European Heart Journal (2014) **35**, 2980–2987
doi:10.1093/eurheartj/ehu052

Association of exhaled carbon monoxide with subclinical cardiovascular disease and their conjoint impact on the incidence of cardiovascular outcomes

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Received 1 July 2013; revised 20 November 2013; accepted 29 December 2013; online publish-ahead-of-print 25 February 2014

Aims Whereas endogenous carbon monoxide (CO) is cytoprotective at physiologic levels, excess CO concentrations are associated with cardiometabolic risk and may represent an important marker of progression from subclinical to clinical cardiovascular disease (CVD).

Methods and results In 1926 participants of the Framingham Offspring Study (aged 57 ± 10 years, 46% women), we investigated the relationship of exhaled CO, a surrogate of blood CO concentration, with both prevalent subclinical CVD and incident clinical CVD events. Presence of subclinical CVD was determined using a comprehensive panel of diagnostic tests used to assess cardiac and vascular structure and function. Individuals with the highest (>5 p.p.m.) compared with lowest (≤4 p.p.m.) CO exposure were more likely to have subclinical CVD [odds ratios (OR): 1.67, 95% CI: 1.32–2.12; $P < 0.001$]. During the follow-up period (mean 5 ± 3 years), 193 individuals developed overt CVD. Individuals with both high CO levels and any baseline subclinical CVD developed overt CVD at an almost four-fold higher rate compared with those with low CO levels and no subclinical disease (22.1 vs. 6.3%). Notably, elevated CO was associated with incident CVD in the presence [hazards ration (HR): 1.83, 95% CI: 1.08–3.11; $P = 0.026$] but not in the absence (HR: 0.80, 95% CI: 0.42–1.53; $P = 0.51$) of subclinical CVD ($P_{interaction} = 0.019$). Similarly, subclinical CVD was associated with incident CVD in the presence of high but not low CO exposure.

Conclusion Our findings in a community-based sample suggest that elevated CO is a marker of greater subclinical CVD burden and, furthermore, a potential key component in the progression from subclinical to clinical CVD.

Keywords Carbon monoxide • Subclinical vascular disease • Cardiovascular outcomes

Introduction

The progression from traditional risk factor exposures to subclinical and, eventually, clinical cardiovascular disease (CVD) remains incompletely understood. Substantial efforts have been made to identify common biological pathways underlying the development of atherosclerotic CVD, and to investigate these pathways across model experimental systems and in humans. A growing body of evidence suggests that carbon monoxide (CO) is a potentially important modulator of CVD risk,[1–3] motivating attempts to examine the

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clinical relevance of endogenous CO.[4,5] Carbon monoxide represents a potential mechanistic link between metabolic disease and CVD. Although endogenous CO is known to be cytoprotective at physiologic levels, excess concentrations promote hypertension and endothelial dysfunction in experimental models of obesity and metabolic syndrome.[6,7] Although data in humans are limited, we and others have reported that increased exhaled CO (a marker of endogenous levels) is associated with traditional CVD risk factors cross sectionally, and with the development of metabolic syndrome and incident CVD prospectively, even in the absence of smoking.[8,9] Thus, variation in endogenous CO may represent an important prognostic as well as pathogenic biomarker.

The mechanisms by which CO can lead to the development of CVD have not been fully investigated. On the one hand, increased CO may represent vascular and metabolic stress that coincides with the presence of subclinical CVD, a known precursor to clinical CVD events.[10] Alternately, or in addition, elevated CO may itself promote the development of subclinical CVD *per se*, and thereby augment the risk for incident CVD in at-risk individuals. To further examine the role of endogenous CO in the progression from risk factor exposure to subclinical and overt clinical CVD, we studied the association between CO exposure, measureable subclinical CVD burden, and risk for incident CVD in a large community-based cohort of ambulatory individuals. We hypothesized that endogenous CO exposure is related positively to the presence of subclinical CVD cross sectionally, and that higher levels of CO are associated with a further increased risk of future CVD events among individuals who have evidence of subclinical disease.

