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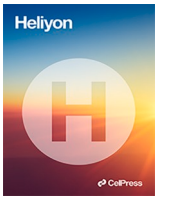

The effect of homocysteine-lowering therapy on the formation of carotid atherosclerosis: A follow-up study in the rural areas of northwest China

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ABSTRACT

Objective: To explore the risk factors for carotid atherosclerosis in the rural areas of Northwest China, and to assess whether reducing serum homocysteine can prevent carotid atherosclerosis in subjects with hyperhomocysteinemia.
Method: This observational study with 767 participants aged 40 years or older was conducted over 3 years. Color Doppler ultrasonography was performed to measure carotid atherosclerosis. We recorded biochemical indexes and carotid ultrasound results in the first and fourth years.
Result: A total of 767 individuals (48.5 % men) were analyzed. Older age, hypertension, and higher levels of baseline low-density lipoprotein cholesterol (LDL-C) were risk factors for increased carotid intima-media thickness (CIMT), carotid plaque, and carotid stenosis (All $P < 0.05$). No association was found between decreased serum homocysteine (Hcy) levels and CIMT, carotid plaque, or carotid stenosis in individuals with hyperhomocysteinemia.
Conclusion: Older age, hypertension, and higher baseline levels of LDL-C were independent risk factors for carotid atherosclerosis. Reducing serum Hcy levels may not prevent carotid atherosclerosis in the general population with hyperhomocysteinemia.

1. Introduction

Carotid atherosclerosis accounts for a substantial proportion of the global burden of disease [1]. At least 15–20 % of all cases of ischemic stroke (IS) is attributed to carotid atherosclerosis [2]. Studies also showed that carotid atherosclerosis is associated with a higher risk of dementia and cognitive impairment [3,4]. Therefore, physicians should pay more attention to carotid atherosclerosis, whose treatment has become a hot spot in recent years. Carotid atherosclerosis is associated with advanced age, male sex, smoking, and hypertension [1]. Homocysteine (Hcy) is a sulfur-containing amino acid that is generated during methionine metabolism. Elevated serum levels of Hcy can be caused by vitamin B12 or folate deficiency [5,6]. Some cross-sectional studies indicated that increased serum level of Hcy is related to carotid atherosclerosis [7–10], but other studies did not support these findings [11–13]. In addition, whether Hcy level reduction by folic acid and B12 vitamin can prevent the progression of carotid atherosclerosis was not unanimously determined by previous studies. Some randomized controlled trials showed that reducing Hcy levels does not prevent the progression

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of carotid atherosclerosis [14,15]; however, a meta-analysis indicated that folic acid supplementation is effective in reducing CIMT [16]. Most current studies have focused on the progression of carotid atherosclerosis rather than on its formation. The prevalence of hyperhomocysteinemia was high in Northern areas of China, especially in rural areas [17,18]. Therefore, we retrospectively collected data from subjects at high risk of stroke in Shaanxi Province, China. We evaluated the risk factors for CIMT increasing, carotid plaque formation, and carotid stenosis in the rural areas of Northwest China. We also explore the relationship between decreased serum Hcy levels and CIMT increasing, carotid plaque formation, or carotid stenosis in subjects with hyperhomocysteinemia.

2. Methods

2.1. Participants

We were responsible for the Screening and Intervention Programs for People at High Risk of Stroke in the Shaanxi province of China. All participants have volunteered for this program. In this program, people with baseline hyperhomocysteinemia were given 800 μg folic acid and 500 μg vitamin B_{12} daily to reduce their serum levels of Hcy, and they were followed for three years. A specialist supervised them via telephone to regularly take their medication. In total, 1017 participants aged ≥40 years from Liulin town of Hanzhong city in the Shaanxi Province of China of this program were initially included in this study. They lived there for more than 1 year. Among them, we excluded 95 subjects who had no baseline carotid ultrasound data and 150 subjects who had no 3-year follow-up carotid ultrasound data. In addition, 3 subjects lost follow-up, 2 subjects died, and 767 subjects were included in the final analysis (Fig. 1). All participants signed an informed consent form, and the study was approved by the Ethics Committee of Xijing Hospital, the First Affiliated Hospital of the Fourth Military Medical University. The ethics number is KY20212114.

