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Side-Stream Cigarette Smoke Induces Dose–Response in Systemic Inflammatory Cytokine Production and Oxidative Stress

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Side-stream cigarette smoke (SSCS), a major component of secondhand smoke, induces reactive oxygen species, which promote oxidative damage in tissues and organs. Inflammatory cytokines play an important role in the pathogenesis of atherosclerosis and heart failure. The present 4-month study examined the effect of various chronic SSCS exposure levels on splenic inflammatory cytokine secretion, heart contractile function, and pathology at 60- and 120-min per day, 5 days per week, for a total of 16 weeks. Tissue vitamin E level and lipid peroxide production also were tested to estimate the oxidative stress. The study found that the pro-inflammatory cytokines, interleukin (IL)-6, tumor necrosis factor (TNF)-α, and IL-1β, significantly increased in 120-min SSCS-exposed mice. Decreased stroke volume and increased peripheral arterial resistance were observed in mice exposed to 120-min SSCS per day. Heart pathology was only found in 120-min SSCS-exposed mice. Cardiac and hepatic antioxidant vitamin E levels were decreased as a result of oxidative stress. Hepatic lipid peroxides were increased upon 60-min SSCS exposure. The data also demonstrated that the cardiac α-tocopherol level has a strong correlation with stroke volume; splenic IL-1β has a strong negative correlation with stroke volume; splenic TNF-α has a very strong negative correlation with stroke volume. In conclusion, SSCS exposure induced systemic inflammatory responses. SSCS exposure also accentuated systemic lipid peroxidation with depletion of cardiac and hepatic antioxidant vitamin E level. Finally, SSCS exposure at 120 min per day decreased stroke volume and increased vascular resistance. Systemic IL-1β and TNF-α production are responsible for heart contractile dysfunction. Free radicals may be responsible for the progression to heart contractile dysfunction induced, in part, by SSCS. Oxidized lipoprotein could contribute to the vascular functional changes. Exploring the mechanism of vascular dysfunction in mice is warranted. A more precise quantification of the smoking exposure dose in mice needs to be determined as well. Exp Biol Med 227:823–829, 2002

Key words: pro-inflammatory cytokines; cardiac vitamin E; arterial resistance

Side-stream cigarette smoke (SSCS) is a major component of second-hand smoke, which contains thousands of different chemical constituents (1). The toxicity of tobacco smoke is due to nicotine, cadmium, benzopyrene, oxidants, and inducers of reactive oxygen species (ROS) like NO, NO_2, peroxynitrite, and nitrosamines that initiate, promote, or amplify oxidative damage (2). Free radicals disturb biological systems by reacting with a variety of their constituent molecules. Lipids are one of the potential targets for the oxidative attack of radicals. Polyunsaturated fatty acid residues in lipoproteins have a chemical structure that makes them particularly vulnerable targets for free radical oxidation (or lipid peroxidation; 3). Pregnant women smokers had significantly lower plasma α-tocopherol levels than nonsmokers (4). Prolonged *in vitro* exposure of plasma to cigarette smoke depletes vitamin E (5). Therefore, tissue vitamin E level and lipid peroxidation are indicators of oxidative stress induced by tobacco smoke.

Inflammatory cytokines, including tumor necrosis factor-α (TNF-α) and other cytokines, including interleukin-6 (IL-6) and interleukin-1beta (IL-1β), play an important role in the pathogenesis of atherosclerosis and heart failure (6–9). Elevated levels of TNF-α, IL-6, IL-1β, vascular cell adhesion molecule-1, and intracellular adhesion molecule-1 were detected in the plasma and in the myocardium of patients with heart failure. These elevated levels apparently are correlated with the severity of heart failure (10–12).

SSCS is a significant risk factor for cardiovascular disease. Epidemiologic studies demonstrated an increase in coronary artery disease risk and mortality with exposure to secondhand smoke (13). There is growing concern about the potential impact of second-hand smoke on cardiovascular disease. However, research on the pathologic mechanisms

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that SSCS promotes the development of cardiovascular disease is limited. SSCS increased myocardial infarct size in a rat model of ischemia and reperfusion (14). It also increased experimental atherosclerosis in cholesterol-fed rabbits (15). The present study examined the effect of various chronic SSCS exposure levels at 60- and 120-min per day, 5 days per week, for a total of 16 weeks in mice, on splenic inflammatory cytokine secretion and heart contractile function. Tissue vitamin E level and lipid peroxide production in mice were tested as well to estimate the oxidative stress.

