Maternal Prenatal Stress and Offspring Wheeze in Dyads Experiencing High Adversity

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Abstract

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Abstract

Significant disparities in childhood asthma rates exist across racial and socioeconomic backgrounds. Evidence links *in utero* exposure to maternal psychological stress with childhood wheezing and asthma, but mechanisms for this association remain poorly elucidated. Furthermore, pregnant women facing high adversity are often missing from clinical trials limiting available data to examine potential relationships between maternal stress and childhood respiratory health. We report results from secondary analyses of data from the Vitamin C to Decrease Effects of Smoking in Pregnancy on Infant Lung Function (VCSIP) study. VCSIP is a randomized double-blind controlled trial of vitamin C supplementation (500mg/daily) during pregnancy to decrease some of the harmful effects of tobacco smoke on offspring pulmonary function, measured at 3 months of life. First, we describe associations between prenatal maternal hair cortisol concentration (HCC) and any report of childhood wheeze through 12 months of age among 158 women facing high adversity and who smoke cigarettes. Hairs from a subset of 33 nonsmokers were available to examine the effect of smoking on HCC. Second, we report VCSIP

study procedures, descriptive statistics of recruitment and retention, and provide a 4 - Step plan to recruitment and retention of pregnant women from marginalized populations into randomized clinical trials.

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Chapter 1

Maternal Prenatal Stress and Offspring Wheeze in Dyads Experiencing High Adversity

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KEYWORDS
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Abstract

Background: We examined associations between prenatal maternal hair cortisol concentration (HCC) and any report of childhood wheeze through 12 months of age among 158 U.S. women facing high adversity and who smoke cigarettes. In addition, hairs from a subset of 33 nonsmokers were available to examine the effect of smoking on HCC.

Methods: Data are from the Vitamin C to Decrease Effects of Smoking in Pregnancy on Infant Lung Function (VCSIP) study Hair, a randomized controlled trial of vitamin C supplementation during pregnancy to decrease some of the harmful effects of tobacco smoke on offspring pulmonary function. Hair was collected between 13 – 23 gestational weeks (T1) and at delivery (T2). Cortisol was extracted from 3 cm long strands of hair, via overnight methanol and measured using liquid chromatography - mass spectrometry. Using chi square and logistic regression, we examined relationships between maternal characteristics and HCC and any child wheeze, adjusting for covariates (e.g., maternal body-mass index [BMI], maternal asthma, and demographics). Interactions between maternal HCC and asthma on child wheeze were assessed using logistic regression.

Result: Women were 77% White, 57% had $\leq$ high-school education, 86.7% received Medicaid, and 33% were obese (BMI $\geq$30 kg/m^2); 46% of mothers reported wheezing in children. Women with log-transformed HCC > 4.5 pg/mg at T2 were 3.45 times more likely to have a child who experienced wheeze (p = 0.009). Maternal asthma was associated with child wheezing (p = 0.02); BMI and demographics were not. Maternal non-White race and smoking were associated with elevated HCC.

Conclusion: Among pregnant women experiencing high adversity, maternal HCC was associated with child wheezing, that was not diminished after consideration for potential

confounding factors. Pathways linking maternal chronic stress and asthma to childhood wheeze may operate through different mechanisms. Future studies are needed to confirm the associations observed here.

INTRODUCTION

Childhood asthma is a major public health issue in the United States (US) contributing to 10.5 million lost school days each year and more than 1.59 billion of annual healthcare costs.[1,2] Maternal prenatal psychological stress and correlates of stress (i.e., maternal depression and anxiety) increase the risk of wheezing and asthma in children[3], but the mechanisms for these associations remain unclear. Products of the maternal stress response, adrenergic hormones and glucocorticoids (i.e., cortisol) released via activation of the sympathetic nervous system and hypothalamic-pituitary-adrenal (HPA) axis may promote childhood wheezing and asthma by impacting fetal lung growth and maturation[4], programming the immune system[5], and altering gene expression[6]. Furthermore, animal models demonstrate that fetal and newborn lung structure-function impairments persist into adulthood.[7]

Evidence supports a link between HPA axis disruption, indexed by altered cortisol secretion, and poor birth outcomes including reduced gestational length and low birth weight.[8] However, limited available studies comparing prenatal serum or salivary cortisol measurements and childhood respiratory health outcomes have reported mixed results.[9-11] Cortisol secretion is pulsatile and follows circadian rhythms, and is highly influenced by circumstantial factors (e.g., menstrual cycle phase).[12] Thus, extrapolating immediate serum and saliva cortisol levels to chronic stress is challenged by high intra and inter-individual variation necessitating multiple samplings.[13]

Hair cortisol concentration (HCC) has emerged as a valid measure of average circulating cortisol levels over time.[14] Circulating cortisol is likely incorporated through passive diffusion into hair that grows, on average, one cm per month.[15] Thus, one cm of new hair growth is considered representative of cortisol levels over the prior month. HCC increases in response to

long periods of stress[16] and correlates with measures of salivary and serum cortisol.[17] As expected, HCC rises with increased circulating cortisol in the last trimester of pregnancy.[14] Although investigators recently reported finding no link between maternal HCC or psychological stress and childhood atopic dermatitis[18], an inflammatory skin condition commonly associated with wheezy illnesses, to our knowledge, no study has investigated whether maternal prenatal HCC maternal prenatal HCC is related to childhood wheeze or asthma.

The mechanisms linking maternal psychological stress and childhood wheezing and asthma are complicated, and may be influenced by maternal history of asthma or atopy[19], maternal adiposity[10], and maternal smoking.[20] However, results are not consistent and study population characteristics and timing of cortisol measurement are important considerations. Wright and colleagues[10] measured salivary cortisol over three days in mid-late gestation in a majority Hispanic, less educated US population and showed that maternal obesity (BMI $\geq$ 30 kg/m^2) and elevated evening cortisol were independently associated with increased odds of wheezing through 2 years of life; obese mothers had uniquely flatter afternoon cortisol declines. Conversely, de Vries et al. reported no association of a single first trimester serum cortisol level and obesity, or childhood wheeze among 2,227 Dutch women.[9]

Given the gaps in understanding maternal bio-psycho-social phenotypes associated with childhood respiratory disease, our primary aim was to examine the relationship between early and late prenatal HCC and maternal reports of wheezing in children through 12 months of age among a sample of 158 US women facing high adversity and who report cigarette smoking. Our secondary aim was to describe the correlates of HCC during pregnancy and early childhood wheezing. A comparison group of 33 nonsmokers was available to examine the effect of cigarette smoking on HCC. We hypothesized that higher concentrations of hair cortisol during

early and late pregnancy, and health and behaviors such as history of asthma and obesity would be associated with increased reported wheezing among children. Because previous studies found links between adiposity and cortisol, and between asthma and race, we examined whether inclusion of maternal asthma, race, or BMI in the model attenuated effects of HCC on childhood wheeze or whether associations remained independent. We also tested whether maternal asthma and race modified the effects of HCC on child wheeze. Mechanisms between in utero exposures and childhood wheeze and asthma are complex. Thus, elucidating relationships between maternal factors including a chronically activated stress response and early childhood wheeze may assist to differentiate stress-induced pathways to respiratory pathology.

METHODS

Study Design and Participants

We analyzed data from the double-blind, randomized controlled intervention trial, Vitamin C to Decrease Effects of Smoking in Pregnancy on Infant Lung Function (VCSIP).[21] Briefly, between December 2013 and May 2016, 252 English-speaking pregnant women at least 15 years of age, with singleton gestation, and who reported smoking cigarettes were recruited from clinics delivering at Oregon Health & Science University (OHSU) in Portland, OR, Peace Health Southwest Medical Center in Southwest, WA, and from Indiana University and Wishard Hospital in Indianapolis, IN; 74% of eligible women participated. Exclusion criteria included: major fetal congenital anomalies; current use of illicit drugs or alcohol abuse; history of kidney stones; complex maternal medical conditions; pregnancy by in-vitro fertilization or planned termination. Participants completed a trial medication adherence period prior to allocation to study arm. No apparent differences in demographics were seen between women completing the trial period versus those who did not (n = 42).

As part of a VCSIP sub-study protocol to examine placental blood-flow, hair samples from 33 healthy nonsmokers with singleton pregnancies were collected to confirm negative nicotine exposure. These samples were available for cortisol analysis to compare HCC among smokers and nonsmokers. Wheeze outcomes among nonsmokers are not described here, as the sub-study is ongoing and follows a different postpartum protocol.

Participation in VCSIP was voluntary and informed consent was obtained. The study was approved by the institutional review boards at the participating hospitals and universities. Notably, women were counseled in smoking cessation at every interaction with VCSIP research

personnel using the 5 A's[22] and pregnancy-specific smoking cessation pamphlets. The study design was intention to treat; a date was recorded for women able to quit smoking and they continued to study protocol completion.

