Longitudinal Impact of Smoking and Smoking Cessation on Inflammatory Markers of Cardiovascular Disease Risk

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Objective—To evaluate longitudinal changes in 6 inflammatory markers that predict cardiovascular disease events among smokers making a quit attempt and to characterize their cross-sectional associations between smoking and smoking heaviness.

Approach and Results—In a longitudinal cohort study of contemporary smokers (n=1652), we evaluated (1) independent associations of smoking heaviness markers (exhaled carbon monoxide, cigarettes/d, pack-years) with inflammatory markers (C-reactive protein, D-dimer, fibrinogen, urinary F_2 isoprostane:creatinine [F_2:Cr] ratio, white blood cell [WBC] count, myeloperoxidase) and (2) the effects of smoking cessation and continued smoking on these inflammatory markers after 1 year, among the 888 smokers who made an aided quit attempt as part of a randomized comparative effectiveness trial or standard care. There were strong, independent associations between smoking heaviness markers and the F_2:Cr ratio, WBC, and myeloperoxidase (all P_{adj}<0.001), but not high-sensitivity C-reactive protein, D-dimer, or fibrinogen. Participants were mean (SD) 49.6 years old (11.6), 54% women, 34% non-white, and smoked 16.8 cigarettes/d (8.5) for 27.3 pack-years (18.6). After 1 year, the 344 successful abstainers gained more weight (4.0 [6.0] versus 0.4 [5.7] pounds; P<0.001) and had larger increases in insulin resistance scores (P=0.02) than continuing smokers. Despite these increases, abstainers had significant decreases in F_2:Cr ratio (P<0.001) and WBC counts (P<0.001). Changes in other markers were not related to quitting.

Conclusions—Smoking heaviness is associated with increased F_2:Cr ratio, myeloperoxidase, and WBC counts. Cessation improves the F_2:Cr ratio and WBC counts independent of weight change, suggesting reduced inflammation related to less oxidant stress. (***Arterioscler Thromb Vasc Biol*. 2017;37:374-379. DOI: 10.1161/ATVBAHA.116.308728.)**

Key Words: carbon monoxide ■ cardiovascular diseases ■ inflammation ■ oxidative stress ■ smoking

In the United States, cigarette smoking contributes to nearly a half a million deaths annually with significant morbidity and mortality attributable to cardiovascular disease (CVD).[1,2] Indeed, smoking accounts for greater than one third of the population-attributable risk for myocardial infarction.[1] Smoking cessation is linked to lower CVD mortality and reductions in thrombotic events, most notably acute myocardial infarction.[3–6]

Atherosclerosis is a response to arterial injury, mediated by inflammation.[7–9] Clinical CVD events have been linked to elevated levels of inflammatory markers such as C-reactive protein (CRP),[10–12] D-dimer, fibrinogen, myeloperoxidase, white blood cell (WBC) count,[13–15] and urinary F_2 isoprostanes, a lipid peroxidation end product.[16] Tobacco-induced activation of inflammatory pathways, lipid oxidation, hypercoagulability, and vascular dysfunction is among the primary mechanisms by which cigarette smoking promotes CVD.[17,18] Previous cross-sectional studies have demonstrated that active cigarette smokers have higher levels of inflammatory markers such as CRP, WBC count, fibrinogen, D-dimer,[10,19,20] and F_2 isoprostanes.[21,22] However, no studies that we are aware of have investigated the longitudinal effects of smoking cessation and continued smoking on inflammatory markers associated with CVD risk.[20,23] Furthermore, the observational studies published to date have had important limitations: some were small, they often did not adjust for confounders that affect inflammatory marker levels (ie, age, sex, adiposity, and insulin resistance), did not study newer inflammatory markers, and importantly, participants likely were not representative of contemporary smokers who tend to be more overweight than historical cohorts.[24]

To address this critical gap in our understanding of smoking-associated arterial disease, we analyzed the cross-sectional and longitudinal relationships between smoking burden, smoking cessation, and 6 inflammatory markers that predict CVD events (CRP, D-dimer, fibrinogen urinary F_2 isoprostane:creatinine [F_2:Cr] ratio, myeloperoxidase, and WBC count) in a large cohort of contemporary smokers.

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Nonstandard Abbreviations and Acronyms	
CVD	cardiovascular disease
CO	carbon monoxide
CRP	C-reactive protein
WBC	white blood cell

Materials and Methods

Materials and Methods are available in the online-only Data Supplement.