Materials and Methods

Animals and Study Design. All animal studies were performed with approval by the University of Arizona animal review committee. C57BL/6 female mice, 4–5 months old, were purchased from Charles River Laboratories (Wilmington, DE). They were housed in transparent plastic cages with stainless wire lids (4 mice per cage) in the animal facility of the Arizona Health Science Center. The housing facility was maintained at 20–22°C and 60%–80% relative humidity with a 12-hr light-dark cycle. The mice had free access to water and semipurified feed (4% mouse diet, #7001, Teklad, Madison, WI). After 2 weeks of housing, the mice were randomly divided into three groups containing 12 mice each: sham control, 60-min SSCS exposure per day; and 120-min SSCS exposure per day. All treatments were performed 5 days per week. By the end of 16-week-exposure, the heart contractile function of sham control and those exposed to 60- and 120-min were investigated. The splenocyte cytokine production and cardiac vitamin E level were examined in all three groups.

Side-Stream Cigarette Exposures. SSCS is produced from the lit end of a burning cigarette, unfiltered and generated from tobacco combustion at lower temperature. It has the potential to produce similar damages as mainstream cigarette smoke (16). Standard research cigarettes (IR4F, University of Kentucky Smoking & Health Effects Laboratory, Lexington, KY) were used in this study. The methodology for SSCS exposure was adapted from a previous study in mouse model with minor modifications (17). The mice were exposed to SSCS through a 24-port nose-only exposure chamber (IN-TOX, Albuquerque, NM) using a constant vacuum (15 l/min; See Fig. 1 illustration). The

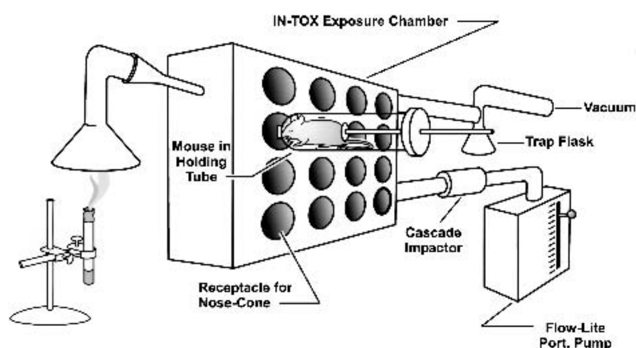

Figure 1. An illustration of SSCS exposure.

time-integrated mass of SSCS total particulate matter delivered to the mice was 2 mg/exposure averaged, at a concentration of 0.4 mg/m^3, as measured by a seven-stage multi-jet impactor (IN-TOX, Albuquerque, NM). The chosen concentration of SSCS was referred from realistic measurements of indoor air concentrations of total suspended particles (TSPs) (range from 10 to 1000 g/m^3) in smoker-occupied residences; up to approximately 2 mg respirable suspended particulates/m^3 in restaurants (18). The first four cigarettes were placed upright in a clamp 2.5 cm below the bottom edge of an inverted 220 cm^3 funnel and allowed to burn for 10-min. The second four cigarettes were ignited after 10-min to replace the first four cigarettes. This sequence was repeated until the various times were reached, at which time the exposed mice were removed. The sham control was treated in a similar manner except that the cigarettes were not lit. All the exposures were conducted 5 days a week.

Determination of α-Tocopherol Levels. α-Tocopherol levels in heart and liver were measured by HPLC, as described previously, with few modifications (19). Briefly, 0./~0.2 g of wet tissue was homogenized in 1.0 ml of distilled water. Butylated hydroxytoluene was added to prevent oxidation of α-tocopherol from the homogenate. Pentane, ethanol, and sodium dodecyl sulfate were used to extract α-tocopherol from the homogenate. Extracts were evaporated under a steady flow of nitrogen gas at 20°C and then redissolved in 1.0 ml of methanol injection onto a C18 column (3.9 × 150 mm NovaPak, Millipore, Bedford, MA). A mobile phase which is composed of methanol, distilled water in the ratio of 93: 7 (by volume) with a flow rate of 0.8 ml/min. α-Tocopherol, eluting at 26 min, was monitored by an HP 1046A programmable fluorescence detector (HP Company, Wilmington, DE) at 290 nm excitation and 340 nm emission wavelengths. A set of α-tocopherol standard concentrations was analyzed to make a standard curve and to verify calibration.

Lipid Peroxidation Assays. Liver tissues were removed gently and stored at −70°C. Quantitative determination of lipid peroxides in liver was done using the LPO-CC K-ASSAY (Kamiya Biomedical Company, Seattle, WA). Phospholipids were extracted from approximately 0.2 g liver in chloroform/methanol (2:1, v/v). After centrifuging, the chloroform layer was mixed with 0.6 ml of saline to separate protein. The chloroform layer was evaporated in a steady flow of nitrogen gas, and lipid residues were dissolved with 100 μl of isopropanol. Test samples, standards, and controls were added in triplicate in the same 96-well microplate. In principle, in the presence of hemoglobin, lipid hydroperoxides are reduced to hydroxyl derivatives (lipid alcohol) and the MCDP (10-*N*-methylcarbamoyl-3,7-dimethylamino-10*H*-phenothiazine) chromogen is oxidatively cleaved to form methylene blue in an equal molar reaction. Lipid peroxidase are quantitated by colorimetrically measuring the methylene blue at 675 nm. Lipid peroxide (LPO) values were calculated by the following equation:

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LPO value in nmol/ml = (sample absorbance − blank absorbance) × 50.0 (absorbance of 50 nmol/ml standard −blank absorbance). LPO value was converted to nmol/g-wet tissue for illustration.