Maternal Cortisol

VCSIP collected hair samples from women at two time-points during pregnancy for evaluation of nicotine exposure; 13 – 23 weeks gestation (T1) and at delivery (T2). Hair was cut close to the root from the posterior aspect of the head and tied at the cut end to identify the most recent portion of hair growth. Samples were wrapped in laboratory grade antistatic tissue paper, zip-locked, placed in a light occlusive envelope, labeled with study identification only and stored. Date of most recent hair dying or treatment was recorded at each sample collection. Cortisol is incorporated into hair, growing on average, one cm per month.[15] We extracted cortisol from 3 cm long segments of hair, considered representative of the prior 3 months of circulating cortisol levels (i.e., the first and last trimesters [T1 & T2]). A caveat to this secondary research was constraints on the quantity of hair available for analysis. To allow for future nicotine and cotinine analysis to meet VCSIP study objectives, samples were limited to 5 strands and processed in singlets (versus duplicates). Cortisol has been measured from minimal amounts of hair.[23]

We followed a similar extraction and measurement protocol reported by others.[24] Briefly, samples of 5 strands were selected, cut to 3 cm in length from the root end, and weighed in milligrams (mg). Cortisol was extracted from 0.22 mg to 2.44 mg samples of whole, nonpulverized hair using 2 ml of methanol in the presence of 2.0 ml cortisol-d4 as an internal

standard, and then subjected to overnight extraction at 55C in an ultrasonic water-bath. Dried samples were reconstituted in 25:75 methanol: water. Cortisol was measured using liquid chromatography - mass spectrometry (LC – MS / MS) on a Shimadzu Nexera - LCMS 8050 (Shimadzu, Canby, OR) with heated electrospray ionization (hESI) in positive ion mode. Data were processed and analyzed using LabSolutions Software, V5.72. Assay quantifiable range was 1.044 picograms (pg) per sample to 4275 pg per sample. Intraassay and interassay coefficients of variation were less than 6%.

Childhood Wheeze

Maternal report of wheeze was ascertained via respiratory questionnaires (RQ)[25] administered at least quarterly by phone or in-person through 12 months of age as part of VCSIP. A positive report of wheeze was defined as any positive response to the question, "Since [birth/the last time we talked] has your child had wheezing or whistling in his/her chest?".

Maternal Adiposity

Maternal body mass index (BMI) was calculated as weight (kg) divided by height^2 (m) and measurements were collected from medical records at enrollment. BMI was categorized following the Centers for Disease Control guidelines (underweight <18 kg/m^2, normal $\geq$18 to <25 kg/m^2, overweight $\geq$25 to <30 kg/m^2, obese $\geq$30 kg/m^2).[26] Maternal obesity was categorized (obese $\geq$30 kg/m^2 versus not obese < 30 kg/m^2).

Maternal smoking

Inclusion criteria for the VCSIP study was maternal report of smoking at least one cigarette in the two weeks prior to enrollment. Maternal smoking status was indexed as positive at baseline if a woman reported smoking at least one cigarette per day (CPD) at enrollment and as positive during postpartum if a woman reported smoking at least one CPD on any respiratory questionnaires through child's first year of life. CPD at enrollment was categorized as (0 CPD, 1-10 CPD, >10 CPD). The median CPD at enrollment was 5 (interquartile range [IQR] = 7) and only six mothers reported less than one CPD on post-partum respiratory questionnaires; postpartum smoker status was not associated with report of wheeze ($p = 0.96$). The 33 nonsmoking mothers participating in the VCSIP sub-study protocol reported zero CPD at enrollment.

Socioeconomic, demographic and health factors

The VCSIP study collected maternal and labor and delivery outcome data through interviews and electronic medical records. At enrollment, baseline characteristics were collected from maternal report unless otherwise indicated, and included: maternal race and ethnicity (White/Caucasian vs non-White/non-Caucasian); maternal education (< or = high school diploma or GED vs some college or more); maternal age in years; type of insurance (government assisted vs other); maternal relationship status (single or divorced vs married or significant other); maternal history of asthma ([y/n] ascertained from medical records); maternal non-insulin dependent diabetes diagnosis ([y/n] ascertained from medical records). Maternal and infant characteristics ascertained at delivery from medical records included: mode of delivery (C-

section vs vaginal); infant sex (male vs female); gestational week (term [$\geq 37^{th}$] vs not term [< 37^{th}]).

Analysis

Baseline characteristics and HCC were summarized descriptively for the total study population. Pearson chi-square and logistic regression were used to examine relationships between wheeze and maternal and infant characteristics for categorical and continuous variables, respectively. HCC was assessed for normality and due to skewness was log transformed. Hair cortisol values were dichotomized using optimal cut-points identified through maximum selection chi square analyses. Dichotomized hair cortisol values were evaluated as predictors because predictability was higher and more interpretable using this parameterization instead of the continuous parameterization.[27] Dichotomized HCC was fit in a logistic regression model to predict the categorical wheeze outcome (Yes/No). These models were run adjusted and unadjusted for potential confounding variables identified *a* priori: maternal history of asthma; maternal obesity (BMI > or = 30 kg/m^2); maternal race. Additional models of interactions among potential covariates were assessed for significance *p-value* < 0.05. Analyses were performed using R language software (R Core Team, R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

A total of 252 women participated in the VCSIP study and hair samples were available for 181 participants. The median gestational week at T1 hair sampling was 16 (IQR = 5]) and 39 weeks at T2 (IQR = 1). Maternal report of wheezing at any time during the first year of life was missing for one child whose mother was lost-to-follow up (n = 180). Mothers were primarily White/Caucasian (23% non-White/non-Caucasian), had low socioeconomic status (98% received Medicaid), and 34% had history of asthma; 46% of children experienced wheezed in the first year of life. Children who experienced wheezing were significantly more likely to have mothers with a history of asthma ($p = 0.02$). No mothers quit smoking prenatally and only six reported zero CPD after birth; postnatal smoking was not associated with wheeze (Table 1.1). Among the sub-set of 33 nonsmokers, women were nearly exclusively White (99%), had higher socioeconomic status (100% carried private insurance and 94% $\geq$ HS diploma), and were older at enrollment (median age 31 years vs 27 years among smokers, $p = 0.03$).

Maternal Characteristics and Prenatal Cortisol

We included observations within the assay quantifiable range of 1.044 to 4275 pg/sample. This eliminated 141 samples from T1 and T2 total, of which, 139 were less than 1.044 pg/sample; only 26 women were excluded completely for T1 and T2 observations outside the quantifiable range, leaving 187 for statistical analysis of characteristics associated with HCC. Among non-smokers vs smokers, 15 vs 71 samples at T1 and 5 vs 27 samples at T2 were outside the quantifiable range.

Median non-transformed HCC at T1 was 13.69 pg/mg (IQR = 48.84; n = 109) and at T2 was 23.6 pg/mg (IQR = 52.77; n = 130) (Figure 1.1). At T1, smokers were more likely to be in the high cortisol group (cortisol $\geq$ 2.62 pg/mg, $p = 0.02$, n = 127, smokers = 18), but not at T2. Younger women with lower education were somewhat more likely to have higher cortisol at T1 ($p = 0.045$ and $p = 0.09$, respectively). Maternal obesity (BMI $\geq$ 30 kg m^2) or BMI as a continuous measure was not associated with high cortisol level. Maternal race other than White was associated with elevated T1 and T2 HCC ($p = 0.006$ and $p = 0.045$) (Table 1.1).

Maternal Prenatal Cortisol and Offspring Wheeze

Distribution of HCC by childhood wheeze is depicted by figure 1.2. Table 1.3 shows unadjusted and adjusted logistic regression model outputs for the relationship between maternal HCC (continuous and dichotomized <= 4.5 pg/mg) and childhood wheeze. Cortisol metrics at T1 and T2 where positively associated with childhood wheeze. Women with log-transformed HCC above 4.5 pg/mg at T2 were 3.45 times more likely to have a child who experienced wheeze (95% confidence interval [CI], 1.40 - 9.15; $p = 0.009$). The relationship at T1 was less significant ($p = 0.013$); the slope (T2 - T1) was not associated with wheeze ($p = 0.94$, n = 98). The relationship between HCC at T2 and wheeze remained significant after adjustment for HCC at T1.

The addition of adjustment variables did not significantly change effect estimates (> 0.10). In congruence with other studies, we found maternal asthma to be associated with childhood wheeze ($p = 0.02$), but not with HCC. If maternal asthma and elevated cortisol shared a causal pathway to childhood wheeze, we would expect attenuation toward the null, assuming

there are no unmeasured confounders, instead effect estimates were constant suggesting that asthma and psychological stress are independently associated with wheeze. Additionally, we found no significant interactions among HCC and maternal race or asthma history as the exposures on the outcome of wheeze.

DISCUSSION

Among maternal-infant dyads facing high adversity, in utero exposure to elevated maternal cortisol during early and late pregnancy was associated with child wheezing through 12 months of age. These results are congruent with other studies showing an association between maternal prenatal psychological stress and childhood wheeze and asthma[3], and contribute new information to the literature. To our knowledge, this is the first prospective study to compare maternal prenatal HCC with occurrence of wheeze in children. Furthermore, main effects modeling showed maternal history of asthma and HCC were independently associated with child wheezing and additional analyses indicated no significant interactions. We would expect attenuation of effects or significant interactions if maternal asthma and chronically elevated cortisol shared a causal pathway to childhood wheeze or asthma, assuming no unmeasured confounders[28], and that was not the case suggesting maternal asthma and psychological stress may impact childhood wheeze through differing mechanisms.