Results

Subject Characteristics

Baseline subject characteristics are shown in Table 1. The 1652 smokers from the longitudinal study (54% women, 66% white) were 49.6 years old (11.7), smoked 16.8 cigarettes/d (8.5), and had a smoking burden of 27.3 pack-years (18.6) with carbon monoxide (CO) levels of 14.4 ppm (8.3). Their mean body mass index was 29.4 kg/m^2 (6.7). The use of lipid-lowering and antidiabetic medications was reported by 18% and 8.7% of participants, respectively. Baseline subject characteristics for the subset of participants (n=888) who made

Table 1. Baseline Characteristics for All Smokers (n=1652) and for Smokers Who Made an Aided Quit Attempt (n=888)

	All Smokers (n=1652)		Smokers Who Made an Aided Quit Attempt and Completed Year 1 Assessments (n=888)			
			Smokers at Year 1 (n=544)		Abstainers at Year 1 (n=344)	
	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range
Age, y	49.6 (11.7)	18–91	49.3 (11.1)	18–88	49.6 (12.4)	18–91
Sex (% female)	54.2		53.5		56.1	
Race (% white)	65.6		61.9		70.6	
Body mass index	29.4 (6.7)	15.9–60.8	29.4 (6.7)	15.9–60.8	29.3 (6.7)	16.1–56.7
Weight, kg	85.7 (21.1)	41.6–180.5	85.3 (20.6)	41.6–180.5	85.5 (21.6)	46.3–179.8
Markers of smoking heaviness						
Current smoking, cigarettes/d	16.8 (8.5)	1–75	17.3 (8.9)	1–75	15.5 (8.1)	1–60
Carbon monoxide, ppm	14.4 (8.3)	0–67	15.4 (8.4)	2–66	13.1 (7.4)	2–48
Smoking burden, pack-years	27.3 (18.6)	0.5–165	28.0 (19.1)	0.5–165	25.4 (18.7)	1.55–144
Inflammatory markers						
C-reactive protein, mg/L	4.6 (8.1)	0.2–115.3	4.7 (8.0)	0.2–96.1	4.5 (6.8)	0.2–64.1
D-dimer, μgFEU/mL	0.3 (0.5)	0–8.1	0.3 (0.5)	0–8.1	0.3 (0.3)	0.0–3.1
Fibrinogen, mg/dL	286.4 (81.1)	101–764	280.4 (78.0)	101–764	283.4 (80.6)	102–579
Urinary F_2 isoprostane:creatinine ratio, ng/mg	0.8 (0.6)	0–5.6	0.8 (0.6)	0–5.6	0.7 (0.5)	0–4.8
Myeloperoxidase, pmol/L	279.7 (185.2)	0-4091	277.7 (173.0)	0-2792	272.0 (150.3)	0-2261
White blood cell count, cells/mL	7.5 (2.2)	2.2–20.1	7.6 (2.3)	3.0–20.1	7.4 (2.1)	2.5–14.2
Systolic blood pressure, mm Hg	126.77 (17.3)	79–197	125.3 (16.3)	79–197	124.3 (17.2)	86–191.5
Diastolic blood pressure, mm Hg	76.1 (10.0)	53–117	75.5 (10.1)	53–113	75.3 (9.0)	53–117
Antihypertensive medication use, %	29.6		32.2		29.9	
Lipids, mg/dL						
Total cholesterol	192.1 (41.0)	84–452	189.8 (39.7)	84–397	191.9 (38.6)	98–366
High-density lipoprotein cholesterol	50.5 (17.6)	16–162	49.8 (16.5)	19–121	50.2 (18.5)	19–149
Low-density lipoprotein cholesterol	113.8 (34.8)	17–302	113.4 (35.2)	38–302	113.5 (31.6)	24–236
Triglycerides	141.4 (126.6)	30–2774	134.5 (74.0)	36–506	140.8 (98.2)	31–801
Lipid medication use, %	18.3		20.8		20.6	
Creatinine, mg/dL	0.85 (0.23)	0.33–4.58	0.85 (0.20)	0.45–2.01	0.84 (0.18)	0.43–1.79
Diabetes mellitus medication use, %	8.7		10.3		9.0	
Hemoglobin A_1C, %	5.9 (0.9)	4–14.4	5.8 (0.9)	4.3–13.6	5.8 (0.9)	4.7–11.3
Glucose, mg/dL	121.3 (26.7)	68–367	121.0 (25.0)	77–344	121.0 (24.7)	88–276
Insulin, pg/mL	9.9 (8.8)	0–85	9.2 (7.9)	0–70.1	9.5 (8.7)	1–85

an aided quit attempt and completed year 1 assessments also are in Table 1.

