

## Age and Gender Specific Reference Values of Brachial Flow-mediated Dilation and Carotid Intima-media Thickness in CATHAY Study: Atherosclerosis Surrogate Markers in Chinese Adults

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### Abstract

#### Background

Atherosclerotic diseases are currently important health hazard, and surrogate biomarkers (brachial flow-mediated dilation FMD and carotid intima-media thickness IMT) have been advocated for the documentation of early atherogenesis, epidemiological comparison and preventive measures.

#### Subjects and Methods

To establish the reference values of brachial FMD and carotid IMT, we studied 2163 (male 983, female 1180) asymptomatic Han Chinese, aged 20-70 years in Southern and Northern China, and in Overseas Chinese (1996-2007). Fasting glucose, lipid profile (LDL-C, HDL-C, TG), hemoglobin A1-C, folate and vitamin B12 were measured. PM2.5 exposure, brachial

FMD and carotid IMT (ultrasound-derived) were evaluated.

### Results

The male and female subjects were similar in their mean age, blood glucose, HBA1-C and metabolic status (MS) ( $p>0.1$ ), but males had higher smoking status, BMI, DBP, LDL-C and TG, and lower HDL-C, folate, vitamin B12 and PM2.5 exposure than the females ( $p<0.0002$ ). Brachial FMD ( $8.43\pm 2.58$ , 95% CI 8.21-8.65%) was significantly greater, but carotid IMT ( $0.63\pm 0.13$ , 95% CI 0.62-0.64) significantly lower in females than in the males ( $7.55\pm 2.74$ , 95% CI 7.39-7.78, and  $0.67\pm 0.16$ , 95% CI 0.66-0.68mm respectively)  $p<0.0001$ . The age specific percentile curves confirmed significant impairment of FMD but obvious increase in IMT after 40 years of age, in the males in particular.

On multivariate regression FMD was inversely related to age ( $\beta=-0.166$ ,  $p<0.0001$ ), gender ( $\beta=0.152$ ,  $p<0.0001$ ) and PM2.5 ( $\beta=-0.486$ ,  $p=0.005$ ), independent of smoking, and carotid IMT was related to age ( $\beta=0.454$ ,  $p<0.0001$ ), gender ( $\beta=0.116$ ,  $p<0.0001$ ) and PM2.5 ( $\beta=0.204$ ,  $p=0.025$ ), independent of BMI, SBP, DBP, LDL-C and MS. In comparison with the French, Italian and Latin American populations, carotid IMT in overall Chinese cohort was significantly lower.

### Conclusions

The age and gender specific reference values of early atherogenesis, brachial FMD and carotid IMT, in the Chinese were established, and carotid IMT was lower than those in the Caucasian and Latin-American populations.

**Keywords:** Reference Values; Brachial Flow-mediated Dilation; Carotid Intima-media Thickness; Atherosclerotic Surrogates; Native vs Overseas Chinese; Comparative Epidemiology

## Introduction

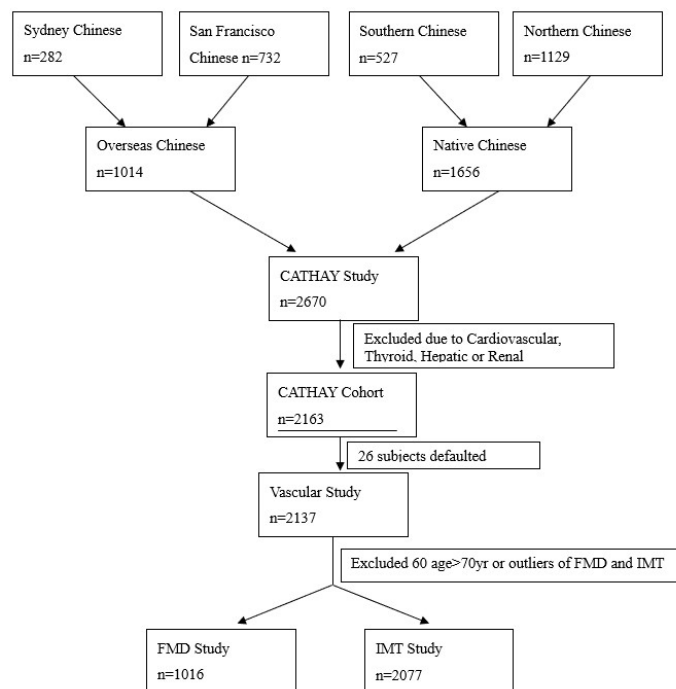
Atherosclerosis is currently a great health hazard, giving rise to heart attacks, stroke and peripheral vascular diseases [1,2]. Pathogenesis of atherosclerosis involves long process for decades of atherogenesis, starting with endothelial dysfunction, in early age, intima-media thickening, plaque formation with or without rupture and ultimately leading to angina, myocardial infarction, heart failure and ischemic stroke [3]. Early preventive measure before the onset of full-blown atherosclerotic diseases is the most important strategy. Advance in ultrasonic imaging has facilitated high resolution vessel-imaging noninvasively, in particular with automatic edge-detection and measurement, and become the gold standard, readily affordable and safe imaging modality. On this issue, two surrogate markers (brachial flow-mediated dilation FMD and carotid intima-media thickness IMT) have been advocated for the documentation of early atherogenesis and evaluation of preventive measures, as well as for comparative epidemiological studies [4-12]. These include the prevention modalities for smoking, hyperlipidemia, hypertension, diabetes mellitus, physical inactivities and air pollution (PM2.5) exposure [13-22].

Reference values of these 2 markers have been reported and derived mostly from the Caucasians [23-31], with uncertain applicability to other ethnic groups, including Chinese, due to possible inter-ethnic differences. Hence, in the present study, we aimed to establish the reference values of brachial FMD and carotid IMT in Chinese subjects aged 20-70 years, as well as the determinant factors for these 2 markers in Chinese population.

## Subjects and Methods

We studied 2670 asymptomatic Han Chinese, aged 20-70 years in Southern China (Hong Kong, Macau, Pan Yu), Northern China (Three Gorges Territories of Yangtze River, Yu County in Shanxi), and Overseas Chinese (San Francisco in USA, and Sydney in Australia) in years 1996-2007, as our multinational collaborative Chinese Atherosclerotic Study in the Aged and Young (CATHAY Study) community project (Figure 1). The project study protocol has been outlined and the preliminary findings of Chinese cohorts reported previously [32-40]. The majority of native Chinese were either born locally, or a minority had migrated to the locations from other Chinese cities for over

20 years, while the overseas Chinese were either born overseas or had migrated and stayed overseas for at least 15 years.