2.2. Demographic and clinical data collection

Physicians at Hanzhong Central Hospital collected information on demographic characteristics and the history of diseases including hypertension, diabetes mellitus, atrial fibrillation, stroke, and transient ischaemic attack (TIA) via a face-to-face interview at the participants' first visit. The incidence of IS or coronary heart disease (CHD) was recorded on the second visit after 3 years. Hypertension was defined as the presence of a history of hypertension, or an SBP ≥140 mmHg and/or a DBP ≥90 mmHg. Diabetes mellitus was defined as a self-reported history of diagnosis in the department of endocrinology, or fasting blood glucose level ≥7.0 mmol/L (126 mg/dL). Atrial fibrillation was defined as absolute arrhythmia or current medical treatment. Stroke, TIA, and CHD were defined as self-reported history. Smoking was defined as continuous smoking for 6 months or more and within 30 days before the survey [19]. Fasting blood was collected in the morning by experienced nurses, and serum triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and Hcy were measured in the Hanzhong Central Hospital clinical laboratory. The blood samples collected at the participants' first visit and collected again on the second visit after 3 years. All laboratory measurements were performed using a Modular P800 automated biochemistry analyzer (Roche, Switzerland, Germany). Hyperhomocysteinemia was defined as an Hcy level greater than 15 μmol/L in the serum.

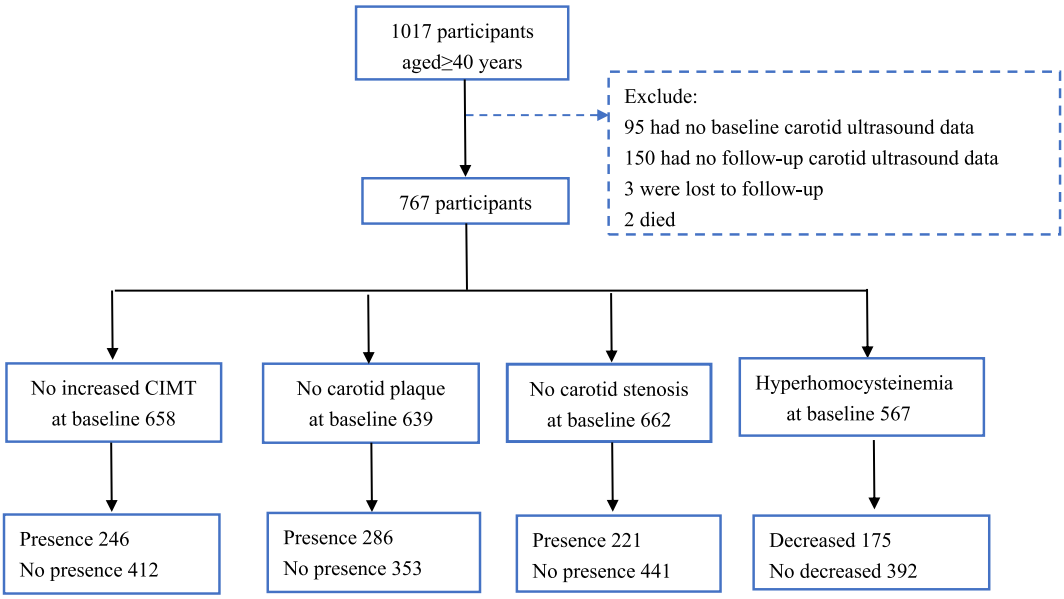

Fig. 1. The flow diagram.

Table 1
Characteristics of included participants.

