Secondhand Smoke Exposure and Preclinical Markers of Cardiovascular Risk in Toddlers

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Objective To investigate relationships between secondhand smoke exposure in young children and several preclinical markers of cardiovascular risk that have been established as relevant to adult populations.

Study design There were 139 children, 2-5 years of age, enrolled in a cross-sectional study. Secondhand smoke exposure was objectively determined by hair nicotine level; a comprehensive panel of clinical markers (morning blood pressure, fasting glucose and insulin, lipid profiles, inflammation) and research markers (markers of oxidation, endothelial stress, and endothelial repair) of cardiovascular risk status were assessed. Univariate and multivariate linear regression were used to evaluate relationships between secondhand smoke exposure and cardiovascular risk markers.

Results Hair nicotine levels were correlated directly with blood pressure and serum C-reactive protein, and inversely correlated with serum high-density lipoprotein cholesterol and endothelial cell progenitor cell prevalence. In multivariate analyses, these relationships remained when controlled for age, sex, body mass index *z*-score, maternal education, and method of payment. Additionally, in multivariate analyses, hair nicotine level was significantly negatively correlated with total antioxidant capacity.

Conclusions These results support the view that secondhand smoke exposure in the very young has a detectable relationship with several markers of cardiovascular risk, long before the emergence of clinical disease. Further studies to define mechanisms and strategies to prevent and mitigate these risks early in life are warranted. *(J Pediatr 2017;189:155-61).*

The latest report from the US Centers for Disease Control and Prevention (CDC) shows that, despite an overall reduction in secondhand smoke exposure from 1999 to 2015, 25% of the US population remains exposed.[1] Of special concern are children from low-income homes and African American children, because they have the highest rates of biologically measured secondhand smoke exposure.[1] More than 40% of US children aged 3-11 were exposed to tobacco smoke from 2011 to 2012, based on a biological marker of exposure, serum cotinine levels.[1] An inverse relationship between socioeconomic status and secondhand smoke exposure has been well-documented,[2,3] and recent analyses have shown that for every decrease in family income ratio, serum cotinine levels increased by 1.18 ng/L among children.[2] This evidence, coupled with a burden of lifetime exposure, make young children of utmost concern. Better defining health risks of secondhand smoke in young children from low-income settings may help to underscore this health risk disparity and help pediatricians to enhance strategies to mitigate these risks.

Links between secondhand smoke and cardiovascular disease (CVD) and death in adults are very well established.[4-7] Secondhand smoke is a known risk factor for the development of atherosclerotic heart disease and increases the risk of CVD by about 30% in nonsmoking adults.[5-7] In contrast with the compelling evidence in adults, studies demonstrating cardiovascular implications of secondhand smoke exposure during childhood are less defined. Young children are at particular risk for secondhand smoke exposure.[2,8] More important, in our previous work, we demonstrated that toddlers had higher hair nicotine levels when compared with older children with equivalent survey measures of secondhand smoke exposure.[9]

BMI	Body mass index
CDC	US Centers for Disease Control and Prevention
CRP	C-reactive protein
CVD	Cardiovascular disease
EPC	Endothelial progenitor cell
HDL	High-density lipoprotein cholesterol
MDA	Malondialdehyde
TAC	Total antioxidant capacity
TNF	Tumor necrosis factor

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A challenge for studying cardiovascular risk in pre-adulthood is the fact that children and adolescents very rarely exhibit clinical manifestations of acquired (noncongenital) heart disease. Most adult forms of CVD are either related to or driven by inflammatory processes,[4] and such processes are apparently initiated in childhood. However, few studies have assessed the concurrent relationship between secondhand smoke exposure and cardiovascular risk markers in children <6 years of age.[10]

Our goal was to better define the relationship between secondhand smoke exposure in toddlers age 2-5 years of age and preclinical CVD risk. We investigated several surrogate markers of CVD risks in relation to secondhand smoke exposure and included traditional clinical measures as well as research measures recognized as relevant and mechanistically involved in adult settings. Clinical measures were blood pressure, glucose metabolism, blood lipids, and markers of inflammation, and "research measures" were markers of oxidation, and vascular endothelial stress and endothelial repair. Each of these indicators has been linked to secondhand smoke exposure and CVD in adult or older pediatric populations,[7,11-14] but has not been investigated in very young children. Prior studies have not focused on this age group where the cardiovascular implications of secondhand smoke may be importantly under-recognized.

