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Thyroid Hormone Levels Associated with Active and Passive Cigarette Smoking

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Background: Active and passive cigarette smoking are a risk factor among women of reproductive age—leading to reproductive health morbidity, including fetal and infant death and developmental problems with the newborn. However, the underlying physiological mechanisms for these ill-effects are not fully understood. Smoke exposure may affect various metabolic and biological processes, including hormone biosynthesis and secretion, interfere with thyroid hormone release, binding, transport, storage, and clearance, associated with adverse effects on the thyroid resulting in changes in circulating hormone concentrations. We measured and compared serum thyroid hormone and thyroid stimulating hormone (TSH) concentrations in active, passive, and nonsmokers and determined their association with cigarette tobacco smoke exposure. We use a comprehensive approach to assess the interrelationships between active and passive tobacco smoke exposure and thyroid hormone levels by employing innovative hormone analysis techniques.
Methods: Serum was obtained from women (18–44 years of age). Thyroxine (T4), triiodothyronine (T3), and cotinine concentrations were quantified using isotope dilution high performance liquid chromatography tandem mass spectrometry, and TSH concentrations by chemiluminescence.
Results: Serum concentrations of the various hormones of active smokers, passive smokers, and nonsmokers (nonexposed), respectively, were as follows. Median TSH concentrations were 1.02, 1.06, and 1.12 mIU/L ($p < 0.001$ for the comparison of each group with the other two groups), and mean TSH levels were 1.40 mIU/L, confidence interval (CI) 0.07–6.83 mIU/L; 1.30 mIU/L, CI 0.25–3.01 mIU/L; and 1.50 mIU/mL, CI 0.71–4.00 mIU/L. Median serum T4 concentrations were 7.6, 7.9, and 8.7 μg/dL, and median serum T3 concentrations were 92.0, 84.0, and 102.0 ng/dL ($p < 0.0001$). Mean T3 levels were 99.1 ng/dL, CI 52.1–204.3 ng/dL; 87.6 ng/dL, CI 40.1–160.2 ng/dL; and 106.6 ng/dL, CI 46.4–175.0 ng/dL. Pair-wise comparisons of the three study groups indicate statistically significant differences in serum T4 ($p < 0.01$) and T3 ($p < 0.001$) means for the comparison of each group with the other two groups.
Conclusions: This study is unique in examining the association of serum cotinine and thyroid hormone concentrations using liquid chromatography tandem mass spectrometry in women smokers, passive smokers, and nonsmokers. Active and passive exposure to cigarette tobacco smoke is associated with a mild inhibitory effect on the thyroid reflected in higher serum T4 and T3 in nonsmokers compared to smokers in this cohort of women.

Introduction

The thyroid hormones are critical in cellular metabolism and in the coordination of physiological and behavioral responses (e.g., growth, maturation, and differentiation to biological stimuli) (1). In women of reproductive age, thyroid hormone insufficiency can impact fertility and reproduction, resulting in pregnancy loss and low birth weight (2). Moreover, thyroid hormone sufficiency during pregnancy is critical for normal fetal neurodevelopment (2,3).

The effects of cigarette tobacco smoke on the thyroid have been of concern. Cigarette smoke contains over 4800 compounds, including at least 200 toxicants or endocrine disruptors and 80 known or suspected carcinogens (4). Further, the International Agency for Research on Cancer classifies cigarette tobacco smoke itself as a known human carcinogen

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(5). A burning cigarette emits both mainstream smoke, which originates from the mouth piece of the cigarette and is inhaled and exhaled by the smoker, and sidestream smoke, which emanates from the tip of a smoldering cigarette. Second-hand smoke (SHS), to which passive smokers are exposed, is comprised of both mainstream (11%) and sidestream (85%) smoke, along with other contaminants (4). Thus, passive smokers are exposed to a different spectrum of toxicants than the active smokers. In the United States, approximately 30% of women of reproductive age smoke cigarettes (6), while many others exposed to SHS are passive smokers (7).

