Exhaled Carbon Monoxide and Risk of Metabolic Syndrome and Cardiovascular Disease in the Community

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Background—Endogenous carbon monoxide (CO) at physiological concentrations is cytoprotective, whereas excess levels reflect underlying oxidative stress, inflammation, and vascular pathology and portend adverse clinical sequelae. However, the relation of exhaled CO to metabolic/vascular risk in the community is unknown.

Methods and Results—We related exhaled CO, a surrogate measure of blood CO concentration, to the risk of developing new-onset metabolic syndrome and incident cardiovascular disease following 14 943 routine examinations (4139 unique participants; mean age, 46 years, 53% women) in the Framingham Heart Study. Baseline exhaled CO was associated with the presence of cardiometabolic risk factors (including smoking) and prevalent metabolic syndrome (odds ratio, 1.09 per log CO; 95% confidence interval, 1.02 to 1.17; \( P = 0.01 \)). During up to 4 years of follow-up, 1458 participants developed new-onset metabolic syndrome, and 416 experienced a first cardiovascular disease event. Compared with individuals in the lowest quartile of exhaled CO, those in the highest quartile were more likely to develop metabolic syndrome (odds ratio, 1.48; 95% confidence interval, 1.25 to 1.76; \( P < 0.0001 \)) and cardiovascular disease events (hazard ratio, 1.66; 95% confidence interval, 1.14 to 2.40; \( P = 0.008 \)) in multivariable analyses that included adjustment for smoking status.

Conclusion—In our community-based sample, higher exhaled CO levels predicted the development of metabolic syndrome and future cardiovascular disease events, underscoring the importance of this endogenous second messenger in the pathogenesis of metabolic and vascular risk. (Circulation. 2010;122:1470-1477.)

Key Words: cardiovascular diseases • carbon monoxide • epidemiology • metabolic syndrome • risk factors

Carbon monoxide (CO) is a ubiquitous gas that is produced both exogenously by the incomplete combustion of hydrocarbons and endogenously by the enzymatic degradation of heme and nonheme compounds. Endogenous CO is produced predominantly as an essential byproduct of heme oxygenase (HO) activity. Accumulating evidence suggests that endogenous CO is not only structurally but also biologically similar to nitric oxide (NO) in its ability to modulate vascular function. In light of numerous studies highlighting the fundamental role of NO as a signaling molecule in both physiological and disease states, CO has now also been identified as a key gaseous second messenger of special relevance to the cardiovascular system. At physiological levels, endogenous CO is cytoprotective and exerts antioxidant, anti-inflammatory, and antiapoptotic properties. In addition, experimental studies indicate that endogenous CO has the ability to vasodilate arteries, to regulate vascular smooth muscle cell growth, and to inhibit platelet aggregation. Conversely, excess endogenous CO, even at low levels, can reflect underlying inflammatory, oxidative, and vascular pathology. Clinical studies indicate that exhaled CO, which closely reflects total blood CO concentration, is increased among individuals with respiratory infections, hyperglycemia, and critical illness, regardless of smoking status. Functionally, excess endogenous CO can lead to the formation of reactive oxygen species, can impair NO-mediated vasodilation, and can promote adverse vascular remodeling. Thus, despite exhibiting protective activity at physiological concentrations, chronically elevated levels of CO may reflect underlying oxidative stress and inflammation, as well as more specific vascular processes that predispose to cardiovascular disease (CVD).

Clinical Perspective on p 1477

Exhaled CO closely reflects an individual’s total blood CO, which largely reflects the activity of pathways involved in

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1470
endogenous CO cycling. Therefore, we hypothesized that exhaled CO is associated with both metabolic and cardiovascular risk in the general population. We tested this hypothesis by investigating the relation of exhaled CO to the presence of cardiometabolic risk factors cross-sectionally and to the incidence of metabolic syndrome and CVD longitudinally in a large community-based sample.