Variable	No increased CIMT at baseline (n = 658)		*P*-value	No plaque at baseline (n = 639)		*P*-value	No carotid stenosis at baseline (n = 662)		*P*-value
	Presence 246 (37.4 %)	No presence 412 (62.6 %)		Presence 286 (44.8 %)	No presence 353 (55.2 %)		Presence 221 (33.4 %)	No presence 441 (66.6 %)	
Age, years (median, IQR)	70 (64–75)	65 (55–73)	<0.001	71 (65–75)	62 (54–71)	<0.001	70 (64–76)	65 (55–72)	<0.001
Gender, male (n,%)	133 (54.1)	183 (44.4)	0.017	159 (55.6)	149 (42.2)	0.001	122 (55.2)	189 (42.9)	0.003
Previous stroke (n,%)	23 (9.3)	29 (7.0)	0.288	36 (12.6)	16 (4.5)	<0.001	22 (10.0)	36 (8.2)	0.442
Previous TIA (n,%)	14 (5.7)	23 (5.6)	0.953	16 (5.6)	17 (4.8)	0.658	10 (4.5)	29 (6.6)	0.291
Atrial fibrillation (n,%)	5 (2.0)	7 (1.7)	0.993	5 (1.7)	6 (1.7)	1.000	4 (1.8)	12 (2.7)	0.472
Hypertension (n,%)	148 (60.2)	198 (48.1)	0.003	172 (60.1)	160 (45.3)	<0.001	135 (61.1)	216 (49.0)	0.003
Diabetes mellitus (n,%)	62 (25.2)	96 (23.3)	0.580	75 (26.2)	77 (21.8)	0.193	60 (27.1)	98 (22.2)	0.161
Smoking (n,%)	86 (35.0)	103 (25.0)	0.006	92 (32.2)	89 (25.2)	0.052	78 (35.3)	109 (24.7)	0.004
IS occurred (n,%)[a]	16 (6.5)	10 (2.4)	0.009	20 (7.0)	5 (1.4)	<0.001	12 (5.4)	12 (2.7)	0.079
CHD occurred (n,%)[b]	11 (4.5)	10 (2.4)	0.149	15 (5,2)	5 (1.4)	0.006	6 (2.7)	8 (1.8)	0.636
Baseline LDL-C (mmol/L)	3.24 (2.89–3.77)	3.17 (2.69–3.65)	0.017	3.22 (2.85–3.76)	3.14 (2.70–3.66)	0.070	3.25 (2.92–3.73)	3.15 (2.70–3.68)	0.022
Baseline HDL-C (mmol/L)	1.34 (1.14–1.51)	1.34 (1.13–1.54)	0.554	1.34 (1.13–1.53)	1.32 (1.13–1.50)	0.814	1.34 (1.14–1.56)	1.32 (1.13–1.51)	0.580
Baseline Hcy (μmol/L)	19.05 (15.18–25.53)	17.60 (14.20–22.98)	0.005	19.25 (15.10–24.63)	17.50 (13.80–22.55)	<0.001	19.60 (15.05–24.80)	17.60 (14.25–23.30)	0.004
Baseline TG (mmol/L)	1.40 (1.04–2.13)	1.53 (1.07–2.23)	0.361	1.41 (1.00–2.15)	1.53 (1.09–2.18)	0.232	1.32 (0.99–2.15)	1.51 (1.08–2.17)	0.226

Data are shown as number (percentile), median (interquartile range).
Abbreviations: CIMT = carotid intima-media thickness; TIA = transient ischaemic attack; IS = ischemic stroke; CHD = coronary heart disease; LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol; Hcy = homocysteine; TG = triglyceride.
[a] IS occurred during the follow-up.
[b] CHD occurred during the follow-up.

