INTERNATIONAL DOCTORAL SCHOOL
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Mouriño Castro

PhD Thesis

Relation of exposure to secondhand tobacco smoke during pregnancy and early childhood with adolescent's body composition and cardiometabolic health: a cohort study

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Doctoral Programme in Epidemiology and Public Health

DOCTORAL THESIS

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Nerea Mouriño Castro

INTERNATIONAL PHD SCHOOL OF THE UNIVERSITY OF SANTIAGO DE COMPOSTELA

PHD PROGRAMME IN EPIDEMIOLOGY AND PUBLIC HEALTH

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DECLARACIÓN DEL AUTOR DE LA TESIS

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Relation of exposure to secondhand tobacco smoke during pregnancy and early childhood with adolescent's body composition and cardiometabolic health: a cohort study

Ms. Mónica Pérez Ríos

STATES:

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SUPERVISOR AUTHORISATION

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Mr. Joseph M. Braun

STATES:

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In Santiago de Compostela, 8 of September, 2022

Joseph M. Braun

DECLARATION OF CONFLICTS OF INTERESTS

Relation of exposure to secondhand tobacco smoke during pregnancy and early childhood with adolescent's body composition and cardiometabolic health: a cohort study

Ms. Nerea Mouriño Castro

The doctoral candidate declares no conflicts of interest related to her thesis

Santiago de Compostela, 8 de Septiembre de 2022

Nerea Mouriño Castro

"A prudent person profits from personal experience, a wise one from the experience of others"

- Dr. Joseph Collins

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PRESENTATION

This thesis is the culmination of a three-year research period within the Department of Preventive Medicine and Public Health at the University of Santiago de Compostela (Spain), in collaboration with the Department of Epidemiology at the Brown University School of Public Health (Providence, Rhode Island, USA).

The core of this thesis, presented by compendium of articles, is to examine the serum cotinine cut-points used to classify secondhand tobacco smoke (SHS) exposure in children under 5 years, the suitability of age-specific cut-points, and the association of pre- and postnatal exposure to SHS with adolescent's body composition and cardiometabolic (CM) risk. This was achieved through the compilation of five scientific studies, three of them already published in international peer-reviewed journals indexed to the Journal Citation Reports (JCR); regarding the remaining two studies, they are undergoing peer review after being submitted to international journals indexed to the JCR (Table 1).

Table 1. Compendium of the five papers included in the thesis

	PAPER TITLE	AIM	PAPER STATUS	JOURNAL (JCR CATEGORY/ IMPACT FACTOR)
1st	Serum cotinine cut-points for secondhand smoke exposure assessment in children under 5 years: A systematic review	Identification of the serum cotinine cut-points used to classify SHS exposure in children from birth through first 4 years of life	Published	PLoS One (Q2/3.75)
2nd	Secondhand tobacco smoke exposure among children under 5 years old: questionnaires versus cotinine biomarkers: a cohort study	Assessment of the concordance between maternal self-reported exposure and serum cotinine when classifying children's SHS exposure from birth through first 4 years of life, and ascertainment of the suitability of age-specific cotinine cut-points, compared with the cotinine assay limit of detection	Published	BMJ Open (Q2/3.02)
3rd	Pre- and postnatal exposure to secondhand tobacco smoke and body composition at 12 years: periods of susceptibility	Assessment of the effects of pre- and postnatal SHS exposure on body composition at age 12 years	Published	Obesity (Q1/9.30)

	PAPER TITLE	AIM	STATUS
4th	Pre- and postnatal exposure to secondhand tobacco smoke and cardiometabolic risk at 12 years: periods of susceptibility	Assessment of the effects of pre- and postnatal SHS exposure on CM risk at age 12 years	Submitted (under peer review)
5th	Influence of maternal smoking in pregnancy on blood pressure during childhood and adolescence: systematic review and meta-analysis	Identification of the influence of maternal smoking in pregnancy on blood pressure figures during childhood and adolescence	Submitted (under peer review)

The document of this thesis is written in English, and it is composed of the following 9 sections: introduction, justification of the research and objectives, methods, results, discussion, conclusions, implications, references and annexes.

By way of presentation, this thesis reflects the importance of using age-specific cut-points when classifying young children's SHS exposure with cotinine, and evaluates the adverse effects of pre- and postnatal exposure to SHS on body composition and CM risk during adolescence, while identifying periods of heightened susceptibility and differences by adolescent's sex.

Exposure to SHS, defined as the involuntary inhalation of mainstream and sidestream tobacco smoke produced by an active smoker, is a global public health concern and there is no apparent safe threshold of exposure (1). SHS includes in its composition more than 5,000 chemical compounds, 70 of which are recognized as human carcinogens by the International Agency for Research on Cancer (IARC) (2).

Although a decline in the prevalence of SHS exposure has been observed in the United States of America (USA) since 1988 as a result of combined tobacco control policies, approximately 1 in 4 US children is still exposed to this toxicant (3). Of note, approximately 72% Spanish children younger than 12 years were exposed to SHS in 2016, according to parental self-report (4, 5). Pediatric populations may be especially vulnerable to its effects due to their narrower bronchi, faster respiratory rate, and immature detoxification systems (6).

In 2004, 165,071 deaths worldwide among children under 5 years old were attributed to SHS exposure (7). However, these estimations were considered to be prone to bias because

exposure data was mainly derived from surveys. Accurate estimation of the children who are exposed to SHS is important for determining its attributable burden of disease and mortality. Although parental self-report questionnaires have been widely used to assess both pre- and postnatal SHS exposure among their offspring, underestimation of the prevalence of exposure has been found when compared with that obtained with biomarkers; inaccurate estimations from parental self-report could be due to subjectivity, linked to differences in perception, ignorance of SHS exposures, or recall and social desirability biases (8, 9). Therefore, the ideal approach would be to have objective indicators obtained from the measurement of specific biomarkers of exposure, such as cotinine, which is the main metabolite of nicotine (10). Nonetheless, the determination of SHS exposure is rather challenging as currently there is no standardized consensus regarding the optimal cut-point to be used when classifying SHS exposure among young children; importantly, developmental changes in child's behavior, anatomy, and physiology during the first years of life, support the need for age-specific cotinine cut-points to accurately estimate SHS exposure in this target group.

Deleterious effects derived from SHS exposure on children's health have been documented since the early 1970s and it is currently associated with an increased risk of sudden infant death syndrome, otitis, acute respiratory symptoms (such as cough, phlegm, wheeze and breathlessness), asthma, cognitive deficits and cardiovascular (CV) disease (CVD) (1, 11-13). Few studies have assessed the impact of both pre-and postnatal exposure to SHS on adolescent's CV health. This is critical as the prevalence of adolescence obesity and related CM disorders are increasing worldwide (14, 15). CM disorders begin early in life and have lifelong health consequences as they increase the risk of all-cause mortality, CVD morbidity and mortality, and type 2 diabetes mellitus in adulthood (16).

In 2003, a prospective and ongoing pregnancy and birth cohort study, the Health Outcomes and Measures of the Environment (HOME) Study, was initiated with the objective of assessing the impact of pre- and postnatal exposure to low-level environmental toxicants on multiple child's health endpoints from infancy to adolescence (17, 18). From 2003 to 2006, 389 pregnant women living in a nine county region of the Cincinnati, Ohio (OH) metropolitan area and Northern Kentucky were eligible to participate. Follow-up visits were conducted in mothers and their offspring at the delivery hospital, the study clinic, or participants' homes. Pre- and postnatal SHS exposure was determined with maternal self-report and mothers' and children's serum cotinine from pregnancy through the first four years

of life. Specifically, when children were on average 12.4 years of age (range: 11-14.1), weight, height, waist circumference, total body and regional fat mass, visceral fat, lean mass, and several CM risk components were measured.

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Regarding participation in the development of the studies that form part of this thesis, the doctoral student declares to have been responsible for the conception and planning of the studies; analysis and interpretation of the results; writing of the first version of the manuscripts and editing of subsequent versions until the final version of the manuscripts. All other authors provided critical comments, contributed significantly to the drafts of successive versions of the manuscript and approved the final version of the studies.

The tables and figures included in this thesis have been prepared by the authors and therefore, it has not been necessary to request permission for their inclusion in this dissertation document

Authors have complied with the Principles of the Ethical Practice of Public Health. One of the authors, Joseph M. Braun, was financially compensated for his services as an expert witness for plaintiffs in litigation related to PFAS-contaminated drinking water. The other authors declare no competing interests.

ABSTRACT

Relation of exposure to secondhand tobacco smoke during pregnancy and early childhood with adolescent's body composition and cardiometabolic health: a cohort study

Exposure to secondhand tobacco smoke (SHS) is a worldwide public health problem and children are a particularly vulnerable population due to their anatomical, physiological, and behavioral characteristics. Cotinine concentrations in saliva, blood, and hair have become the most widely used biomarkers of SHS in children. Currently, there is no consensus regarding the optimal cut-point for the classification of SHS exposure among young children. Indeed, the systematic review conducted as part of this thesis shows that serum cotinine cut-points have varied remarkably since 1985 and across the countries; the most recently used cut-point to assess SHS exposure in US children has been 0.015 ng/mL, which derives from the assay limit of detection (LOD) used by the National Health and Nutrition Examination Survey (NHANES). Considering that cotinine metabolism and clearance may vary by age, and that children's physiology is quite different within the 0-5 years old age range, age-specific cut-points could be useful to accurately classify nonsmoking children as exposed or unexposed to SHS in a standardized manner.

This thesis includes the first study estimating age-specific cut-points to classify SHS exposure among children under 5 years old. Interestingly, when applying these serum cotinine cut-points, compared to the assay LOD derived cut-point, results showed that prevalence of children's SHS exposure declined at all ages, and that concordance between mother-reported SHS exposure and children's serum cotinine improved considerably.

In the last two decades there has been an evolution in epidemiological and clinical research on the consequences of SHS, with particular emphasis on adverse CV effects. Prevention of CVD remains a major public health issue, with a lack of studies examining the association of SHS exposure during pregnancy and/or childhood with the onset of CV risk factors in adolescence or adulthood. The metabolic syndrome (MetS) is a cluster of CV risk factors that are associated with CM disorders, including heart disease, stroke and diabetes.

This thesis includes the first studies evaluating associations of serum cotinine concentrations, from pregnancy through the first four years of life, with body composition and CM risk at age 12 years, while using multiple informant models in order to identify periods of heightened susceptibility. Results suggest that postnatal exposure to SHS could have greater influence on adolescent's body composition and CM risk than prenatal exposure, and associations may be sex-specific. Specifically, postnatal cotinine concentrations were associated with higher BMI z-score, lean mass index z-score, and CM risk score, particularly visceral fat area. When considering adolescents' sex, postnatal cotinine was more strongly associated with some adiposity and CM risk measures among girls compared to boys, specifically, greater body fat mass index z-score and visceral fat area.

To date, there are contradictory results about the potential effects of prenatal exposure to SHS on blood pressure (BP) during childhood and adolescence. The results from the meta-analysis conducted as part of this thesis show that smoking in pregnancy increases systolic BP (SBP) during childhood and adolescence, though the mean difference in SBP from children or adolescents who were exposed, compared to those unexposed, is small (under 1 mmHg).

Studies included in this thesis reinforce the need for age-specific cut-points when assessing SHS exposure among young children, and for ongoing public health interventions to discourage parents and caretakers from smoking, and to minimize their children's exposure to SHS. Future cohorts should verify if early-life exposures to SHS are associated with increased adiposity and CM risk during adolescence, while examining possible differences by exposure period and adolescent's sex.

Keywords: secondhand tobacco smoke, cotinine, reference value, children, adolescents, body composition, cardiometabolic risk factors

RESUMO

Relación entre exposición a fume ambiental de tabaco durante o embarazo e primeira infancia coa composición corporal e saúde cardiometabólica na adolescencia: un estudo de cohortes.

Esta tese é a culminación dun período de investigación de tres anos dentro do Departamento de Medicina Preventiva e Saúde Pública da Universidade de Santiago de Compostela (España), en colaboración co Departamento de Epidemioloxía da Escola de Saúde Pública da Universidade de Brown (Providence, Rhode Island, EE.UU.).

O núcleo desta tese, presentada por compendio de artigos, é examinar os puntos de corte de cotinina sérica utilizados para clasificar a exposición ao fume de segunda mano ou fume ambiental de tabaco (FAT) en nenos menores de 5 anos, a idoneidade de puntos de corte específicos por idade, e a asociación da exposición pre- e postnatal ao FAT coa composición corporal e o risco cardiometabólico (CM) dos adolescentes. Para iso, recompiláronse cinco estudos científicos, tres deles xa publicados en revistas internacionais revisadas por pares e indexadas no Journal Citation Reports (JCR). Os obxectivos desta tese foron os seguintes:

1. Avaliar a concordancia entre a exposición ao FAT declarada pola nai e a exposición baseada en biomarcadores en nenos estadounidenses menores de 5 anos, e determinar a idoneidade dos puntos de corte específicos por idade para caracterizar a exposición ao FAT dos nenos, en comparación co punto de corte derivado do límite de detección (LD) da técnica analítica empregada para cuantificar a cotinina sérica.
2. Revisar os puntos de corte da cotinina sérica utilizados ata o de agora para avaliar a exposición ao FAT en nenos menores de 5 anos, examinando ao mesmo tempo os cambios nos valores ao longo do tempo e en diferentes países.
3. Avaliar a asociación entre a exposición pre- e postnatal ao FAT e as alteracións na composición corporal do adolescente, á vez que se consideran as modificacións do efecto segundo o período de exposición e o sexo do adolescente.

4. Avaliar a asociación entre a exposición pre- e postnatal ao FAT e as alteracións na saúde CM do adolescente, tendo en conta as posibles modificacións do efecto segundo o período de exposición e o sexo do adolescente.
5. Analizar a evidencia actual sobre o efecto que o tabaquismo materno no embarazo ten sobre a tensión arterial diastólica (TAD) e/o tensión arterial sistólica (TAS) dos seus fillos durante a infancia ou adolescencia.

A exposición ao FAT, o tabaquismo pasivo, defínese como a inhalación involuntaria do fume de tabaco da corrente principal e da corrente secundaria producido por un fumador activo. O fume da corrente secundaria é unha combinación de fume procedente de produtos do tabaco que arden entre e durante as caladas, e de compoñentes do fume que se difunden a través do papel dos cigarros; o fume da corrente lateral comprende aproximadamente o 85% do FAT e, segundo a súa composición química, é máis tóxico que o fume da corrente principal. A exposición ao FAT é un problema de saúde pública a nivel mundial, e non existe un limiar de exposición seguro. O FAT inclúe na súa composición máis de 5.000 compostos químicos, 70 dos cales están recoñecidos como carcinógenos humanos do grupo 1 pola IARC; algunhas destas substancias químicas poden atravesar a placenta e causar efectos adversos duradeiros nas funcións neurales, endocrinas e fisiolóxicas do neno.

Os nenos están expostos ao FAT e son unha poboación especialmente vulnerable debido ás súas características anatómicas, fisiolóxicas, de comportamento, e á súa falta de control sobre a contorna. Os efectos adversos derivados da exposición ao FAT na saúde dos nenos foron documentados desde 1970, e actualmente asóciase a un maior risco de síndrome de morte súbita do lactante, otite, síntomas respiratorios agudos (tose, flemas, pitos e disnea), asma, déficits cognitivos e enfermidades cardiovasculares (ECV).

Aínda que desde 1988 se observou un descenso na prevalencia da exposición ao FAT nos Estados Unidos como resultado das políticas combinadas de control do tabaco, en 2012 máis do 40% dos nenos de 3 a 11 anos tiñan niveis de cotinina sérica que reflectían exposición ao FAT. Cabe destacar que no ano 2016, aproximadamente o 72% dos nenos españois estiveron expostos ao FAT segundo a autodeclaración dos pais. A estimación precisa dos nenos expostos ao FAT é importante para determinar a carga de enfermidade atribuíble á poboación e avaliar o impacto das leis de control do tabaco, e os programas educativos destinados a reducir a exposición ao FAT neste grupo vulnerable. A estimación da prevalencia da

exposición ao FAT entre os nenos a partir da autodeclaración dos pais podería estar nesgada debido ás diferenzas de percepción, a ignorancia ou o nesgo de deseabilidade social. Por tanto, o enfoque ideal sería contar con indicadores obxectivos obtidos a partir da medición de biomarcadores específicos de exposición, como a cotinina.

A cotinina é o principal metabolito da nicotina e é un biomarcador preciso para avaliar a exposición ao FAT debido á súa alta especificidade e sensibilidade, e á súa longa vida media. Pode medirse en diversas mostras biolóxicas como o sangue (soro/plasma), os ouriños, o pelo, a saliva, o cordón umbilical, o líquido amniótico, o leite materno ou o meconio. Co tempo, a cotinina sérica converteuse nun dos biomarcadores de exposición ao FAT máis utilizados entre os nenos. A cotinina sérica permite diferenciar aos fumadores activos dos pasivos e dos non fumadores. Con todo, a determinación da exposición ao FAT é difícil, xa que actualmente non existe un consenso estandarizado sobre o punto de corte óptimo de cotinina sérica que debe utilizarse para clasificar a exposición ao FAT entre os nenos pequenos.

Esta tese inclúe a primeira revisión sistemática sobre os puntos de corte de cotinina sérica utilizados ao longo do tempo para clasificar a exposición ao FAT en nenos, desde o nacemento ata os primeiros 4 anos de vida. Este estudio mostra que os puntos de corte da cotinina sérica variaron notablemente desde 1985, e nos diferentes países; o punto de corte máis recentemente utilizado para avaliar a exposición ao FAT nos nenos estadounidenses foi de 0,015 ng/mL, que deriva do LD da técnica analítica e foi amplamente utilizado pola Enquisa Nacional de Saúde e Nutrición Americana (NHANES). Varios estudos, incluíndo aqueles con datos da NHANES, que mediu a exposición ao FAT desde 1988 mediante cuestionarios cuestionarios de autodeclaración e cotinina sérica, observaron concentracións de cotinina máis elevadas entre os nenos en comparación cos adolescentes e os adultos non fumadores cunha exposición similar ao FAT. Así, un dos estudos estadounidenses que incluíu a nenos de entre 18 meses e 17 anos de idade, observou que a prevalencia de exposición ao FAT foir maior entre os nenos de 1 e 3 anos. Con todo, só un estudo estadounidense publicado en 2012, que incluía a nenos e adolescentes de 1 a 16 anos, calculou dous puntos de corte específicos por idade empregando a curva característica operativa del receptor (ROC), fixando a idade de referencia nos 12 anos; cabe destacar que se utilizou o mesmo punto de corte (0,9 ng/mL) para clasificar a exposición ao FAT en nenos durante os primeiros 12 anos de vida sen ter en conta os cambios que se producen, desde a infancia ata a nenez, na fisioloxía e anatomía do neno, na frecuencia respiratoria, no seu comportamento, nivel de

exposición ao FAT, e no metabolismo ou aclaramento da cotinina. Ao ter en conta que o metabolismo e o aclaramento da cotinina poden variar en función da idade, e que a fisioloxía dos nenos é moi diferente dentro do rango de idade de 0 a 5 anos, os puntos de corte específicos por idade poderían ser útiles para clasificar con precisión aos nenos non fumadores como expostos ou non expostos ao FAT dunha maneira estandarizada.

Ao avaliar a exposición dos nenos ao FAT, algúns dos estudos incluídos na revisión atoparon discrepancias entre a autodeclaración dos pais sobre a exposición e as concentracións séricas de cotinina dos nenos. Algúns investigadores concluíron que a autodeclaración materna sobre a exposición ao FAT dos seus fillos podería subestimar drasticamente a súa exposición. Aínda que un dos estudos considerou a posibilidade dunha clasificación errónea debido ao emprego dun punto de corte de cotinina inadecuado, a maioría dos estudos asumiron que as nais non informan con exactitude da exposición ao FAT dos seus fillos; isto podería deberse á ocultación do seu consumo de tabaco para evitar ser xulgadas socialmente, ou ao descoñecemento sobre exposicións insignificantes, transitorias ou procedentes doutras fontes de FAT na contorna do seu fillo, distintas do fume do tabaco.

En 2003, iniciouse un estudo americano de cohortes prospectivo en Cincinnati, the Health Outcomes and Measures of the Environment (HOME), co obxectivo de avaliar o impacto da exposición pre- e postnatal a diferentes tóxicos ambientais, entre eles o FAT, na saúde do neno durante a infancia e adolescencia. De 2003 a 2006, 389 mulleres embarazadas foron elixibles para participar. As visitas de seguimento realizáronse ás nais e aos seus fillos no hospital do parto, no centro clínico do estudo ou nos fogares das participantes. A exposición pre- e postnatal ao FAT determinouse coa autodeclaración materna e a cotinina sérica das nais e os nenos, desde o embarazo ata o primeiros catro anos de vida. Cando os nenos tiñan unha media de 12,4 anos de idade (rango: 11-14,1), mediuse o seu peso, a altura, índice de masa corporal (IMC), o perímetro da cintura, a masa graxa total e rexional, a graxa visceral, a masa magra, e varios compoñentes de risco CM.

Para o segundo estudo desta tese doutoral, utilizáronse datos da cohorte HOME co fin de caracterizar a concordancia entre a exposición ao FAT declarada pola nai e as concentracións séricas de cotinina dos seus fillos ao nacer e aos 1, 2, 3 e 4 anos de idade, tras aplicar o LD da técnica analítica e os puntos de corte específicos para cada idade. En particular, esta tese inclúe o primeiro estudo que estima os puntos de corte de cotinina sérica específicos por idade entre nenos menores de 5 anos empregando curvas ROC. Curiosamente, ao aplicar estes

puntos de corte de cotinina sérica, en comparación co punto de corte derivado do LD da técnica analítica de 0,015 ng/mL, os resultados mostraron que a prevalencia de exposición ao FAT dos nenos diminuíu en todas as idades, e que a concordancia entre a exposición ao FAT declarada pola nai e a obtida a partir da cotinina sérica dos nenos mellorou considerablemente. Por tanto, as diferenzas atopadas na prevalencia da exposición ao FAT durante o período postnatal poderían estar influídas pola sensibilidade e a idoneidade dos puntos de corte utilizados para a clasificación da exposición ao FAT dos nenos, ao ter en conta as diferenzas por idade no nivel de exposición ao FAT ou no metabolismo e aclaramento da cotinina.

Nas dúas últimas décadas produciuse unha evolución na investigación epidemiolóxica e clínica sobre as consecuencias do FAT, con especial énfase nos efectos CV adversos. A ECV segue sendo a principal causa de morte e discapacidade en todo o mundo. Aínda que, faltan estudos que examinen a asociación da exposición ao FAT durante o embarazo e/o a infancia coa aparición de factores de risco CV na adolescencia ou na idade adulta. A exposición ao FAT está relacionada co estrés oxidativo que é crucial na programación fetal dos trastornos metabólicos e as ECV, xa que se asocia coa resistencia á insulina, elevación das cifras de TA e dos niveis séricos de triglicéridos e leptina, e coa diminución dos niveis séricos de colesterol HDL e adiponectina.

O síndrome metabólico (SM) é un trastorno multifactorial caracterizado por factores de risco CM que tenden a agruparse, como a obesidade central, a alteración do metabolismo da glicosa, a dislipidemia e a elevación da TA. Aínda que a prevalencia do SM entre os adolescentes estadounidenses mantívose estable durante as dúas últimas décadas (15), a prevalencia da obesidade central e da hiperglucemia aumentou. A prevalencia da obesidade case se triplicou desde 1975 a nivel mundial. En 2016, máis de 330 millóns de nenos e adolescentes tiñan sobrepeso ou obesidade. En 2019, entre o 10,3% e o 23,4% dos adolescentes estadounidenses eran obesos.

Esta tese inclúe os primeiros estudos que avalían as asociacións das concentracións séricas de cotinina, desde o embarazo ata os primeiros catro anos de vida, coa composición corporal e o risco CM aos 12 anos, e que identifica períodos de maior susceptibilidade mediante os modelos de informantes múltiples. Os modelos de informantes múltiples utilizan ecuacións de estimación xeneralizada para estimar conxuntamente as asociacións entre exposición e variables resultado para cada período de exposición definido, e para probar se as

asociacións varían entre os períodos. Creáronse dúas estimacións conxuntas separadas, unha para o período de exposición prenatal e outra para o período de exposición postnatal. A hipótese nula é que as asociacións son as mesmas nos dous períodos; os valores p de interacción (período de exposición × cotinina) < 0,05 consideráronse como evidencia de que polo menos una das asociacións entre a cotinina e as variables resultado difire do resto. Os posibles efectos modificadores do sexo do adolescente examináronse utilizando un termo triplo de interacción (período de exposición × cotinina × sexo do adolescente). Todos os modelos de informantes múltiples axustáronse por idade materna, raza/etnia, estado civil, educación, IMC previo ao embarazo e duración da lactación. Para as variables resultado que non eran puntuacións z, os modelos axustáronse adicionalmente polo sexo do adolescente, o estadio puberal e a idade. Realizáronse análise de sensibilidade axustando ademais pola dieta e actividade física do adolescente, e tras excluír ás nais fumadoras activas durante o embarazo (concentracións de cotinina sérica iguais ou superiores a 3 ng/mL ás 16 ou 26 semanas de xestación).

Os resultados do terceiro e cuarto estudo desta tese suxiren que a exposición postnatal ao FAT podería ter unha maior influencia na composición corporal e no risco CM dos adolescentes, en comparación coa exposición prenatal, e que as asociacións poderían variar en función do sexo. En concreto, as concentracións de cotinina postnatal asociáronse cunha maior puntuación z do IMC, puntuación z do índice de masa magra e unha maior puntuación de risco CM, debida principalmente ao incremento de graxa visceral. Outros investigadores atoparon resultados controvertidos ao valorar como a exposición pre- e postnatal ao FAT podía afectar a saúde CM dos nenos. As inconsistencias nas asociacións da exposición ao FAT coa composición corporal ou o risco CM entre estudos previos poden estar relacionadas coas diferenzas nos deseños dos estudos, as características dos participantes (idade, raza/etnia e hormonas), a avaliación da exposición ao FAT (definición, intensidade, momento da medición, método da medición: autoinforme fronte a biomarcadores), os biomarcadores empregados, o punto de corte do biomarcador, a valoración das variables resultado (método de medición, definición de SM, compoñentes de risco CM incluídos, puntos de corte utilizados ao avaliar as medidas de adiposidade ou ao definir niveis elevados dos compoñentes de risco CM nos nenos), e nas covariables incluídas nos modelos de axuste.

Ao considerar o sexo dos adolescentes, a cotinina postnatal asociouse máis fortemente con algunhas medidas de adiposidade e de risco CM entre as mozas, en comparación cos

mozos, especificamente, cunha maior puntuación z do índice de masa graxa corporal e graxa visceral. A modificación das asociacións entre exposición e variables resultado segundo o sexo do adolescente podería explicarse polas diferenzas no nivel de exposición ao FAT como resultado do maior período de tempo que as mozas adoitan pasar nos fogares, e no metabolismo e a eliminación da cotinina; ademais, tamén podería haber diferenzas por sexo no modo en que os compoñentes do fume do tabaco afectan o sistema endocrino (hormonas gonadais, esteroides, tiroideas e leptina), ao metabolismo, ao crecemento e á distribución/deposición da graxa nas mozas fronte aos mozos. As hormonas gonadais poden desempeñar un papel importante na modulación dos compoñentes de risco CM máis aló dos seus efectos sobre a acumulación de graxa visceral e a resistencia á insulina, e por este motivo, o estadio puberal, que se correlacionou positivamente coas concentracións séricas de hormonas gonadais obtidas aos 12 anos, incluíuse como posible covariable no modelo.

É importante destacar que os modelos de informantes múltiples non axustan polas exposicións ao FAT que puideron ocorrer noutros momentos e, por tanto, os resultados significativos da cotinina postnatal poderían ser un reflexo da exposición máis prolongada e constante ao FAT ao longo da vida, tras o nacemento.