Methods

Study Sample

The sampling design and enrollment criteria of the offspring cohort \( n = 5124 \) of the Framingham Heart Study have been described previously. The present investigation included offspring cohort participants who attended at least 1 quadrennial examination from cycles 2 (1979 to 1982) through 6 (1996 to 1997). Of the 19,086 participant visits at these examinations with available follow-up data, we excluded 1,618 visits with prevalent CVD and 1,635 with prevalent lung disease (chronic bronchitis, emphysema, or asthma, plus 890 visits with unavailable exhaled CO measures). Individuals with and without exhaled CO measures were similar with respect to age, sex, body mass index (BMI), and smoking status \( P > 0.24 \). After the exclusions, 14,943 participant visits (4139 individual participants) were included in the analysis. At each Heart Study examination, participants undergo standardized measurements of blood pressure, anthropometry, medical history, physical examination, and laboratory assessment of risk factors. All participants gave written informed consent, and the study protocol was approved by the Institutional Review Board at Boston University Medical Center.

Exhaled CO Assessment

In the absence of acute or chronic pulmonary disease, exogenous and endogenous CO concentrations effectively extricate across the alveolar-capillary barrier, and exhaled CO accurately reflects blood concentrations of carboxyhemoglobin. Exhaled CO was measured in each participant in a resting state with the Ecolyzer (2000 series) instrument (Energetics Science Inc, Elmsford, NY). This device uses an electrochemical sensor to quantify the level of CO gas in samples, and smoking status \( P > 0.24 \). After the exclusions, 14,943 participant visits (4139 individual participants) were included in the analysis. At each Heart Study examination, participants undergo standardized measurements of blood pressure, anthropometry, medical history, physical examination, and laboratory assessment of risk factors. All participants gave written informed consent, and the study protocol was approved by the Institutional Review Board at Boston University Medical Center.

Clinical Assessment of Risk Factors

Systolic (SBP) and diastolic (DBP) blood pressures were defined as the average of 2 physician-obtained measurements (with a mercury sphygmomanometer) on participants who had rested for \( > 5 \) minutes in a sitting position. Hypertension was defined as an SBP \( \geq 140 \) mm Hg, DBP \( \geq 90 \) mm Hg, or the use of antihypertensive medication. Current smoking was self-reported and defined as regular cigarette smoking within the year preceding the Heart Study examination. Serum triglycerides, total and high-density lipoprotein (HDL) cholesterol, and blood glucose were measured after an overnight fast. Diabetes mellitus was defined by fasting glucose \( \geq 126 \) mg/dL or the use of oral hypoglycemic agents or insulin. Among participants without diabetes mellitus, metabolic syndrome was defined as the presence of 3 or more of the following: waist circumference \( \geq 40 \) inches in men or \( \geq 35 \) inches in women; serum triglycerides \( \geq 150 \) mg/dL or taking medication for hypertriglyceridemia; HDL cholesterol \( < 40 \) mg/dL in men, \( < 50 \) mg/dL in women, or taking medication for reduced HDL cholesterol; SBP \( \geq 130 \) mm Hg, DBP \( \geq 85 \) mm Hg, or taking antihypertensive medication; and fasting glucose \( \geq 100 \) mg/dL. BMI \( \geq 30 \) kg/m\(^2\) was substituted for increased waist circumference at examinations 2 and 3 because waist measurements were not obtained at those visits. As previously described, high-sensitivity C-reactive protein (CRP) was measured on examination cycle 2 and 6 specimens with a Dade Behring nephelometer; CRP was measured on examination 5 specimens with the Hemagen assay.

Follow-Up and Outcomes

The outcomes of interest included incidence of metabolic syndrome and a first CVD event during a follow-up period of 4 years after the index examination. Major CVD events were fatal and nonfatal coronary heart disease (myocardial infarction, coronary insufficiency, and angina pectoris), stroke or transient ischemic attack, intermittent claudication, and heart failure. Criteria for these events have been described previously. CVD events were adjudicated by a panel of 3 experienced investigators based on a review of medical histories, physical examinations at the Framingham Heart Study, and hospitalization and personal physician records. The incidence of metabolic syndrome was defined on the basis of values for the components at the Heart Study examination.

Statistical Analyses

Exhaled CO values \( \geq 50 \) ppm were censored, and the remaining values were log-transformed for all analyses because of a right-skewed distribution.