2.3. Carotid ultrasound

Two professional radiologists at the Hanzhong Central Hospital measured bilateral common carotid artery (CCA), carotid bulb, and internal carotid artery (ICA) using color Doppler ultrasonography (IE33, Netherlands Philips). Participants were examined in the supine position and the anterior region of the neck was fully exposed. CIMT was defined as the distance between the lumen-intima interface and the media-adventitia interface in the non-plaque areas of bilateral common carotid arteries. According to the Mannheim carotid intima-media thickness consensus [20], CIMT ≥1.0 mm was defined as increased CIMT. The number of plaques in 3 regions, including CCA, carotid bulb, and ICA, was counted bilaterally. The plaque was defined as a focal protrusion if its thickness was 50 % greater than the surrounding intima-media thickness (IMT) or IMT ≥1.5 mm [20]. According to the diagnostic criteria established by the American Radiological Association in 2003, the stenosis rate detected in CCA, carotid bulb, and ICA was classified as 0–50 %, 50–69 %, 70%–99 %, and occlusion [21].

2.4. Statistical analysis

Normally distributed continuous data are presented as mean ± standard deviations (SD), otherwise median with interquartile range (IQR). Groups with normal distribution were compared by the Student's t-test, otherwise compared by the Mann-Whitney *U* test. Categorical variables are described as frequencies and percentages. Two groups were compared using the x^2 or Fischer exact test as appropriate. Univariate analysis was applied to find significant differences in participant characteristics between the two groups. Subsequently, binary logistic regression was applied for multivariate analysis. Analyses were performed using SPSS v22.0. All tests were 2-sided, and a p-value less than 0.05 was considered statistically significant.

3. Results

3.1. Demographic and baseline information

There were 767 participants eventually included in the analysis, the median (interquartile range (IQR)) age was 68 (58–74) years, and a total of 51.5 % of the included participants were female. There were 38 (5 %) participants who had a new ischemic stroke during the follow-up, 567 (73.9 %) participants with hyperhomocysteinemia at baseline (Supplementary Table S1).

3.2. Factors associated with increased CIMT

There were 658 (85.5 %) individuals without increased CIMT at baseline, and 246 (37.4 %) of them developed after 3 years, including 133 males (54.1 %) and 113 females (45.9 %). In the univariate analysis, increased CIMT was associated with male gender, older age, hypertension, smoking, incident IS, high baseline level of Hcy, and high level of baseline LDL-C (All P < 0.05, Table 1). In the multivariable logistic regression analysis, after adjusting for gender, age, hypertension, smoking, incident IS, baseline LDL-C, and baseline Hcy, the results showed that older age (OR 1.05, 95%CI 1.03–1.07, P < 0.001), hypertension (OR 1.51, 95%CI 1.07–2.13, P = 0.018), incident IS (OR 2.64, 95%CI 1.15–6.09, P = 0.022) and high level of baseline LDL-C (OR 1.37, 95%CI 1.09–1.71, P = 0.007, Table 2) were independent predictors of increased CIMT. However, gender, smoking, and baseline level of Hcy were not significantly associated with increased CIMT.

3.3. Factors associated with carotid plaque

There were 639 (83.3 %) participants without carotid atherosclerotic plaque at baseline, and 286 (44.8 %) of them developed after 3 years. The plaque was present in 55.6 % of males and 44.4 % of females. In the univariate analysis, the presence of carotid plaque was

Table 2
Multivariate analysis of risk factors for carotid atherosclerosis.

Variable	Increased CIMT		Carotid plaque		Carotid stenosis	
	OR (95%CI)	*P*-value	OR (95%CI)	*P*-value	OR (95%CI)	*P*-value
Age	1.05 (1.03–1.07)	< 0.001	1.07 (1.05–1.09)	< 0.001	1.06 (1.04–1.08)	< 0.001
Male	1.14 (0.78–1.68)	0.496	1.48 (0.99–2.22)	0.060	1.22 (0.82–1.82)	0.324
Previous stroke	–		1.87 (0.88–4.01)	0.104	–	
Hypertension	1.51 (1.07–2.13)	0.018	1.63 (1.15–2.30)	0.007	1.52 (1.07–2.15)	0.021
Smoking	1.50 (0.98–2.28)	0.061	1.12 (0.72–1.74)	0.609	1.49 (0.97–2.29)	0.068
IS occurred	2.64 (1.15–6.09)	0.022	2.51 (0.76–8.25)	0.130	–	
CHD occurred	–		2.24 (0.73–6.89)	0.156	–	
Baseline LDL-C	1.37 (1.09–1.71)	0.007	1.35 (1.07–1.71)	0.010	1.32 (1.05–1.67)	0.017
Baseline Hcy	1.01 (0.99–1.03)	0.207	1.01 (0.99–1.02)	0.492	1.00 (0.99–1.02)	0.620