Material and methods

The study design and enrolment criteria of the Offspring Cohort of the Framingham Heart Study have been described previously. Individuals eligible for the present investigation included Offspring Cohort participants who attended the sixth examination cycle (1995–98) and underwent standardized anthropometric measurements, a routine medical history, physical examination, and laboratory assessment of CVD risk factors ($n = 3532$). Individuals attending this examination cycle also underwent measurement of exhaled CO and testing for the presence of subclinical CVD, as described below. We excluded individuals from the present analysis based on the following criteria: prevalent CVD ($n = 412$), unavailable exhaled CO measures ($n = 11$), unavailable electrocardiographic data ($n = 6$), unavailable urinary albumin measurement ($n = 454$), unavailable ankle-brachial blood pressure ($n = 49$), unavailable or inadequate carotid ultrasonography data ($n = 69$), unavailable or inadequate echocardiographic data for determining left ventricular (LV) mass ($n = 588$), and unavailable smoking status ($n = 2$). Of the remaining 1941 individuals, 1926 had at least two serial exhaled CO measures collected over the course of quadrennial Offspring examinations, beginning with the second cycle and leading up to and including the sixth examination cycle (second examination cycle, 1979–83; third examination cycle, 1983–87; fourth examination cycle, 1987–91; fifth examination cycle, 1991–95). These 1926 individuals constituted the study sample for analyses.

All participants provided written informed consent and the study protocol was approved by the Institutional Review Board at Boston University Medical Center.

Assessment of exhaled carbon monoxide

Exogenous and endogenous CO concentrations equilibrate across the alveolar-capillary barrier such that exhaled CO reflects blood concentrations of carboxyhemoglobin.[11] At the second through sixth examination cycles, exhaled CO was measured in resting participants using the Ecolyzer (2000 series) instrument (Energetics Science Inc., Elmsford, NY, USA), which employs an electrochemical sensor to quantify CO in a sample of exhaled breath (with CO levels ranging from 1 to 100 p.p.m.). A canister of CO gas containing exactly 50 p.p.m. was used to calibrate the Ecolyzer to the midpoint of the scale on each day of testing. The average of two sequential Ecolyzer readings obtained from each participant was recorded. The exhaled CO level was then calculated as this average value minus the base rate of the ambient CO level in the testing room at the Heart Study. This method of determining exhaled CO level has been shown to be reproducible[12] and predominantly reflective of endogenous CO, with minimal local environmental (ambient) contamination.[13] Reproducibility of sequential Ecolyzer 2000 measurements of exhaled CO has been previously reported, with intra-individual correlations ranging from ≥ 0.89 to 0.94.[12,14]

Assessment of subclinical cardiovascular disease

The presence of subclinical vascular disease and target organ damage was determined at the sixth examination using a comprehensive series of non-invasive diagnostic tests, as previously detailed (Supplementary material online).[15] Presence vs. absence of subclinical abnormalities detected by each these diagnostic tests was defined using validated cutpoints and criteria (Supplementary material online, *Table S1*), and used to calculate a subclinical CVD score ranging from 0 to 5, where 1 point each was assigned for the presence of any component of the following abnormalities:[15] LV hypertrophy (either by electrocardiography or echocardiography), LV systolic dysfunction (by echocardiography), carotid artery disease (based on abnormal intima-media thickness or presence of stenosis on ultrasound), peripheral arterial disease (based on decreased ankle-brachial index), and glomerular endothelial dysfunction (based on urinary albumin excretion rate).

Cardiovascular outcomes

All study participants were under longitudinal surveillance for incident CVD events, as previously described.[15] Briefly, all events were adjudicated by an endpoints review committee of three investigators following an appraisal of outcomes data collected from examination visits, health history updates, and hospitalization and physician office medical records. The primary outcome in the present analysis was incidence of a first CVD event, defined as a composite of coronary heart disease, stroke or transient ischaemic attack, heart failure, and intermittent claudication (as defined previously[16]). The mean follow-up period was 9.2 ± 2.1 years.