Baseline Associations of Smoking Heaviness Markers with Inflammatory Markers

Associations of the 6 inflammatory markers with smoking heaviness parameters (exhaled CO, cigarettes/d, and pack-years) for all smokers are shown in Table 2, adjusted for age, sex, race, body mass index, total cholesterol, high-density lipoprotein cholesterol, systolic blood pressure, diabetes mellitus status, antihypertensive medication use, and lipid medication use. These models indicate strong, independent associations between smoking heaviness markers and the urinary F_2:Cr ratio, WBC counts, and myeloperoxidase (P<0.001). No statistically significant associations were observed between CRP, D-dimers, and fibrinogen levels and any smoking heaviness marker in the adjusted models. These models were not affected substantively when adjusted by alcohol use or alternative models of adiposity (data not shown).

Table 2. Baseline Associations of Smoking Heaviness and Inflammatory Markers for All Smokers

	n	Standardized Coefficient (β)	*P* Value
C-reactive protein (log-transformed)			
Carbon monoxide	1442	0.016	0.519
Cigarettes/d	1404	0.045	0.064
Pack-years	1398	0.055	0.055
D-dimer (log-transformed)			
Carbon monoxide	1437	0.024	0.381
Cigarettes/d	1399	0.019	0.487
Pack-years	1393	0.026	0.403
Fibrinogen			
Carbon monoxide	1438	–0.024	0.336
Cigarettes/d	1400	0.011	0.645
Pack-years	1394	0.029	0.324
Urinary F_2 isoprostane:creatinine ratio (log-transformed)			
Carbon monoxide	1360	0.105	<0.001
Cigarettes/d	1322	0.089	0.001
Pack-years	1317	0.130	<0.001
Myeloperoxidase (log-transformed)			
Carbon monoxide	1434	0.075	0.006
Cigarettes/d	1396	0.077	0.004
Pack-years	1390	0.085	0.007
White blood cell count			
Carbon monoxide	1438	0.103	<0.001
Cigarettes/d	1400	0.103	<0.001
Pack-years	1394	0.129	<0.001

Models adjusted for age, sex, race, body mass index, total cholesterol, high-density lipoprotein cholesterol, systolic blood pressure, diabetes mellitus status, antihypertensive medication use, lipid medication use.

Inflammatory Markers After Smoking Cessation

Of the 888 participants who made an aided quit attempt and completed the year 1 assessments, 344 (29.7%) participants had biochemically confirmed 7-day point-prevalence abstinence at 1 year. Their characteristics at the year 1 visit are in Table I in the online-only Data Supplement. At the baseline visit, there were no significant differences in sex, race, or anthropometric measures between successful abstainers and continuing smokers; however, eventual abstainers smoked fewer cigarettes/d (t=2.95; P=0.003) and had lower CO levels (t=4.17; P<0.001) at baseline than continuing smokers. Abstainers gained more weight than continued smokers (4.0 kg [6.0] versus 0.4 kg [5.7]; P<0.001) and had greater increases in homeostasis model of insulin resistance scores (15.4 U [47.3] versus 7.2 U [48.1]; P=0.02). Continuing smokers at year 1 had similar measures of smoking heaviness as at baseline. We observed statistically significant correlations between changes in CO with changes in urinary F_2:Cr ratio (P=0.002) and leukocyte counts (P<0.001), but no significant correlations with the other inflammatory biomarkers after 1 year. Table 3 describes unadjusted changes in the inflammatory markers and other variables after 1 year among smokers who made an aided quit attempt. These models were not affected substantively by adjusting for alcohol use or alternative models of adiposity (data not shown).

Despite greater weight gain and increases in insulin resistance, abstinence at 1 year—when compared with continued smoking—was associated independently with decreases in the urinary F_2:Cr ratio (P<0.001) and WBC counts (P<0.001) in models adjusting for the baseline inflammatory marker value, change in weight, and change in homeostasis model of insulin resistance. These results are consistent with the simple correlations described above and continued to be statistically significant (P<0.001) after further adjusting for age, sex, race, total cholesterol, high-density lipoprotein cholesterol, systolic blood pressure, diabetes mellitus status, antihypertensive medication use, lipid medication use. Results of models evaluating the effects of successful abstinence compared with continued smoking on the inflammatory markers after 1 year among smokers who made an aided quit attempt (n=888) are shown in Table 4. These models accounted for 24% to 48% of the variance in the inflammatory markers.