**Figure 1:** Schematic Diagram of Enrollment in CATHAY Study, Recruiting 2163 Native and Overseas Chinese adults

All enrolled subjects were apparently healthy, asymptomatic and had no known major cardiovascular, hepatic, thyroid or renal disorders, and were not taking any regular medications or supplementary vitamins. Excluded from the study (n=507) were those discovered after a physical examination and blood test to have cardiovascular, thyroid or renal derangements. (Figure 1) After fasting for over 12 hours and provision of written informed consent, their cardiovascular profiles were evaluated, including smoking status, body mass index (BMI), waist circumference and waist-hip ratio (WHR), systolic and diastolic blood pressures (SBP and DBP). Fasting blood samples were taken once at recruitment, for lipid profiles (low density lipoprotein cholesterol LDL-C, high density lipoprotein cholesterol HDL-C and triglycerides TG), glucose, hemoglobin A1-C, folate, vitamin B-12 and creatinine. Blood glucose was assessed by hemstix, folate and vitamin B-12 were evaluated by enzymatic immune assays (Abbott X analyses, Abbott Park, Illinois, USA). Blood were assayed in batches at the Prince of Wales Hospital in Hong Kong, the Centro Hospitalas Corde San Januario in Macau, and the Second Hospital of Chongqing Medical University in Chongqing, all cur-

rently accredited by USA laboratory centers. The long-term particulate matters <math><2.5\mu\text{m}</math> aerodynamic diameter (PM2.5) exposure over China, Sydney and San Francisco Bay Area were evaluated by satellite remote sensing technology, with a root-mean-squared error of  $8.9\mu\text{g}/\text{m}^3$ , a correlation coefficient of  $>0.9$  and a mean absolute percentage error within  $\pm 20\%$ , against the ground observation [41-43]. Metabolic syndrome (MS) was diagnosed according to the International Diabetes Federation (IDF) criteria [44-45].

### Vascular Studies

Prognostic atherogenesis surrogate markers, brachial FMD and IMT, were studied once at recruitment (2137, 26 defaulted), using high-resolution B-mode ultrasonography as outlined previously [32-40]. In brief, we used a linear array transducer (L10-5) with a medium frequency of 7.5MHz and a standard Advanced Technology Laboratories (ATL 3000, USA) or Sonosite (model Micromaxx, Bothell, USA) system to measure the diameter of the brachial artery. A forearm tourniquet cuff was applied to induce reactive hyperemia upon deflation. The scans were acquired at rest and during reactive hyperemia for flow-medi-

ated endothelium-dependent dilation FMD and after 200 $\mu$ g sublingual glyceryltrinitrate (GTN, an endothelium-independent dilation). FMD and GTN were expressed as percentage dilation of the vessel diameter from the baseline. Hyperemia, as a stress indicator to the endothelium, was calculated as the maximum increase in the blood flow after cuff deflation, compared with the baseline.

Carotid IMT of both carotid arteries were measured, using a standardized scanning protocol as described by Salonen and Salonen, Bots, and Touboul [8,9,11]. Images of the far wall of the distal 10 mm of the common carotid artery were acquired. All scans were measured offline by a verified automatic edge-detecting and measurement software package. The intra-observer reproducibility for mean IMT was 0.03 $\pm$ 0.01mm, the coefficient of variation was 1% (R=0.99), while the inter-observer variability was optimally maintained (R=0.95).

### Statistical Analyses

The project database was processed to derive the mean values, standard deviations and 95% confidence intervals (CI) when appropriate. Standard testing of the normality of distribution was used for assessment of the normal distribution. An independent sample Students' t-test and an one-way ANCOVA model were used for the evaluation of the intergroup differences. 60 subjects aged >70 years or recognized obviously outliers of FMD or IMT values were excluded from the final analyses. The primary study endpoints were carotid IMT and brachial FMD. The other outcome variables were compared after a Bonferroni adjustment for multiple comparisons [46]. Percentile curves for brachial FMD and carotid IMT were constructed using the LMS standard methods. The LMS method evaluates the measurement centiles in terms of three age – and gender-specific cubic spline curves: the Box-Cox power to transform the normally distributed data (L curve), the median (M curve) and the coefficient of variation (S curve).

(link: <https://www.healthforallchildren.com/shop-base/shop/software/lmschartmaker-light/>)

Multiple regression analyses were carried out to assess the determinants of the major risk factors for FMD and IMT in the CATHAY subjects respectively, including age, gender, smoking status, BMI, SBP, DBP, LDL-C, MS, PM2.5 and locations (Overseas, Southern or Northern China). The beta effect was evaluated by mixed effect. The variables with significant standard beta coefficients (beta value with 2p<0.05) as a measure of the contribution to the model were identified, and the insignificant variables (2p>0.05) was subsequently discarded from the regression model. A group difference with an error probability of less than 5% (2p<0.05) was considered to be statistically significant.

Sample size was calculated in terms of the standard deviation of the 100th centile ( $c_{100}$ ,  $\alpha=0.05$  for 95th percentile) using the sample size planning formula developed by Healy [47]. To obtain an extreme centile, i.e. the 97th centile, with an error of 5.0% and a power of 85%, the estimated sample size of subjects for FMD was 1002 and for IMT 1992 respectively. The statistical analyses were performed with SPSS version 25.

The project complied with the 1995 and 2003 Declaration of Helsinki for human studies. The research study and written informed consent form were approved by our institutional research ethics committee at the Chinese University of Hong Kong and Eastern New Territories of Hong Kong (CREC 2000-108 & CREC 2018-157).

### Results

A total of 2163 subjects were enrolled and included, 1180 were female and 983 were male (Table 1). They were similar in their mean age, fasting blood glucose, HBA1-C and metabolic syndrome status. The male subjects had higher smoking status (p<0.0001), BMI (p<0.0001), SBP (p=0.047), DBP (p<0.0001), creatinine (p<0.0001), LDL-C (p<0.0001) and TG (p<0.0001), but lower blood HDL-C (p<0.0001), folate (p<0.0001), vitamin B12 (p<0.0001) and PM2.5 pollution exposure (p<0.0002), compared with female subjects. 78.1% of females were native Chinese, compared with 74.8% of male subjects (p=0.1).