Statistical analyses

Exhaled CO values >50 p.p.m. ($n = 4$) were considered equivalent to 50 p.p.m. for all analyses. For each participant, all available exhaled CO measurements performed at serial examinations leading up to and including the sixth examination were collected and averaged. Each participant was then grouped into a CO category based on the calculated average value of all available CO levels for that participant, including individual values that may have been above or below the final averaged CO category. These averaged CO values, representing antecedent CO exposure, were categorized into three groups (≤ 4, >4 and ≤ 5, and >5 p.p.m.) based on their distribution in the study sample. Categorical thresholds of average CO were determined as whole number approximations of tertiles. For all analyses, exhaled CO was analyzed with the lowest category (≤ 4 p.p.m.) serving as the referent.

In the first stage, we performed analyses focused on determining the extent to which CO is related cross-sectionally with subclinical CVD.

In age- and sex-adjusted logistic regression analyses, we examined the relations of exhaled CO with any prevalent subclinical CVD, defined as presence of ≥1 abnormality detected on testing for subclinical disease (i.e. score ≥1). We also evaluated multivariable models adjusting for the following standard risk factors in addition to age and sex: body mass index, systolic blood pressure (SBP), anti-hypertensive medication use, total/HDL cholesterol ratio, and presence of diabetes. To account for the possible effect of active smoking on exhaled CO levels, we also performed analyses adjusting for current smoking status in addition to other traditional risk factors included in the multivariable models. To assess for potential non-linearity of the relation between exhaled CO and subclinical CVD, above or below any particular cut point, we also examined generalized additive models using penalized splines adjusting for multiple variables including age, sex, SBP, diabetes, anti-hypertensive treatment, and total/HDL cholesterol ratio.

In the second stage, we performed analyses focused on determining the extent to which presence vs. absence of subclinical CVD modifies the prognostic significance of exhaled CO on incidence of CVD events. We calculated age- and sex-adjusted incidence rates of CVD for each CO group overall, and then for each group stratified by subclinical disease status. We used Cox proportional hazards regression in a series of hierarchical models constructed to examine the association of exhaled CO, subclinical disease, and risk of incident CVD. We assessed and confirmed the assumption of proportionality of hazards of the models used in analyses. All models adjusted for age and sex in addition to standard CVD risk factors (SBP, anti-hypertensive treatment, diabetes, total/HDL cholesterol ratio) and smoking status. Model A did not include adjustment for subclinical disease; Model B adjusted for subclinical disease modelled as a dichotomous variable (any vs. none); Model C adjusted for subclinical disease modelled as an ordinal variable; Model D stratified individuals according to CO category and by the presence vs. absence of subclinical disease. For Models A, B, and C, we also used multiplicative interaction terms to assess for the presence of effect modification by subclinical disease status on the association between CO and risk for CVD. Additionally, we used penalized splines to assess the relation between CO and incident CVD while adjusting for presence of baseline subclinical disease. In secondary analyses, we assessed for effect modification of sex and smoking status on the association between CO and risk for CVD.

The null hypothesis was rejected for a two-tailed *P*-value of <0.05, and all analyses were performed using SAS 9.2 (SAS Institute, Cary, NC, USA).

Results

The characteristics of the study sample at examination cycle 6 are shown in *Table 1*. Individuals with higher levels of CO exposure were more likely to be younger, men, and current smokers.

Exhaled carbon monoxide and subclinical vascular disease

Individuals with greater CO exposure had a higher frequency of prevalent subclinical disease, as represented by the overall subclinical disease score as well as by abnormal values for many of the component subclinical disease measurements (*Table 1*). Compared with individuals in the lowest category of CO exposure, individuals with average exhaled CO levels >5 p.p.m. had significantly greater odds of having prevalent subclinical disease (*Table 2*) in age- and sex-adjusted logistic regression analyses; these results remained unchanged in analyses adjusted for standard cardiovascular risk factors including active smoking (*Figure 1*).

