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Relation of Interleukin-6 and Vascular Cellular Adhesion Molecule-1 Levels to Functional Decline in Patients with Lower Extremity Peripheral Arterial Disease

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Abstract

We determined whether persistently high levels of interleukin-6 (IL-6) or soluble vascular adhesion molecule (sVCAM-1) are associated with faster functional decline, compared to fluctuating or persistently low biomarker levels, among 255 participants with peripheral arterial disease (PAD). Participants underwent baseline and at least two annual follow-up measures of IL-6 and sVCAM-1. Participants were categorized as follows: Category 1- annual levels of IL-6 (or sVCAM-1) were in the lowest tertile for at least three study visits. Category 3- annual levels of IL-6 (or sVCAM-1) were in the highest tertile for at least three visits. Category 2- levels of IL-6 (or sVCAM-1) did not meet criteria for Groups 1 or 3. Six-minute walk, fastest paced four meter walking velocity, and the short physical performance battery (SPPB) were measured annually. Results were adjusted for age, sex, race, comorbidities, statins, physical activity, the ankle brachial index, and other confounders. Across IL-6 categories, average annual declines in six minute walk performance were Category 1: -21.4 feet, Category 2:-49.2 feet, and Category 3:-76.8 feet (p trend = 0.013) and average annual declines in the SPPB score were -0.18, -0.45, and -0.62, respectively (p trend = 0.022). Similar associations of IL-6 categories with decline in fastest paced walking velocity were observed (p trend = 0.034). There were no significant associations of sVCAM-1 categories with functional decline. In conclusion, among PAD participants, persistently high IL-6

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levels are associated with faster functional decline compared to those with fluctuating or persistently low IL-6 levels.

Keywords

Inflammation; physical functioning; peripheral arterial disease; intermittent claudication

Persistently high levels of inflammation may promote functional decline in patients with lower extremity peripheral arterial disease (PAD), if persistent inflammation impairs skeletal muscle function or promotes more rapid progression of lower extremity atherosclerosis (1-3). We studied associations of persistently high levels of Interleukin-6 (IL-6) and soluble vascular cellular adhesion molecule-1 (sVCAM-1), respectively, with average annual functional decline in participants with PAD.

Methods

The protocol was Institutional Review Board-approved by Northwestern University Feinberg School of Medicine and Catholic Health Partners Hospitals. Participants gave informed consent. Participants were men and women with PAD in the Walking and Leg Circulation Study II (WALCS II) (4) who had blood drawn during at least three of their four annual WALCS II visits.

The WALCS II cohort consisted of 368 PAD participants completing their fourth annual follow-up visit for original WALCS cohort and 402 newly identified PAD participants (4,5). PAD participants were age ≥ 59 at baseline and were identified from among consecutive patients in the non-invasive vascular laboratories at Chicago-area hospitals. Baseline data were collected between November 2002 and May, 2004. Follow-up data were collected annually through October 31, 2009.

PAD was defined as an ankle brachial index < 0.90 at baseline (4,5). Patients with recent major surgery were excluded. At the time of enrollment for WALCS and WALCS II, patients with dementia, nursing home residents, wheelchair-bound patients, and patients with foot or leg amputations or critical limb ischemia were excluded (4,5). Non-English-speaking patients were excluded because investigators were not fluent in non-English languages.

Participants rested supine for five minutes prior to the ankle brachial index measurement. Using a handheld Doppler Probe (Nicolet Vascular Pocket Dop II, Nicolet Biomedical Inc., Golden, Colorado), systolic blood pressures were measured in the following order: right brachial artery, right dorsalis pedis and posterior tibial arteries, left dorsalis pedis and posterior tibial arteries, and left brachial artery. Two sets of measurements were obtained. The mean of the dorsalis pedis and posterior tibial pressures in each leg was divided by the mean of all four brachial pressures to obtain the ankle brachial index value (6). Subclavian stenosis was suspected if the right or left brachial pressure was higher in both measurement sets, and there was a difference of at least 10 mm Hg between the two brachial pressures in either measurement set. In these cases, the average brachial pressure in the arm with highest pressure was used to calculate the ankle brachial index (7).

