Original investigation

Oxidative Stress in Youth and Adolescents With Elevated Body Mass Index Exposed to Secondhand Smoke

Judith A. Groner MD^{1,2}, Hong Huang MD, PhD³, Nicholas Eastman BS⁴, Luke Lewis BS⁵, Mandar S. Joshi PhD³, Brandon L. Schanbacher MS³, Lisa Nicholson PhD⁶, John A. Bauer PhD³

¹Nationwide Children's Hospital, Ohio State University College of Medicine, Columbus, OH; ²American Academy of Pediatrics, Julius B. Richmond Center of Excellence, Elk Grove Village, IL; ³Department of Pediatrics, University of Kentucky College of Medicine, Lexington, KY; ⁴College of Medicine, University of Toledo, Toledo, OH; ⁵College of Medicine, University of Cincinnati, Cincinnati, OH; ⁶Institute for Health Research and Policy, University of Illinois at Chicago, Chicago, IL

Corresponding Author: Judith A. Groner, MD, Nationwide Children's Hospital, Ohio State University College of Medicine, 700 Children's Drive, Columbus, OH 43205, USA. Telephone: 614-722-4957; Fax: 614-722-4966; E-mail: judith.groner@nationwidechildrens.org

Abstract

Introduction: Our objective was to investigate the relationships between secondhand smoke (SHS) exposure and oxidative stress in a group of youth and adolescents with elevated body mass index.

Methods: Participants in this cross sectional study were healthy nonsmoking youth and adolescents ages 9 to 18 years old. Three-quarters of the participants were either overweight or obese. SHS exposure was determined by survey and hair nicotine level. Markers of oxidation were total antioxidant capacity and protein malondialdehyde adducts (MDA).

Results: Ninety subjects were studied; adequate hair samples were available for 86. The mean hair nicotine level was 0.75 ng/mg, the median was 0.58 ng/mg and the range was 0.09–2.88 ng/mg. There was a significant relationship between MDA and the three survey questions regarding smoke exposure ([mother smokes, $r = 0.29$, $P = .006$], [smoker lives in the home, $r = 0.31$, $P = .004$], and [number of smokers in the home, $r = 0.36$, $P = .002$]). There was a significant positive relationship between log-hair nicotine and MDA (Pearson $r = 0.233$, $P = .031$), which remained significant after controlling for age, sex, race, and method of insurance. No relationship was found between log-hair nicotine and total antioxidant capacity. However, there was a significant relationship between number of smokers in the home ($r = 0.24$, $P = .042$) and total antioxidant capacity.

Conclusions: We have demonstrated a significant positive relationship hair nicotine level and MDA in a group of youth with a high proportion of overweight/obese subjects.

Implications: We have shown a significant relationship between objectively measured SHS exposure and one marker of oxidative stress in a sample of youth and adolescents with a high proportion of overweight/obese subjects, and who were nonsmokers with relatively low tobacco exposure. This finding remains significant after controlling for age, sex, race, and type of medical insurance. Since the cardiovascular effects of SHS exposure are related to oxidative stress, this finding adds to our knowledge that the sequence of deleterious effects of tobacco exposure on the cardiovascular system begins long before clinical disease is evident.

Background

Despite an encouraging overall decrease in secondhand smoke (SHS) exposure among children,¹ a subgroup of vulnerable children persists who are at risk for the multiple health consequences of this exposure.² National Health and Nutrition Examination Survey (NHANES) data from 2007 to 2008 suggests that half of 3–19 years old had detectable levels of a nicotine metabolite, cotinine, in their blood.³ Smoking prevalence varies inversely with socioeconomic status with exposure rates in low income communities as high as 79%.⁴

Adult cardiovascular disease is now considered to be a progressive inflammatory disease initiated in childhood.^{5–7} Exposure to SHS increases the risk of cardiovascular disease to about 30% in nonsmoking adults.^{8–10} However, their exposure to nicotine from tobacco smoke is less than 1% of the exposure of an active smoker of 20 cigarettes per day.⁸ It has been hypothesized that the most likely cause of elevated cardiovascular disease risk among SHS-exposed nonsmokers is oxidant gas exposure^{11,12} from the burning cigarette. Free radicals, oxides of nitrogen, metabolites of polycyclic aromatic hydrocarbons, metals such as cadmium, acrolein (which induces the formation of free radicals) and particulate matter are produced by many burning SHS constituents.¹³ With this exposure, endogenous antioxidants may be overwhelmed by reactive oxygen species and free radicals.¹⁴ Oxidative stress can cause damage to DNA, proteins, and lipids.^{15,16} The exposure to oxidants can be significant with SHS, leading to inflammation and subsequent endothelial dysfunction, with eventual cardiovascular disease ensuing.¹¹