**Table 1:** Clinical Characteristics of 2163 Subjects in CATHAY Study

	Female(n= 1180)	Male(n= 983)	Overall(n=2163)	P-value*
Age (yr)	46.5±11.6	47.6±11.8	47±11.7	0.28
Smoking Status (%)	4.8	38.6	20.8	<0.0001
BMI	23.0±3.8	23.7±3.4	23.3±3.6	<0.0001
WHR	0.83±0.07	0.87±0.06	0.85±0.07	<0.0001
SBP (mmHg)	121.0±17.9	122.5±16.6	121.8±17.3	0.047
DBP (mmHg)	77.5±10.4	80.0±10.7	78.7±10.6	<0.0001
Glucose (mmol/l)	5.4±1.0	5.4±1.0	5.4±1.0	0.53
Creatinine (µmol/l)	61.8±15.2	83.1±18.9	71.3±20.0	<0.0001
LDL-C (mmol/l)	2.92±0.95	3.09±1.01	2.99±0.48	<0.0001
HDL-C (mmol/l)	1.33±0.36	1.17±0.33	1.25±0.86	<0.0001
Triglycerides (mmol/l)	1.26±1.05	1.48±1.09	1.36±1.08	<0.0001
HbA1c (%)	5.4±0.69	5.7±1.1	5.6±1.0	0.1
Folate (mmol/l)	27.1±15.2	21.0±12.3	24.0±14.1	<0.0001
B12 (mmol/l)	334.4±247.9	274.8±171.7	303.8±214.2	<0.0001
MS (%)	20.4	17.8	19.7	0.6
PM2.5 (µg/m <sup>3</sup> )	52.4±26.8	48.8±27.0	50.8±26.9	<0.0002

\*P value: Comparing Female &amp; Male

47.0±11.7

BMI: Body Mass Index; B12: Vitamin B12; DBP: Diastolic Blood Pressure; HbA1c: haemoglobin A1c; HDL-C: High Density Lipoprotein Cholesterol; LDL-C: Low Density Lipoprotein Cholesterol; MS: Metabolic Syndrome; PM2.5: Particulate Matter<2.5µm in Diameter; SBP: Systolic Blood Pressure; WHR: Waist-Hip Ratio

## Vascular Parameters

Mean diameter of brachial artery in the females (2.98±0.56 mm) was significantly lower than that in the males (3.70±0.61 mm, p=0.0005), while the hyperemia (705.6±257.1% vs 664.9±265.1%) were similar (p=0.07), and GTN slightly greater in the females (18.8±4.2) than in the

males (17.6±3.6), p=0.0005. The mean endothelium-dependent dilation FMD were significantly greater in the females (8.43±2.58, 95% CI 8.21-8.65%) than in the males (7.59±2.24, 95% CI 7.39-7.78), p<0.0001. The mean carotid IMT, however, was significantly lower in the females (0.63±0.13, 95% CI 0.62-0.64 mm) compared with the male subjects (0.67±0.16, 95% CI 0.66-0.68mm), p=0.047.

**Table 2:** Vascular Parameters of CATHAY Subjects

	Female	Male	P-value
Vessel Diameter (mm)	2.98±0.56	3.70±0.61	<0.0001 (0.0005)*
Hyperemia (%)	705.6±257.1	664.9±265.1	0.014 (0.07)*
GTN (%)	18.8±4.2	17.6±3.6	0.0001 (0.0005)*
FMD (%) (95% CI)	8.43±2.58(8.21-8.65)	7.59±2.24(7.39-7.78)	<0.0001
IMT (mm) (95% CI)	0.63±0.13(0.62-0.64)	0.67±0.16(0.66-0.68)	0.047

\*After Bonferroni Adjustment

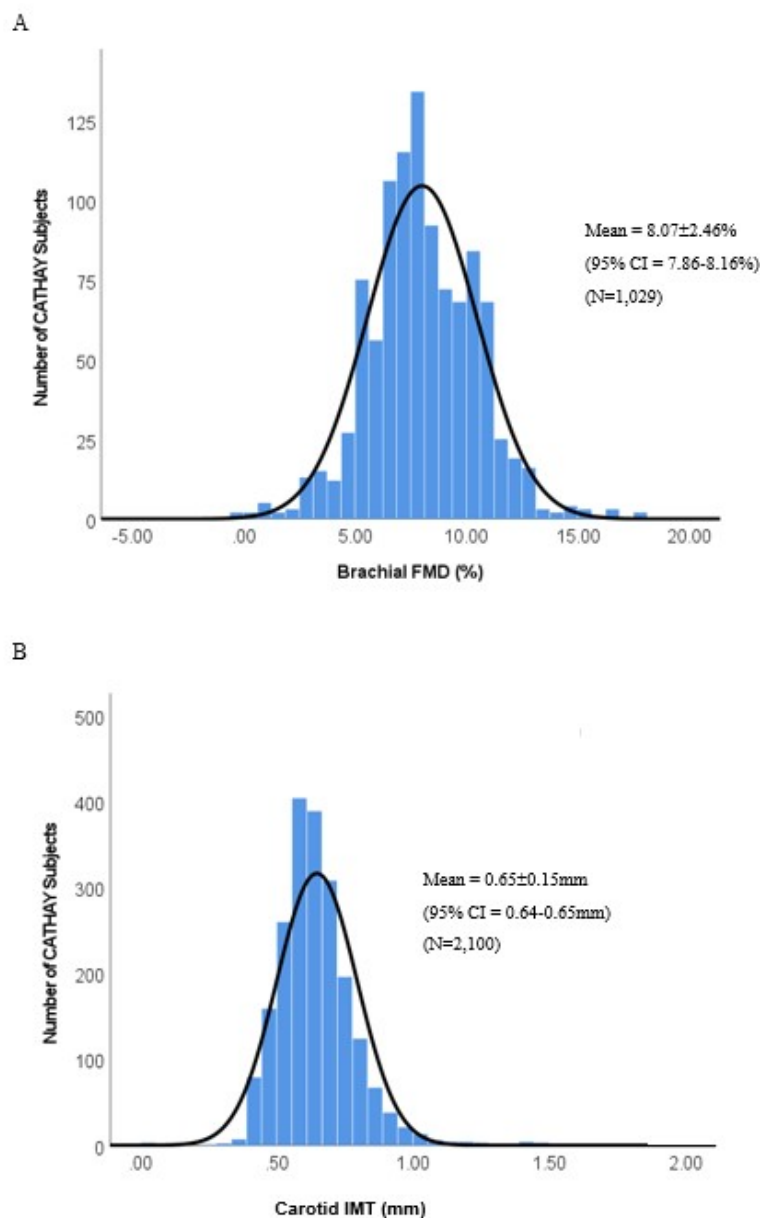
95% CI: 95% Confidence Interval; FMD: Brachial flow-mediated Dilation; GTN: Glyceryltrinitrate-induced Dilation; IMT: Intima-media thickness