Functional performance was measured annually with the six-minute walk, four meter walking velocity at usual and fastest pace, and the short physical performance battery (4,5,8). The six-minute walk measures walking endurance and correlates with physical activity levels in patients with PAD (9). Participants walk up and down a 100 foot hallway for six minutes after instructions to cover as much distance as possible (8,9). Walking

velocity was measured with a four-meter walk performed at “usual” and “fastest” pace. Each walk was performed twice. The faster walk in each pair was used in analyses (4,5,10).

The short physical performance battery (SPPB) is a global measure of leg functioning that predicts mobility loss, nursing home placement, and mortality (10). To calculate the SPPB, a 0-4 score is assigned for performance on three measures of lower extremity functioning: usual paced four-meter walking velocity, time to rise from a seated position five times, and standing balance, respectively. Individuals receive a zero score for each task they are unable to complete. One to four scores for each task are assigned based upon quartiles of performance for over 6,000 participants in the Established Populations for the Epidemiologic Study of the Elderly (10). Scores are summed to obtain the SPPB, ranging from 0 to 12. For the five timed repeated chair rises, participants sit in a straight-backed chair with arms folded across their chest and stand five times consecutively as quickly as possible (4,8,10). Time to complete five chair rises is measured. For standing balance, participants were asked to hold three increasingly difficult standing positions for ten seconds each: standing with feet together side-by-side and parallel (side-by-side stand), standing with feet parallel with the toes of one foot adjacent to and touching the heel of the opposite foot (semi-tandem stand), and standing with one foot directly in front of the other (tandem stand) (4,8,10).

Blood specimens were collected at baseline and at each annual follow-up visit. Blood specimens were immediately processed for storage at -70 degrees Celsius until the time of measurement. IL-6 and sVCAM-1 were measured by ELISA assays from R & D Systems (Minneapolis, MN). Coefficient of variation percent values were calculated from a randomly selected subset of samples in WALCS II at baseline and were 4.21 for IL-6 and 5.61 for sVCAM-1. Similar variability was observed for follow-up visits. Total cholesterol levels were measured on blood samples stored at -70 degrees Celsius, using enzymatic reaction with peroxidase/phenol-4-aminoiphenazone indicator reaction (11). High Density Lipoprotein cholesterol was measured using a direct enzymatic colorimetric assay (12).

Algorithms developed for the Women's Health and Aging Study and the Cardiovascular Health Study were used to document comorbidities. These algorithms combine data from patient report, physical examination, medical record review, medications, laboratory values, and a primary care physician questionnaire (13). Comorbidities assessed were angina, diabetes mellitus, myocardial infarction, stroke, heart failure, pulmonary disease, cancer, spinal stenosis, and disk disease. History of hypertension was based on patient report of physician-diagnosed hypertension or results from the primary care physician questionnaire.

The study principal investigator (MMM), blinded to all other patient data, reviewed lists of medications at each visit and identified presence vs. absence of use of statins, diabetes medications, cilostazol, pentoxifylline, non-steroidal anti-inflammatory drugs, and steroids. Height was measured at baseline and weight was measured at each study visit. Body mass index was calculated as weight (kg)/(height (meters))². Walking exercise and cigarette smoking were based on patient report (14). Physical activity was assessed with patient report of the number of blocks walked in the past week (15). Leg symptoms were measured with the San Diego claudication questionnaire, based on previous study (16).

Tertiles of IL-6 and sVCAM-1 were defined for the baseline visit and at each annual follow-up visit, respectively, for up to four visits. Participants were classified according to the number of visits at which their IL-6 level fell within the highest and lowest tertile, respectively. Participants whose IL-6 value fell within the lowest tertile of IL-6 in at least three of the four study visits (i.e. persistently low inflammation) were classified in Category 1 for IL-6. Participants whose IL-6 value fell within the highest tertile of IL-6 in at least

three study visits (persistently high inflammation) were classified in Category 3 for IL-6. Remaining participants, who had intermittently high or low levels of each biomarker and did not meet criteria for Category 1 or Category 3, were classified in Category 2. Similar categories were defined for sVCAM-1 values. We used tertiles to define persistently high and low inflammation because tertiles allowed us to distinguish between participants with high vs. low levels of the biomarkers while also maximizing sample size in the three categories of inflammation defined.