Ata a data, existen resultados contraditorios sobre os posibles efectos da exposición prenatal ao FAT na TA durante a infancia e a adolescencia. Aproximadamente o 4,5% dos nenos estadounidenses tiñan hipertensión arterial (HTA) en 2016. A HTA infantil, definida como unha TAS ou TAD igual ou superior ao percentil 95, por sexo, idade e estatura, é unha afección cada vez máis frecuente; a miúdo tende a ser asintomática e está infradiagnosticada polos profesionais sanitarios durante a infancia ou a adolescencia. Algúns investigadores avaliaron como a exposición ao FAT durante o período fetal podería afectar o desenvolvemento da HTA máis adiante na vida, tendo en conta o efecto ateroxénico deste carcinóxeno. As probas fisiopatolóxicas e epidemiolóxicas actuais suxiren que a HTA durante a infancia aumenta o risco de HTA esencial, así como de eventos CV máis adiante na vida.

O quinto estudo desta tese, a revisión sistemática e o metaanálise realizados sobre o consumo materno de tabaco durante o embarazo e a TA dos seus fillos, mostrou un aumento das cifras de TAS durante a infancia e a adolescencia dos nenos expostos no período fetal; con todo, a diferenza nas cifras medias de TAS dos nenos ou adolescentes que estiveron expostos ao FAT durante o embarazo, en comparación cos non expostos, é pequena (inferior a 1 mmHg). Con respecto á TAD, non se observou o efecto do tabaquismo materno, pero

poucos estudos avaliaron a TAD e a heteroxeneidade entre eles foi elevada. Observouse un maior aumento das cifras medias de TAS no subgrupo de estudos que completaron o recrutamento antes de 1990, que se realizaron en zonas continentais non europeas, que utilizaron a esfigmomanometría estándar de mercurio ou manual, que axustaron por peso ao nacer e no subgrupo de estudos clasificados como de baixa calidade. Con todo, a heteroxeneidade entre estudos foi substancial nalgúns subgrupos.

Finalmente, os estudos incluídos nesta tese reforzan a necesidade de establecer puntos de corte específicos por idade á hora de avaliar a exposición ao FAT entre os nenos, e de intervencións de saúde pública encamiñadas a disuadir aos pais e coidadores de fumar, e a minimizar a exposición dos seus fillos ao FAT. Futuras cohortes deberían verificar se as exposicións ao FAT nas primeiras etapas da vida asócianse cun aumento da adiposidade e do risco CM durante a adolescencia, á vez que examinan as posibles diferenzas segundo o período de exposición e o sexo do adolescente.

Palabras chave: fume de tabaco ambiental, cotinina, valor de referencia, nenos, adolescentes, composición corporal, factores de risco cardiometabólico

LIST OF ABBREVIATIONS

AHT: Arterial hypertension

AUSC: Area under ROC curves

BMI: Body mass index

BP: Blood pressure

CCHMC: Cincinnati Children's Hospital Medical Center

CDC: Centers for Disease Control and Prevention

CI: Confidence interval

CM: Cardiometabolic

CV: Cardiovascular

CVD: Cardiovascular disease

DAGS: Directed acyclic graphs

DBP: Diastolic blood pressure

DXA: Dual energy x-ray absorptiometry

ELISA: Enzyme-linked immunosorbent assay

GC/MS: Gas-chromatography mass spectrometry

GC: Gas chromatography

GEE: Generalized estimating equations

GLC/NPD: Gas-liquid chromatographic procedure with nitrogen-phosphorus specific detector

GRADE: Grading of Recommendations, Assessment, Development and Evaluation

HDL-C: High-density lipoprotein cholesterol

HEI: Healthy Eating Index

HIV: Human immunodeficiency virus

HOMA-IR: Homeostatic model assessment for insulin resistance

HOME: Health Outcomes and Measures of the Environment

IARC: International Agency for Research on Cancer

ICC: Intraclass correlation coefficient

IRBs: The institutional review boards

JCR: Journal Citation Reports

LC/MS: Isotope dilution-liquid chromatography-tandem mass spectrometry

LDL: Low density lipoprotein

LOD: Limit of detection

Mets: Metabolic syndrome

MmHg: Millimetre of mercury

N/A: Not available

Ng/mL: Nanogram/milliliter

NHANES: National Health and Nutrition Examination Survey

NIH: National Institutes of Health

NPV: Negative predictive values

OH: Ohio

PECO: Participants, Exposure, Comparator and Outcomes

PPV: Positive predictive values

RIA: Radioimmunoassay

ROC: Receiver Operating Characteristic

SBP: Systolic blood pressure

SD: Standard desviation

SHS: Secondhand tobacco smoke

TG: Triglycerides

THS: Third-hand smoke

TNF-α: Tumour necrosis factor alpha

USA: United States of America

Vs.: Versus

WHO: World Health Organization

INDEX OF PUBLICATIONS INCLUDED IN THIS THESIS

Doctoral Thesis by compendium of publications in international peer-reviewed journals indexed to the JCR (article 41 of the Regulations for Doctoral Studies of the University of Santiago de Compostela):

1. Mourino N, Ruano-Raviña A, Varela Lema L, et al. **Serum cotinine cut-points for secondhand smoke exposure assessment in children under 5 years: A systemic review**. PLoS One. 2022;17(5):e0267319. https://doi.org/10.1371/journal.pone.0267319
    - Specific contribution to the publication: conceptualization, data curation, investigation, methodology, validation, visualization, and writing (original draft, review and editing).
    - PLoS One: Q2 journal with an impact factor of 3.75 and occupying the 29/73 position among multidisciplinary sciences journals in JCR. https://jcr.clarivate.com/jcr-jp/journal-profile?journal=PLOS%20ONE&year=2021&fromPage=%2Fjcr%2Fhome.
      The journal allows the reuse of the article by the doctoral student as part of her thesis.

2. Mourino N, Pérez-Ríos M, Santiago-Pérez MI, Lanphear B, Yolton K, Braun JM. **Secondhand tobacco smoke exposure among children under 5 years old: questionnaires versus cotinine biomarkers: a cohort study.** BMJ Open. 2021;11(6):e044829. http://dx.doi.org/10.1136/bmjopen-2020-044829
    - Specific contribution to the publication: conceptualization, data curation, investigation, methodology, validation, visualization, and writing (original draft, review and editing).

- BMJ Open: Q2 journal with an impact factor of 3.02 and occupying the 85/172 position among medicine, general and internal journals in JCR. https://jcr.clarivate.com/jcr-jp/journal-profile?journal=BMJ%20OPEN&year=2021&fromPage=%2Fjcr%2Fsearch-results.

  The journal allows the reuse of the article by the doctoral student as part of her thesis.

3. Mourino N, Pérez-Ríos M, Yolton K, Lanphear BP, Chen A, Buckley JP, Kalkwarf HJ, Cecil KM, Braun JM. **Pre- and postnatal exposure to secondhand tobacco smoke and body composition at 12 years: periods of susceptibility.** Obesity (Silver Spring). 2022;30(8):1659-1669. https://doi.org/10.1002/oby.23480
   - Specific contribution to the publication: conceptualization, data curation, investigation, methodology, validation, visualization, and writing (original draft, review and editing).
   - Obesity: Q1 journal with an impact factor of 9.30 and occupying the 14/146 position among endocrinology and metabolism journals in JCR. https://jcr.clarivate.com/jcr-jp/journal-profile?journal=OBESITY&year=2021&fromPage=%2Fjcr%2Fhome.

     The journal allows the reuse of the article by the doctoral student as part of her thesis.

Papers under peer review in international journals indexed to the JCR:

1. Mourino N, Pérez-Ríos M, Yolton K, Lanphear BP, Chen A, Buckley JP, Kalkwarf HJ, Cecil KM, Braun JM. **Pre- and postnatal exposure to secondhand tobacco smoke and cardiometabolic risk at 12 years: periods of susceptibility.**
   - Specific contribution to the publication: conceptualization, data curation, investigation, methodology, validation, visualization, and writing (original draft, review and editing).

2. Mourino N, Varela-Lema L, Ahluwalia JS, Rey-Brandariz J, Candal-Pedreira C, Ruano-Ravina A, Vila-Farinas A, Torres A, Pérez-Ríos M. **Influence of**

maternal smoking in pregnancy on blood pressure during childhood and adolescence: systematic review and meta-analysis.

- Specific contribution to the publication: conceptualization, data curation, investigation, methodology, validation, visualization, and writing (original draft, review and editing).

CONTENTS

PART ONE: INTRODUCTION

INTRODUCTION

Exposure to SHS (i.e., passive smoking) is defined as the involuntary inhalation of mainstream and sidestream tobacco smoke produced by an active smoker. Sidestream smoke is a combination of smoke from smoldering tobacco product between and during puffs and smoke components which diffuse through cigarette paper (1, 19); sidestream smoke comprises approximately 85% of SHS, and based on its chemical composition, is more toxic than mainstream smoke (20, 21). Exposure to SHS is a global public health concern, and there is no apparent safe threshold of exposure (1). SHS includes in its composition more than 5,000 chemical compounds, 70 of which are recognized as group 1 human carcinogens by the IARC (2); some of these chemicals can cross the placenta and cause long-lasting adverse effects on child's neural, endocrine, and physiological functions (22).

Pediatric populations are exposed to SHS and may be especially vulnerable to its effects due to their narrower bronchi, faster respiratory rate, and immature detoxification systems (6). Deleterious effects derived from SHS exposure on children's health have been documented since the 1970s. In 1986, the first United States (US) Surgeon General's Report on the health effects of involuntary smoking concluded that children whose parents smoke, compared to those of nonsmoking parents, had an increased frequency of respiratory symptoms and infections, and worse lung function (21). In 2006, The US Surgeon General's Report associated SHS exposure with increased risk of sudden infant death syndrome, acute respiratory symptoms (cough, phlegm, wheeze and breathlessness) and ear infections in children (1). Moreover, some investigators have emphasized that even very low levels of SHS exposure can cause adverse effects on cognitive outcomes among children (12).

Although a decline in the prevalence of SHS exposure has been observed in the USA since 1988 as a result of combined tobacco control policies, over 40% of children aged 3-11 years had serum cotinine levels consistent with SHS exposure in 2012 (23). Accurate estimation of the children exposed to SHS exposure is important for determining the population attributable burden of disease and evaluating the impact of tobacco control laws and educational programs aimed at reducing SHS exposure on this target group. Of note, estimating the prevalence of exposure to SHS among children from parental report could be biased due to differences in perception, ignorance, or social desirability bias (24, 25). Thus,

the ideal approach would be to have objective indicators obtained from the measurement of specific biomarkers of exposure, such as cotinine (8).

Cotinine is the primary metabolite of nicotine and it is an accurate biomarker for assessing exposure to SHS due to its high specificity and sensitivity, and its long half-life (8, 10). It can be measured in various biological samples such as blood (serum/plasma), urine, hair, saliva, umbilical cord, amniotic fluid, maternal milk or meconium (10, 26). Although the collection of serum or plasma cotinine is more invasive than that from other samples, it is not influenced by renal function, urine dilution, and urinary pH. Over time, serum cotinine has become one of the most widely used biomarker of SHS exposure among children (1, 8). Serum cotinine allows for differentiating active smokers from passive smokers and non-smokers (27). Nonetheless, the determination of SHS exposure is rather challenging as currently there is no standardized consensus regarding the optimal cut-point to be used when classifying SHS exposure among young children.

Some studies have assessed SHS exposure among children by using the serum cotinine cut-points of 0.05 ng/mL or 0.015 ng/mL, which derive from the assay limit of detection (LOD) (25, 27, 28); these cut-points could reflect transient and accidental SHS exposure, exposure from certain foodstuffs or third-hand smoke (THS) exposure, i.e., involuntary inhalation, ingestion or cutaneous absorption of residual nicotine particles from SHS deposited on clothing, sofas, furniture, carpets or other surfaces which remain there after smoke is gone (29). There is evidence that, at similar exposures to SHS, young children have higher serum cotinine concentrations than adolescents or nonsmoking adults. This could be due to differences in cotinine metabolism and clearance, faster respiratory rate, greater physical proximity to their smoking fathers or mothers, breastfeeding, or other sociodemographic and behavioral characteristics (23-25, 30). It is noteworthy that prior studies involving children have not taken into account these facts, and have used the same cut-point as in adolescents or adults (31, 32).

In the last two decades there has been an evolution in epidemiological and clinical research on the consequences of SHS exposure, with particular emphasis on adverse cardiovascular (CV) effects (23). In 1986, Barker published a seminal paper regarding the influence of adverse intrauterine conditions on the development of adult's CV disease (CVD) (33). Since then, a compelling body of evidence suggests that the development of obesity and cardiometabolic (CM) disease during childhood or adolescence could be related to

intrauterine growth restriction induced by maternal undernutrition, early life exposures, including lifestyle, nutritional factors or exposure to environmental chemicals, such as those found in SHS (34-40).

Prevention of CVD remains a major public health issue, with a lack of studies examining the association of SHS exposure during pregnancy and/or childhood with the onset of CV risk factors in adolescence or adulthood (41). The prenatal, early childhood, and adolescence periods have been proposed as critical periods for the development of obesity and related CM disorders, with the latter being the leading cause of death and disability in the USA (42, 43). The metabolic syndrome (MetS), which often emerges in childhood, predicts future CVD and metabolic disorders such as heart disease, stroke and diabetes (41, 44). The MetS is a multifactorial disorder characterized by physiological CM risk factors that tend to cluster together, such as central obesity, impaired glucose metabolism, dyslipidemia, and elevated blood pressure (BP) (45-47). Although the prevalence of MetS among US adolescents has remained stable during the last two decades (15), the prevalence of central obesity and hyperglycemia has increased. Worldwide, the prevalence of obesity has nearly tripled since 1975 (48). In 2016, more than 330 million children and adolescents were overweight or obese (48). In 2019, between 10.3% and 23.4% of US adolescents were obese (14).

Both pre- and postnatal SHS exposure has been associated with increased body mass index (BMI) and MetS risk during childhood and adolescence (11, 13, 49-54). Prenatal SHS exposure has been associated with fetal growth retardation, as a result of vasoconstriction and hypoxemia (55); restricted fetal growth may lead to rapid postnatal compensatory weight gain resulting in increased BMI and adiposity during childhood and adolescence (22, 52, 53). Fetal nicotine exposure could alter central and peripheral sympathetic norepinephrine systems, key regulators of appetite and adipose metabolism (22). Alterations in peripheral signals of satiety and adiposity, which arise from adipose, liver, pancreas, and gastrointestinal tissues, could be altered by the effect of SHS constituents on glucose, peptides (leptin, adiponectin, insulin, cholecystokinin, and tumor necrosis factor alpha (TNF-α)), lipid-derived molecules (prostaglandins, triglycerides (TG), low density lipoprotein (LDL)), eicosanoids, and fatty acids (56, 57). Other chemicals found in SHS, including polycyclic aromatic hydrocarbons and nitrosamine 4 (methylnitrosamino)-1-(3-pyridyl)-1-butanone, can directly impact the hypothalamic neuropeptides and amygdala volume and in turn, alter child's appetite, food intake, and eating preferences (58-60). Moreover, there is evidence that SHS exposure

induces oxidative stress, lipoprotein modification, vascular inflammation, endothelial dysfunction, platelet activation, and CV function impairment (61). Specifically, nicotine has been associated with insulin resistance and dyslipidemia, and acrolein, which persist for longer in SHS, with dyslipidemia, thrombosis and arterial hypertension (AHT) (23).

Approximately 4.5% of US children had AHT in 2016 (62). Children's AHT, defined as systolic BP (SBP) or diastolic BP (DBP) equal to or higher than the 95th percentile, by sex, age and height, is an increasingly frequent condition (63). It often tends to be asymptomatic, and underdiagnosed by health professionals during childhood or adolescence (64, 65). Some investigators has assessed how SHS exposure during the fetal period might affect the development of AHT later in life, taking into account the atherogenic effect of this carcinogen (66). Current physiopathologic and epidemiologic evidence suggests that AHT during childhood increases the risk of essential AHT, as well as CV events later in life (67).

To our knowledge, no previous studies have identified periods of heightened susceptibility to the potential effects of SHS exposure, during pregnancy and childhood, on overall body composition and CM risk in adolescents. This is critical as CVD remains the leading cause of death and disability worldwide (62).

PART TWO: JUSTIFICATION OF THE RESEARCH AND OBJECTIVES

JUSTIFICATION OF THE RESEARCH AND OBJECTIVES

Exposure to SHS is a worldwide public health problem and children are a more vulnerable population due to their anatomical, physiological, and behavior characteristics (6) and their lack of control over their environments. Accurate measurements of exposure to SHS, which are difficult to obtain in children, are required to attain a valid estimation of the health risks associated with SHS exposure. The prevalence of SHS exposure obtained from maternal-report could underestimate children's exposure compared to that obtained with cotinine biomarkers (8, 9). However, discordance could be related to inadequate selection of serum cotinine cut-points. Of note, no systematic reviews have been conducted to identify the serum cotinine cut-points used over time to classify children's SHS exposure, and no studies have calculated serum cotinine age-specific cut-points to ascertain such exposure among young children. The adverse health consequences derived from any level of SHS exposure, and the developmental changes in child's behavior, anatomy, and physiology during the first years of life, support the need for age-specific cut-points for health research and public health purposes aimed at accurately estimating SHS exposure and the attributable burden disease to such exposure.

The detrimental effects of SHS exposure among children have been documented since the early seventies of the last century (1). In the last two decades there has been an evolution in epidemiological and clinical research on the consequences of SHS exposure, with particular emphasis on adverse CM effects (23). Exposure in childhood has been associated with an increase in obesity, insulin resistance, dyslipidemia and AHT (23). However, there is insufficient information about the possible effects that prenatal and postnatal exposure to SHS may have on adolescent's body composition and CM risk. Of note, to our knowledge, no studies have identified periods of heightened susceptibility to the potential effects of SHS exposure, during pregnancy and childhood, on CV health. This is critical as the prevalence of childhood CM disorders is increasing worldwide (48, 68), and it has lifelong health consequences in terms of increased risk of premature morbidity and mortality.

The precise role played by smoking during pregnancy in the development of AHT in childhood and adolescence has not been established yet, and controversy remains with mixed findings from studies (69, 70). Two previous reviews with meta-analysis were identified, but

most of the studies were cross-sectional, and among the cohort studies, these had a short follow-up time (52, 69). Furthermore, one of the reviews drew no distinction between maternal smoking and exposure to SHS (69).

Taking into account the aforementioned aspects, the following objectives were established:

1. Assessment of the concordance between mother-reported SHS exposure and biomarker-based exposure in US children under 5 years old, and ascertain the suitability of age-specific cut-points to characterize children's SHS exposure, compared with the serum cotinine assay LOD.
2. Review of the serum cotinine cut-points used so far to assess SHS exposure in children under 5 years old, while examining the changes in values over time and across different countries.
3. Assessment of the association between pre- and postnatal exposure to SHS and alterations in adolescent's body composition, while considering effect modifications by period of exposure and adolescent's sex.
4. Assessment of the association between pre- and postnatal exposure to SHS and alterations in adolescent's CM health, while considering possible effect modifications by period of exposure and adolescent's sex.
5. Analyze current evidence about the effect that maternal smoking during pregnancy has on children's or adolescent's DBP and/or SBP.

PART THREE: METHODS

METHODS

This thesis is comprised of five scientific studies, based on two systematic reviews, one of them accompanied with meta-analysis, and three original studies with data from a prospective and ongoing pregnancy and birth cohort, the Health Outcomes and Measures of the Environment (HOME) Study. Regarding the five papers included in this thesis, three have been published in international peer-reviewed journals indexed to the JCR, and the remaining two studies are undergoing peer review after being submitted to international journals indexed to JCR:

1. Mourino N, Ruano-Raviña A, Varela Lema L, et al. **Serum cotinine cut-points for secondhand smoke exposure assessment in children under 5 years: A systemic review**. PLoS One. 2022;17(5):e0267319. Q2 journal with an impact factor of 3.75 and occupying the 29/73 position among multidisciplinary sciences journals in JCR.

   This systematic review was conducted to identify the serum cotinine cut-points used to classify SHS exposure in children from birth through first 4 years of life.
2. Mourino N, Pérez-Ríos M, Santiago-Pérez MI, Lanphear B, Yolton K, Braun JM. **Secondhand tobacco smoke exposure among children under 5 years old: questionnaires versus cotinine biomarkers: a cohort study.** BMJ Open. 2021;11(6):e044829. Q2 journal with an impact factor of 3.02 and occupying the 85/172 position among medicine, general and internal journals in JCR.

   Data from the HOME study was used to examine the concordance between maternal self-reported exposure and biomarkers (serum cotinine) when classifying children's SHS exposure from birth through first 4 years of life.
3. Mourino N, Pérez-Ríos M, Yolton K, Lanphear BP, Chen A, Buckley JP, Kalkwarf HJ, Cecil KM, Braun JM. **Pre- and postnatal exposure to secondhand tobacco smoke and body composition at 12 years: periods of susceptibility.** Obesity (Silver Spring). 2022;30(8):1659-1669. Q1 journal with an impact factor of 9.30 and occupying the 14/146 position among endocrinology and metabolism journals in JCR.

Data from the HOME study was used to longitudinally assess the effects of pre- and postnatal SHS exposure on body composition at age 12 years.

4. Mourino N, Pérez-Ríos M, Yolton K, Lanphear BP, Chen A, Buckley JP, Kalkwarf HJ, Cecil KM, Braun JM. **Pre- and postnatal exposure to secondhand tobacco smoke and cardiometabolic risk at 12 years: periods of susceptibility.** Under peer review after submission to an international journal indexed to JCR.

   Data from the HOME study was used to longitudinally assess the effects of pre- and postnatal SHS exposure on CM risk at age 12 years.

5. Mourino N, Varela-Lema L, Ahluwalia JS, Rey-Brandariz J, Candal-Pedreira C, Ruano-Ravina A, Vila-Farinas A, Torres A, Pérez-Ríos M. **Influence of maternal smoking in pregnancy on blood pressure during childhood and adolescence: systematic review and meta-analysis.** Under peer review after submission to an international journal indexed to JCR.

   This systematic review, accompanied with meta-analysis, was conducted to identify the influence of maternal smoking in pregnancy on BP figures, both SBP and DBP, during childhood and adolescence.

3.1 SERUM COTININE CUT-POINTS FOR SECONDHAND SMOKE EXPOSURE ASSESSMENT IN CHILDREN UNDER 5 YEARS: A SYSTEMATIC REVIEW

A bibliographic search was conducted in Medline (PubMed) and Embase databases. The search protocol was registered on PROSPERO database (International Prospective Register of Systematic Reviews; registration number: CRD42021251263). Methods of the systematic review were reported following the PRISMA 2020 (**P**referred **R**eporting **I**tems for **S**ystematic Reviews and **M**eta-**A**nalyses) guidelines (71).

Pre-designed search strategy were developed in January 2020 by three expert reviewers in the matter and updated up to April 2021. The search terms included both MeSH and free terms. “serum cotinine”, “tobacco smoke pollution” (MeSH), “secondhand smoke” and “environmental tobacco smoke” were used as key words. Age filters were set (newborns-infant-preschool child). No restrictions were applied in terms of country, study period, study design, or language.

After excluding duplicate articles, the title and abstract of the records were screened individually by two independent reviewers to ascertain that they met eligibility criteria. In the case of papers considered potentially relevant, the full text was read to ensure that they fulfilled the inclusion/exclusion criteria. Differences of opinion about the inclusion or exclusion of any given paper were settled by consensus. To ensure the inclusion of all possible studies, the references selected were screened manually.

The review covered studies that assessed prenatal SHS exposure in newborns and/or postnatal SHS exposure in children under 5 years by using serum cotinine assays in addition or in lieu of parental self-report. Investigations with mother-child pairs and those grouping children under 5 with older ones, adolescents or adults were included; however, when the data from the studies were stratified by age group, only those that referred to the youngest age group, including children under 5 years of age, were selected for the review. Studies with serum cotinine obtained just from mothers, without cut-point values to distinguish between exposed and unexposed children, and those measuring cotinine from other biological fluids as urine, saliva, hair, meconium or maternal milk were excluded. No reports, communications to congresses, simulation studies, or retracted studies were included. Studies published in English, Spanish, French, and German were considered for inclusion.

Data from those studies meeting eligibility criteria were extracted by two reviewers using a data-extraction sheet designed in Microsoft Excel; discrepancies in interpretation were

resolved by consensus. The data extracted for analysis were: study characteristics (author, publication year, design, period of the study, use of questionnaire in conjunction with cotinine (yes/no) and country); population characteristics (sample size, age and race); information regarding the analytical technique used to quantify serum cotinine (radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), gas chromatography (GC), gas-chromatography mass spectrometry (GC/MS), gas-liquid chromatographic procedure with nitrogen-phosphorus-specific detector (GLC/NPD) and isotope dilution liquid chromatography tandem mass spectrometry (LC/MS)); serum cotinine cut-point values to classify SHS exposure in children and method for the selection of the cut-points (assay LOD, previous studies or other such as separation point in bimodal distribution of serum cotinine in smokers and nonsmokers, median cotinine levels of the cohort, Receiver Operating Characteristic (ROC) curves or percentiles).

A modified Newcastle-Ottawa scale (72) was used to assess appropriateness of the representativeness of the sample, participation rate, ascertainment of the exposure, consideration of child's race, and the adequacy of the method used for the selection of the cut-points. Studies were scored from 0 to 8 by each researcher. Score disagreements were resolved by consensus between two researchers, and a final agreed-upon rating was assigned to each study. In the event of any difference of opinion, a third researcher was consulted. Studies that obtained a score under 5 points were rated as poor-quality, those with a score of 5 points or more as high-quality.

3.2 ORIGINAL STUDIES USING DATA FROM THE HEALTH OUTCOMES AND MEASURES OF THE ENVIRONMENT (HOME) STUDY

Home Study: main characteristics

The HOME Study is a prospective and ongoing pregnancy and birth cohort that recruited pregnant women between 2003 and 2006, and conducted follow-up visits with the mothers (or children's caregivers) and their children through age 12 years (17, 18). From 2003 to 2006, 401 pregnant women were eligible to participate in the study; among these, 389 delivered singletons, 9 had sets of twins and 3, delivered stillbirths. From 2003 to 2014, up to 11-in person follow-up visits were conducted on 410 eligible children (390 singleton and 10 twin sets) at the delivery hospital, the study clinic, or participants' homes when infants/children

were approximately age 1 day, 4 weeks, and 1, 2, 3, 4, 5, and 8 years. From June 2016 to April 2019, 431 mothers and their 441 offspring were invited to return to the study clinic, including those whose mothers had dropped out during the run-in phase of the randomized trial, when they were on average 12.4 years (range: 11-14.1). Thirty-seven mothers were not contacted because the fetus or child was deceased, did not provide prenatal biospecimens or they indicated that they did not want to be contacted for future study visits (18). Follow-up was finally completed on 256 children (242 singletons and 7 twin sets) (Figure 1).

