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Segment-specific association between plasma homocysteine and carotid artery intima-media thickness in the Framingham Heart Study

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Abstract

Background—Higher plasma total homocysteine (tHcy) is an established risk factor for cardiovascular disease. The relation between tHcy and carotid artery intima-media thickness (IMT) at the internal carotid artery (ICA)/bulb-IMT and common carotid artery (CCA)-IMT has not been systematically examined. Since the ICA/bulb segment is more prone to plaque formation than the CCA segment, differential associations with tHcy at these sites might suggest mechanisms of tHcy action.

Methods—We examined the cross-sectional segment-specific relations of tHcy to ICA/bulb-IMT and CCA-IMT in 2,499 participants from the Framingham Offspring Study, free of cardiovascular disease.

Results—In multivariable linear regression analysis, ICA/bulb-IMT was significantly higher in the fourth tHcy quartile category compared to the other quartile categories, in both the age- and sex-adjusted and in the multivariable-adjusted model (P for trend <0.0001 and <0.01, respectively). We observed a significant age by tHcy interaction for ICA/bulb-IMT (P=0.03) and therefore stratified the analyses by median age (58 years). There was a significant positive trend between tHcy and ICA/bulb-IMT in individuals 58 years of age or older (P-trend <0.01), but not in the younger individuals (P-trend=0.24). For CCA-IMT, no significant trends were observed in any of the analyses.

Conclusions—The segment-specific association between elevated tHcy levels and ICA/bulb-IMT suggests an association between tHcy and plaque formation.

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Keywords

carotid artery; intima-media thickness; homocysteine; atherosclerosis; Framingham Offspring Study

1. Introduction

Higher circulating total homocysteine (tHcy) levels have been shown to be associated with increased risk for peripheral vascular, cerebrovascular and coronary heart disease¹⁻³. Higher tHcy levels have also been associated with increased carotid artery intima-media thickness (IMT)⁴⁻⁶, an index of early subclinical atherosclerosis⁷. IMT is commonly measured at various segments of the carotid artery including the common carotid artery (CCA), the internal carotid artery (ICA), and the bifurcation segment (bulb), using B-mode ultrasonography⁸. In addition to elevated tHcy, known determinants of IMT thickening include a number of cardiovascular disease (CVD) risk factors such as older age, elevated blood pressure, cholesterol, and smoking⁹⁻¹².

Increased IMT thickness can be due to the thickening of the wall only or thickening of the wall with plaque deposits. Focal plaque formation occurs mostly in the carotid artery bulb and the proximal internal carotid artery (ICA/bulb) of the internal carotid artery sinus, whereas the CCA segment is more prone to wall thickening without plaque formation¹³. Explanations for these regional differences in susceptibility to plaque formation versus wall thickening include differences in blood flow these segments are exposed to, as well as histological differences in the artery segments. Blood flow in the CCA is mostly laminar and produces shear stress that can lead to wall thickening, whereas blood flow in the ICA/bulb area show cyclical variations in low-shear stress that predisposes this segment to early plaque formation¹⁴⁻¹⁷. The CCA is an elastic artery segment, whereas the ICA is a muscular artery segment¹³. Such histological differences might also underlie differing risk of plaque formation in these segments in response to CVD risk factors^{12, 18}.

The association of higher tHcy to increased carotid IMT has been described previously in several reports^{4-6, 19, 20}. However, none of these studies investigated segment-specific associations. In some of these studies, mean IMT was measured in the common carotid artery segment (CCA) only^{5, 6}. In other reports, maximum IMT was measured at various segments but then reported as a combined measurement, or results from only one segment were reported^{4, 19, 20}. These prior studies do not provide information about the relations of different carotid segments to potential pathogenic effects of higher circulating tHcy levels.

Given the propensity of plaque to form in ICA-IMT, and not in CCA-IMT, the existence of differential associations between tHcy levels and specific carotid segments could indicate whether or not the associations seen between elevated tHcy and IMT are a function of wall thickening or of plaque formation. To examine this potential for segment-specific differences, we investigated the associations between tHcy and ICA-IMT and CCA-IMT among participants in the Framingham Offspring Study.