Preparation of Splenocytes. Mitogen-stimulated splenocytes were cultured in triplicate in 96-well microtiter plates as described previously (20). Briefly, spleens were removed and gently teased with cell strainers (Falcon #2340, Lincoln Park, NJ) in culture medium (RPMI 1640 containing 10% fetal bovine serum, 2 mmol/L glutamine, 1 × 10^5 units/l of penicillin and streptomycin), producing suspension of spleen cells. Red blood cells were lysed by the addition of a lysis buffer (0.16 mol/l ammonia chloride Tris buffer, pH 7.2) at 37°C for 3 min. Then, the cells were washed twice with culture medium. Cell viability exceeded 95% by trypan blue exclusion. Splenocyte concentrations were adjusted to 1 × 10^6 cells/100 μl. Splenocyte suspension was added 100 μl per well in triplicate on 96-well flat-bottom culture plates (Falcon #3072, Lincoln Park, NJ) with culture medium. Splenocytes in 96-well microtiter plates were incubated for 24 h after addition of lipopolysaccharide (1 × 10^{-2} g/L. Gibco, Grand Island, NY) for the induction of cytokines. After incubation, the plates were centrifuged for 10 min at 800*g* Supernatants were collected and stored at −70°C until analysis.

Enzyme-Linked Immunosorbent assay (ELISA). To measure the quantity of murine IL-6, TNF-α, and IL-1β in supernatants of splenocyte cultures, a specific solid-phase ELISA assay using the multiple antibody sandwich principle was used (20). Rat anti-murine IL-6, TNF-α, and IL-1β purified antibodies; rat anti-murine IL-6, TNF-α, and IL-1β biotinylated antibodies; and recombinant murine IL-6, TNF-α, and IL-1β were obtained from Pharmingen (Endogen, Woburn, MA). All tests were performed according to the manufacturer's instructions. All tests were performed in triplicate in 96-well microtiter plates.

Heart Contractile Function. Mice were tested for heart contractile function as described by Yang *et al.* (21). All mice were anesthetized with urethane in saline (1000 mg/kg, ip) and α–chloralose in propylene glycol (50 mg/kg ip). The mice were ventilated through a tracheostomy connected to a pressure-controlled respirator (RSP 1002, Kent, CT) at a rate of 120 times/min, FIO_2 of 1.0. The mice were placed on a surgical table maintained at 37.5°C. The external jugular vein was cannulated with a #23-gauge butterfly, and volume administration was limited to 300 μl of hetastarch (6% hetastarch in 0.9% saline) Abbott Laboratories, N. Chicago, IL). Precise volume management was adhered to as described in detail by Yang *et al.* (21). The apical portion of the heart and the interior vena cava were exposed through a substemal-transverse incision. A conductance catheter 1.4 Fr (Millar Corporation, Houston, TX) was inserted into the apex of the left ventricle, with the distal electrode in the aortic root and the proximal electrode in the LV apex. The parameters of contractility were expressed.

Statistical Analysis. Statistical analyses were performed by using the SAS 8.1 Windows (SAS Institute, Cary, NC) statistical package. All data were reported as mean ± SE. Variables were compared using a one-way analysis of variance (ANOVA), followed by a two-tailed Student's *t* test for comparison between two groups. Differences between two groups were considered significant at $P < 0.05$. Pearson correlation was conducted between tissue vitamin E, lipid peroxide, splenic cytokines and heart function parameters.

Results

Cardiac Vitamin E Level. Vitamin E level in the heart was significantly reduced 55% by 120-min SSCS exposure compared with sham control (Fig. 2A, dotted line). SSCS exposure of 120 min further decreased cardiac vitamin E by 47% compared with SSCS exposure of 60-min. However, there is no significant difference between 60-min SSCS exposure and sham control.

