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Examining the validity of self-reported primary and secondary exposure to cigarette smoke in adolescent girls: The utility of salivary cotinine as a biomarker

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Abstract

Background: Studies of cigarette use and exposure often rely on either self-report or cotinine assay. In adolescence it is not clear how well assays and self-report correspond, or what effect estrogen exposure has on cotinine.

Objectives: This study sought to identify optimal cut-points for salivary cotinine thresholds for girls with primary, secondary, and no smoke exposure, and whether menarche and hormone contraceptive use are important for interpreting salivary cotinine.

Methods: This longitudinal prospective study recruited 262 healthy adolescent girls who participated in for three annual interviews across 24 months. Salivary cotinine assays and self-report of primary and secondary smoke exposure, menarcheal status, and hormone contraceptive use were collected.

Results: No adolescents reported primary smoke exposure without secondary exposure. Optimal cut-points for distinguishing primary smoke exposure from secondary-only and no smoke exposure were 1.05 ng/ml and 3.01 ng/ml respectively based on receiver operator curves (ROC); no reliable cut-point for secondary-only versus no smoke exposure was identified. The ideal salivary cotinine cut-point to distinguish primary smoke exposure varied by hormone contraceptive use and was 2.14 ng/ml for those using progesterone contraceptives, higher than that of girls using estrogen contraceptives and those not using hormone contraceptives.

Conclusions: This study is the first to examine variance in salivary cotinine cut-points based on hormone exposure for adolescent girls, with findings indicating that hormone contraceptive use in particular may be a key consideration when identifying adolescent smoking. The use of previously recommended salivary cotinine cut-points of 3.85 ng/ml or higher may overestimate non-smokers.

Keywords

cotinine; adolescent; hormone contraceptives; smoking; secondary smoke exposure

Recent research has taken advantage of smoking biomarkers (e.g., cotinine, carbon monoxide, thiocyanate) to examine the validity of self-reported smoking in adolescent samples. Overwhelmingly, these studies have demonstrated the usefulness of biomarkers in verifying self-reported smoking behavior, with assessments of sensitivity (i.e., correctly detecting adolescents who smoke) of salivary cotinine compared to self-reported smoking between 71% and 98% (Dolcini, Adler, Lee, & Bauman, 2003; Kumar et al., 2008; McNeill, Jarvis, West, Russell, & Bryant, 1987). Unfortunately, studies examining cotinine biomarkers compared to reports of smoking in adolescents have been limited in at least three ways. First, studies introduced a wide range of cut-off values for salivary cotinine to indicate smoking behavior in adolescents. Second, in general, studies have emphasized differentiating primary smoke exposure (i.e., adolescents who smoke) from no smoke exposure (i.e., adolescents who do not smoke) without accounting for secondary smoke exposure (i.e., adolescents who do not smoke, but spend time with people who do smoke). Third, variation in levels of estrogen among teen girls due to pubertal changes (e.g., onset of menses) and use of hormone contraception may modify how nicotine is metabolized, subsequently altering cotinine levels.

Wide ranges of salivary cotinine levels have been used as cut-points to distinguish no smoking from primary smoking. These cut-points range from 5–15 ng/ml (Binnie et al., 2004; Bramer & Kallungal, 2003; Dolcini et al., 2003; Kumar et al., 2008; McNeill et al., 1987; Ronchetti et al., 1994; Yamamoto et al., 2005). Unfortunately, no studies to date have reported differences in the sensitivity or specificity (i.e., cotinine correctly identifying adolescents who do not smoke) of self-reported smoking and different cut-points for salivary cotinine within the same sample. The bulk of studies addressing this issue have used serum cotinine instead, and cotinine is known to be lower in serum compared to saliva (Bernert, McGuffey, Morrison, & Pirkle, 2000). For example, Benowitz and colleagues (2009) found that 2.99 ng/ml was an optimal cut-point when using serum cotinine to distinguish adolescent non-smokers from smokers, based on their analyses of sensitivity and specificity. Using the formula proposed by Bernert and colleagues (2000) to convert serum cotinine levels to saliva cotinine, this would correspond to a cut-point of approximately 3.85 ng/ml for distinguishing non-smokers from smokers using salivary cotinine in adolescence.