Exhaled carbon monoxide, subclinical vascular disease, and cardiovascular outcomes

Of the 1926 individuals in the study, 193 (46% women) developed incident CVD during the follow-up period. The age- and sex-adjusted incidence of CVD increased with rising CO exposure (*Figure 2*), particularly among individuals with subclinical disease present at baseline (*Table 3*). Accordingly, individuals with baseline subclinical disease had the highest rates of incident CVD overall. The CVD risk conferred by CO, as reflected by the cumulative incidence of events over the follow-up period, was observed to be stable over time (*Figure 3*).

In age- and sex-adjusted and in multivariable-adjusted Cox proportional hazards analyses, higher average CO was associated with an increased risk for CVD in models without adjustment for subclinical disease (*Table 4*, Model A). In models adjusting for subclinical disease, as either a dichotomous or ordinal variable, the association of CO with CVD risk was attenuated but remained statistically significant in the multivariable models not including smoking as a covariate (*Table 4*, Models B and C). In the multivariable models additionally adjusting for current smoking, the association of CO with CVD risk was attenuated and became borderline significant. Results were similar in analyses adjusting for each component measure of subclinical CVD separately, either as dichotomous or continuous variables (Supplementary material online, *Tables S2* and *S3*). Notably, there was significant evidence of effect modification by subclinical disease on CO exposure and the risk for incident CVD in the main multivariable model ($P = 0.018$) and in the model additionally adjusting for current smoking ($P = 0.019$). Accordingly, in models stratifying CO exposure groups by the presence vs. absence of subclinical disease at baseline (*Table 4*, Model D), individuals with both elevated CO (average CO >5 p.p.m.) and baseline subclinical disease experienced an approximately two-fold risk for CVD compared with individuals in the lowest CO group without subclinical disease. In addition, presence of subclinical disease was associated with incident CVD among individuals with moderate CO exposure (average CO >4 and ≤5 p.p.m.) but not among individuals in the lowest CO exposure group. Results were similar in analyses adjusting for number of cigarettes smoked per day, instead of current vs. non-current smoker (Supplementary material online, *Table S4*). In Models A, B, and C, there was no significant effect modification by smoking status on the association between CO and CVD (data not shown). In all models, there was also no significant effect modification by sex on the association between CO and CVD (data not shown). In analyses of the association between CO and the specific outcome of incident coronary heart disease ($n = 109$ events), with and without adjustment for subclinical CVD, results were similar (Supplementary material online, *Table S5*).

Discussion

The main findings of this study were four-fold. First, higher mean levels of CO exposure were associated cross-sectionally with a higher prevalence of subclinical CVD detected using a comprehensive panel of diagnostic tests. Second, on prospective follow-up, individuals with both high CO levels and evidence of subclinical disease

Table 1 Sample Characteristics

Characteristic	CO category			
	≤4 (n = 713)	>4 and ≤5 (n = 568)	>5 (n = 645)	P-value
Clinical features				
Age (years)	58.6 ± 9.5	57.6 ± 9.7	56.0 ± 9.0	<0.0001
Women (%)	76	50	47	<0.0001
Body mass index (kg/m^2)	26.5 ± 4.5	27.8 ± 4.7	27.4 ± 4.5	<0.0001
Systolic blood pressure (mmHg)	127 ± 20	127 ± 19	125 ± 18	0.096
Diastolic blood pressure (mmHg)	75 ± 9	76 ± 9	75 ± 9	0.493
Hypertension (%)	35	36	34	0.706
Diabetes (%)	5	6	7	0.137
Current smokers (%)	0	1	41	<0.0001
Subclinical disease measures				
LV hypertrophy by ECG or echocardiography				
LV hypertrophy by Cornell criteria (%)	1	3	1	0.269
LV mass-to-height ratio (g/m)				
In men	107 ± 24	107 ± 22	108 ± 23	0.856
In women	84 ± 18	87 ± 19	86 ± 18	0.067
LV hypertrophy by echocardiography (%)	18	20	21	0.002
LV systolic dysfunction by echocardiography				
Fractional shortening	0.38 ± 0.05	0.38 ± 0.05	0.37 ± 0.05	<0.0001
LV systolic dysfunction (%)	2	2	5	0.022
Carotid ultrasound abnormality				
Increased carotid artery IMT (%)	17	18	26	<0.0001
Extreme increase of common carotid artery IMT (%)	3	5	5	0.164
Carotid artery stenosis ≥25% (%)	11	13	18	0.0002
Peripheral arterial disease				
Ankle-brachial index ≤0.9 (%)	1	1	3	0.009
Glomerular endothelial dysfunction				
Microalbuminuria (%)	9	7	10	0.212
Composite of subclinical disease measures				
At least one (%)	40	41	47	0.023
At least two (%)	10	10	17	0.0001
Three or more (%)	2	1	4	0.002
Mean score	0.52 ± 0.73	0.53 ± 0.72	0.69 ± 0.89	<0.0001