Methods

The Nationwide Children's Hospital Institutional Review Board approved the study. Participants were children aged 2-5 years, and parents provided informed consent. They were recruited via convenience sampling through recruiting in Nationwide Children's Hospital Primary Care Network (Columbus, Ohio), and advertising via an internal hospital e-mail system. The Primary Care Network primarily serves low-income, urban children in Columbus. Inclusion criteria were healthy children both exposed and unexposed to secondhand smoke by parental report. Exclusion criteria were the presence of ≥1 of the following: acute febrile illness or other active infections, congenital heart disease, diabetes (type 1 or 2), elevated fasting glucose (>100 mg/dL), family history of elevated cholesterol, use of oral or inhaled (anti-inflammatory) steroids within 11 month of testing, and/or not having enough hair for hair sampling of secondhand smoke exposure (hair nicotine level). This approach to enrollment thus avoided children with persistent asthma, because of the use of daily anti-inflammatories, which we considered confounding for this study.

The study was introduced to the mother or caregiver at a clinic visit. Subjects were subsequently scheduled for testing at a research site in the morning between 8 and 10 a.m., after overnight fasting. The protocol was carried out as follows: (1) Study procedures were described with parental informed consent and youth or teen assent and consent obtained, (2) anthropomorphic measurements obtained, (3) structured interview with subject and parent (demographics and secondhand smoke exposure history), (4) hair sample obtained, and (5) the blood sample of 7 mL was collected for biomarkers and covariates. After serum sample collection all assays were stored on ice and used within 12 hours of collection (24-hour for flow cytometry studies).

Trained research staff obtained height and weight from each subject using a Tanita BWB800 scale (Tanita Corporation of America, Inc, Arlington Heights, Illinois) and Seca stadiometer (Seca, Hamburg, Germany). Weights were recorded to the nearest 0.1 kilogram. Heights were measured to the nearest 0.5 cm. Body mass index (BMI) was determined according the CDC guidelines (BMI = weight [kg]/ height [m^2]), and percentile norms to define normal weight, overweight, and obese were from CDC guidelines (available at: http://www.cdc.gov/healthyweight/assessing/bmi/childrens_bmi/about_childrens_bmi.html).

Measured Variables

We assessed a comprehensive panel of clinically relevant and research-based outcome variables that have been used by others to reflect cardiovascular status and related disease risks. Brief descriptions of methods and rationale are provided.

Hair nicotine was used as a biological marker of secondhand smoke exposure to assess long-term evaluation of smoke exposure because the nicotine is incorporated into the growing hair shaft over several months. Samples are easy to obtain, handle, and store. Approximately 20-40 hair shafts from the occipital area were cut at the root and 2-3 cm in length. Hairs were stored and later sent for assay at an established contract research facility (Johns Hopkins School of Public Health). Between 10 and 30 mg of each hair sample were washed using 3 mL of dichloromethane and sonicated (Model 250HT, Aquasonic, Hayward, California) for 30 minutes before nicotine extraction and analysis. This process removed any nicotine adherent to the surface of the hair, limiting measurement of nicotine to that accumulated by inhalation, ingestion, and dermal absorption, and subsequent incorporation into the growing hair. Hair nicotine analysis was performed using gas chromatography/mass spectrometry (GC-17/MS-QP5000, Shimadzu, Canby, Oregon) in selected ion monitoring and splitless modes. For quality control, approximately 10% of the hair samples were subjected to duplicate analyses. Content is expressed as ng/mg of hair.

Blood pressure was measured via a Critikon-Dinamap Compact T monitor (Critikon Inc, Tampa, Florida). The subject was fasting and allowed upright (typically in a parent's lap) for ≥5 minutes; the measurement is taken on the subject's left arm. Normalized blood pressure (percentile of normative values) was derived from National Heart, Lung, and Blood Institute tables, accounting for height, age, and sex (http://www.nhlbi.nih.gov/guidelines/hypertension/child_tbl.htm).

Fasting lipid profiles and glucose were measured via our hospital core laboratory facility. Insulin was determined by enzyme immunoassay (GenWay Biotech Inc, San Diego, California, Cat# 40-056-205011).

Biomarkers of Inflammation

C-Reactive Protein. C-reactive protein (CRP) was measured as a classical and clinically relevant measure of systemic inflammation that has been linked to secondhand smoke