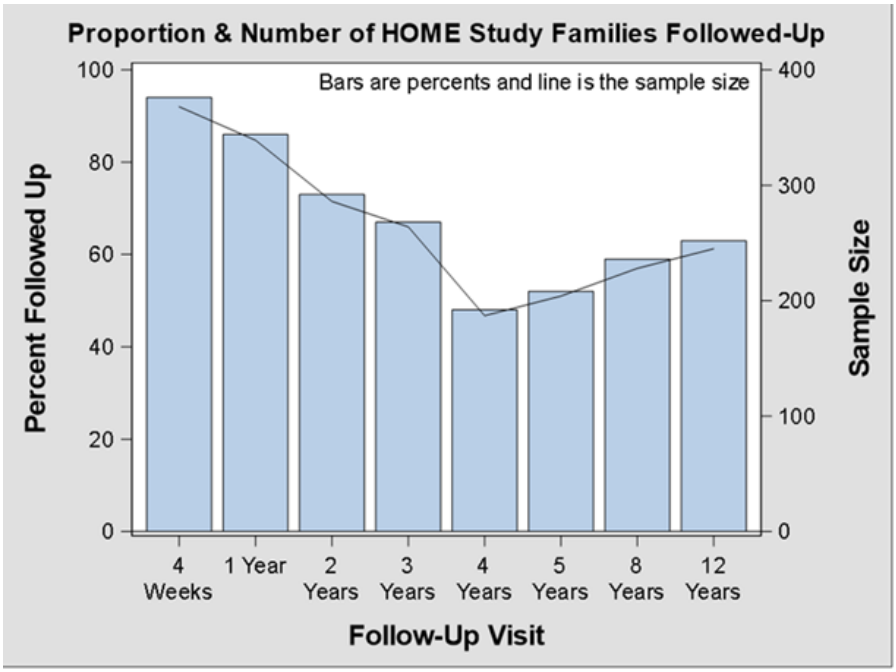

Figure 1. Proportion and number of HOME Study families followed up from 2003-2019.

The principal objective of the HOME Study was to evaluate the impact of pre- and postnatal exposures to low-level prevalent environmental toxicants on multiple child's health endpoints from infancy to adolescence.

The recruitment strategy was developed in collaboration with a local Community Advisory Board and a National Oversight Panel who reviewed the study and provided guidance in addressing research ethics, study design and reporting concentrations of environmental chemical biomarkers and clinically significant results to participants and their medical providers.

Pregnant women were identified through medical scheduling systems from 9 obstetric clinics affiliated with 3 hospitals in Cincinnati. A total of 5,512 letters were e-mailed to

pregnant women. Of these, 5,184 women were screened for eligibility: at least 18 years old, between 13 to 19 weeks of gestation, residing in a house built before 1978 within the study area (Butler, Clermont, Hamilton and Warren counties in Ohio metropolitan area; and Campbell, Brenton and Boone counties in Northern Kentucky), human immunodeficiency virus (HIV)-negative, not taking thyroid or epilepsy medication, no diagnosis of diabetes, schizophrenia, bipolar disorder and not undergoing chemotherapy or radiation therapy). Of the 1,263 eligible women, 468 initially agreed to participate. Sixty-seven women dropped out during a run-in phase of a nested, randomized controlled trial conducted to test the efficacy of lead hazard controls on blood lead concentrations and neurodevelopment, and the efficacy of injury hazard controls on injury incidence and severity (17).

Beginning in 2022, the HOME Study began conducting a new visit to examine the role of air pollution on mental health, as well as endocrine disrupting chemicals on bone health and density measures at age 16-20 years (Figure 2). Of note, The HOME study is not currently recruiting new participants but continues to follow those who were previously enrolled.

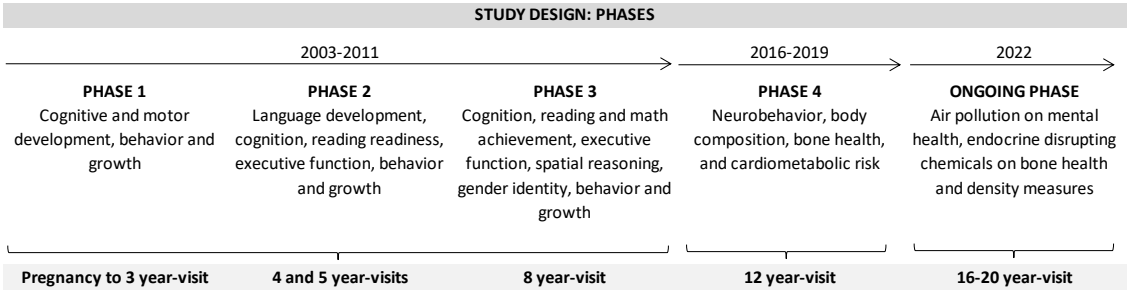

Figure 2. Phases of the Health and Outcomes Measures of the Environment (HOME) Study (2003-ongoing).

The institutional review boards (IRBs) at Cincinnati Children's Hospital Medical Center (CCHMC) and participating delivery hospitals approved the HOME Study protocols. Brown University and the Centers for Disease Control and Prevention (CDC) IRBs deferred to the CCHMC IRB. All mothers provided informed consent for themselves and their children at all visits; children provided informed assent at the 12-year visit.

3.2.1 Secondhand tobacco smoke exposure among children under 5 years old: questionnaires versus cotinine biomarkers: a cohort study

Concordance between maternal self-reported SHS exposure and children's biomarkers of SHS exposure was assessed using Cohen's kappa index for two observers, considering two categories (exposed/unexposed). To classify children's SHS exposure, maternal self-report regarding tobacco consumption and children's SHS exposure was ascertained via standardized questionnaires. Second, child's serum cotinine concentrations (ng/mL) at birth, 1, 2, 3 and 4 years were quantified. Third, children were classified as exposed/unexposed considering both the assay LOD threshold for cotinine and age-specific serum cotinine cut-points.

HOME Study participants

Data from mothers and their offspring (from birth to age 4 years) participating in the HOME Study were included. Analysis was restricted to children with mother-reported information (both on tobacco consumption and SHS exposure) and serum cotinine samples (N=280 newborns, and 270, 197, 196, and 150 children at ages 1, 2, 3 and 4 years, respectively).

Assessment of tobacco smoke exposure with maternal self-report via questionnaires

Maternal-reported tobacco consumption and children's SHS exposure was obtained by using standardized face-to-face interviews administered by a trained interviewer. The questionnaire was administered during pregnancy and at four different points during the follow-up when children were ages 1, 2, 3 and 4 years. At each interview, trained research staff surveyed the women about their smoking of cigarettes, cigars and pipes as well as the smoking of these products by other members of the household. Women were also asked about their SHS exposure and that of their child at home (living with a smoker who smokes at home), in the car or in other frequently visited homes and places (such as grandmother's home or daycare). Each mother was classified as either a smoker, exposed (non-smoker with SHS exposure), or unexposed (non-smoker with no SHS exposure). Each child was classified as exposed if the mother reported either being a smoker or living with a smoker who smokes

at home or if the mother reported that her child was exposed to SHS at home, in the car or in other homes and places ever, sometimes or seldom. Otherwise, children were classified as unexposed.

Assessment of tobacco smoke exposure with children's serum cotinine concentrations

Cotinine concentrations were measured in children's venous serum samples at birth (newborns' umbilical cord) and at age 1, 2, 3, and 4 years obtained via venipuncture. The samples were stored at or below -80ºC until analysis. Serum cotinine concentrations were determined by the CDC and Prevention Environmental Health Laboratories using high performance LC/MS (73).

The distribution of serum cotinine concentrations (range; 25^{th}, 50^{th}, and 75^{th} percentiles and geometric mean with 95% confidence interval (CI)) was ascertained. Considering the assay LOD threshold for cotinine, 0.015 ng/mL (73), children were classified as unexposed if their cotinine concentration was below the LOD and exposed if it was equal to or above the LOD (27).

Estimation of age-specific serum cotinine cut-points

Cotinine concentrations were $\log_{10}$-transformed prior to analysis to reduce the potential influence of outliers. Pairwise correlation between and reproducibility of children's $\log_{10}$-transformed serum cotinine concentrations between ages 1 and 4 years were estimated with Spearman correlation coefficients (rho) and intraclass correlation coefficient (ICC), respectively.

ROC curves were used to identify the optimal cut-points for discriminating SHS exposure from non-exposure in children at 1, 2, 3 and 4 years. Information reported by mothers regarding children's SHS exposure was considered to validate the discriminatory capacity of the ROC curve. Area under ROC curves (AUCs) was calculated for each age-specific cut-points. Cotinine cut-points were selected that maximized both sensitivity and specificity. Specificity-sensitivity and positive-negative predictive values (PPV-NPV) were calculated for each age-specific cut-point. Estimations were accompanied by 95% CI. Analysis was performed with Stata V.14.2.

After calculating children's age-specific cut-points, they were classified as unexposed if their cotinine concentration was below cut-points, and exposed if it was equal to or above the age-specific cut-points.

Concordance between self-reported exposure and serum cotinine measures

Kappa concordance coefficient accompanied by 95% CI and the percentage of agreement (%) were estimated considering both the assay LOD threshold and age-specific cut-points at 1, 2, 3 and 4 years of age.

3.2.2 Pre- and postnatal exposure to secondhand tobacco smoke and body composition at 12 years: periods of susceptibility

Multiple informant models were used to examine the associations of pre- and postnatal SHS exposure with adolescent's body composition measures, and to identify whether the estimates varied by period of exposure. To this effect, maternal serum cotinine at 16 and 26 weeks pregnancy, and children's serum cotinine at age 1, 2, 3 and 4 years were first averaged to estimate prenatal and postnatal exposure. Second, the 12-year visit anthropometry and dual energy x-ray absorptiometry (DXA) measures were determined. Third, mothers' and adolescents' characteristics were ascertained to adjust the models for potential confounders.

HOME Study participants

Data from mothers and their offspring participating at the 12-year HOME study visit were included. Final analysis was restricted to 217 adolescents with at least one serum cotinine measurement from 16 weeks pregnancy to age 4 years, one anthropometry or DXA measures at age 12 years, and with complete information on covariates.

Assessment of pre- and postnatal tobacco smoke exposure with serum cotinine concentrations

The distribution (overall, boys' and girls' median, 25th and 75th percentiles) of maternal serum cotinine at 16 and 26 weeks pregnancy (including active smoking and nonsmoking

mothers who could be exposed to SHS), and children's serum cotinine at age 1, 2, 3 and 4 years was ascertained. Average pre- and postnatal cotinine concentrations were log_{10}-transformed to reduce the potential influence of outliers. Spearman's rho was calculated to assess correlation between maternal log_{10}-transformed serum cotinine concentrations from 16 and 26 weeks pregnancy, and between children's cotinine at different moments between ages 1 and 4 years. The ICC was calculated to assess reproducibility of pre- and postnatal log_{10}-transformed cotinine concentrations at each time point. The ICC between repeated maternal cotinine concentrations (analysis restricted to 220 mothers with cotinine measures at 16 and 26 weeks pregnancy) was 0.96 (95% CI 0.94 to 0.97); the ICC between the four childhood cotinine concentrations (analysis restricted to 65 children with cotinine measures at 1, 2, 3 and 4 years) was 0.95 (95% CI 0.93 to 0.97). Given the excellent agreement, available maternal serum cotinine concentrations (range = 1-2 measures), and children's serum cotinine concentrations (range = 1-4 measures) were averaged to assess pre- and postnatal exposure, respectively, in further analysis. The ICC between average pre- and postnatal cotinine concentrations was also calculated.

Assessment of adolescent's body composition with anthropometry and DXA measures

When adolescents were on average 12.4 years old (range: 11-14 years), trained staff measured weight, height, and waist circumference to the nearest 0.01 kg or 0.1 cm in triplicate following standardized protocols (18). Children wore hospital scrubs and removed their shoes and head coverings during anthropometric assessments. BMI in kg/m^2 from weight and height was calculated and age- and sex-specific BMI z-scores were obtained based on CDC growth curves. Childhood overweight and obesity were defined as BMI z-scores above 1 and 2, respectively based on CDC growth reference data for 5-19 years (74).

At the same visit, trained and experienced technicians measured body composition with DXA using a Hologic Horizon Densitometer. Body fat mass index was calculated as whole body fat mass (kg) divided by height $(m)^2$. Body lean mass index, a measure of skeletal muscle mass and lean soft tissues that does not include bone mineral content, was calculated as lean body mass (kg) divided by height $(m)^2$. Age- and sex-specific fat mass index and lean mass index standardized z-scores were calculated based on the pediatric reference curves generated from the NHANES 1999−2004 (75). Body fat mass percent, android and gynoid fat

percent, and android to gynoid percent fat ratio (percent fat measured in the android region divided by percent fat measured in the gynoid region) were estimated. The whole-body DXA scan also yielded a measure of visceral fat area (cm^2), highly correlated with visceral fat area estimated by computed tomography (76). Scans were analyzed using NHANES body composition analysis calibration.

The distribution (overall, boys' and girls' mean (standard desviation (SD)) or N (%)) of the 12-year visit anthropometry measures (weight, height, BMI z-scores, waist circumference), and DXA measures (body fat mass index z-score, body fat mass percent, body lean mass index z-score, android fat percent, gynoid fat percent and android/gynoid fat percent ratio) was determined. Spearman's rho between anthropometry and DXA measures were estimated.

Assessment of mothers' and adolescents' characteristics as potential confounders

Based on previous literature and a directed acyclic graphs (DAGs) (77), potential confounders that may be associated with both serum cotinine concentrations and adolescent's body composition were identified (Figure 3 and Figure 4).

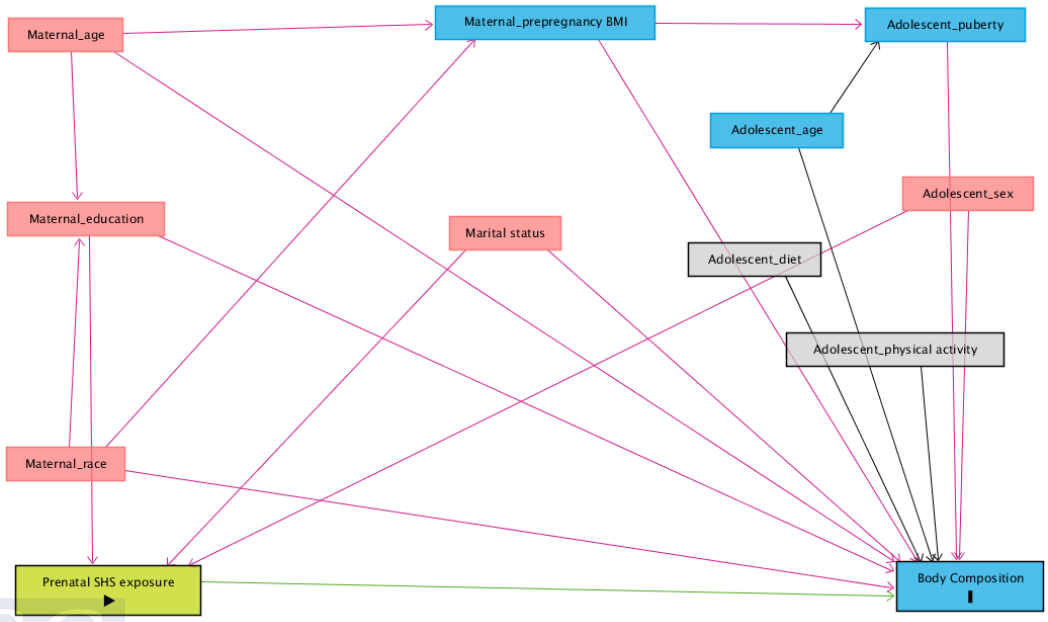

Figure 3. Directed acyclic graph of potential confounders of the association between prenatal serum cotinine concentrations and adolescent's body composition.

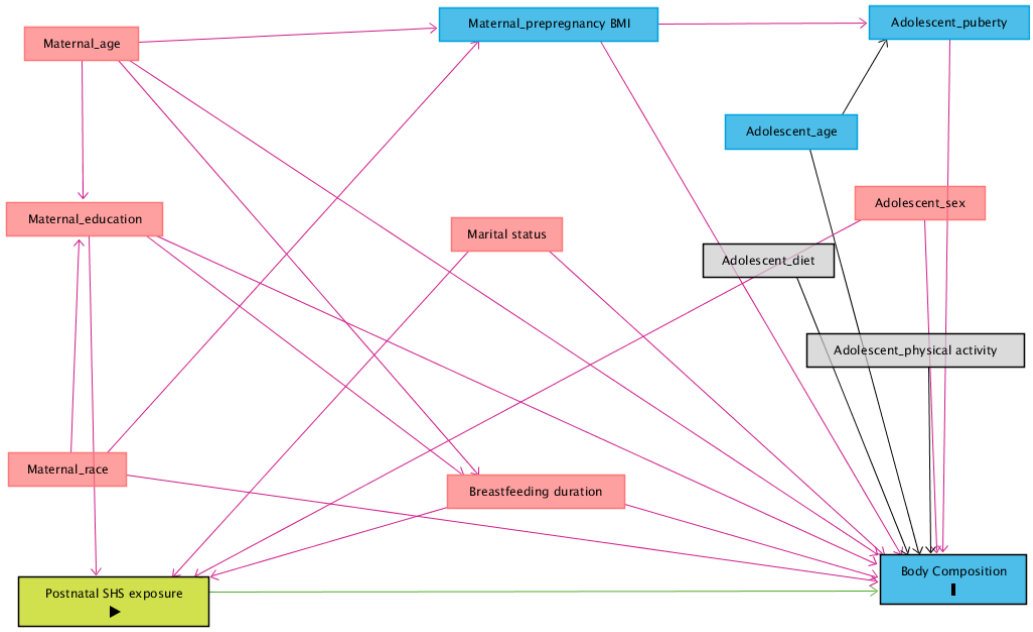

Figure 4. Directed acyclic graph of potential confounders of the association between postnatal serum cotinine concentrations and adolescent's body composition.

Trained research staff collected sociodemographic covariates using standardized computer-assisted interviews, including maternal age, race/ethnicity, marital status, and education in the second or third trimester of pregnancy, and breastfeeding throughout the first three years of life. Self-reported weight and height (or imputed weight if missing) was used to calculate maternal pre-pregnancy BMI (78). Child's sex was obtained from medical records. Pubertal status was self-evaluated using Tanner stage (I-V) based on pubic hair development in both sexes at the 12-year study visit (79), and it was positively correlated with serum concentrations of gonadal hormones from this visit (80).

Trained research staff at the National Institutes of Health-funded Clinical Translational Research Center Bionutrition Core collected 24-hour food recalls from adolescents (2 weekdays and 1 weekend day). Food recalls were analyzed using the Nutrition Data systems for Research software and foods database (University of Minnesota, MN); the Healthy Eating Index (HEI) score (2010) was calculated as a measure of diet quality in terms of conformance with federal dietary guidance (81). Adolescent's physical activity level was also assessed by administering the validated Physical Activity Questionnaire for Older Children and calculating the activity summary score (82).

Multiple informant models, sensitivity analyses, and Poisson regression

Covariate-adjusted differences in each anthropometry and DXA measures associated per 1-unit increase in pre- and postnatal average log_{10}-transformed serum cotinine concentrations (β and 95% CI) were estimated by using multiple informant models.

The multiple informant model utilizes generalized estimating equations (GEE) to jointly estimate the exposure-outcome association for each defined exposure period and test whether association vary across the periods (83, 84). Two separate joint estimates were created, one for prenatal exposure period and another for postnatal exposure period. The period-specific cotinine effect estimates were generated from a model with an interaction term between exposure period and serum cotinine. The interaction p-value obtained is for the two-way interaction term between exposure period and serum cotinine. The null hypothesis is that associations are the same across the two-time period; interaction p-values < 0.05 were considered as evidence that at least one of the cotinine-outcome associations differed from the rest. Potential modifying effects of adolescent's sex was examined by using a three-way interaction term of exposure period, cotinine and adolescent's sex. All multiple informant models were adjusted for maternal age, race/ethnicity, marital status, education, pre-pregnancy BMI and breastfeeding duration. For outcome variables that were not z-scores, models were additionally adjusted for adolescent's sex, pubertal stage, and age. Sensitivity analyses were conducted by further adjusting for adolescent's HEI 2020 total scores and physical activity summary scores and after excluding active smoking mothers during pregnancy (serum cotinine concentrations equal to or above 3 ng/mL at 16 or 26 weeks gestation (27)).

Risk of overweight/obesity at age 12 years with increasing pre- and postnatal cotinine concentrations was estimated by using modified Poisson regression with robust standard errors. Overweight/obesity was defined as having an age- and sex- standardized BMI z-score above 1 (85).

Analyses were performed using R statistical software, version 4.1.2 (R Core Team, Vienna, Austria).

3.2.3 Pre- and postnatal exposure to secondhand tobacco smoke and cardiometabolic risk at 12 years: periods of susceptibility

Multiple informant models were used to examine the associations of pre- and postnatal SHS exposure with adolescents' body composition measures, and to identify whether the estimates vary by period of exposure and adolescent's sex. To this effect, maternal serum cotinine at 16 and 26 weeks pregnancy, and children's serum cotinine at age 1, 2, 3 and 4 years were first ascertained. Second, the 12-year visit CM risk components were determined. Third, mothers' and adolescents' characteristics were ascertained to adjust the models for potential confounders.

HOME Study participants

Data from mothers and their offspring participating at the 12-year HOME Study visit were included. The final analysis was restricted to 190 adolescents with at least one serum cotinine measurement from 16 weeks pregnancy to age 4 years, one CM risk component measurement at age 12 years, and with complete information on covariates.

Assessment of pre- and postnatal tobacco smoke exposure with serum cotinine concentrations

Assessment was conducted in the same manner as mentioned in section 3.2.2.

Assessment of adolescent's CM health with the CM risk summary score and individual CM risk components

Overnight-fasting blood sample from adolescents was obtained via venipuncture. Serum glucose (mg/dL), insulin (mIU/L), TG (mg/dL), high-density lipoprotein cholesterol (HDL-C) (mg/dL), leptin and adiponectin (ng/mL) concentrations were measured by using valid immunoassays. Trained laboratory technicians in the CCHMC National Institutes of Health (NIH)-funded Clinical Translational Research Center Core Laboratory conducted all assays; based on 22 blinded duplicates, the coefficient of variations for glucose, HDL-C, TG, insulin, leptin (ng/mL) and adiponectin were 2.0%, 3.0%, 6.5%, 9.5%, 11.1% and 13.1%, respectively. Three sitting BP measurements, each one minute apart, were obtained with an

oscillometric monitor (Dinamap Pro100) (86, 87). Iliac waist circumference was measured around a horizontal plane defined by the iliac crests. Visceral fat area was measured by using DXA (Hologic Horizon Densitometer) (88).

The distribution (overall, boys' and girls' mean (SD) or N (%)) of the 12-year visit CM risk measures (adolescent's homeostatic model assessment for insulin resistance (HOMA-IR= fasting insulin x fasting glucose/405) (89), TG to HDL-C ratio, leptin to adiponectin ratio, average of the 2nd and 3rd measures of SBP (90) and visceral fat area (cm^2)) was determined. Spearman's rho between CM risk components were estimated.

Linear regression models of each individual CM risk component (dependent variable) with age and sex as predictors were run to derive the age and sex-specific z-scores after standardization of the residuals (89). BP z-scores were sex-, age-, and height-standardized according to the Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in children and adolescents (45). HOMA-IR, TG to HDL-C ratio, leptin to adiponectin ratio, and visceral fat area were not normally distributed (right skewed) and thus, they were log_2-transformed in the model before standardization. After summing the standardized z-scores for the corresponding individual CM risk components, a continuous CM risk summary score was constructed for each adolescent, with higher values indicative of worse MetS profile compared to lower values (11) CM risk score (mean (SD)) was presented according to mothers' and adolescents' characteristics.

Assessment of mothers' and adolescents' characteristics as potential confounders

The same potential confounders as abovementioned in prior study (section 3.2.2) were identified. Pregnancy-induced hypertensive disorders obtained from medical records were also included in the analysis (Figure 5 and Figure 6).

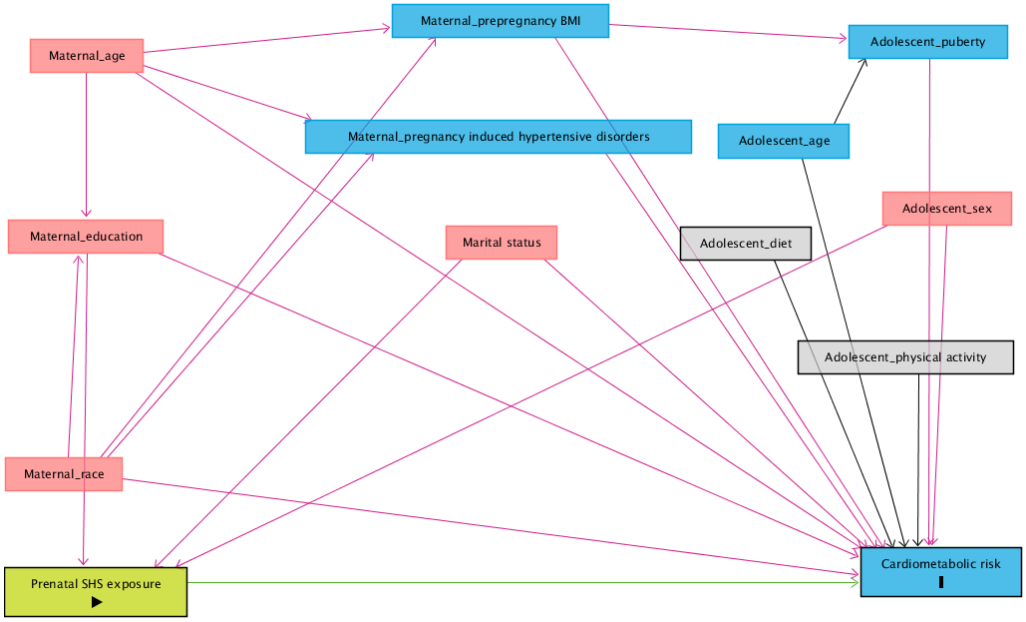

Figure 5. Directed acyclic graph of potential confounders of the association between prenatal serum cotinine concentrations and adolescent's cardiometabolic risk.

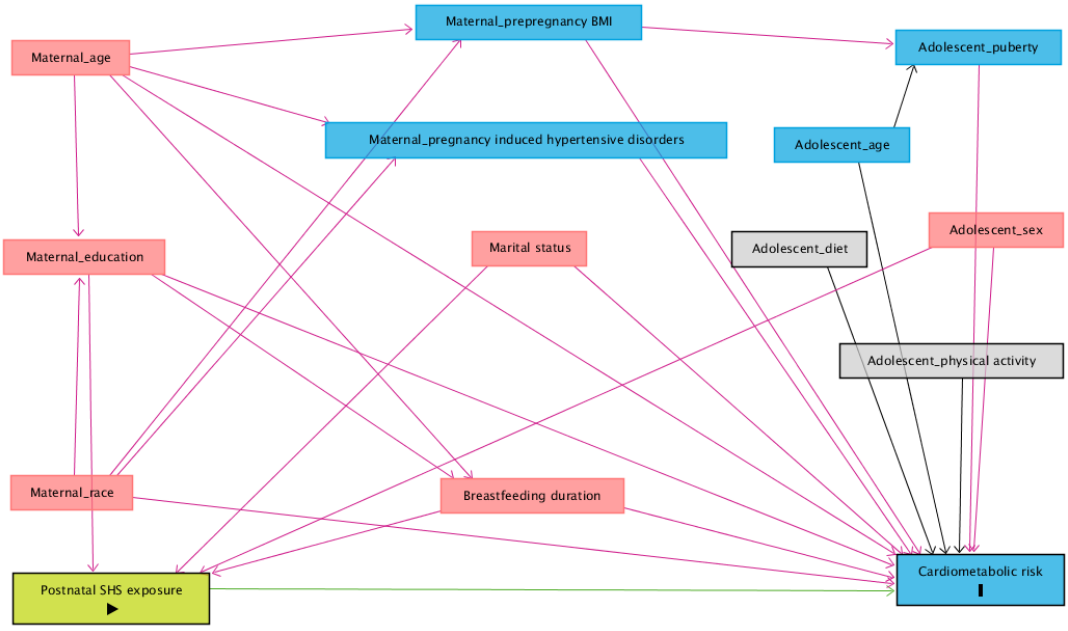

Figure 6. Directed acyclic graph of potential confounders of the association between postnatal serum cotinine concentrations and adolescent's cardiometabolic risk.

