Journal of the American Heart Association

ORIGINAL RESEARCH

# Association of Exhaled Carbon Monoxide With Ideal Cardiovascular Health, Circulating Biomarkers, and Incidence of Heart Failure in the Framingham Offspring Study

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**BACKGROUND:** Exhaled carbon monoxide (eCO) is directly associated with traditional cardiovascular disease risk factors and incident cardiovascular disease. However, its relation with the cardiovascular health score and incidence of heart failure (HF) has not been investigated.

**METHODS AND RESULTS:** We measured eCO in 3521 Framingham Heart Study Offspring participants attending examination cycle 6 (mean age 59 years, 53% women). We related the cardiovascular health score (composite of blood pressure, fasting plasma glucose, total cholesterol, body mass index, smoking, diet, and physical activity) to eCO adjusting for age, sex, and smoking. Higher cardiovascular health scores were associated with lower eCO ($\beta$=–0.02, $P$<0.0001), even among nonsmokers. Additionally, C-reactive protein, plasminogen activator inhibitor-1, fibrinogen, growth differentiation factor-15, homocysteine, and asymmetrical dimethylarginine were positively associated with eCO ($P\leq$0.003 for all). The age- and sex-adjusted and multivariable-adjusted heritabilities of eCO were 49.5% and 31.4%, respectively. Over a median follow-up of 18 years, 309 participants (45% women) developed HF. After multivariable adjustment, higher eCO was associated with higher risk of HF (hazards ratio per SD increment: 1.39; 95% CI, 1.19–1.62 [$P$<0.001]) and with higher risk of HF with reduced ejection fraction (N=144 events; hazard ratio per SD increment in eCO: 1.43; 95% CI, 1.15–1.77 [$P$=0.001]).

**CONCLUSIONS:** In our community-based sample, higher levels of eCO were associated with lower cardiovascular health scores, an adverse cardiovascular biomarker profile, and a higher risk of HF, specifically HF with reduced ejection fraction. Our findings suggest that carbon monoxide may identify a novel pathway to HF development.

**Key Words:** carbon monoxide ■ cardiac biomarkers ■ cardiovascular health ■ heart failure

Carbon monoxide (CO) is a gasotransmitter produced endogenously in the body, as the by-product of heme metabolism.[1] At physiological concentrations, CO is cytoprotective and may serve as a potential signaling mediator.[2] However, at excess concentrations, elevated endogenous CO levels have been shown to impair nitric oxide (NO)–mediated vasodilation,[3] foster oxidative stress,[4] lead to the formation of reactive oxygen species,[5] and promote adverse vascular remodeling.[6] The paradoxical effects of CO have been evaluated in human studies. As CO equilibrates quickly across the alveolar-capillary membrane, exhaled CO (eCO) reflects endogenous CO production.[7] Investigators from the FHS (Framingham Heart Study) have reported that eCO is directly associated with traditional cardiovascular disease (CVD) risk factors, presence of the metabolic syndrome,[8] and subclinical CVD cross-sectionally.[9] Additionally, higher

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Supplementary Material for this article is available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.120.016762

For Sources of Funding and Disclosures, see page 10.



*JAHA* is available at: www.ahajournals.org/journal/jaha

## CLINICAL PERSPECTIVE

### What Is New?

- In the community-based Framingham Heart Study sample, higher levels of exhaled carbon monoxide were associated with lower cardiovascular health scores and adverse cardiovascular biomarker profiles cross-sectionally, and a higher risk of heart failure, specifically heart failure with reduced ejection fraction, prospectively.

### What Are the Clinical Implications?

- Exhaled carbon monoxide, reflecting endogenous carbon monoxide, may identify a novel pathway to the development of heart failure.

### Nonstandard Abbreviations and Acronyms

| | |
|---|---|
| **ADMA** | asymmetrical dimethylarginine |
| **AHA** | American Heart Association |
| **CVH** | cardiovascular health |
| **eCO** | exhaled carbon monoxide |
| **FHS** | Framingham Heart Study |
| **GDF-15** | growth differentiation factor-15 |
| **HFpEF** | heart failure with preserved ejection fraction |
| **HFrEF** | heart failure with reduced ejection fraction |
| **NT-proANP** | N-terminal pro–atrial natriuretic peptide |
| **PAI-1** | plasminogen activator inhibitor-1 |
| **SBP** | systolic blood pressure |
| **SDMA** | symmetric dimethylarginine |
| **SOLAR** | Sequential Oligogenic Linkage Analysis |

levels of eCO are associated with a higher risk of incident stroke,[10] incident metabolic syndrome, and incident CVD prospectively.[9]

The American Heart Association (AHA) created an ideal cardiovascular health (CVH) score, which promoted 7 health behaviors and factors, including abstinence from smoking, ideal body mass index (BMI), regular physical activity, healthy diet, low untreated serum total cholesterol concentrations, and optimal blood pressure and glucose levels to reduce CVD deaths and improve CVH.[11] Previous studies have reported poor CVH to be associated with a higher risk of CVD (including heart failure [HF]) and CVD mortality.[11–14] The inverse relation between the CVH score and incident CVD may partly be caused by its favorable impact on CVD biomarker levels.[13] In that context, no study to our knowledge has evaluated an association between the CVH score and eCO.

While there are well-established associations of risk factors and biomarkers with HF, there are still gaps in our understanding of the underlying molecular mechanisms of HF. As such, it is of great importance to continue evaluating new biomarkers to help clinicians better identify high-risk individuals, to diagnose swiftly and accurately, and to prognosticate patients appropriately.[15] Multiple biomarkers that represent different biological pathways have been shown to be positively associated with the development of HF.[16] Accordingly, we investigated eCO and its association with incident HF.

We hypothesized that the CVH score is inversely associated with eCO and that select biomarkers, associated with higher risk of CVD, will be directly associated with eCO. We postulated that eCO is heritable with a moderate portion of variability being explained by genetic information. Last, we hypothesized that higher values of eCO are associated with a higher risk of HF, as well as with its subtypes HF with preserved ejection fraction (HFpEF) and HF with reduced ejection fraction (HFrEF).

## METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

### Study Sample

The study design and enrollment criteria of the FHS Offspring Study have been previously described.[17] The current investigation included Offspring participants who attended the sixth examination cycle (1995–1998).

For investigating the relation between the CVH score and eCO, of the 3521 individuals who had at least 1 eCO reading (base sample), we excluded those with prevalent CVD (n=407), serum creatinine ≥2 mg/dL (n=9), BMI <18.5 kg/m$^2$ (n=13), and missing data on the CVH score components (n=1031), resulting in a sample size of 2061 (sample 1, Figure S1).

For investigating the association of biomarkers with eCO, we excluded from the base sample individuals with missing values on any of the 15 biomarkers of interest (n=286) and missing covariates (n=19), resulting in a subsample size of 3216 (sample 2, Figure S2).

To examine the heritability of CO, we excluded from the base sample individuals with missing values on covariates (n=67), resulting in a sample size of 3454 (sample 3).

When investigating the association of eCO with incidence of HF, we excluded from the base sample individuals with prevalent HF at examination cycle 6 (n=39) and missing values on covariates (n=66), resulting in a sample size of 3416 (sample 4, Figure S3).

For analyses using the HF subtypes, we further excluded individuals with missing data on ejection fraction (n=57), resulting in a subsample size of 3359 (sample 5, Figure S3). All participants gave written informed consent, and the protocol was approved by the institutional review board at Boston University Medical Center.