→ Thickness

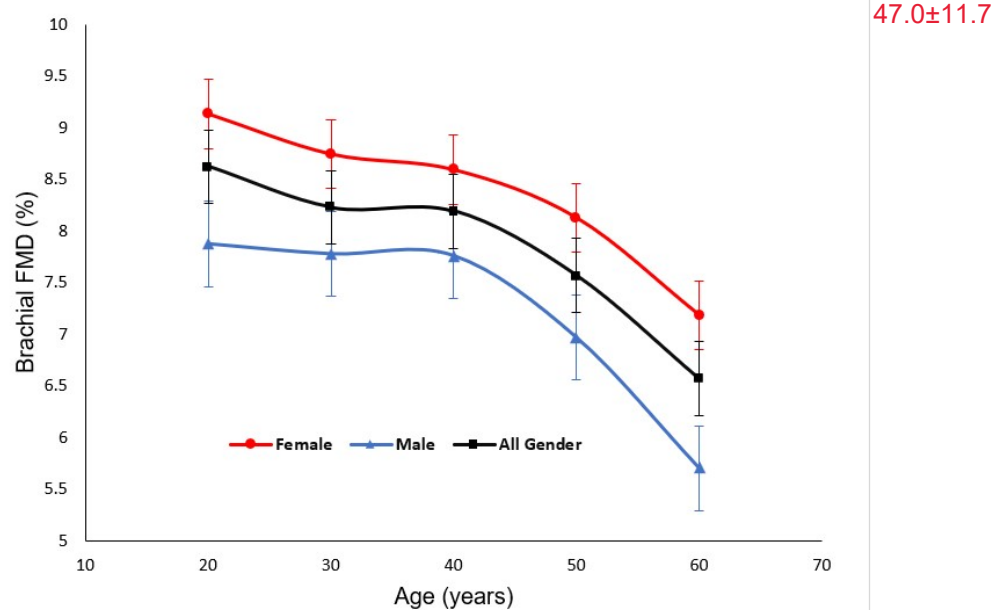
## Reference Values of Brachial FMD and Carotid IMT

The brachial FMD and carotid IMT curves were normally distributed (Figure 2). Mean FMD for the CATHAY cohort was  $8.0 \pm 2.5\%$ , and mean carotid IMT was  $0.65 \pm 0.15\text{mm}$ . The gender and age-specific values of

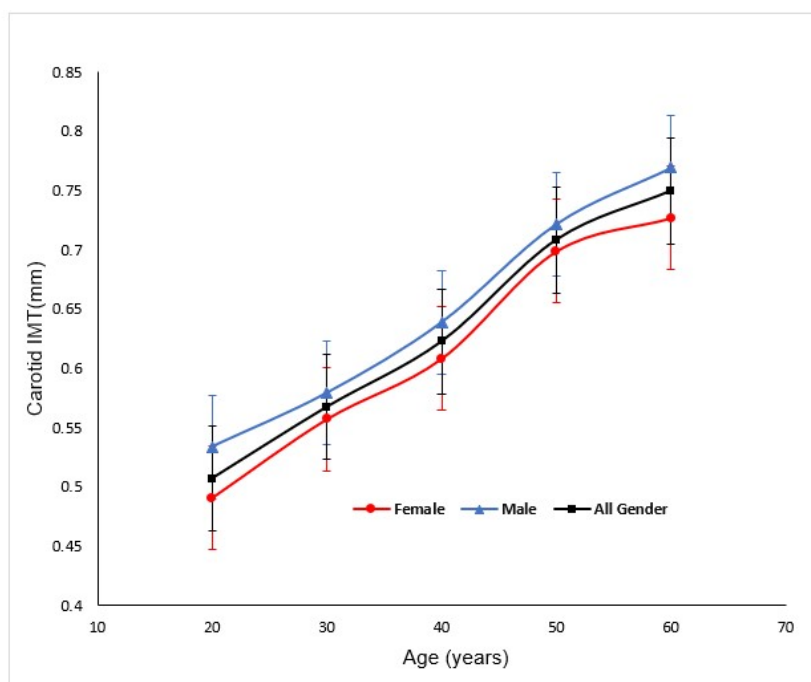
brachial FMD and carotid IMT were shown (Figure 3-4). The age and gender specific percentile curves of brachial FMD (Figure 5) and carotid IMT (Figure 6) were displayed. These all showed age-related decrease in brachial FMD and increase in carotid IMT, in particular in the males and age over 40 years.



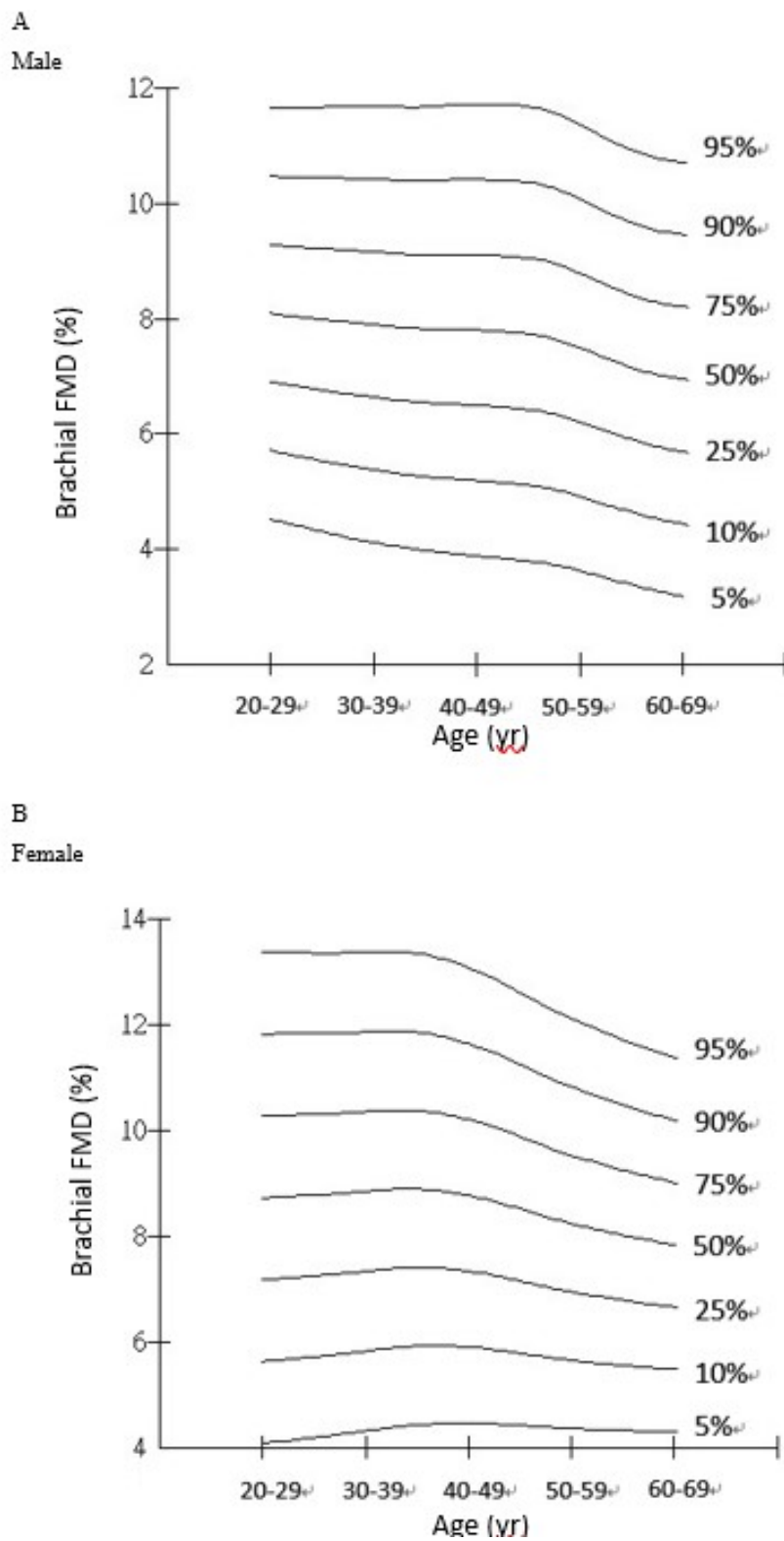
**Figure 2:** Normal distribution of (A) brachial flow-mediated dilation (FMD) and (B) carotid intima-media thickness (IMT) in CATHAY Cohort