There is currently a gap in knowledge regarding the effects of cigarette smoke on thyroid hormone levels in women of reproductive age. Exposure to cigarette tobacco smoke has been previously shown to have variable effects on thyroid function, reflected by an increase or decrease in serum thyroxine (T4)/triiodothyronine (T3) concentrations (8–17). Active and passive smoking can result in smoke-induced interference with thyroid hormone homeostasis (18,19). Moreover, T4 biosynthesis, release, binding, and transport have been associated with disturbances in circulating hormone levels (18,19). Cigarette smoking is associated with changes in maternal thyroid function throughout the pregnancy and in fetal thyroid function reflected in cord blood levels. In a meta-analysis of 25 studies ($n = 62$–208) that examined the association between smoking and thyroid diseases (including Graves' disease, Graves' ophthalmopathy, toxic nodular goiter, nontoxic goiter, postpartum thyroid disease, Hashimoto's thyroiditis, or hypothyroidism), an odds ratio (OR) of 3.30 (95% confidence interval [CI] 2.09–5.22) was found among current smokers, and OR = 1.41 (95% CI 0.77–2.58) among previous smokers (20). In addition, cigarette smoking has been associated with an increased production of T3 and thyroglobulin, indicating decreasing levels of T4 (21,22), while thyroid-stimulating hormone (TSH) levels have been found to be significantly lower in cigarette smokers compared to nonsmokers (23,24). Further, serum T4 and T3 quantification has been controversial due to unreliable or inconsistent results because of inherent problems associated with the nature of immunoassays (25–27).

To resolve some of the controversies regarding the association between thyroid hormone levels and tobacco smoke exposure, we measured T3 and T4 levels using state-of-the-art liquid chromatography tandem mass spectrometry (LC/MS/MS) technology and determined individual serum concentrations associated with serum cotinine levels as well as by self-reported smoking status. The harmful effects of smoking on the immune system are known (28,29). Our analysis also included considerations of autoimmune antibodies and dietary intake that could influence T3 and T4 levels and increase the adverse effects of cigarette tobacco smoke.

Materials and Methods

The study was approved by the Georgetown University Medical Center (GUMC) Institutional Review Board. The study population included active smokers, passive smokers (SHS-exposed), and nonsmokers. After obtaining consent, all participants provided blood samples and completed a structured in-person interview conducted by a trained clinical research assistant. The interviewer collected detailed information by completing a validated questionnaire on tobacco smoke exposure and other potential risk factors. Blood samples were processed and analyzed in our laboratory at the Lombardi Comprehensive Cancer Center and the Bioanalytical Core Laboratory, GUMC. Serum cotinine analysis was conducted at Harvard University.

Subject recruitment and consent

The current prevalence of women who smoke in the Washington metropolitan area ranges from 13.6% to 23.6% depending on age and race (Centers for Disease Control and Prevention [CDC] prevalence data). We recruited 237 subjects who met the inclusion/exclusion criteria from the greater Washington, DC, metropolitan area using flyers (internal GUMC) and advertisements in the health section of local newspapers to solicit telephone calls from interested subjects. Women who met the eligibility criteria were invited to the GUMC and were consented before sample collection and administration of the questionnaire. Baseline characteristics of study participants are presented in Table 1.

Inclusion and exclusion criteria

Participants were healthy, nonpregnant women with regular menstrual cycles, between the ages of 18 and 44 years. Women were ineligible to participate in the study if they had any major illness, infection, immunological or endocrinological disorders within the previous 6 months, or surgery with general anesthesia within the previous 3 months, or if they used birth control pills, steroids, or immunosuppressive medications within the previous 2 months. By design, all men, postmenopausal women, and women with irregular menstrual cycles were excluded. Only women from Maryland, the District of Columbia, and nearby counties of Virginia (Arlington, Alexandria, Fairfax, and Loudoun) were recruited.

