosis remains largely unexplored. Following on previous research linking a low prolactin response to serotonergic pharmacological probes with cardiovascular risk factors, here we tested the hypothesis that a blunted central serotonergic response would be associated further with preclinical atherosclerosis. In a relatively large sample of community volunteers receiving no medications affecting cardiovascular risk factors or serotonergic

function, prolactin response to intravenous citalopram was inversely related to maximum carotid artery IMT. Prolactin response was also related, albeit less robustly, to mean IMT and carotid artery plaque (the latter finding being limited by the low prevalence of plaque in this sample). The effect size of +0.009 to +0.016 mm greater IMT per 1 SD lower prolactin response compares to the average +0.008 mm yearly increase in carotid artery thickness in adults.²⁰ Supplementary analyses indicated that the association between central serotonergic responsivity and preclinical vascular disease was partially mediated by components of the metabolic syndrome.

Interpretation of these findings rests on the specificity and reliability of neuropharmacologic challenges as indices of serotonergic function.^{2,3} In this regard, serotonergic drug challenges increase prolactin release in a dose-dependent manner that is blocked by serotonergic receptor (particularly 5-HT₂) antagonists^{21,22} and varies in magnitude with genetic polymorphisms affecting 5-HT synthesis and release.^{23,24} In addition, prolactin responses covary across different serotonergic pharmacological probes and exhibit reasonable test-retest reliability.^{16,17} Finally, the pharmacological agent used in this study, citalopram, binds with very high specificity to the 5-HT transporter protein.¹⁴

The observed association between blunted central serotonergic responsivity and preclinical atherosclerosis could be the result of cerebrovascular disease, but this is unlikely since there was minimal atherosclerosis and no significant carotid stenosis in the study sample. Alternatively, blunted central serotonergic responsivity and atherosclerotic disease may share an etiologic factor, in which case no causal connection may exist between serotonergic function and vascular disease. Candidate “third factors” include shared genetic determinants,²⁵ chronic systemic inflammation, and dietary omega-3 fatty acid deficiency. The latter 2 factors are generally viewed as modifiable causes of ASCVD,^{26,27} and both chronic systemic inflammation and dietary omega-3 fatty acid deficiency can affect the brain. However, the extent to which these systemic conditions may influence central serotonergic function is unclear.²⁸

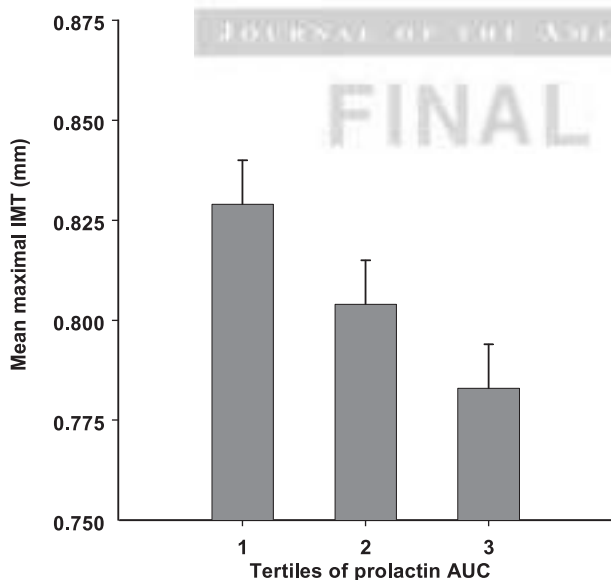


Figure 2. Maximum carotid artery thickness as a function of prolactin response. Bars reflect the mean maximum IMT in subjects grouped by tertile of increasing prolactin AUC after citalopram infusion. Values are adjusted for age, gender, race, baseline prolactin concentration, and citalopram AUC. *P*=0.004 for trend.

Several lines of evidence support a causal role of 5-HT in ASCVD. Polymorphic variation in the promoter regions of the 5-HT_{2A} and 2C receptors are associated with abdominal obesity, type 2 diabetes, and prevalent coronary artery disease.^{10,29,30} Similarly, a functional polymorphism in the 5-HT transporter gene promoter is associated with asymptomatic carotid artery morphology and with prevalent and incident coronary disease.^{9,31,32} The current study findings suggest partial mediation by the metabolic syndrome and, in this regard, several clinical trials have examined the effects of SSRI treatment on glucose metabolism in individuals with type 2 diabetes, impaired glucose tolerance, or obesity. Though not uniform, trial findings include lowered fasting blood glucose or glycosylated hemoglobin with SSRI treatment,^{33,34} improved insulin sensitivity assessed by euglycemic clamp,³⁵ reduced hepatic glucose production, and enhanced peripheral glucose uptake.³⁶ Moreover, preliminary data from pharmacoepidemiologic investigations and randomized clinical trials suggests that treatment of depression with SSRIs may reduce major heart disease events.^{37–40}

Such evidence, however, does not inform us regarding the biological or behavioral mechanisms through which serotonergic dysregulation might affect ASCVD. Within the CNS, serotonergic circuits affect eating behavior,⁴¹ and certain serotonergic agonists are useful as appetite suppressants.⁴² Central 5-HT also regulates sleep, and various sleep disturbances may predispose to atherosclerosis.^{43,44} Neuroanatomic and neuropharmacologic studies indicate that CNS 5HT modulates sympathetic and parasympathetic outflow affecting heart rate and blood pressure,^{45,46} as well as glucose and energy homeostasis.^{47,48} Therefore, the influence of CNS serotonergic circuits on autonomic activity could affect atherosclerosis via several internal mechanisms.

Finally, the current findings could be a reflection of a role of peripheral, not central, 5-HT in ASCVD. The 5-HT concentration is low in blood because platelets avidly sequester it in their dense granules. Nonetheless, 5-HT is a potent vasoconstrictor and may have pathogenic roles in both plaque rupture and atherogenesis.^{49–51} A novel serotonergic antagonist holds promise as an anti-atherosclerotic agent,⁵² and SSRIs appear to reduce platelet 5-HT content⁵³ and platelet activation.⁵⁴ However, because elevated plasma 5-HT levels and platelet release appear to promote atherogenesis, one would have to postulate that central serotonergic responsiveness, as measured in the current investigation, correlates inversely with peripheral 5-HT indices. There is little theoretical, functional, and empirical support for such a relationship.⁵⁵

The central limitation of the current study is its cross-sectional design. This feature leaves unresolved whether serotonergic dysfunction might contribute to atherogenesis or is a marker of some other, correlated pathogenic process. Nonetheless, the evidence that 5-HT-related polymorphisms are associated with central obesity, diabetes, and coronary disease, and that SSRI treatment tends to improve glycemic control and reduce coronary events supports the general plausibility of a causal association. Future studies are warranted to examine the reported association prospectively, elucidate the mechanism or mechanisms linking central serotonergic

function to ASCVD, and further test the efficacy of 5-HT-based pharmacological interventions on atherosclerosis.

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Disclosures

Dr Pollock serves on consultant/advisory boards of Lundbeck and Forest pharmaceutical companies. The other authors have nothing to disclose.

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