In analyses of prevalent disease and risk factor burden, we used multivariable linear regression to examine the relation of exhaled CO with the following variables: age, sex, BMI, SBP, DBP, ratio of total to HDL cholesterol, log triglycerides, diabetes mellitus, self-reported smoking status (never, prior, current), and examination cycle. Among individuals without diabetes mellitus, we also examined the relations of exhaled CO with the presence of metabolic syndrome in analyses adjusting for age. All analyses were performed with generalized estimating equations based on an autoregressive correlation structure to account for correlations within each participant in the study sample (SAS PROC GENMOD). We performed these analyses in both the total sample and the sample of nonsmokers (including never and prior smokers) to elucidate correlates influenced by the effects of active smoking. For all analyses, exhaled CO was analyzed as a continuous log-transformed variable and as quartiles, with the lowest quartile as the referent. We also examined the correlation of exhaled CO with serum iron (a byproduct of HO activity), serum total bilirubin (a powerful antioxidant), and high-sensitivity CRP (an inflammatory marker) at examination 2, the cycle at which these measurements were available contemporaneously with exhaled CO.

In analyses of incident disease, we used the method of pooling repeated observations to assess the relation of exhaled CO with outcomes over consecutive 4-year intervals after confirming that the assumption of proportionality of hazards was met. Thus, Cox regression models were constructed for each 4-year follow-up interval, and participants were eligible to reenter the analyses if they remained free of events and did not meet the exclusion criteria at each examination visit. Individuals with prevalent metabolic syndrome or diabetes mellitus were additionally excluded from analyses of incident metabolic syndrome (leaving 9573 eligible attendees) but remained eligible for analyses of incident CVD (participants with prevalent CVD were excluded at the outset). Multivariable analyses of incident metabolic syndrome adjusted for age (as a result of collinearity of metabolic syndrome with other conventional risk factors). In multivariable analyses of incident CVD, model 1 adjusted for age and sex; model 2 further adjusted for BMI, SBP, DBP, ratio of total to HDL cholesterol, diabetes mellitus, and examination cycle; and model 3 adjusted for the covariates in model 2 in addition to self-reported smoking status. All analyses were performed in both the total sample and a sample of nonsmokers. To assess for any potential nonlinearity of relations between exhaled CO and CVD incidence existing above or below any particular quartile cut point, we also examined multivariable generalized additive models using penalized splines.

In secondary analyses, we repeated analyses in the sample of participants with BMI in the narrower range of \( \geq 22 \) and \( \leq 28 \) kg/m\(^2\).
To assess the role of inflammation on the association of CO with cardiometabolic risk, we also repeated the main analyses while adjusting for circulating CRP concentrations. For analyses of incident metabolic syndrome, additional adjustment was made for BMI, SBP, and smoking status.

To determine the relative contributions of CO and metabolic syndrome to CVD risk, we performed analyses that included both CO and metabolic syndrome as covariates in the same model. We also used a multiplicative interaction term to assess for effect modification of each covariate on the relation of the other to incident CVD.

All analyses were performed with SAS version 9.1.3 (SAS, Cary, NC). The display of multivariable-adjusted hazard ratio (HR) of CVD risk versus CO was generated with S-Plus. A 2-sided value of $P<0.05$ was considered significant.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

### Results

#### Correlates of Exhaled CO

The baseline characteristics of our sample are shown in Table 1. A rug plot displaying the distribution of exhaled CO in our sample is shown in Figure 1. In the total sample, exhaled CO was associated positively with diabetes mellitus, ratio of total to HDL cholesterol, and smoking status and inversely associated with age and examination cycle (Table 2). In the subset of nonsmokers, CO was directly associated with diabetes mellitus and BMI and inversely associated with age and examination cycle. At examination cycle 2, exhaled CO was modestly correlated with serum iron ($r=0.13$, $P<0.001$), serum total bilirubin ($r=0.10$, $P=0.001$ in nonsmokers; and $r=-0.11$, $P<0.001$ in current/past smokers), and CRP ($r=0.18$, $P<0.001$) in unadjusted Pearson correlation analyses of log-transformed values of each biomarker.

#### Relations of Exhaled CO With Metabolic Syndrome

Higher CO was positively associated with prevalent metabolic syndrome in both the total sample (Table 3) and the subset of nonsmokers (odds ratio [OR], 1.38; $P<0.001$). In longitudinal analysis, exhaled CO was modestly correlated with metabolic syndrome ($r=0.12$, $P<0.001$).