There is limited evidence that exposure to smoke among nonsmokers is associated with biomarkers of oxidative stress. Megson and colleagues demonstrated this relationship among adults hospitalized with myocardial infarctions,¹⁷ showing increased levels of protein carbonyl and protein malondialdehyde adducts (MDA) among smoke exposed nonsmoking adults. The strongest relationship between smoke exposure and markers of oxidative stress was with malondialdehyde. Aycicek¹⁸ demonstrated higher antioxidant levels (total antioxidant capacity) and other measures, among young infants ages 6–28 weeks exposed to five cigarettes per day. School children (ages 9–13) who were exposed to at least 10 cigarettes per day were shown to have higher oxidative stress index than unexposed children.¹⁹ These studies relied on self-report for SHS exposure, which is frequently misclassified. Block and colleagues²⁰ examined smoking status by serum cotinine levels, and found a significant independent relationship between MDA and smoking and smoke exposure. Bono and colleagues^{21,22} found a direct correlation between 15-F_{2t} isoprostane, a marker of oxidation, and SHS exposure (measured by serum cotinine) and with urbanization in a group of Italian teenagers.

Elevated body mass index (BMI) has been associated with increased oxidative stress in adults.²⁰ Research on childhood obesity and inflammation is robust; data on obesity and oxidative stress in childhood obesity is emerging.²³ This relationship is most likely a result of chronic low grade inflammation induced by obesity.^{23,24} These processes appear to be related to abnormalities in the adipocytes and the dysregulation of adipocytokines, which are secreted by adipose tissue.²⁴ Obesity induced oxidative stress in childhood may lead to decreased nitric oxide availability²³ and subsequently to endothelial dysfunction, a precursor of adult cardiovascular disease. Thus while the “insults” of SHS exposure and obesity are different, the end pathways and resultant risks for adult disease are similar.

Our objective was to investigate relationships between SHS exposure and two established markers of oxidative stress. Our

hypothesis was that there is a relationship between an objective marker of smoke exposure (hair nicotine levels) and oxidative stress in a sample of youth and adolescent with a high proportion of overweight/obese subjects and varying exposure to SHS.

Methods

Participants and Recruitment

Participants in this cross sectional study were youth and adolescents ages 9 to 18 years old. They were recruited via convenience sampling through recruiting in Nationwide Children’s Hospital (NCH; Columbus, OH) Primary Care Network, the NCH Center for Healthy Weight and Nutrition and via advertising in the NCH internal hospital e-mail system. The Primary Care Network serves low-income, urban children in Columbus, Ohio and the Center for Healthy Weight and Nutrition is a multidisciplinary referral center for obese children and adolescents. The protocol was approved by the NCH IRB; parents provided informed consent and participants provided assent. We oversampled obese youth and teens from a clinic which served this population because obesity is an important variable of interest for our measures and outcomes. Relationships between elevated BMI and some markers of oxidative stress have been previously described.^{20,23,25} The inclusion criteria were healthy children and adolescents (ages 9–18), both exposed and unexposed to SHS by parental report. The exclusion criteria were presence of one or more of the following: active smoker (defined as one puff of a cigarette or more in the past 7 days), acute febrile illness or other active infections, congenital heart disease, diabetes (Type 1 or 2), (elevated fasting glucose [>100 mg/dl]), use of oral or inhaled steroids within 1 month of testing family history 1 week of testing, caffeine within 2 days of testing, and not having enough hair for hair sampling for nicotine.