## Assessment of eCO

eCO reflects endogenous CO as CO equilibrates quickly across the alveolar-capillary membrane.[7] eCO was measured at rest using the Ecolyzer (2000 series) instrument (Energetics Science Inc.). This instrument employs an electrochemical sensor to quantify CO, with a range from 1 to 100 ppm. Each day of the testing, a canister of CO gas containing exactly 50 ppm was used to calibrate the Ecolyzer to the midpoint of the scale. The average of 2 readings from each participant was recorded. eCO level was calculated by taking this averaged Ecolyzer reading and subtracting from it the base rate of the ambient CO level in the testing room. This method has been shown to be reproducible[18] and predominantly reflective of endogenous CO with minimal local contamination by ambient exposure.[19]

## Assessment of the CVH Score

For each participant, a CVH score was created as a composite of 7 metrics, including smoking status, BMI, serum total cholesterol, blood pressure, fasting plasma glucose, physical activity, and diet (Table S1). Each metric was assigned a score of 0 to indicate poor status, 1 to indicate intermediate status, or 2 to indicate ideal status, with the CVH score for each participant ranging from 0 (reflecting poor CVH) to 14 (indicating ideal CVH). Fasting blood glucose, blood cholesterol, resting blood pressure, BMI, and self-reported smoking status were obtained during the routine examination. Adapted from the AHA guidelines[11] and consistent with previous FHS publications,[13,20,21] dietary quality was assessed using a food frequency questionnaire. Physical activity was assessed using a questionnaire-based physical activity index, as follows: 1×sleep hours per day+1.1×sedentary hours per day+1.5×slight activity hours per day+2.4×moderate activity hours per day+5×heavy activity hours per day, as previously described.[13,20,21] The top quartile of this score was used to indicate ideal physical activity, which corresponds qualitatively to the definition used by the AHA.[11]

## Circulating Biomarker Measurements

We measured the concentration of biomarkers using venous blood drawn in the morning on participants who had fasted overnight, with biosamples stored at −80°C until assayed. Biomarkers of interest included the following that were assayed at the sixth examination cycle: CRP (C-reactive protein), NT-proANP (N-terminal pro–atrial natriuretic peptide), BNP (B-type natriuretic peptide), renin, aldosterone, plasminogen activator inhibitor-1 (PAI-1), D-dimer, fibrinogen, homocysteine, asymmetrical dimethylarginine (ADMA), L-arginine, symmetric dimethylarginine (SDMA), high-sensitivity troponin I, ST-2, and growth differentiation factor-15 (GDF-15). ADMA, L-arginine, and SDMA were measured using a validated liquid chromatography-tandem mass spectrometry method with high quality that has been previously reported.[22] CRP, NT-proANP, BNP, renin, aldosterone, PAI-1, D-dimer, fibrinogen, homocysteine, high-sensitivity troponin I, ST-2, and GDF-15 were assayed with routine methods as previously reported, with excellent reproducibility.[23,24]

## Outcomes of Interest

The outcomes of interest for this investigation were incidence of HF and its subtypes HFpEF and HFrEF. All study participants were under continuous surveillance for the development of CVD outcomes (including HF), which were determined after review of data collected from examination visits, health history updates, and hospitalization and physician office medical records. All events were adjudicated by an end point review committee of 3 physicians using the FHS criteria for HF.[25] HF events were classified as HFrEF if left ventricular ejection fraction was <50% and HFpEF if left ventricular ejection fraction was ≥50%.

## Statistical Analysis

eCO values >50 ppm (n=13) were considered equivalent to 50 ppm for all analyses. eCO was calculated as the average of eCO values available from examinations 2 (1979–1983) through 6 (1995–1998) and then natural logarithmically transformed to normalize its skewed distribution.

Using sample 1, we related the CVH score (independent variable) to eCO (dependent variable) in a linear regression model adjusting for: (1) age and sex; and (2) age, sex, and smoking status. In sensitivity analyses, we related the CVH score with eCO among nonsmokers adjusting for age and sex. Least square means of eCO were calculated by tertiles of the CVH score, adjusting for age, sex, and smoking status. Tertile 1 included the CVH scores 0 to 7, tertile 2 included scores 8 and 9, and tertile 3 included scores 10 to 14.

Using sample 2, we natural log-transformed values of arginine, CRP, NT-proANP, BNP, renin,

**Table 1. Baseline Characteristics of Study Sample**

| | Men (n=1626) | Women (n=1828) |
|---|---|---|
| Clinical characteristics | | |
| Age, y | 59±10 | 59±10 |
| BMI, kg/m$^2$ | 28.5±4.4 | 27.4±5.7 |
| SBP, mm Hg | 130±17 | 127±20 |
| DBP, mm Hg | 77±9 | 74±9 |
| Hypertension medication, n (%) | 509 (31.3) | 464 (25.4) |
| Total serum cholesterol, mg/dL | 199±41 | 212±39 |
| HDL cholesterol, mg/100 mL | 43±12 | 58±16 |
| LDL cholesterol, mg/100 mL | 127±32 | 128±35 |
| Current smoker, n (%) | 238 (14.6) | 290 (15.9) |
| Diabetes mellitus, n (%) | 196 (12.1) | 146 (8.0) |
| Circulating biomarkers | | |
| Aldosterone, ng/100 mL median (quartile 1, quartile 3) | 9 (7, 13) | 10.5 (7, 15) |
| Arginine, µmol/L median (quartile 1, quartile 3) | 77.7 (66.5, 90.4) | 75.7 (64.0, 88.5) |
| Asymmetrical dimethylarginine, µmol/L | 0.6±0.1 | 0.5±0.1 |
| BNP, pg/mL median (quartile 1, quartile 3) | 6.7 (4.0, 16.7) | 10.2 (4.1, 20.5) |
| CRP, mg/L median (quartile 1, quartile 3) | 1.9 (0.9, 3.8) | 2.4 (1.0, 5.7) |
| D-dimer, mg/mL median (quartile 1, quartile 3) | 299.5 (181, 471) | 336 (232, 486) |
| Fibrinogen, mg/dL median (quartile 1, quartile 3) | 326 (288, 376) | 336 (296, 381) |
| Growth differentiation factor-15, ng/L median (quartile 1, quartile 3) | 1069 (820, 1426) | 1024 (812, 1307) |
| High-sensitivity troponin I, pg/mL median (quartile 1, quartile 3) | 1.6 (1.1, 2.7) | 1.2 (0.8, 1.9) |
| Homocysteine, µmol/L median (quartile 1, quartile 3) | 9.8 (8.3, 11.9) | 8.4 (7.0, 10.3) |
| N-terminal pro-atrial natriuretic peptide, pmol/L median (quartile 1, quartile 3) | 292 (197, 442) | 353 (254, 501) |
| Plasminogen activator inhibitor-1, ng/mL | 25.4 (17.0, 36.1) | 20.3 (12.2, 31.9) |
| Renin, µU/mL median (quartile 1, quartile 3) | 14 (8, 25) | 11 (6, 19) |
| ST-2, ng/mL median (quartile 1, quartile 3) | 23.6 (19.2, 29.3) | 18.8 (15.2, 23.2) |
| Symmetric dimethylarginine, µmol/L | 0.4±0.1 | 0.4±0.1 |
| eCO* | | |
| ln(eCO) | 1.8±0.6 | 1.7±0.6 |
| eCO, ppm median (quartile 1, quartile 3) | 5 (4.3, 9.5) | 4.3 (3.8, 7.3) |
| eCO ≤4 ppm | 347 (21.2) | 815 (44.6) |
| eCO 4 ppm ≤ eCO <5 ppm | 560 (31.1) | 475 (25.0) |
| eCO 5 ppm ≤ eCO | 719 (47.8) | 556 (30.4) |

All values shown are mean±SD or n (%) unless otherwise specified. BMI indicates body mass index; BNP, B-type natriuretic peptide; CRP, C-reactive protein; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; and SBP, systolic blood pressure.

*All values for exhaled carbon monoxide (eCO) are the average values taken from examinations 2 through 6.

aldosterone, PAI-1, D-dimer, fibrinogen, homocysteine, high-sensitivity troponin I, ST-2 and GDF-15 to normalize their skewed distributions. ADMA and SDMA were not logarithmically transformed since their values were normally distributed. Spearman rank partial correlation coefficients for the association between each biomarker and eCO were estimated adjusting for age and sex (Table S2). Then, we used linear regression models to relate each of the biomarkers (independent variables, separate model for each) with eCO (dependent variable), adjusting for age, sex, BMI, smoking status, total cholesterol/high-density lipoprotein (HDL), systolic blood pressure (SBP), hypertension medication, diabetes mellitus, and history of CVD. Bonferroni correction was used to account for multiple testing, resulting in a statistical significance level of 0.0033 (=0.05/15 biomarkers).