Abbreviations: CIMT = carotid intima-media thickness; IS = ischemic stroke; CHD = coronary heart disease; LDL-C = low-density lipoprotein cholesterol; Hcy = .
Homocysteine; OR = odds ratio.

Table 3
Characteristics of serum Hcy levels decreased group and no decreased group in participants with hyperhomocysteinemia.

Variable	No increased CIMT at baseline (n = 481)		*P*-value	No plaque at baseline (n = 464)		*P*-value	No carotid stenosis at baseline (n = 484)		*P*-value
	Hcy decreased 148 (30.8 %)	Hcy no decreased 333 (69.2 %)		Hcy decreased 137 (29.5 %)	Hcy no decreased 327 (70.5 %)		Hcy decreased 141 (29.1 %)	Hcy no decreased 343 (70.9 %)	
Age, years (median, IQR)	70 (58–74)	69 (60–75)	0.940	69 (57.5–74)	69 (60–75)	0.715	68 (57.5–74)	69 (60–75)	0.486
Gender, male (n,%)	65 (43.9)	199 (59.8)	0.001	63 (46.0)	195 (59.6)	0.007	66 (46.8)	193 (56.3)	0.058
Previous stroke (n,%)	19 (12.8)	23 (6.9)	0.033	19 (13.9)	23 (7.0)	0.019	21 (14.9)	26 (7.6)	0.014
Previous TIA (n,%)	13 (8.8)	16 (4.8)	0.091	9 (6.6)	15 (4.6)	0.379	10 (7.1)	19 (5.5)	0.513
Atrial fibrillation (n, %)	2 (1.4)	8 (2.4)	0.731	2 (1.5)	7 (2.1)	1.000	4 (2.8)	9 (2.6)	0.895
Hypertension (n,%)	80 (54.1)	183 (55.0)	0.855	72 (52.6)	177 (54.1)	0.756	74 (52.5)	193 (56.3)	0.447
Diabetes mellitus (n, %)	37 (25.0)	75 (22.5)	0.553	32 (23.4)	74 (22.6)	0.865	33 (23.4)	81 (23.6)	0.960
Smoking (n,%)	35 (23.6)	129 (38.7)	0.001	30 (21.9)	128 (39.1)	<0.001	30 (21.3)	131 (38.2)	<0.001
Baseline LDL-C (mmol/L)	3.34 (2.88–3.85)	3.21 (2.74–3.68)	0.071	3.26 (2.83–3.80)	3.18 (2.73–3.65)	0.136	3.26 (2.89–3.80)	3.21 (2.73–3.68)	0.164
Baseline HDL-C (mmol/L)	1.29 (1.11–1.56)	1.35 (1.14–1.51)	0.286	1.28 (1.10–1.56)	1.34 (1.14–1.51)	0.303	1.30 (1.11–1.56)	1.35 (1.14–1.51)	0.363
Baseline Hcy (μmol/L)	25.65 (20.00–32.70)	19.30 (16.80–23.70)	<0.001	25.00 (19.40–31.50)	19.30 (17.00–23.80)	<0.001	25.00 (19.30–32.25)	19.70 (17.00–24.00)	<0.001
Baseline TG (mmol/L)	1.50 (1.06–2.15)	1.39 (1.03–2.04)	0.371	1.50 (1.06–2.15)	1.38 (1.03–2.01)	0.276	1.50 (1.08–2.18)	1.51 (1.08–2.17)	0.188
3-year TG (mmol/L)[a]	1.73 (1.08–2.43)	1.80 (1.14–2.73)	0.313	1.75 (1.09–2.39)	1.76 (1.12–2.67)	0.498	1.75 (1.09–2.39)	1.76 (1.12–2.67)	0.518
3-year LDL-C (mmol/L)	3.14 (2.69–3.69)	3.13 (2.69–3.68)	0.797	3.09 (2.69–3.69)	3.12 (2.68–3.68)	0.597	3.11 (2.72–3.69)	3.13 (2.69–3.68)	0.721
3-year HDL-C (mmol/L)	1.35 (1.16–1.58)	1.34 (1.16–1.55)	0.415	1.32 (1.16–1.58)	1.34 (1.15–1.55)	0.736	1.34 (1.16–1.59)	1.34 (1.15–1.55)	0.607
3-year Hcy (μmol/L)	19.40 (16.30–23.35)	25.20 (20.95–32.25)	<0.001	19.30 (16.30–23.25)	25.30 (21.20–32.40)	<0.001	19.30 (16.10–23.05)	25.40 (21.20–32.70)	<0.001