Study Procedures

The study was introduced to most subjects (except those recruited via email advertising) at a clinic visit. Subjects were subsequently scheduled for testing at a research site in the morning between 8:00 AM to 10:00 AM, after overnight fasting. The protocol was carried out as follows: (1) Study procedures were described with parental informed consent and youth/teen obtained by trained IRB-approved personnel, (2) anthropomorphic measurements obtained, (3) followed by structured interview with youth and teen, and parent to obtain demographics and SHS exposure history, (4) hair sample obtained, and (5) the blood sample of approximately 7 ml was collected for measures of oxidative stress. After serum sample collection all assays were stored on ice and used within 12 hours of collection. In all cases, replicate samples and known standards of authentic analyte were included to verify the reliability of the assay conditions.

Clinical Measures

Trained research staff obtained height and weight from each subject using a Tanita BWB800 scale and Seca stadiometer. Weights were recorded to the nearest 0.1 kilogram. Heights were measured to the nearest 0.5 cm. BMI was determined according the CDC guidelines ($\text{BMI} = \text{weight [kg]} / \text{height [m]}^2$), and percentile norms to define normal weight, overweight, and obese were from CDC guidelines (www.cdc.gov/healthyweight/assessing/bmi/childrens_bmi/about_childrens_bmi.html).

SHS exposure was assessed by questionnaire and hair nicotine because a secondary aim of the study, not reported here, was to determine relationships between parental report of children’s SHS

exposure and an objective measure of this exposure. Exposure to tobacco smoke was defined as living in a home with a smoker regardless of whether the smoker claims “indoor” or “outdoor” smoking. A smoker was defined as an individual who has smoked at least one cigarette per day during the previous 7 days. We asked the following survey questions (1) “is the mother of the child a smoker? Yes, No,” (2) “does your child live with someone who smokes? Yes, No,” and (3) if “yes” to the previous item, “please tell us the number of number of people the child lives with who are smokers (number).”

Hair Nicotine Measurement

Hair nicotine was used as a biological marker of SHS exposure because this measure provides a long-term evaluation of this exposure, since the nicotine is incorporated in the growing hair shaft over several months.²⁶ Additionally, samples are easy to obtain, handle, and store. Approximately 20–40 shafts of hair, 2–3 cm in length were cut at the root at the occipital area. Hairs were stored and later sent for assay at established contract research facility (Specialist Biochemistry Laboratory, Wellington Hospital, Wellington, New Zealand). The hair nicotine assay involves washing the hair sample prior to analysis, and therefore is designed to measure inhaled nicotine, and not ambient nicotine which has adhered to hair.²⁶ The method is reversed-phase high-performance liquid chromatography with electrochemical detection as described previously.²⁶ All samples were run in duplicates. Hair nicotine level is expressed as ng/mg of hair. The lowest sensitivity of the assay is 0.01 ng/mg hair when 2 mg of hair is used.

Measures of Oxidation

Total antioxidant capacity is a nonspecific measure of “antioxidant reserve” in plasma. The role of the antioxidant network is to scavenge free radicals and other oxidizing species. Measurement of total antioxidant capacity is an indication of the overall capability to counteract reactive oxygen species and combat oxidative stress related diseases and has been shown to be changed in many settings of chronic inflammation/oxidation.^{27,28} We would expect an inverse relationship between increased oxidation and total antioxidant capacity. Total plasma antioxidant capacity was measured with a commercial assay kit (Biovision, Cat#K274-100). Both small molecule and protein antioxidants were measured, and results were presented as mM Trolox equivalent.

Protein MDA is a measure of lipid peroxidation in serum.²⁹ Lipid peroxidation well-established mechanism of cellular injury and is used as indicator of oxidative stress in cells and tissues. Malondialdehyde is a highly reactive three-carbon dialdehyde byproduct of fatty acid peroxidation and arachidonic acid metabolism. It readily combines with several functional groups on molecules including proteins. Plasma malondialdehyde–protein adducts were measured by ELISA (Cell Biolabs, INC, San Diego, CA, Cat#STA-332). Total protein concentration in plasma was measured by Bradford Reagent. Each sample was diluted to 10 µg protein/mL in 1× PBS. One-hundred microliter of the diluted samples was assayed with pre-coated ELISA plates in triplicates. The results was calculated from MDA-BSA standards and reported as pmol malondialdehyde per mg protein. The analytical sensitivity of this assay is less than 2 pmol/mg. Intra and inter-assay variations are less than 5% and 7%, respectively.