Baseline characteristics of participants were compared across the three categories of IL-6 and sVCAM-1, respectively, using analysis of covariance, adjusting for age. In comparing change in functional performance across the groups, a longitudinal or repeated measures analysis of covariance was carried out using a mixed model approach, where the within subject correlations are modeled with a subject-specific random effect. The primary dependent variables for each analysis were the successive annual differences in the particular functional performance measure of interest (i.e., the difference in functioning between baseline and the first follow-up visit, between the first and second follow up visit, and between the second and third follow-up visit). Associations of each IL-6 category with decline in each functional performance measure were compared using analysis of covariance, adjusting for baseline covariates (gender, age, race, comorbidities, body mass index, pack-years of smoking, High Density Lipoprotein cholesterol, total cholesterol, statin use, walking exercise, and physical activity) and prior year performance (Model 1). Covariates in Model 1 were selected because they were potential confounders of the associations of the inflammation categories with average annual decline in functional performance. Model 1 analyses were repeated with additional adjustment for time dependent ankle brachial index, smoking status, statin use, physical activity, diabetes medication use, and walking exercise (Model 2). These additional time-dependent covariates in Model 2 were selected to determine whether changes in lower extremity atherosclerosis severity and participant behaviors overtime influenced the significant associations identified in Model 1 analyses. For Models 1 and 2, pairwise comparisons between Category 1 and Category 3 were performed using analysis of covariance, adjusting for covariates as defined for Models 1 and 2 above. Model 2 analyses were repeated with additional adjustment for cilostazol, pentoxifylline, non-steroidal anti-inflammatory medications, and steroid medications. Model 1 and Model 2 analyses were repeated for the sVCAM-1 categories. Statistical analyses were performed using SAS Statistical Software version 9.2 (SAS Inc, Cary, NC).

Results

Among 478 WALCS II participants with PAD, 255 had blood drawn for at least 3 annual study visits and were included in analyses. WALCS II participants excluded because they did not have at least three annual blood draws were older (75.9 ± 8.99 vs. 74.4 ± 7.54 , $p=0.049$) and walked fewer blocks during the past week (17.7 ± 31.1 vs. 37.5 ± 74.0 , $p<0.001$), compared to WALCS II participants included in analyses.

Fifty-seven participants met criteria for persistently low IL-6 levels (Category 1), 150 met criteria for Category 2, and 48 met criteria for persistently high IL-6 levels (Category 3). Sixty-five participants met criteria for persistently low sVCAM-1 levels (Category 1), 121 met criteria for Category 2, and 69 met criteria for persistently high sVCAM-1 levels (Category 3). Average age of participants in Categories 1, 2, and 3 for IL-6 were 72.1 ± 7.3 , 74.9 ± 7.7 , and 75.7 ± 6.9 years (p trend = 0.011). Average age of participants in Categories 1, 2, and 3 for sVCAM-1 were 70.4 ± 6.9 , 74.0 ± 7.3 , and 78.8 ± 6.1 years (p trend < 0.001).

Persistently high levels of IL-6 were associated with higher body mass index, greater smoking pack-years, and a greater number of cardiovascular diseases, compared to

persistently lower levels of IL-6, adjusting for age (Table 1). Persistently high levels of sVCAM-1 were associated with a higher prevalence of men and a lower prevalence of African-Americans, compared to persistently low levels of sVCAM-1, adjusting for age (Table 1).

Figure 1 shows associations of IL-6 categories with average annual decline in six-minute walk. Adjusting for age, sex, race, prior year performance, comorbidities, high density lipoprotein, total cholesterol, smoking, body mass index, ankle brachial index, statins, and physical activity (Model 1, Figure 1), participants with persistently high levels of IL-6 (Category 3) had greater average annual decline in six-minute walk performance, compared to participants with fluctuating IL-6 levels (Category 2) and persistently low levels of IL-6 (Category 1). After additional adjustment of Model 1 for annual changes in ankle brachial index, statin use, walking exercise, smoking status, physical activity, and diabetes medication use, persistently high levels of IL-6 remained associated with faster decline in six-minute walk performance (Figure 1, Model 2). In Model 2, those in Category 3 for IL-6 had significantly greater average annual decline in the six-minute walk compared to those in Category 1.