Abbreviations: Hcy = homocysteine; TIA = transient ischaemic attack; LDL-C = low-density lipoprotein cholesterol; HDL-C = highdensity lipoprotein cholesterol; Hcy = homocysteine; TG = triglyceride.
[a] The concentration measured after 3 years.

associated with male gender, older age, previous history of stroke, hypertension, incident IS, incident CHD, and high level of baseline Hcy (All P < 0.05, Table 1). In a multivariable logistic regression model, older age (OR 1.07, 95%CI 1.05–1.09, P < 0.001), hypertension (OR 1.63, 95%CI 1.15–2.30, P = 0.007), and high level of baseline LDL-C (OR 1.35, 95%CI 1.07–1.71, P = 0.010, Table 2) were associated with higher risk of carotid plaque. Gender, previous stroke, incident IS or CHD, baseline Hcy and smoking were not significantly associated with carotid plaque in multivariable analysis.

3.4. Factors associated with carotid stenosis

There were 662 (86.3 %) individuals without carotid stenosis at baseline, and 221 (33.4 %) of them appeared after 3 years, including 122 (55.2 %) males and 99 (44.8 %) females. In the univariate analysis, carotid stenosis was associated with male gender, older age, hypertension, smoking, and higher levels of baseline Hcy and higher levels of baseline LDL-C (All P < 0.05, Table 1). In the multivariable logistic regression analysis, older age (OR 1.06, 95%CI 1.04–1.08, P < 0.001), hypertension (OR 1.52, 95%CI 1.07–2.15, P = 0.021), and higher levels of baseline LDL-C (OR 1.32, 95%CI 1.05–1.67, P = 0.017, Table 2) were independent risk factors of carotid stenosis. Gender, smoking, and baseline levels of Hcy were not independent risk factors for carotid stenosis.

3.5. The relationship between decreased serum hcy and carotid atherosclerosis in participants with hyperhomocysteinemia

Of the 567 (73.9 %) participants with hyperhomocysteinemia at baseline, 481 had not increased CIMT, 464 had no carotid plaque, and 484 had no carotid stenosis at baseline (Table 3). Of the 481 participants without increased CIMT at baseline, 148 (30.8 %) had varying degrees of serum Hcy level decline after 3 years and 333 (69.2 %) had no decline. Participants with decreased serum Hcy were likely to be female (56.1 % vs. 40.2 %, P = 0.001) and more likely to have a prior stroke (12.8 % vs. 6.9 %, P = 0.033) and had a higher level of baseline Hcy and a lower level of 3-year Hcy (P < 0.001). There were fewer smokers among subjects with decreased Hcy levels (23.6 %) compared with those without Hcy level decrease (38.7 %, P = 0.001). Among 464 participants without plaque at baseline, Hcy level decreased in 137 (29.5 %). Subjects with decreased Hcy were more likely to be female (54.0 % vs. 40.4 %, P = 0.007), more likely to have a previous stroke (13.9 % vs. 7.0 %, P = 0.019), and less likely to be a smoker (21.9 % vs. 39.1 %, P < 0.001), and had a higher level of baseline Hcy and a lower level of 3-year Hcy (P < 0.001). Of the 484 participants without carotid stenosis at baseline, Hcy decreased in 141 (29.1 %) participants. Participants with decreased Hcy were more likely to have a previous stroke (14.9 % vs. 7.6 %, P = 0.014) and less likely to be a smoker (21.3 % vs. 38.2 %, P < 0.001) and had a higher level of baseline Hcy and a lower level of 3-year Hcy (P < 0.001).