We used sample 3 to estimate the heritability of eCO. eCO was inverse-normal transformed to account for high residual kurtosis. Heritability for eCO was calculated for 2 models: (1) adjusting for age and sex; and (2) adjusting for age, sex, BMI, smoking status, total cholesterol/HDL, SBP, hypertension medication,

**Table 2. Association Between 14-Point CVH Score and eCO**

| | Estimate | Standard Error | *P* Value |
|---|---|---|---|
| Model 1: adjusted for age and sex | −0.07 | 0.005 | <0.0001 |
| Model 2: adjusted for age, sex, and smoking | −0.02 | 0.004 | <0.0001 |
| Model 3: nonsmokers, adjusted for age and sex | −0.02 | 0.004 | <0.0001 |

Estimate is change in ln(exhaled carbon monoxide [eCO]) per 1-unit increase in cardiovascular health (CVH) score. Estimate of −0.07 can be interpreted as e^(−0.07)=0.93-fold change, ie, 7% decrease in eCO per unit increase in CVH. The eCO value is the average of values taken from examinations 2 through 6.

diabetes mellitus, and history of CVD. The statistical methods for assessing heritability for biomarkers have been previously described.[26] Sequential Oligogenic Linkage Analysis (SOLAR) was used to estimate the heritability of eCO.

Using samples 4 and 5, we related eCO (independent variable) to time to HF, HFpEF, and HFrEF (separate model for each outcome) with Cox proportional hazards regression models, after confirming that the proportional hazards assumption was met. Four different models were estimated: (1) adjusting for age, sex, BMI, smoking status, total cholesterol/HDL, SBP, hypertension medication, diabetes mellitus, and history of CVD (not including HF); (2) same as model 1 but sample was restricted to nonsmokers (n=2892); (3) same as model 1 plus further adjusting for interim myocardial infarction (MI) and ADMA, CRP, fibrinogen, GDF-15, homocysteine, PAI-1, BNP, high-sensitivity troponin I, and ST-2 (n=3180); and (4) same as model 3 but sample was restricted to nonsmokers (n=2699).

For models 3 and 4 above, biomarkers were chosen for inclusion because of their statistically significant association with eCO in our investigation or their previously reported association with HF.[24,27] PROC LIFETEST was used to create Kaplan–Meier curves to show survival time free of HF (including HFrEF and HFpEF) by tertiles of eCO. All analyses were performed using SAS version 9.4 (SAS Institute Inc).

## RESULTS

Baseline characteristics of the study sample are shown in Table 1. Our study sample consisted of mostly middle-aged to older participants, and over half of the sample were women. Most of the participants had a BMI in the overweight to obese range, and the majority of the participants were nonsmokers. The median eCO value for men was 5.0 and 4.3 ppm for women.

We observed a significant inverse relation between CVH score and eCO, which also held among nonsmokers (Table 2, Figure 1; *P* for trend <0.0001). For example, adjusting for age and sex, an estimate of −0.07 was observed. This can be interpreted as an $e^{-0.07}$=0.93-fold change, ie, a 7% decrease in eCO per unit increase in CVH. Additionally, blood concentrations of CRP, PAI-1, fibrinogen, GDF-15, homocysteine, and ADMA were directly associated with eCO, adjusted for age, sex, BMI, smoking status, total cholesterol/HDL, SBP, hypertension medication, diabetes mellitus, and history of CVD (Table 3). We did not observe a statistically significant association of eCO with NT-proANP, arginine, D-dimer, ST-2, renin, SDMA, high-sensitivity troponin I, aldosterone, or BNP.

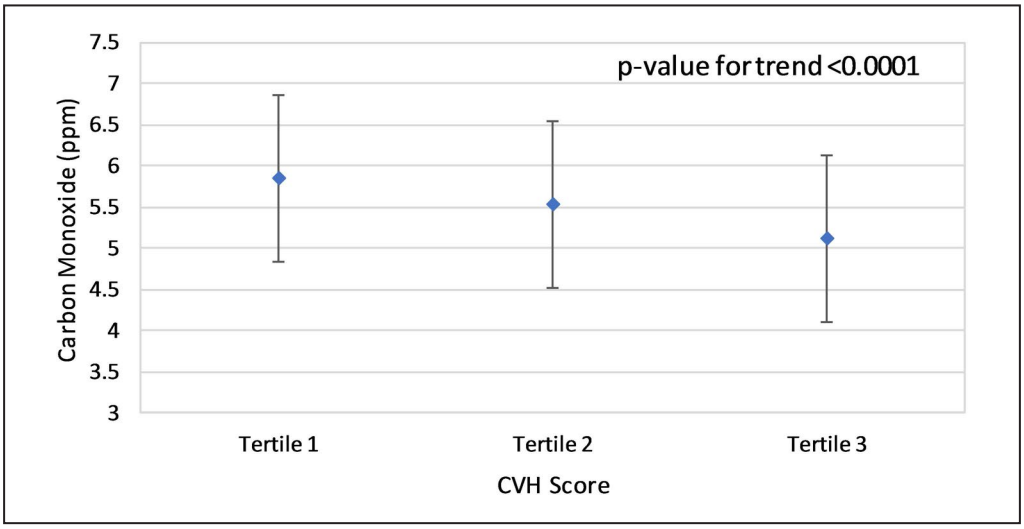


**Figure 1. Least square means of exhaled carbon monoxide per tertile of cardiovascular health (CVH) score adjusting for age, sex, and smoking status.**
Tertile 1: CVH score 0 to 7, tertile 2: CVH score 8 to 9, tertile 3: CVH score 10 to 14.

**Table 3. Associations Between Biomarkers and eCO**

| Biomarker | Estimate | Standard Error | Fold Change in eCO | *P* Value |
|---|---|---|---|---|
| ln(CRP), mg/L | 0.04 | 0.01 | 1.05 | <0.0001 |
| ln(PAI-1), ng/mL | 0.07 | 0.01 | 1.07 | <0.0001 |
| ln(fibrinogen), mg/dL | 0.19 | 0.04 | 1.21 | <0.0001 |
| ln(GDF-15), ng/L | 0.13 | 0.02 | 1.14 | <0.0001 |
| ln(homocysteine), µmol/L | 0.07 | 0.02 | 1.08 | 0.002 |
| ADMA, µmol/L | 0.17 | 0.06 | … | 0.003 |

Includes statistically significant biomarkers at the 0.0033 level (Bonferroni correction for multiple tests). Each biomarker was included in a separate model. All models were adjusted for age, sex, smoking, body mass index, total cholesterol/high-density lipoprotein, systolic blood pressure, antihypertensive medication, diabetes mellitus, and history of cardiovascular disease. Estimate is change in ln(average exhaled carbon monoxide [eCO]) per 1-unit increase of ln(biomarker) when transformed, and change in ln(average eCO) per 1-unit increase of biomarker when not transformed. ADMA indicates asymmetrical dimethylarginine; CRP, C-reactive protein; CVH, cardiovascular health; GDF-15, growth differentiation factor-15; and PAI-1, plasminogen activator inhibitor-1.

## Heritability

The age- and sex-adjusted heritability of eCO was 49.5% and the multivariable-adjusted heritability estimate was 31.4%, adjusted for age, sex, BMI, smoking status, total cholesterol/HDL, SBP, hypertension medication, diabetes mellitus, and history of CVD.

## Relation of eCO With Incident HF

Over a median follow-up period of 18 years, there were a total of 309 (45% women) incident HF events, with 108 HFpEF, 144 HFrEF, and 57 nonclassifiable (attributable to missing EF values at the time of HF onset).

Adjusting for age, sex, BMI, smoking status, total cholesterol/HDL, SBP, hypertension medication, diabetes mellitus, and history of CVD (excluding HF), higher levels of eCO were associated with a higher risk of HF overall and of HFrEF (Table 4). We did not observe a significant association between eCO and risk of HFpEF (*P*=0.07) (Table 4). When further adjusting for MI and biomarkers, higher levels of eCO were also only associated with a higher risk of HF and HFrEF. In the subset of nonsmokers, higher levels of eCO were associated with higher risks of HF, HFpEF, and HFrEF. However, the association with HFpEF did not hold upon further adjusting for MI and biomarkers among nonsmokers.

Figures 2 through 4 depict the association between tertiles of eCO and time to HF, HFpEF, and HFrEF, respectively. Compared with individuals with the highest levels of eCO, the individuals in the bottom tertile of eCO had significantly lower risks of developing HF and, more specifically, HFrEF.