By design, our sample population was homogenous for multiple factors associated with childhood wheeze and asthma (i.e., low socioeconomic status and smoking). The relationship between maternal HCC and childhood wheeze above and beyond these competing predictors strongly suggests a role for chronically elevated maternal prenatal cortisol in the development of childhood wheezing and asthma. In that context, HCC presents as a reasonable biomarker of the contribution of stress to childhood respiratory health among populations with multiple risk factors. Our findings are corroborated by studies correlating prenatal HCC with the natural surge of cortisol during pregnancy[29] and of expected stress exposures.[30] However, this area of research is growing and results from limited studies among pregnant and non-pregnant populations

remain mixed, potentially owing to heterogeneity in sample collection timing, population demographics, and cortisol extraction and measurement methods.[31]

In our sample consisting of predominantly White women (67% White/Caucasian, 16% African American, 5% multiracial, and 1% Native American), we found significantly higher cortisol levels among women identifying as a race other than White. Such findings corroborate other studies reporting associations between higher stress and disrupted cortisol among African American women, and adverse birth outcomes.[32] However, a relationship between maternal race and maternal report of childhood wheezing was not found, nor were the variables maternal race and maternal history of asthma associated. These results counter data from nationally representative samples, where African Americans are disproportionally burdened by asthma.[1] Determining the reason for these discrepancies is challenging. We acknowledge the potential limitation of maternal report of wheeze and that definitive outcomes such as provider diagnosed asthma or pulmonary function would improve precision. However, maternal report of wheeze is widely considered a reasonable proxy for future respiratory health issues.[3] A potential scenario is that differential reporting of wheeze occurred between White and non-White mothers. We postulate a more likely scenario, that among generalized US sample populations women identifying as racial minorities are also disproportionally burdened by lower socioeconomic status and race is serving as a proxy for unmeasured confounders of socioeconomic status, which are less influential among our sample of smokers.

Average log-transformed hair cortisol concentration at T1 was higher among the population of women who reported smoking cigarettes (2.17 ± SD 1.29 vs 3.28 ± SD 1.65 for non-smokers vs smokers, respectively). While this finding adds to the literature, as no consistent relationship between smoking and hair cortisol has been established,[31] influences on HCC by

differences in socioeconomic and demographic makeup between smokers and nonsmoker cannot be excluded.

Contradictory to other studies among pregnant[10] and non-pregnant populations[33] our analysis did not show an association between maternal prepregnancy BMI and HCC or early childhood wheeze. Children in our population who wheezed had mothers with slightly lower prepregnancy BMI compared to those who did not wheeze (difference of means = -0.08) although this was not significant (n = 180, $p = 0.43$). Across the literature, the results appear mixed regarding hair cortisol and adiposity, with some studies finding no relationship between varying measures of adiposity (BMI, waist/hip ratio, waist circumference) among diverse adult populations, and others reporting associations with at least one measure.[31] Furthermore, evidence suggests cortisol levels and adiposity and asthma are influenced by body fat distribution (e.g., visceral v waist/hip ratio)[34], which we were unable to explore.

The mean hair cortisol concentration among our population at T2 was 24.78 pg/mg SD ± 4.9 pg/mg. These results are consistent with others reported in the literature.[35] We found no indication that women excluded for HCC less than or greater than quantifiable levels were different from women included. Ideally, hair collection for cortisol analysis would be the primary objective, but as is reported across the literature methods for cortisol analysis vary widely and often data are obtained secondarily.[31]

An important question is how to determine the subjective experience of stress among pregnant women. Although the VCSIP study did not capture perceived stress directly, we leveraged available and detailed demographic and behavioral data along with HCC to characterize the chronic maternal bio-psycho-social factors potentiating the development of wheeze in offspring. Additionally, among this homogenous sample confounding was limited and

factors of interests stood apart (e.g., cortisol and asthma). Moreover, limited and inconstant results exist across the literature regarding perceived stress and hair cortisol.[36] As evidence by Braig and colleagues, who found significant correlations between maternal demographic and behavioral factors with HCC among 768 women, while no such correlation existed with self-report of stress.[37,38] Therefore, the benefit of including a perceived stress measure such as those reported by others is unclear. Future studies with a qualitative component (i.e., focus-groups) may better elucidate modifiable stressors.

Given the public health burden of childhood asthma and the gaps in knowledge of prevention, a priority is to differentiate bio-psycho-social phenotypes associated with respiratory pathology. These data support a role for in utero exposure to chronic psychological stress in the development of childhood wheeze. Additionally, our data support the use of HCC as a biomarker of cortisol levels during pregnancy. Elevated cortisol and asthma did not seem to operate on the same path to child wheeze, and we found no evidence that maternal minority race was associated with maternal asthma or child wheeze among this population homogenous for low socioeconomic status. Generally, maternal-infant dyads facing high adversity are disproportionately burdened by wheezing and asthma[39], further studies among similar populations exploring links between biomarkers of stress and maternal asthma on development of childhood wheeze may be particularly important to identify women at the highest risk.

Table 1.1 Maternal and infant characteristic associated with wheezing

	All maternal-infant dyads (n=180)	Any Wheeze		
Enrollment characteristic	N (%) or Median (IQR)	No (%)	Yes (%)	P
Maternal Age (years)	27.0 ± 5.6	27.7 ± 5.5	26.1 ± 5.7	0.06
Low Level of Education (≤ 12 years)				0.23
Low[b]	103 (57.2)	51 (52.6)	52 (62.7)	
High	77 (42.8)	46 (47.4)	31 (37.4)	
Currently married				0.18
Single	57 (31.7)	26 (26.8)	31 (37.4)	
Other	123 (68.3)	71 (73.2)	52 (62.7)	
Maternal race				0.57
White/Caucasian	139 (77)	77 (79.4)	62 (74.7)	
Non-White/non-Caucasian	41 (23)	20 (20.6)	21 (25.3)	
Asthma or atopy				0.02[a]
Asthma	61 (33.9)	25 (25.8)	36 (43.4)	
No asthma	119 (66.1)	72 (74.2)	47 (56.6)	
BMI (kg/m^2)	29.3 ± 7.1	29.7 ± 7.3	28.9 ± 6.9	0.43
Cortisol T1 (pg/mg)[c]	3.3 ± 1.7	3.0 ± 1.4	3.7 ± 1.90	0.04[a]
Cortisol T2 (pg/mg)[d]	3.5 ± 1.3	3.2 ± 1.2	3.8 ± 1.5	0.02[a]
Postnatal smoking status				0.96
Smoker	174 (96.7)	101 (96.2)	73 (97.3)	
Nonsmoker	6 (3.3)	4 (3.8)	2 (2.7)	
Child's sex				0.28
Male	93 (51.7)	46 (47.4)	47 (56.6)	
Female	87 (48.3)	51 (52.6)	36 (43.4)	
Term birth[e]				0.69
Term	162 (90)	86 (88.7)	76 (91.6)	
Not term	18 (10)	11 (11.3)	7 (8.43)	

[a] alpha <0.05
[b] Trade school such as hair/beauty school included
[c] included only women with quantifiable results at T1 n=109
[d] included only women with quantifiable results at T2 n=130
[e] term birth ≥37 weeks GA

Table 1.2. Maternal and Infant characteristics associated with HCC

	HCC Dichotomized T1 (> 2.62pg/mg)[a]			HCC Dichotomized T2 (> 3.16pg/mg)[a]		
	High (n=64)	**Low** (n=63)		**High** (n=79)	**Low** (n=79)	
Characteristic	n (%) or mean (SD)		P	n (%) or mean (SD)		P
Age (years)	26.6 ± 5.7	28.7 ± 5.6	0.05	27.62 ± 5.88	27.6 ± 5.3	0.98
Education[c]						
High	24 (37.5)	34 (54.0)	0.19	35 (44.3)	47 (59.5)	0.08
Low	40 (62.5)	29 (46.0)		44 (55.7)	32 (40.5)	
Insurance						
Government	50 (78.1)	42 (66.7)	0.21	59 (74.7)	53 (67.1)	0.38
Other	14 (21.9)	21 (33.3)		20 (25.3)	26 (32.9)	
Relationship						
Single/divorced	26 (40.6)	16 (25.4)	0.10	27 (34.2)	22 (27.9)	0.49
Other	38 (59.4)	47 (74.6)		52 (65.8)	57 (72.2)	
Race						
White	36 (56.3)	57 (90.5)	<0.001	55 (69.6)	70 (88.6)	0.006
Others	28 (43.8)	6 (9.5)		24 (30.4)	9 (11.4)	
Smoker						
Smoker	60 (93.8)	49 (77.8)	0.02	69 (87.34%)	61 (77.2)	0.15
Nonsmoker	4 (6.3)	14 (22.2)		10 (12.66%)	18 (22.8)	
CPD (prenatal)[c]						
0	17 (26.6)	25 (39.7)		26 (32.9)	36 (45.6)	0.24
1-10	41 (64.1)	30 (47.6)	0.17	46 (58.2)	36 (45.6)	
>10	6 (9.4)	8 (12.7)		7 (8.9)	7 (8.9)	
Maternal Asthma						
Yes	22 (34.4)	21 (33.3)	1	27 (34.2)	27 (34.2)	1
No	42 (65.6)	42 (66.7)		52 (65.8)	52 (65.8)	
BMI[d]	30.6 ± 6.8	28.7 ± 6.6	0.2	30.3 ± 7.7	28.6 ± 6.2	0.21
Obesity						
BMI <30 kg/m^2	37 (57.8)	40 (63.5)	0.64	46 (58.2)	50 (63.3)	0.63
BMI ≥30 kg/m^2	27 (42.2)	23 (36.5)		33 (41.8)	29 (36.7)	
Diabetes						
Yes	2 (3.1)	0 (0)	0.51	2 (2.5)	0 (0)	0.5
No	62 (96.8)	63 (100)		77 (97.5)	79 (100)	
Delivery type						
Cesarean	18 (28.1)	18 (28.6)	0.73	22 (27.9)	25 (31.7)	1
Vaginal	46 (71.9)	45 (71.4)		57 (72.2)	54 (68.4)	
Infant sex						
Female	33 (51.6)	37 (58.7)	0.53	42 (53.2)	37 (46.8)	0.52
Male	31 (48.4)	26 (41.3)		37 (46.8)	42 (53.2)	
Infant term						
Term	57 (89.1)	58 (92.1)	0.78	70 (88.6)	73 (92.4)	0.58
Not term	7 (10.9)	5 (7.9)		9 (11.4)	6 (7.6)	