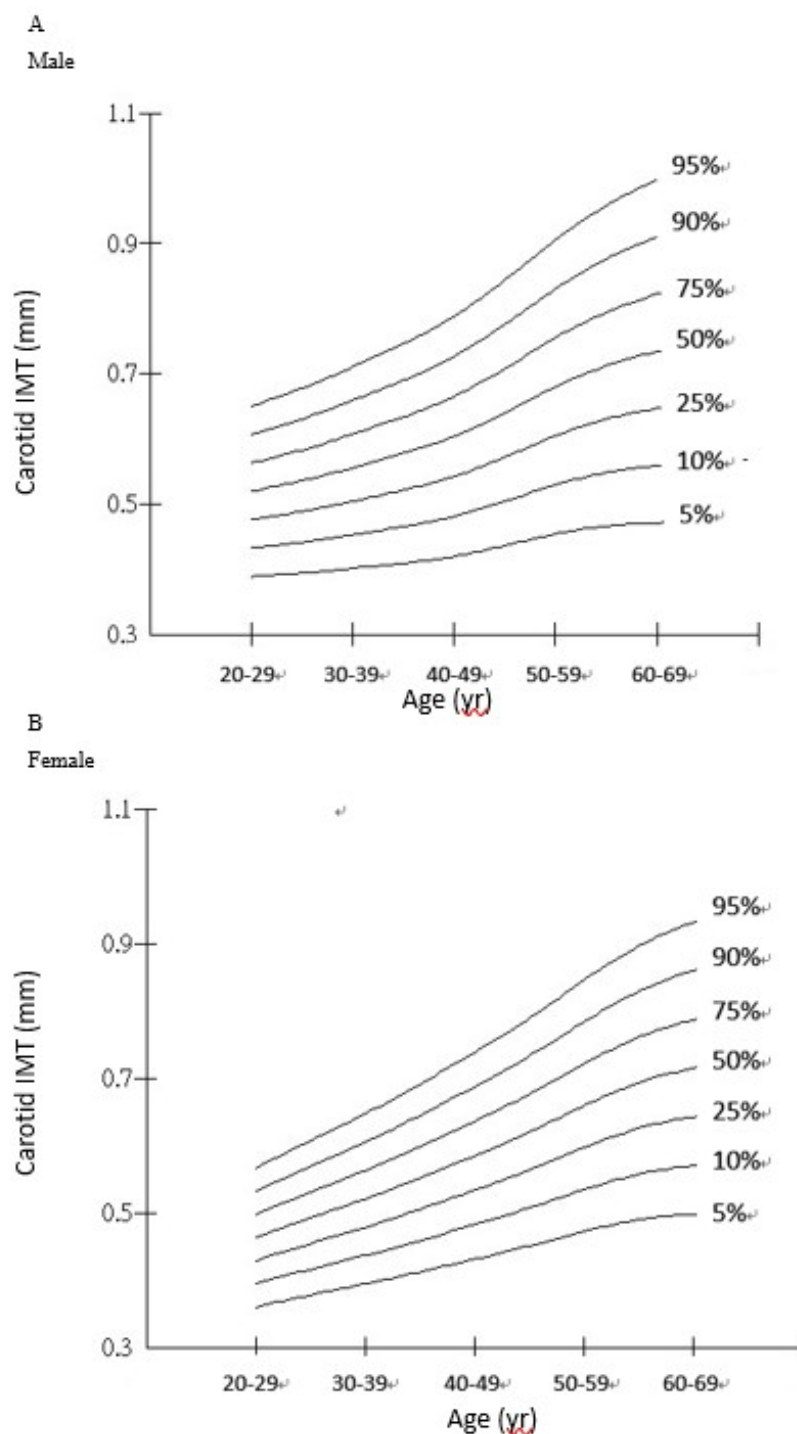
**Figure 3:** Gender-specific brachial flow-mediated dilation (FMD) in CATHAY subjects according to age, with display of the mean and standard deviation (%) in each age group.



**Figure 4:** Gender-specific carotid intima-media thickness (IMT) in CATHAY subjects according to age, with display of the mean and standard deviation (mm) in each age group.



**Figure 5:** Smoothed brachial flow-mediated dilation (FMD) in centiles 5%-95% of CATHAY subjects according to age in the males (A) and the females (B).



**Figure 6:** Smoothed carotid IMT in centiles in CATHAY subjects in centiles 5%-95% of CATHAY subjects according to age in the males (A) and the females (B).

### Determinants of Brachial FMD and Carotid IMT

On multivariate regression analyses, brachial FMD was significantly related to age ( $\beta=-0.166$ ,  $p<0.0001$ ), gender ( $\beta=0.152$ ,  $p<0.0001$ ) and PM2.5 ( $\beta=-0.486$ ,  $p=0.005$ ), independent of smoking status or locations ( $p<0.03$ ), but not to BMI, SBP, DBP, LDL-C or MS (Table

3). Carotid IMT was significantly related to age ( $\beta=0.454$ ,  $p<0.0001$ ), gender ( $\beta=0.116$ ,  $p<0.0001$ ) and PM2.5 ( $\beta=0.204$ ,  $p=0.025$ ), independent of BMI, SBP, DBP, LDL-C and MS, but not to smoking status or locations (Table 4).