Multiple informant models and sensitivity analyses

Covariate-adjusted differences in CM risk score and their individual components associated per 1-unit increase in pre- and postnatal average log_{10}-transformed serum cotinine concentrations (β and 95% CI) were estimated by using multiple informant models and sensitivity analysis in the same manner as explained in prior study (section 3.2.2). For this study, a further sensitivity analysis was conducted after excluding those mothers with pregnancy induced hypertensive disorders (maternal SBP equal to or above 140 mmHg and/or DBP equal to or above 90 mmHg after 20 weeks of pregnancy) (91).

3.3 INFLUENCE OF MATERNAL SMOKING IN PREGNANCY ON BLOOD PRESSURE DURING CHILDHOOD AND ADOLESCENCE: SYSTEMATIC REVIEW AND META-ANALYSIS

Systematic review

A bibliographic search was conducted in Medline (PubMed) and Embase databases. The search protocol was registered on PROSPERO database (registration number: CRD42021247824). Methods and results of the systematic review were reported following the PRISMA 2020 guidelines (71). Pre-designed search strategy was drawn up in January 2021 by three expert reviewers in the matter and updated up to March 2022. The search terms included both MeSH and free terms: “tobacco smoke pollution”, “smoking”, “tobacco smoking”, “cigarette smoking”, “pregnancy”, “hypertension”, “blood pressure”, "passive smoking", "secondhand smoke", "environmental tobacco smoke", smok*, "maternal smoking", "paternal smoking", "arterial pressure", "hypertension" "systolic pressure", "diastolic pressure", and "mean blood pressure". No restrictions were applied in terms of country, study period, study design, or language.

After excluding duplicate articles from the search, the title and abstract of the records were screened individually by three independent reviewers to ascertain that they met eligibility criteria. In the case of papers considered potentially relevant, the full text was read to ensure that they fulfilled the inclusion/exclusion criteria. Differences of opinion about the

inclusion or exclusion of any given paper were settled by consensus. To ensure the inclusion of all possible studies, the references selected were screened manually.

The review covered studies that evaluated the association between smoking in pregnancy and the BP of offspring aged 3 through 17 years. Thus, we used the PECO statement (Participants, Exposure, Comparator and Outcomes) to answer the question: "Among children or adolescents, what is the effect of maternal tobacco consumption during pregnancy, compared to those whose mothers did not smoke?". Difference in children's and/or adolescents' mean DBP and/or SBP figures (in mmHg), according to maternal smoking during pregnancy, was considered as the outcome variable. Prospective cohort studies that provided the necessary data to calculate differences in means and their 95% CIs were included. When different papers based on the same study were identified, those that had the largest sample size and with the most up-to-date data were selected. In the case that the outcome variable was adjusted for different covariates, the model with the largest adjustment was included. When studies provided exclusively children's and/or adolescents' mean DBP and/or SBP figures, according to maternal smoking, EPIDAT program was used to calculate the coefficient of the difference between means along with the 95% CI.

The studies with the following characteristics were excluded: studies including women who consumed exclusively e-cigarettes or other tobacco products; studies that reported combined results (women's use of other tobacco products in addition to cigarettes); studies that evaluated exposure to SHS during pregnancy, not exclusively due to maternal smoking; studies that estimated mean DBP and/or SBP figures without adjustment for possible covariates; and studies that included smoking children and/or adolescents. Furthermore, communications to congresses, letters to the editor, opinion articles, narrative reviews, case-control studies, cross-sectional studies, case series, simulation studies, or studies which had been retracted were excluded. Studies published in Spanish, English or Portuguese were considered for inclusion.

Data from those meeting eligibility criteria were extracted by two reviewers, and both sets of extractions were then reviewed by a third. Differences of opinion were discussed and settled by consensus. From each paper, data were extracted on: author and year of publication of the study; period of recruitment of pregnant women; country of study; data source (hospital, clinic, or general population); age of children in whom BP was evaluated; sample size; definition of maternal smoking in pregnancy; number of BP measures; BP measurement

method (oscillometry, digital sphygmomanometry and standard mercury or manual sphygmomanometry); difference in mean SBP/DBP figures (in mmHg) along with the 95% CI, considering the maternal smoking in pregnancy; and the variables included in the adjusted models.

Meta-analysis

To perform the meta-analysis, the difference in mean SBP/ DBP figures in children or adolescents, according to whether their mothers had or had not been smokers during pregnancy, was calculated. A random effects model was applied, and separate analyses were performed for the DBP and/or SBP figures, using the study's covariate-adjusted results.

Between-study heterogeneity was evaluated using the p-value of the Cochran's Q test and the I^2 statistic. A p-value < 0.1 indicates the presence of heterogeneity, (substantial heterogeneity if I^2 higher than 50%) (92-94). Presence of publication bias was analyzed using a funnel plot, Egger's regression test for funnel plot asymmetry, and Begg's test (95, 96).

A leave one out analysis to ascertain the influence of each study, and the following meta-analyses by subgroups were performed: recruitment period (1958-1989; 1990-2000; 2001-2007); continent (European or non-European); BP measurement method (digital sphygmomanometry or oscillometry versus (vs.) standard mercury or manual sphygmomanometry); study quality (low vs. medium/high); and studies that adjusted for children's birth weight (yes vs. no); All analyses were performed using the STATA statistical analysis software program v17.

Assessment of quality and level of evidence

A modified Newcastle-Ottawa scale (2) was used to assess the quality of the studies included in the meta-analysis. Two researchers screened each study separately by reference to the following domains (97): recruitment strategy, selection criteria, exposure assessment, covariate-adjustment, and outcome assessment. Studies were scored from 0 to 10 by each researcher. Score disagreements were resolved by consensus between two researchers, and a final agreed-upon rating was assigned to each study. In the event of any difference of opinion, a third researcher was consulted. Studies that obtained a score below 5 points were rated as

low-quality, those with a score of 5-6 points as moderate-quality, and those with a score of 7 points or more as high-quality. Although no studies were excluded on the basis of the evaluation of risk of bias, a sensitivity analysis was performed with the lowest and highest quality studies. Evidence levels were rated using the GRADE (**G**rading of **R**ecommendations, **A**ssessment, **D**evelopment and **E**valuation) system. The GRADE system allows for classification of evidence into four grades of evidence (high quality, moderate, low, and very low) attending to the risk of bias, inconsistency, uncertainty, inaccuracy, publication bias and other considerations (98).

PART FOUR: RESULTS

RESULTS

PAPER 1

Mourino N, Ruano-Raviña A, Varela Lema L, et al. **Serum cotinine cut-points for secondhand smoke exposure assessment in children under 5 years: A systemic review**. PLoS One. 2022;17(5):e0267319. Q2 journal with an impact factor of 3.75 and occupying the 29/73 position among multidisciplinary sciences journals in JCR.

A total of 247 articles were obtained after the systematic review in PubMed and Embase and 51, published between 1985 and 2020, fulfilled the inclusion criteria. Figure 7 and Table 2 show the main characteristics of the included articles. Thirty-three out of 51 papers measured postnatal exposure to SHS with serum cotinine; 17 prenatal SHS exposure with newborns' umbilical cord serum cotinine, and one both pre- and postnatal exposure. Among the 33 studies assessing postnatal exposure, 30 grouped children under 5 years with older, adolescents and adults; the remaining 3 papers, measured exposure in children aged 4 months-3 years with sample sizes ranging from 132 to 504 children (Table 2).

In order to ascertain SHS exposure among children, cut-point values ranged between 0.015-35 ng/mL, except in one study that used 100 ng/mL. The most commonly used threshold was 0.05 ng/mL (17 out of 51), which derives from the assay LOD used by the NHANES (Table 2). LC/MS was used in 35 of the selected papers, and 26 studies mentioned the assay LOD threshold as cut-point. One study calculated age-specific cut-point with ROC curves (52): 0.9 ng/mL (under 12 years) vs. 0.6 ng/mL (12 years and older). However, none of the selected articles proposed sex, race or age-specific serum cotinine cut-points in children under 5 years.

When applying the modified Newcastle-Ottawa scale, 2 cohort studies and one cross-sectional study, which used data from NHANES, were rated as being high-quality (99-101), and thirty-six studies were rated as being low-quality. The most frequent reason for the low quality assigned was insufficient data on the characterization of SHS exposure and unsatisfactory participation rate.

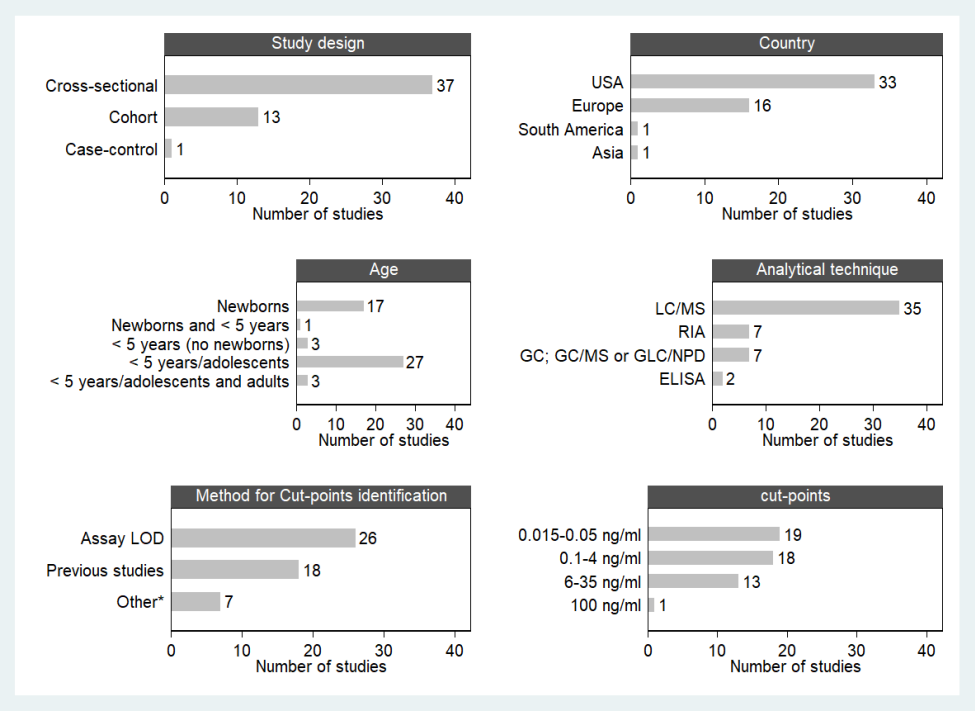

Figure 7. Study and population characteristics (design, country, and age); analytical technique for serum cotinine quantification, methods for cut-point identification (assay limit of detection, previous studies or other (separation point in the bimodal distribution, median cotinine levels of the cohort, receiver operating characteristic curves or percentiles)) and cut-point values range (ng/mL).

LC/MS, isotope dilution-liquid chromatography-tandem mass spectrometry; RIA, radioimmunoassay; GC, gas chromatography ; GC/MS, gas-chromatography mass spectrometry; GLC/NPD, gas-liquid chromatographic procedure with nitrogen-phosphorus-specific detector; ELISA, enzyme-linked immunosorbent assay (ELISA); LOD, limit of detection.

Table 2. Study and population characteristics; analytical technique for serum cotinine quantification; cotinine cut-points (ng/mL) and method for their selection.

STUDY CHARACTERISTICS				STUDY CHARACTERISTICS			CUT-POINTS SELECTION		
Author,yr (reference)	**Design, period**	**Questio -nnaire**	**Country**	**N**	**Age**	**Race**	**Assay**	**Cut-point**	**Method**
Luck et al, 1985(102)	Cross-sectional N/A	Yes	Germany	8	Newborns	N/A	GLC/NPD	**5**	Assay LOD
Ahlsten et al, 1989(103)	Cross-sectional N/A	Yes	Sweden	39	Newborns	N/A	GC	**1**	Assay LOD
Etzel et al, 1992(104)	Cohort study 1964-1983	No	USA	132	4 months-3 years	White, black or mixed race	RIA	**2.5**	ROC curves
Bardy et al, 1993(105)	Cross-sectional 1991	Yes	Finland	1,237	Newborns	N/A	GC/MS	**6**	Assay LOD
CDC,1993 (106)	Cross-sectional (NHANES, 1988-1991)	Yes	USA	800†	4-91 years	N/A	LC/MS	**10/ 15**	Separation point in bimodal distribution
Bardy et al, 1994(107)	Cross-sectional 1991	Yes	Finland	1,323	Newborns	N/A	GC/MS	**35**	Assay LOD
Martinez et al, 1994(108)	Cohort study 1980-1984	Yes	USA	175	Newborns	Hispanic or other non-Anglo	RIA	**1**	Assay LOD
Ruhle et al, 1995(109)	Cross-sectional N/A	Yes	Germany	75	Newborns	N/A	RIA	**15**	Data from previous study
Pirkle et al, 1996(30)	Cross-sectional (NHANES, 1988-1991)	Yes	USA	1,793‡	4-11 years	Non-Hispanic white, non-Hispanic black, Mexican American or other	LC/MS	**10/ 15**	Separation point in bimodal distribution
Nafstad et al, 1996(110)	Cohort study 1992-1993	Yes	Norway	202	Newborns	N/A	RIA	**14**	Data from previous study
Bearer et al, 1997(111)	Case-control 1991-1992	Yes	USA	70	Newborns	Non-Hispanic white, non-Hispanic black or other (Hispanic and Asian)	GC/MS	**1**	Assay LOD
Pichini et al, 2000(99)	Cohort study 1997-1998	Yes	Spain	429	Newborns	N/A	RIA	**1,78**	ROC curves

Mannino et al, 2001(112)	Cross-sectional (NHANES, 1988-1994)	Yes	USA	1,533	4-6 years	Non-Hispanic white, non-Hispanic black or Mexican American	LC/MS	**0.05**	Assay LOD
Strauss, 2001(113)	Cross-sectional (NHANES, 1988-1991)	Yes	USA	2,968‡	4-18 years	N/A	LC/MS	**2**	Data from previous study
Mannino et al, 2001(101)	Cross-sectional (NHANES, 1989-1994)	Yes	USA	5,653‡	4-16 years	Non-Hispanic white, non-Hispanic black or Mexican American	LC/MS	**0.05**	Assay LOD
Lieu et al, 2002(114)	Cross-sectional (NHANES, 1989-1994)	Yes	USA	1,825‡	4-12 years	Non-Hispanic white, non-Hispanic black or Mexican American	LC/MS	**10/ 20**	Data from previous study
Mannino et al, 2002(115)	Cross-sectional (NHANES, 1989-1994)	No	USA	523‡	4-16 years	Non-Hispanic white, non-Hispanic black or Mexican American	LC/MS	**0.05**	Assay LOD
Aligne et al, 2003(116)	Cross-sectional (NHANES, 1989-1994)	No	USA	3,531‡	4-11 years	Non-Hispanic white, non-Hispanic black, Mexican American or other	LC/MS	**0.05**	Assay LOD
Rubin et al, 2004(117)	Cross-sectional (NHANES, 1989-1994)	Yes	USA	6,153‡	4-16 years	N/A	LC/MS	**2.9**	Data from a previous study
Pino et al, 2004(118)	Cross-sectional 1995-1997	No	Chile	504	1 year	N/A	RIA	**100**	Assay LOD (from a study conducted in 1987)
Wilkinson et al,2006 (119)	Cross-sectional (NHANES, 1989-1991)	Yes	USA	2,516‡	4- 16 years	Non-Hispanic white, non-Hispanic black, Mexican American or other	LC/MS	**0.05**	Assay LOD

Pirkle et al, 2006(28)	Cross-sectional (NHANES, 1989-2002)	Yes	USA	1988-91: 1,839‡ 1991-94: 2,090‡ 1999-2000: 1,065‡ 2001-02: 1,278‡	4-11 years	Non-Hispanic white, non-Hispanic black, Mexican American or other	LC/MS	**0.05 0.015**	Assay LOD
Franchini et al, 2008(120)	Cross-sectional 2004-2005	Yes	Italy	979	Newborns	Italian or foreign	GC/MS	**1**	Data from previous studies
Puig et al, 2008(121)	Cohort Study 1996-1998	Yes	Spain	487	Newborns	Spanish or non-Spanish	RIA	**1**	Data from previous study
Max et al, 2009(122)	Cross-sectional (NHANES, 1999-2006)	Yes	USA	1999-2000: 1,179‡ 2001-02: 1,423‡ 2003-04: 1,265‡ 2005-06: 1,300‡	3-11 years	Non-Hispanic white, non-Hispanic black, Mexican American, other Hispanic or other	LC/MS	**0.05 0.015**	Assay LOD
Dixon et al, 2009(123)	Cross-sectional (NHANES, 1999-2004)	Yes	USA	829‡	1-5 years	Non-Hispanic white, non-Hispanic black, Hispanic or other	LC/MS	**0.05 0.015**	Assay LOD
CDC (Vital signs), 2010(124)	Cross-sectional (NHANES, 1999-2008)	Yes	USA	N/A	3-11 years	Non-Hispanic white, non-Hispanic black, Mexican American or other	LC/MS	**0.05**	Assay LOD
Dove et al, 2010(125)	Cross-sectional (NHANES, 1999-2006)	Yes	USA	1,582‡	3- 5 years	Non-Hispanic white, non-Hispanic black, Mexican American or other	LC/MS	**0.05**	Assay LOD
Vesper et al, 2010(126)	Cross-sectional (NHANES, 2003-2004)	No	USA	N/A	3-11 years	Non-Hispanic white, non-Hispanic black or Mexican American	LC/MS	**10**	Data from a previous study
Xu et al, 2010(127)	Cross-sectional (NHANES, 2001-2002)	No	USA	4,508‡	4-15 years	Non-Hispanic white, non-Hispanic black, Mexican American	LC/MS	**0.035**	33rd and 67th percentiles (serum cotinine levels)

						or other			
Preston et al, 2010(128)	Cross-sectional, 2004-2005	Yes	USA	30	Newborns	Asian, Caucasian or African American	LC/MS	**1**	Data from a previous study
Sharief et al, 2011(31)	Cross-sectional (NHANES, 2005-2006)	No	USA	3,136†	1-21 years	Non-Hispanic white, non-Hispanic black, Mexican American or other	LC/MS	**2.9**	Data from a previous study
Spanier et al, 2011(100)	Cohort study 2003-2006	Yes	USA	Newborn: 273 1 year: 275 2 years: 206	Newborn-2 years	Non-Hispanic White, non-Hispanic Black or other	LC/MS	**0.015**	Assay LOD
Cardwell et al, 2012(129)	Cross-sectional N/A	No	USA	220‡	1-16 years	N/A	LC/MS	**0.9** **0.6**	ROC curves
Dempsey et al, 2012(25)	Cross-sectional with a matched case control substudy 2009-2010	Yes	USA	274‡	8 months-17 years (70% were under 3 years)	Latino, African American, Asian, White non-Hispanic or other	LC/MS	**0.05**	Assay LOD
Wang et al, 2013(130)	Cohort study N/A	Yes	China	14	Newborns	N/A	LC/MS	**0,12**	Median cotinine levels
Andersen et al, 2013(131)	Cross-sectional 1988-1990	No	Denmark	133	Newborns	N/A	Immulite 2000	**5**	Data from previous study
Kit et al, 2013(132)	Cross-sectional (NHANES, 1988-2010)	Yes	USA	1988-1994: 248‡ 1999-2004: 336‡ 2005-10: 392‡	4-11 years	Non-Hispanic White, non-Hispanic Black, Mexican American or other	LC/MS	**0.05**	Assay LOD
Florath et al, 2014(133)	Cohort study 2000-2001	Yes	Germany	972	Newborns	German or non-German	LC/MS	**14**	Data from previous studies
Howrylak et al, 2014(134)	Cohort Study 2010-2011	Yes	USA	619	1-16 years	White, African American, Multiracial or other	LC/MS	**0.1**	Assay LOD
Mason et al, 2015(32)	Cross-sectional (NHANES, 2007-2010)	No	USA	N/A†	3 or more years	N/A	LC/MS	**0.05** **0.015**	Assay LOD

West et al, 2015(135)	Cohort study 1980-2014	Yes	Finland	1,578‡	3-18 years	N/A	GC	**3**	Data from a previous study
Merianos et al, 2017(136)	Cross-sectional (NHANES, 2009-2012)	No	USA	2,707‡	3-11 years	Non Hispanic White, non Hispanic-Black, Hispanic, other races/multi racial	LC/MS	**0.05**	Assay LOD
Shenassa et al, 2017(137)	Cross-sectional (NHANES, 1999-2012)	Yes	USA	2,679‡	3-5 years	Non-Hispanic White, non-Hispanic Black, Mexican American or other	LC/MS	**10**	Data from a previous study
Yilmaz et al, 2018(138)	Cross-sectional 2012-2013	No	Turkey	150	1-3 years	N/A	ELISA	**3**	Data from a previous study
Hedengran et al, 2018(139)	Cohort study 2003-2004	Yes	Denmark	263	Newborns	N/A	LC/MS	**0.2**	Data from a previous study
Nwosu et al, 2018(140)	Cross-sectional (NHANES, 2009-2010)	No	USA	1,013‡	3-9 years	Non-Hispanic White, Mexican American, Other Hispanics, African American or other	LC/MS	**0.05**	Assay LOD
Chelchowska et al, 2019(141)	Cohort study 2013-2015	Yes	Poland	80	Newborns	Caucasian	ELISA	**13.7**	Data from a previous study
Brody et al, 2019(3)	Cross-sectional (NHANES, 2013-2016)	Yes	USA	2,833‡	3-11 years	Non-Hispanic White, non-Hispanic Black, non-Hispanic Asian or Hispanic	LC/MS	**0.05**	Assay LOD
Biren et al, 2020(142)	Cross-sectional (NHANES, 2015-2016)	Yes	USA	257	3-5 years	Non-Hispanic white, non Hispanic-black, Mexican-American, other Hispanic or other/multi race.	LC/MS	**0.05**	Assay LOD

Rovio et al, 2020(143)	Cohort study 1980-2011	Yes	Findland	1,504‡	3-18 years	N/A	LC/MS	**3**	Data from a previous study

†Sample sizes includes children (3-11 years), adolescents (12-19 years) and adults (older than 19 years).

‡Sample size includes children aged 5 years and older.

N/A, not available; GLC/NPD, gas-liquid chromatographic procedure with nitrogen-phosphorus-specific detector; LOD, limit of detection; GC, gas chromatography; USA, United States of America; RIA, radioimmunoassay; ROC, receiver operating characteristic; GC/MS,Gas- chromatography mass spectrometry; NHANES, National Health and Nutrition Examination Survey; LC/MS, isotope dilution-liquid chromatography-tandem mass spectrometry; SHS, secondhand smoke; ELISA, Enzyme-linked immunosorbent assay.

PAPER 2

Mourino N, Pérez-Ríos M, Santiago-Pérez MI, Lanphear B, Yolton K, Braun JM. **Secondhand tobacco smoke exposure among children under 5 years old: questionnaires versus cotinine biomarkers: a cohort study**. BMJ Open. 2021;11(6):e044829. Q2 journal with an impact factor of 3.02 and occupying the 85/172 position among medicine, general and internal journals in JCR.

At baseline (N=384), 31% of pregnant women were between ages 30 and 34 years, 62% were non-Hispanic white, 75% had more than high school education, 81% were employed, 78% lived with a spouse or partner and 71% had private health insurance. The characteristics of the final sample at age 4 years (N=187) did not differ from those of the participants at baseline.

Children's geometric mean serum cotinine concentrations from 1 to 4 years was higher than newborns' geometric mean umbilical cord serum concentrations (Table 3).

Table 3. Descriptive statistics of serum cotinine concentrations at birth and at 1, 2, 3 and 4 years: N, range, quartiles and geometric mean with 95% CI.

			Quartiles			Geometric mean		
	N	Range (ng/mL)	P25	P50	P75	Mean	95% CI	
Newborns†	280	0.00001 - 261.0	0.003	0.017	0.088	0.022	0.015	0.032
Children†								
1 year	270	0.00030 - 35.3	0.023	0.063	0.357	0.093	0.073	0.118
2 years	197	0.00126 - 10.5	0.020	0.046	0.212	0.070	0.053	0.092
3 years	196	0.00032 - 21.6	0.012	0.033	0.199	0.046	0.034	0.064
4 years	150	0.00024 - 14.9	0.013	0.027	0.249	0.047	0.033	0.067

†Participants with both maternal-reported data and cotinine measures. N, number of observations; ng/ml, nanogram/milliliter; CI, confidence interval; P, percentile.

Serial measures of log-transformed children's serum cotinine concentrations from 1 to 4 years were highly correlated, Spearman's rho= 0.81 (2–3 years) to 0.72 (1–3 years). The ICC between repeated serum cotinine concentrations (analysis restricted to 71 children with cotinine measures at 1, 2, 3 and 4 years) was 0.72 (95% CI 0.63 to 0.80) reflecting good agreement between measurements.

Newborns and children whose mothers reported SHS exposure had higher serum cotinine concentrations than children whose mothers reported no SHS exposure (Figure 8). At 1 and 2

years of age, 83% and 80% of children whose mothers reported that they were not exposed had cotinine values higher than the LOD (0.015 ng/mL), and the percentage was 66% and 65% at 3 and 4 years of age. Moreover, the distribution of serum cotinine concentrations was similar among children whose mothers were active smokers and nonsmoking mothers who reported SHS exposure (Figure 8); for this reason, these categories were combined in further analysis.

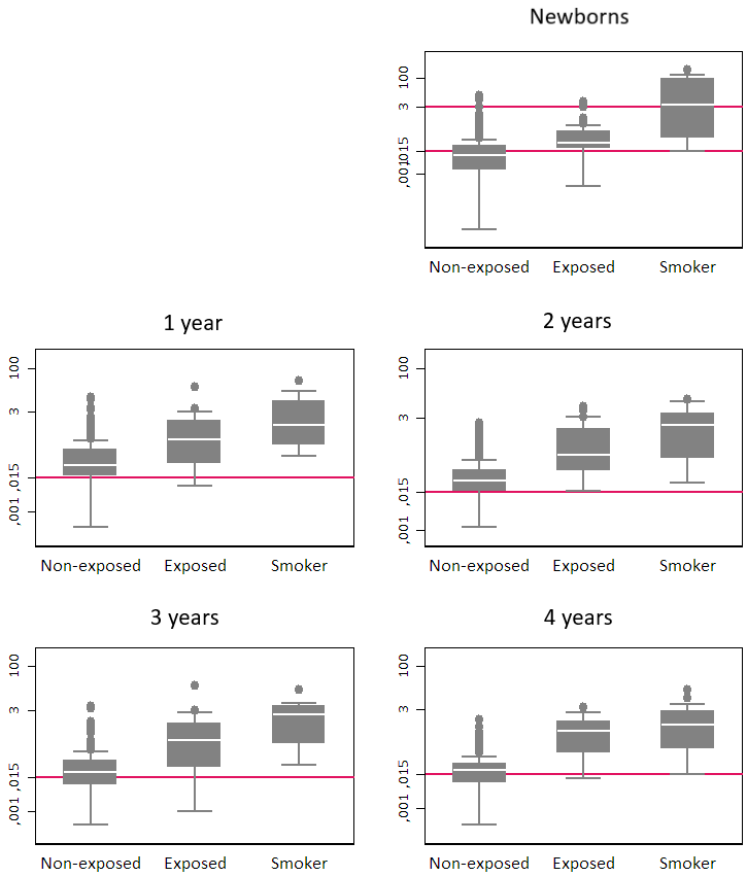

Figure 8. Distribution of cotinine concentrations (ng/mL), as logarithm, from newborn's umbilical cord blood and child's serum at 1, 2, 3 and 4 years according to maternal self-report on tobacco consumption and offspring's SHS exposure.