Figure 2 shows associations of IL-6 categories with average annual decline in the short physical performance battery. Adjusting for age, sex, race, prior year performance, comorbidities, High Density Lipoprotein cholesterol, total cholesterol, smoking, body mass index, ankle brachial index, statins, and physical activity (Model 1, Figure 2), participants with persistently high levels of IL-6 had greater average annual decline in the SPPB, compared to participants with fluctuating IL-6 levels and persistently low IL-6 levels. After additional adjustment of Model 1 for annual changes in ankle brachial index, statin use, walking exercise, smoking status, physical activity, and diabetes medication use, (Model 2, Figure 2), participants with persistently high levels of IL-6 (Category 3) had greater decline in the short physical performance battery compared to those in Categories 1 and 2. In this fully adjusted model, those in Category 3 had significantly greater decline in the SPPB compared to those in Category 1.

Figure 3 shows associations of IL-6 categories with average annual decline in fastest-paced four-meter walking velocity. Adjusting for age, sex, race, prior year performance, comorbidities, cholesterol levels, smoking, body mass index, ankle brachial index, statins, and physical activity (Model 1, Figure 3), participants with persistently high levels of IL-6 (Category 3) had greater average annual decline in fastest-paced walking velocity compared to participants with fluctuating levels and persistently low levels of IL-6. After additional adjustment of Model 1 for annual changes in ankle brachial index, statin use, walking exercise, smoking status, physical activity, and diabetes medication use (Model 2, Figure 3), participants with persistently high levels of IL-6 (Category 3) had greater average annual decline in fastest walking velocity compared to participants with fluctuating and persistently low levels of IL-6. In pair-wise analyses, participants in Category 3 had greater decline in fastest-paced walking velocity compared to those in Category 1. Results shown in Figures 1-3 were not substantially changed when Model 2 was additionally adjusted for cilostazol, pentoxifylline, non-steroidal anti-inflammatory medications, and steroid medications.

Adjusting for age, sex, race, prior year performance, baseline comorbidities, High Density Lipoprotein cholesterol, total cholesterol, smoking, body mass index, ABI, statins, diabetes medication use, and physical activity, there were no differences in average annual decline in usual-paced four-meter walking velocity across the three inflammation categories (-0.033, -0.040, and -0.052 meters/second, respectively, p trend = 0.124). However, after additional adjustment of Model 2 for cilostazol, pentoxifylline, non-steroidal anti-inflammatory medications, and steroid medications, associations of the inflammation categories with

decline in usual-paced four-meter walking velocity became statistically significant (-0.28, -0.41, and -0.51 meters/second, respectively, p trend=0.045).

Persistently high levels of sVCAM-1 (Category 3) were not associated with greater average annual decline in functional performance, compared to participants in Category 2 and Category 1 (Table 2).

Discussion

Findings reported here demonstrate that among participants with PAD, persistently high levels of IL-6 are associated with faster decline in six minute walk performance, the short physical performance battery, and fast-paced four-meter walking velocity compared to PAD participants with fluctuating or persistently low levels of IL-6 at three year follow-up. These associations were independent of baseline participant characteristics and changes in characteristics over time including the ankle brachial index, smoking, physical activity, statin use, and walking exercise behavior. The differences in rates of decline in six-minute walk performance, SPPB score, and fast-paced four-meter walking velocity between participants with persistently low IL-6 levels (Category 1) and those with persistently high IL-6 levels (Category 3) are consistent with clinically meaningful change, based on prior study (17). To our knowledge no prior studies have assessed associations of persistently elevated inflammatory biomarker levels with functional decline in men and women with or without PAD.

The association of inflammation on the rate of functional decline may be related to the length of inflammation exposure and may become clinically important only when high levels of inflammation are maintained over long periods of time. There are several potential explanations for associations of persistently high levels of IL-6 with faster decline in functional performance measures. First, inflammation promotes progression of atherosclerosis (3). In the Edinburgh Artery Study, higher baseline levels of IL-6 were associated with a higher incidence of PAD at 5 and 12-year follow-up (18). Persistently high levels of IL-6 may be associated with more significant progression of lower extremity atherosclerosis, which may promote faster declines in walking performance. However, our findings did not change substantially after additional statistical adjustment for changes over time in the ankle brachial index, making this hypothesis less likely. A second potential explanation is that inflammation directly impairs skeletal muscle function, for example, by inhibiting muscle repair after injury (1,2). Consistent with this hypothesis, previous study demonstrates that higher levels of IL-6 and sVCAM-1 are each associated with smaller muscle area and other adverse calf muscle characteristics in participants with PAD (19). Inflammation-related adverse calf skeletal muscle characteristics may contribute to faster functional decline in participants with PAD. Third, persistently high levels of inflammatory biomarkers may minimize measurement error associated with single inflammatory biomarker measures.