For 1 standard deviation (SD) of these significant determinants, brachial FMD decreased by 0.4-0.6% (Figure

7). For 1 SD of age increase (11.7 years), IMT increased by 0.07mm (95% CI 0.06-0.08mm), for 1 SD increase in LDL-C (0.96 mmol/l), IMT increased by 0.01mm (95% CI 0.006-0.019mm), and for 1 SD increase in PM2.5 (26.9  $\mu\text{g}/\text{m}^3$ ), IMT increased by 0.012mm (95% CI 0-0.027mm) (Figure 8).

**Table 3:** Determinants of Risk Factors for FMD in CATHAY Chinese

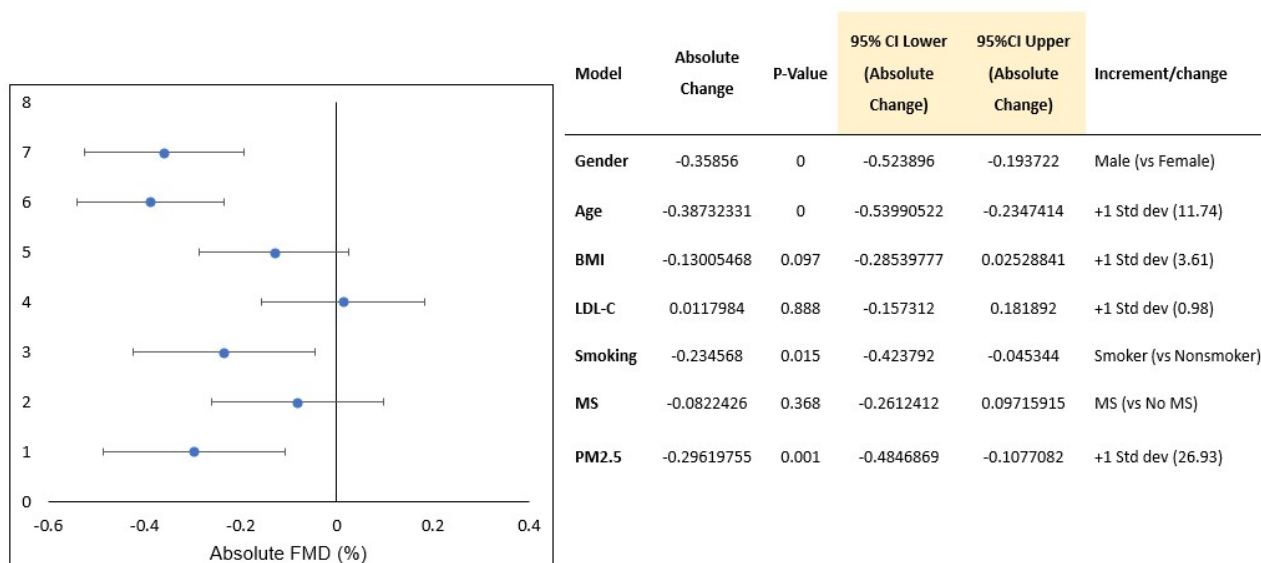
	Estimated CoefficientBeta Value	P-value
Age (yr)	-0.166	<0.0001
Gender	0.152	<0.0001
Smoking Status (%)	-0.083	0.020
BMI	-0.051	0.166
SBP (mmHg)	-0.030	0.549
DBP (mmHg)	-0.038	0.418
LDL-C (mmol/l)	0.021	0.570
MS (%)	-0.019	0.612
PM2.5 ( $\mu\text{g}/\text{m}^3$ )	-0.486	0.005

BMI: Body Mass Index; DBP: Diastolic Blood Pressure; FMD: Flow-Mediated Dilation; LDL-C: Low Density Lipoprotein Cholesterol; MS: Metabolic Syndrome; PM2.5: Particulate Matter<2.5um in Diameter; SBP: Systolic Blood Pressure

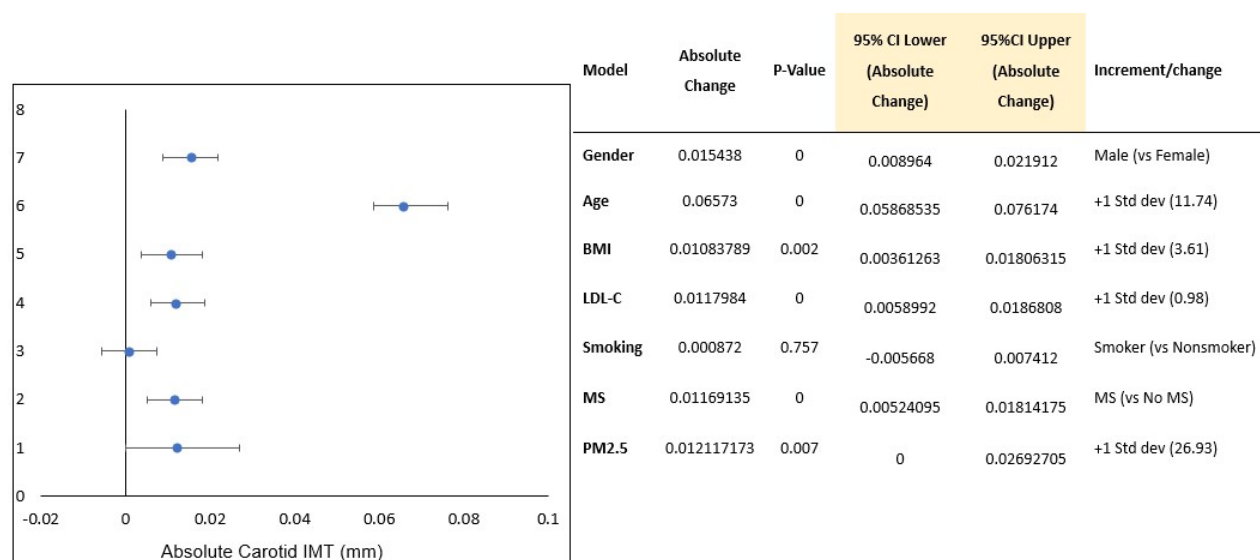
**Table 4:** Determinants of Risk Factors for Carotid IMT in CATHAY Chinese