**Table 4. Association of eCO With Risk of Heart Failure**

| Model | HF Type | No. of Events/No. at Risk | Standardized HR (95% CI) | *P* Value |
|---|---|---|---|---|
| All participants | | | | |
| Adjusting for covariates | HF | 309/3416 | 1.39 (1.19–1.62) | <0.0001 |
| | HFpEF | 108/3359 | 1.30 (0.98–1.74) | 0.07 |
| | HFrEF | 144/3359 | 1.43 (1.15–1.77) | 0.001 |
| Adjusting for MI and biomarkers | HF | 283/3180 | 1.29 (1.09–1.53) | 0.003 |
| | HFpEF | 98/3125 | 1.18 (0.87–1.60) | 0.29 |
| | HFrEF | 130/3125 | 1.36 (1.07–1.71) | 0.01 |
| Nonsmokers | | | | |
| Adjusting for covariates | HF | 264/2892 | 1.44 (1.22–1.71) | <0.0001 |
| | HFpEF | 92/2844 | 1.44 (1.06–1.95) | 0.02 |
| | HFrEF | 124/2844 | 1.42 (1.12–1.79) | 0.004 |
| Adjusting for MI and biomarkers | HF | 241/2699 | 1.34 (1.11–1.61) | 0.002 |
| | HFpEF | 82/2653 | 1.30 (0.92–1.84) | 0.14 |
| | HFrEF | 113/2653 | 1.36 (1.05–1.75) | 0.02 |

All models were adjusted for age, sex, smoking, body mass index, total cholesterol/high-density lipoprotein, systolic blood pressure, antihypertensive medication, diabetes mellitus, and history of cardiovascular disease. Biomarkers include asymmetrical dimethylarginine (ADMA), B-type natriuretic peptide, C-reactive protein, high-sensitivity troponin I, fibrinogen, growth differentiation factor-15, homocysteine, plasminogen activator inhibitor-1 and ST-2. All biomarkers were log-transformed except for ADMA. Hazard ratios (HRs) are per SD increase in ln(average exhaled carbon monoxide [eCO]). HF indicates heart failure; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; and MI, myocardial infarction.

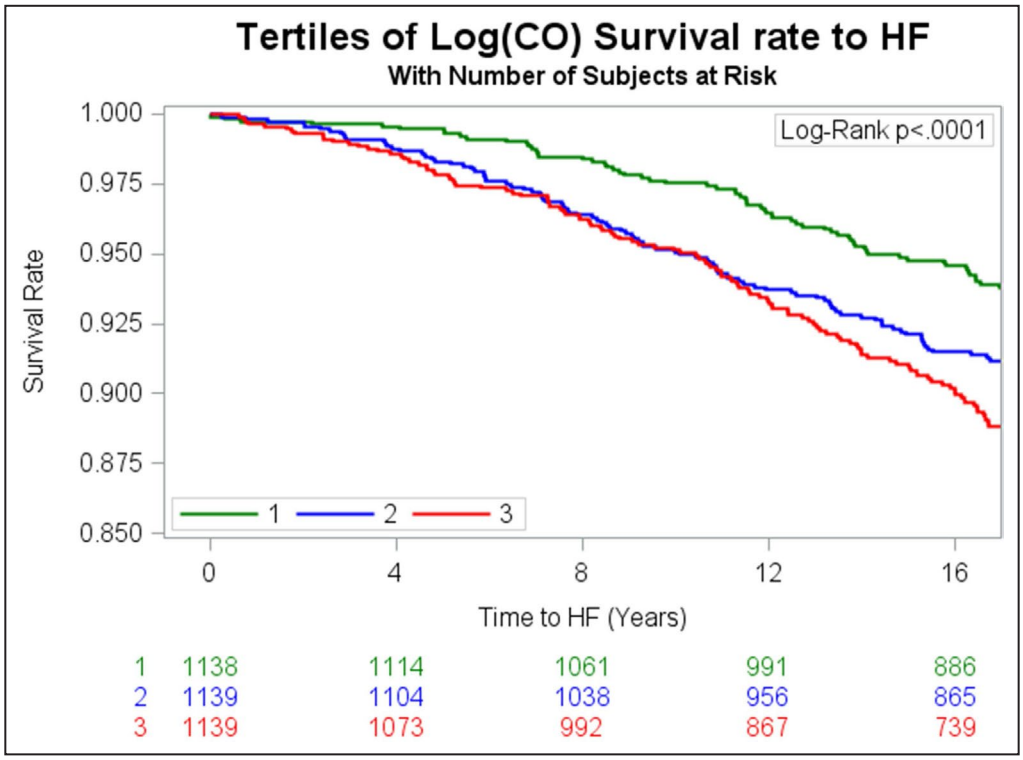


**Figure 2. Association between tertiles of exhaled carbon monoxide (eCO) and heart failure (HF) (Kaplan–Meier plot).**
The tertiles are based on separating the sample into 3 groups, each ordered by their eCO value. Group 1 (green) represents the tertile with the lowest levels of eCO and group 3 (red) represents the tertile with the highest levels of eCO. Tertile 1: log (carbon monoxide [CO]) <1.3863; tertile 2: 1.3863 < log(CO) <1.6864; tertile 3: 1.6864 < log(CO).

# DISCUSSION

## Principal Findings

First, we observed that CVH score was inversely related to eCO. Second, blood concentrations of CRP, PAI-1, fibrinogen, GDF-15, homocysteine, and ADMA were positively associated with eCO. Third, there was a moderate heritability observed with regards to eCO. Fourth, higher levels of eCO were associated with a higher risk of HF and HFrEF.

## Comparison With the Literature

Several individual components of the CVH score have been shown to be associated with eCO. For example, levels of eCO among smokers are significantly higher than in nonsmokers.[8,28–30] Additionally, eCO values are directly associated with presence of diabetes mellitus, increased total cholesterol levels, and greater BMI.[8] Exercise has also been shown to increase the levels of eCO, although the levels of eCO return to pre-exercise levels within several minutes after cessation of exercise.[31] However, to our knowledge, no prior studies have studied the association of the composite CVH score with eCO. Therefore, we expanded these findings and showed that a higher CVH score was associated with lower levels of eCO. Interestingly, the CVH score remained inversely associated with eCO even among nonsmokers.

We have previously reported that serum iron, serum total bilirubin, and CRP are directly associated with eCO.[8] In our current investigation, we demonstrated that, in addition to CRP, biomarkers of endothelial dysfunction (homocysteine and ADMA), hemostasis (PAI-1 and fibrinogen), and the pleiotropic growth factor GDF-15 are directly associated with eCO. These associations are in accord with previous understanding of the mechanisms by which the above-mentioned biomarkers may contribute to the development of CVD. For example, homocysteine and ADMA contribute to endothelial dysfunction by reducing NO bioavailability[32] and inhibiting NO synthesis,[33] respectively. Similarly, CO has been shown to block NO-induced vasorelaxation.[34] GDF-15 is a stress-responsive cytokine that is produced by a variety of cells in response to inflammation, injury, and oxidative stress.[35–37] Furthermore, endogenous CO is the byproduct of heme degradation by heme oxygenase, whose activity is induced by oxidative stress and inflammatory cytokines.[1] Last, elevated PAI-1 levels increase the risk of atherothrombotic