Table 1.3 Odds Ratio (95% CI) of wheeze in models including HCC T1 & T2 and Potential Adjustment Variables

		T1		T2	
Model	Hair Cortisol Measures (adjustment variables)	OR	95% CI	OR	95% CI
0	Cortisol continuous	1.28	1.01 – 1.64	1.38	1.06 – 1.84
1	Cortisol continuous (Asthma)	1.38	1.05 – 1.85	1.38	1.05 – 1.85
2	Cortisol continuous (Cortisol T1 and asthma)	-	-	1.48	1.10 – 2.05
4	Cortisol dichotomized[a]	5.56	1.58 – 26.01	3.45	1.40 – 9.15
5	Cortisol dichotomized[a] (Asthma)	5.63	1.58 – 26.60	3.40	1.35 – 9.17
6	Cortisol dichotomized[a] (Cortisol T1 and asthma)	-	-	4.32	1.48 – 10.21
7	Cortisol dichotomized[a] (Asthma, BMI, race and relationship)	5.83	1.55 – 28.71	3.84	1.46 – 10.97

Definition of abbreviations: HCC = hair cortisol concentration; CI = confidence interval; OR = odds ratio
[a] Dichotomized by optimal cut-point for T1 = 5.77 pg/mg and T2 = 4.54 pg/mg

Figure 1.1 Hair Cortisol Concentration at T1 and T2 Raw vs Log-transformed

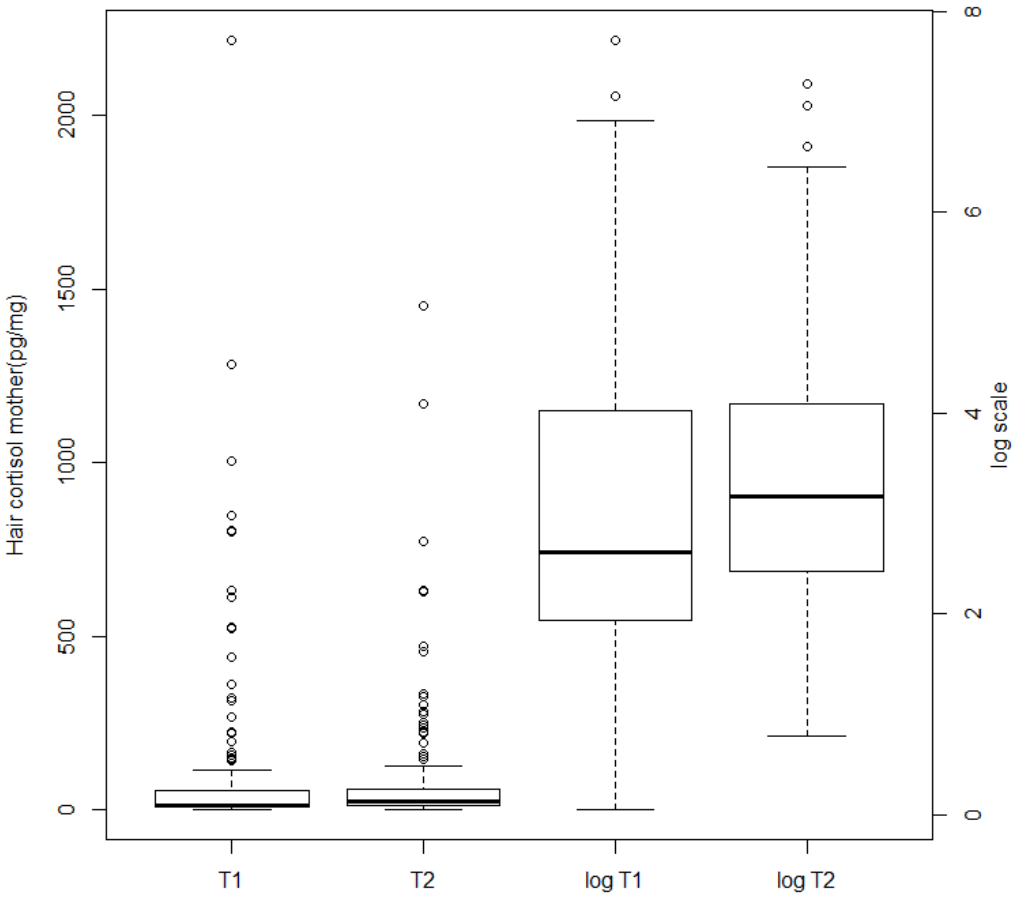
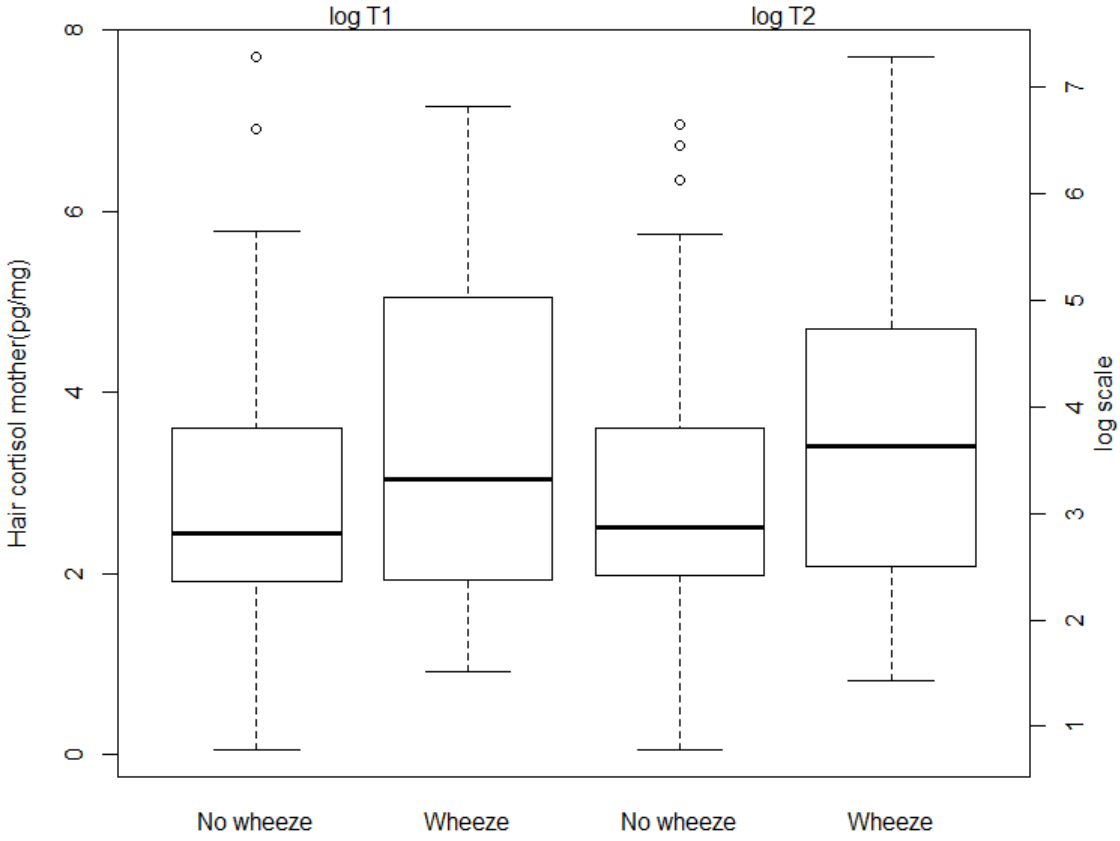


Figure 1.2 Report of wheeze by Hair Cortisol Concentration at T1 and T2



Chapter 2

Strategies for Recruiting and Retaining Pregnant Women Facing High Amounts of Adversity into Randomized Clinical Trials.

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Abstract

Pregnant women from marginalized groups facing high adversity are systemically underrepresented in medical research. Failing to obtain data from a representative sample can compromise the validity and generalizability of a study. Few roadmaps exist to successful recruitment and retention of this vulnerable population into randomized trials with challenging protocols. We report successful recruitment and retention methods for The Vitamin C to Decrease Effects of Smoking in Pregnancy on Infant Lung Function (VCSIP) study, a double-blind randomized controlled trial that recruited pregnant smokers during the years 2013 – 2015 in Indianapolis, IN and in the Pacific Northwest region of the United States. Women who report smoking during pregnancy are disproportionately from marginalized groups and face adverse conditions that contribute to limited participation in clinical trials. VCSIP investigators developed strategies to overcome barriers to recruitment and retention, successfully enrolling 333 pregnant smokers over 17 months and completing follow-up of 86.5% of the offspring through 1 year of life. We also include our recruitment and retention methods disseminated into a 4-step plan spanning pre-recruitment to completion of secondary outcomes.