Data are means ± SD (for continuous variables) or percentages (for categorical variables).

developed new-onset CVD at rates that were nearly four times those of individuals with low CO levels and no subclinical disease. Third, when adjusting for traditional risk factors, CO conferred increased risk for incident CVD in the presence of subclinical disease but not in its absence (consistent with the observed statistical interaction between CO and subclinical disease for incident CVD). Finally, participants with subclinical CVD were more likely to develop clinical CVD in the setting of high but not low CO levels. Taken together, these findings indicate that higher CO levels could represent an aggregate marker of greater subclinical disease burden affecting different end organs and, furthermore, a critical component in the progression from subclinical to clinical CVD.

Endogenous CO has garnered attention as a gaseous second messenger that is biologically similar to nitric oxide. Like nitric oxide, CO is also a diatomic small molecule implicated in multiple vasoactive, inflammatory, and oxidative pathways.[1,17–19] Compared with nitric oxide, however, endogenous CO may exhibit even more involvement in metabolic pathways.[20] In experimental models, CO has been shown to stimulate insulin and glucagon release from islet cells[21] as well as modulate insulin sensitivity and glucose tolerance in obesity and diabetes.[22] Thus, CO represents a plausible mechanistic link between metabolic and vascular disease. Indeed, we and others have previously shown that elevation of CO levels in humans is associated with metabolic risk factors, diabetes, and

Table 2 Relation of carbon monoxide with any evidence of subclinical vascular disease

	Age- and sex- adjusted		[a]Multivariable-adjusted		[b]Multivariable-adjusted including smoking	
	OR (95%CI)	*P*-value	OR (95%CI)	*P*-value	OR (95%CI)	*P*-value
Average CO ≤4	Referent	–	Referent	–	Referent	–
Average CO >4 and ≤5	1.12 (0.88–1.43)	0.37	1.11 (0.86–1.43)	0.42	1.10 (0.85–1.42)	0.48
Average CO >5	1.67 (1.32–2.12)	<0.0001	1.69 (1.32–2.17)	<0.0001	1.37 (1.03–1.83)	0.033
Trend	–	<0.0001	–	<0.0001	–	0.038

Values are odds ratios (95% CI).
[a]Adjusted for age, sex, SBP, anti-hypertensive therapy, diabetes, and total/HDL cholesterol.
[b]Adjusted for age, sex, SBP, anti-hypertensive therapy, diabetes, total/HDL cholesterol, and smoking.

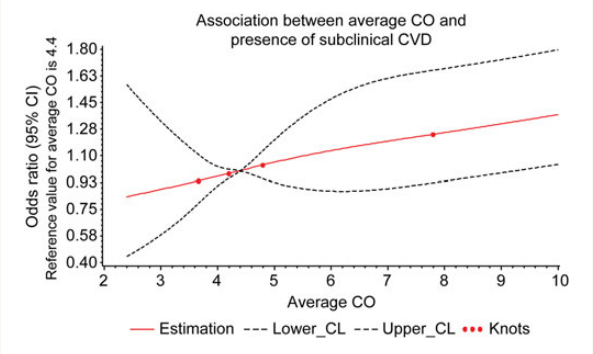

Figure 1 Multivariable-adjusted spline graph displaying the relation of carbon monoxide exposure with prevalent subclinical cardiovascular disease. Bold lines show the association between average exhaled carbon monoxide and presence of subclinical disease; upper and lower 95% confidence limits are plotted as dashed lines, with knots corresponding to carbon monoxide categories (where knots are the points connecting adjoining pieces of the polynomial spline function).