The box plots depict the distribution of serum cotinine concentrations (ng/mL), as logarithm, from newborn's umbilical cord (upper line=3 ng/mL and bottom line=0.015 ng/mL) and child's serum at 1, 2, 3 and 4 years (line=0.015 ng/mL) depending on children's SHS exposure reported by mothers (unexposed/exposed/mother smoker).

The AUC of various serum cotinine thresholds ranged from 0.80 to 0.89. Cut-points for distinguishing child's SHS exposure (defined as having a mother who smokes or living with a smoker who smokes at home, or as being exposed to SHS in the car or in other frequently

visited homes and places) from no exposure decreased with child's age, and were set at 0.11 ng/mL at 1 year, 0.08 ng/mL at 2, 0.05 ng/mL at 3 and, at 0.04 ng/mL at 4 years (Table 4). The sensitivity and specificity corresponding to these cut-points were above 72%, and NPV was over 87%.

Table 4. Area under ROC curves, new age-specific cut-points for each age (ng/mL) with its sensitivity, specificity, positive predictive value and negative predictive value.

	1 year	2 years	3 years	4 years
N	270	197	196	150
Exposed to SHS, N (%)	72 (26.7)	54 (27.4)	51 (26.0)	47 (31.3)
AUC (95% CI)	0.80 (0.74 - 0.86)	0.83 (0.76 - 0.90)	0.84 (0.77 - 0.91)	0.89 (0.82 - 0.95)
Cut-points (ng/mL)†	**0.11**	**0.08**	**0.05**	**0.04**
Sensitivity (95% CI)	72.20 (60.40 - 82.10)	75.90 (62.40 - 86.50)	74.50 (60.40 - 85.70)	83.00 (69.20 - 92.40)
Specificity (95% CI)	72.70 (66.00 - 78.80)	76.20 (68.40 - 82.90)	74.50 (66.60 - 81.40)	82.50 (73.80 - 89.30)
PPV (95% CI)	49.10 (39.20 - 59.00)	54.70 (42.70 - 66.20)	50.70 (38.90 - 62.40)	68.40 (54.80 - 80.10)
NPV (95% CI)	87.80 (81.80 - 92.40)	89.30 (82.50 - 94.20)	89.30 (82.30 - 94.20)	91.40 (83.80 - 96.20)

†Age-specific cut-points (bold values) are those that maximised the AUC, that is to say, those that minimise the difference between sensitivity and specificity. These values were calculated with ROC curves and children's SHS exposure reported by their mothers was considered the gold standard. Children's serum cotinine concentrations above these cut-point values will reflect SHS exposure. ROC, receiver operating characteristics; SHS, secondhand tobacco smoke; AUC, area under receiver operating characteristic curves; NPV, negative predictive value; PPV, positive predictive value.

The prevalence of children exposed to SHS, based on maternal report, varied between 26.8% and 31.3%. After applying the assay LOD derived cut-point of 0.015 ng/mL, the difference between the maternal self-reported prevalence of SHS exposure and that estimated from child's serum cotinine at ages 1, 2, 3 and 4 years was nearly 50 percentage points at any age (Figure 9). However, after using the age-specific serum cotinine cut-points, this difference diminished with the greatest being nearly 13 percentage points at age 1 year (Figure 9).

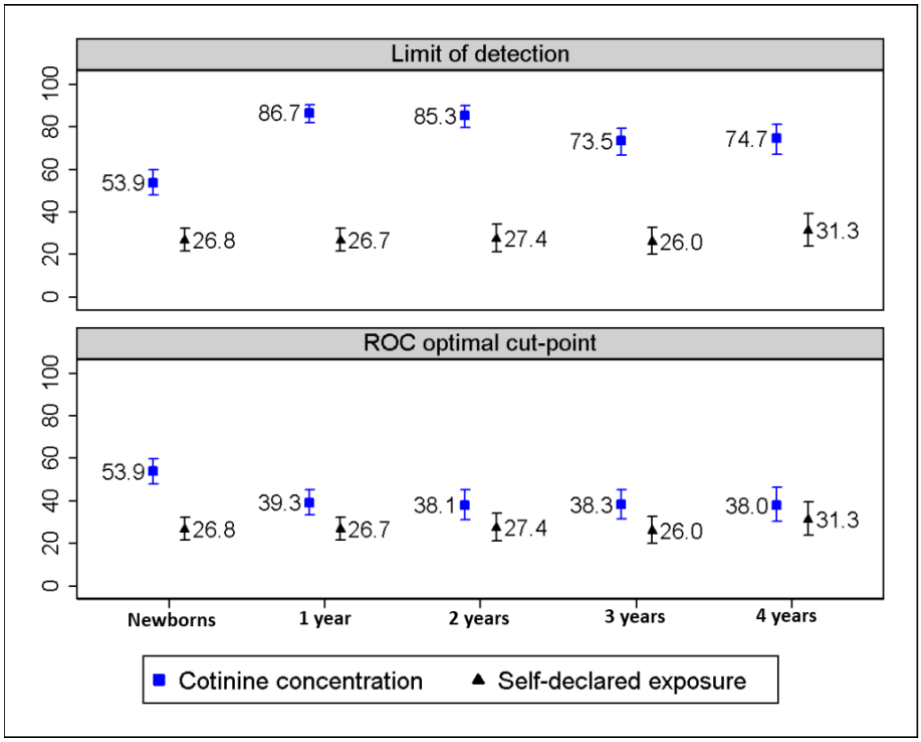

Figure 9. Prevalence of SHS exposure among newborns and children at 1, 2, 3 and 4 years based on serum cotinine concentrations or maternal self-report.

Prevalence of secondhand tobacco smoke exposure among children is derived from maternal self-report (exposed/unexposed), depicted with a triangle, and also from serum cotinine concentrations, depicted with a square, applying assay limit of detection derived cut-point of 0.015 ng/mL (upper) and age-specific cut-points of 0.11 ng/mL at 1 year; 0.08 ng/mL at 2 years; 0.05 ng/mL at 3 years; and 0.04 ng/mL at 4 years (bottom). SHS, secondhand tobacco smoke; ROC, receiver operating characteristic.

The kappa coefficient estimated to assess the concordance between mother-reported exposure and child's serum cotinine concentrations was below 0.22 in each of the four time periods when using the LOD as threshold. In contrast, when age-specific cut-points were used, the kappa coefficient improved considerably (Table 5). Taking Landis and Koch criteria into account in assessing the kappa coefficient, the concordance between maternal-reported and children's serum cotinine concentrations after delivery, when using the assay LOD of 0.015 ng/mL as cut-point, was insignificant at 1, 2 and 3 years and low at 4 years. Of note, when using the age-specific cut-points, concordance improved with age, being low at 1 year, but moderate at 2 and 3, and high at 4 years (Table 5).

Table 5. Kappa concordance coefficient between maternal-reported SHS (exposed/ unexposed) and child´s serum cotinine concentrations accompanied with the percentage of agreement when using the assay LOD threshold and age-specific cut-points at 1, 2, 3 and 4 years of age.

	1 year	2 years	3 years	4 years
Assay LOD threshold†				
Agreement (%)	38.52	42.13	49.49	54.00
Kappa (95% CI)	0.08 (0.04 - 0.13)	0.12 (0.07 - 0.17)	0.18 (0.10 - 0.25)	0.22 (0.13 - 0.32)
Age-specific cut-points‡				
Agreement (%)	72.59	76.14	74.49	82.67
Kappa (95% CI)	0.39 (0.28 - 0.50)	0.47 (0.34 - 0.59)	0.43 (0.30 - 0.56)	0.62 (0.49 - 0.75)

† Assay LOD threshold to discriminate between children exposed and unexposed to SHS: 0.015 ng/mL.
‡ Age-specific cut-points (ng/mL) to discriminate between children exposed and unexposed to SHS calculated with ROC curves, 1 year: 0.11 ng/mL; 2 years: 0.08 ng/mL; 3 years: 0.05 ng/mL; 4 years: 0.04 ng/mL.
SHS, secondhand tobacco smoke; LOD, limit of detection; ROC, receiver operating characteristic.

PAPER 3

Mourino N, Pérez-Ríos M, Yolton K, Lanphear BP, Chen A, Buckley JP, Kalkwarf HJ, Cecil KM, Braun JM. **Pre- and postnatal exposure to secondhand tobacco smoke and body composition at 12 years: periods of susceptibility.** Obesity (Silver Spring). 2022;30(8):1659-1669. Q1 journal with an impact factor of 9.30 and occupying the 14/146 position among endocrinology and metabolism journals in JCR.

The mean age of the 217 children included in the analysis was 12.3 years (SD: 0.7, range 11-14); 54% were girls, and 89% were stage 2 or higher for pubic hair development. Among the mothers, 57% were overweight or obese before pregnancy and 42% breastfed their children for 6 months or longer. Characteristics did not differ from those of the participants at baseline.

Thirty-four percent adolescents were overweight or obese at age 12 years. With the exception of the visceral fat area, body composition measures were higher among girls (Table 6).

Table 6. Distribution of adolescent's anthropometry and DXA measures at age 12 years.

	Overall		Boys		Girls	
Variable	**N**	**Mean (SD) or N [%]**	**N**	**Mean (SD) or N [%]**	**N**	**Mean (SD) or N**
Anthropometry measures						
Height for age z-score	255	0.47 (1.13)	113	0.47 (1.18)	142	0.47 (1.09)
Weight for age z-score	255	0.52 (1.25)	113	0.33 (1.22)	142	0.67 (1.25)
Normal/underweight	255	169 [66.27%]	113	85 [75.22%]	142	84 [59.15%]
Overweight (>1 SD)†	255	69 [27.06%]	113	24 [21.24%]	142	45 [31.69%]
Obese (>2 SD)†	255	17 [6.67%]	113	4 [3.54%]	142	13 [9.16%]
BMI z-Score	255	0.38 (1.20)	113	0.18 (1.16)	142	0.55 (1.20)
Waist circumference (cm)	237	78.12 (13.88)	105	75.71 (11.54)	132	80.04 (15.26)
DXA measures						
Body fat mass index z-score	237	0.23 (0.86)	105	0.24 (0.74)	132	0.22 (0.95)
Body fat mass percent	237	32.36 (7.07)	105	29.70 (6.6)	132	34.48 (6.71)
Body lean mass index z-score	237	-0.32 (1.22)	105	-0.46 (1.15)	132	-0.21 (1.27)
Visceral fat area (cm^2)	237	45.75 (21.20)	105	50.82 (14.70)	132	41.72 (24.52)
Gynoid fat percent (%)	237	36.32 (6.58)	105	33.34 (6.53)	132	38.69 (5.60)
Android/gynoid fat percent ratio	237	0.81 (0.12)	105	0.80 (0.11)	132	0.82 (0.13)

†Overweight and obesity were defined as BMI z-scores above 1 SD and 2 SD, respectively based on CDC growth reference data for 5-19 years. N, number of observations; SD, standard deviation; DXA, dual energy x-ray absorptiometry.

Anthropometry or DXA measures at age 12 years were weakly to strongly correlated with each other with correlation coefficients ranging from 0.01 (gynoid fat percent, height-for-age z-score) to 0.96 (gynoid fat percent, body fat mass percent). Median prenatal serum cotinine concentrations were similar to median postnatal concentrations (0.04 vs. 0.05 ng/mL, respectively; p-value < 0.001). Girl's median postnatal cotinine concentrations were similar to boy's concentrations (0.06 vs. 0.04 ng/mL) (Figure 10). Serum cotinine concentrations were strongly correlated with each other during the prenatal period and postnatal period (rho= 0.76); the ICC between pre- and postnatal concentrations was 0.84 (95% CI: 0.81, 0.88).

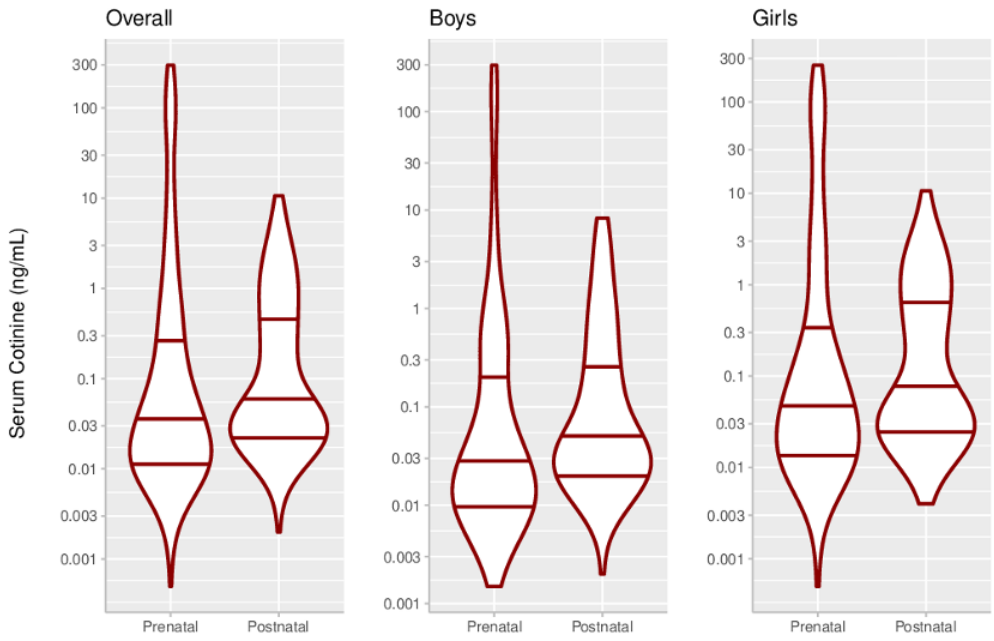

Figure 10. Violin plots of prenatal and postnatal serum cotinine concentrations (ng/mL)[†].

[†]The horizontal lines of the violin, which is a density plot (width=frequency), represent the 25, 50 and 75 percentile. Prenatal period includes maternal serum cotinine concentrations at 16 and 26 weeks pregnancy. Postnatal period includes children's serum cotinine concentrations at ages 1, 2, 3 and 4 years. Ng/mL, nanogram/milliliter.

After adjusting for covariates, associations of serum cotinine concentrations with most anthropometry or DXA measures at age 12 years differed by exposure period (cotinine × exposure period interaction term under 0.05), except for height for age z-score and gynoid fat percent (Table 7). Associations with prenatal serum cotinine were null. Postnatal cotinine was associated with higher adiposity measures (Table 7). Specifically, each increase in $\log_{10}$-transformed postnatal cotinine concentrations were associated with greater BMI z-score (β=0.27; 95% CI: 0.05, 0.49) and body lean mass index z-score (β= 0.27; 95% CI: 0.04, 0.50) at age 12 years (Table 7).

When considering the three-way interaction term for exposure period × serum cotinine × adolescent's sex from the multiple informant model, we observed no clear pattern for prenatal cotinine but there was suggestive evidence that postnatal cotinine concentrations were more strongly associated with some adiposity measures among girls compared to boys. Specifically, each increase in $\log_{10}$-transformed postnatal cotinine was associated with higher body fat mass index z-score and visceral fat area in girls compared to boys [β=0.26 (95% CI: 0.06, 0.47) vs. β=0.09 (95% CI: -0.12, 0.29) and β=0.27 (95% CI: 0.10, 0.45) vs. β=-0.09 (95% CI: -0.25, 0.07), respectively] (Table 8).

Table 7. Adjusted associations (β (95% CI)) of increase in measures of log_{10}-transformed serum cotinine concentrations (ng/mL) with anthropometry and DXA measures at age 12 years by adolescent's sex[†].

Variable	N	Coefficients (95% CI)		Interaction p-value[‡]
		Prenatal	Postnatal	
Height for age z-score	217	0.01 (-0.15 ; 0.17)	-0.03 (-0.34 ; 0.28)	0.90
Weight for age z-score	217	-0.01 (-0.17 ; 0.15)	0.20 (-0.04 ; 0.45)	0.02
BMI z-score	217	-0.02 (-0.18 ; 0.14)	0.27 (0.05 ; 0.49)	<0.01
Body fat mass index z-score	205	-0.07 (-0.19 ; 0.05)	0.18 (-0.01 ; 0.37)	<0.01
Body fat mass percent	205	-0.59 (-1.59 ; 0.42)	1.15 (-0.50 ; 2.79)	0.02
Body lean mass index z-score	205	-0.02 (-0.15 ; 0.12)	0.27 (0.04 ; 0.50)	<0.01
Waist circumference (cm)	205	-0.82 (-2.63 ; 0.98)	2.58 (-1.16 ; 6.33)	0.02
Visceral fat area (cm^2)	205	-0.05 (-0.14 ; 0.04)	0.11 (-0.04 ; 0.27)	0.04
Android fat percent (%)	205	-0.04 (-0.10 ; 0.02)	0.04 (-0.06 ; 0.13)	0.03
Gynoid fat percent (%)	205	-0.56 (-1.46 ; 0.34)	0.79 (-0.70 ; 2.28)	0.06
Android/gynoid fat percent ratio	205	-0.02 (-0.06 ; 0.01)	0.00 (-0.05 ; 0.06)	0.04

[†]Adjusted for maternal age, race/ethnicity, marital status and education, prepregnancy BMI, breastfeeding duration; and adolescent's sex, puberty and age for all variables that are not z-scores. Visceral fat area, android fat percent and android/gynoid fat percent ratio were not normally distributed and thus were log2-transformed in the model. The period-specific cotinine effect estimates were generated from a model with an interaction term between exposure period, serum cotinine and adolescent's sex. P-value is for the two-way interaction term between exposure period and serum cotinine. Interaction p-values < 0.05 were considered as an evidence that at least one of the cotinine-outcome associations differed between prenatal and postnatal exposure periods. Ng/mL, nanogram/milliliter; N, number of observations; DXA, dual energy x-ray absorptiometry; BMI, body mass index.

Table 8. Adjusted associations (β (95% CI)) of increase in measures of log_{10}-transformed serum cotinine concentrations (ng/mL) with anthropometry and DXA measures at age 12 years by adolescent's sex[†].

Variable	β coefficients (95% CI)	
	Boys	Girls
Height for age z-score		
Prenatal	-0.10 (-0.28 ; 0.09)	0.07 (-0.09 ; 0.23)
Postnatal	-0.18 (-0.51 ; 0.14)	0.10 (-0.21 ; 0.41)
Interaction p-value	0.05	
Weight for age z-score		
Prenatal	-0.03 (-0.22 ; 0.17)	0.01 (-0.16 ; 0.18)
Postnatal	0.14 (-0.13 ; 0.41)	0.27 (0.00 ; 0.53)
Interaction p-value	0.03	
BMI z-score		
Prenatal	-0.01 (-0.20 ; 0.19)	-0.02 (-0.20 ; 0.16)

Postnatal	0.25 (0.00 ; 0.49)	0.30 (0.05 ; 0.54)
Interaction p-value	0.09	
Body fat mass index z-score		
Prenatal	-0.12 (-0.26 ; 0.03)	-0.05 (-0.18 ; 0.09)
Postnatal	0.09 (-0.12 ; 0.29)	0.26 (0.06 ; 0.47)
Interaction p-value	0.03	
Body fat mass percent		
Prenatal	0.79 (-0.46 ; 2.03)	-1.24 (-2.38 ; -0.10)
Postnatal	2.40 (0.47 ; 4.32)	0.25 (-1.61 ; 2.10)
Interaction p-value	0.09	
Body lean mass index z-score		
Prenatal	-0.06 (-0.24 ; 0.13)	0.007 (-0.14 ; 0.15)
Postnatal	0.18 (-0.07 ; 0.44)	0.34 (0.09 ; 0.59)
Interaction p-value	0.06	
Waist circumference (cm)		
Prenatal	-0.49 (-2.71 ; 1.73)	-0.89 (-2.88 ; 1.11)
Postnatal	2.35 (-1.49 ; 6.19)	2.96 (-1.08 ; 7.00)
Interaction p-value	0.01	
Visceral fat area (cm^2)		
Prenatal	-0.20 (-0.33 ; -0.08)	0.03 (-0.09 ; 0.15)
Postnatal	-0.09 (-0.25 ; 0.07)	0.27 (0.10 ; 0.45)
Interaction p-value	0.01	
Android fat percent (%)		
Prenatal	0.04 (-0.04 ; 0.11)	-0.08 (-0.14 ; -0.01)
Postnatal	0.11 (0.00 ; 0.22)	-0.02 (-0.13 ; 0.08)
Interaction p-value	0.28	
Gynoid fat percent (%)		
Prenatal	0.99 (-0.28 ; 2.26)	-1.30 (-2.36 ; -0.24)
Postnatal	2.32 (0.48 ; 4.16)	-0.33 (-2.04 ; 1.38)
Interaction p-value	0.19	
Android/gynoid fat percent ratio		
Prenatal	-0.01 (-0.05 ; 0.03)	-0.03 (-0.06 ; 0.01)
Postnatal	0.01 (-0.05 ; 0.07)	-0.00 (-0.06 ; 0.06)
Interaction p-value	0.42	

† Adjusted for maternal age, race/ethnicity, marital status and education, prepregnancy BMI, breastfeeding duration; and adolescent's sex, puberty and age for all variables that are not z-scores.

Visceral fat area, android fat percent and android/gynoid fat percent ratio were not normally distributed and thus were log2-transformed in the model.

The period-specific cotinine effect estimates were generated from a model with an interaction term between exposure period, serum cotinine, and adolescent's sex.

P-value is for the three-way interaction term of exposure period × serum cotinine × adolescent's sex. Interaction p-values < 0.05 were considered as evidence that at least one of the cotinine-outcome associations differed between prenatal and postnatal exposure periods, and by adolescent's sex.

Ng/ml, nanogram/milliliter; DXA, dual energy x-ray absorptiometry; BMI, body mass index.

In sensitivity analysis, our results did not meaningfully change after further adjusting by HEI scores and physical activity level scores.

After adjusting for all covariates, including HEI scores and physical activity level scores, the risk of overweight/obesity increased by 1.75 (95% CI: 1.12 ; 2.73) per each 1-unit

increase in postnatal average log_{10}-transformed serum cotinine concentrations. Prenatal serum cotinine concentrations were not associated with risk of overweight/obesity.

PAPER 4

Mourino N, Pérez-Ríos M, Yolton K, Lanphear BP, Chen A, Buckley JP, Kalkwarf HJ, Cecil KM, Braun JM. **Pre- and postnatal exposure to secondhand tobacco smoke and cardiometabolic risk at 12 years: periods of susceptibility.** Under peer review after submission to an international journal indexed to JCR.

The mean age of the 212 children included in the analysis was 12.4 years (SD: 0.7, range 11-14). Characteristics and serum cotinine concentrations did not differ from those of the participants from previous study (pre- and postnatal exposure to secondhand tobacco smoke and body composition at 12 years: periods of susceptibility).

On average and compared to other groups, CM risk score was higher among adolescents who were at more advanced pubertal stage at age 12 years, born to non-Hispanic Black mothers aged 26 to 29 or older than 35 years, with less education (high school or less), pre-pregnancy obesity (BMI 30 kg/m^2 or higher) and who breastfed for less than 6 months (Table 9).

Table 9. Cardiometabolic (CM) risk scores at age 12 years according to maternal and adolescent's characteristics.

	CM risk score	
Overall	N	Mean (SD)
	190	0.17 (3.43)
MATERNAL CHARACTERISTICS		
Age (years)		
18-25	44	0.13 (2.97)
>25-29	49	0.51 (4.07)
>29-34	56	-0.24 (3.15)
>34	26	0.32 (3.71)
Race/ethnicity		
White, non-Hispanic	113	-0.31 (3.36)
Black, non-Hispanic	67	1.12 (3.46)
All others	10	-0.72 (3.01)
Marital status		
Married	108	-0.21 (3.46)
Not married, living with someone	21	0.01 (3.23)

Not married, living alone	46	0.83 (3.20)
Education		
High school or less	41	1.32 (3.22)
Technical school	34	-0.17 (3.24)
Some college	53	-0.69 (3.78)
Bachelor's or more	56	0.29 (3.19)
Pregnancy-induced hypertensive disorders		
Yes	2	-0.16 (0.58)
No	180	0.16 (3.47)
Prepregnancy BMI (kg/m^2)		
Underweight/normal (<24.9)	71	-0.67 (3.06)
Overweight (25.0 - 29.9)	62	-0.28 (3.60)
Obese (≥30)	45	1.80 (3.26)
Breastfeeding duration (months)		
<6	108	0.24 (3.23)
≥6	71	-0.12 (3.73)
ADOLESCENT'S CHARACTERISTICS		
Sex		
Girls	103	0.08 (3.79)
Boys	87	0.28 (2.97)
Pubic hair		
Stage 1	17	-0.64 (3.51)
Stage 2	50	-0.42 (3.67)
Stage 3	53	0.26 (3.36)
Stage 4	40	0.96 (3.46)
Stage 5	29	0.59 (2.90)

Compared to boys, girls had higher mean HOMA-IR, TG, and leptin to adiponectin ratio, but lower TG to HDL-C ratio, and visceral fat area (Table 10).

Table 10. Mean (SD) of age- and sex- specific cardiometabolic (CM) risk components and summary score at age 12 years, stratified by adolescent's sex.

Measurement	All		Boys		Girls	
	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)
CM risk score	190	0.17 (3.43)	87	0.28 (2.97)	103	0.09 (3.79)
HOMA-IR	203	3.43 (2.38)	98	2.70 (1.72)	105	4.11 (2.70)
TG to HDL-C ratio	203	1.86 (1.60)	98	1.91 (1.91)	105	1.81 (1.26)
Leptin to adiponectin ratio	195	1.60 (2.44)	91	1.04 (1.70)	104	2.09 (2.86)
SBP z-score†	246	-0.56 (0.79)	110	-0.54 (0.71)	136	-0.57 (0.86)
Visceral fat area (cm^2)	237	45.75 (21.20)	105	50.82 (14.70)	132	41.72 (24.52)

†BP z-scores were sex-, age-, and height-standardized according to the Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in children and adolescents.

HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; SBP, systolic blood pressure.

CM risk was highly correlated with visceral fat area (rho= 0.90), and moderately correlated with HOMA-IR (rho= 0.76).

After adjusting for covariates, associations of serum cotinine concentrations with CM risk score and visceral fat area at age 12 years differed by exposure period (cotinine × exposure period interaction term < 0.05). Unlike the prenatal cotinine concentrations, postnatal concentrations were associated with higher CM risk components levels. However, the 95% CI of all the point estimates included the null value (Table 11).

Table 11. Adjusted associations (β (95% CI)) of log_{10}-transformed serum cotinine concentrations (ng/mL) with cardiometabolic risk outcomes at age 12 years†.

Variable	N	β coefficients (95% CI)		Interaction p-value‡
		Prenatal	Postnatal	
CM risk score[a]	170	-0.06 (-0.62 ; 0.50)	0.57 (-0.32 ; 1.45)	0.04
HOMA-IR	181	-0.02 (-0.15 ; 0.12)	0.09 (-0.13 ; 0.31)	0.07
TG to HDL-C ratio	181	-0.03 (-0.15 ; 0.09)	0.14 (-0.08 ; 0.35)	0.10
Leptin to adiponectin ratio	174	-0.09 (-0.36 ; 0.18)	0.07 (-0.34 ; 0.48)	0.28
SBP z-score	212	0.01 (-0.10 ; 0.12)	0.03 (-0.15 ; 0.22)	0.35
Visceral fat area (cm^2)	205	-0.05 (-0.14 ; 0.04)	0.11 (-0.04 ; 0.27)	0.04

†Adjusted for maternal age, race/ethnicity, marital status, education, prepregnancy BMI, pregnancy-induced hypertensive disorders, breastfeeding duration, and child's sex, puberty and age. The period-specific cotinine effect estimates were generated from a model with an interaction term between exposure period and serum cotinine.

‡Interaction p-value is for the two-way interaction term between exposure period and serum cotinine. HOMA-IR, TG to HDL-C ratio, leptin to adiponectin ratio, and visceral fat area were not normally distributed and thus, were log2-transformed in the model. SBP z-scores were sex-, age-, and height- standardized according to the Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents.