Studies examining cotinine in adolescence have also generally failed to account for secondary smoke exposure. In one of the few studies that has examined whether salivary cotinine may be used to identify secondary smoke exposure in adolescence, results suggest that salivary cotinine of less than 3 ng/ml indicates secondary smoke exposure, while levels of 3 ng/ml or higher indicate active smoking (Huntington-Moskos, Rayens, Hall, & Hahn, 2016). The study did not include a sample of adolescent smokers, and was therefore unable to validate this cut-off for primary smoke exposure. However, a separate study of salivary cotinine levels for adolescent non-smokers and smokers that accounted for smoking in the home reported that 8 ng/ml was the ideal cut-point to distinguish smoking and non-smoking in teens from non-smoking homes, while 12 ng/ml was ideal for distinguishing smokers from non-smokers in homes where other individuals also smoked (Jarvis, Fidler, Mindell, Feyerabend, & West, 2008). An explicit comparison of sensitivity and specificity for non-smokers and those with secondary smoke exposure only was not conducted. Understanding the levels of cotinine that may reflect secondary rather than primary smoke exposure is

essential for understanding where to target interventions – misclassification of these youth may lead to conclusions about youth (and even implementing interventions) that are not appropriate. The use of biomarkers to indicate secondhand smoke exposure has primarily occurred in younger pediatric populations where primary smoking is less prevalent compared to older ages. Within that literature, having undetectable levels of cotinine (usually between .1 and .7 ng/ml) is often considered an indication of low or no exposure to secondhand smoke, with cotinine levels anywhere from .1 to 2 ng/ml used to indicate secondhand smoke exposure (Jarvis, Sims, Gilmore, & Mindell, 2011; Kumar et al., 2008). In children, child-report and parent-report of parent smoking in the home are associated with increased cotinine levels in children when compared to children with non-smoking parents (Jarvis et al., 2011; Kumar et al., 2008; Scherer, Meger-Kossien, Riedel, Renner, & Meger, 1999). With adults, there is also evidence of significantly increased salivary cotinine levels in non-smokers who are exposed to secondhand smoke in their home (Binnie et al., 2004). Importantly, cotinine levels in children indicate exposure to secondhand smoke as much as four times greater than what is reported by parents (Dempsey et al., 2012) which may reflect both a lack of awareness with regard to exposure to secondhand smoke and intentional under-reporting by parents.

Finally, changing levels of estrogen due to puberty (e.g., onset of menses) and use of hormone contraception adds to the complexity of using cotinine as a biomarker in adolescence. Research has indicated that estrogen exposure contributes to more rapid metabolism of cotinine (Benowitz, Lessov-Schlaggar, Swan, & Jacob, 2006) and that effects persist when women are compared to men, pre- and post-menopausal women are compared, and for the impact of estrogen-based and combination hormone contraception compared to progesterone-only contraception (Benowitz et al., 2006; Berlin, Gasior, & Moolchan, 2007). Thus, it is essential for cotinine research to account for the use of hormone contraceptives and menarcheal status when examining salivary cotinine. Importantly, no studies were found in the literature where estrogen exposure was considered by including endogenous measures (e.g., puberty) and exogenous measures (e.g., use of hormonal contraception) in an adolescent sample.

The primary purpose of this manuscript is to address three gaps in the existing literature by examining levels of self-reported primary (i.e., teens report smoking, regardless of whether friends or family members smoke), secondary (i.e., teens report not smoking, but have friends or family members who smoke), and no smoke exposure as they correspond to salivary cotinine in a sample of girls ranging from ages 11–17 at the beginning of the study. Girls were followed for two years, allowing for the examination and replication of relations between type of smoke exposure and salivary cotinine. Having longitudinal data on this topic is novel and also allowed us to examine potential differences in sensitivity and specificity of cotinine levels by factors that may be more crucial in an adolescent sample [i.e., menarcheal status (i.e., pre versus post-menarcheal) and hormone contraception use (e.g., none, estrogen or combined contraceptive, and progesterone-only contraceptive)] in order to identify any potential factors that might influence cotinine thresholds for primary and secondary smoke exposure.

Methods

Study Design and Participants

This secondary analysis draws on data from 262 healthy girls between the ages of 11 and 17 years at baseline (M age = 14.35, SD = 2.16) who were recruited from a primary care teen clinic at Cincinnati Children's Hospital Medical Center and the surrounding community. Girls were white non-Hispanic (62%) or African American (38%). The goal of the parent study was to examine the impact of smoking and depressive symptoms on reproductive and bone health in adolescent girls. Exclusion criteria of the parent study were: A) pregnancy or breastfeeding within 6 months, B) primary amenorrhea (menarche >16 years), C) secondary amenorrhea (<6 cycles/year; not due to hormonal contraception), D) body mass index <1st percentile or weight greater than 300 pounds (limitation of DXA machine), E) medications or medical disorder influencing bone health (primary outcome for the study), and F) psychological or developmental disorders impairing comprehension or compliance of the study. The sample therefore represents typically developing adolescent girls.

Study Protocol

The study was approved by the Institutional Review Board of the associated children's hospital. Girls were purposively sampled based on age and smoking status. Parents provided informed consent and adolescents provided assent. Girls attended a total of 3 annual on-site visits conducted in the Clinical Translational Research Center. Visits for the parent study included a battery of questionnaires and interviews, anthropometry, a physical examination, phlebotomy, collection of saliva for cotinine assay, and bone density measurements. Baseline visits were conducted from December 2003 to October 2007. Girls completed surveys (including self-reported cigarette use) alone in a private study room to ensure confidentiality. Saliva cotinine data were available for all girls at the first timepoint; 74% of girls provided cotinine samples for all 3 time points, and 89% of girls provided samples for two time points. Cotinine levels at baseline were statistically similar for girls who were present versus were not present for subsequent timepoints ($p > .05$). For post-menarcheal girls, study visits were scheduled to occur during menstrual cycle days 5–9 in order to ensure that all hormones for the parent study were collected during the follicular phase of the girls' menstrual cycles.