Questionnaire

A questionnaire was developed to assess variables related to both active and passive cigarette tobacco smoke exposure. The questionnaire, based on the validated Transdisciplinary Tobacco Use Research Centers questionnaire (30), comprised 11 sections that detail subjects' cigarette tobacco smoke exposure profiles, occupational exposure, hobbies, and alcohol

Table 1. Baseline Characteristics of Study Participants

	Analysis variable	%
Education	Up to 12th Grade	26
	Some College/Technical School	18
	College or Professional School	56
Race/Ethnicity	White	59
	Black	14
	Hispanic	21
	Other	5
Alcohol consumption	Yes	51
	No	49
Body mass index	<25	83
	25–30	11
	>30	6
Household income	<49.99 thousand	47
	>50 thousand	53

and drug consumption. In addition, there are sections on individual and family smoking history, weight change in adulthood, medical and reproductive health history, and socioeconomic factors. Nutritional health history was extensively evaluated using a food frequency questionnaire.

Biological specimen collection and processing

For this study, it was important that all women provided biosamples such that data could be evaluated accurately. For the assessment of hormone concentrations, all samples were collected at the follicular phase and at a similar time of day to minimize diurnal variation. The blood collected was processed and separated into multiple labeled (ID number only) aliquots of sera. All aliquots were frozen at -80°C until analysis.

Classification of subjects

The women were classified into one of the three study groups—active smokers, passive smokers, or nonsmokers—using two classification methods based on self-reported smoking status and on serum cotinine concentrations.

Self-reported smoking status. (a) Active smokers were defined as subjects who had smoked at least one cigarette in the previous 5 days; (b) passive smokers were defined as subjects who had been regularly exposed to SHS within the previous 6 months; (c) nonsmokers were defined as subjects who had neither smoked a tobacco product within the previous 5 days nor been exposed to SHS within the previous 6 months.

The subjects were also classified according to serum cotinine concentrations as a biomarker for cigarette smoke exposure (31). The stability of cotinine (half-life, $t_{1/2} = 18$–24 hours) makes it a more suitable surrogate for nicotine exposure, which is comparatively unstable ($t_{1/2} = 0.5$ hour). Cotinine levels therefore reflect smoking activity in the past few days before serum measurements (it is possible to detect serum cotinine for up to 5 half-lives). (a) Active smokers were defined as those subjects who had serum cotinine concentration greater than or equal to 15 ng/mL; (b) passive smokers were those whose serum cotinine levels fell between 0.54 and 15 ng/mL; (c) nonsmokers had serum cotinine concentrations below 0.5 ng/mL (32). Serum cotinine allows the verification of exposure to cigarette tobacco smoke in the immediate 48 hours before testing.

Serum cotinine. Since we were interested primarily in endogenous hormonal changes, we confirmed endogenous smoke exposure by measuring serum cotinine concentrations to reliably reflect nicotine exposure. Cotinine concentrations were quantified using LC/MS/MS (33) after a simple extraction(API–3000; Applied Biosystems, Foster City, CA). While most immunoassays for cotinine are only sensitive to 20 ng/mL, LC/MS/MS is sensitive to at least 0.5 ng/mL, enabling us to distinguish active smokers, passive smokers, and nonsmokers.

Hormone analysis

Thyroid hormones were analyzed using isotope dilution LC/MS/MS API-4000 (Applied Biosystems) equipped with an electrospray ionization source, with deuterium-labeled internal standards as described previously (34–36). The measurement of T4 and T3 was precise and accurate down to 30 pg/mL. Within run and interassay imprecision, as well as the coefficients of variation for replicate quality control samples were 4–6%. TSH concentrations were measured using chemiluminescence (Dade RXL Dimention; Siemens, Willmington, DE) according to the manufacturer's instructions. Luteal hormone (LH) and follicle-stimulating hormone (FSH) concentrations were measured according to the manufacturer's instructions using immunoassays (Immulite 2000; Siemens, Wilmington, DE). LH and FSH were determined to confirm that each subject was in the follicular phase of her menstrual cycle at the time of the blood draw (follicular phase: LH $<20\ \mu$IU/mL and FSH 2.5–10.2 μIU/mL).