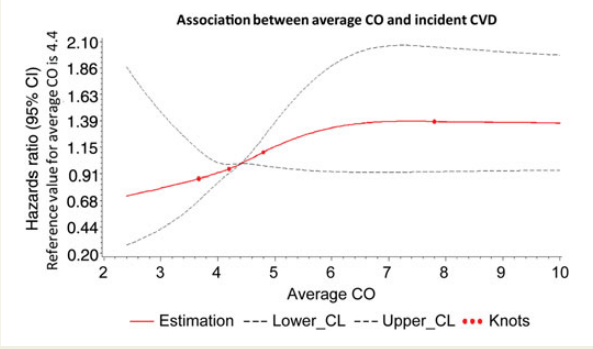

Figure 2 Spline graph displaying the relation of carbon monoxide exposure with incident cardiovascular events, adjusted for baseline presence of subclinical cardiovascular disease. Bold lines show the association between average exhaled carbon monoxide and risk for cardiovascular events; upper and lower 95% confidence limits are plotted as dashed lines, with knots corresponding to carbon monoxide categories.

incident CVD.[8,9] Findings from the present study now further demonstrate an association between CO and multiple measures of subclinical CVD, including carotid atherosclerosis, peripheral arterial disease, and LV hypertrophy.

There are several mechanisms by which CO may be related to the development of subclinical CVD. Endogenous CO is primarily produced from heme catabolism via the enzymatic activity of heme-oxygenases (HO), which have wide tissue distribution.[19] On the one hand, excess levels of HO activity and circulating CO may directly promote subclinical atherosclerosis, given that CO is involved in the regulation of vascular smooth muscle cell growth[23] as well as the inhibition of nitric oxide-induced vascular relaxation.[24] On the other hand, elevated CO levels could represent an endogenous compensatory response to worsening subclinical atherosclerosis, given that physiologic concentrations of CO have been shown to promote large vessel and myocardial angiogenesis[25,26] as well as exhibit anti-oxidant,[27] anti-inflammatory,[28,29] anti-thrombotic,[30] and anti-apoptotic[31,32] properties. Accordingly, investigations of human vascular tissue have observed increased levels of HO expression in relation to features of vulnerable atheromatous plaque, including increased thrombogenecity and MMP-9 levels.[33] Notably, HO-1 dependent production of CO may be closely interrelated with NO synthase-dependent production of NO in pathways related to endothelial cell protection or angiogenesis. Experimental studies indicate that NO upregulates HO-1 activity; in turn, high levels of CO can inhibit NO synthase activity while low levels of CO can induce NO release.[34]

To investigate the extent to which CO may be a marker or promoter of CVD, we examined the association of CO with incident CVD among individuals with and without evidence of subclinical disease. We observed that in the absence of subclinical disease, CO exposure was not significantly associated with risk for CVD in multivariable analyses. Thus, elevated CO may be regarded, at the very least, as a marker of prevalent subclinical disease. However, we also observed that subclinical disease was not associated with incident CVD in the setting of very low CO levels. Together, these findings suggest that CO could be involved not only in the development

Table 3 Incidence of CVD

Characteristic	No. events/No. at risk	Person-years at risk	Age- and sex-adjusted incidence rate[a] (95% CI)
Average CO ≤4			
All	52/713	6675	7.64 (5.13–10.03)
No subclinical disease	25/431	4099	6.32 (2.98–9.50)
Any subclinical disease	27/282	2576	9.86 (5.58–13.88)
Average CO >4 and ≤5			
All	62/568	5225	10.80 (7.54–13.88)
No subclinical disease	20/337	3203	5.67 (2.51–8.68)
Any subclinical disease	42/231	2022	18.07 (11.57–23.93)
Average CO >5			
All	79/645	5755	13.70 (9.90–17.24)
No subclinical disease	19/345	3221	5.99 (2.57–9.23)
Any subclinical disease	60/300	2534	22.13 (15.07–28.37)

[a]Data shown are number per 100 person years.