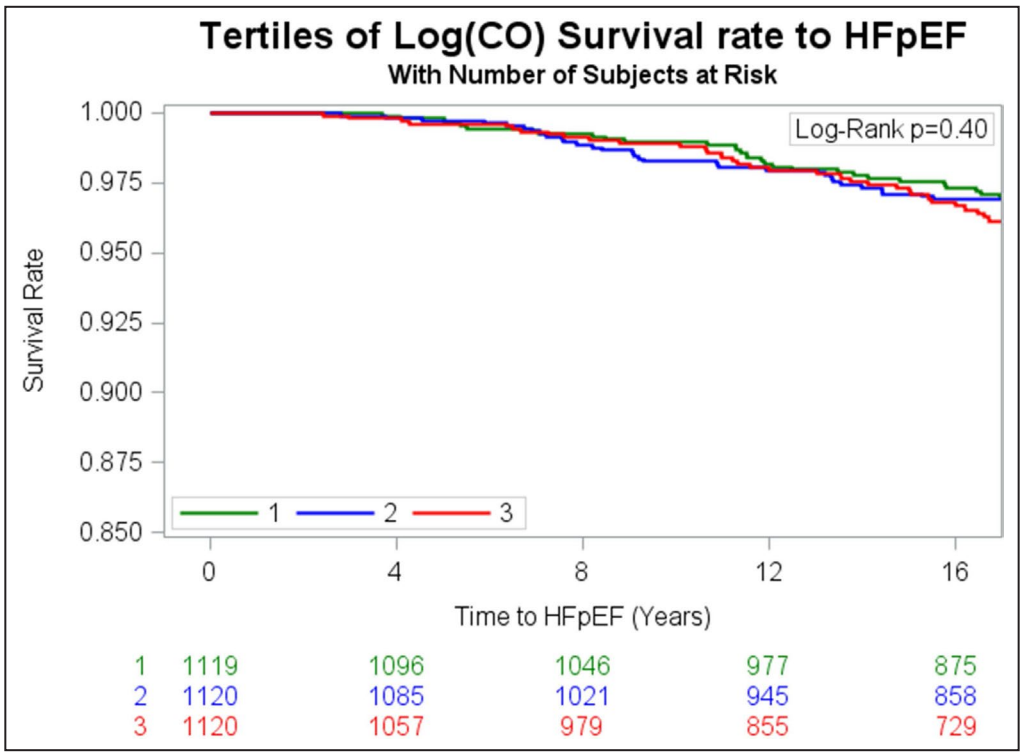


**Figure 3. Association between tertiles of exhaled carbon monoxide (eCO) and heart failure with preserved ejection fraction (HFpEF; Kaplan–Meier plot).**
The tertiles are based on separating the sample into 3 groups, each ordered by their eCO value. Group 1 (green) represents the tertile with the lowest levels of eCO and group 3 (red) represents the tertile with the highest levels of eCO. Tertile 1: log(carbon monoxide [CO]) <1.3863; tertile 2: 1.3863 ≤ log(CO) <1.6864; tertile 3: 1.6864 ≤ log(CO).

events[38] and fibrinogen is a well-described coagulation factor involved in the thrombotic complications of CVD. High levels of CO reduce the antithrombogenic activity of the endothelial layers through inhibition of the production of endothelial NO.[39]

The heritability for cardiovascular biomarkers such as CRP and fibrinogen has been reported and shown to have a moderate proportion of variability explained by genetic factors.[40] Our investigation reported moderate heritability estimates for eCO. To our knowledge, this is the first report of heritability of eCO.

## eCO and HF Incidence

A multitude of biomarkers have been identified to be associated with the development of HF, many with the development of HFrEF and fewer with HFpEF.[16] However, the differences in the factors that precede the 2 subtypes are poorly understood. In our investigation, higher levels of eCO were associated with a higher risk of HF, even among nonsmokers, adjusting for standard HF risk factors (BMI, total cholesterol/HDL, SBP, hypertension treatment, diabetes mellitus, and history of CVD). When HF subtypes were evaluated separately, eCO was associated with a higher risk of HFrEF. By contrast, eCO was only significantly associated with a higher risk of HFpEF in the model using data from nonsmokers. The exact mechanism by which CO contributes to the development of HF and HFrEF remains unclear. CO may contribute to the development of HF through generation of reactive oxygen species and promoting oxidative stress.[4,5] On the other hand, CO may simply represent an endogenous compensation to worsening subclinical atherosclerosis.[9] Investigations of atherectomy biopsy from patients with clinical carotid artery disease have observed increased levels of heme oxygenase expression in relation to features of vulnerable human atheromatous plaque, including macrophage and lipid accumulation.[41] This may explain the direct association between eCO and incident HFrEF. Interestingly, in our investigation, adjusting for MI and biomarkers attenuated the association of eCO with HF and HFrEF to some extent. This supports the idea that the association of eCO with HF and HFrEF may partially be mediated by mechanisms involved in the pathophysiology of MI, a common cause of HFrEF, and the biological pathways involved with the above-mentioned biomarkers. However, there remains a direct association of eCO with HF and HFrEF despite adjusting for

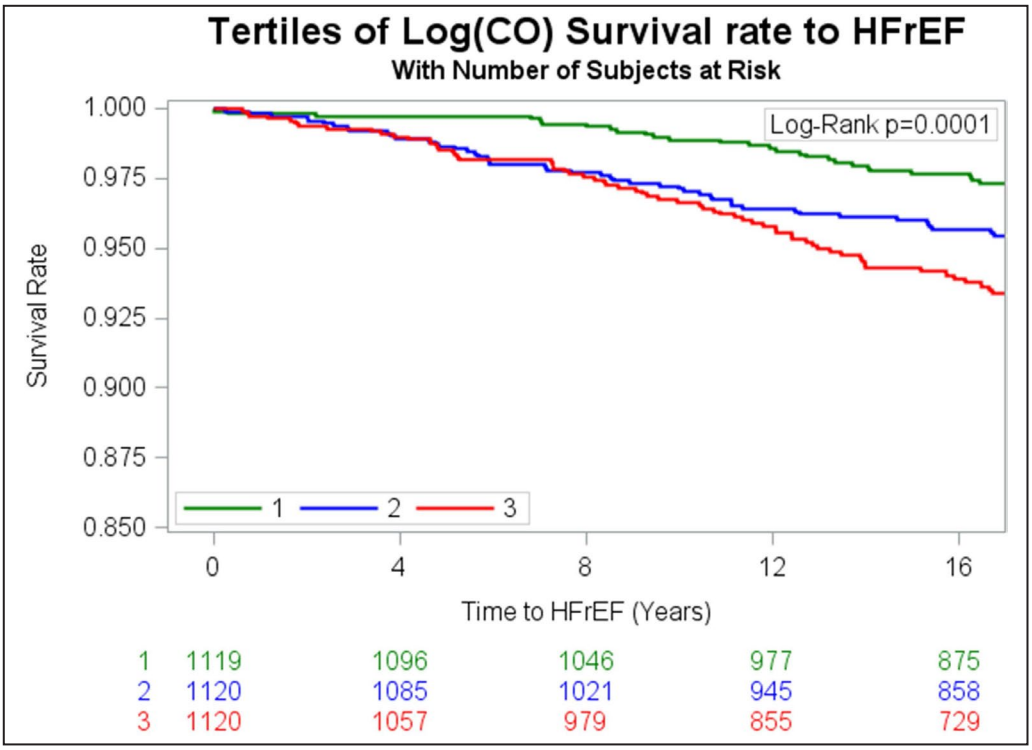


**Figure 4. Association between tertiles of exhaled carbon monoxide (eCO) and heart failure with reserved ejection fraction (HFrEF; Kaplan–Meier plot).**
The tertiles are based on separating the sample into 3 groups, each ordered by their eCO value. Group 1 (green) represents the tertile with the lowest levels of eCO and group 3 (red) represents the tertile with the highest levels of eCO. Tertile 1: log(carbon monoxide [CO]) <1.3863; tertile 2: 1.3863 ≤ log(CO) <1.6864; tertile 3: 1.6864 ≤ log(CO).

MI and biomarkers, suggesting that CO may promote HF through mechanisms that remain to be elucidated. Our finding of a significant association between eCO and risk of HFpEF was somewhat unexpected. A paradigm that has been proposed for HFpEF identifies a systemic inflammatory state that induces oxidative stress in the coronary microvascular endothelium, ultimately leading to high diastolic left ventricular stiffness and HF development.[42] One explanation is that factors other than oxidative stress may play a major role in the development of HFpEF, highlighting the complexity of the pathophysiology of HFpEF. Further studies are warranted to evaluate the usefulness of eCO as a clinical biomarker for future HF risk in select individuals.