[a]CM risk score was the sum of the standardized z-scores for HOMA-IR (fasting insulin (mIU/L) x fasting glucose (mg/dL)/405) , TG to HDL-C ratio, leptin to adiponectin ratio, SBP, and visceral fat area.

Ng/ml, nanogram/milliliter; CM, cardiometabolic; N, number of observations; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; SBP, systolic blood pressure.

When considering the three-way interaction term for exposure period × serum cotinine × adolescent's sex from the multiple informant model, we observed some evidence of sex-specific associations of serum cotinine concentrations with visceral fat area (p-value: 0.01). This was largely driven by the stronger association of postnatal concentrations with visceral fat area in girls (β: 0.27; 95% CI: 0.10, 0.45) compared to boys (β: -0.09; 95% CI: -0.25, 0.07). Prenatal cotinine concentrations were not associated with CM risk scores in either sex (Table 12).

Table 12. Adjusted associations (β (95% CI)) of log_{10}-transformed serum cotinine concentrations (ng/mL) with cardiometabolic risk outcomes at 12 years[†]

Variable	β coefficients (95% CI)	
	Boys	Girls
CM risk score[‡]		
Prenatal	-0.38 (-1.06 ; 0.30)	0.11 (-0.52 ; 0.74)
Postnatal	0.15 (-0.76 ; 1.06)	0.91 (-0.10 ; 1.93)
Interaction p-value	0.14	
HOMA-IR		
Prenatal	0.09 (-0.08 ; 0.27)	-0.08 (-0.22 ; 0.07)
Postnatal	0.16 (-0.07 ; 0.40)	0.04 (-0.21 ; 0.29)
Interaction p-value	0.05	
TG to HDL-C ratio		
Prenatal	-0.01 (-0.18 ; 0.15)	-0.03 (-0.16 ; 0.10)
Postnatal	0.19 (-0.03 ; 0.42)	0.08 (-0.17 ; 0.33)
Interaction p-value	0.16	
Leptin to adiponectin ratio		
Prenatal	0.16 (-0.21 ; 0.52)	-0.22 (-0.53 ; 0.09)
Postnatal	0.32 (-0.11 ; 0.75)	-0.10 (-0.61 ; 0.40)
Interaction p-value	0.06	
SBP z-score		
Prenatal	-0.05 (-0.18 ; 0.08)	0.04 (-0.09 ; 0.17)
Postnatal	-0.02 (-0.20 ; 0.17)	0.07 (-0.15 ; 0.30)
Interaction p-value	0.97	
Visceral fat area (cm^2)		
Prenatal	-0.20 (-0.33 ; -0.08)	0.03 (-0.09 ; 0.15)
Postnatal	-0.09 (-0.25 ; 0.07)	0.27 (0.10 ; 0.45)
Interaction p-value	0.01	

[†]Adjusted for maternal age, race/ethnicity, marital status, education, prepregnancy BMI, pregnancy-induced hypertensive disorders, breastfeeding duration, and adolescent's sex, puberty and age. HOMA-IR, TG to HDL-C ratio, leptin to adiponectin ratio, and visceral fat area were not normally distributed and thus, were log2-transformed in the model. The period-specific cotinine effect estimates were generated from a model with an interaction term between exposure period, serum cotinine and adolescent's sex.

‡CM risk score was the sum of the standardized z-scores for HOMA-IR (fasting insulin (mIU/L) x fasting glucose (mg/dL)/405) , TG to HDL-C ratio, leptin to adiponectin ratio, SBP, and visceral fat area.

P-value is for the three-way interaction term of exposure period × serum cotinine × adolescent's sex. Interaction p-values < 0.05 were considered as evidence that at least one of the cotinine-outcome associations differed between prenatal and postnatal exposure periods, and by adolescent's sex.

Ng/mL, nanogram/milliliter; CM, cardiometabolic; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; SBP, systolic blood pressure.

When excluding mothers who smoked during pregnancy (N=32), or further adjusting for HEI scores, physical activity scores, or both, associations of cotinine with CM risk score and visceral fat area did not meaningfully change in magnitude; however, exposure period × serum cotinine interaction term was not significant (p-value: ≥ 0.05). After excluding those mothers with pregnancy induced hypertensive disorders (N=4), results did not meaningfully change.

PAPER 5

Mourino N, Varela-Lema L, Ahluwalia JS, Rey-Brandariz J, Candal-Pedreira C, Ruano-Ravina A, Vila-Farinas A, Torres A, Pérez-Ríos M. **Influence of maternal smoking in pregnancy on blood pressure during childhood and adolescence: systematic review and meta-analysis.** Under peer review after submission to an international journal indexed to JCR.

A total of 11,608 papers were obtained after the systematic review in Pubmed and Embase. Twenty-one studies fulfilled the inclusion criteria, and 15 were included in the meta-analysis. Main characteristics of the 21 studies are shown in Table 13. Most studies recruited pregnant women before or during the 1990s (n=12), were conducted in non-European continental areas (n=12), and used oscillometry to measure BP (n=11). Six studies were excluded from the meta-analysis because they did not adjust the BP figures for covariates, and because the data came from the same study (Generation R) as another paper with a larger sample size (Table 13).

Table 13. Main characteristics of the cohort studies included in the systematic review (n=21).

Author, year (reference)	Recruit-ment period	Country	Source	Age (years)	N	Definition of maternal smoking (pregnancy)	BP measures (method)[a]	Adjusted (SBP/DBP)
Law, 1991 (144)	1984-1985	UK	Hospital	4-4.5	405	Daily cigarette use	3 x SBP (1)	Yes
Morley†, 1995 (145)	Not shown	UK	Hospital	7.5-8	618	Daily cigarette use	3 x SBP+DBP (1)+(2)[b]	Yes/Yes
Williams, 1999 (146)	1972-1973	New Zealand	Hospital	9	795	Use during pregnancy (yes/no)	3 x SBP+DBP (2)	Yes/No
Bergel†, 2000 (147)	1987-1990	Argentina	Clinical profile	5-9	518	Use during pregnancy (yes/no)	3 x SBP+DBP (2)	Yes/No
Blake*, 2000 (148)	1989-1992	Australia	Hospital	6	702	Use during pregnancy (yes/never)	2 x SBP+DBP (1)	No/No
Lawlor, 2004 (149)	1981-1984	Australia	Hospital	5	3,299	Use during pregnancy (yes/never)	3 x SBP (3)	Yes
Oken, 2005 (150)	1999-2002	USA	Clinical profile	3	689	Use during beginning of pregnancy	5 x SBP (1)	Yes
Brion, 2007 (151)	1991-1992	UK	Population	7	6,509	Use in any trimester (yes/no)	2 x SBP+DBP (1)	Yes/Yes
Laura*, 2010 (152)	1993	Brazil	Population	11	4,452	Use during pregnancy (yes/no)	2 x SBP+DBP (3)	No/No
Wen,2011 (153)	1959-1965	USA	Hospital	7	30,461	Use during pregnancy (never, moderate, intense)	1 x SBP (2)	Yes
Ayer, 2011 (154)	1997-1999	Australia	Hospital	8	405	Use during pregnancy (yes/no)	3 x SBP+DBP (1)	Yes/No
Belfort†, 2012 (155)	1984-1985	USA	Hospital	6.5	694	Use during pregnancy (yes/no)	3 x SBP (1)+(2)[b]	Yes
Leary, 2013 (156)	1991-1997	UK	Population	15	4,723	Use during pregnancy (yes/no)	2 x SBP (1)	Yes
Rob Taal, 2013 (157)	2002-2006	The Netherlands	Population	6	5,447	Continued smoking during pregnancy	4 x SBP+DBP (3)	Yes/Yes
Van den Berg, 2013 (158)	2003-2004	The Netherlands	Hospital	5-6	3,024	Use during pregnancy (yes/no)	2 or 3 x SBP+DBP (1)	Yes/Yes
Yang, 2013 (159)	1996-1997	Bielorrusia	Hospital	6,5	12,196	Use during pregnancy (yes/no)	2 x SBP+DBP (1)	Yes/Yes
Rauschert*, 2019 (160)	1989-1999	Australia	Hospital	17	740	Use during the 18th and 34th week of gestation (yes/no)	[n]SBP+DBP (not shown)	No/No

Xie, 2020 (161)	GECKO/ ABCD: 2003-2007	The Netherlands	Population	5-6	1,613/ 2052‡	Use during pregnancy (yes/no)	3 x SBP+DBP (1)	Yes/Yes
De Smidt*, 2020 (162)	2006-2017	Africa	Hospital	5	500	Use during pregnancy (yes/no)	3 x SBP+DBP (3)	No/No
Grouleff*, 2021 (163)	2019-2020	Greenland	Hospital	3,5-5,5	76	Use during pregnancy (yes/no)	ⁿSBP+DBP (not shown)	No/No
Cajachagua -Torres*, 2021 (164)	2002-2006	The Netherlands	Population	10	4,792	Continued smoking during pregnancy	3 x SBP+DBP (3)	No/No

†Data sourced from clinical trials

‡Sample size of 2 cohorts, GEYCKO and ABCD, respectively.

ⁿNot specified

*Studies excluded from the meta-analysis

[a](1): oscillometry; (2): standard mercury or manual sphygmomanometry; (3): digital sphygmomanometry.

[b]Used both methods to measure BP; however, authors specified that most of the readings were taken with oscillometry.

N, number of observations; UK, United Kingdom; USA, United States of America; SBP, systolic blood pressure; DBP, diastolic blood pressure.

All the 15 studies included in the meta-analysis evaluated the difference in the adjusted SBP figures of children and/or adolescents, aged 3 to 15 years, according to maternal use/non-use of tobacco during pregnancy (N=73,448); six studies also evaluated the difference in the adjusted DBP figures (N=31,459). Adjustment for covariates differed by studies; however, the most commonly used covariates were child's sex and age, and parental socioeconomic status (Table 14).

Table 14. Adjustment variables used in the studies included in the meta-analysis (n=15)

Author	Child/adolescent	Mother	Father/Partner	BP measurement
Law et al	Weight at age 4 years	SBP		
Morley et al	Birth weight and current age and weight	Socioeconomic level, single or multiple pregnancy		Measurement method
Williams et al	Sex, birth weight, current weight, height and BMI	Height, single or multiple pregnancy, marital status, and socioeconomic status by educational level	Socioeconomic status by educational level	
Bergel et al	Sex, age, current height and BMI	Calcium supplementation during pregnancy		
Lawlor et al	Sex, birth weight, current age, weight, and height	Age, BMI prior to pregnancy, child's order of birth, educational level, and income during pregnancy		
Oken et al	Sex, birth weight, current age, height and BMI	Socioeconomic status by educational level and income, SBP in 3rd trimester, race/ethnicity, and parity		BP measurement conditions (order of readings, cuff size, arm position and child's activity)

Brion et al	Sex, current age, and BMI	Age at the child's birth, parity, height, BMI, breastfeeding, and socioeconomic status by social class and educational level	Age, height, BMI, socioeconomic status by social class, and educational level at child's birth	
Wen et al	Sex and gestational age	Age at pregnancy, race/ethnicity, marital status, socioeconomic status, and parity		
Ayer et al	Current exposure to SHS at home			
Belfort et al	Sex, current age and height	Age, educational level, race/ethnicity, and household annual income		Measurement method, and child's behavior during measurement
Leary et al	Sex, birth weight, and current age, height and BMI	Age, height, BMI prior to pregnancy, history of AHT, parity, socioeconomic status by educational level and social class (job occupation)	Age, height, and BMI prior to child's birth	Room temperature, time of day, and child not talking during measurement and cuff size
Rob Taal et al	Sex, gestational age, birth weight, current age and BMI	Age, parity, educational level, race/ethnicity, BMI prior to pregnancy, BP, and breastfeeding		
Van der Berg et al	Sex, age, height, and race/ethnicity	Adequacy of income, and socioeconomic status by educational level		
Yang et al	Sex and age	Age, height, BMI, educational level, occupation, marital status at child's birth, child's order of birth (number of older siblings in household), alcohol consumption during pregnancy	Age, height, BMI, educational level, occupation, marital status at child's birth, and maternal smoking	
Xie et al	Sex, gestational age, birth weight, current age, height and BMI, and early increase in BMI	Educational level, BMI prior to pregnancy, AHT, and breastfeeding		

SBP, systolic blood pressure; BMI, body mass index; SHS, secondhand tobacco smoke; AHT, arterial hypertension.

When applying the modified Newcastle-Ottawa scale, five studies were rated as being high-quality, 5 as moderate, and 5 as low-quality. Low quality studies (144-146, 155, 159) displayed bias related to the design and analysis such as selection, sample size, classification of the prenatal exposure to maternal tobacco smoke, and confounding. Confounding bias was the most widely observed limitation in low and moderate-quality studies due to failure to

adjust for important variables which could have confounded the association, or due to adjustment for potential causal intermediates which could result in an underestimation of the total effect of prenatal SHS exposure on child's BP, such as birth weight or gestational age.

The GRADE level of evidence was low for those studies assessing SBP (n=15), and very low for those assessing DBP (n=6).

Maternal smoking in pregnancy significantly increased SBP figures during childhood or adolescence (β=0.31mmHg; 95% CI: 0.14-0.49) (Figure 11). The Cochran's Q test indicated that there might be inter-study heterogeneity (p-value < 0.1) but that such heterogeneity was not substantial (I^2=0.00%). The leave one out analysis showed that none of the studies significantly modified the results. The funnel plot and Egger's test suggested that there might be publication bias (p-value < 0.1).

No significant associations were found for DBP (β=-0.16mmHg; 95% CI: -0.75-0.43) and inter-study heterogeneity was high (I^2=73.10% and p-value of the Q test < 0.1).

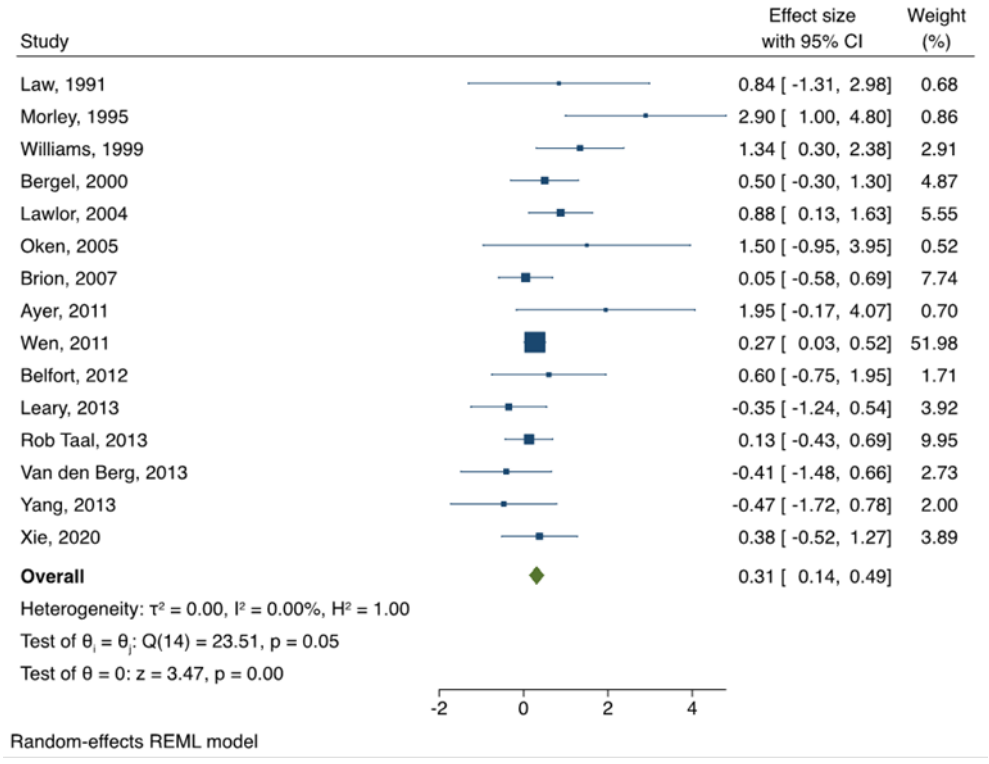

Figure 11. Forest plot of studies which measured difference in mean adjusted systolic blood pressure figures (in mmHg) between children and adolescents exposed or not exposed to maternal smoking in pregnancy.

Regarding the meta-analyses conducted by subgroups, a greater increase in mean SBP figures was observed in the subgroup of studies that completed the recruitment before 1990, were conducted in non-European continental areas, used standard mercury or manual sphygmomanometry, adjusted for birth weight, and in the lowest quality subgroup. However, inter-study heterogeneity was substantial for some subgroups (Table 15). Subgroup analyses were not deemed for studies measuring DBP due to the small number (n=6) and marked inter-study heterogeneity.

Table 15. Results of meta-analyses conducted by subgroups

	Adjusted SBP			
	N	β	95% CI	I^2 (%)
Overall	15	0.31	0.14-0.49	0.00
Subgroups				
Quality of the study[†]				
Low	5	0.94	-0.09-1.97	59.99
Moderate-High	10	0.26	0.08-0.45	0.00
Adjustment for birth weight				
Model adjusted for birth weight	7	0.69	0.06-1.32	64.25
Model without adjustment for birth weight	8	0.25	0.04-0.45	0.00
Recruitment period				
1959-1989	6	0.87	0.28-1.47	57.21
1990-2000	5	0.10	-0.31-0.50	0.00
2001-2007	4	0.14	-0.28-0.57	0.00
Continent				
European	8	0.09	-0.22-0.40	0.00
Non-European	7	0.67	0.25-1.09	36.94
Method for blood pressure measurement				
Oscillometry or digital sphygmomanometry	10	0.33	-0.10-0.77	0.00
Standard mercury or manual sphygmomanometry	5	0.40	0.06-0.75	26.75

[†]Quality of the study was rated by applying a modified Newcastle-Ottawa scale. Studies that obtained a score < 5 points were rated as low-quality, those with a score of 5-6 points as moderate-quality, and those with a score of 7 points or more as high-quality.

PART FIVE: DISCUSSION

DISCUSSION

5.1 DISCUSSION OF THE METHODOLOGICAL ASPECTS

In the systematic review of the literature on the serum cotinine cut-points used to assess SHS exposure in children under 5 years old, the identification of studies involved an exhaustive search of databases after applying a pre-designed search strategy, drawn up by three expert reviewers in the matter, and inspection of the bibliographic references from the selected articles to ensure the inclusion of all possible studies.

Fifty one studies were examined in detail to extract several data regarding study and population characteristics, analytical technique for cotinine quantification, cut-point values and method for their selection. Some of the studies mentioned serum cotinine cut-points to classify children as exposed or unexposed to SHS, though their main objective was not to assess such exposure. Distinction was made between those which assessed prenatal exposure with newborns' umbilical cord serum, postnatal exposure with child's serum cotinine, or both.

Analysis of data from nationally representative group of US smokers and nonsmokers reflected that the optimal cut-point, which maximizes sensitivity and specificity while minimizing the rate of misclassification of self-reported smoking status, regardless of race/ethnicity, is a serum cotinine concentration of 3.08 ng/mL for adults and 2.99 ng/mL for adolescents (27). Unfortunately, no previous studies have performed a similar analysis to accurately differentiate between children exposed and unexposed to SHS.

Initially, the main objective was to review the age-specific serum cotinine cut-points used over time to classify postnatal SHS exposure in children under 5 years. However, only three studies measured postnatal SHS exposure exclusively in this age group with serum cotinine, and none of them estimated age-specific cut-points. Although there is compelling evidence that young children have higher cotinine concentrations compared to adolescents and nonsmokers adults at similar exposures to SHS (1, 28, 165, 166), the majority of the studies grouped children aged under 5 years with older and adolescents, and three papers did not specify participants' sample sizes (32, 124, 126). Importantly, when data on population

characteristics and SHS exposure assessment from the studies included in the systematic review were stratified by age groups, only those that referred to children under 5 years were selected.

Notably, some papers did not specify the period of the study (102, 103, 109, 129, 130), and this could influence the cut-point value selected to classify SHS exposure considering the decline in SHS exposure observed in some countries since 1988. Moreover, the method for the selection of the cut-points varied across studies. Some investigators did not justify the value of the cut-point selected, and simply indicated the reference of another study on which they were based to select it; thus, this involved examining other papers to obtain more detailed information.

Three studies provided the serum cotinine cut-points in pg/mL, nmol/L and µg/mL (112, 118, 134); yet, these units of measurement were converted to ng/mL to homogenize the results. Regarding the Chilean study that used serum cotinine concentrations in µg/mL (118), it mentioned two different thresholds in the methods and results sections (0.1 and 2 µg/mL, respectively). After consulting the value with the corresponding author via email and obtaining no response, the value provided in the methods section was selected for our review; surprisingly, when converted to ng/mL, the resulting value was more than 6,600 times that of the lowest used in US studies (100 vs. 0.015 ng/mL).

Biomarker assessment of SHS exposure provides an advantage over questionnaires assessment with respect to their accuracy. When estimating the prevalence of SHS exposure among US children participating in the HOME Study, considerable differences were found between prevalence of SHS based on maternal report and that based on serum cotinine when using the assay LOD threshold of 0.015 ng/mL. Therefore, optimal serum cotinine cut-points were identified with ROC curves in order to accurately distinguish between exposed and unexposed children at 1, 2, 3 and 4 years of age.

Concordance between maternal report and cotinine concentrations was used as another way of validating the discriminatory capacity of the ROC curves. Any maternal misreporting regarding offspring's SHS exposure would be expected to predominately affect the sensitivity of report (few women would report exposure in its absence); however, some mothers could ignore some sources of SHS, other than tobacco smoke, in their child's environment (167). Importantly, mother's report could have been misclassified if the child was exposed in other locations and the mother was unaware of that exposure. However, maternal misreporting of

SHS exposure is likely reduced considering that 75% of the mothers had more than high school education in this study (168).

There are various methods to optimize cotinine cut-points. Importantly, the three criteria most often used in biostatistics were tested: maximizing the Youden index, the identification of the point on the curve with minimum distance from the left-upper corner of the unit square, and minimizing the difference between sensitivity and specificity; while the three methods provided similar results, the third approach was used to identify the optimal age-specific cut-point to minimize misclassification either as exposed or unexposed, based on cotinine concentration.

Importantly, the use of inadequate cut-points when classifying SHS exposure among vulnerable population, such as children, could lead to an inaccurate estimate of the prevalence of SHS exposure and consequently, of the attributable burden of disease to such exposure.

Since Barker's hypothesis, growing evidence showed that fetal exposure to SHS during pregnancy, mainly due to maternal tobacco consumption, may have obesogenic, atherogenic, and thrombotic effects that could negatively affect CV health later in life (50, 52, 53, 169, 170). However, prior studies did not examine the possibility of periods of heightened susceptibility to the potential effects of such exposure. Interestingly, when data from the HOME Study were used with the aim of longitudinally assessing the effects of pre- and postnatal SHS exposure on body composition and CM risk at 12 years of age, the results showed that SHS exposure may have a greater influence compared to prenatal SHS exposure.

Some studies have recently used multiple informant models to assess critical periods of susceptibility in the association between exposure to environmental chemicals, other than SHS, and child's health (171-173). Regarding this thesis, multiple informant models were used in two studies to estimate the associations of the SHS exposure with the outcome (body composition and CM risk score) for each exposure period (pre- and postnatal), and test whether exposure-outcome associations differed by exposure periods and adolescent's sex (p-value: < 0.05). SHS exposure was measured with cotinine as a continuous variable to examine the effects that each 1-unit increase in $\log_{10}$-transformed serum cotinine had on the different outcomes. Maternal serum cotinine was used to characterize prenatal SHS exposure given the fewer newborns' cord blood measures available, compared to mothers' cotinine measures obtained at 16 and 26 weeks pregnancy (183 vs. 251 and 232, respectively), and the high correlation between cord blood cotinine and maternal cotinine (Spearman's rho=0.80 and

0.82, respectively). Both maternal active smoking exposure and maternal passive smoking exposure were considered when assessing children's SHS exposure. Importantly, multiple informant models were adjusted for an extensive set of covariates, including breastfeeding duration, child's pubertal status, diet quality, and physical activity. However, models were not adjusted for potential intermediates such as birth weight and gestational age since they could result in an underestimation of the total effect of the prenatal exposure on adolescent's body composition or CM risk.

Regarding the study assessing the association between pre- and postnatal SHS exposure and adolescent's body composition, anthropometric measures and also direct measures of total, central and gynoid adiposity (DXA) were included, in contrast to prior published studies which used indirect measures of adiposity (37, 51, 174) such as BMI, which does not accurately distinguish between body fat mass and lean mass (175)**.** Importantly, association between postnatal cotinine and body lean mass would have been missed if just BMI had been included as in previous studies.

Although overweight and obesity are considered established risk factors for the MetS, individuals with BMI-defined normal weight could also develop this syndrome (176). Different thresholds have been used to classify overweight and obesity in children and adolescents and this could have led to an inaccurate estimation of the prevalence (177). In the third paper of this thesis, overweight and obesity were defined as BMI z-scores above 1 SD and 2 SD, respectively, based on CDC growth reference data for 5-19 years (74). These cut-points seem to be more sensitive and suitable to classify overweight/obesity in adolescents compared to other used cut-points (z-score above 1.036 SD and 1.645 SD, respectively) (177, 178).

Since 1988, new criteria have been adopted to define the MetS, evolving from an insulinocentric to an obesocentric perspective as a diagnostic key to this syndrome (179). Definitions of MetS are problematic based on the use of arbitrary cut-points, without considering sex, age or race/ethnicity differences, when assessing several outcomes related to CM risk as they can lead to some overestimation or underestimation of such risk (180-182). Indeed, definitions continue to change and differ between international authorities (183). To our knowledge, the fourth study included in this thesis is the second that has constructed a score using five detailed CM risk factors (HOMA-IR, TG to HDL-C ratio, leptin to

adiponectin ratio, SBP z-scores and visceral fat area) to assess the effects of pre- and postnatal exposure to environmental chemicals on CM risk at age 12 years (173).

Previous studies assessing CM risk in adolescents defined central adiposity based on waist circumference, which cannot disentangle subcutaneous fat from visceral fat (181). Visceral fat area plays a key role in the MetS, regardless of waist circumference and BMI, mainly due to its association with insulin resistance (184, 185). Insulin resistance has a major influence on the onset and perpetuation of the pathological manifestations of MetS (186). However, this is the second study (187) which has used HOMA-IR as an indicator of insulin resistance when measuring CM effects derived from SHS exposure in children (188, 189), rather than simply measuring levels of fasting insulin or glucose (13, 169, 190, 191).

There is evidence that TG to HDL-C ratio and the leptin to adiponectin ratio, or adiponectin to leptin ratio, are better predictors of insulin resistance and MetS in adolescents, compared to individual TG/HDL-C and adipocytokines (192-194). Nevertheless, prior studies included individual components to examine the effect of SHS exposure on CM risk during adolescence, rather than those ratios; specifically, just one study included leptin and adiponectin concentrations as potential CM risk components (57).

The size of the BP cuff, the calibration of the equipment, the adolescent's previous activity and activity during the measurement, the setting and time of day of the reading, and the staff training and experience, could have influenced the proper reading of the three SBP measurements obtained from the adolescents participating in the 12-year visit of the HOME study (86). Notwithstanding, an oscillometric device was used which, in contrast to the standard mercury sphygmomanometer, is not subject to observer biases (86).

In the systematic review of the literature on the maternal smoking in pregnancy and BP during childhood and adolescence, the identification of studies involved an exhaustive search of databases after applying a pre-designed search strategy, drawn up by three expert reviewers in the matter, and inspection of the bibliographic references from the selected articles to ensure the inclusion of all possible studies. Of note, papers assessing the effects of postnatal exposure on children's or adolescents' BP were not included due to the small number of studies found (n=6) (191, 195-199), and the cross-sectional nature of these studies.