2. Methods

2.1. Study population

The study design and selection criteria of the Framingham Heart Study, a population based study on risk factors for CVD, have been described elsewhere²¹. Participants for the present investigation were members of the Framingham Offspring Study which began in 1971 with the recruitment of 5,124 men and women who were offspring, and spouses of offspring, of

the above mentioned original Framingham Heart Study cohort. The Framingham Offspring cohort has undergone repeat examinations at approximately 3–4-years intervals to assess the occurrence of vascular disease. Offspring study participants who underwent B-mode carotid ultrasonography during the 6th (1995–1998) examination cycle, who were free of CVD at cycle 6, and had plasma tHcy measured in the 5th (1991 to 1995) examination, were included. Although plasma tHcy measures were available for cycle 6, FDA-mandated folic acid fortification of enriched cereal-grain products in the US was phased in during the course of examination cycle 6. This resulted in a dramatic reduction of tHcy concentrations which respond quickly to changes in folic acid intake 22, 23. However, we would not expect rapid changes for potential clinical consequences of elevated tHcy, such as carotid artery IMT, over a relatively short exposure period (the median exposure time to fully implemented fortification for those participants included in the analyses using IMT from cycle 6 was only 2 months). Therefore, we used plasma tHcy data from the 5th examination cycle, because it reflects a more typical tHcy exposure level of the participants since it is uninfluenced by folic acid fortification.

Exclusion criteria included prevalent cardiovascular disease (CVD) at examination 6, missing tHcy examination 5 data, missing IMT examination 6 data, or missing covariate examination 6 data: 3,532 participants had an examination 6 visit. Of those, 383 participants were ineligible due to pre-existing cardiovascular disease (CVD) at exam 6. Of the remaining 3,149 eligible participants, 650 were excluded due to missing data. This resulted in 2,499 participants available to be included in the analyses.

To investigate whether bias might be introduced by exclusion of participants with missing data (n=650), we performed paired t-tests to compare the main characteristics of the included versus the excluded participants. The characteristics on which all of the 650 participants had data available (age, sex, smoking) were compared. Only age was significantly different (P=0.03). Although the difference was statistically significant, the absolute difference was only half a year (included: 58.2±9.6 yrs; excluded: 57.3±9.7 yrs (mean ± SD)). The study protocol was approved by the Institutional Review Board (IRB) at Tufts Medical Center and Boston Medical Center.

2.2. Carotid ultrasonography

Ultrasound imaging was conducted using a high-resolution 7.5 MHz transducer for the common carotid artery and a 5.0 MHz transducer (the –3dB point of the 5 MHz transducer being 6.7 MHz) for the carotid bulb and internal carotid artery (Toshiba Medical Systems). One image was obtained at the level of the distal left and right side common carotid artery. Two separate images were taken at the carotid artery bulb and separately in the proximal 2 cm of the internal carotid artery on the left and right side. All images were synchronized at end-diastole (R-wave of an electrocardiogram).

Of the 3,532 participants with examination cycle 6 visits, 3,407 participants (96.5%) had ultrasonographic IMT measurements. For the common carotid artery, at least 1 near wall IMT measurement was made in 99.1% of the participants, and for the far wall in 99.6% of the participants. For the internal/bulb carotid artery IMT, at least one measurement was made on the near wall in 93.1% and for the far wall in 97.9% of the participants. A single trained sonographer who was blinded to all clinical information made measurements and was over-read by one of the investigators (J.F.P.).

Common carotid artery IMT (CCA-IMT) was defined as the mean of the mean IMT measurement of the near and far walls of the right and left common carotid arteries. Internal carotid artery/bulb IMT (ICA/bulb-IMT) was defined as the maximum of the maximal IMT

measurement of the near and far walls in either the carotid bulb or the proximal internal carotid artery.