Hepatic Vitamin E Level. In contrast, the liver tissue vitamin E level decreased during SSCS exposure (Fig. 2A,

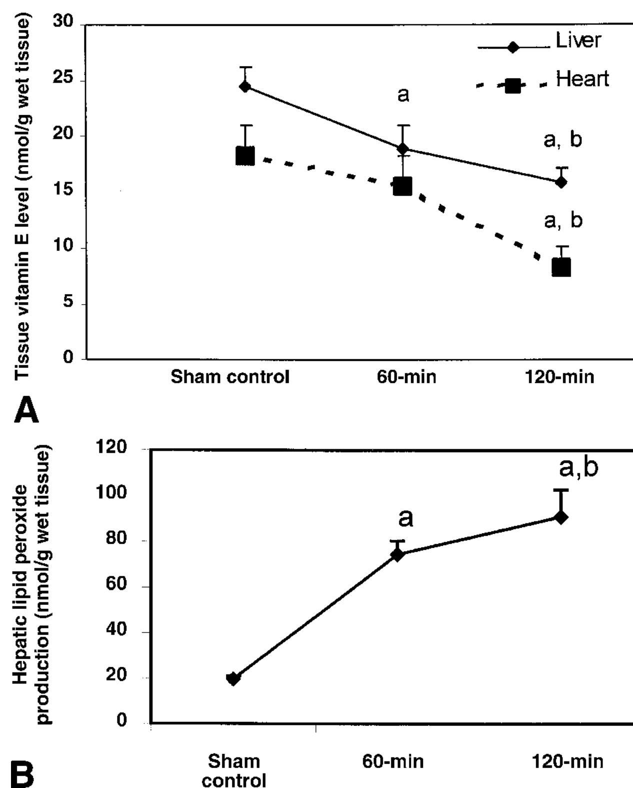

Figure 2. Effects of different doses of side-stream cigarette exposure on tissue vitamin E levels and hepatic lipid peroxide production. (A) Tissue vitamin E level was used as an indicator for oxidative stress. The assay was performed by HPLC. The solid line represents hepatic vitamin E; the dotted line represents cardiac vitamin E. (B) Lipid peroxide was determined with 0.2 g of mouse liver tissue. Phospholipids in the liver tissue were extracted by the $CHCl_3$/methanol 2:1, v/v) method. LPO was measured with a spectrophotometer at 675 nm. Values were mean ± SE of 10 to 12 mice in each group. Sham control = no smoke exposure; 60-min = 60-min smoke exposure; 120-min = 120-min smoke exposure. a, compared with sham control mice significantly different at $P < 0.05$; b, compared with 60-min smoking mice significantly different at $P < 0.05$.

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solid line). Vitamin E level in liver was significantly reduced 23% and 35%, respectively, by 60- and 120-min SSCS exposure ($P < 0.05$). Also, SSCS exposure of 120-min significantly reduced hepatic vitamin E level compared with that of 60-min ($P < 0.05$). SSCS exposure of 60-min significantly reduced hepatic vitamin E level than sham control ($P < 0.05$).

Hepatic Lipid Peroxides Production. The liver is a major organ for tissue lipid peroxidation. Hepatic lipid peroxides were significantly increased by 60- and 120-min SSCS exposure compared with sham controls ($P < 0.05$; Fig. 2B). SSCS exposure of 120-min produced higher hepatic lipid peroxides than that of 60 min ($P < 0.05$).

Splenocyte Pro-Inflammatory Cytokines Production. *In vitro* production of the pro-inflammatory cytokines was stimulated in splenocytes from SSCS-exposed mice. IL-1β production was 26.9 ± 5.8 pg/ml and 43.5 ± 4.6 pg/ml, respectively, in cells from mice given 60- and 120-min SSCS exposure compared with 20.9 ± 3.6 pg/ml in cells from sham control mice (Fig. 3A). IL-6 was 2126.9 ± 125.5 pg/ml and 2619.1 ± 122.8 pg/ml, respectively, in 60- and 120-min SSCS-exposed mice, whereas it was 1821.9 ± 291.1 pg/ml in sham control mice. TNF-α was 1034.8 ± 57.2 pg/ml and 1453.2 ± 208.6 pg/ml, respectively, in cells from mice given 60- and 120-min SSCS exposure, whereas it was 855.8 ± 68.5 pg/ml in sham control mice (Fig. 3B). In 120-min SSCS-exposed mice, IL-1β, IL-6, and TNF-α were more significantly increased than in both sham control and 60-min SSCS-exposed mice ($P < 0.05$). TNF-α in 60-min SSCS-exposed mice was more significantly increased than in sham control mice ($P < 0.05$) whereas IL-1β and IL-6 in 60-min SSCS-exposed mice did not show significant difference compared to sham control mice (Fig. 3, A and B).