KEYWORDS

Randomized clinical trial
Recruitment
Retention
Pregnancy
Socioeconomically disadvantaged

Introduction

Pregnant women remain underrepresented in medical research despite recommendations by the United States Food and Drug Association (US) (FDA)[40] and pressure from leaders including the Institute of Medicine[41] and the American College of Obstetricians and Gynecologists.[42] Sixty-four percent of US women receive a prescription medication in the 270 days prior to delivery,[43] yet pregnant women are excluded from 95% of phase IV pharmaceutical trials.[44] Furthermore, women from marginalized groups who face high amounts of adversity (i.e., women of minority race or low level of education) lack representation in clinical trials regardless of pregnancy status.[45] Failing to obtain data from samples representative of the target population can compromise validity and generalizability of the results of randomized clinical trials (RCTs).[46] Excluded groups are deprived from potential health benefits of an intervention and healthcare providers are less inclined to implement novel interventions without population specific evidence.[47]

Limited strategies exist within the literature for recruitment and retention of pregnant women who face high amounts of adversity into RCTs. General recruitment and retention plans to increase enrollment of subjects from marginalized groups[48] may not transfer to pregnant women, and reported strategies specific to pregnant populations[49] may not be effective for RCTs featuring lengthy, demanding protocols that include technically complicated outcomes measured physically in offspring across multiple time-points. Such protocols may elicit parental concern and require greater participant engagement. Mistrust of research investigators by marginalized groups in response to negative experiences with healthcare and public memory of horrific practices such as the Tuskegee Syphilis Study is discussed extensively in the literature.[50] Challenging this narrative, Wendler et al.[51] argued that little difference in participation rates exist

among racial groups and those of minority race are potentially more likely to participate in research, but are less likely to be invited. Given the above context, successful retention and recruitment plans would likely be specific to the proposed intervention, target population, and setting. The population of interest described hereinafter is of pregnant women with low socioeconomic status and who report smoking cigarettes.

This paper presents recruitment and retention strategies employed by the Vitamin C to Decrease Effects of Smoking in Pregnancy on Infant Lung Function (VCSIP) study. VCSIP is a randomized double-blinded controlled trial of vitamin C supplementation (500mg/daily) during pregnancy to decrease some of the harmful effects of tobacco smoke on offspring pulmonary function, measured at 3 months of life. We describe study procedures, descriptive statistics of recruitment and retention rates, and a 4-step recruitment and retention plan, designed to achieve *buy-in* from the key players involved in the success of the study: (a) participants; (b) recruitment center staff; (c) research team.

Methods

Data used in this report are from VCSIP, a double-blinded randomized controlled trial of vitamin C supplementation (500 mg/day) during pregnancy to block harmful effects of maternal prenatal smoking on offspring lung function (ClinicalTrials.gov [NCT01723696]). VCSIP is the second of two consecutive trials of vitamin C supplementation by the same investigators and follows a similar study design as the previous trial, both are referred to hereinafter as VCSIP-1[20] and VCSIP-2. The VCSIP-2 study has two important differences. First, a clinical site set among a more racially diverse population in Indianapolis, Indiana was added to increase sample diversity and thus generalizability; second, a more sensitive and complex measure of offspring lung function at 3 and 12 months of age was included to strengthen the validity of intervention effects on lung function.

Healthy pregnant women >15 years old who were between $13^{0/7}$ and $22^{6/7}$ weeks gestation and who reported smoking were recruited at prenatal visits from a mixture of urban and suburban area hospitals and clinics in Portland, Oregon, Vancouver, Washington, and Indianapolis, Indiana. The study was approved by the institutional review boards of participating hospitals and clinics, and funded by the National Heart, Lung, and Blood Institute and the Office of Dietary Supplements.

Pregnant women meeting the inclusion criteria who signed informed consent, and completed the run-in period were randomized to receive either 500 mg vitamin C daily or placebo. Study investigators, staff, and participants were blinded to treatment allocation. The primary outcome was forced expiratory flows, specifically forced expiratory flows at 75% of forced vital capacity (FEF_{75}) at 3 months of age in the offspring. Secondary outcomes were the incidence of wheeze through 12 months of age and the measurement of FEF_{75} at 12 months, and forced expiratory

flows at 3 and 12 months in the offspring. Data were maintained electronically (REDCap™), and a Data Coordinating Center (DCC) and Data Safety Monitoring Board (DSMB) appointed by the National Institute of Health assured data integrity and quality, and participant safety. Smoking cessation counseling as per the American Congress of Obstetricians and Gynecologists guidelines[52] occurred at enrollment and at each study visit. Daily doses of up to 2000 mg of vitamin C are likely to pose no risks of adverse effect[53] during pregnancy, and the VCSIP-1 study[20] showed no difference in adverse events between placebo-group and pregnant women receiving 500mg vitamin C daily.

Participants were instructed to take study medication daily and were seen by research staff at their prenatal visits where adherence was assessed via pill counts, and questionnaires and biological samples were collected (Table 1). At delivery, samples were collected from mother and offspring to measure ascorbic acid levels and nicotine exposure, and for genetic analysis. From delivery to 12 months postpartum, the mother completed quarterly questionnaires regarding their offspring's respiratory health and maternal smoking. Pulmonary function testing was performed on offspring at 3 and 12 months of age. Pulmonary function testing was non-invasive and followed the guidelines of the American Thoracic Society[54] requiring procedural sedation for which informed maternal consent was obtained per each institution's guidelines. Total participation in the study could be from $13^{0/7}$ weeks gestation to 15 months after delivery. Monetary compensation of $25.00 was provided for sample collection and of $75.00 for infant pulmonary function testing; other compensation included $10.00 per respiratory questionnaire (birth – 12 months of age) and coverage of transportation costs.

Recruitment and Retention

Employee *buy-in* is a sense of ownership developed through aligning of company and employee values[55] and is widely discussed in the private sector as crucial to managerial and company-wide success; VCSIP-2 investigators sought to apply this concept. Thus, participant recruitment and retention strategies were multifaceted stemming from an overarching theme of achieving *buy-in* from the key players involved in the success of the study: (a) participants; (b) recruitment center staff; (c) research team (*see* Appendix A).

Based on experience from the VCSIP-1 study among a similar population, and from reported literature,[56-58] VCSIP-2 investigators identified the following as potential barriers to the recruitment and retention plan: (a) limited interaction with pregnant smokers; (b) intermittent cell phone coverage; (c) unstable housing; (d) drug and alcohol relapse; (e) unreliable or no transportation; (f) removal of enrolled offspring from custody of study participant by the state or voluntary transfer of custody to another guardian; (g) maternal depression or other mental health diagnosis; (h) interpersonal conflict; (i) poor rapport between study staff and participant.

Two RAs at 1.0 full-time equivalent (FTE), based on 40 hr/week, were employed at each Oregon Health & Science University (OHSU) in Portland, OR and at Indiana University (IU) in Indianapolis, IN. RAs and investigators were experienced with clinic and hospital staff because of other prior studies (at OHSU and IU) and the VCSIP-1 (OHSU only). As demographic makeup of pregnant smokers in the US is well established, recruitment was limited to healthy English speaking women who reported smoking at least 1 cigarette in the 2 weeks prior to enrollment, and receiving care and/or delivering at one of the participating hospitals clinics. Electronic medical record (EMR) queries of all pregnant women who reported smoking during a prenatal care visit at OHSU and IU were received by RAs biweekly for screening. At clinics

outside the university hospital systems, contact cards, study brochures and tear-off fliers were provided. In addition, healthcare providers and a lead clinic staff member, typically a nurse or medical assistant, were trained on the study inclusion criteria and encouraged to contact RAs or the site primary investigator (PI) when eligible patients expressed interest in the study. Traditional methods of recruitment were utilized in hospitals and clinics including tear-off fliers, business cards, and a website (www.VCSIP.org).

Based on the VCSIP-1 experience, VCSIP-2 investigators were concerned with the transient nature of the sample population (unstable housing, transportation, and methods of communication). To limit recruitment of women clearly unable to complete the study protocol, an exclusion criterion of "Unable to demonstrate stable method of communication or incarcerated" was established. Such criterion was not intended to exclude women who were highly motivated to join the study, and RAs were advised to use alternative stable contact methods when available (e.g., Facebook messenger). Two emergency contacts were obtained at enrollment including postal and e-mail addresses, a VCSIP-2 Facebook page was created to communicate via private messaging, and encrypted study cellphones were utilized for text and voice calls between RAs and subjects. Permission to contact participants via each mode was established at enrollment, and women could check Y/N.