Discussion

In this large cohort of contemporary smokers, we observed independent associations between smoking heaviness and three inflammatory markers that are associated with CVD risk—the urinary F_2:Cr ratio, myeloperoxidase, and WBC count—but not with CRP, fibrinogen or D-dimer. After 1 year, successful abstainers experienced statistically significant, independent reductions in urinary F_2:Cr ratios and WBC counts, despite weight gain and increased insulin resistance, whereas differences in myeloperoxidase, CRP, D-dimer, and fibrinogen were not observed.

In some population-based studies, active cigarette smoking has been associated with elevated levels of each of the 6 inflammatory markers we investigated[19–23,25–27]; however, these studies have had conflicting results, especially for the most

Table 3. Unadjusted Changes in Inflammatory and Other Markers at 1 Year (Year 1-Baseline) Among Smokers Who Made an Aided Quit Attempt (n=888)

	Continued Smoker (n=544)	Abstainer (n=344)	*P* Value
Inflammatory markers			
C-reactive protein	0.03 (9.9)	0.4 (9.1)	0.53
D-dimer	0.1 (0.9)	0.1 (0.3)	0.20
Fibrinogen	42.6 (80.7)	38.2 (83.7)	0.46
Urinary F_2 isoprostane:creatinine ratio	−0.02 (0.5)	−0.1 (0.5)	0.06
Myeloperoxidase	−3.3 (156.0)	13.5 (267.0)	0.02
WBC count	0.2 (1.8)	−0.6 (1.9)	<0.001
Weight	0.4 (5.7)	4.0 (5.9)	<0.001
Lipids			
Total cholesterol	2.4 (29.3)	4.8 (31.9)	0.27
High-density lipoprotein cholesterol	2.6 (10.4)	4.4 (11.0)	0.02
Low-density lipoprotein cholesterol	−0.5 (25.6)	−0.2 (26.7)	0.09
Triglycerides	2.0 (69.6)	8.6 (103.9)	0.27
Creatinine	0.01 (0.1)	0.03 (0.1)	0.02
Hemoglobin A_1C	0.03 (0.5)	0.1 (0.6)	0.06
Homeostasis model of insulin resistance score	7.2 (48.1)	15.4 (47.3)	0.02

commonly studied markers, CRP[20,23] and WBC count.[20,25] These discrepant findings may have been because of the evolution of the population of smokers, imprecise assessment of smoking status and burden, and changes in laboratory measurement technology over time. The characteristics of smokers have changed since the first population-based studies that found associations between smoking and inflammation >30 years ago. Contemporary smokers are older, weigh more, are less educated, and have more medical and psychiatric comorbidities.[2,28] They also smoke less but seem to gain more weight after smoking cessation.[2,28,29] Our assessment of smoking status (biochemically confirmed 7-day point prevalence abstinence) was state of the art and avoids misclassification bias. Also, bioassays for several of the inflammatory markers have changed over time, yielding differing and presumably, more precise results. The results of this study reflect the relationships between smoking and inflammatory processes in a contemporary sample of smokers using the best contemporary research approaches.

Differences in the cross-sectional relationships between smoking heaviness and inflammatory markers and longitudinal changes in these same markers with successful abstinence versus continued smoking may reflect distinct biological processes. For example, CRP is a nonspecific marker of inflammation that in large part reflects adiposity and responses to adipocytokines.[30] In our study and in others, CRP levels seem to be more related to weight and weight gain than changes in smoking heaviness with strong observed associations between CRP levels and measures of adiposity.[20,31] WBC count is also a nonspecific marker that has consistently been associated with smoking and smoking heaviness,[20,32] likely reflecting different inflammatory pathways less closely linked to adiposity. The urinary F_2:Cr ratio primarily reflects oxidant stress because of lipid peroxidation,[21,33] which promotes atherogenesis.[34] Like WBC count, the urinary F_2:Cr ratio had strong, independent cross-sectional associations with each marker of smoking heaviness, and levels improved with cessation, suggesting a causal relationship. An association between increased levels of urinary isoprostanes and smoking has been described[27]; however, to our knowledge, this is the first to prospectively demonstrate its reduction with smoking cessation. Myeloperoxidase also contributes to oxidative damage and has been hypothesized to be a catalyst for low-density lipoprotein oxidation in vivo.[35] Like the urinary F_2:Cr ratio and WBC, it showed significant cross-sectional associations with each smoking heaviness marker; however, it did not improve with smoking cessation.