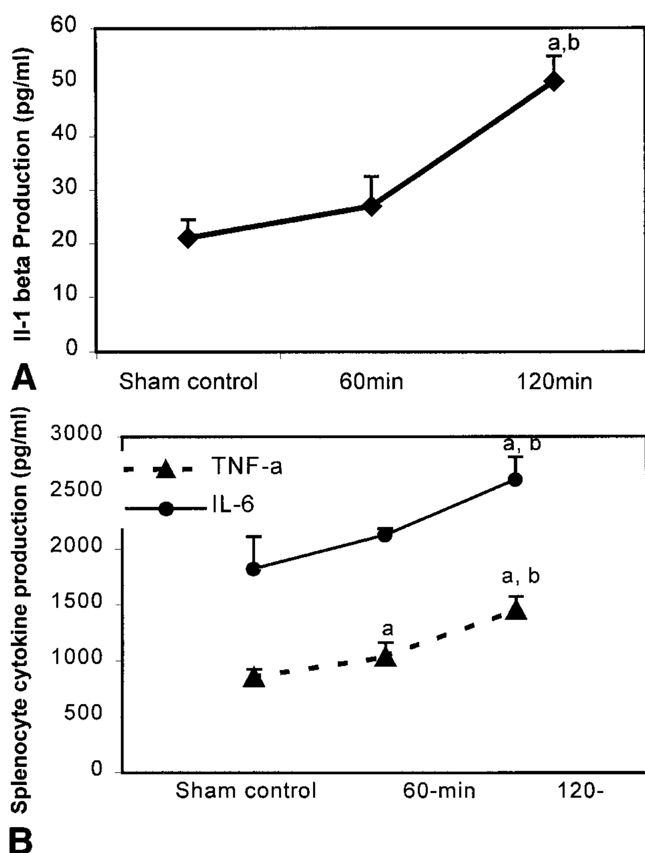

Figure 3. *In vitro* splenocyte IL-1β, TNF-α, and IL-6 production upon different dose of side-stream cigarette smoke exposure. The assay was conducted by ELISA. Values were mean ± SE of 12 mice in each group. Sham control = no smoke exposure; 60-min = 60-min smoke exposure; 120-min = 120-min smoke exposure; a, compared with sham control mice significantly different at $P < 0.05$; b, compared with 60-min smoke exposure mice significantly different at $P < 0.05$. (A) IL-1β; (B) Solid line represents IL-6; dotted line represents TNF-α.

Heart Contractile Function. The left ventricle functional parameters, cardiac output index, stroke volume index, preload recruitable stroke work, and derivative of change in systolic pressure over time, showed no significant difference in 60- and 120-min SSCS-exposed mice compared with sham controls (data not shown). However, stroke volume (SV) was significantly reduced in 120-min SSCS-exposed mice compared with sham controls ($P < 0.05$; Table I). Vascular impedance (arterial elastance, Ea) of smoke-exposed mice was significantly increased in those exposed to 120-min/d for 16 weeks ($P < 0.05$; Table I).

Correlation Between Tissue α-Tocopherol, Lipid Peroxide, Splenic Cytokines, and Heart Function Parameters. Results from Table II show that the cardiac α-tocopherol level has a strong correlation with stroke volume ($P < 0.05$); splenic IL-1β has a strong negative correlation with stroke volume ($P < 0.05$); and splenic TNF-α has a very strong negative correlation with stroke volume ($P = 0.01$).

Discussion

Cigarette smoke affects various systems, including immune and cardiovascular systems. Cigarette smoke contains vast amounts of both carbon- and oxygen-centered free radicals, directly or indirectly initiating lipid peroxidation. Lipid-soluble vitamin E (α-tocopherol), as an integral part of cell membranes, acts as cytosolic antioxidant, scavenges the free radicals that promote peroxidative chain reactions (22). Thus, reduced hepatic and cardiac vitamin E levels indicate that vitamin E is involved in antioxidant defense against oxidative stress. A decrease in antioxidant defense in myocytes can promote oxidative stress. Dhalla and Singal found that the production of superoxide in cardiac tissue is increased as a result of the reduced antioxidant reserve in heart failure (23). However, the liver is a major organ subjected to the free radical attack. Hepatic lipid peroxide production is higher in the SSCS exposure. It has been shown that antioxidant depletion would initiate and accelerate cytotoxic or mutagenic events during excessive production of ROS (24).

Increased pro-inflammatory cytokine production was associated with increased SSCS exposure. ROS can activate redox-sensitive transcription factors, nuclear factor-kappa B, and activator protein-1, activating the genes of pro-

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Table I. Left Ventricular Contractile Parameters[a]

	Units	Sham control	60 min	120 min	*P* value
Numbers of mice		8	9	11	0 vs 120
Age of mice (months)		9, 10	9, 10	9, 10	
CO	ml/min	6.19 ± 0.6	5.63 ± 0.6	4.77 ± 0.52	
SV	μl	11.91 ± 0.98	11.04 ± 0.99	9.15 ± 0.73*	0.042
Pes	mm Hg	73.95 ± 3.33	72.43 ± 3.95	71.39 ± 1.83	
Ped	mm Hg	2.79 ± 0.45	2.38 ± 0.16	2.28 ± 0.21	
Ea	mm Hg/μl	6.54 ± 0.59	6.39 ± 0.57	8.53 ± 0.72*	0.047

[a] Values were expressed as mean ± SE. Sham control = no smoke exposure; 60 min = 60-min smoke exposure; 120 min = 120-min smoke exposure.
* Compared with nonsmoke exposure significantly different at $P < 0.05$.
CO, cardiac output = heart rate × stroke volume; SV, stroke volume = end diastolic volume − end systolic volume; Pes, end-systolic pressure; Ped, end-diastolic pressure; Ea, effective arterial elastance = End-systolic pressure/stroke volume.