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**Table 1. Baseline Characteristics of Study Participants (n=4139)**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total Sample</th>
<th>First CO Quartile</th>
<th>Second CO Quartile</th>
<th>Third CO Quartile</th>
<th>Fourth CO Quartile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>45.6±10.2</td>
<td>46.5±10.4</td>
<td>46.2±10.6</td>
<td>45.8±10.1</td>
<td>44.6±10.0</td>
</tr>
<tr>
<td>Women, %</td>
<td>53</td>
<td>71</td>
<td>53</td>
<td>45</td>
<td>47</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.9±4.7</td>
<td>25.1±4.6</td>
<td>26.1±4.5</td>
<td>26.6±4.9</td>
<td>25.6±4.5</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>35.2±5.8</td>
<td>33.4±5.5</td>
<td>35.1±5.6</td>
<td>36.0±5.8</td>
<td>35.8±5.7</td>
</tr>
<tr>
<td>Blood pressure, mm Hg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP</td>
<td>122±17</td>
<td>122±17</td>
<td>124±17</td>
<td>124±16</td>
<td>121±17</td>
</tr>
<tr>
<td>DBP</td>
<td>78±10</td>
<td>77±9</td>
<td>79±10</td>
<td>79±10</td>
<td>77±10</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>25</td>
<td>22</td>
<td>27</td>
<td>28</td>
<td>22</td>
</tr>
<tr>
<td>Antihypertensive therapy, %</td>
<td>11</td>
<td>10</td>
<td>11</td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td>Serum cholesterol, mg/dL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>206.3±40.2</td>
<td>203.7±40.8</td>
<td>205.4±39.4</td>
<td>206.7±40.2</td>
<td>208.2±40.3</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>49.8±14.7</td>
<td>53.9±15.0</td>
<td>50.6±14.8</td>
<td>50.0±14.3</td>
<td>46.8±14.1</td>
</tr>
<tr>
<td>Total/HDL ratio</td>
<td>4.5±1.7</td>
<td>4.1±1.5</td>
<td>4.4±1.6</td>
<td>4.5±1.7</td>
<td>4.8±1.8</td>
</tr>
<tr>
<td>Serum triglycerides (median, IQR), mg/dL</td>
<td>90.0 (54.8, 125.2)</td>
<td>80.3 (48.9, 111.7)</td>
<td>86.5 (55.0, 118.0)</td>
<td>91.2 (54.5, 128.0)</td>
<td>96.2 (57.4, 135.0)</td>
</tr>
<tr>
<td>Blood glucose, mg/dL</td>
<td>94.7±22.5</td>
<td>93.2±19.5</td>
<td>95.0±23.1</td>
<td>95.3±24.6</td>
<td>95.1±22.1</td>
</tr>
<tr>
<td>Metabolic syndrome, %</td>
<td>25</td>
<td>19</td>
<td>25</td>
<td>26</td>
<td>27</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Smoking status, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>32</td>
<td>7</td>
<td>7</td>
<td>12</td>
<td>78</td>
</tr>
<tr>
<td>Past</td>
<td>33</td>
<td>43</td>
<td>46</td>
<td>43</td>
<td>12</td>
</tr>
<tr>
<td>Never</td>
<td>34</td>
<td>50</td>
<td>46</td>
<td>44</td>
<td>10</td>
</tr>
</tbody>
</table>

IQR indicates interquartile range. Values are presented as mean±SD or percentages unless otherwise indicated.

Figure 1. A rug plot displaying the frequency distribution of exhaled CO in the study sample.
The presence of metabolic syndrome as a covariate did not significantly alter the relation of exhaled CO to CVD risk in multivariable analyses adjusting for traditional risk factors (HR, 1.64; 95% CI, 1.41 to 1.90; P=0.0001) and additionally for smoking status (HR, 1.48; 95% CI, 1.18 to 1.85; P=0.0006). Metabolic syndrome was a significant predictor of CVD in analyses adjusting for age, sex, smoking status, and examination cycle (HR, 2.22; 95% CI, 1.81 to 2.73; P<0.0001), and this relation remained statistically significant in analyses additionally adjusting for exhaled CO (HR, 2.21; 95% CI, 1.80 to 2.71; P<0.0001). The multiplicative term