Forty-nine studies were examined in detail to extract several data regarding study and population characteristics, maternal smoking in pregnancy, BP measures, including both SBP and DBP, and covariates. Meta-analysis was limited to 15 prospective cohort studies that

provided the necessary longitudinal data to calculate the coefficient of the differences between children's or adolescents' mean DBP and/or SBP figures according to their prenatal exposure from maternal smoking.

Findings from the meta-analysis could be influenced by the heterogeneity of the included studies. Part of this heterogeneity could be due to differences on the definition of maternal smoking habit during pregnancy, recruitment period, inclusion criteria, BP measurement method, and covariates included in the adjustment model. Importantly, a random effects model was applied, leave one out analyses were performed to ascertain the influence of each of the studies, and meta-analyses by subgroups were conducted for those studies measuring SBP, based on factors that could have influenced the results obtained, such as recruitment period, continent where the study was conducted, BP measurement method, study quality and adjustment for child's birth weight.

5.2 DISCUSSION OF THE RESULTS

To start with, this thesis includes the first systematic review on serum cotinine cut-points used over time to classify SHS exposure among children under 5 years old.

Commonly, cotinine cut-points were established attending the assay LOD or based on previously defined adolescent's and adult's cut-points without accounting for population characteristics, analytical technique or prevalence of SHS exposure in the country of the study.

Several studies, including the NHANES which has measured SHS exposure since 1988 using questionnaires and serum cotinine, have observed higher cotinine concentrations among children compared to adolescents and nonsmokers adults at similar exposure to SHS (28, 122, 166, 200). Thus, one of the US studies which included children between 18 months and 17 years of age (25), observed the highest prevalence of SHS exposure among those aged 1 and 3 years. However, just one US study published in 2012, which included children and adolescents aged 1-16 years, calculated two age-specific cut-points with ROC curves setting the reference age at 12 years (129). Of note, the same cut-point (0.9 ng/mL) was used to classify SHS exposure in children during the first 12 years of life, without taking into account changes in the child's physiology, respiratory rate, anatomy, behavior, level of exposure, and cotinine metabolism/clearance that occur from infancy to childhood (30, 200-202).

Serum cotinine cut-point values varied remarkably across the countries since 1985, being the highest value mentioned in a Chilean study conducted in 1990s, more than 6,600 times and 2,000 times that of the lowest and most widely used cut-points to classify SHS exposure among the US studies (100 ng/mL vs. 0.015 ng/mL and 0.05 ng/mL, respectively). European studies used values between 0.2-35 ng/mL. We only found three studies assessing postnatal exposure exclusively among children under 5 years, between 1-3 years, with disparate cut-points ranging from 2.5 ng/mL to 100 ng/mL (104, 118, 138).

One Spanish study conducted at the end of the 1990's (99), obtained a cut-point of 1.78 ng/mL from the ROC curves, with a sensitivity and specificity of 60%, to discriminate between newborns unexposed and exposed to SHS. It is noteworthy that newborn's cotinine could reflect SHS exposure during the period of time in which women were hospitalized (120, 139) and thus, characterization of prenatal SHS exposure from cord serum cotinine samples may be imprecise. There is evidence that newborns' cord serum cotinine concentrations may be lower than children's serum cotinine concentrations for the same exposure levels, considering the placental transfer of cotinine to fetus and the higher metabolism and faster clearance of cotinine in pregnant women (1, 200). However, the most widely used cord blood cotinine cut-point to classify SHS exposure among newborns was 1 ng/mL, which exceeds some of those used to classify exposure among children. The unique study that measured both pre- and postnatal SHS exposure used the same cut-point, based on the assay LOD (0.015 ng/mL), for newborns and children aged 1 and 2 years (100).

Twenty-six studies mentioned the assay LOD threshold as cut-point, with the LC/MS being the most commonly assay for the determination of serum cotinine concentrations. Beginning in 2001-2002, the LC/MS LOD threshold was lowered from 0.05 to 0.015 ng/mL as a result of the introduction of a more sensitive mass spectrometer (28), being these two the most widely used cut-points to classify exposure to SHS in the USA. Whilst lower cut-points could be recommended from a health perspective to avoid ignoring low level or accidental exposures to SHS (203), they could lead to an overestimation of the population exposure due to a trade-off between sensitivity and specificity. The lack of consensus with respect to the most appropriate cut-point has important implications with regards to the comparison of data and trend analysis, the calculations of SHS exposure in a given population, and the estimated attributable burden of disease.

Of note, it should be acknowledged that serum cotinine, like other biomarkers, has an important limitation, as it does not provide information on the route and source of exposure. Although the main health risks associated with SHS are related to inhalation of fine particulate and gases from sidestream smoke (19, 20), values around 0.015 ng/ml could reflect not only transient and accidental SHS exposure (sometimes unnoticed by children's parents or caregivers), but also THS or exposure from other sources such as food. Thus, a child ingesting nicotine laden dust through crawling or hand-to-mouth activity may have high serum cotinine from ingesting that dust, but could have experienced very little exposure to SHS. Previous studies reported that certain foodstuffs such as potatoes, tomatoes, eggplant, cauliflower, green peppers and black tea have measurable levels of nicotine (204, 205). Whilst food consumption levels of dietary nicotine are insignificant compared to moderate SHS exposure, the consumption of high quantities might contribute to low-level elevations in serum cotinine (e.g. 80 g of eggplant is equivalent to approximately 0.01 ng/mL of serum cotinine) (10). Further studies in children could verify this hypothesis using detailed dietary information and sensitive cotinine biomarkers; this information could be useful to propose high level cotinine cut-points that could allow for a better characterization of the SHS exposure in children, differentiating significant exposure from minor or accidental exposure.

Some of the studies included in the review found discrepancies between self-report and serum cotinine when assessing child's SHS exposure. Previous investigators have concluded that maternal report could dramatically underestimate children's SHS exposure. Although one of the studies considered the possibility of misclassification due to inadequacy of the cut-point (116), most studies have assumed that mothers do not accurately report their offspring's SHS exposure (25, 108, 121) due to concealment to avoid social judgement or ignorance about negligible and transient low-level nicotine exposures (24, 25, 206).

Data from the HOME Study was used with the purpose of characterizing the concordance between mother-reported SHS exposure and offspring's serum cotinine concentrations at birth, and at ages 1, 2, 3 and 4 years, after applying the assay (LC/MS) LOD threshold and age-specific cut-points. Notably, this thesis includes the first study estimating serum cotinine age-specific cut-points for children under 5 years.

As expected, median prenatal cotinine concentrations, obtained from newborns' umbilical cord, was lower than median postnatal cotinine concentrations obtained from children's serum, and this could be due to lower level of exposure or tobacco consumption in the days

prior to delivery, or just a reflection of the higher maternal cotinine metabolism and faster clearance during pregnancy (200). When considering the assay LOD of 0.015 ng/mL as cut-point to classify SHS exposure among children, the prevalence of SHS was higher than that obtained from maternal self-report at all ages, but especially at 1 and 2 years, period in which important developmental and behavioral changes occur. Although prevalence decreased when children were 4 years, it was still higher than that obtained from those population studies which classified SHS exposure in US children aged 3-11 years during the period of enrollment and follow-up based on the previous assay LOD threshold of 0.05 ng/mL (74.7% vs. 50.9% and 40.6%, respectively) (122, 166).

It is noteworthy that cotinine concentrations decreased as age increased even among those HOME Study children whose mothers declared they were not smokers at the four follow-up periods and that their offspring were not exposed to SHS. Therefore, differences found in the prevalence of SHS exposure during the postnatal period could be influenced by the sensitivity and suitability of the cut-points used for child's SHS exposure classification. In order to test this hypothesis, and in view of the lack of scientific evidence, age-specific serum cotinine cut-points were further estimated at ages 1, 2, 3 and 4 years with ROC curves. Age-specific cut-points were higher than the assay LOD threshold of 0.015 ng/mL at all ages; at age 3 years, the age-specific cut-point was 0.05 ng/mL, which has been the most commonly used value to assess SHS exposure over time among US children aged 3 years or older. Indeed, the percentage of children misclassified as unexposed decreased when using the age-specific cut-points, compared to the latest assay LOD threshold of 0.015 ng/mL (65%-83% vs. 17%-27%, respectively), and hence, concordance between serum cotinine concentrations and mother-reported exposure to SHS improved, with increasing concordance as child's age increased. Findings indicate that discordance found by prior investigators could be partly due to failure to account for age-related differences in the level of exposure or cotinine metabolism/clearance.

Third and fourth papers of this thesis, are the first studies that have assessed the association of repeated measures of serum cotinine concentrations from pregnancy to age 4 years with adolescents' body composition and CM risk, while identifying periods of heightened susceptibility and potential differences by adolescent's sex. Findings, which seem more convincing for body composition than CM risk outcomes, suggest that cotinine-outcome

associations may differ by exposure period, with postnatal exposure having greater influence than prenatal exposure.

The exposure period-specific association for SHS and body composition was driven by weight for age z-score, BMI z-score, body fat mass index z-score, body fat mass percent, body lean mass index z-score, waist circumference, visceral fat area, android fat percent, and android/gynoid fat percent ratio. Strong postnatal cotinine-outcome associations were found with lean mass index z-score, which is not a measure of adiposity, and BMI z-score, which does not distinguish between body fat mass and lean mass.

The correlation between fat mass index z-score and lean mass index z-score was moderate (Spearman's rho=0.64) and in turn, these two components were highly correlated with BMI (Spearman's rho=0.89); this is consistent with the observation that BMI measures both lean and fat mass, and fat mass is less correlated with BMI at lower levels of adiposity (207). The relation between postnatal exposure and increased body lean mass and body fat mass during early adolescence could be explained by changes that occur in the growth pattern, fat distribution (208), or possibly, due to confounding related to differences in cotinine metabolism and clearance during the first years of life among children who are at risk of overweight/obesity during adolescence compared to those who are not (200). Although it is possible that postnatal exposure to SHS increases lean mass during adolescence (208), the observed association could be merely a reflection of its correlation with BMI z-scores.

Postnatal serum cotinine concentrations were associated with increased risk of overweight or obesity at age 12 years. Increased risk of obesity in adolescents exposed to SHS may result from endocrine disruption or by extrinsic factors such as maternal socioeconomic characteristics, method of feeding during the first year, and unhealthy lifestyle habits frequently observed among children of smoking mothers (209, 210).

Visceral obesity increases oxidative stress as a consequence of excessive production of free fatty acids in liver (211). Exposure to SHS is linked to oxidative stress, which seems to be crucial in the fetal programming of metabolic disorders and CVD, as it is associated with insulin resistance, higher levels of BP, serum TG and leptin, and lower levels of serum HDL-C and adiponectin (55, 179, 212-214). The etiology of MetS is still a matter of debate but visceral obesity and insulin resistance seem to play a major role in initiating and perpetuating the pathologic manifestations of MetS (186). In the fourth study of this thesis, the exposure period-specific association for SHS and overall CM risk score was driven by visceral fat.

Although the interaction p-values for the associations between cotinine and the other CM risk components were > 0.05, unlike prenatal cotinine, postnatal cotinine was associated with higher TG to HDL-C ratio, visceral fat area, and HOMA-IR; however, 95% CI of all point estimates included the null value.

In sensitivity analyses, when smoking mothers were excluded or models were further adjusted for HEI scores or HEI scores and physical activity, associations of cotinine with CM risk score and visceral fat area were less precise and the interaction p-values were ≥ 0.05.

Higher levels of serum TG and lower levels of HDL-C have been found in previous studies among adolescents exposed to postnatal SHS exposure, compared to those unexposed (13, 182, 215-217). Interestingly, when smoking mothers during pregnancy were excluded in the sensitivity analysis, TG to HDL-C ratio differed by exposure period (interaction p-value: 0.04), with a higher increase among those adolescents exposed to postnatal exposure, compared to prenatal exposure. It is difficult to find a possible explanation since no prior studies have included both smoking mothers and nonsmoking but exposed mothers, and TG to HDL-C ratio when assessing associations of cotinine with CM risk. In line with the findings from the fourth study of this thesis, a prior study observed an adverse association between postnatal SHS exposure and 10-year-old children's HOMA-IR (187). Further studies examining the CM effects derived from SHS exposure should include HOMA-IR as an indicator of insulin resistance (188, 189), rather than simply measuring levels of fasting insulin or glucose (13, 169, 190, 191).

Findings from the third and fourth papers of this thesis suggested a null association of prenatal exposure with body composition and CM risk during adolescence. However, prior studies found that prenatal SHS exposure was associated with increased risk of overweight/obesity and MetS, and with higher body fat mass and lean mass (38, 50, 52, 212).

Controversial results have been found when assessing the effects of pre- and postnatal SHS exposure on the CM risk score and individual components in children (13, 136, 164, 169, 190, 191, 215, 218, 219). Inconsistencies in the associations of SHS exposure with body composition or CM risk across studies may relate to differences in the study designs, participants' characteristics (age, race/ethnicity, and hormones), assessment of SHS exposure (definition, intensity, timing, method to measure exposure: self-report vs. biomarkers), biomarkers cut-points to classify SHS exposure, outcomes assessment (measurement methods, MetS definition, CM risk components included, cut-points used when assessing

adiposity measures or defining increased levels of CM risk components in children), and in the covariates included in the adjustment models (169, 179, 187, 220-222).

The sex-specific association for SHS and outcomes was driven by body fat mass index z-score and visceral fat area (p-values: 0.03 and 0.01, respectively), with greater increase among girls exposed to postnatal SHS, compared to boys. Prenatal cotinine concentrations were not associated with body composition or CM risk scores in either sex. The modification of the exposure-outcome associations by adolescent's sex could be explained by differences in the level of SHS exposure as a result of the longer period of time that girls often spend indoors, and in cotinine metabolism and clearance (28, 223); in addition, there could also sex differences in the way tobacco smoke constituents affect the endocrine system (gonadal, steroid, thyroid, and leptin hormones), metabolism, growth, and fat distribution/deposition in girls vs. boys (42, 175, 210). Gonadal hormones may play a significant role in further modulating CM risk components beyond their effects on visceral fat accumulation and insulin resistance (164). Importantly, pubertal status, which was positively correlated with serum concentrations of gonadal hormones at the 12-year study visit (18), was included as potential covariate.

To date, there are contradictory results about the potential effects of prenatal exposure to SHS on BP during childhood and adolescence (69, 70). The fifth paper of this thesis, the systematic review and meta-analysis conducted on tobacco maternal exposure and children's BP, showed that smoking in pregnancy increases SBP figures during childhood and adolescence; however, the difference in mean SBP figures from children's or adolescents' who were exposed to SHS during pregnancy, compared to those unexposed was small (below 1 mmHg). With respect to DBP, effect from maternal smoking was not observed, but few studies assessed DBP and their results were heterogeneous.

Findings from this meta-analysis are consistent with those of a previous one conducted in 2008 (70), which observed a higher increase in BP figures among those children exposed to SHS, compared to unexposed; however, it drew no distinction between SBP and DBP (increase in covariate-adjusted BP: 0.62mmHg; 95% CI: 0.19-1.05) (70). The results also coincide with those of another meta-analysis conducted in 2017 (69), which observed that passive exposure to tobacco (parental smoking or exposure to SHS from other smokers) significantly increased SBP (β=0.26mmHg; 95% CI: 0.12-0.39) but not DPB (β=0.07mmHg; 95% CI: -0.15-0.29).

The definition of maternal self-reported smoking during pregnancy varied across the studies included in the meta-analysis with most of them considering exclusively smoker status (yes/no). Prior investigators found a dose-dependent association between maternal cigarette smoking during pregnancy and children's or adolescents' BP (69, 158). Unfortunately, this could not be tested in this meta-analysis since just two studies recorded the daily cigarette consumption (144, 145).

The increase in SBP figures of those children and adolescents exposed to maternal tobacco smoke, compared to those unexposed, differed by the period of recruitment, continent where the study was conducted, BP measurement method, and adjustment for child's birth weight.

Increased SBP was greater in the group of children and adolescents exposed to maternal tobacco smoke who participated in studies whose recruitment was conducted during 1959-1985 (144-146, 149, 153, 155), compared to those with recruitment after 1985, and in that from non-European studies (146, 147, 149, 150, 153-155), compared to European. These findings could be due to greater cigarette consumption by smoking mothers during pregnancy, as a consequence of lower awareness and social concern about the harmful effects for the fetus (148, 224-226), worse diagnosis and treatment of gestational hypertension (227, 228) or lower prevalence and/or shorter duration of maternal breastfeeding (149, 229). Alternatively, maternal socioeconomic status, parity and age at childbirth, and child's BMI could also have influenced the results (144, 149, 158, 229, 230); however, when examining the available data from the meta-analyzed studies, it was observed that the majority of the mothers were multiparous, between 25 and 35 years old, had average household income, and their children's BMI ranged from 15 to 17 kg/m^2. The difference in mean SBP was significant only in children and adolescents from studies in which BP was measured with standard mercury or manual sphygmomanometry (146, 147, 151, 153, 154). Previous studies displayed discrepancies in terms of overestimation or underestimation of SBP figures according to the measurement method (oscillometry vs. standard mercury or manual sphygmomanometry) (231-233). Accuracy of children's BP reading could be influenced by the equipment (cuff size and calibration), subject (previous activity and activity during measurement, age, and height), setting and time of day of BP reading, and measurement technique (standard mercury vs. manual sphygmomanometry, staff training and experience) (64, 86). Interestingly, only three of the meta-analyzed studies adjusted for some of these covariates (145, 150, 156).

The heterogeneity among the study participants could also affect BP estimates. Children's age differed by studies (3 through 15 years); however, this should not have influenced the results from the meta-analysis since most of them adjusted BP figures for children's age at the time of measurement (145, 147, 149-151, 155-159, 161). Interestingly, three of the meta-analyzed studies (146, 152, 156) measured BP during puberty and observed that the association between mothers' smoking in pregnancy and BP in their offspring did not vary according to whether measurements were obtained before or during initiation of puberty.

Some of the meta-analyzed studies excluded twins, children with congenital heart disease or kidney abnormalities, premature births, and newborns with low or high birth weight for gestational age, which could have underestimated the total effect of tobacco smoke exposure on child's BP figures (144, 146, 153, 157, 159, 234). The unique study that included exclusively premature children and children with low birth weight (155), reported no statistically significant increase in mean SBP of children exposed to maternal tobacco smoke, compared to those unexposed. In the subgroup analysis, the difference in mean SBP figures was greater in the group of studies that adjusted for birth weight with respect to those that did not. A previous study concluded that the direct effect of birth weight on children's and adolescents' BP could be overestimated, when taking into account the indirect effect of this variable on children's height and BMI; in this case, all the studies which adjusted for birth weight, with the exception of three (145, 149, 157), also did so for children or adolescents' height and BMI. Specifically, eight studies adjusted BP figures for child's height (146, 147, 149, 150, 155, 156, 158), and all but five studies (153-155, 158, 159), for child's weight or BMI.

5.3 STRENGTHS

Although prior investigations have identified the cotinine cut-points to discriminate between smokers and nonsmokers, this thesis includes the first systematic review that has identified the cotinine cut-point values used so far across different countries to assess SHS exposure in children, and also the first study that has calculated age-specific serum cotinine cut-points in one of the largest samples of children aged under 5 years, with the aim of validating SHS exposure using both parental report and biomarkers. Importantly, maternal self-report was obtained by standardized face-to-face interviews administered by a trained interviewer during pregnancy and at four different time points after birth, taking into consideration exposure from tobacco consumption by mother and other smokers in several settings.

Furthermore, this thesis includes the first cohort studies that have used multiple informant models to assess the associations of serum cotinine concentrations, from pregnancy to early childhood, with body composition and CM risk at age 12 years, while identifying periods of heightened susceptibility and possible adolescent's sex differences. The multiple informant models make it possible to reduce the number of fitted regression models. When assessing the effect of SHS exposure on body composition outcomes, both anthropometry and DXA measures were included. Considering that CM risk components tend to cluster together, and the lack of consensus both on the definition of MetS and on the appropriate cut-points to define increased concentrations of its components, a CM risk summary score was constructed of five validated biomarkers, which seem to be good predictors of future CM health (41), including fasting serum biomarkers and direct measures of central obesity (DXA visceral fat). Moreover, models to assess the associations between cotinine and outcomes were adjusted for an extensive set of covariates, including breastfeeding duration, adolescent's pubertal status, diet quality, and physical activity. Notwithstanding, models were not adjusted for potential causal intermediates, such as gestational age and birth weight, since they could result in an underestimation of the total effect of the prenatal SHS exposure on adolescent's body composition and CM risk.

Regarding the meta-analysis, differences in mean BP figures were evaluated, rather than the risk of AHT, thereby reducing the risk of incorrect classification, considering the different criteria for defining AHT in children and adolescents (64, 161). Moreover, inclusion criteria were strict, and results made it possible to examine the covariate-adjusted difference in SBP and DBP figures, across almost five decades, for a total of 73,448 children and adolescents

whose mothers smoked during pregnancy. Considering that the covariate sets differed across studies, subgroup analyses made it possible to control for factors that could influence the increase in children or adolescents' BP figures.

Finally, in addition to using PubMed and EMBASE databases, the search was expanded with a manual review of the references cited in all the papers included in the two systematic reviews of this thesis, with the aim of ensuring that no relevant work, which could meet the inclusion criteria, was missing.

5.4 LIMITATIONS

To start with, some data were missing from the studies included in the systematic review on serum cotinine cut-points used to assess SHS exposure among children aged under 5 years, such as period of study and sample size. To the best of our knowledge, just one paper published in Polish was excluded after limiting the selection to English, Spanish, French and German. The main limitation was that the majority of the studies grouped children under 5 years with older and adolescents.

One limitation from the HOME study is the attrition rate of study participants over the course of follow-up (42% at 12-year study visit); yet, loss to follow-up was not associated with any measured sociodemographic characteristic (18). Another aspect to consider is the modest sample size of the third and fourth papers included in this thesis, as it may have reduced statistical power to estimate small effect sizes, and limit the ability to identify distinct periods of heightened susceptibility and sex-specific cotinine-outcome. Given the fact that 95% CI of some point estimates included the null value, findings should be interpreted with caution. Importantly, multiple informant models do not adjust for SHS exposure at other time and therefore, significant findings from postnatal cotinine could be a reflection of the longer and consistent exposure to SHS after birth. Unfortunately, SHS exposure measures were only obtained until children were 4 years old; more recent measures might be more relevant when assessing the effects of SHS exposure on adolescents' body composition and CM risk.

Particularly, regarding the fourth paper of this thesis, it was assumed that each individual CM risk component had equal weights/contribution to the overall CM risk; however, it is possible that some of them are more important predictors of CVD risk than the other, such as visceral fat area or HOMA-IR (179).

Young children are more likely to be exposed to THS than older ones due to their age-associated behaviors and interactions with their environment. Regrettably, questions to evaluate THS exposure were not included in the HOME Study. Future studies should assess THS exposure considering that it could be more toxic than SHS (multiple entry routes, much longer duration of exposure, and novel pollutants not found in SHS) (235).

Although findings from the studies using data from the HOME cohort may not be generalizable to other populations; reassuringly, serum cotinine concentrations among HOME Study participants were very similar to those of other pregnant women and children in the USA during the time of enrolment and follow-up (236, 237). The limited number of smoking mothers in the cohort (N=32) precludes the replication of prior studies of prenatal smoking vs. nonsmoking mothers.

Regarding the meta-analysis on maternal smoking and offspring's BP, some papers, mainly Russian, Polish and German studies, were excluded due to language (n=35). One study, which fulfilled the inclusion criteria, could not be included due to the unfeasibility to estimate the difference in mean figures (238). Of note, increase in child's SBP could be influenced by current SHS exposure as some mothers could continue smoking; however, just one of the studies included in the meta-analysis adjusted BP figures for this covariate (154). Furthermore, subgroup analyses could not be performed for DBP due to the small number of studies measuring these figures, and their marked heterogeneity. However, it should be borne in mind that AHT in children is mainly due to elevated SBP (158).

PART SIX: CONCLUSIONS

CONCLUSIONS

- No studies have calculated serum cotinine age-specific cut-points to ascertained SHS exposure among children under 5 years. The adverse health consequences derived from SHS exposure, and the developmental changes in child behavior, anatomy and physiology during the first years of life, support the need for age-specific cut-points for health research and public health purposes.
- The difference between the maternal self-reported prevalence of SHS exposure and that estimated from children's serum cotinine diminished when age-specific cut-points were used, compared to the widely used cut-point of 0.015 ng/mL. Age-specific cut-points should be validated in future cohorts in order to accurately assess SHS exposure among children.
- Postnatal exposure to SHS could have greater influence on adolescents' body composition and CM risk than prenatal exposure, and associations may be sex-specific. Future cohorts should verify these findings.
- Identification and management of CM risk factors during childhood and adolescence are warranted to avoid the onset or reduce the progression of CVD or CM disorders during adulthood, which could lead to premature death.
- Maternal smoking in pregnancy could increase offspring's SBP figures during childhood and adolescence. Future cohorts should examine the effect of pre- and postnatal SHS exposure on SBP and DBP figures, after adjusting for potential covariates.
- Identification and management of high BP or AHT during childhood or adolescence are warranted to reduce CV risk during adulthood.
- Ongoing public health interventions are needed to discourage parents and caretakers from smoking, and to minimize their children's exposure to SHS. Finally, 100% smoke-free policies should be implemented worldwide, both in homes, private vehicles, and public settings, to protect children's harmful effects of SHS exposure.

PART SEVEN: IMPLICATIONS

IMPLICATIONS

According to the 2020 Surgeon General's report on smoking cessation, over 41,000 US persons die of SHS exposure each year (239). In Spain, the most recent study, showed that 1,028 deaths were attributable to SHS exposure in 2011 (240). Since the first report issued by the US Surgeon General in 1964, tobacco control efforts aimed at minimizing active and passive smoking, have contributed to a reduction of 8 million premature smoking-attributable deaths in USA (241). Exposure to SHS, which remains an alarming public health hazard in the 21st century, significantly increases the risk for all-cause mortality, cancer, and CVD (36).

Children who are exposed to SHS are more likely to have school absenteeism, hospital admissions and hospitalizations, compared to those unexposed (136, 242). Children have less control over home and social environments, compared to adolescents or adults; hence, children are more likely to be involuntarily exposed to SHS. Specifically, during 2013-2016, more than one-third of US nonsmoking children (38.1%) and adolescents (32.1%) were exposed to SHS, as measured by serum cotinine (3). In many cases, estimates of children's SHS exposure are outdated, include children older than 3 years, and are based on parental self-report, which may lead to inaccurate measure of SHS exposure due to parental reluctance to disclose their smoking status. Therefore, further studies measuring young children's SHS exposure with biomarkers, such as cotinine, are needed in order to update the impact of such exposure on different children's health endpoints health. Furthermore, considering the developmental changes in young children's behavior, anatomy and physiology, these studies should examine age differences in cotinine concentrations, and propose age-specific cotinine cut-points to accurately estimate SHS exposure and thus, the burden of disease attributed to SHS.

Data suggests that parental smoking is a major source of SHS exposure for nonsmoking children and adolescents, with the home and private vehicles remaining the most important target settings for reducing their exposure (1). Considering that lockdown during the COVID-19 pandemic impacted tobacco smoking behavior worldwide (243), children could have been more highly exposed to SHS at home from smoking parents and cohabitants, or smokers from

other dwellings. In this respect, it would be interested to assess whether the pandemic has led to an increase in the prevalence of children's SHS exposure. Protecting children from SHS by promoting smoke-free homes has been the focus of the US Environmental Protection Agency's parental outreach and educational programs for the last decades (1). Smoke-free policies are the most cost-effective and efficient approach to provide protection from SHS exposure and consequently, improve health outcomes, specifically at the CV level (244); thus, reinforcement of 100% smoke-free policies is warranted not only in public places, but also in private homes and vehicles.