Table II. Correlation Analysis

Parameters	Pearson correlation	*P* value
Cardiac vitamin E versus Ea	−0.95	0.10
Hepatic vitamin E versus Ea	−0.73	0.24
Hepatic lipid peroxide versus Ea	0.63	0.28
IL-1β versus Ea	0.97	0.08
TNF-α versus Ea	0.94	0.12
IL-6 versus Ea	0.83	0.18
SV versus Ea	0.92	0.13
Cardiac vitamin E versus SV	0.99	0.03*
Hepatic vitamin E versus SV	0.94	0.11
Hepatic lipid peroxide versus SV	−0.88	0.16
IL-1β versus SV	−0.99	0.04*
TNF-α versus SV	−0.99	0.01**
IL-6 versus SV	−0.98	0.06

* Cardiac α-tocopherol level has a strong correlation with stroke volume ($P < 0.05$); Splenic IL-1β has a strong negative correlation with stroke volume ($P < 0.05$).
** Splenic TNF-α has a very strong negative correlation with stroke volume ($P = 0.01$).

inflammatory mediators TNF-α, IL-1β, and IL-6 (25, 26). We previously observed that 13-month-old C57BL/6 female mice exposed to SSCS for 30-min per day, 5 days a week for 16 weeks exhibited increased IL-6 production in splenocytes (19). In the present study, the dosage of 120-min per day is sufficient to significantly increase all three pro-inflammatory cytokines IL-1β, IL-6, and TNF-α. These pro-inflammatory cytokines are closely linked with pathology in a wide range of diseases and conditions that have an inflammatory basis.

The heart contractile function data showed SV decreased significantly upon 120-min SSCS exposure. SV is determined by three parameters: preload, afterload, and inotropic effects. The correlation analysis indicated that systemic elevated TNF-α and IL-1β was associated with decreased SV. TNF-α and IL-1β can both depress myocardial function (27). Oral and co-workers demonstrated that TNF-α induced an early depression mediated by sphingosine (28). Sphingosine is rapidly produced via sphingomyelin degradation after the intermediate formation of ceramide upon cardiomyocyte exposure to TNF-α (29, 30). Sphingosine decreases calcium transients by blocking the ryanodine receptor, thereby impeding calcium-induced calcium release from the sarcoplasmic reticulum. A common pathway that TNF-α and IL-1β can induce myocardial functional depression is to activate inducible nitric oxide synthase and induce nitric oxide-dependent myofilament desensitization to calcium (27). IL-1β, however, can also directly inhibit aerobic energy metabolism and myocardial contractility via a direct inhibition of the mitochondria enzyme activities (31). Cardiac vitamin E content was significantly correlated with SV, as indicated in Table II. The beneficial effects if vitamin E in protecting from depression of left ventricular function may be occurring though the reduction of oxidative stress (32).

The heart function data suggest that 120-min per day of SSCS affects arterial elastance (Ea). Ea is defined as end-systolic pressure over stroke volume; it is the diameter of afterload. The augmentation of arterial elastance, reflecting the resistance of peripheral arteries, suggests that SSCS exposure could induce cellular alterations in the peripheral arteries. If Ea is high, the resistance is high, and the heart needs to do more work to pump the same amount of blood. Vascular impedance of smoke-exposed mice was significantly increased during 120-min SSCS exposure. Cigarette smoking is a highly significant risk factor for development of peripheral arterial occlusive disease (33). SSCS impaired endothelium-dependent relaxation of isolated rabbit arteries (34). Endothelial dysfunction of the peripheral vasculature contributes to the elevated peripheral vascular resistance in patients with heart failure (35). Free radical-mediated oxidative damage can also take place in lipoproteins. Oxidized lipoprotein is an important factor in predisposing SSCS-exposed mice to uptake foam cells in the vascular wall. These events have been correlated with the initiation and progression of coronary heart disease. The most often proposed mechanism for endothelial dysfunction related to ROS activity in heart failure is the enhanced biodegradation of nitric oxide by ROS. Elevated levels of ROS deplete bioavailable nitric oxide and exacerbate local oxidant stress by reacting directly with nitric oxide to form peroxynitrite, which, in turn, imparts further oxidative injury to the endothelium (36). Free radicals and oxidized lipoproteins that are

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oxidized by free radicals derived from cigarette smoke can inhibit endothelium-dependent vasodilation; the antioxidant diet reversed the impairment of the endothelial function (37, 38). The precise component of cigarette smoke that contributes to the pathogenesis of vascular disorder is unclear. Nicotine is a major component besides reactive oxygen species. We cannot exclude the effect of nicotine on vascular dilation at this point. Mayhan and Patel found that nicotine contributed to endothelium-dependent arteriolar dilatation *in vivo* (39).