We used the ultrasonographic measurements of the maxima of ICA/bulb-IMTs as an estimate of plaque height^{24, 25}. This more closely parallels the plaque measurements made by previous investigators^{4, 5, 25}. We chose the mean IMT in the common carotid artery since this measure is believed by many investigators to represent a robust surrogate measurement of intima-media thickness^{24–26}. Images were analyzed according to a standard protocol and image processing software^{27, 28}. Based upon 38 randomly selected readings by two separate readers, the intra-class correlation coefficient for the maximum ICA/bulb-IMT was 0.77 (95% CI 0.58, 0.87; $p < 0.0001$) and 0.94 (95% CI 0.89, 0.97; $p < 0.0001$) for the mean CCA-IMT.

2.3. Laboratory measurements

At the fifth and sixth offspring cohort examination, fasting (>10 h) blood samples were obtained for determination of tHcy, total cholesterol, high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol. Plasma tHcy was measured by HPLC with fluorometric detection²⁹. Plasma total cholesterol and HDL cholesterol were measured with enzymatic methods^{30, 31}.

2.4. Statistical Analysis

Multiple linear regression analyses were performed for investigating the association of circulating tHcy with IMT. ICA/bulb-IMT and CCA-IMT were modeled as continuous dependent variables with natural logarithmic transformation for normalization of the distributions. The independent variable plasma tHcy was coded as sex-specific quartiles (quartiles 1, 2, 3, and 4). For the trend analysis, tHcy was treated as a continuous variable with the P-value for tHcy regression coefficient used as the P for trend (Table 2). Potential effect modification by sex and age was examined. Our primary analysis, included participants of all ages combined. Because we observed a statistically significant interaction between tHcy and age for ICA/bulb-IMT, based on categorization of age using the median value ($P = 0.03$), we also performed secondary analyses with age dichotomized at the median age of 58 years. Although there was no evidence of any interaction between age and tHcy for CCA-IMT ($P = 0.48$), for consistency, we also conducted the age stratified analysis for CCA-IMT. For both dependent variables (ICA/bulb-IMT and CCA-IMT), two linear regression models were constructed, one adjusting for age and sex only, and one multivariable model adjusting for age, sex, current smoking, systolic blood pressure, body mass index, total to HDL cholesterol ratio, hypertension treatment, creatinine, and diabetes. All analyses were performed with SAS statistical software version 8.2³².

3. Results

Table 1 lists the characteristics of the study individuals by all ages combined and by <58 and ≥ 58 years of age at examination cycle 6. The mean age of all study participants at the Framingham Offspring examination cycle 6 was 58 years of age (range 29 to 86 years). Forty-five percent of the participants were male and the mean BMI was 28 kg/m^2 in all three groups (all participants combined, < and ≥ 58 years of age). As expected, the mean total plasma homocysteine was higher in the older than in the younger age group (10.4 ± 3.8 and $9.4 \pm 3.6 \text{ nmol/ml}$, respectively), and higher in males compared to females (all participants combined: 10.7 ± 4.0 and $9.2 \pm 3.3 \text{ nmol/ml}$, respectively). Other expected differences in the older compared to the younger age group were thicker mean ICA/bulb-IMT, CCA-IMT, higher systolic blood pressure, total cholesterol, serum creatinine, and a higher percentage of participants having diabetes mellitus type 2 and being treated for high blood pressure. As

also expected, fewer participants in the older age group were current smokers compared to the younger age group.

Table 2 shows the results of the linear regression models adjusting for age and sex as well as the results from the multivariable-adjusted models, for both dependent variables (ICA/bulb-IMT and CCA-IMT), for all ages combined, and for < and $\geq$ 58 years of age, with tHcy as categorical quartile variable.

In all participants combined, ICA/bulb-IMT was significantly higher in the fourth tHcy quartile category compared to the other tHcy quartile categories, in both the age- and sex-adjusted and in the multivariable-adjusted models (P for trend <0.0001 and <0.01, respectively). For CCA-IMT, no significant trends were observed in the age and sex adjusted or in the multivariable models (P for trend 0.24 and 0.90, respectively).

In the younger age group (<58 years), there was a borderline non-significant trend for ICA/bulb-IMT across tHcy quartile categories (P for trend = 0.07) in the age- and sex-adjusted model. After multivariable adjustment the trend was non-significant (P for trend = 0.24). Plasma tHcy was not significantly associated with CCA-IMT in the age-and sex-adjusted or in the multivariable-adjusted models (P for trend 0.15 and 0.47, respectively).