### Table 2. Multivariable-Adjusted Cross-Sectional Correlates of Exhaled CO*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Regression Coefficient (SE)</th>
<th>P</th>
<th>Regression Coefficient (SE)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (per 10 y)</td>
<td>-0.025 (0.005)</td>
<td>&lt;0.0001</td>
<td>-0.018 (0.004)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Women</td>
<td>-0.133 (0.010)</td>
<td>&lt;0.0001</td>
<td>0.143 (0.008)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI (per 5 kg/m²)</td>
<td>-0.001 (0.005)</td>
<td>0.88</td>
<td>-0.028 (0.004)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total/HDL cholesterol</td>
<td>0.031 (0.006)</td>
<td>&lt;0.0001</td>
<td>-0.003 (0.005)</td>
<td>0.56</td>
</tr>
<tr>
<td>Log triglycerides</td>
<td>-0.008 (0.010)</td>
<td>0.43</td>
<td>0.011 (0.009)</td>
<td>0.21</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0.045 (0.018)</td>
<td>0.014</td>
<td>0.039 (0.014)</td>
<td>0.006</td>
</tr>
<tr>
<td>Current smoker</td>
<td>1.088 (0.018)</td>
<td>&lt;0.0001</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Former smoker</td>
<td>0.036 (0.007)</td>
<td>&lt;0.0001</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

*Exhaled CO is log-transformed, and all models are run according to the generalized estimating equation. The model for the total sample adjusted for age, sex, BMI, SBP, DBP, ratio of total to HDL cholesterol, log triglycerides, diabetes mellitus, smoking status, and examination cycle. The model for the nonsmoker sample is adjusted for these same covariates except smoking status.

†Regression coefficients represent the change in natural log CO for an increase in the value of the predictor variables shown. Thus, women had a 13% lower CO (in ppm) relative to men (e^{-0.133}=0.87).

<table>
<thead>
<tr>
<th>Exhaled CO Variable</th>
<th>Prevalent Metabolic Syndrome</th>
<th>Incident Metabolic Syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>Events/Individuals at Risk, n*</td>
</tr>
<tr>
<td>Log CO†</td>
<td>1.09 (1.02–1.17)</td>
<td>1458/3199</td>
</tr>
<tr>
<td>First quartile‡</td>
<td>Referent</td>
<td>333/663</td>
</tr>
<tr>
<td>Second quartile</td>
<td>1.10 (1.01–1.20)</td>
<td>390/778</td>
</tr>
<tr>
<td>Third quartile</td>
<td>1.11 (1.01–1.22)</td>
<td>404/769</td>
</tr>
<tr>
<td>Fourth quartile</td>
<td>1.18 (1.05–1.33)</td>
<td>331/989</td>
</tr>
</tbody>
</table>

All analyses were adjusted for age. Analyses of prevalent metabolic syndrome were performed with the general estimating equation.

†Risk estimates correspond to 1-unit change in log CO (in ppm).

‡Exhaled CO quartiles 1 through 4 are defined as 0 to <4, ≥4 to <5, ≥5 to <6, and ≥6 ppm, respectively.

Over the follow-up period, there were 416 incident CVD events/1000 person years, with higher CO also conferring a greater risk for CVD in multivariable analyses (adjusted HR, 1.13 to 1.34; P=0.008). Accordingly, nonsmokers with the highest compared with lowest quartile of CO had a lower age-adjusted HR of 1.64 for CVD (95% CI, 1.06 to 2.54; P=0.027). Regression splines (Figure 3) suggested a progressive escalation in CVD risk with increasing values of CO.

### Secondary Analyses

In analyses restricted to participants with a BMI of 22 to 28 kg/m², results of the main analyses were unchanged (data not shown). In analyses adjusting for CRP in the total sample, exhaled CO remained significantly associated with prevalent metabolic syndrome (OR, 1.15; 95% confidence interval [CI], 1.05 to 1.25; P=0.003) and incident (OR, 1.17; 95% CI, 1.03 to 1.33; P=0.0001) metabolic syndrome. Exhaled CO also remained significantly associated with incident CVD in multivariable analyses adjusting for CRP in addition to traditional risk factors (HR, 1.54; 95% CI, 1.26 to 1.88; P<0.0001) and smoking status (HR, 1.34; 95% CI, 1.00 to 1.79; P=0.05).

The relation of CO with incident metabolic syndrome remained significant in analyses adjusting for age, BMI, SBP, and smoking status (OR, 1.17; 95% CI, 1.02 to 1.34; P=0.03).