Taken together, SSCS exposure at 120-min per day induced systemic inflammatory responses. SSCS exposure at both 60-min and 120-min per day accentuated systemic lipid peroxidation with depletion of cardiac and hepatic antioxidant vitamin E levels. Finally, SSCS exposure at 120-min per day decreased stroke volume and increased vascular resistance. Systemic IL-1β and TNF-α production are responsible for heart contractile dysfunction. Free radicals may be responsible for the progression to heart contractile dysfunction induced, in part, by SSCS.

There are some limitations in the present study. Oxidative stress should be not only observed in major organs (such as heart and liver), but also in the vessel wall. Oxidized lipoprotein may contribute to the vascular functional changes. SSCS is very complex in chemical composition. Exploring the defined mechanism of vascular dysfunction in mice is warranted. A more precise quantification of the smoking exposure dose in mice needs to be determined so that it can be comparable to human exposure.

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1. Stedman RL. The chemical composition of tobacco and tobacco smoke. Chemistry Rev **68:**153–207, 1968.
2. Church DF, Pryor WA. Free-radical chemistry of cigarette smoke and its toxicological implications. Environ Health Perspect **64:**111–126, 1985.
3. Evstigneeva RP, Volkov IM, Chudinova VV. Vitamin E as a universal antioxidant and stabilizer of biological membranes. Membrane Cell Biol **12:**151–172, 1998.
4. Daube H, Scherer G, Riedel K, Ruppert T, Tricker AR, Rosenbaum P, Adlkofer F. DNA adducts in human placenta in relation to tobacco smoke exposure and plasma antioxidant status. J Cancer Res Clin Oncol **123:**141–151, 1997.
5. Handelman GJ, Packer L, Cross CE. Destruction of tocopherols, carotenoids, and retinol in human plasma by cigarette smoke. Am J Clin Nutr **63:**559–565, 1996.
6. Azzawi M, Hasleton P. Tumour necrosis factor alpha and the cardiovascular system: Its role in cardiac allograft rejection and heart disease. Cardiovasc Res **43:**850–859, 1999.
7. Ikonomidis I, Andreotti F, Economou E, Stefanadis C, Toutouzas P, Nihoyannopoulos P. Increased proinflammatory cytokines in patients with chronic stable angina and their reduction by aspirin. Circulation **100:**793–798, 1999.
8. Dinarello CA. Interleukin 1 and interleukin 1 receptor antagonism. Blood **77:**1627–1652, 1991.
9. Kishimoto T. The biology of interleukin-6. Blood **71:**1–10, 1989.
10. Deswal A, Peterson NJ, Feldman AM, Young JB, White BG, Mann DL. Cytokines and cytokine receptors in advanced heart failure: An analysis of the cytokine database from the Vesnarinone Trial (VEST). Circulation **103:**2055–2059, 2001.
11. Adamopoulos S, Parissis J, Kroupis C, Georgiadis M, Karatzas D, Karavolias G, Koniavitou K, Coats AJ, Kremastinos DT. Physical training reduces peripheral markers of inflammation in patients with chronic heart failure. Eur Heart J **22:**791–797, 2001.
12. Oyama J, Shimokawa H, Morita S, Yasui H, Takeshita A. Elevated interleukin-1beta in pericardial fluid of patients with ischemic heart disease. Coron Artery Dis **12:**567–571, 2001.
13. Office of Environmental Health Hazard Assessment CEPA. Health Effects of Exposure to Environmental Tobacco Smoke. Final Draft for Scientific, Public and SRP Review **8:**71, 1997.
14. Zhu BQ, Sun YP, Sievers RE, Glantz SA, Parmley WW, Wolfe CL. Exposure to environmental tobacco smoke increases myocardial infarct size in rats. Circulation **89:**1282–1290, 1994.
15. Zhu BQ, Sun YP, Sievers RE, Isenberg WM, Moorehead TJ, Parmley WW. Effects of etidronate and lovastatin on the regression of atherosclerosis in cholesterol-fed rabbits. Cardiology **85:**370–377, 1994.
16. Zhang Z, Araghiniknam M, Inserra P, Jiang S, Lee J, Chow S, Breceda V, Balagtas M, Witten M, Watson RR. Vitamin E supplementation prevents lung dysfunction and lipid peroxidation in nude mice exposed to side-stream cigarette smoke. Nutr Res **19:**75–84, 1999.
17. Pryor WA, Dooley MM, Church DF. Human alpha-1-proteinase inhibitor is inactivated by exposure to sidestream cigarette smoke. Toxicol Lett **28:**65–70, 1985.
18. Respiratory Health Effects of Passive Smoking. Lung Cancer and Other Disorders. Washington DC: Off. Health Environ. Assess., US EPA (EPA/600/6-90/006F), 1992.
19. Zhang J, Jiang S, Watson RR. Antioxidants prevent oxidation and immune dysfunction induced by side-stream cigarette smoke in Old Mice. Environ Health Perspect **109:**1007–1009, 2001.
20. Chouaib S, Welte K, Mertelsmann R, Dupont T. PGE2 acts at two distinct pathways of T lymphocyte activation: Inhibition of IL-2 production and down regulation of transferring expression. J Immunol **135:**1172–1179, 1985.
21. Yang B, Larson DF, Kelley R, Beischel J, Watson RR. Conductivity: An issue for the application of the conductance catheter system in mice. Cardiovasc Eng **5:**57–60, 2000.
22. Galli C, Socini A. Biological actions and possible uses of vitamin E. Acta. Vitaminologica et Enzymologica **4:**245–251, 1982.
23. Dhalla AK, Singal PK. Antioxidant changes in hypertrophied and failing guinea pig hearts. Am J Physiol **266:**H1280–H1285, 1994.
24. Pryor WA. Cigarette smoke radicals and the role of free radicals in chemical carcinogenicity. Environ Health Perspect **105:**875–882, 1997.
25. MacNee W. Oxidants/antioxidants and COPD. Chest **117:**303S–317S, 2000.
26. Grimble RF. Modification of inflammation aspects of immune function by nutrients. Nutr Res **18:**1297–1317, 1998.
27. Cain BS, Meldrum DR, Dinarello CA, Meng X, Joo KS, Banerjee A, Harken AH. Tumor necrosis factor-alpha and interleukin-1beta synergistically depress human myocardial function. Crit Care Med **27:**1309–1318, 1999.
28. Oral H, Dorn GW II, Mann DL. Sphingosine mediates the immediate negative inotropic effects of tumor necrosis factor-alpha in the adult mammalian cardiac myocyte. J Biol Chem **272:**4836–4842, 1997.
29. Meldrum DR. Tumor necrosis factor in the heart. Am J Physiol **274:**R577–R595, 1998.
30. Dressler KA, Mathias S, Kolesnick RN. Tumor necrosis factor-alpha activates the sphingomyelin signal transduction pathway in a cell-free system. Science **255:**1715–1718, 1992.
31. Tatsumi T, Matoba S, Kawahara A, Keira N, Shiraishi J, Akashi K, Kobara M, Tanaka T, Katamura M, Nakagawa C, Ohta B, Shirayama T, Takeda K, Asayama J, Fliss H, Nakagawa M. Cytokine-induced nitric oxide production inhibits mitochondrial energy production and impairs contractile function in rat cardiac myocytes. J Am Coll Cardiol **35:**1338–1346, 2000.