Women were typically enrolled at regular clinical prenatal appointments, although enrollment could occur at a setting of the participants' choosing. Women were provided a magnet containing study contact information and branded with the VCSIP-2 logo, along with the

placebo run-in medication. A run-in period is designed to limit randomization of potentially non-adherent participants.[59] VCSIP-2 required

women to take ≥75% of one placebo pill per day for 14 ± 7 days (e.g., a 14 day run-in period requires ≥11 pills consumed). Randomization appointments were scheduled at enrollment and the RAs encouraged women to create an appointment reminder (e.g., phone calendar or note). Women were contacted ≥1 time before the randomization appointment, generally 24-48 hrs post enrollment and 24 hrs prior to randomization appointment. Those who did not respond to the RA contact attempt between enrollment and randomization were placed on a "watch list" and multiple contact attempts were made. Women unable to return for randomization visit or who failed run-in (took <75% of 14 + 7 pills) were not eligible to participate in the study. The DCC monitored randomization and only women meeting inclusion/exclusion criteria and passing run-in were randomized. At randomization, tote bags sporting the study logo (Image 2.1) and a monetary incentive for time and travel required at the visit were provided. Small monetary incentives were provided at subsequent appointments requiring maternal/offspring sample collection including newborn pulmonary function tests (PFT). To increase comfort, RAs offered women a snack after any fasting blood samples were obtained. Prior to subsequent study visits subjects were contacted and arrangements were made to answer questionnaires or collect samples at the next prenatal visits. A log created using Microsoft Excel auto-populated the sample collection period windows (shown in Table 2.1) and RAs prospectively planned for sample collection in consultation with participants.

Table 2.1 Summary of Sample Collection for all Time Points

Specimen	Randomization	24-28 GA	30-34 GA	Delivery	3 Months	12 months
Plasma[a]	X	X	X			
Whole blood[b]	X					
Urine[c]	X	X	X			
Exhaled carbon monoxide	X	X	X	X	X	X

Maternal hair[d]	X			X	X	X
Smoking questionnaire**	X	X	X	X	X	X
Cord blood – plasma[a]				X		
Cord blood – whole blood[b]				X		
Placenta samples[e]				X		
Baby hair[d]				X	X	X
Buccal swabs[f]				X	X	X
PFT*					X	X
Respiratory Questionnaire***					X	X

[a] maternal/infant ascorbic acid levels
[b] maternal/infant genome sequencing
[c] cotinine
[d] maternal/infant hair nicotine
[e] ascorbic acid and genomics
[f] genomics
*offspring pulmonary function test
**every study encounter & monthly post delivery
***at least quarterly post delivery
GA = gestational age weeks

To maximize rapport and compliance, RAs offered to meet women outside of normal work hours and at locations of their choosing. Instituting time windows to complete study protocol activities furthered flexibility, the window was ±2 weeks for prenatal sample collection. Completion of the primary outcome could occur between 10 - 24 weeks of post term age. At every study visit, preferred method of contact was reviewed and current contact information was reconfirmed. The offspring's first and surname as well as medical record number were recorded at delivery. All identifying information was maintained in REDCap™ and site-specific. No identifiable information was passed to other sites or to DCC. Every subject had a 6-letter randomization ID name (e.g., HIPPIE) and only that ID name was used in research team discussions or appointment reminders. These unique ID names minimized potential labeling mistakes and maximized patient-staff interactions.

RAs and investigators from the Pacific Northwest and from Indiana convened weekly via Skype to discuss triumphs and pitfalls of recruitment and retention. Continuity of care was important for rapport-building, but alternate RAs were encouraged to offer suggestions for reconnecting with subjects placed on the watch list. Yearly in-person protocol training boosters included team-building activities. PIs drove research staff buy-in through weekly check-ins praising staff for achieving study goals for recruitment and retention, arranging team-building activities, promptly addressing RA concerns, and implementing suggested improvements when appropriate. Recruitment site staff was a source of assistance during the clinical trial, and investigators worked hard to show appreciation by providing bi-yearly updates on completed milestones (e.g., 25% of patients randomized, 50% randomized, or first primary outcome completed) and name-tag lanyards.

Analyses

This report does not include the trial's outcomes, as the objective of this paper is to describe successful strategies for recruitment and retention of pregnant women from socio-economically disadvantaged groups, a vulnerable population that is considered challenging to retain. We performed descriptive statistics on recruitment and retention, which are compared to projections based upon the VCSIP-1 study.[20] Demographic and economic data were obtained from participants (e.g., highest level of education completed, government assisted or private insurance, race/ethnicity etc.), and mode of contact and recruitment were abstracted from REDCap™.

Results

Study Population

VCSIP-2 screened 1231 subjects (698 in Pacific Northwest & 533 in Indiana) and recruited 333 eligible pregnant women who reported smoking, 252 of whom were allocated to vitamin C or placebo upon satisfactory completion of the run-in period. There were 153 participants randomized in Oregon and Washington and 99 participants randomized in Indiana. The study population is primarily non-Hispanic white followed by African American, which reflects the demographics of clinical site locations and of women who report smoking during pregnancy per the discussion above. Sixty-eight percent of the patients had ≤ high school education/GED and 88% had government assisted insurance, again reflecting the literature that shows rates of prenatal smoking are differentially distributed across socioeconomic groups (Table 2.2).

Table 2.2. Maternal Characteristics at Baseline

Characteristic	All Subjects Enrolled (N=333[a]) n (%)
Race	
Caucasian	259 (77.8)
Black or African American	50 (15)
American Indian or Alaska Native	5 (1.5)
Native Hawaiian, Pacific Islander or other	19 (5.7)
Ethnicity	
non-Hispanic	315 (94.6)
Hispanic	17 (5.1)
Not reported	1 (0.3)
Health Insurance Type	
Government	294 (88.3)
Private	36 (10.8)
None or self-pay	3 (0.9)
Highest Education Level	
≤12 years	209 (62.7)
Some college[b]	113 (33.9)
Bachelor's degree[c]	11 (3.3)

[a] includes all enrolled subjects, 252 subjects were randomized
[b] trade school such as hair/beauty school included
[c] no participants reported earning higher than a bachelor's degree.

Retention Timeline

The recruitment period lasted 17 months. The first VCSIP-2 participant was enrolled in December 2013 and the last in May 2015. Average actual enrollment per month was 11.5 participants; the projected range was 9-13 participants enrolled per month. The last infant was delivered in December 2015, with completion of the primary outcome, offspring PFT at 3 months of age, in April 2016. The retention period to secondary aims (PFT at 12 months of age) spanned 24 months.

Recruitment and Retention Rates

Actual cohort loss from enrollment to randomization was nearly double that of the projected percent based on the VCSIP-1 trial (24.3% actual vs. 12.6% projected). Of the 81 not randomized, 67 failed run-in (placebo adherence <75% or did not return for randomization visit) or withdrew consent and 4 women experienced fetal loss (Table 2.3). Conversely, from randomization to delivery actual cohort loss was half of the projected percent (3.2% actual vs. 6.3% projected) suggesting an effective run-in period. Regardless, overall run-in effectiveness, real-time monitoring of recruitment by investigators and the DCC resulted in concern for cohort loss to affect study power, and the randomization goal was increased from 242 to the final sample size of 252. This latter goal was met, and the final sample size completing the primary

outcome exceeded projections (222 infants actual vs. 218 infants projected) while meeting study timeline goals.

Table 2.3 Subject Retention per Protocol

Population	n (%)[a]
Completed the Study as per protocol	
Delivery	244 (96.8)
3 Months Post-delivery PFT	222[b] (88.1)
12 Months Post-delivery PFT	218[c] (86.5)
Withdrew Prior to Study Completion[d]	
Failed Run-in/Withdrew prior to randomization	81 (24.3)
Withdrew before delivery but post randomization	6 (2.4)
Withdrew after delivery but before Month 3	2 (0.8)
Withdrew after delivery but before Month 12	6 (2.4)

[a] enrolled (N=333) and randomized (N=252)
[b] projection 218 completed
[c] projection 212 completed
[d] withdrew / enrolled or randomized (post randomization)

Recruitment and Retention Logistics

VCSIP-2 staff required personal transportation to attend patient appointments at the various recruitment sites or at participants' homes; mileage reimbursement was provided. RAs were at recruitment sites 5 days per week until completion of study protocol for entire sample, either enrolling new women or meeting enrolled participants for sample and questionnaire collection or study drug pill-count and refill. Informed consent lasted 45 - 60 minutes from initial introductions to completion. The study is complicated since we are allocating pregnant women to an intervention, but the primary outcomes are measured in the offspring through 12 months of age. Successful cohort retention depended on a thorough explanation of the study protocol and

confidence that participants comprehend the offspring PFT procedures, which will occur roughly 7 months into the future. The latter is a critical since it is the primary outcome and the PFT requires infant sedation, a potential factor increasing cohort loss.

Strategies to improve efficiency and patient satisfaction were shared at weekly team meetings. Strategies included calling ahead to request the per-protocol amount of blood be drawn for VCSIP-2 study during a routine prenatal glucose measurement to limit additional venipuncture, or consulting clinic staff regarding available space to reduce potential conflicts between study visits and clinical care. For post-delivery questionnaires, RA's called at the first of the month when participants likely had cell-phone minutes, and Facebook Private Message (PM) was readily used, as it requires only Wi-Fi. RAs also contributed their time in tasks not directly related to study ascertainment, such as assisting participants in obtaining car seats or other childcare supplies when necessary and available through local organizations.