Relation of Exhaled CO With Incident CVD

Over the follow-up period, there were 416 incident CVD events (128 in women) among 14,943 individuals at risk. A total of 125 individuals were censored because of death (n=124) or being lost to follow-up (n=1). Higher CO was independently associated with higher CVD incidence in the total sample, even after adjustment for smoking status (Table 4). This trend was particularly evident in the highest compared with lowest quartile of CO (Figure 2). In the subset of nonsmokers, increasing CO was similarly associated with greater risk for CVD in multivariable analyses (adjusted HR, 1.50; 95% CI, 1.07 to 2.10; P=0.018). Accordingly, nonsmokers with the highest compared with lowest quartile of CO had a adjusted HR of 1.64 for CVD (95% CI, 1.06 to 2.54; P=0.027). Regression splines (Figure 3) suggested a progressive escalation in CVD risk with increasing values of CO.
representing the interaction between metabolic syndrome and exhaled CO was statistically nonsignificant in analyses of incident CVD adjusting for traditional risk factors, including smoking status ($P = 0.45$). Accordingly, the association of exhaled CO with incident CVD was similar between individuals with (HR, 1.40; 95% CI, 1.03 to 1.89; $P = 0.03$) and without (HR, 1.54; 95% CI, 1.10 to 2.16; $P = 0.01$) the metabolic syndrome.

**Discussion**

Our principal findings were 3-fold. First, exhaled CO was inversely associated with age but positively associated with several conventional cardiometabolic risk factors. Second, higher exhaled CO was directly related cross-sectionally to the prevalence of the metabolic syndrome. Third, higher CO was associated with both future development of the metabolic syndrome and CVD incidence. Notably, individuals with the highest quartile of exhaled CO had an $\approx 1.7$-fold higher risk for CVD than those with the lowest quartile of CO exposure. Overall, these findings suggest that interindividual variation in CO is associated with metabolic derangements and the development of manifest CVD.

**Clinical Correlates of Exhaled CO**

In our community-based sample, exhaled CO levels were significantly higher in younger compared with older individuals and in men compared with women. These age- and sex-based differences are consistent with findings from smaller samples and may be related to demographic variations in long-term exposure to microenvironmental CO. The inverse association of CO with age and the differences in CO between men and women may also be related to the dependence of endogenous CO production on HO activity. In animal models, HO activity is known to be reduced in older age, more so in men than in women, potentially because of decreased constitutive expression and inducibility of HO as a result of yet undetermined mechanisms.

**Cardiometabolic Correlates of Exhaled CO**

Importantly, exhaled CO remained significantly associated with several specific cardiovascular risk factors after adjustment for age, sex, examination cycle, and even smoking.

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**Table 4. Relation of Exhaled CO With Incident Cardiovascular Disease**

<table>
<thead>
<tr>
<th>Exhaled CO Variable</th>
<th>Events/Individuals at Risk, n*</th>
<th>Model 1, HR (95% CI)</th>
<th>$P$</th>
<th>Model 2, HR (95% CI)</th>
<th>$P$</th>
<th>Model 3, HR (95% CI)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log CO†</td>
<td>416/4139</td>
<td>1.72 (1.50–1.98)</td>
<td>&lt; 0.0001</td>
<td>1.62 (1.40–1.88)</td>
<td>&lt; 0.0001</td>
<td>1.46 (1.18–1.82)</td>
<td>0.0006</td>
</tr>
<tr>
<td>First quartile‡</td>
<td>92/836</td>
<td>Referent</td>
<td>...</td>
<td>Referent</td>
<td>...</td>
<td>Referent</td>
<td>...</td>
</tr>
<tr>
<td>Second quartile</td>
<td>80/922</td>
<td>0.76 (0.56–1.02)</td>
<td>0.070</td>
<td>0.75 (0.55–1.01)</td>
<td>0.057</td>
<td>0.74 (0.55–1.00)</td>
<td>0.052</td>
</tr>
<tr>
<td>Third quartile</td>
<td>109/991</td>
<td>1.12 (0.84–1.48)</td>
<td>0.43</td>
<td>1.05 (0.78–1.39)</td>
<td>0.76</td>
<td>1.03 (0.77–1.37)</td>
<td>0.84</td>
</tr>
<tr>
<td>Fourth quartile</td>
<td>135/1390</td>
<td>2.19 (1.66–2.89)</td>
<td>&lt; 0.0001</td>
<td>1.94 (1.45–2.58)</td>
<td>&lt; 0.0001</td>
<td>1.66 (1.14–2.40)</td>
<td>0.008</td>
</tr>
</tbody>
</table>

*No. of attendees at risk: 14,943 in the total sample; 3674, 4037, 3871, and 3361 across increasing quartiles of exhaled CO, respectively.
†Risk estimates correspond to 1-unit change in log CO (in ppm).
‡Exhaled CO quartiles 1 through 4 are defined as 0 to <4, ≥4 to <5, ≥5 to <6, and ≥6 ppm, respectively.