We found significant associations of persistently elevated inflammation with functional decline for IL-6, but not for sVCAM-1. The reason for this discrepancy is not clear. IL-6 may be a more non-specific inflammatory biomarker that better represents the cumulative level of systemic inflammation than sVCAM-1. Vascular cellular adhesion molecule-1 (VCAM-1) is a transmembrane glycoprotein that binds β 1 integrins on lymphocytes and monocytes. VCAM-1 tends to be located on activated endothelial cells in lesion-prone area and is more common on neovasculature of intima than on the arterial luminal surface of complex atherosclerotic lesions (20,21). sVCAM-1, the soluble form of VCAM-1, is shed into the circulation. As compared to IL-6, sVCAM-1 may represent a more specific step in atherosclerotic disease progression that is less relevant to functional impairment and decline.

Alternatively, the assay measuring IL-6 may be more valid than the assay measuring sVCAM-1.

This study has limitations. First, the study is observational. Thus, we are not able to make causal inferences about associations of persistently high inflammation with functional decline in persons with PAD. We cannot rule out the possibility that declining functional status contributed to higher levels of IL-6. Second, 18.8% of WALCS II PAD participants did not have at least two blood draws at annual visits and were excluded from analyses. Findings may not be generalizable to these excluded individuals.

In summary, among PAD participants, persistently high IL-6 levels are associated with faster functional decline compared to those with fluctuating or persistently low IL-6 levels.

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Figure 1. Adjusted associations of interleukin-6 categories with decline in six-minute walk performance at three-year follow-up among participants with peripheral arterial disease
 Category 1- Participants' annual interleukin-6 (IL-6) values were in the lowest tertile for at least three of four study visits. Category 2- participants did not meet criteria for Category 1 or Category 3. Category 3- participants' annual IL-6 values were in the highest tertile for at least three of four study visits.

Model 1- adjusts for age, sex, race, prior year performance, comorbidities, high density lipoprotein cholesterol, total cholesterol, smoking, body mass index, ankle brachial index, statins, and physical activity.

Model 2- adjusts for covariates in Model 1 and annual changes in ankle brachial index, statin use, physical activity, smoking status, diabetes medication use, and walking exercise behavior.

Figure 2. Adjusted associations of interleukin-6 categories with decline in the short physical performance battery at three-year follow-up among participants with peripheral arterial disease
 Category 1- Participants' annual interleukin-6 (IL-6) values were in the lowest tertile for at least three of four annual study visits. Category 2- participants did not meet criteria for Category 1 or Category 3. Category 3- participants' annual IL-6 values were in the highest tertile for at least three of four annual study visits.

Model 1- adjusts for age, sex, race, prior year performance, baseline comorbidities, high density lipoprotein cholesterol, total cholesterol, smoking, body mass index, ankle brachial index, statins, and physical activity.

Model 2- adjusts for covariates in Model 1 and annual changes in ankle brachial index, statin use, physical activity, smoking status, diabetes medication use, and walking exercise.

Figure 3. Adjusted associations of interleukin-6 categories with decline in fast-paced four-meter walking velocity at three-year follow-up among participants with peripheral arterial disease
 Category 1- Participants' annual interleukin-6 (IL-6) values were in the lowest tertile for at least three of four annual study visits. Category 2- participants did not meet criteria for Category 1 or Category 3. Category 3- participants' annual IL-6 values were in the highest tertile for at least three of four annual study visits.

Model 1- adjusts for age, sex, race, prior year performance, comorbidities, high density lipoprotein cholesterol, total cholesterol, smoking, body mass index, ankle brachial index, statins, and physical activity.

Model 2- adjusts for covariates in Model 1 and annual changes in ankle brachial index, statin use, physical activity, smoking status, diabetes medication use, and walking exercise.