In the older age group ($\geq$ 58 years), ICA/bulb-IMT increased significantly with increasing plasma tHcy quartile levels in the age- and sex-adjusted model (P for trend <0.001). In the multivariable adjusted model, individuals in the fourth tHcy quartile had significantly higher ICA/bulb-IMT than individuals in the second and in the third quartiles (P<0.01, for both) and the trend was significant (P for trend <0.01). Plasma tHcy was not significantly associated with CCA-IMT in the age-and sex-adjusted or in the multivariable-adjusted models (P for trend 0.71 and 0.44, respectively).

4. Discussion

We investigated the association between plasma tHcy and IMT of the internal carotid artery/bifurcation (ICA/bulb) and the common carotid artery segment (CCA) of the extracranial artery, adjusting for other major risk factors for atherosclerosis. We observed a significant positive association between elevated tHcy levels and ICA/bulb-intima-media thickness (IMT) in individuals of all ages and in individuals 58 years old and older. The levels of circulating tHcy associated with increased ICA/bulb-IMT were similar to or higher than those associated with an increased risk for cardiovascular disease in other studies^{33–37}. No significant positive association between tHcy levels and ICA/bulb-IMT was observed in individuals in the younger age group. It is possible that ICA/bulb-IMTs in the young age group were too low and less variable compared to those in the older age group to see a significant association with tHcy. No significant associations were observed between tHcy and the CCA-IMT segment after adjusting for important CVD risk factors.

Several other studies have investigated tHcy as a risk factor for increased IMT in the extracranial carotid artery^{4–6, 19, 38}. However, in these studies, IMT was either measured only at the CCA or it was measured at two or three different segments of the artery (ICA, bulb, CCA) and the measures were then combined. No conclusions about segment specificity of the effect of high circulating tHcy can be drawn from these studies.

Studies relating cardiovascular risk factors (other than higher tHcy levels) with regard to IMT or plaque of the extracranial carotid artery have noted that the associations may vary according to the segment of the artery evaluated. Rubba et al.³⁹, for example observed high body mass index (BMI) and systolic blood pressure (SBP) to be significantly associated with plaques in the common carotid artery in middle-aged women, while high LDL cholesterol

levels and smoking were significantly associated with lesions at the bifurcation. In a study by Ebrahim et al. 40 systolic blood pressure and age were determinants of increased IMT at the common carotid artery in both men and women. Cigarette smoking was associated with increased IMT and plaques at the bifurcation. Results from these studies indicate that age, systolic blood pressure and BMI may be primarily associated with artery wall changes in the CCA segment, whereas smoking and higher LDL cholesterol may be risk factors that are associated with wall changes in the bifurcation segment. However, results are not completely consistent across various studies examining carotid artery segment-specific relations of these vascular risk factors.

Risk factors that are associated with wall changes at one specific segment might share common mechanisms leading to these changes, whereas changes at different segments might be a result of differing mechanisms associated with the specific structure of the artery segments. The artery segments differ in geometry, cellular composition and functions and are exposed differently to shear stress. The internal carotid artery is a muscular artery whereas the common carotid artery is an elastic artery¹³ and the carotid wall at the level of the bifurcation contains more macrophages than the common carotid artery wall⁴¹.

It has been observed that atherosclerotic lesions occur mainly in the ICA/bulb segment and are rare in the CCA segment²⁴. Therefore, our observed segment specific effect of higher circulating tHcy on the ICA/bulb segment may suggest that higher plasma tHcy levels are associated with plaque formation, rather than with wall thickening per se.

Several mechanisms for homocysteine as a risk factor for the development of atherosclerosis have been proposed, which include inducing endothelial dysfunction, supporting the oxidation of low-density lipoproteins initiating the lipid peroxidation cascade, and increasing vascular smooth-muscle cell proliferation via the activation of the transcription factor NFκB⁴². However, questions about specific mechanistic actions still remain⁴³.