Among participants with hyperhomocysteinemia and normal CIMT at baseline, 58 (39.9 %) participants with decreased Hcy and 133 (39.3 %) participants without decreased Hcy had increased CIMT after 3 years of follow-up (Table 4). Among participants with hyperhomocysteinemia and no carotid plaque at baseline, 68 (49.6 %) participants with decreased Hcy and 152 (46.5 %) participants without decreased Hcy had carotid plaque after 3 years of follow-up (Table 4). Among participants with hyperhomocysteinemia and no carotid stenosis at baseline, 48 (43.0 %) participants with decreased Hcy and 122 (35.6 %) participants without decreased Hcy had stenosis after 3 years of follow-up (Table 4). After adjusting for confounding factors, no association was found between decreased serum Hcy and any of the three outcomes (Table 4).

We also analyzed the relationship between hyperhomocysteinemia and ischemic stroke risk. Univariate analysis showed that there was no significant difference in baseline hyperhomocysteinemia between the new IS occurrence group and the no new IS occurrence group, and there was no relationship between hyperhomocysteinemia and new IS (Supplementary Table S2 and Table S3).

4. Discussion

In this 3-year observational retrospective study conducted in the rural areas of Northwest China, we observed that older age, hypertension, and higher levels of baseline LDL-C are independent risk factors for increased CIMT, carotid plaque, and carotid stenosis.

Table 4
Association between serum Hcy levels decreased and the formation of increased CIMT, carotid plaque, and carotid stenosis.

	Prevalence (%)	Crude OR (95%CI)	*P*-value	Adjusted OR (95%CI)	*P*-value
Increased CIMT					
Hcy no decreased	133 (39.3 %)	Ref		Ref	
Hcy decreased	58 (39.2 %)	0.97 (0.65–1.44)	0.877	0.95 (0.56–1.60)[a]	0.842
Carotid plaque					
Hcy no decreased	152 (46.5 %)	Ref		Ref	
Hcy decreased	68 (49.6 %)	1.14 (0.76–1.69)	0.535	0.91 (0.54–1.55)[b]	0.726
Carotid stenosis					
Hcy no decreased	122 (35.6 %)	Ref		Ref	
Hcy decreased	48 (43.0 %)	0.91 (0.62–1.41)	0.749	0.87 (0.51–1.49)[c]	0.617

Abbreviations: OR = odds ratio; CIMT = carotid intima-media thickness; Hcy = homocysteine.
[a] Adjusted for Gender、Previous stroke、Smoking、Baseline Hcy、3-year Hcy.
[b] Adjusted for Gender、Previous stroke、Smoking、Baseline Hcy、3-year Hcy.
[c] Adjusted for Previous stroke、Smoking、Baseline Hcy、3-year Hcy.

In participants with hyperhomocysteinemia, we observed that 3-year treatment with vitamin B12 and folic acid did not prevent CIMT increasing, carotid plaque formation, and carotid stenosis.