Despite a wealth of evidence linking cigarette smoking with CVD, the exact mechanisms that contribute to this association remain unclear. Our findings suggest that smoking increases risk by increasing oxidant stress that improves on successful cessation, despite the weight gain and worsening insulin resistance that are common after smoking cessation.

Table 4. Effect of Abstinence on Inflammatory Markers After 1 Year Among Smokers Who Made an Aided Quit Attempt (n=888)

Year 1 Inflammatory Marker	Adjusted R^2 for Model	*F* Value	Partial Eta Squared	*P* Value for Abstinence Status at Year 1*
C-reactive protein (log-transformed)	0.47	0.45	0.001	0.50
D-dimer (log-transformed)	0.24	0.64	0.001	0.42
Fibrinogen	0.25	0.22	0.000	0.64
Urinary F_2 isoprostane:creatinine ratio (log-transformed)	0.39	18.05	0.024	<0.001†
Myeloperoxidase (log-transformed)	0.42	0.23	0.000	0.63
White blood cell count	0.48	36.39	0.043	<0.001†

*Analysis of covariance models adjusted for baseline inflammatory marker value, change in weight, and change in homeostasis model of insulin resistance.

†*P* values remained <0.001 when further adjusted for age, sex, race, total cholesterol, high-density lipoprotein cholesterol, systolic blood pressure, diabetes mellitus status, antihypertensive medication use, and lipid medication use.

F_2:Cr ratio and myeloperoxidase—inflammatory markers that reflect oxidative stress—are likely targets to enhance CVD risk prediction and for responses to therapeutic interventions in smokers.

Limitations

This was a longitudinal, observational study conducted in the context of a randomized comparative effectiveness trial of smokers highly motivated to quit. Eventual quitters and continuing smokers differed in many factors that may have affected the relationships between the inflammatory markers studies and smoking heaviness markers, so residual confounding cannot be excluded. However, the strong associations, multiple factors we adjusted for, and consistent findings after 1 year, at least for the urinary F_2:Cr ratio and WBC count, strongly suggest that our findings are accurate and that oxidant stress plays an important role in the inflammatory component of smoking-related CVD risk. As commonly observed in studies of smoking cessation, ≈26% of subjects did not return for their 1-year follow-up visit. This is comparable drop-out rates in other recent clinical trials of smoking cessation pharmacotherapy.[36,37] We measured CO to ascertain quit status. Some investigators prefer serum cotinine; however, it may also be influenced by environmental exposure such as second-hand smoke. From a tobacco science perspective, the use of CO to verify smoking status is common and in our opinion, is preferable, as does not capture nicotine from nicotine replacement products. Participants from Milwaukee were older, more often were non-white, and had lower socioeconomic status. A sensitivity analysis that adjusted for recruitment site did not reveal any substantive differences in the standardized coefficients or levels statistical significance, for which changes were minimal and at the thousandth decimal point (data not shown). Finally, we cannot exclude the possibility that there may have been differences in undiagnosed infections, inflammatory diseases, or malignancies between continuing smokers and abstainers that may have confounded our findings.

Conclusions

In this large cohort of contemporary smokers, smoking heaviness was associated independently with urinary F_2:Cr ratio, WBC counts, and myeloperoxidase levels, but not with CRP, D-dimer, or fibrinogen levels. After 1 year, smoking cessation lead to significant reductions in the urinary F_2:Cr ratio and WBC counts despite weight gain and worse insulin resistance, suggesting that oxidant stress may mediate increased inflammation and CVD risk in smokers.

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Disclosures

None.

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Highlights

- There are strong, independent associations between smoking heaviness markers and the urinary F_2 isoprostane:creatinine ratio, white blood cell, and myeloperoxidase but not high-sensitivity C-reactive protein, D-dimer, or fibrinogen.
- Successful abstainers from smoking gain more weight and have larger increases in insulin resistance scores than continuing smokers.
- Despite weight gain, successful abstainers have significant decreases in urinary F_2 isoprostane:creatinine ratio and white blood cell counts.
- These findings suggest reduced inflammation in successful smokers who successfully quit, related to less oxidant stress.