Quality control

All subjects were asked to respond to the same structured interview by trained clinical interviewers. Several types of analyses were used to detect potential interviewer bias and data errors not picked up during data entry. Range and validity checks were incorporated in the program to identify discrepancies. Any inconsistencies in questionnaire completion were directed to the interviewer for resolution. All hormone measurements and biomarker analyses were blinded and conducted in batches. Quality control samples were run at several concentrations with each batch of samples. In addition, external proficiency testing samples were run daily.

Statistical analysis

The data were examined for the main effect of tobacco smoke exposure on hormone concentrations (37). Hormone concentrations were compared, using *t*-tests and analysis of variance (ANOVA), between active smokers, passive smokers, and nonexposed study subjects separately and then overall. A one-way ANOVA was used to examine the difference in means of a normally distributed variable (hormone) between more than two groups (smoking groups). When the normality assumption was violated, the Kruskal-Wallis test, which is the nonparametric analog of the one-way ANOVA, was used. The Bonferroni correction was used to adjust for multiple comparisons. Due to the fact that Statistical Analysis Software (SAS®) does not currently support multiple test correction for the Kruskal-Wallis test, values of variables whose distribution was not normal were ranked. A one-way ANOVA was then used to examine statistically significant differences among the ranks of the three smoking groups. Adjusted geometric means and 95% CIs were calculated using linear regression on log-transformed hormone concentrations, adjusting for age (linear), body mass index (BMI), race/ethnicity (non-Hispanic white, African American, or other), hormone therapy use (yes or no), herbal hormone use (yes or no), tobacco smoke exposure (smoking or nonexposed women).

Correlations of all factors were tested, so that no substantially corelated variables (e.g., age and duration of smoking) were included in the model, improving model stability. If two variables were correlated, their relationship with the outcome hormone concentrations was tested individually, and the variable that explains the most variation in the hormone levels was the one included in the model. The associations of hormone changes and other potential risk factors, including age, race/ethnicity, BMI, alcohol consumption, socioeconomic status, and education, were also examined.

Power calculations for various hormone levels in smokers, passive smokers, and nonsmokers were based on the methods of Gallicchio *et al.* (38). These levels were measured in women of ages 45–54 years, whereas we studied women whose estrogen levels are expected to be much higher since they are in their reproductive years. The relative differences between smokers and nonsmokers should be similar in these younger women. We have further assumed that $\alpha = 0.05$ and that there are equal numbers of subjects in each group.

Results

Serum LH and FSH concentrations confirmed that each subject included in our analysis was in the follicular phase of her menstrual cycle at the time of the blood draw (follicular phase: LH $<20\,\mu$IU/mL and FSH 2.5–10.2 μIU/mL). The number of subjects in each of the three study groups varied depending on the classification method used (based on either self-report or serum cotinine concentrations). However, cotinine has a half-life of less than 18 hours, and therefore any reported passive exposure that did not have any measurable serum cotinine concentration did not necessarily reflect nonexposure, but probable nonexposure in the past 48 hours. According to serum cotinine analysis, self-reported nonsmokers were most accurate in self-classification, while the status of most self-reported active smokers and some of the self-reported passive smokers was confirmed by serum cotinine measurements.

Hormone profiles for T3, T4, and cotinine were determined by LC/MS/MS for all subjects. Mean, median, and 95% CIs for each of these analytes are presented in Table 2.