## Strengths and Limitations

We used rigorously acquired and standardized measures of eCO in a large, well-characterized community-based sample with long follow-up. The eCO measurements were obtained using instrumentation that was routinely calibrated with the use of a standardized protocol to reduce the intraindividual and interindividual variability. Several limitations of the present investigation warrant consideration. Gas chromatography is the gold standard for estimating blood CO concentrations. However, eCO has been validated as a surrogate measure that can capture variation in CO levels.[19] Additionally, the study participants did not have concurrent measures of exhaled or endogenous NO, a possible confounder in our analyses.[1] Current smoking status was obtained by questionnaire, but misclassification caused by misreporting of current smoking or previous smoking history, may be possible. Our results are based on observational analyses; therefore, causal relations cannot be inferred. Finally, our study was composed of mostly middle-aged white individuals of European descent, so the generalizability of our findings to other racial and ethnic groups is limited.

# CONCLUSIONS

In our community-based sample, higher levels of eCO were associated with lower CVH scores and adverse cardiovascular biomarker profiles cross-sectionally, and a higher risk of HF, specifically HFrEF, prospectively. Our findings suggest that CO may identify a novel pathway to HF development. Future investigations are warranted to evaluate whether modulation of the CO-related pathways may lower HF risk.

## ARTICLE INFORMATION

Received April 18, 2020; accepted August 25, 2020.

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### Sources of Funding

This work was supported by National Heart, Lung, and Blood Institute contracts NO1-HC-25195, HHSN268201500001I, and 75N92019D00031. Vasan was also funded by the Evans Foundation and Jay and Louise Coffman Endowment at Boston University.

### Disclosures

None.

### Supplementary Material

**Tables S1–S2**
**Figures S1–S3**

## REFERENCES

1. Wu L, Wang R. Carbon monoxide: endogenous production, physiological functions, and pharmacological applications. *Pharmacol Rev*. 2005;57:585–630.
2. Ryter SW, Choi AM. Carbon monoxide in exhaled breath testing and therapeutics. *J Breath Res*. 2013;7:017111.
3. Thorup C, Jones CL, Gross SS, Moore LC, Goligorsky MS. Carbon monoxide induces vasodilation and nitric oxide release but suppresses endothelial nos. *Am J Physiol*. 1999;277:F882–F889.
4. Thom SR, Xu YA, Ischiropoulos H. Vascular endothelial cells generate peroxynitrite in response to carbon monoxide exposure. *Chem Res Toxicol*. 1997;10:1023–1031.
5. Zhang J, Piantadosi CA. Mitochondrial oxidative stress after carbon monoxide hypoxia in the rat brain. *J Clin Invest*. 1992;90:1193–1199.
6. Peyton KJ, Reyna SV, Chapman GB, Ensenat D, Liu XM, Wang H, Schafer AI, Durante W. Heme oxygenase-1-derived carbon monoxide is an autocrine inhibitor of vascular smooth muscle cell growth. *Blood*. 2002;99:4443–4448.
7. Jones RH, Ellicott MF, Cadigan JB, Gaensler EA. The relationship between alveolar and blood carbon monoxide concentrations during breathholding; simple estimation of COHb saturation. *J Lab Clin Med*. 1958;51:553–564.
8. Cheng S, Lyass A, Massaro JM, O'Connor GT, Keaney JF Jr, Vasan RS. Exhaled carbon monoxide and risk of metabolic syndrome and cardiovascular disease in the community. *Circulation*. 2010;122:1470–1477.
9. Cheng S, Enserro D, Xanthakis V, Sullivan LM, Murabito JM, Benjamin EJ, Polak JF, O'Donnell CJ, Wolf PA, O'Connor GT, et al. Association of exhaled carbon monoxide with subclinical cardiovascular disease and their conjoint impact on the incidence of cardiovascular outcomes. *Eur Heart J*. 2014;35:2980–2987.
10. Nayor M, Enserro DM, Beiser AS, Cheng S, DeCarli C, Vasan RS, Sudha S. Association of exhaled carbon monoxide with stroke incidence and subclinical vascular brain injury. *Stroke*. 2016;47:383–389.
11. Lloyd-Jones DM, Hong Y, Labarthe D, Mozaffarian D, Appel LJ, Van Horn L, Greenlund K, Daniels S, Nichol G, Tomaselli GF, et al.; American Heart Association Strategic Planning Task F and Statistics C. Defining and setting national goals for cardiovascular health promotion and disease reduction: the American Heart Association's strategic Impact Goal through 2020 and beyond. *Circulation*. 2010;121:586–613.
12. Folsom AR, Yatsuya H, Nettleton JA, Lutsey PL, Cushman M, Rosamond WD. Community prevalence of ideal cardiovascular health, by the American Heart Association definition, and relationship with cardiovascular disease incidence. *J Am Coll Cardiol*. 2011;57:1690–1696.
13. Xanthakis V, Enserro DM, Murabito JM, Polak JF, Wollert KC, Januzzi JL, Wang TJ, Tofler G, Vasan RS. Ideal cardiovascular health: associations with biomarkers and subclinical disease and impact on incidence of cardiovascular disease in the Framingham Offspring Study. *Circulation*. 2014;130:1676–1683.
14. Yang Q, Cogswell ME, Flanders WD, Hong Y, Zhang Z, Loustalot F, Gillespie C, Merritt R, Hu FB. Trends in cardiovascular health metrics and associations with all-cause and CVD mortality among US adults. *JAMA*. 2012;307:1273–1283.
15. Vasan RS. Biomarkers of cardiovascular disease: molecular basis and practical considerations. *Circulation*. 2006;113:2335–2362.
16. de Boer RA, Nayor M, de Filippi CR, Enserro D, Bhambhani V, Kizer JR, Bla-ha MJ, Brouwers FP, Cushman M, Lima JAC, et al. Association of cardiovascular biomarkers with incident heart failure with preserved and reduced ejection fraction. *JAMA Cardiol*. 2018;3:215–224.
17. Kannel WB, Feinleib M, McNamara PM, Garrison RJ, Castelli WP. An investigation of coronary heart disease in families: the Framingham Offspring Study. *Am J Epidemiol*. 1979;110:281–290.
18. Hedblad B, Ogren M, Engstrom G, Wollmer P, Janzon L. Heterogeneity of cardiovascular risk among smokers is related to degree of carbon monoxide exposure. *Atherosclerosis*. 2005;179:177–183.
19. Kharitonov SA, Barnes PJ. Exhaled markers of pulmonary disease. *Am J Respir Crit Care Med*. 2001;163:1693–1722.
20. Nayor M, Enserro DM, Vasan RS, Xanthakis V. Cardiovascular health status and incidence of heart failure in the Framingham Offspring Study. *Circ Heart Fail*. 2016;9:e002416.
21. Pase MP, Beiser A, Enserro D, Xanthakis V, Aparicio H, Satizabal CL, Himali JJ, Kase CS, Vasan RS, DeCarli C, et al. Association of ideal cardiovascular health with vascular brain injury and incident dementia. *Stroke*. 2016;47:1201–1206.
22. Schwedhelm E, Maas R, Tan-Andresen J, Schulze F, Riederer U, Boger RH. High-throughput liquid chromatographic-tandem mass spectrometric determination of arginine and dimethylated arginine derivatives in human and mouse plasma. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2007;851:211–219.
23. Wang TJ, Gona P, Larson MG, Tofler GH, Levy D, Newton-Cheh C, Jacques PF, Rifai N, Selhub J, Robins SJ, et al. Multiple biomarkers for the prediction of first major cardiovascular events and death. *N Engl J Med*. 2006;355:2631–2639.
24. Wang TJ, Wollert KC, Larson MG, Coglianese E, McCabe EL, Cheng S, Ho JE, Fradley MG, Ghorbani A, Xanthakis V, et al. Prognostic utility of novel biomarkers of cardiovascular stress: the Framingham Heart Study. *Circulation*. 2012;126:1596–1604.
25. McKee PA, Castelli WP, McNamara PM, Kannel WB. The natural history of congestive heart failure: the Framingham study. *N Engl J Med*. 1971;285:1441–1446.
26. Dupuis J, Larson MG, Vasan RS, Massaro JM, Wilson PW, Lipinska I, Corey D, Vita JA, Keaney JF Jr, Benjamin EJ. Genome scan of systemic biomarkers of vascular inflammation in the Framingham Heart Study: evidence for susceptibility loci on 1q. *Atherosclerosis*. 2005;182:307–314.
27. Wang TJ, Larson MG, Levy D, Benjamin EJ, Leip EP, Omland T, Wolf PA, Vasan RS. Plasma natriuretic peptide levels and the risk of cardiovascular events and death. *N Engl J Med*. 2004;350:655–663.
28. Deveci SE, Deveci F, Acik Y, Ozan AT. The measurement of exhaled carbon monoxide in healthy smokers and non-smokers. *Respir Med*. 2004;98:551–556.
29. Hung J, Lin CH, Wang JD, Chan CC. Exhaled carbon monoxide level as an indicator of cigarette consumption in a workplace cessation program in Taiwan. *J Formos Med Assoc*. 2006;105:210–213.
30. Fabricius P, Scharling H, Lokke A, Vestbo J, Lange P. Exhaled CO, a predictor of lung function? *Respir Med*. 2007;101:581–586.
31. Yasuda Y, Ito T, Miyamura M. Effect of ramp bicycle exercise on exhaled carbon monoxide in humans. *J Physiol Sci*. 2011;61:279–286.
32. Lai WK, Kan MY. Homocysteine-induced endothelial dysfunction. *Ann Nutr Metab*. 2015;67:1–12.
33. Sibal L, Agarwal SC, Home PD, Boger RH. The role of asymmetric dimethylarginine (ADMA) in endothelial dysfunction and cardiovascular disease. *Curr Cardiol Rev*. 2010;6:82–90.
34. Marazioti A, Bucci M, Coletta C, Vellecco V, Baskaran P, Szabo C, Cirino G, Marques AR, Guerreiro B, Goncalves AM, et al. Inhibition of