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**Figure 2.** Kaplan–Meier curves showing the cumulative incidence of CVD by exhaled CO quartiles over the follow-up period from the baseline examination (in years).

**Figure 3.** Multivariable-adjusted spline graph displaying the relation of exhaled CO with cardiovascular events. Bold gray lines show the association between exhaled CO and event risk; upper and lower 95% CIs are plotted as fine gray lines. The horizontal dashed line is the line of no association.
status. Notably, each of these risk factors is also considered a metabolic trait: increased ratio of total to HDL cholesterol, diabetes mellitus, and greater BMI. Accordingly, exhaled CO was also significantly associated with both prevalent and incident metabolic syndrome. The association of CO with metabolic syndrome and its components may be due to several mechanisms. In particular, oxidative stress and inflammatory cytokines are known to increase expression of HO, which has a wide tissue distribution and is the primary contributor of enzymatic heme degradation to form endogenous CO, in addition to biliverdin and iron products. This corresponds with the observed positive correlation of exhaled CO with serum iron and CRP in our sample. We also observed a correlation between exhaled CO and bilirubin that was positive in nonsmokers but negative in current and past smokers. Consistent with the inverse relation of bilirubin to smoking status seen in prior studies, it is possible that excess levels of oxidative stress in smokers lead to a relative decrease in conversion of biliverdin to bilirubin via reduction, in favor of increased bilirubin-to-biliverdin conversion via oxidation. The HO-dependent response to oxidative stress, in particular, may be a main common pathway by which CO is associated with metabolic traits. In a small physiological study of 24 individuals with diabetes mellitus and 37 healthy control subjects, Paredi and colleagues observed that exhaled CO was higher in the presence of diabetes mellitus, regardless of smoking status. Both hyperglycemia and the progressive accumulation of advanced glycation end-products in diabetes mellitus can be drivers of increased oxidative activity and, in turn, long-term activation of HO. Similarly, the relation of dyslipidemia with excess CO levels may be related to the fact that oxidized lipids also activate HO. This association could also be part of a regulatory pathway whereby the non-CO byproducts of HO activity, biliverdin and bilirubin, are capable of preventing oxidative modification of LDL cholesterol. Among the nonsmokers in our sample, exhaled CO was also strongly associated with BMI even after adjustment for diabetes mellitus. This association could be driven by the presence of metabolic derangements that precede overt diabetes mellitus but similarly predispose to oxidative stress. Notably, long-term elevations in endogenous CO may be the both the product and cause of oxidative stress because excess levels of CO can disrupt the mitochondrial electron transport chain, resulting in the generation of reactive oxygen species.

**Relations of Exhaled CO and Incidence of Cardiovascular Disease**

Beyond its relation with metabolic traits, exhaled CO was also significantly associated with incident CVD. Elevated CO may be related to CVD risk in a dose-dependent fashion through a variety of mechanisms. At low physiological levels, endogenous CO production reflects vasoprotective HO activity. In response to stress, endothelial and vascular smooth muscle cells increased HO expression, leading to increased production of CO, which acts as a potent vasodilator. Endogenous CO can also inhibit platelet aggregation, vascular cell apoptosis, and inflammatory cytokine release. Conversely, overexpression of HO with excess CO production may decrease NO-mediated vasodilation and promote adverse vascular remodeling by inhibiting vascular proliferation. Recent experimental data suggest that unopposed oxidative stress may cause endogenous CO to exert vasoconstrictive rather than vasodilatory activity. In addition to these vascular effects, it is well recognized that excess total CO exposure can lead to tissue hypoxia by preferentially binding circulating hemoglobin to form carboxyhemoglobin.