Table 2. Hormone Concentrations of Thyroid-Stimulating Hormone, Triiodothyronine, and Thyroxine in Active Smokers, Passive Smokers, and Nonsmokers

Analyte	*TSH (mIU/L)*	*T4 (μg/dL)*	*T3 (ng/dL)*
Reference interval	0.5–3.5	4.7–12.0	78–217.4
Active smokers ($n = 79$)			
Mean ± SD	1.40	7.8 ± 2.31	99.1 ± 36.9
Median	1.02[a]	7.6[b]	92.0
95% CI	0.07–6.83	3.9–13.3	52.1–204.3
Passive smokers ($n = 53$)			
Mean ± SD	1.30	8.1 ± 2.62	87.6 ± 30.5
Median	1.06	7.9	84.0
95% CI	0.25–3.01	4.5–12.3	40–160.2
Nonsmokers (nonexposed) ($n = 105$)			
Mean ± SD	1.5	8.9 ± 2.3	106.6 ± 32.9
Median	1.12	8.7	102.0
95% CI	0.71–4.00	3.7–15.5	46.4–175.0

Pair-wise comparisons of the three study groups indicate statistically significant differences in serum T4 ($p < 0.01$) and T3 ($p < 0.001$) means for the comparison of each group with the other two groups.

[a]Median TSH $p < 0.001$ for the comparison of each group with the other two groups.

[b]Median T4 $p < 0.0001$ for the comparison of each group with the other two groups.

TSH, thyroid-stimulating hormone; T3, triiodothyronine; T4, thyroxine; CI, confidence interval; SD, standard deviation.

All serum cotinine samples of those reporting nonsmoking status did not show measurable serum cotinine. Overall, active and passive smokers defined by serum cotinine had the lowest median levels of TSH and the lowest median value for T3 and T4. However, *p*-values comparing these subjects did not yield any significant difference in T3 levels (not shown). Median T4 levels were higher in serum cotinine–defined nonsmokers in comparison to smokers, but again, *p*-values did not demonstrate a significant difference between the groups. For the purpose of our analysis we used serum cotinine measurements to categorize volunteers into the three smoking groups (Table 2). The decrease in TSH proved to be significant when comparing serum cotinine–defined active smokers versus nonsmokers ($p < 0.01$) and also comparing serum cotinine passive smokers versus nonsmokers ($p < 0.05$). However, no significant difference in TSH levels was determined between serum cotinine active smokers and passive smokers.

In comparison, the self-reported smoking status groupings also yielded trends that were statistically significant. Mean and median T3 and TSH levels in passive smokers yielded the lowest values for TSH and T3. Because of the agreement between the self-reported exposure and three exposure groups defined by serum cotinine levels, we report in Table 2 only serum cotinine–defined data.

Median serum TSH was low-normal and lower in active smokers compared to nonsmokers (nonexposed) (1.02 mIU/L vs. 1.12 mIU/L, $p < 0.001$). Median serum T4 concentrations were similar in active and passive smokers and lower than in nonsmokers (median 8.68 μg/dL). Serum T4 concentrations were consistently lower in active smokers (Quartile (Q)1 = 5.81 μg/dL and Q3 = 9.56 μg/dL) and higher in nonsmokers (Q1 = 7.66 μg/dL and Q3 = 10.10 μg/dL). Serum T3 concentrations were consistently lower in passive smokers (Q1 = 62.0 ng/dL and Q3 = 102.0 ng/dL) and higher in nonsmokers (Q1 = 84.0 ng/dL and Q3 = 128.0 ng/dL). Median serum T3 was higher in the nonsmokers (102.0 ng/dL versus 92.0 ng/dL in active smokers, $p < 0.0001$) and lower in passive smokers (84.0 versus 102.0 ng/dL, $p < 0.0001$). Table 3 gives the mean of the T4/T3 hormone ratios by smoking group.

Table 3. Ratios of Thyroxine/Triiodothyronine Hormone Concentrations

Smoking status	*Stat*	*Ratio T4/T3*
Active smokers		
	Min	0.03
	Max	0.20
	Mean	0.09
	SD	0.03
Passive smokers		
	Min	0.04
	Max	0.23
	Mean	0.10
	SD	0.04
Nonexposed		
	Min	0.02
	Max	0.25
	Mean	0.09
	SD	0.04

Min, minimum; Max, Maximum.