Table 1
Age-Adjusted Associations of Inflammatory Biomarker Categories with Baseline Characteristics among Men and Women with Peripheral Arterial Disease (n=255)

Interleukin-6 (IL-6) Levels				
Variable	Persistently low (n=57)	Fluctuating (n=150)	Persistently high (n=48)	Trend P value
Male	61%	50%	67%	0.562
African-American	16%	18%	9%	0.372
Ankle Brachial Index	0.64 ± 0.02	0.63 ± 0.01	0.64 ± 0.02	0.979
Body Mass Index (Kg/M ²)	27.11 ± 0.63	28.05 ± 0.39	29.00 ± 0.68	0.044
Diabetes Mellitus	28%	31%	41%	0.197
Current Smoker	9%	11%	17%	0.190
Number of cardiovascular diseases (range- 0-4) [†]	0.56 ± 0.15	1.13 ± 0.09	1.31 ± 0.17	<0.001
Pulmonary disease	35%	42%	45%	0.336
Cancer	26%	19%	18%	0.344
Statin use	56%	55%	54%	0.878
Blocks walked during the past week	46.7 ± 9.8	34.9 ± 6.0	34.6 ± 10.6	0.384
Classic intermittent claudication	24.6%	24.7%	27.1%	
Asymptomatic PAD	17.5%	21.3%	20.3%	0.912 ***
Exertional leg symptoms other than intermittent claudication	58.0%	54.0%	52.6%	
Soluble Vascular Adhesion Molecule-1				
Variable	Persistently low (n=65)	Fluctuating (n=121)	Persistently high (n=69)	Trend P value
Male	47%	54%	66%	0.048
African-American **	29%	13%	8%	0.005
Ankle Brachial Index	0.60 ± 0.02	0.64 ± 0.01	0.64 ± 0.02	0.145
Body Mass Index (kg/M ²)	27.08 ± 0.61	28.38 ± 0.43	28.26 ± 0.60	0.189
Diabetes Mellitus	25%	32%	39%	0.113
Current Smoker	14%	11%	12%	0.764
Number of cardiovascular diseases (range- 0-4)	0.89 ± 0.15	1.03 ± 0.11	1.20 ± 0.15	0.171
Pulmonary Disease	34%	43%	42%	0.384
Cancer	20%	25%	13%	0.265
Statin	49%	62%	48%	0.943
Blocks walked during the past week.	36.3 ± 9.5	39.4 ± 6.7	35.2 ± 9.3	0.935
Intermittent claudication	30.8%	24.0%	21.7%	
Asymptomatic	9.2%	18.2%	33.3%	0.061 ***
Exertional leg symptoms other than intermittent claudication.	12.3%	14.1%	10.1%	

Asymptomatic refers to no exertional leg symptoms.

* P = 0.005 for Pair-wise comparison between Category 1 and Category 3.

[†] P = 0.001 for Pair-wise comparison between Category 1 and Category 3.

**
P =0.002 for Pair-wise comparison between Category 1 and Category 3.

P value represents overall P value for the association of the inflammation category with leg symptoms.

Table 2
Adjusted Associations of soluble Vascular Cellular Adhesion Molecules-1 Categories with Average Annual Functional Decline among Men and Women with Lower Extremity Peripheral Arterial Disease* (n=253)

Functional Performance Measure	Persistently Low (n=65)	Fluctuating (n=119)	Persistently High (n=69)	P Trend
Six-minute walk (feet)	-32.8	-59.2	-42.2	0.615
Short Physical Performance Battery (0-12 scale, 12= best)	-0.34	-0.41	-0.53	0.318
Four-Meter Walking Velocity (usual pace) (Meters/second)	-0.03	-0.04	-0.04	0.288
Four-Meter Walking Velocity (fastest pace) (Meters/second)	-0.05	-0.05	-0.07	0.235

* Analyses adjust for age, sex, race, prior year performance, comorbidities, smoking history, body mass index, ankle brachial index, total cholesterol, High Density Lipoprotein cholesterol, statin use and blocks walked during the past week.

Category 1- Participants' annual soluble vascular cellular adhesion molecule- 1 values were in the lowest tertile for at least three of four annual study visits. Category 2- participants did not meet criteria for Category 1 or Category 3. Category 3- participants' annual soluble vascular cellular adhesion molecule- 1 values were in the highest tertile for at least three of four annual study visits.