Statistical Analysis

All analyses were performed using statistical software STATA S.E. Version 13.1 (StataCorp. 2013. *Stata Statistical Software: Release 13*. College Station, TX: StataCorp LP). Hair samples were missing or

quantity not sufficient for four subjects, with 86 subjects remaining in the analysis. The independent variable hair nicotine was non-normally distributed, and was therefore was log transformed. The log-transformed measure was used in all analyses. Malondialdehyde and total antioxidant capacity were both found to be normally distributed and were therefore used in their original forms. Key covariates included age (continuous), Medicaid status (1 = yes; 0 = no), sex (1 = female, 0 = male); and a multi-categorical measure of race/ethnicity (non-Hispanic whites were the omitted reference, non-Hispanic blacks, Hispanics, and multi-race/other race). Pearson correlations were first used to examine the associations among hair nicotine, malondialdehyde, total antioxidant capacity, and all covariates. Supplemental Pearson correlation analyses also examined associations among log-hair nicotine, a continuous measure of BMI, malondialdehyde, and total antioxidant capacity to check for potential confounding. *t* tests were used to test for mean differences in continuous measures by whether or not there was smoker in the home; chi-square tests were used to evaluate associations with categorical variables. Multivariate ordinary least squares regression analysis was performed to examine the association between log-hair nicotine and the outcomes malondialdehyde and total antioxidant capacity. All key covariates listed above were included in the multivariate models.

Based on a series of power analyses it was determined that approximately 80 subjects were needed to yield a power of 0.8 in testing hypotheses concerning both the continuous research outcome (MDA and total antioxidant capacity) variable and the categorical research variables. The nominal alpha level is 0.05.

Results

Demographics

Eighty-six subjects, with slightly more females (54.4%) were studied (Table 1).

Almost half of the subjects were insured by private insurance (48.9%); 42.2% were insured by Medicaid. The racial composition of the population was: African American (21%), white (46.7%), multiracial (22.2%), with the rest either not answering this questions (6.7%) or endorsing Asian (3.3%); with 13 (15%) endorsing Hispanic ethnicity. Since one of our recruitment sites was a pediatric clinic which served low income children and teens where smoking prevalence among parents is high, it is not surprising that subjects from lower income and educational backgrounds were highly represented. Slightly less than half (46.7%) of subjects reporting a family income have less than \$40 000 year. The prevalence of obesity in this group was high; 57 (66.3%) were obese, 8 (9.3%) were overweight, and 24.4% normal weight by CDC standards for age and sex.

Higher Hair Nicotine Levels in SHS-Exposed Subjects

Twenty-one subjects (24.4%) lived in a home with a mother who smoked and 33 (38.3%) reported that they lived in a home with at least one smoker. The overall mean hair nicotine level was 0.75 ng/mg, the median was 0.58 ng/mg and the range was 0.09–2.88 ng/mg. Subjects who were exposed to at least one smoker in the home by report had higher mean hair nicotine levels than children who were not exposed, 1.23 (*SD* 0.64) versus 0.47 (*SD* 0.23), *P* < .05. Log-hair nicotine was significantly and positively correlated with maternal smoking (*r* = 0.47, *P* = .0001), at least one smoker in the home (*r* = 0.61, *P* = .0001), and number of smokers in the home (*r* = 0.52, *P* = .0001; Table 2).

Table 1. Description of Population by Exposure to Secondhand Smoke

	Full sample (<i>n</i> = 86)		Unexposed (<i>n</i> = 52)		Exposed (<i>n</i> = 33)	
	<i>N</i> (%)		<i>N</i> (%)		<i>N</i> (%)	
Gender						
Male	38 (44.2)		24 (46.2)		13 (39.4)	
Female	48 (55.8)		28 (53.9)		20 (60.6)	
Race						
African American	19 (22.1)		15 (28.6)		4 (12.1)	
Hispanic	13 (15.1)		10 (19.2)		3 (9.1)	
Non-Hispanic white	40 (46.5)		25 (48.1)		15 (45.5)	
Multiracial or other race	14 (16.3)		2 (3.60) ^b		11 (33.3)	
Medicaid	37 (43.0)		18 (34.6) ^b		19 (57.6)	
Overweight or Obese	65 (75.6)		38 (73.1)		27 (81.8)	
	Mean (range)	<i>SD</i>	Mean (range)	<i>SD</i>	Mean (range)	<i>SD</i>
Age (y)	13.4(9–19)	2.6	12.90 (9–18) ^a	2.62	14.13 (9–19)	2.36
Malondialdehyde adducts (pmol MDA/mg proteins)	11.68 (3.86–22.77)	4.12	10.73 (4.86–19.29) ^a	3.04	13.30 (3.86–24.77)	5.07
Total antioxidant capacity (nmoles Trolox equivalent)	5.90 (3.48–8.81)	1.15	6.03 (3.48–8.32)	1.20	5.74 (4.50–8.81)	1.07
Hair nicotine (ng/mg hair)	0.75 (0.09–2.89)	0.57	0.47 (0.09–1.02) ^a	0.23	1.23 (0.09–2.89)	0.64