Participant completion of the protocol was maximized through consistency in research staff and maintaining multiple modes of communication. RAs became familiar with communication habits of participants. Generally, participants were placed on the "watch list" when (a) they "no-showed" a prenatal appointment; (b) regular method of contact was disconnected; (c) no response after 1 week post three contact attempts. At times RAs would forego the general guidelines and place a subject on the watch list if communication habits changed. Until completion of the primary outcome at 3 months of age VCSIP-2 employed two RAs at 1.0 FTE at each site, for a total of 4 RAs. Time to complete study visits post-delivery varied by participant. At best, a participant answered phone calls or responded quickly to text messages or voicemails. At worst, a participant was without custody of the enrolled infant and consent from legal or current guardian was sought, requiring weeks or months of transactions.

Discussion

VCSIP-2 successfully randomized 252 pregnant women who reported smoking during pregnancy, of whom 222 women and their offspring were retained until completion of the primary outcome at 3 months post-delivery. Despite mid-study concern, sample size goals met or exceeded projections, suggesting proposed recruitment and retention plans of medical record queries, run-in phase, and multiple methods of contact, as well as real-time adaptive strategies of early-month phone calls and Wi-Fi capable modes of communication were successful. Notably, <10 participants were recruited via fliers (traditional methods); instead, most enrollments were RA-initiated and guided by EMR queries and clinic staff liaison.

VCSIP-2 cohort retention to the primary and secondary outcomes at 3 and 12-months post- delivery was 88.1% and 86.5%, respectively. RCTs with prenatal and postpartum activities among comparable US populations range in retention rates 74%-95% for any postpartum follow-up.[49,50,60,61] Few randomized trials attempt placebo-controlled interventions among highly vulnerable populations of pregnant women with outcomes measured in offspring.[48]

RAs led real-time adaptive retention strategies. After delivery, women in our study most commonly experienced changes in healthcare, housing, their own mental health including drug relapse and depression, and interpersonal conflict with partners and family. These events influenced how RAs operated: visits to homeless shelters; three-way communication among participants and state agencies or other guardians of enrolled infants removed from participant's custody; securing subject's transportation to study appointments were among the actions taken to limit attrition. Snowball recruitment occurred in limited numbers, but without influence by VCSIP-2 staff. Beside social support and peer pressure, this method may be effective among

women who potentially feel more ownership over participation in a study within a community of like-individuals.[62]

REDCap™ and Microsoft Excel proved to be useful tools for tracking subject progression through the study protocol, and DCC staff queried RAs when subjects were nearing end of sample collection windows. Had the study performed tracking of communication attempts, participant phones number and address changes, and removals from maternal home of enrolled infant there would be further objective evidence of difficult life circumstances affecting this population.

Eighty percent of African American participants were recruited from the Indianapolis, Indiana site who recruited 37% of the total participants (129 vs. 204 at Oregon/Washington sites). The difference in recruitment potentially limited participation of African American women in the study. Potential reasons for the difference between regional sites are threefold: the exclusion criteria "participating in another research study" was cited in 12 women in Indiana vs. none of the women in Oregon and Washington; inclement weather in the winters of 2013 and 2014 in Indiana that limited access to the hospital and participating clinics; differences in the population other than race/ethnicity unaccounted for by baseline demographics. Despite differences in recruitment, the sociodemographic and economic status of the final sample reflect expectations for pregnant women who report smoking in the US.

We acknowledge that aside from successful completion of study recruitment and retention goals, measuring the success of our buy-in themed recruitment and retention plan would benefit from a qualitative component. Anecdotal measures include a continued and responsive following on Facebook by 70 participants, and regular inquiries from recruitment center staff regarding study outcomes. Although we did not find interpersonal conflict between

staff and participants to be an issue, again, qualitative assessment of study participants' perspectives would assist in evaluating the effectiveness of rapport building strategies. Additionally, we found that the life situations exemplified throughout this paper made it difficult for participants to stay in contact with the study staff and women expressed relief when RAs made the effort to contact them. Such experiential feedback from the RAs' points-of-view remains one-dimensional without a complete qualitative assessment of participants' perspectives.

Frequent, consistent, and personal contact is critical to retain this vulnerable population in a research protocol. The four 1.0 FTE RAs relied heavily on WI-FI capable communication such as Facebook PM. Interval phone calls or text messages or Emails to patients occurred approximately monthly, and investigators often utilized multiple contact phone numbers and addresses (including those of relatives and friends). Time windows to complete study protocol activities, availability of RAs via study cell phone outside of normal work hours, and small reimbursements for sample collection, time and travel assisted in retaining the VCSIP-2 cohort of pregnant women facing high adversity who report smoking cigarettes.

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Appendix A. 4 - Step Recruitment and Retention Plan

Step 1. Pre-recruitment

1a. VCSIP-2 primary investigators (PI) worked as clinicians at most recruitment sites and personally and frequently communicated the background, objectives and protocol to staff in the year before recruitment began.

1b. Formal luncheons were held at each recruitment center once before recruitment began and recruitment materials were provided in advance to allow for discussion among site staff and the research team regarding practical methods for consenting on-site (e.g., private rooms or spaces to obtain informed consent and best practices for sharing the crowded clinic workspace).

1c. Recruitment site staff were provided with VCSIP-2 logo branded items including a name-tag lanyard with orange-print material.

Step 2. Recruitment

2a. Foremost, research assistants (RAs) were available at recruitment sites to enroll participants 5 days/week. RAs responsibilities at the recruitment sites also included maintaining relationships with the site staff, RAs fielded questions, and reminded site staff to refer pregnant smokers.

2b. Screening occurred on-site and RAs received weekly medical record queries of upcoming prenatal appointments of women who report smoking. RAs would contact

women to schedule the initial study visit prior to or after the prenatal appointment: non-responders were still approached at prenatal appointment.

2c. Recruitment site staff were provided with bi-yearly updates on VCSIP-2 progress toward recruitment goals in conjunction with vitamin C themed cookies and thank-you baskets containing teas, chocolate, oranges etc.

2d. During informed consent RAs had women repeat back the general concept of the pulmonary function test (PFT) procedures, which required procedural sedation. This occurred prior to discussion of monetary incentives.

Step 3. **Retention to Birth**

3a. Multiple methods of contact were exchanged at enrollment: participant's cellphone; email; address; employer; two emergency contacts (e.g., full name, address, phone, and email); permission to contact via social media private messenger. Participants were provided with the RA's study cellphone numbers and Facebook contact information.

3b. Enrolled participants completed a placebo drug adherence trial (run-in period) prior to blinded allocation to study arm. Only women who returned having consumed 75% or more of expected quantity of placebo tablets were randomized (1 tablet/day for 14 days +-7 days).

3c. At the randomization study visit women were required to fast for 8 hours prior, and RAs provided snacks and a VCSIP-2 logo branded tote bag with magnets and business cards inside.

3d. Upon completion of the randomization visit women were provided with $25.00 and an additional $25.00 for subsequent visits requiring sample collection.

3e. Based on participant's preference, RAs sent text messages and/or called participants prior to prenatal appointments and met with them during regularly scheduled prenatal visits at least monthly until delivery. RAs also made themselves available to meet other times.

3f. At delivery RAs personally visited participants and provided a VCSIP-2 logo branded infant onesie.

3g. Preferred method of contact and housing status were confirmed at each participant encounter.

Step 4. **Retention to primary and secondary aims**

4a. Participants were provided with $10.00 for each monthly respiratory questionnaire completed and $75.00 for completion of the PFT at 3 and 12 months. When necessary,

participants received monetary assistance for transportation to the hospital to complete the 3 and 12 month PFTs.

4b. Approximately 2-4 weeks after delivery, RAs mailed VCSIP-2 logo branded Postcards congratulating mothers on the birth and reminding them of the 3-month appointment.

4c. Weekly video conferencing calls occurred between RAs and PIs at the two different clinical sites. All participants were reviewed regarding pending study procedures (using study ID only). Participants with pending procedures and participants the RAs were unable to contact for two consecutive weeks were placed on a "watch list". Strategies to communicate with these participants included: text message and private Facebook message; calls outside of workday hours and on weekends; certified letters to addresses.

4d. RA's reviewed participant's medical charts for upcoming maternal or child clinical appointments and would meet participants at clinics to complete questionnaires or schedule 3 or 12 month appointments for PFTs.

4e. RAs worked hard to build rapport with family members or foster care providers in scenarios where children were no longer with the mother. Occasionally RAs met participants in the community setting at homes or shelters.

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Jackson, K., Tepper, R.S., Morris, C.D. (In press). Vitamin C to Decrease the Effects of Smoking in Pregnancy on Infant Lung Function (VCSIP): Rationale, design, and methods of a randomized, controlled trial of vitamin C supplementation in pregnancy for the primary prevention of effects of in utero tobacco smoke exposure on infant lung function and respiratory health. Contemporary Clinical Trials.