Pair-wise comparisons of the three self-identified smoking groups for T4 and T3 show a statistically significant difference in mean T4 levels among the three self-identified smoking groups (one-way ANOVA test, $p < 0.01$), which was driven by a statistically significant difference in means of T4 levels between the active and not exposed groups ($p < 0.01$), after multiplicity corrections. Further, a statistically significant difference in mean T4 levels among the three self-identified smoking groups (one-way ANOVA test, $p < 0.001$) was driven by a statistically significant difference in means of T3 levels between the passive and not exposed groups ($p < 0.001$), after multiplicity corrections.

The relationship between the hormone levels for each of the study arms and BMI were explored for T4 and T3. Of the three exposure groups, only passive smokers had a significant negative relationship between BMI and T4 and T3. The BMI $\pm$ standard deviation of the subjects in the highest quintile of T4 was $25.85 \pm 1.06\,kg/m^2$, compared to $22.10 \pm 1.51\,kg/m^2$. Further, the BMI $\pm$ standard deviation of the subjects in the highest quintile of T3 was $25.85 \pm 1.06\,kg/m^2$, compared to $22.10 \pm 1.51\,kg/m^2$. No other relationships were found to be significant.

Discussion

Bias in reporting is inherent to all studies of smoking behavior; in fact, previous studies have concluded that self-reporting of smoking status is unreliable (39–41). Further, self-reporting lacks objective guidelines for determining smoking status. For example, the definition of smoking used in the Youth Risk Behavior Surveillance System is self-reported "current smoking and the use of cigarettes on at least 20 of the previous 30 days" (42), while the definition of smoking used in the Behavioral Risk Factor Surveillance System is self-reported "current smoking and having smoked more than 100 cigarettes in a lifetime" (used by the CDC).

One of the strengths of our study design was the use of cotinine as an objective serum biomarker to determine the smoking status, instead of self-reporting, atmospheric nicotine measurements, or ecological parameters. More than 90% of nicotine is metabolized to cotinine, and serum cotinine is proportional to the amount of exposure to tobacco smoke (32). However, serum cotinine can only be measured for a limited period after exposure to tobacco smoke (2–3 days). Thus, classifications based strictly on cotinine analysis may overlook chronic exposure that may have ended as recently as 3–4 days before sample procurement (43). Therefore, self-reports cannot be completely discounted, but should be used in conjunction with biomarker data to form reasonable conclusions.

Our study findings suggest that active and passive smoking was associated with a higher risk of having a significantly lower TSH levels compared to nonsmokers, suggesting that there was an inhibitory effect of cigarette smoke exposure on the thyroid. Mean TSH and thyroid hormone levels were within the normal intervals. By employing a laboratory measurement of cotinine to assess exposure to cigarette tobacco smoke, the study design may decrease recall bias. Although our smoking groups were not large, the use of LC/MS/MS afforded a more specific measurement of the hormones, and we were able to detect differences in TSH and thyroid hormone levels between the smoking and nonsmoking groups. A larger sample size will assist in confirming the current findings.

Our study results are strengthened by observations from other studies. An analysis of some parameters measured by the Third National Health and Nutrition Examination Survey III (United States 1988–1994) assessed the relationship between varying degrees of cigarette smoke exposure and thyroid abnormalities. For every 10 ng/mL increase in serum cotinine, the odds of having an elevation of TSH levels were decreased by 1.4%. Moreover, subjects with serum cotinine levels between 0.5–15 ng/mL (passive smokers according to their and our definition) were associated with 40% lower odds of elevated TSH levels compared to having a serum cotinine level of 0.00 (OR = 0.6, 95% CI, 0.4–0.7) (24). The mild hyperthyroidism finding is similar to our low normal TSH measurements in some individuals in our smoke-exposed groups. It was suggested that smoking decreased thyroid autoantibodies and increased the risk of Graves' hyperthyroidism in a dose- and time-dependent manner.