nitric oxide-stimulated vasorelaxation by carbon monoxide-releasing molecules. *Arterioscler Thromb Vasc Biol*. 2011;31:2570–2576.
35. Zimmers TA, Jin X, Hsiao EC, Perez EA, Pierce RH, Chavin KD, Koniaris LG. Growth differentiation factor-15: induction in liver injury through p53 and tumor necrosis factor-independent mechanisms. *J Surg Res*. 2006;130:45–51.
36. Kempf T, Zarbock A, Widera C, Butz S, Stadtmann A, Rossaint J, Bolomini-Vittori M, Korf-Klingebiel K, Napp LC, Hansen B, et al. GDF-15 is an inhibitor of leukocyte integrin activation required for survival after myocardial infarction in mice. *Nat Med*. 2011;17:581–588.
37. Schlittenhardt D, Schober A, Strelau J, Bonaterra GA, Schmiedt W, Unsicker K, Metz J, Kinscherf R. Involvement of growth differentiation factor-15/macrophage inhibitory cytokine-1 (GDF-15/MIC-1) in oxLDL-induced apoptosis of human macrophages in vitro and in arteriosclerotic lesions. *Cell Tissue Res*. 2004;318:325–333.
38. Vaughan DE. PAI-1 and atherothrombosis. *J Thromb Haemost*. 2005;3:1879–1883.
39. Thorup C, Jones CL, Gross SS, Moore LC, Goligorsky MS. Carbon monoxide induces vasodilation and nitric oxide release but suppresses endothelial NOS. *Am J Physiol*. 1999;277:F882–F889.
40. Schnabel RB, Lunetta KL, Larson MG, Dupuis J, Lipinska I, Rong J, Chen MH, Zhao Z, Yamamoto JF, Meigs JB, et al. The relation of genetic and environmental factors to systemic inflammatory biomarker concentrations. *Circ Cardiovasc Genet*. 2009;2:229–237.
41. Cheng C, Noordeloos AM, Jeney V, Soares MP, Moll F, Pasterkamp G, Serruys PW, Duckers HJ. Heme oxygenase 1 determines atherosclerotic lesion progression into a vulnerable plaque. *Circulation*. 2009;119:3017–3027.
42. Paulus WJ, Tschope C. A novel paradigm for heart failure with preserved ejection fraction: comorbidities drive myocardial dysfunction and remodeling through coronary microvascular endothelial inflammation. *J Am Coll Cardiol*. 2013;62:263–271.

# SUPPLEMENTAL MATERIAL

**Table S1. Components and definition of the Cardiovascular Health score.**

| Goal/Metric | Poor Health | Intermediate Health | Ideal Health |
|---|---|---|---|
| **Smoking** | Current Smoker | Quit ≤ 12 months | Never or quit ≥ 12 months |
| **Body mass index** | ≥30 kg/m$^2$ | 25−<30 kg/m$^2$ | <25 kg/m$^2$ |
| **Physical activity score*** | < Median value | Median value − top quartile | Top quartile |
| **Healthy diet score†** | 0 components | 1 component | ≥ 2 components |
| **Total serum cholesterol** | ≥240 mg/dL | 200−<240 mg/dL or treated to goal | <200 mg/dL |
| **Blood pressure** | SBP≥140 mmHg or DBP≥90 mmHg | SBP 120−<140 mmHg or DBP 80−<90 mmHg or treated to goal | SBP<120 mmHg and DBP<80 mmHg and not on hypertension treatment |
| **Fasting plasma glucose** | ≥126 mg/dL | 100−<126 mg/dL or treated to goal | <100 mg/dL and not on diabetes treatment |

SBP, systolic blood pressure; DBP, diastolic blood pressure

*Physical activity score was calculated using the formula:
1*sleep hrs/day + 1.1*sedentary hrs/day + 1.5*slight activity hrs/day + 2.4*moderate activity hrs/day + 5*heavy activity hrs/day. This definition is qualitatively similar to the AHA physical activity metric.[11]

† Healthy diet score components, adapted from AHA:[11]

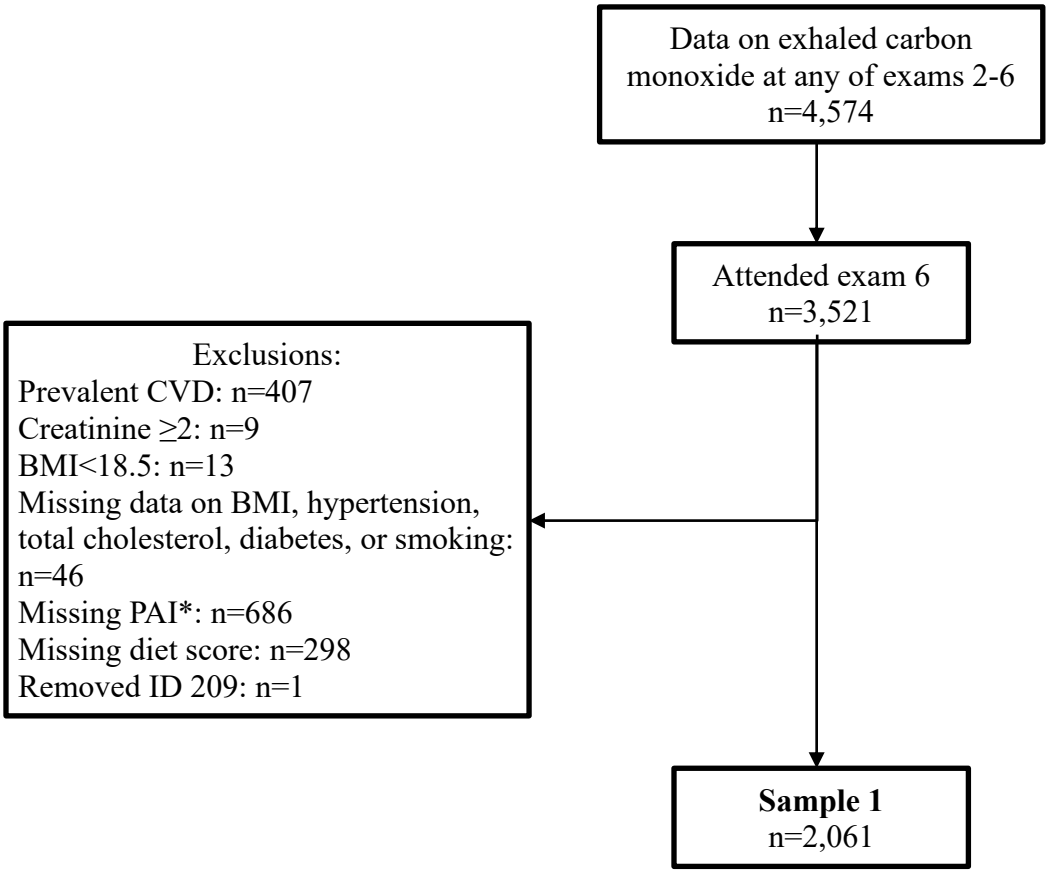
≥4.5 cups/day of fruits and vegetables
≥two 3.5 oz servings/week of fish
<1500 mg/day of sodium
<36 oz/week of sugar-sweetened beverages
≥three 1-oz equivalent servings/day of fiber-rich whole grains