Unexposed is defined as having no smokers in the home; exposed is defined as having one or more smokers in the home. There is one missing value on smokers in the home.

^aSignificantly different by *t* test, *P* < .05 two-tailed.

^bSignificantly different by chi-square test, *P* < .05.

Table 2. Pearson Correlations Among Secondhand Smoke Variables, Malondialdehyde Adducts (MDA), and Total Antioxidant Capacity (TAC)

	Log-hair nicotine	Mom smokes	Smoker in the home	Number of smokers in the home
Log-hair nicotine	1.00	0.47 (<i>P</i> = .0001)	0.61 (<i>P</i> = .0001)	0.52 (<i>P</i> = .0001)
MDA	0.23 (<i>P</i> = .031)	0.29 (<i>P</i> = .006)	0.31 (<i>P</i> = .004)	0.36 (<i>P</i> = .002)
TAC	−0.05 (<i>P</i> = .650)	0.07 (<i>P</i> = .534)	−0.12 (<i>P</i> = .261)	−0.24 (<i>P</i> = .042)

SHS Exposure and Markers of Oxidative Stress

There was a significant correlation between MDA and the three survey questions regarding smoke exposure (Table 2; [mother smokes, $r = 0.29$, $P = .006$], [smoker lives in the home, $r = 0.31$, $P = .004$], and [number of smokers in the home, $r = 0.36$, $P = .002$]). The first two questions did not show a correlation with total antioxidant capacity, but the last one showed a significant inverse correlation (number of smokers in the home, $r = 0.24$, $P = .042$).

Relationships With BMI

There was no relationship between BMI and MDA or total antioxidant capacity. There were no significant differences in mean values of any predictor or confounder variables between overweight/obese subjects versus those of normal weight.

Hair Nicotine Levels and MDA

There was a significant positive relationship between log-hair nicotine and MDA (Pearson $r = 0.233$, $P = .031$). There was no relationship between log-hair nicotine and total antioxidant capacity ($r = -0.05$, $P = .650$). The relationship between log-hair nicotine and MDA remained significant even after controlling for age, sex, race, and method of insurance (Medicaid vs. private pay; Table 3). The final adjusted model has a coefficient 1.36 and standard error 0.65, ($P = .040$). A one-unit increase in log-hair nicotine was associated

Table 3. Relationship Between Malondialdehyde Adducts (MDA) and Log-Hair Nicotine, Adjusted for Age, Sex, Race/Ethnicity, and Method of Payment

	Unstandardized coefficient <i>b</i>	Standard error <i>SE</i>
MDA		
Log-hair nicotine	1.36*	0.65
Sex	0.96	0.91
Age	0.13	0.19
Race/ethnicity		
Hispanic	1.01	1.40
African American	2.33	1.16
Multiracial	0.67	1.36
Medicaid insurance	−0.45	1.01
Cons	9.51	2.55

**P* < .05 statistically significant.

with a 1.36 unit increase in malondialdehyde. The adjusted R^2 value of the model is 0.0463; therefore log-hair nicotine and the additional covariates explains roughly 5% of the variance in malondialdehyde.

Discussion

Our results confirm our hypothesis that there was a significant relationship between an objective marker of SHS exposure (hair

nicotine) and MDA. Additionally, we found a significant positive correlation between MDA and three survey questions regarding the subjects' smoke exposure, and a significant positive correlation between one of those survey items (number of smokers in the home) and total antioxidant capacity.