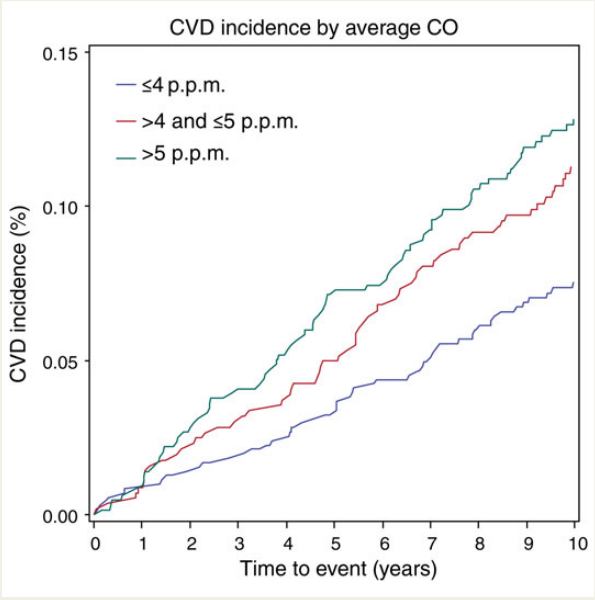

Figure 3 The cumulative incidence of cardiovascular events is shown over the follow-up period by carbon monoxide category.

of subclinical CVD but, importantly, also in the progression from subclinical to clinical CVD. Alternately, or in addition, elevated CO could indicate the presence of more severe rather than less severe subclinical disease. In this regard, the activity of HO enzymes and their by-products, which are known to play a central role in endogenous CO cycling,[18] may be closely related to the pathogenesis of clinical CVD. Thus, the clinical importance of the specific factors involved in endogenous CO cycling (including HO and its other metabolites) remains the focus of ongoing cardiovascular investigations.[35]

Several limitations of the present investigation merit comment. The reference standard for measuring circulating concentrations of CO in humans is gas chromatography; however, exhaled CO has been validated as a surrogate measure that can reliably capture variation in CO levels.[13,36] Furthermore, the exhaled CO measurements in this study were performed in the controlled clinic setting of the Framingham Heart Study and using instrumentation that was routinely calibrated with ambient indoor CO levels, serving to reduce excess intra- and inter-individual variability in measurements. Measured levels of exhaled CO can be influenced by micro- and macro-environmental exposure to products of combustion.[37] In our study, reliable measures were not available for second-hand smoke, occupational smoke exposure, or environmental sources such as areas with a high density of automobile traffic. In addition, neither individual nor regional level data on atmospheric CO levels were available at time points contemporaneous with our study.[38] Residual confounding from additional unmeasured covariates is also possible. Individuals in our study sample also did not have concurrent measures of exhaled or endogenous nitric oxide, a theoretical potential confounder in the present analyses.[18] Because our results are based on observational analyses, causal relationships cannot be inferred. Furthermore, our study sample was limited to individuals with available data for all subclinical CVD measures and, thus, excluded individuals missing any single component of the subclinical CVD score. Because participants who were able to complete separate diagnostic tests for multiple subclinical CVD measure likely represent a healthier sample at baseline, with less variation in both CO levels and subclinical CVD measures, sampling in our study likely biased our results to the null. Finally, our study was conducted in a cohort that included predominantly middle-aged white individuals of European ancestry; thus, the extent to which our findings are generalizable to other ethnicities is not known.