**Table S2. Spearman Rank partial correlation coefficients of ln(biomarkers) with ln(eCO), adjusting for age and sex.**

| Biomarker | Correlation Coefficient |
|---|---|
| ln(GDF15) | 0.241* |
| ln(CRP) | 0.173* |
| ln(Fibrinogen) | 0.173* |
| ln(PAI) | 0.142* |
| ln(HCYST1) | 0.130* |
| ADMA | 0.101* |
| ln(NT-proANP) | -0.065* |
| ln(D_Dimer) | 0.063* |
| ln(Arginine) | 0.051* |
| ln(Renin) | 0.040* |
| ln(ST2) | 0.038* |
| ln(BNP) | 0.026 |
| ln(CTNI) | -0.007 |
| ln(Aldosterone) | 0.004 |
| SDMA | -0.003 |

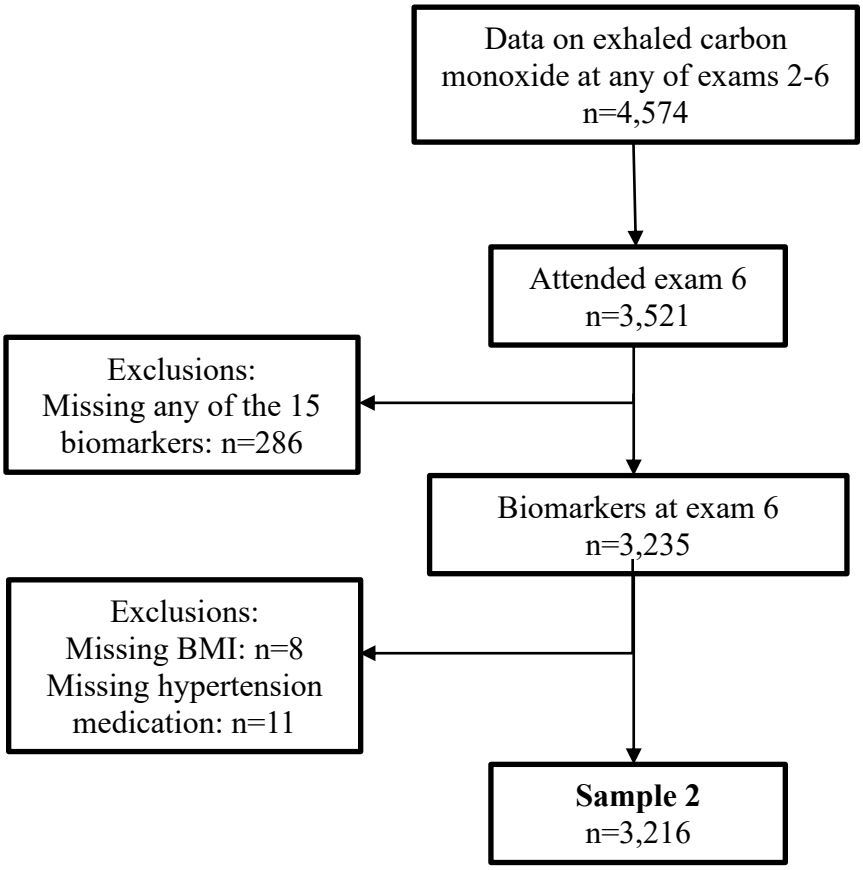
*Significant at the 0.05 level.
Ordered by correlation coefficient, largest to smallest.

**Figure S1. Flow diagram of Sample 1.**



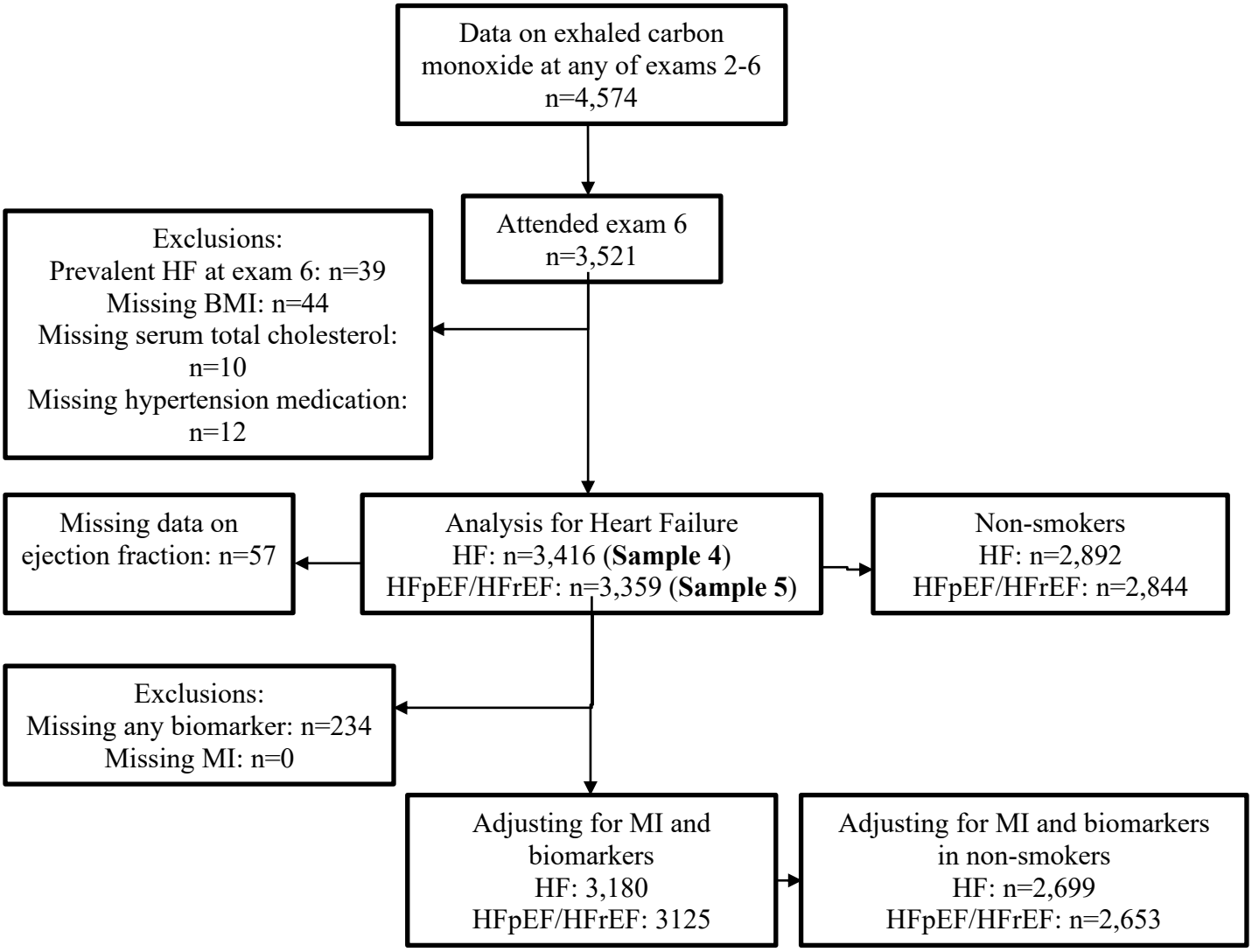

CVD=cardiovascular disease
BMI=body mass index
PAI=physical activity index

**Figure S2. Flow diagram of Sample 2.**




BMI=body mass index

**Figure S3. Flow diagram of Sample 4 and Sample 5.**




HF=heart failure
BMI=body mass index
HFpEF=heart failure with preserved ejection fraction
HFrEF=heart failure with reduced ejection fraction
MI=myocardial infarction