This work confirms Block's findings regarding the relationship between smoking and elevated MDA.²⁰ Our data specifically support this relationship with SHS per se, as distinct from active smoking, and among a younger population than Block studied. Furthermore, the relationship remains after adjusting for sex, age, race, and method of payment. We did not find a relationship between overweight/obesity and either marker oxidative stress, unlike the findings of Block²⁰ and others^{23,24} where such relationships were found.

Although we observed a significant relationship between smoke exposure (hair nicotine and all three survey questions) and plasma malondialdehyde, total antioxidant capacity was only related to one survey question (number of smokers in the home). Total antioxidant capacity determines the overall antioxidant defense in the plasma. Thus, it accounts for various enzymatic antioxidant defense systems such as catalase, superoxide dismutase, glutathione peroxidase as well as nonenzymatic components such as vitamin C and Vitamin E. On the other hand, MDA are a marker of lipid peroxidation. Lipid peroxidation is considered to be a consequence of increased plasma oxidants. One of the possible explanations for our findings is that total antioxidant capacity gives us an overall picture of antioxidant status. Thus, it is likely that exposure to SHS may adversely affect one of antioxidant systems, but there is compensation from another component of the enzymatic or nonenzymatic antioxidant components. In our present study, we did not collect any information related to nutrition and dietary intake, which are known to affect overall oxidant status as well as plasma total antioxidant capacity. These unmeasured factors may have contributed to the lack of a consistent relationship (like that observed with MDA) between total antioxidant exposure and SHS exposure.

One potential limitation of this study is that we did not interview adolescents separately regarding their own personal tobacco use, so it is possible that there were active smokers who did not reveal their smoking status in front of their parents during the research interview. This could skew the data in the sense that we would misattribute the results of SHS exposure to what would actually be active smoking. Indeed, we did find that unexposed subjects were significantly younger than exposed subjects (Table 1). However, this observation is unlikely to be due to subjects who were truly active smokers, because the mean hair nicotine of exposed subjects was 1.23, which is less than the lower cut point hair nicotine observed in active smokers (2.77).³⁰

Although this study is a cross sectional study that can only describe a relationship, it is notable that even within this relatively small sample size, the relationship between hair nicotine and malondialdehyde was found and remained after controlling for several variables. Although we oversampled obese youth and teenagers, we did not find a relationship between either markers of oxidation and BMI. However, because our population was so skewed to elevated BMI, the generalizability of our findings to a nonobese population is limited. We examined a limited set of covariates/potential confounders (age, race, Hispanic ethnicity, method of insurance, gender). It is possible that unmeasured confounders such as quality of diet and intake of antioxidants, exercise, exposure to smoke in utero, and exposure to air pollution, among others, contributed to our findings.

Of note is that the subjects in our sample had relatively low levels of smoke exposure as measured by hair nicotine. For example, among bar and restaurant workers with no smoking ban, median hair nicotine was 1.7 ng/mg among nonsmokers compared to 0.58 ng/mg in our population.³¹ Despite the relatively low level of hair nicotine in our sample, we found a measureable and statistically significant relationship between hair nicotine and MDA. The clinical significance of the relationship between SHS exposure and increasing MDA cannot be precisely defined, as this marker of oxidation is not used in clinical medicine for diagnostic or therapeutic decision making. SHS exposure is one contributing factor for oxidative stress starting in youth, resulting in a lifetime burden of preventable cardiovascular disease. While smoke exposure only accounted for a small amount of the variance in malondialdehyde, such exposure is a finite and modifiable risk factor. As young people who are exposed to SHS have increased risk of becoming active smokers themselves,³² they have the potential in adulthood to have multiple accumulating risk factors for developing cardiovascular disease as adults.

Funding

The research was supported by National Institutes of Health R21ES0116883 (co-PIs JAG and JAB), the Flight Attendant Medical Research Institute 052392 (PI: JAG), and the American Academy of Pediatrics Julius B. Richmond Center of Excellence (co-PIs: JAG and JAB), which is funded by grants from the Flight Attendant Medical Research Institute and Legacy. The findings and conclusions are those of the authors and do not necessarily represent the official position of any of these institutions.

Declaration of Interests

None declared.