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Longitudinal Impact of Smoking and Smoking Cessation on Inflammatory Markers of Cardiovascular Disease Risk

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Materials and Methods

Study Participants and Design

We analyzed baseline and 1-year follow-up data from a longitudinal study (Wisconsin Smokers Health Study-2) designed to examine the natural history of smoking and smoking cessation (clinicaltrials.gov registration number NCT01553084).[1] Participants were smokers recruited from communities in or around Madison and Milwaukee, Wisconsin and included individuals that previously participated in the Wisconsin Smokers Health Study-1.[2] The vast majority participated in a randomized, comparative effectiveness smoking cessation trial designed to evaluate the efficacy of 3 smoking cessation pharmacotherapies (nicotine patch, varenicline, and nicotine patch + nicotine lozenge) or received usual care smoking cessation treatment. Key inclusion criteria for new participants and those wishing to participate in the cessation trial were: age ≥18 years old, smoking ≥5 cigarettes per day, desire to quit smoking but not engaged in smoking treatment, willingness to used tested cessation treatments, and willingness to not use e-cigarettes.[1] Key exclusion criteria were hemodialysis, increased suicide risk; diagnosis of or treatment for psychosis; moderately severe depression; untreated hypertension; current use of bupropion; or recent hospitalization for stroke, myocardial infarction, congestive heart failure, or diabetes mellitus. This study was approved by the institutional review board at the University of Wisconsin School of Medicine and Public Health. All subjects provided written informed consent.

Study Procedures

Participants were screened for eligibility, provided written informed consent, completed baseline assessments, and the vast majority received smoking cessation treatment. All participants completed additional assessments one year later. The baseline and 1-year visits included measurement of anthropometric data, fasting laboratory tests, and completion of validated questionnaires and interviews that assessed smoking burden. Specific measures of burden included current cigarette smoking (cigarettes per day), current pack-years (current cigarettes/day * number of years smoked, which reflects smoking burden), and exhaled CO (which reflects smoking efficiency, recent smoking, and recent smoke exposure). Participants provided fasting blood samples to assess the 6 inflammatory markers (urinary F_2:Cr ratio, WBC count, CRP, MPO, D-dimer, and fibrinogen) at both visits. Seven-day, CO-confirmed (<6 ppm), point-prevalence abstinence was assessed 1 year after the target quit date.

Measurements of Inflammatory Markers

Fasting blood samples were obtained by venipuncture and refrigerated. Plasma aliquots were isolated by centrifugation and frozen at −70°C. All inflammatory markers were analyzed at Cleveland Heart Lab, Inc. (Cleveland, OH) except for WBC count which was measured at the University of Wisconsin Hospital and Clinics using Lab by standard techniques on Sysmex XE 2100 analyzers (Sysex, Inc., Mundelein, IL). High-sensitivity CRP, D-dimer and fibrinogen were measured a using immunoturbidometric methods, MPO by an enzyme-linked immunosorbance assay method, creatinine using a photometric method, and insulin using an electrochemiluminescence immunoassay, each on a Cobas 600 c501 analyzer (Roche Diagnostics, Inc., Indianapolis, IN). Urinary F_2 isoprostanes were measured by liquid chromatography and mass spectroscopy. Homeostasis model of insulin resistance (HOMA-IR), which has been validated to estimate β-cell function and insulin resistance from basal glucose and insulin concentrations, was calculated as insulin * glucose * 22.5.[3]

Statistical Analysis

Means (standard deviations) and ranges were used for descriptive statistics. Evaluating the effects of smoking burden and smoking cessation on the 6 inflammatory markers were pre-

specified secondary analyses of this study. To examine the relations between smoking burden and inflammatory markers, linear regression models were created for each smoking burden variable, adjusting for variables that had correlations with $p<0.10$ for each inflammatory marker (age, sex, race, body-mass index [BMI], total cholesterol, high-density lipoprotein cholesterol, systolic blood pressure, antihypertensive use, lipid-lowering medication use, and diabetes mellitus status). Age was the only independent predictor of successful abstinence that we could adjust for in the smoking cessation efficacy analysis[1]; the others were CO level (which is used in our outcome definition) and self-efficacy (a psychological construct, not a biological parameter). Milwaukee vs. Madison, WI recruitment site was considered as an additional covariate in a sensitivity analysis. We also explored the effects of chronic alcohol use (no alcohol use, moderate alcohol use [≤1 drink/day in women; ≤2 in men], or heavy drinking) and two additional measures of adiposity: waist circumference and a novel body shape index.[4] We additionally looked at simple correlations between changes in CO levels and changes in the biomarkers after one year.