The mechanism by which the hyperthyroidism would be brought about is unknown (44). A possible mechanism may involve T4 binding globulin (TBG), a glycoprotein produced in the liver that binds T4 with high affinity. Approximately 75% of serum T4 is bound to TBG; nearly all the rest is bound to transthyretin or albumin, so that less than 0.1% remains free or unbound. The effect of cigarette tobacco smoking on the capacity and affinity of binding of T3 and T4 to TBG is not documented. However, it has been shown that oral contraceptives increase serum TBG concentrations by as much as 50% due to the increase in estradiol levels (45). It is known that cigarette smoking has major effects on the reproductive potential of humans and an antiestrogenic effect in women. This is probably due to changes in hepatic estrogen metabolism induced by cigarette tobacco smoke. Smoking has a powerful effect on the 2-hydroxylation pathway of estradiol metabolism leading to the increased production of 2-hydroxyestrogens. Further, in nonsmokers about 2% of circulating estrogens are free, since about 38% are avidly bound to sex hormone binding globulins, and about 60% are loosely bound to albumin, but in smokers, since sex hormone binding globulin concentrations are higher, there are lower serum concentrations of biologically active estrogens.

In normal women, the smoke-induced decrease in serum estrogens should induce a decrease in serum TBG because of an increase in the glycosylation of TBG, which slows its clearance, and to a decrease in liver TBG production. The immediate effect of a decrease in TBG must be to decrease T4 binding and therefore to increase the amount of free thyroxine (FT4). This increase should result in a small decrease in TSH secretion, and therefore in T4 secretion, until the serum FT4 is back to normal. Indeed we report here a relative decrease in serum TSH associated with smoking—these associations are of small magnitude because the changes are gradual but they are statistically significant.

Another mechanism may involve iodide transport; smoking appears to be associated with an alteration of thyroid autoimmunity, which may be caused by the interference of smoke with iodide transport and organification. Further, smoking cessation has been shown to lower the risk for the development of Graves' hyperthyroidism (46).

Smoking-related changes in maternal thyroid function during pregnancy may be associated with changes in thyroid

hormone levels potentially associated with having adverse neurocognitive and neurobehavioral impact on the fetus. In a cohort of pregnant women, those who smoked had lower levels of serum TSH and higher levels of T3 than nonsmokers (47). Similar to the results we report, a difference in TSH of about 0.15 mIU/L existed in the first trimester cohort (1.02 in smokers vs. 1.17 mIU/L in nonsmokers), as well as the third trimester cohort (1.72 vs. 1.90 mIU/L). The babies born to the smokers who participated in the pregnancy study also had lower serum TSH concentrations in cord blood (47). However, while FT4 remained stable, median FT3 was higher in the smokers in both 1st and 3rd trimester cohorts. In our study both median T3 and T4 were lower in the group of smokers.

Discrepancies between serum cotinine levels and self-report status are most apparent for passive smokers; the discrepancies suggest that when the timing between cigarette tobacco smoke exposure and cotinine analysis is longer than 24 hours, serum cotinine does not provide a reliable reporting of cigarette smoke exposure. Additional discrepancies may be the result of an overestimation of one's exposure to cigarette tobacco smoke, whether conscious or not.

In summary, we applied a novel approach that provides accurate simultaneous data on serum cotinine, total T4, total T3, and TSH to explore the effect of cigarette smoking, to determine the association with thyroid hormone and TSH levels in women of reproductive age. In addition to a lifestyle and environmental exposure questionnaire, serum cotinine concentrations served as an internal measure to determine smoke exposure. A larger sample size is needed to confirm the current findings. Further, it would be of interest to determine the duration of tobacco-smoke-associated serum hormone concentrations related to active and passive smoking.

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