Acknowledgments

JAG conceptualized and designed the study, drafted the initial manuscript, revised the manuscript, and approved the final manuscript as submitted. HH carried out the investigations, performed laboratory assays, performed data analyses, wrote sections of the manuscript, reviewed and revised the manuscript, and approved the final manuscript as submitted. NE performed laboratory analyses, data analyses, reviewed, revised, and approved the final manuscript as submitted. LL performed laboratory assays, data analyses, reviewed and approved the final manuscript as submitted. BLS performed laboratory analyses and reviewed, revised, and approved the final manuscript as submitted. MSJ performed data analyses, wrote sections of the manuscript, reviewed and revised the manuscript and approved the final manuscript as submitted. LN performed data analyses, statistical modeling, wrote sections of the manuscript, reviewed and revised the manuscript and approved the final manuscript as submitted. JAB conceptualized the study, wrote sections of the manuscript, reviewed and revised the manuscript, and approved the final manuscript as submitted.

References

1. Soliman S, Pollack HA, Warner KE. Decrease in the prevalence of environmental tobacco smoke exposure in the home during the 1990s in families with children. *Am J Public Health*. 2004;94(2):314–320.
2. Quinto KB, Kit BK, Lukacs SL, Akinbami LJ. Environmental tobacco smoke exposure in children aged 3–19 years with and without asthma in the United States, 1999–2010. *NCHS Data Brief*. 2013;(126):1–8.
3. Centers for Disease C, Prevention. Vital signs: nonsmokers' exposure to secondhand smoke --- United States, 1999–2008. *MMWR Morb Mortal Wkly Rep*. 2010;59(35):1141–1146.