SHS exposure may be equally or even more harmful to CV health than active smoking due to increasing concentrations of some chemicals that can promote inflammation and oxidation as SHS exposure persists over time (23). A meta-analysis showed that SHS exposure, self-reported or objectively measured with cotinine, was associated with 23% increased risk of CVD, including stroke and coronary heart disease (myocardial infarction, acute coronary syndrome, or other ischemic heart diseases), and 18% increased risk of total mortality in never smoking adults exposed to SHS (36). Importantly, the estimated direct costs of CVD in the USA increased from $103.5 billion in 1996-97 to $226.2 billion in 2017-18 (239). Considering the economic burden imposed on health systems by CVD, which remains the leading cause of death and disability in USA (43, 135), healthcare professionals, such as pediatricians and family physicians, should offer smoking cessation counseling and parental education about the harmful effects that SHS exposure may have on their offspring's health, with particular attention to smoking women of childbearing age, and families of a lower socioeconomic status (1, 245). Furthermore, in view of the fact that parental smoking may later influence their offspring's smoking behavior (246), government should promote health education in the schools by helping develop and disseminate programs designed to emphasize the adverse effects of tobacco (247). Therefore, robust health strategies, targeting both parents and children, are warranted to diminish SHS exposure among children, and consequently, prevent the onset or progression of CVD during adulthood.

In summary, exposure to SHS is a modifiable risk factor for the development of CVD (36). Primary prevention is the most effective strategy to decrease the prevalence of smoking and consequently, SHS exposure. However, to meet the goals of primary prevention, more scientific evidence is required to widen the knowledge on critical periods of susceptibility to SHS exposure on children's CV health, and the magnitude of its effects. Methods used in the

studies included in this thesis could be applied in future studies aimed at assessing SHS exposure with biomarkers, and identifying periods of heightened susceptibility to the potential effects of environmental exposures on multiple child's health endpoints.

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PART NINE: ANNEXES

ANNEXES

7.1 HOME STUDY DATA AND SPECIMEN REQUEST FORM

HOME Study Data and Specimen Request Form

*** Please modify spacing on the following pages as needed to accommodate any descriptions or information provided ***

Date of request: November 22nd 2019
Short Title: Impact of SHS exposure in adolescent body composition and cardiometabolic health

I. Contact Information
Individual placing request

Name: Nerea Mouriño Castro Institution: University of Santiago de Compostela
Specialty area: Epidemiology
Degree: Nursing degree, MSc
Position: PhD student Department/Division: Epidemiology and Public Health
Telephone: 0034 881812278 Email:nerea.mourino.castro@rai.usc.es

Primary co-Investigator/Mentor (if applicable)

Name: Mónica Pérez Ríos Institution: University of Santiago de Compostela
Position: Associate Professor Department: Preventive Medicine and Public Health
Telephone: 0034881812277 Email: monica.perez.rios@usc.es

Name: Joseph Braun Institution: Brown University
Position: Associate Professor Department/Division: Epidemiology
Telephone: 401-863-2139 Email: joseph_braun@brown.edu

Study Staff

Name: Yingying Xu Institution: CCHMC
Position: Biostatistician Role on project: Data Management
Telephone: Email:

Name: Kimberly Yolton Institution: CCHMC
Position: Professor Role on project: Consultant
Telephone: Email:

Name: Institution:
Position: Role on project:
Telephone: Email:

Lead Statistician (if applicable)

Name: Isolina Santiago Institution: Galician Directorate for Public Health
Position: Statistician Department/Division: Epidemiology
Telephone: 0034881540044 Email:soly.santiago.perez@sergas.es

Additional Authors to be Invited:

Aimin Chen
Bruce Lanphear
Heidi Kalkwarf
Kim Cecil

II. Project Information

Proposed Study Title: "**Relation between exposure to SHS during pregnancy and early childhood with adolescent body composition and cardiometabolic health: a cohort study**".

Project goal:

☒ Data analysis and manuscript
☐ Grant application
☐ Other (explain)

Primary Hypotheses/Research Questions (expand section as needed or insert Specific Aims page here):

1. Exposure to SHS during pregnancy and early childhood is associated with alterations in adolescent body composition.
2. Exposure to SHS during pregnancy and early childhood is associated with adverse cardiometabolic consequences in adolescents

Target Journal or Funding Agency
Tobacco Induced Diseases; Nicotine & Tobacco Research, Environmental Research, Environmental Health Perspectives

Aims:
To investigate the association of prenatal and early childhood exposure to SHS with 1) body composition and 2) cardiometabolic health in adolescents (aged 12 years). As an exploratory aim, we will ascertain whether effect modification by sex was present.

Background and Significance:
Tobacco smoke is an environmental carcinogen. Secondhand Tobacco Smoke (SHS) exposure is a leading public health threat worldwide and it is an important preventable cause of morbidity and mortality. In the past 50 years, more than 100,000 children have died as a result of parental tobacco use. Tobacco consists of 5,000 chemical compounds and some of them, have specific cardiometabolic effects. Nicotine alone is associated with hemodynamic alterations, dyslipidemia and insulin resistance; acrolein causes oxidative stress, inflammation and is linked to hypertension, dyslipidemia, arrhythmia and thrombosis; crotonaldehyde is an atherogenic compound of tobacco which induces plaque instability, increases thrombosis and

may have direct negative inotropic effects; cadmium causes inflammation and facilitates atherosclerosis.
The consequences associated with SHS exposure in children have been documented since 1970. Prenatal exposure is acknowledged to have both short and long-term adverse effects, including not only well-known respiratory complications but also cardiovascular consequences. Since the last publication of the American Heart Association Statement concerning the health of children exposed to SHS, there has been epidemiological and clinical research concerning SHS and cardiovascular disease risk in children.
If a fetus or a child are exposed to SHS, even in absence of other atherosclerosis promoting risk factors, there is evidence that SHS has adverse cardiovascular consequences as early as the first decade of life. Childhood SHS exposure has been associated with clustering of cardiometabolic risk factors such as obesity, dyslipidemia and insulin resistance. Childhood obesity is associated with a range of health problems which may last until adult life and cause premature morbidity and mortality. To date, there are substantial gaps in our knowledge about the potential effects of prenatal and early life exposure to SHS on adiposity and cardiometabolic health in adolescence. The purposes of this study are to investigate the association between prenatal and early childhood exposure to SHS through repeated cotinine analysis during childhood and 1) body composition, 2) cardiometabolic health in adolescents (age 12 years) and to ascertain whether effect modification by sex was present.

Methods:
In order to achieve the previous objectives, we will analyse cohort data on SHS exposure from prenatal to age 12.
Primary dependent variables: adolescent´s body composition and cardiometabolic health at age 12.

To assess body composition at 12 we will consider:

- Child adiposity, BMI, weight, height, waist/hip circumferences and DXA measures of body composition.

To assess cardiometabolic health at 12 we will consider:

- Glucose, insulin, and hemoglobin A1C.
- Leptin/adiponectin.
- Lipids
- Blood pressure

Independent covariable: child cotinine serum concentrations at birth (maternal serum sample) and at 12-24-36-48 months.

Selection of covariates:

- Maternal demographic characteristics at baseline: race/ethnicity, level of education or health care access; maternal health related behaviours: tobacco, alcohol or illicit drug consumption during pregnancy; maternal medical history of cardiometabolic disease.
- Child sex, health, diet, physical activity, sleep habits and pubertal self-staging.

Human Subjects Risks/Benefits: No risks are identified.
Benefits: To extend the research on the effects of the exposure to SHS during childhood.
Data Collection Type:
Secondary analysis of existing data

Primary analysis to include analysis of new samples and/or outcomes
Other

Project Duration and Time Line:24 months

Objective 1 (SHS exposure effects in adolescents body composition)

Data request: 1 month.
Bibliography review: 2 months.
Data analysis: 2 months.
Manuscript draft: 2 months
Review of the manuscript: 1 month.
Manuscript: 2 months.

Objective 2 (SHS exposure effects in adolescents cardiometabolic health)

Data request: 1 month.
Bibliography review: 2 months.
Data analysis: 2 months.
Manuscript draft: 2 months.
Review of the manuscript: 1 month.
Manuscript: 2 months.

III. Funding

Compilation of data sets and samples requires time and effort from the HOME Study staff. Please identify any funds available to support the data management staff who will compile the necessary data sets, and the research staff who will retrieve any requested samples for analysis and process IRB applications at CCHMC. Staff and faculty time can be written into grant applications or can be paid through internal funds. Costs can be estimated at approximately $150/hour for creation of data sets and assembly of samples but will vary depending on the type of data and samples requested. A data query fee of $200 is required to check the availability of specific samples and variables for analyses. Investigators who are supporting the study through other funding mechanism may be exempt from supplying additional funds for new projects as long as funding support is being continued.

Funding Type: Federal Foundation Internal/Institutional Applying for Funds
No Funding plan Other (specify):_____

IV. Regulatory

Studies conducted with HOME Study data require IRB review at CCHMC as CCHMC is the owner of the data. However, IRB approval from your institution or a request for reliance upon CCHMC IRB may be necessary.

Do you already have IRB approval or reliance documentation from your institution for your analysis?

Yes - The project will be covered under the currently approved study IRB
No - Independent IRB approval will be sought

Who will have access to the data that is transferred?
Nerea Mouriño Castro
Joseph M. Braun
Mónica Pérez Ríos
Isolina Santiago

Do you plan to use any participant identifiers for the purposes of your project?
No

V. Data Requests
Indicate what types of data/variables you will need to complete the project. Specific details can be worked out when meeting with the RPC.
Survey Data (Clinical, Recruitment, Follow-up)
If survey data is requested, you will be supplied with the appropriate survey and asked to identify specific questions needed to meet the needs of the project.

Breastfeeding Experience
Child Care Arrangement
Child Development Milestones
X Child Diet 12y diet recall
X Child Health (specify): **Blood pressure and physical activity.**
Child Injury
Child Injury Behavior Check List
Child Mouthing Behaviors
Child Sleep Habits
Demographics
Environmental Exposures – BPA/phthalates
Environmental Exposures – Lead
Environmental Exposures – Other (specify): _____
Environmental Exposures - PBDEs/PFCs
Environmental Exposures – Pesticides
X **Environmental Exposures – Tobacco: SHS exposure from survey - prenatal period to age 12y**
Home Renovations
Injury Hazards in Home
X Maternal Alcohol Use during Pregnancy
Maternal Diet during Pregnancy
X Maternal Drug Use during Pregnancy: Marijuana only
X Maternal Medical History of cardiometabolic disease (family history if possible).
Maternal Supervision
Media Exposure
Neighbourhood Rating
Neurobehavioral Data: none
Standardized Assessments administered to Child
Bayley (1,2,3y) Specify age(s) _____
CELF-P (4y)
Delay of Gratification (4y)
Grooved Pegboard (12y)
KCPT (5y) CPT (8y)
NEPSY Visuospatial core (4y) NEPSY Tower (8y)
NNNS (delivery & 4wk postpartum)
Shape School (4y)
Trails-P (4y)
Virtual Morris Water Maze (8y)

Woodcock-Johnson (5y)
WPPSI-III (5y) WISC-IV (8y)
WRAT-4 (8y)
ChAMP (12y)

Child Surveys: **none**
Behavior Assessment System for Children-2 (12y)
Brief Report Inventory of Executive Function (12y)
Child Depression Inventory – II (8,12y)
Playmate and Playstyles Preferences Inventory (8y)
Spence Children's Anxiety Scale (8,12y)
Behavior Assessment System for Children-3 (12y)
SCARED (12y)
Social Problem Solving (12y)
Social Skills Improvement System (SSIS) (12y)

Parent Surveys about Child: **none**
Behavior Assessment System for Children-2 (2,3,4,5,8,12y) Specify age(s) 0,1,2,3,4_____
Brief Report Inventory of Executive Function (2,3,4,5,8,12y) Specify age(s) _____
Gender Identity Questionnaire (8y)
HOME Inventory (assessment of quality of home environment) (1,3y)
Specify age(s) _____
Parenting Relationship Questionnaire (8,12y)
Social Responsiveness Survey (4,5,8,12y) Specify age(s) _____
Social Skills Improvement System (SSIS) (12y)
Young Child DISC (5y)
Parent Surveys/Assessments about Self/Other: **none**
Beck Depression Inventory-II (Prenatal, 4wk, 1,2,3,4,5,8,12y)
CAARS (self) CAARS (other) *The CAARS is a measure of Adult ADHD (8y)
Parenting Stress Index (1,2,3,4,5,8y)
SCL-90 * The SCL-90 is a measure of mental health (4,5 or 8y, 12y)
WASI (IQ) 2-subscale IQ was administered at 1y, Block Design was administered at 8y

Child Growth:
X **BMI** (derived): **only at 12 years**
Head circumference (4wk, 1,2,3,4,5y)
X **Length/Height (**4wk, 1,2,3,4,5,8,**12y)**
X **Waist circumference (**4,5,8,**12y)**
X **Weight (**4wk, 1,2,3,4,5,8,**12y)**
X **Pubertal Self-staging** (**12y**)
Parent Growth:
Maternal Height/Weight (4wk, 4y, 12y)
Paternal Height/Weight (4y, 12y)

Additional Data:
Medical Chart Review: **none**
If chart review data is requested, you will be supplied with the original data collection form and asked to identify specific data fields needed to meet the needs of the project.
Maternal (OB record, Labor and Delivery record)
Infant

HOME Visit Forms:
If home visit forms are requested, you will be supplied with the original data collection form and asked to identify specific data fields needed to meet the needs of the project.

Specimen Requests: none
Please indicate what type samples or processed exposure data you are requesting. Note that some samples may have limited or no availability.
Whole Blood:
Maternal (GA 16wk) Maternal (GA 26wk) Maternal - delivery
Cord 1y 2y 3y 4y 5y 8y 12y
Serum:
Maternal (GA 16wk) Maternal (GA 26wk) Maternal - delivery
Cord 1y 2y 3y 4y 5y 8y 12y
Urine:
Maternal (GA 16wk) Maternal (GA 26wk) Maternal – delivery Maternal – 4wk 1y 2y 3y 4y 5y 8y 12y
Hair:
Maternal (GA 16wk) Maternal – 4wk
1y 2y 3y 4y 5y 8y 12y
Saliva: Maternal (GA 16wk)
Other:
Meconium Vernix Breast milk Infant Formula
DNA – Maternal DNA – CHILD RNA – CHILD (5y only)
Clotted Red Blood Cells (DNA) – Child (8y) Teeth – Child (8-12y) Stool (12y)
Household Dust Wipe:
Baseline 1y 2y 3y
Household Vacuum Dust:
Baseline 1y 2y 3y
Household Water:
Baseline 1y 2y 3y
Household Soil:
Baseline 1y 2y 3y

Please indicate results for exposures or biomarkers:

BPA
CBC (inc hematocrit and/or hemoglobin)
X Cotinine (prenatal and all child ages - 1-4y, 12y to rule out active smokers)

Folate
Genotyping – ADHD

Genotyping - Asthma
Genotyping – Nicotine
Genotyping – Pesticides
X Glucose/Insulin (12y)
Gonadal Hormones (12y)
X Hemoglobin A1C (12y)
IGF-II (12y)
Lead
X Leptin/adiponectin (12y)
Mercury
Organochlorine pesticides
Organophosphates
Other metals (specify) _____
PBDEs
PCBs
PFCs
Phthalates
Pyrethroids
Thyroid hormone 8 year (specify) _____
Thyroid hormone 12 year (specify) _____
Traffic related air pollution
Transportation Noise
Urine Drug Screen (12y)
X Other (specify): **lipids**

X Other data needs (specify)

Notes:

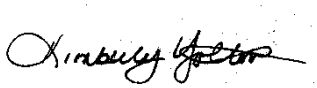
(office notes from review)

This protocol for HOME Study data and specimen use has been reviewed and approved. The PI is reminded to adhere to the Research and Publication Committee approved policy including Authorship Guidelines.

RPC Meeting date: ____01/31/2020_____

Project approval: ☒ YES ☐ NO

Approving Signature:



7.2 APPROVAL OF THE RESEARCH PLAN (DOCTORAL ACADEMIC COMMITTEE)

Datos do plan de investigación

Datos xerais

Neste apartado móstranse os datos xerais dun plan de investigación.

Documento: 54129088Z

Apelidos e nome: Mouriño Castro, Nerea

Programa: [E2041V01] Programa de Doutoramento en Epidemioloxía e Saúde Pública

Rama de coñecemento: Ciencias da Saúde

Estado: Aprobado (29/07/2022)

Versión: 1.1

Data de solicitude de avaliación: 26/04/2022

Curso académico: 2019/2020

Idioma de presentación da tese: Inglés

Idioma do plan de investigación: Inglés

7.3 COMMUNICATIONS TO CONGRESSES

- Mourino N; Pérez-Ríos M; Santiago-Pérez MI; Lanphear B; Yolton K; Braun JM. Exposición a humo ambiental de tabaco y cotinina sérica: nuevos puntos de corte en menores de 5 años. IX Encontro Mocidade Investigadora. Universidad de Santiago de Compostela. 2022.
- Fernández-Villar A; Rey-Brandariz J; Fernández-García A; Pérez-Ríos M; Naveira-Barbeito G; Candal-Pedreira C; Represas-Represas C; Cerdeira-Caramés S; Mourino N; Ruano-Ravina A. Evolución de la mortalidad por EPOC en el periodo 1980-2017: análisis de tendencias de mortalidad. 55º Congreso Nacional de la Sociedad Española de Neumología y Cirugía Torácica (SEPAR). Sociedad Española de Neumología y Cirugía Torácica. 2022. España.
- Rey-Brandariz J; Pérez-Ríos M; Santiag-Pérez MI; López-Vizcaíno E; Provencio-Pulla M; Candal-Pedreira C; Mourino N; Fernández-Villar A; Ruano-Ravina A. Evolución de la tasa de mortalidad por cáncer de pulmón en mujeres en las 17 comunidades autónomas de españa: 1980-2020. 55º Congreso Nacional de la

Sociedad Española de Neumología y Cirugía Torácica (SEPAR). Sociedad Española de Neumología y Cirugía Torácica. 2022. España.

- Fernández-García A; Pérez-Ríos M; Fernández-Villar A; Candal- Pedreira C; Naveira-Barbeito G; Mourino N; Rey-Brandariz J; Represas- Represas C; Malvar-Pintos A; Ruano-Ravina A. Evolución en el tiempo de las causas de hospitalización de los pacientes que ingresan con EPOC y su relación estacional. 55º Congreso Nacional de la Sociedad Española de Neumología y Cirugía Torácica (SEPAR). Sociedad Española de Neumología y Cirugía Torácica. 2022. España.
- Candal-Pedreira C; Lopéz-Pardo ME; Valdés-Cuadrado L; Represas- Represas C; Mourino N; Rey-Brandariz J; Varela-Lema L; Enjo-Barreiro JR; Pérez-Ríos M; Ruano-Ravina A. Factores asociados a la frecuentación extrahospitalaria de los pacientes con EPOC: un estudio de casos y controles. 55º Congreso Nacional de la Sociedad Española de Neumología y Cirugía Torácica (SEPAR). Sociedad Española de Neumología y Cirugía Torácica. 2022. España.
- Mourino N; López-Pardo ME; Valdés-Cuadrado L; Candal-Pedreira C; Rey-Brandariz J; Varela-Lema L; Represas-Represas C; Pérez- Ríos M. Factores relacionados con la asistencia hospitalaria y la mortalidad en pacientes con EPOC: un estudio de casos y controles. 55º Congreso Nacional de la Sociedad Española de Neumología y Cirugía Torácica (SEPAR). Sociedad Española de Neumología y Cirugía Torácica. 2022. España.
- Fernández-García A; Pérez-Ríos M; Fernández-Villar A; Candal- Pedreira; Naveira-Barbeito G; Mourino N; Rey-Brandariz J; Represas- Represas C; Malvar-Pintos A; Ruano-Ravina A. Hospitalizaciones por y con enfermedad pulmonar obstructiva crónica: 20 años de evolución y variaciones en función de edad y sexo. 55º Congreso Nacional de la Sociedad Española de Neumología y Cirugía Torácica (SEPAR). Sociedad Española de Neumología y Cirugía Torácica. 2022. España.
- Mourino N; Curiel-García MT; Pérez-Ríos M; Candal-Pedreiro C; Varela-Lema L; Rey-Brandariz J; Enjo-Barreiro JR; Ruano-Ravina A. La exposición ocupacional a polvo de madera como factor de riesgo de cáncer de pulmón de célula pequeña: una revisión sistemática acompañada de meta -análisis. 55º Congreso Nacional de

la Sociedad Española de Neumología y Cirugía Torácica (SEPAR). Sociedad Española de Neumología y Cirugía Torácica. 2022. España.

- Vila-Farinas A; Pérez-Ríos M; Mourino N; Rey-Brandariz J; Candal-Pedreira C; Varela-Lema L. Consumo de tabaco, cannabis y alcohol: Un programa de deshabituación para embarazadas y sus parejas (PROGRAMA TABACAL). XII CONGRESO - 3º Virtual de Prevención y Control del Tabaquismo CNPT. CNPT. 2022.
- Mourino N; Pérez-Ríos M; Piñeiro-Lamas M; Lanphear B; Yolton K; Braun JM. Exposición pre- y postnatal a humo ambiental de tabaco y adiposidad a los 12 años: identificación de periodos de susceptibilidad. IX Congreso Virtual de Prevención y Control del Tabaquismo del CNPT. Comité Nacional para la Prevención del Tabaquismo. 2021.
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- Rey-Brandariz J, Pérez-Ríos M, Santiago-Pérez MI, Varela-Lema L, Giraldo-Osorio A, Mourino N, Ruano-Ravina A. Smoking-attributable mortality in Spain: A systematic review. Adicciones. 2021;0(0):1619. English, Spanish. doi: 10.20882/adicciones.1619.
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tobacco consumption in Brazil, 2016. Rev Bras Epidemiol. 2020;24:e210007. Spanish, English. doi: 10.1590/1980-549720210007.

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7.5 PUBLISHED OR ACCEPTED PAPERS

Paper 1. Mourino N, Ruano-Raviña A, Varela Lema L, et al. Serum cotinine cut-points for secondhand smoke exposure assessment in children under 5 years: A systemic review. PLoS One. 2022;17(5):e0267319.
https://doi.org/10.1371/journal.pone.0267319

Paper 2. Mourino N, Pérez-Ríos M, Santiago-Pérez MI, Lanphear B, Yolton K, Braun JM. Secondhand tobacco smoke exposure among children under 5 years old: questionnaires versus cotinine biomarkers: a cohort study. BMJ Open. 2021;11(6):e044829.
http://dx.doi.org/10.1136/bmjopen-2020-044829

Paper 3. Mourino N, Pérez-Ríos M, Yolton K, Lanphear BP, Chen A, Buckley JP, Kalkwarf HJ, Cecil KM, Braun JM. Pre- and postnatal exposure to secondhand tobacco smoke and body composition at 12 years: periods of susceptibility. Obesity (Silver Spring). 2022;30(8):1659-1669.
https://doi.org/10.1002/oby.23480

7.6 JOURNAL AUTHORIZATIONS FOR INCLUSION OF THE ARTICLES PUBLISHED

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This is Nerea Mourino, a PhD Student from the University of Santiago de Compostela (Spain). I am working on my final thesis document as part of my PhD in Epidemiology and Public Health. As a requirement to be able to present my doctoral thesis by compendium of articles, I need to include all the papers of my authorship. Therefore, I hereby politely request the favor of authorizing me to include the following article entitled: "Serum cotinine cut-points for secondhand smoke exposure assessment in children under 5 years: A systemic review" (doi: 10.1371/journal.pone.0267319), in my final thesis document.

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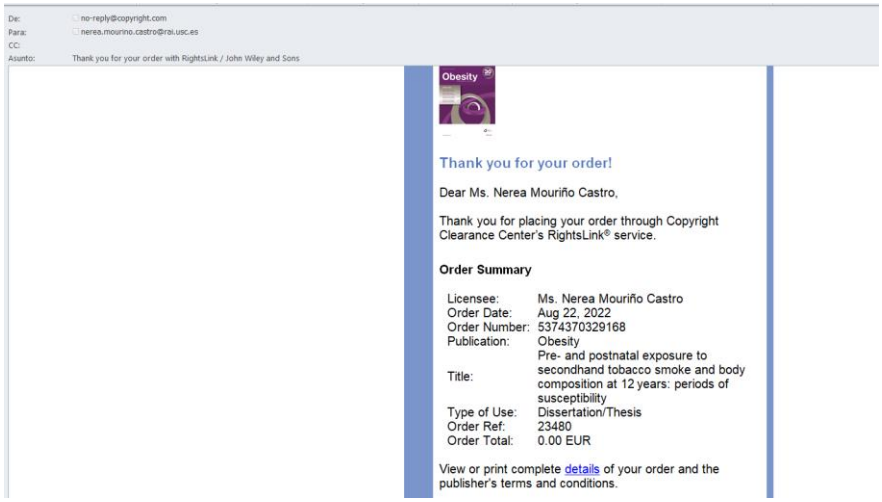

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7.7 RESEARCH STAYS OUTSIDE SPAIN

Confirmation letter from National School of Public Health (ENSP), NOVA University of Lisbon (Portugal).

Escola Nacional de Saúde Pública
UNIVERSIDADE NOVA DE LISBOA

To Whom It May Concern:

Nerea Mourino Castro, PhD candidate from the University of Santiago de Compostela, Department of Preventive Medicine and Public Health, has done a research internship at the National School of Public Health (ENSP), NOVA University of Lisbon, from November 1st, 2021 to 31st January 2022. Nerea was supervised by Prof. Dr. Sofia Belo Ravara and Prof. Dr. Pedro Aguiar and worked on exposure to second-hand tobacco smoke in Portugal. A scientific manuscript entitled Exposure to second-hand tobacco smoke in Portugal: a systematic review is being developed and is about to be submitted to a peer-review international journal.

Sincerely,

Assinado por: **Rui Manuel Candeias Santana**
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Lisbon, 16 th March 2022

Escola Nacional de Saúde Pública, Universidade NOVA de Lisboa

Confirmation letter from Brown University School of Public Health, Brown University (Providence, Rhode Island, USA).

BROWN
School of Public Health

DEPARTMENT OF EPIDEMIOLOGY

Joseph Braun, PhD
Associate Professor
Director, Center for Children's Environmental Health

May 31, 2022

Department of Preventive Medicine and Public Health
University of Santiago de Compostela
Santiago de Compostela 15701
Spain

To whom it may concern:

This note is to confirm that Ms. Nerea Mourino Castro, from the University of Santiago de Compostela, has been with us for a research stay from April 1, 2022 to May 31, 2022. During this period, she has worked with myself, Dr. Joseph M. Braun, Associate Professor of Epidemiology at the Brown University School of Public Health (Brown University, Providence, Rhode Island, USA), on her thesis dissertation, and research related to early exposure to secondhand tobacco smoke exposure and its potential effect on eating behaviors during adolescence, using data from the HOME Study. Moreover, during this time she has also observed and participated in activities related to studies being conducted here at Brown.

Sincerely,

Joseph M. Braun, RN, MSPH, PhD
Associate Professor
Director, Center for Children's Environmental Health
Brown University

Brown University ◘ School of Public Health ◘ Box G-S121-2, Providence, RI 02912 ◘ 401-863-3597 o ◘ 401-863-3713 f ◘ joseph_braun_1@brown.edu

Exposure to secondhand tobacco smoke (SHS) remains an alarming public health hazard in the 21st century, and children are especially vulnerable to its effects.

Serum cotinine has become one of the most widely used biomarkers of SHS in children. The results of this thesis provide insight into the serum cotinine cut-points used to measure SHS exposure in children under 5 years old since 1985 and across the countries, propose age-specific serum cotinine cut-points to accurately estimate their SHS exposure, and assess the effects of early SHS exposure on adolescent's body composition and cardiometabolic health, while identifying periods of heightened susceptibility and differences by adolescent's sex.