Linear regression models were used to identify independent associations between smoking heaviness and log(CRP), log(D-dimer), fibrinogen, log(urinary F_2:Cr ratio), log(MPO) and WBC count. Using data from smokers who made an aided quit attempt, student's t-tests were used to compare Year 1 inflammatory markers between those who successfully quit smoking ("abstainers") and those who were smoking at Year 1. Analyses of covariance were used to examine the impact of smoking abstinence on Year 1 levels of inflammatory markers, controlling for their baseline levels and also adjusting for changes in weight and HOMA-IR. Analyses were performed with SPSS software (Version 22, IBM SPSS Statistics, IBM Corporation).

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Supplemental Table SI: Baseline Characteristics and Year 1 Values for All Smokers Who Made an Aided Quit Attempt (N=888)

	Baseline		Year 1			
	All Smokers (N=888)		Smokers (n=544)		Abstainers (n=344)	
	Mean (standard deviation)	Range	Mean (standard deviation)	Range	Mean (standard deviation)	Range
Body-mass index	29.4 (6.7)	15.9-60.8	29.6 (6.8)	15.7-67.0	30.7 (7.3)	16.5-66.3
Weight (kg)	85.4 (21.0)	41.6-180.5	85.8 (21.0)	40.2-189.2	89.4 (22.8)	44.8-195.0
Markers of smoking heaviness						
Current smoking (cigarettes/day)	16.6 (8.6)	1-75	11.0 (6.7)	0-40	--	--
Carbon monoxide (ppm)	14.5 (8.1)	2-66	14.0 (7.9)	6-70	2.5 (1.3)	0-5
Inflammatory Markers						
C-reactive protein (mg/L)	4.6 (7.5)	0.2-96.1	4.7 (8.8)	0.0-94.4	4.9 (8.4)	0.2-94.5
D-dimer (ugFEU/mL)	0.3 (0.4)	0.0-8.1	0.4 (0.8)	0.0-15.3	0.3 (0.4)	0.0-3.0
Fibrinogen (mg/dL)	281.6 (79.0)	101-764	323.9 (83.1)	107-885	322.3 (83.3)	134-864
Urinary F_2 isoprostane:creatinine ratio (ng/mg)	0.8 (0.6)	0.0-5.6	0.8 (0.6)	0.0-6.4	0.6 (0.5)	0.0-4.2
Myeloperoxidase (pmol/L)	275.5 (164.5)	0-2792	272.5 (104.5)	0-884	288.6 (244.9)	65-3292
White blood cell count (cells/mL)	7.5 (2.2)	2.5-20.1	7.8 (2.4)	2.8-20.5	6.9 (2.0)	1.4-17.5
Lipids						
Total cholesterol (mg/dL)	190.7 (39.3)	84-397	192.1 (40.4)	71-339	197.1 (43.6)	110-523
High-density lipoprotein cholesterol (mg/dL)	50.0 (17.3)	19-149	52.4 (18.0)	19-121	54.6 (20.0)	14-154
Low-density lipoprotein cholesterol (mg/dL)	113.4 (33.9)	24-302	112.8 (36.3)	30-254	113.5 (34.7)	32-253
Triglycerides (mg/dL)	136.9 (84.2)	31-801	136.7 (89.7)	0-729	149.9 (138.5)	30-1908
Creatinine (mg/dL)	0.8 (0.2)	0.4-2.0	0.9 (0.2)	0.5-2.3	0.9 (0.2)	0.5-1.8
Diabetes Mellitus Markers						
Hemoglobin A_1C (%)	5.8 (0.9)	4.3-13.6	5.9 (0.7)	4.2-11.9	6.0 (1.0)	4.8-13.3
Glucose (mg/dL)	121.0 (24.9)	77-344	122.0 (21.2)	74-295	124.2 (27.6)	91-335
Insulin (pg/mL)	9.3 (8.2)	0.0-85.0	10.6 (9.6)	0.2-75.1	11.9 (10.9)	0.4-106.4