4. Cornelius MD, Goldschmidt L, Dempsey DA. Environmental tobacco smoke exposure in low-income 6-year-olds: parent report and urine cotinine measures. *Nicotine Tob Res.* 2003;5(3):333–339.
5. Berenson GS, Srinivasan SR, Hunter SM, et al. Risk factors in early life as predictors of adult heart disease: the Bogalusa Heart Study. *Am J Med Sci.* 1989;298(3):141–151.
6. Berenson GS, Wattigney WA, Bao W, Srinivasan SR, Radhakrishnamurthy B. Rationale to study the early natural history of heart disease: the Bogalusa Heart Study. *Am J Med Sci.* 1995;310(suppl 1):S22–S28.
7. He J, Whelton PK. Passive cigarette smoking increases risk of coronary heart disease. *Eur Hear J.* 1999;20(24):1764–1765. doi:10.1053/ehj.1999.1825.
8. Benowitz NL. Cigarette smoking and cardiovascular disease: pathophysiology and implications for treatment. *Prog Cardiovasc Dis.* 2003;46(1):91–111.
9. Glantz SA, Parmley WW. Passive smoking and heart disease. Mechanisms and risk. *JAMA.* 1995;273(13):1047–1053.
10. Puranik R, Celermajer DS. Smoking and endothelial function. *Prog Cardiovasc Dis.* 2003;45(6):443–458. doi:10.1053/pcad.2003.YPCAD13.
11. Kato T, Inoue T, Morooka T, Yoshimoto N, Node K. Short-term passive smoking causes endothelial dysfunction via oxidative stress in nonsmokers. *Can J Physiol Pharmacol.* 2006;84(5):523–529. doi:10.1139/y06-030.
12. Matturri L, Ottaviani G, Lavezzi AM. Early atherosclerotic lesions in infancy: role of parental cigarette smoking. *Virchows Archiv.* 2005;447(1):74–80. doi:10.1007/s00428-005-1224-4.
13. Church DF, Pryor WA. Free-radical chemistry of cigarette smoke and its toxicological implications. *Environ Health Perspect.* 1985;64:111–126.
14. Yamaguchi Y, Nasu F, Harada A, Kunitomo M. Oxidants in the gas phase of cigarette smoke pass through the lung alveolar wall and raise systemic oxidative stress. *J Pharmacol Sci.* 2007;103(3):275–282.
15. Halliwell B, Cross CE. Oxygen-derived species: their relation to human disease and environmental stress. *Environ Health Perspect.* 1994;102(suppl 10):5–12.
16. Zalata A, Yahia S, El-Bakary A, Elsheikha HM. Increased DNA damage in children caused by passive smoking as assessed by comet assay and oxidative stress. *Mutat Res.* 2007;629(2):140–147. doi:10.1016/j.mrgentox.2007.02.001.
17. Megson IL, Haw SJ, Newby DE, Pell JP. Association between exposure to environmental tobacco smoke and biomarkers of oxidative stress among patients hospitalised with acute myocardial infarction. *PloS One.* 2013;8(12):e81209. doi:10.1371/journal.pone.0081209.
18. Aycicek A, Erel O, Kocyigit A. Decreased total antioxidant capacity and increased oxidative stress in passive smoker infants and their mothers. *Pediatr Int.* 2005;47(6):635–639. doi:10.1111/j.1442-200x.2005.02137.x.
19. Kosecik M, Erel O, Sevinc E, Selek S. Increased oxidative stress in children exposed to passive smoking. *Int. J Cardiol.* 2005;100(1):61–64. doi:10.1016/j.ijcard.2004.05.069.
20. Block G, Dietrich M, Norkus EP, et al. Factors associated with oxidative stress in human populations. *Am J Epidemiol.* 2002;156(3):274–285.
21. Bono R, Bellisario V, Romanazzi V, et al. Oxidative stress in adolescent passive smokers living in urban and rural environments. *Int J Hyg Environ Health.* 2014;217(2–3):287–293. doi:10.1016/j.ijheh.2013.06.008.
22. Bono R, Tassinari R, Bellisario V, et al. Urban air and tobacco smoke as conditions that increase the risk of oxidative stress and respiratory response in youth. *Environ Res.* 2015;137:141–146. doi:10.1016/j.envres.2014.12.008.
23. Montero D, Walther G, Perez-Martin A, Roche E, Vinet A. Endothelial dysfunction, inflammation, and oxidative stress in obese children and adolescents: markers and effect of lifestyle intervention. *Obes Rev.* 2012;13(5):441–455. doi:10.1111/j.1467-789X.2011.00956.x.
24. Codoner-Franch P, Valls-Beltes V, Arilla-Codoner A, Alonso-Iglesias E. Oxidant mechanisms in childhood obesity: the link between inflammation and oxidative stress. *Trans. Res.* 2011;158(6):369–384. doi:10.1016/j.trsl.2011.08.004.
25. Codoner-Franch P, Tavarez-Alonso S, Simo-Jorda R, Laporta-Martin P, Carratala-Calvo A, Alonso-Iglesias E. Vitamin D status is linked to biomarkers of oxidative stress, inflammation, and endothelial activation in obese children. *J Pediatr.* 2012;161(5):848–854. doi:10.1016/j.jpeds.2012.04.046.
26. Al-Delaimy WK. Hair as a biomarker for exposure to tobacco smoke. *Tob Control.* 2002;11(3):176–182.
27. Fabian E, Poloskey P, Kosa L, Elmadfa I, Rethy LA. Nutritional supplements and plasma antioxidants in childhood asthma. *Wein Klin Wochenschr.* 2013;125(11–12):309–315. doi:10.1007/s00508-013-0359-6.
28. Wang Y, Chun OK, Song WO. Plasma and dietary antioxidant status as cardiovascular disease risk factors: a review of human studies. *Nutrients.* 2013;5(8):2969–3004. doi:10.3390/nu5082969.
29. Marnett LJ. Lipid peroxidation-DNA damage by malondialdehyde. *Mutat Res.* 1999;424(1–2):83–95.
30. Kim S, Apelberg BJ, Avila-Tang E, et al. Utility and cutoff value of hair nicotine as a biomarker of long-term tobacco smoke exposure, compared to salivary cotinine. *Int J Environ Res Public Health.* 2014;11(8):8368–8382. doi:10.3390/ijerph110808368.
31. Jones MR, Wipfli H, Shahrir S, et al. Secondhand tobacco smoke: an occupational hazard for smoking and non-smoking bar and nightclub employees. *Tob Control.* 2013;22(5):308–314. doi:10.1136/tobaccocontrol-2011-050203.
32. Selya AS, Dierker LC, Rose JS, Hedeker D, Mermelstein RJ. Risk factors for adolescent smoking: parental smoking and the mediating role of nicotine dependence. *Drug Alcohol Depend.* 2012;124(3):311–318. doi:10.1016/j.drugalcdep.2012.02.004.