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CURRICULUM VITAE

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CERTIFICATION AND LICENSURE

Registered Nurse	Oregon State #201041985
	Washington State #60515085
C.P.R.	American Heart Association expires 10/2018

EDUCATION

2014-PRESENT	University of Washington SON, Seattle, WA, PhD Candidate
2007-2010	Oregon Health & Science University SON, Portland, OR, B.S.N, Cum laude

PROFESSIONAL EXPERIENCE

Research

01/2014 - PRESENT	Oregon Health & Science University Assistant *Vitamin C to Decrease the Effects of Smoking on Infant Lung Function study* PI, Cindy McEvoy, MD, MCR [clinical trials # NCT01723696]	Senior Research
06/2015 - 12/2015	Oregon Health & Science University *Validation of a Novel, Non-Invasive, Accurate Measurement of Skeletal Muscle Mass in Children and Infants* P.I. Brian Scottoline, MD, PhD [Gates Foundation]	Study Coordinator
01/2015 - 03/2015	University of Washington, SON Assist with design of an intervention for breast cancer survivors Supervisor, Kerryn Reding, PhD	Research Assistant
01/2015 - 04/2015	University of Washington, DGH Assist with literature review of PrEP in pregnancy PI, Grace John-Stewart, MD, PhD	Research Assistant
05/2012 - 09/2014	Oregon Health & Science University *A Noninvasive Portable Device to Evaluate Feeding Problems in Infants*	Study Coordinator

PI, Brian Rogers, MD [SBIR grant # 54308]

Teaching

09/2015 - 06/2016	University of Washington, SON *NURS 415A: Nursing of Families: Childbearing and Childrearing* Supervisor, Rizza Cea, CNM & Anne Kalkbrenner, RN, MSN *NURS 419: Transition to Professional Practice* Supervisor, Rebecca O'Connor, RN, PhD	Teaching Assistant
03/2015 - 06/2015	University of Washington, SON *NSG 432: Infants and Young Children, Risk and Resilience* Supervisor, Susan Spieker, PhD	Teaching Assistant
04/2012 - 06/2012	Girls Inc. S.T.E.M. after school curriculum instructor Portland, OR	Program Facilitator
09/2009 - 04/2009	Umpqua Community College School of Nursing Roseburg, OR	Student Tutor

Clinical

09/2010 - 04/2012	Mary's Woods Skilled Nursing Care Lake Oswego, OR	Charge Nurse
07/2010 – 09/2010	GetAFluShot.com Community Immunizations Portland, OR	Nurse

FUNDING

Source:	UW Institute of Translational Health Sciences
Role:	P.I.
Entire Project:	6/2016-6/2017
Direct Costs:	Graduate Tuition, monthly stipend, and $2,600
Title:	"Maternal Hair Cortisol and Offspring Lung Function"

Maternal prenatal stress is a plausible contributing factor to altered lung development and subsequent childhood respiratory disease. Prenatal hair cortisol concentration will be measured across pregnancy and compared to pulmonary function testing at 3 months of age, in a cohort of 252 mother-infant dyads consisting of racially diverse and underserved women recruited for the study, "Vitamin C to Decrease Effects of Smoking in Pregnancy on Infant Lung Function" [NCT01723696].

Source:	Fred Hutchinson Cancer Research Center: Health Disparities Research Center.
Role:	Co-Investigator (Bridgette Hempstead)
Entire Project:	2/2015- 02/2016
Direct Costs:	$7,500
Title:	"Examining the use of peer education to empower African American women to improve their health"

This pilot project tested a train-the-trainer designed intervention intended to improve knowledge of breast health and confidence in communicating with providers among African American women in the Seattle, Washington area.

AWARDS, SCHOLARSHIPS, HONORS

Scholarships		
2016/2017	Catherine H. Thompson Nursing Term Fellowship	$5,000
2016/2017	Jacqueline Vandeman Endowed Fund	$8,000
2016/2017	Katherine Hoffman Fellowship in Nursing Science	$9,000
2015/2016	Christie Endowed Fund, academic conference travel	*$350.00*
2015/2016	Catherine H. Thompson Nursing Term Fellowship	$5,000.00
2014/2015	Christie Endowed Fund, academic conference travel	$350.00
2014/2015	University of Washington School of Nursing Top Scholar Award	Full Tuition
2009/2010	Oregon Health & Science University Pasarow Scholarship	Partial tuition
Honors		
2010	Oregon Health & Science University	Magna Cum Laude
2008	Umpqua Community College Nursing Program	President's List

PUBLICATIONS

Peer Reviewed

McEvoy, C., **Scherman, A.,** Milner, K., Schuff, R., Schilling, D., Tiller, C., Spindel, E., Mitchell, J., Haas, D., Vuylsteke, B., Shorey-Kendrick, L., Peters, D., Metz, J., Tepper, R., Jackson, K., Morris, C. (in press). Vitamin C to Decrease the Effects of Smoking in Pregnancy on Infant Lung Function (VCSIP): Rationale, design, and methods of a randomized, controlled trial of vitamin C supplementation in pregnancy for the primary prevention of effects of in utero tobacco smoke exposure on infant lung function and respiratory health. *Contemporary Clinical Trials*.

Scherman, A., Wiedrick, J., Lang, W., Rdesinski, R., Lapidus, J., Abu-Shamieh, A., Buckley, S., McEvoy, C., Rogers, B., Buist, N. (under review) Maturation of Nutritive Sucking in Preterm and Full Term Infants. *Dysphagia*.

MacDonald KD, Moran AR, **Scherman AJ**, McEvoy CT, Platteau AS. Maternal high-fat diet in mice leads to innate airway hyperresponsiveness in the adult offspring. *Physiological Reports*. 2017;5(5):e13082. doi:10.14814/phy2.13082.

Molina, Y., **Scherman, A.,** Constant, T.H., Hempstead, B., Thompson-Dodd, J., Richardson, S., Weatherby, S.R., Reding, K.W. and Ceballos, R.M., 2016. Medical advocacy among African-American women diagnosed with breast cancer: from recipient to resource. *Supportive Care in Cancer*, pp.1-8.

Invited Presentations

06/2017 American Society for Mass Spectrometry. 65th ASMS Conference on Mass Spectrometry and Allied Topics 2017.
Indianapolis, IN
Poster Presentation
Sensitive quantitative measurement of cortisol from limited human hair samples using LC-MS/MS
David W. Erikson, Amy V. Kaucher, Emily Agan, Andrea J. Winchell, Steven W. Blue, **Ashley Scherman**, Cynthia T. McEvoy, Eliot R. Spindel [*Abstract ID number: 288743*]

05/2017 Pediatric Academic Society Annual Meeting. San Francisco, CA
Poster Presentation
Adverse Experiences in Childhood and Preschool Language Development
Scherman, A., Hash, J., Fleming, Lohr, MJ., C., Oxford, M.

04/2017 Western Institute of Nursing's 50th Annual Communicating Nursing Research Conference. Denver, CO
Poster Presentation
Adverse Experiences in Childhood and Preschool Language Development
Scherman, A., Hash, J., Fleming, C., Lohr, MJ., Oxford, M.

09/2016 The Council for the Advancement of Nursing Science.
Washington D.C.
Podium Presentation
Women's views on a healthy lifestyle: uncovering similarities and differences by race and ethnicity.
Kerryn Reding, **Ashley Scherman**.

10/2015 The National Institute of Nursing Research, Building the Scientific Foundation for Clinical Practice. Bethesda, MD
Poster Presentation
Age-related Quantitation of Sucking Behavior in Normal Infants.
Scherman, A, Lang, WC, Lewis, H, McEvoy, C, Orozco, Abu-Shamsieh, A, Rdesinski, R, Wiedrick, Lapidus, J, Rogers, B, & Buist, N

04/2015 Pediatric Academic Society Annual Meeting. San Diego, CA
Poster Presentation
Age-related Quantitation of Sucking Behavior in Normal Infants.
A Scherman, WC Lang, H Lewis, C McEvoy, V Orozco, R Rdesinski, J Lapidus, and B Rogers. *[E-PAS2015:750480]*

05/2013 Pediatric Academic Society Annual Meeting. Washington, DC
Poster Presentation
Quantification of sucking behavior in late preterm [LPI], and full term [FT] infants at 38-42 weeks post menstrual age [PMA].
Scherman, A., Lewis, H., Lang, WC., Rogers, B., McEvoy, C., Buist N., and Orozco, V.*[E-PAS2013:3840.697]*

Abstracts

11/2014	*Pulmonary Function in Very Low Birth Weight Infants Without BPD Before Hospital Discharge.* Ryan Lam, Diane Schilling, Amanda Hamilton, **Ashley Scherman**, Cindy McEvoy. Publication Trends in Neonatal Respiratory Support *[E-PAS2015:*1599.739*]*

SERVICE, PROFESSIONAL AND COMMUNITY

Present	Psi-at-Large Chapter of the Honor Society of Nursing Sigma Theta Tau International (STTI)	Member
09/15/2016	The Council for the Advancement of Nursing Science (CANS) Podium Session A, *Women's Health* Washington D.C.	Session Moderator
2015 - 2016	Fred Hutchinson Cancer Research Center Bridgette Hempstead of Cierra Sisters Community Based Research Partner (CBRP) Seattle, WA	
2013 - 2014	Oregon Health & Science University Tech Fair Neuroimaging Lab Portland, OR	Neonate Resuscitation
2011 - 2012	Girls Inc. After school program Portland, OR	Co-facilitator
2009 - 2011	Planned Parenthood Portland, OR	Patient Advocate
2007 - 2008	Special Olympics Douglas County, OR	Assistant Coach

SERVICE, ADMINISTRATIVE AND ACADEMIC

2014 - PRESENT	University of Washington **PhD Course Committee School of Nursing** Seattle, WA	Student Representative
2009 - 2010	Oregon Health & Science University **Nursing Students for Choice** Portland, OR	Co-chair
2009 - 2010	Oregon Health & Science University **Student Council School of Nursing**	Class representative

	Portland, OR	
2007 - 2008	Umpqua Community College **Maya Public Health & Stove Building** Xela, Guatemala	Assistant Coordinator