	Estimated CoefficientBeta Value	P-value
Age (yr)	0.454	<0.0001
Gender	0.116	<0.0001
Smoking Status (%)	0.010	0.672
BMI	0.067	0.006
SBP (mmHg)	0.145	<0.0001
DBP (mmHg)	-0.065	0.047
LDL-C (mmol/l)	0.075	0.002
MS (%)	0.066	0.008
PM2.5 ( $\mu\text{g}/\text{m}^3$ )	0.204	0.025

BMI: Body Mass Index; DBP: Diastolic Blood Pressure; IMT: Intima-Media Thickness; LDL-C: Low Density Lipoprotein Cholesterol; MS: Metabolic Syndrome; PM2.5: Particulate Matter<2.5um in Diameter; SBP: Systolic Blood Pressure



**Figure 7:** Forest Plot of Brachial Flow-mediated Dilation (FMD) in CATHAY Subjects



**Figure 8:** Forest Plot of Carotid Intima-media Thickness (IMT) in CATHAY Subjects

## Discussion

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The normal values of brachial FMD ( $8.07 \pm 2.46$ , 95% CI 7.81-8.30%) and carotid IMT ( $0.65 \pm 0.15$ , 95% CI 0.64-0.66mm) as prognostic atherosclerosis surrogates in different age and gender groups in the Chinese ( $n=2163$ ) were established in Northern and Southern China as well as in overseas migrants. These confirmed the findings of our preliminary reports in 410-1656 CATHAY subjects on mean brachial FMD (ranging 7.75-8.15%) and mean carotid IMT (ranging 0.64-0.65mm) [14-15,21-22,32-35,38-40].

The current report included the subjects from all of these preliminary reports. These will be important and mandatory for epidemiological comparison and confirmation of the preventive strategies, both for traditional atherosclerosis risk factors and emerging factors such as PM2.5 air pollution.

FMD was significantly related to age, male gender, smoking and PM2.5 pollution, whereas carotid IMT was related to age, male gender, PM2.5 and traditional risk factors. The gender difference regarding FMD could be mainly

attributed to difference in vessel size, apart from their different neurohormonal status [6,48,49]. Age, gender, PM2.5, BMI, SBP and MS were significant determinant factors of carotid IMT, with 0.02-0.06mm difference in carotid IMT for 1 SD of risk factors. To contextualize these differences, a 0.1mm increase in carotid IMT has been associated with a 41% increase in stroke and 43% increase in acute myocardial infarction over a follow-up period of 2-7 years [9]. The 8% difference in carotid IMT was approximately similar to the kind of difference seen between diabetic and non-diabetic Chinese adults [35].

In comparison with the Italian, French and Latin American cohorts [29-31, 50-52], using similar methodologies of ultrasonic scanning and analysis package, carotid

IMT in the Chinese is identical to those of Lima cohort, but lower (thinner) than those reported in Italian, Buenos Aires Mexico cities and overall Latin American cohorts (Table 5A&5B). Similarly, for those without risk factor, carotid IMT in the Chinese is lower (thinner) than the French cohort (Table 5C). The mean IMT reported from Santiago is slightly lower than our CATHAY cohort.[30] On the same issue, we have previously reported better preservation of brachial FMD in Chinese than the Australian White on exposure to westernization, aging and smoking [32]. By enlarge, our normal values of these two atherosclerosis surrogate markers concur with our previous epidemiological findings of lower prevalence of atherosclerotic diseases in the Chinese compared with the Western peoples.

**Table 5A:** Comparison of Mean Carotid IMT in Overall Chinese versus Other Ethnicities

Study	CATHAY	PARC[31]	PARC-AALA[29]	CARMELLA[30]	VITA[49-50]
Country	Chinese	French	French/ Latin American/Asian	Latin American	Italian
No. of Subjects	2163	5264	2328	10415	2420
Male Age (yr)	47±11.7	50.6±11.6	49.8±8.7	44.7±11.3	54±14.5
Male (%)	45.4	57	48.2	46.6	44.3
Carotid IMT (mm)	0.65±0.15	0.75±0.12	0.69±0.11	0.67±0.11	0.67±0.28

47.6±11.8

Numerical numbers 29, 30, 31 indicate 'references'

, 49-50

**Table 5B:** Comparison of Carotid IMT in Different City/ County

Study Year	No. of Subjects	Overall (mm)	Age Groups (Years)				
			25-34 (mm)	35-44 (mm)	45-54 (mm)	55-64 (mm)	>64 (mm)
CATHAY 2007(Chinese)	2163	0.65±0.15(0.64-0.65)					
Female	1180		0.52±0.07 (0.51-0.53)	0.59±0.09 (0.58-0.60)	0.66±0.12 (0.65-0.67)	0.7±0.17 (0.70-0.73)	0.76±0.18 (0.72-0.81)
Male	983		0.56±0.09 (0.54-0.57)	0.60±0.09 (0.59-0.62)	0.68±0.13 (0.66-0.69)	0.75±0.17 (0.73-0.78)	0.79±0.27 (0.73-0.86)
CARMELLA 2007 [30](Latin America)							
Buenos Aires	1482	0.74(0.74-0.75)					
Female			0.68 (0.67-0.68)	0.71 (0.70-0.72)	0.76 (0.74-0.77)	0.81 (0.80-0.82)	
Male			0.70 (0.68-0.71)	0.74 (0.73-0.75)	0.79 (0.78-0.81)	0.85 (0.83-0.87)	
Mexico City	1722	0.69(0.69-0.70)					
Female			0.67 (0.66-0.68)	0.68 (0.67-0.68)	0.70 (0.69-0.71)	0.73 (0.72-0.75)	

Male			0.68	0.69	0.73	0.76	
			(0.67-0.69)	(0.68-0.69)	(0.72-0.74)	(0.75-0.78)	
Lima	1632	0.63(0.63-0.64)					
Female			0.57	0.62	0.68	0.72	
			(0.57-0.58)	(0.61-0.62)	(0.67-0.69)	(0.71-0.73)	
Male			0.58	0.63	0.68	0.72	
			(0.57-0.59)	(0.62-0.65)	(0.67-0.69)	(0.71-0.74)	
Santiago	1655	0.60(0.59-0.60)					
Female			0.53	0.58	0.62	0.68	
			(0.52-0.54)	(0.57-0.58)	(0.61-0.64)	(0.67-0.69)	
Male			0.55	0.61	0.65	0.70	
			(0.54-0.55)	(0.60-0.62)	(0.64-0.66)	(0.68-0.71)	