CIMT changes occur due to structural alteration in the carotid artery wall and may be a sign of early systemic atherosclerosis and smooth muscle hypertrophy or hyperplasia [22]. Carotid plaque and carotid stenosis are advanced-stage phenotypes of carotid atherosclerosis. 3-Year follow-up in this study has shown that all outcomes were related to older age, hypertension, and elevated baseline serum LDL-C levels. Consistent with previous studies, age and hypertension were predictors of CIMT increasing, carotid plaque formation, and carotid stenosis [23–26]. Consistent with previous findings, our findings also showed that high levels of LDL-C are associated with carotid atherosclerosis [27–29]. We did not find a significant relationship between diabetes mellitus and CIMT increasing, carotid plaque, or carotid stenosis, which was in agreement with some previous studies [24,25]. Some of the previous epidemiological studies have shown that diabetes is a significant risk factor for carotid atherosclerosis [1,30,31]. However, these studies were cross-sectional or explored risk factors for the progression of carotid atherosclerosis, but we focused on the formation of carotid atherosclerosis. Therefore, the relationship between diabetes mellitus and carotid atherosclerosis needs to be further explored. It could also be that we defined diabetes as a self-reported history of diagnosis in the department of endocrinology, so some people with diabetes who didn't go to the endocrinology department were missed, this may influence the result.

Hyperhomocysteinemia can induce oxidative stress, endothelial dysfunction, inflammation, smooth muscle cell proliferation, and endoplasmic reticulum (ER) stress [32]. Some of the previous cross-sectional studies have shown that Hcy is associated with carotid atherosclerosis [7,8,10,33]. However, this conclusion is currently contradictory [11,12,34]. We did not find a significant relationship between serum Hcy and CIMT increasing, carotid plaque formation, and carotid artery stenosis. The relationship between Hcy and carotid atherosclerosis needs to be further elucidated in future studies. Some studies were also conducted to determine whether decreasing serum Hcy levels can prevent carotid atherosclerosis progression. Till et al. found that vitamin supplementation significantly reduces CIMT in patients at high risk of cerebral ischemia [35]. A meta-analysis has shown the benefit of Hcy-lowing therapy on CIMT progression in subjects with hyperhomocysteinemia and chronic kidney disease or increased risk of cardiovascular disease [16]. On the contrary, our 3-year observational study did not show that decreased serum Hcy levels can improve the three outcomes in individuals with hyperhomocysteinemia. Previous studies were conducted on patients with chronic kidney disease and a high risk of cardiovascular disease or ischemic stroke. In contrast, our study focused on the general population with hyperhomocysteinemia. Some of the previous studies on the general population also revealed that Hcy-lowering therapy is not useful [14,15]. Hcy-lowering therapy may not reduce the formation of carotid atherosclerosis in the general population with hyperhomocysteinemia, but subjects with chronic kidney disease or high cardiovascular disease risks may benefit from folic acid supplementation [16]. Our result did not show that hyperhomocysteinemia was related to ischemic stroke risk. It may be that the low incidence of IS in the general population leads to a small number of new IS during the follow-up period, and because of the small sample size of our study, therefore, no correlation was shown between hyperhomocysteinemia and IS risk in this study. Positive results may be obtained in the future by increasing the sample size.

There are several limitations to this study. First, potential confounders were not exhaustively included. For instance, total cholesterol value is a known risk factor for carotid atherosclerosis [36]. Second, geographical limitations and the small sample size may undermine the generalizability of our results. Therefore, the role of Hcy in the development of carotid atherosclerosis should be evaluated in large-scale randomized controlled trials.

In conclusion, our results showed that older age, hypertension, and higher levels baseline LDL-C levels were independent risk factors for the formation of CIMT, carotid plaque, and carotid stenosis in the rural areas of Northwest China. Reducing serum Hcy levels in the general population with hyperhomocysteinemia may not prevent the formation of carotid atherosclerosis.

Author contribution statement

Jianing Wu: Rui Shi: Xiao Zhang: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Hua Li: Contributed reagents, materials, analysis tools or data.

Data availability statement

Data will be made available on request.

Ethical approval and the ethics committee

KY20212114, Ethics Committee of Xijing Hospital, the First Affiliated Hospital of the Fourth Military Medical.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e21548.

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