There are strengths and limitations to the present study. Major strengths of the study include the large number and broad age range of individuals, which allowed us to examine the associations in two age groups and at two different segments of the carotid artery, the inclusion of both men and women, and the rich data set that facilitated adjustment for a large number of potential confounders. A potential limitation of our study is the use of homocysteine levels from examination 5 and the use of IMT measurements from examination 6. This approach was prompted by the implementation of FDA-mandated folic acid fortification of enriched cereal grain products during the 6th examination period at which the carotid IMT was assessed. The homocysteine levels respond rapidly to increased folic acid in fortified foods^{22, 23}, but given the fairly short exposure times to mandatory fortification (as mentioned in the Methods section above, the median exposure time to fully implemented fortification was only 2 months), we do not believe that the increased folic acid would have any significant impact on IMT. IMT data from examination 5 were obtained by an older and less sensitive ultrasonography method and could therefore not be used in association with tHcy examination 5 data for the here presented analyses. Another limitation of our study is the cross-sectional nature of the association between tHcy and IMT as we are unable to demonstrate that a change in homocysteine levels would result in a reduced progression of ICA/bulb-IMT. Also, despite the high sensitivity of the B-mode ultrasonographic method used, it is not possible to differentiate the intima from the media layer of the artery. Therefore, it was not possible to technically distinguish between increased wall thickness and plaque per se. Further, it needs to be noted that although we observed these segment-specific associations between higher circulating tHcy and increased wall intima-media thickness, it is possible that tHcy is a risk marker for chronic disease rather than the cause for it⁴³. In addition, our cohort consisted mainly of Caucasians of

European ancestry. Thus, the presented findings therefore do not allow any conclusions with regard to the effect of race on the association, and the result may not apply to the general population.

Conclusion

In our large community-based sample, individuals with elevated plasma tHcy levels, specifically those at or above age 58 years, had increased IMT in the region of the carotid artery bulb and proximal internal carotid artery. It is well known that plaque formation occurs preferentially in this region of the carotid arteries. The lack of any association between elevated tHcy levels and the common carotid artery suggests that tHcy may play a lesser role in the pathophysiological processes responsible for diffuse thickening of the common carotid artery. Combined, our findings indicate that elevated circulating tHcy levels may be associated with plaque formation rather than with increased wall thickness. Additional studies are needed to confirm our findings and to elucidate the reasons for the observed differences in the segment-specific associations between IMT and elevated tHcy levels.

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Table 1

Participants' characteristics for all individuals combined and dichotomized by <58 and ≥58 years of age

Characteristics*	All individuals	<58 yrs	≥58 yrs
Participant numbers	2,499	1,230	1,269
Sex, % male	45	45	45
Age (yrs) [†]	58±10	50±5	66±6
BMI (kg/m ²)	27.9±5.2	27.9±5.5	27.8±4.9
Plasma tHcy (nmol/ml)			
All individuals	9.9±3.7	9.4±3.6	10.4±3.8
Males	10.7±4.0	10.4±3.9	11.1±4.1
Females	9.2±3.3	8.6±3.1	9.8±3.4
ICA/bulb-IMT (mm)	1.35±0.77	1.10±0.57	1.59±0.86
CCA-IMT (mm)	0.60±0.13	0.55±0.11	0.65±0.14
SBP (mm Hg)	128±19	121±16	135±19
Plasma total cholesterol (mg/dL)	206±37	202±37	209±37
Plasma HDL cholesterol (mg/dL)	52±16	52±15	52±17
Total:HDL cholesterol ratio	4.3±1.5	4.3±1.5	4.4±1.4
Serum creatinine (μmol/L)	101.9±19.0	100.1±16.1	103.5±21.3
Current smoker (%)	15	18	11
Diabetes (%)	8	5	11
Blood pressure treatment (%)	25	15	36

* All characteristics and measures are from the 6th examination cycle of the Framingham Offspring Study, except plasma total homocysteine levels which are from the 5th examination cycle.

[†] Means ± Standard Deviation.

Abbreviations: tHcy=total homocysteine; BMI=Body Mass Index; ICA/bulb-IMT=Internal Carotid Artery/bulb-Intima Media Thickness; CCA-IMT=Common Carotid Artery-Intima Media Thickness; SBP=Systolic Blood Pressure; HDL=High Density Lipoprotein.