( ): 95% Confidence intervals

**Table 5C:** Comparison of Carotid IMT (mm) in Chinese vs French Population Without Risk Factor

Study Year	IMT Percentile	Female				Male			
		30-39	40-49	50-59	60-69	30-39	40-49	50-59	60-69
PARC [31]2009									
French	P05	0.467	0.514	0.553	0.575	0.464	0.528	0.549	0.569
(n=889)	P10	0.492	0.539	0.586	0.612	0.519	0.560	0.585	0.618
	P25	0.537	0.576	0.619	0.665	0.574	0.586	0.637	0.663
	P50	0.588	0.640	0.669	0.752	0.616	0.653	0.701	0.738
	P75	0.635	0.691	0.737	0.817	0.672	0.705	0.787	0.842
	P90	0.670	0.726	0.789	0.865	0.749	0.756	0.836	0.909
	P95	0.687	0.745	0.828	0.906	0.772	0.802	0.914	1.030
CATHAY 2007									
Chinese	P05	0.452	0.455	0.531	0.505	0.410	0.441	0.515	0.521
(n=1029)	P10	0.480	0.490	0.581	0.553	0.450	0.500	0.564	0.580
	P25	0.510	0.540	0.635	0.646	0.510	0.570	0.646	0.688
	P50	0.560	0.600	0.673	0.713	0.550	0.610	0.703	0.750
	P75	0.590	0.659	0.730	0.785	0.610	0.678	0.808	0.796
	P90	0.633	0.706	0.830	0.841	0.640	0.736	0.875	0.966
	P95	0.644	0.730	0.899	0.850	0.675	0.770	0.957	1.034

Brachial FMD and carotid IMT are both prognostic surrogates of atherogenesis related to long term cardiovascular or stroke outcome. FMD measurement is more dynamic and vulnerable to short term variability in physiological status, mental and physical stress, whereas IMT measurement is comparatively more stable [48-50]. We have previously reported carotid IMT is lower in working farmers

than in ex-farmers in the 3-Gorges Dam Territories. Lifestyle changes in ex-farmers is associated with a worse IMT, compared with those remaining in farming work [39]. In modernizing China, PM2.5 pollution is significantly related to worse IMT and to those with metabolic syndrome in particular, with thinner IMT in Southern Chinese, as compared with Northern Chinese and Overseas Chinese mi-

grants [21-22, 38, 53]. Improvement in IMT was demonstrated after folate and vitamin C supplementation in Northern Chinese, and after vitamin B12 correction in the vegetarians in Hong Kong and in Northern Chinese with suboptimal vitamin B12 status, as compared with those with normal B12 status [54]. Similarly adjunctive traditional Chinese medicine treatment in coronary and hypertensive subjects was associated with a significant improvement in FMD and IMT in the randomized placebo-control studies [17,55].

In the present report, two different ultrasonic machine models (ATL3000 or Sonosite) were utilized for brachial and carotid imaging by two investigators (CP and HY). The images were measured and analysed blindly throughout, using the same scanning protocol and automatic edge-detecting analysing packages. Accordingly, variations due to technical differences were kept minimized, with good intra-observer reproducibility and inter-observer variability, despite the relatively long period of study (11 years) over 11 sites in mainland China and Overseas.

For a valid FMD measurement, we need an induction of forearm ischemia (by cuff inflation) and subsequent reactive hyperemia (on cuff deflation). Reactive hyperemia can be monitored by measuring the increase in blood flow compared with basal flow. The resulting flow or shear rate-mediated dilation was measured offline retrospectively, compared with basal brachial diameter. In this regard, the short supply of video-recorder and tape for image storage has recently become a problematic logistic issue. More efficient continuous digital storage onsite for longer period (>2 minutes) in current ultrasonic machines and online measurement of vessel diameters by automatic edge-detecting technology, ought to be developed and popularized for the promotion of FMD measurement in the future. In-service training of ultrasonographer to familiarization of the ultrasonic acquisition and online measurement is equally important and mandatory for the reporting of more accurate and reproducible FMD results. On the other hand, much progress has been made in IMT imaging and analyses in recent years, including instantaneous digital acquisition and storage, automatic edge-detecting facility and measuring packages to facilitate a more user-friendly, practical and highly reproducible IMT measurement.

## Limitation of Study

We acknowledge certain limitations in the present CATHAY Study. Firstly, all subjects recruited were volunteers but not quota samples of specific cities and may not be truly representative of the real general population. Secondly for similar reason, female subjects are believed to be more health-concerned, and therefore we have slightly more females (54.6%) coming up for the study. However, the normal values of FMD and IMT in each gender are still valid for highlighting a gender difference. Thirdly our CATHAY cohort was studied prospectively for demographic factors, ultrasonic imaging and blood tests, although at different time over 11 years, no follow up vascular restudy was planned or arranged. Fourthly only the far wall of both common carotid arteries was measured and registered, which had made the IMT measurement more reproducible and decreased the variability. While the normal values of carotid IMT of both bifurcations may be more significant for reflecting the carotid atherosclerosis progression, their measurement would have increased the standard deviations of our reference values.

## Summary and Clinical Implications

The normal values, age and gender distribution of brachial FMD and carotid IMT for Southern, Northern Mainland China and Overseas Chinese migrants were established. These were significantly related to traditional modifiable and non-modifiable atherosclerotic risk factors. Carotid IMT in the Chinese was lower than those in the French, Italian and most Latin American populations. These values should help adding local public health epidemiological information, and improving clinical efforts to reduce cardiovascular risk and diseases in Chinese population, in mainland China and overseas alike.

## Data Availability

The research data will be available from the corresponding author to the editor and readers on reasonable request.

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## Conflicts of Interest

We declare no financial interests or connections, direct or indirect, or other situations that might raise the question of bias in the work reported or the conclusions, implications or opinions stated - including pertinent commercial or other sources of funding for the individual author(s) or for the associated department(s) or organization(s), personal relationships, or direct academic competition.

## Author Contribution

Woo KS: Project conception and design, research administration, statistical analysis and interpretation of data, drafting and revision of the article and final approval of the version to be published.

Chook Ping: Project conception and design, performance of ultrasonography analysis and interpretation of data, drafting, revision of the article and final approval of the version to be published.

Hu YJ: Research data collection, performance of ultrasonography analysis and interpretation of data, revision of the article and final approval of the version to be published.

Wong MK: Statistical analyses of database and interpretation of the CATHAY data.

Yin YH: Project conception, interpretation of data, revision of the article and final approval of the version to be published.

Li AM: Analyses of data, revision of the paper and final approval of the version to be published.

Chan SW: Project conception and design, research recruitment, administration and interpretation of data, revision of the paper, and final approval of the version to be published.

Kwok CY, Timothy: Design of project, interpretation of data, revision of the article and final approval of the version to be published.

Celemajer DS: Project conception and design, interpretation of data, drafting and revision of the article, and final approval of the version to be published.

All authors agree to be accountable for all aspects of the works.

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