In summary, we observed that measurably elevated levels of CO exposure (assessed in exhaled breath) are associated both with the

Table 4 Carbon monoxide, subclinical vascular disease, and risk of incident cardiovascular disease

	Age- and sex- adjusted		[a]Multivariable-adjusted		[b]Multivariable-adjusted including smoking	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Model A: no adjustment for subclinical disease						
Average CO ≤4	Referent	–	Referent	–	Referent	–
Average CO >4 and ≤5	1.45 (0.99–2.11)	0.054	1.47 (1.01–2.14)	0.045	1.45 (1.00–2.12)	0.053
Average CO >5	1.91 (1.33–2.75)	0.0005	1.95 (1.35–2.82)	0.0004	1.55 (1.02–2.36)	0.042
Trend	–	0.0005	–	0.0004	–	0.034
Model B: Adjustment for subclinical disease as dichotomous variable (any vs. none)						
Average CO ≤4	Referent	–	Referent	–	Referent	–
Average CO >4 and ≤5	1.43 (0.99–2.09)	0.059	1.46 (1.00–2.13)	0.049	1.44 (0.99–2.10)	0.057
Average CO >5	1.77 (1.23–2.55)	0.002	1.84 (1.27–2.66)	0.001	1.48 (0.97–2.25)	0.070
Trend	–	0.002	–	0.001	–	0.056
Model C: Adjustment for subclinical disease as ordinal variable (sum score)						
Average CO ≤4	Referent	–	Referent	–	Referent	–
Average CO >4 and ≤5	1.47 (1.01–2.14)	0.042	1.51 (1.04–2.21)	0.031	1.50 (1.03–2.18)	0.036
Average CO >5	1.67 (1.16–2.40)	0.006	1.79 (1.23–2.59)	0.002	1.48 (0.97–2.26)	0.068
Trend	–	0.007	–	0.002	–	0.052
Model D: Risks by presence vs. absence of any subclinical disease						
Average CO ≤4 with no subclinical CVD	Referent	–	Referent	–	Referent	–
Average CO ≤4 with any subclinical CVD	1.16 (0.67–2.02)	0.60	0.91 (0.52–1.60)	0.75	0.89 (0.50–1.56)	0.68
Average CO >4 and ≤5 with no subclinical CVD	0.95 (0.53–1.73)	0.87	0.95 (0.52–1.72)	0.86	0.93 (0.51–1.69)	0.81
Average CO >4 and ≤5 with any subclinical CVD	2.18 (1.31–3.62)	0.003	1.76 (1.05–2.95)	0.033	1.70 (1.01–2.85)	0.046
Average CO >5 with no subclinical CVD	1.03 (0.56–1.90)	0.92	1.00 (0.54–1.85)	0.99	0.80 (0.42–1.53)	0.51
Average CO >5 with any subclinical CVD	2.77 (1.71–4.47)	<0.0001	2.33 (1.44–3.79)	0.0006	1.83 (1.08–3.11)	0.026

Values are Cox proportional hazards ratios (95% CI).
[a]Adjusted for age, sex, SBP, anti-hypertensive therapy, diabetes, and total/HDL cholesterol.
[b]Adjusted for age, sex, SBP, anti-hypertensive therapy, diabetes, total/HDL cholesterol, and smoking.

presence of subclinical CVD cross-sectionally, and with a greater risk of incident clinical CVD prospectively. The presence of subclinical CVD is conventionally defined as anatomic or functional evidence of end-organ damage, which is known to predate the incidence of clinical CVD events in many but not all affected individuals. In this context, our data suggest that CO could be a marker of pathways that are active in both the development and progression of CVD. Further research is needed to determine the possible mechanisms underlying our results and the extent to which measures of CO may be useful for prognostication among individuals both with and without subclinical CVD.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

Funding

From the Framingham Heart Study of the National Heart, Lung, and Blood Institute of the National Institutes of Health and Boston University School of Medicine. This work was supported by the National Heart, Lung and Blood Institute's Framingham Heart Study (Contract No. N01-HC-25195). This work was supported in part by the National Heart, Lung and Blood Institute's Framingham Heart Study (contract no. N01-HC-25195) and grants R01-NS017950 and K99HL107642 and the American College of Cardiology/Merck Research Fellowship in Cardiovascular Disease and Cardiometabolic Disorders (S.C.).

Conflict of interest: none declared.

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