Taken together, the association of excess CO with both metabolic syndrome and CVD highlights the possibility that CO-related pathways are involved in the pathogenesis of both conditions. Indeed, experimental evidence suggests that CO cycling plays a central role in regulating the endogenous response to a variety of stresses, including oxidative stress, inflammation, and disruption of vascular integrity. Accordingly, interindividual variation in circulating CRP accounted for only a portion of the cardiometabolic risk associated with CO in our analyses. Given its involvement in multiple pathways, an excessive rise in CO could represent a common pathological factor contributing to the clustering of cardiometabolic disorders. Notably, CO was associated with risk for metabolic syndrome at a lower concentration threshold than for CVD, consistent with the hypothesis that accrual of metabolic derangements likely precedes the development of overt vascular disease. Thus, in addition to increasing risk for metabolic syndrome, elevated CO could promote the progression of metabolic syndrome to overt CVD. Exhaled CO and metabolic syndrome also appeared to have largely independent effects on increasing cardiovascular risk in our sample. Indeed, elevated CO was associated with incident CVD among individuals with or without baseline metabolic syndrome.

**Limitations**

Several limitations of the present study merit consideration. Although the reference standard for estimating individual CO concentrations is gas chromatography, exhaled CO is a validated surrogate measure that can similarly capture spatial and temporal variation in CO levels (unlike ambient monitors). Although exhaled CO measurements in this study were obtained at a single point in time per visit, the use of a standardized protocol and routinely calibrated instrumentation likely served to reduce excess intra-individual and inter-individual variability. We did not consider data on current number of cigarettes per day or time between the last cigarette and the CO measurement; however, the consistency of the findings in nonsmokers is reassuring in this regard. Individuals in our study sample did not have concurrent measures of exhaled or endogenous NO, a theoretical potential confounder in the present analyses. Residual confounding from additional unmeasured covariates is also possible. Generalized estimating equations were based on an autoregressive correlation structure to account for multiple observations within each participant but not for between-sibling correlations because these correlations have been associated with minimal effects on outcomes in similar prior Framingham studies. Finally, our sample was predominantly white and of
European ancestry, limiting the generalizability of our findings to other racial/ethnic groups.

Conclusions
In our community-based sample, higher exhaled CO levels were associated with the development of metabolic syndrome and future CVD events. The association of exhaled CO with adverse cardiovascular outcomes was maintained after adjustment for traditional cardiovascular risk factors, including smoking status. Taken together, the relation of CO with both metabolic traits and overall CVD risk suggests that excess CO exposure is associated with cardiovascular risk via mechanisms beyond those related to tissue hypoxia. Abnormal activation of endogenous CO and HO-related pathways could predispose to both metabolic disease and CVD through a variety of mechanisms. Similar to the importance of physiological NO in maintaining vascular homeostasis, endogenous pathways involving the cycling of CO may be critical for modulating not only vasoactive but also oxidative and inflammatory activity. Further investigations of such endogenous CO-related pathways could provide insights into the pathogenesis of metabolic syndrome and CVD.

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Disclosures
None.

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**CLINICAL PERSPECTIVE**

Carbon monoxide (CO) is widely recognized as a potentially toxic byproduct of hydrocarbon combustion. More recently, CO has been recognized as a key second messenger molecule that is normally present in the body at low concentrations and serves to protect the cardiovascular system against oxidative, inflammatory, and vascular stresses. Thus, elevated levels of endogenous CO may reflect the presence of underlying metabolic and cardiovascular pathology. We assessed the association of exhaled CO (a marker of endogenous CO) with the presence of cardiometabolic risk factors cross-sectionally and with the risk of developing future metabolic syndrome and overt cardiovascular disease prospectively among 4139 individuals (contributing 14,943 person-examinations) in the Framingham Heart Study. We observed that exhaled CO was associated with the presence of metabolic syndrome \( (P=0.01) \) in addition to many of its component risk factors. During up to 4 years of follow-up, baseline CO was also associated with future cardiometabolic events, with individuals in the highest quartile of exhaled CO having a \( \approx 1.5 \) odds of developing metabolic syndrome \( (P<0.0001) \) and \( \approx 1.7 \) risk of developing cardiovascular disease \( (P=0.008) \) compared with the lowest quartile, serving as referent. Overall, the findings of this study suggest that elevated CO is an important marker for the development of both metabolic disease and cardiovascular disease. Additional research is warranted to elucidate how CO-related pathways may contribute to cardiometabolic disorders and their associated outcomes.
Exhaled Carbon Monoxide and Risk of Metabolic Syndrome and Cardiovascular Disease in the Community
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