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32. Sethi R, Takeda N, Nagano M, Dhalla NS. Beneficial effects of vitamin E treatment in acute myocardial infarction. J Cardiovasc Pharmacol Ther **5:**51–58, 2000.
33. Kannel WB, Shurtleff D. The Framingham Study: Cigarettes and the development of intermittent claudication. Geriatrics **28:**61–68, 1973.
34. Torok J, Gvozdjakova A, Kucharska J, Balazovjech I, Kysela S, Simko F, Gvozdjak J. Passive smoking impairs endothelium-dependent relaxation of isolated rabbit arteries. Physiol Res **49:**135–141, 2000.
35. Lopez Farre A, Casado S. Heart failure, redox alterations, and endothelial dysfunction. Hypertension **38:**1400–1405, 2001.
36. Moncada S, Higgs A. L-Arginine–nitric oxide pathway. N Engl J Med **329:**2002–2012, 1993.
37. Schwarzacher SP, Hutchison S, Chou TM, Sun YP, Zhu BQ, Chatterjee K, Glantz SA, Deedwania PC, Parmley WW, Sudhir K. Antioxidant diet preserves endothelium-dependent vasodilatation in resistance arteries of hypercholesterolemic rabbits exposed to environmental tobacco smoke. J Cardiovasc Pharmacol **31:**649–653, 1998.
38. Murohara T, Kugiyama K, Ohgushi M, Sugiyama S, Yasue H. Cigarette smoke extract contracts isolated porcine coronary arteries by superoxide anion-mediated degradation of EDRF. Am J Physiol **266:**H874–H880, 1994.
39. Mayhan WG, Patel KP. Effect of nicotine on endothelium-dependent arteriolar dilatation in vivo. Am J Physiol **272:**H2337–H2342, 1997.

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