Table 2
Associations of plasma homocysteine and IMT for all individuals combined and by age group

Age group	IMT variable (mm)	Linear Regression Models	Mean IMT values* [mm] by quartile categories of tHcy [†]				P _{trend} [‡]
			Q1	Q2	Q3	Q4	
All ages	ICA/bulb-IMT	Age, sex	1.16 (1.13,1.20)	1.14 (1.10,1.18)	1.17 (1.13,1.12)	1.26 [¶] (1.22,1.31)	<0.0001
		Multivariable ^{//}	1.17 (1.14,1.21)	1.15 (1.11,1.19)	1.17 (1.14,1.21)	1.23 [‡] (1.19,1.27)	<0.01
<58 yrs	CCA-IMT	Age, sex	0.58 (0.58,0.59)	0.58 (0.57,0.59)	0.58 (0.58,0.59)	0.59 [£] (0.59,0.60)	0.24
		Multivariable	0.59 (0.58,0.59)	0.58 (0.58,0.59)	0.58 (0.58,0.59)	0.59 (0.58,0.60)	0.90
<58 yrs	ICA/bulb-IMT	Age, sex	0.97 (0.93,1.01)	0.97 (0.93,1.01)	1.01 (0.96,1.05)	1.03 (0.98,1.08)	0.07
		Multivariable	0.98 (0.94,1.02)	0.98 (0.94,1.02)	1.01 (0.97,1.06)	1.01 (0.96,1.05)	0.24
≥58 yrs	CCA-IMT	Age, sex	0.53 (0.53,0.54)	0.54 (0.53,0.55)	0.54 (0.53,0.55)	0.55 [#] (0.54,0.56)	0.15
		Multivariable	0.54 (0.53,0.54)	0.54 (0.53,0.55)	0.54 (0.53,0.55)	0.54 (0.53,0.55)	0.47
≥58 yrs	ICA/bulb-IMT	Age, sex	1.39 (1.31,1.47)	1.32 (1.25,1.39)	1.35 (1.28,1.42)	1.52 [‡] (1.45,1.59)	<0.001
		Multivariable	1.40 (1.32,1.48)	1.35 (1.28,1.42)	1.36 (1.29,1.43)	1.49 ^{††} (1.42,1.56)	<0.01
≥58 yrs	CCA-IMT	Age, sex	0.64 (0.62,0.65)	0.62 (0.61,0.63)	0.63 (0.61,0.64)	0.64 ^{//} (0.63,0.65)	0.71
		Multivariable	0.64 (0.63,0.65)	0.63 (0.61,0.64)	0.63 (0.61,0.64)	0.63 (0.62,0.65)	0.44

* IMT values are geometric least square means with 95% Confidence Intervals.

[†] Sex-specific tHcy quartile levels were as follows (means ± SD) in nmol/mL:

Males: Q1 = 7.2 ± 0.9; Q2 = 9.2 ± 0.5; Q3 = 11.0 ± 0.6; Q4 = 15.7 ± 4.9. Females: Q1 = 5.9 ± 0.7; Q2 = 7.8 ± 0.5; Q3 = 9.6 ± 0.6; Q4 = 13.6 ± 3.3.

[‡] Based on continuous tHcy models; the homocysteine quartiles are sex-specific but not age-specific.

^{//} All multivariable adjusted models include adjustment for: age, sex, current smoking, systolic blood pressure, body mass index, total to HDL cholesterol ratio, hypertension treatment, creatinine, and diabetes.

[¶] P<0.01, for all comparisons of fourth tHcy quartile with first, second, and third tHcy quartile.

[‡] P<0.05, for all comparisons of fourth tHcy quartile with first, second, and third tHcy quartile.

[£] P<0.05, for comparison of fourth tHcy quartile with second tHcy quartile.

[#] P<0.05, for comparison of fourth tHcy quartile with first tHcy quartile.

^{††} P<0.01, for both comparisons of fourth with second and third quartile.

/// $P < 0.05$, for comparison of fourth with second quartile.

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