Measures

Self-reported primary smoke exposure was defined as smoking in the 48 hours prior to the study visit, as determined by questionnaire based on Mayhew and colleagues (Mayhew, Flay, & Mott, 2000). Self-reported secondary smoke exposure was also determined by a questionnaire based on Mayhew and colleagues, asking "How many people who live in your home smoke?" with specific prompts for parents or step-parents, brothers or sisters, other adults, and other people. To assess exposure outside the home, girls also answered the question "Of your three closest or best friends, how many of them are regular smokers (at least once a week)?" Responses to these questions were used to identify secondary smoke exposure. Of note, none of the participants reported that they had friends who smoked but did not have someone smoking in their home. Cotinine from saliva was collected by chewing on a cotton dental role then placed in Sarstedt tubes (Sarstedt, 2003–2014), frozen and

assayed via enzyme linked immunoassay (ELISA) using the kit from Salimetrics Inc. (State College, PA). Samples were analyzed by the General Clinical Research Center Core laboratory at Cincinnati Children's Hospital Medical Center. The lower limit of detection (LOD) for the assay was 0.8 ng/ml and individuals with levels below 0.8 were coded as having a value of 0 ng/ml. Menarcheal status was based on adolescent report via clinician interview of the age of first menstrual period. Contraception use was based on the name of the prescribed contraception girls reported using in the two weeks prior to clinician interviews. Hormone contraceptives were classified as either estrogen-based (i.e., estrogen-only or combined contraceptives; e.g., Ortho Evra and Ortho Tri-Cyclen, respectively) or progesterone-only (e.g., Ovrette, Depo-Provera).

Analytic Plan

To identify optimal cotinine thresholds in distinguishing among primary, secondary, and no smoke exposure, we completed receiver operating characteristic (ROC) curve analysis. This technique identifies the optimal cut-point that maximizes sensitivity (i.e., the ability of salivary cotinine to correctly identify self-reported primary smoke exposure and secondary smoke-only) and specificity (i.e., the ability of salivary cotinine to correctly identify no self-reported smoke exposure; Altman & Bland, 1994). Consistent with the work of Benowitz, self-reported smoking status was used as the gold standard in these analyses (N. L. Benowitz et al., 2009). Sensitivity is calculated by dividing the cases where both self-report and cotinine level indicated smoke exposure (for example) by the total number of youth reporting smoke exposure (for example). Specificity is calculated by dividing the cases where both self-report and cotinine level indicate no smoke exposure by the total number of cases where youth report no smoke exposure. All p-values are adjusted to account for multiple analyses using the Bonferroni-Holm method. Analyses were conducted in SAS version 9.3 (SAS Institute, Inc., Cary, North Carolina) using PROC LOGISTIC. ROC analyses reported the area under the curve (AUC), which is another way of classifying the utility of a test (Pencina, D'Agostino, D'Agostino, & Vasan, 2008). The ROC AUC can range from 0 to 1, with 1 representing a perfect test (i.e., all true cases are classified as true, all false cases are classified as false). Using this approach, an AUC between 0.9 and 1.0 is considered excellent, between 0.8 and 0.9 is considered good, between 0.7 and 0.8 is considered fair, and between 0.6 and 0.7 is considered poor (Pencina et al., 2008).

Results

Descriptive statistics for the participants are provided in Table 1. Using self-reports of primary and secondary smoke exposure, girls were classified into one of three categories: primary smoke exposure, secondary smoke-only, and no smoke exposure. At the first time point (T1), 24% reported primary exposure, 43% reported secondary smoke-only, and 33% reported no exposure. Similarities in the distributions of smoking categories across T1-T3 was tested using the McNemar-Bowker test, and found to not differ across time (T1 to T2 $\chi^2(2) = 0.22, p = .90$; T2 to T3 $\chi^2(3) = 5.42, p = .14$). Differences in cotinine levels were examined using analysis of variance with Bonferroni-adjusted p-value (T1 F (2, 257) = 101.55, $p < .001$). Mean levels of cotinine at T1 were significantly higher for those who were exposed to primary smoke compared to secondary smoke-only (Mean difference (MD)

= 143.69, 95% Confidence Interval (CI) = 116.82–170.53, $p < .001$) and no smoke exposure (MD = 148.20, 95% CI = 119.86–176.52, $p < .001$), but did not differ between those who were only exposed to secondary smoke-only compared to no smoke exposure (MD = 4.52, 95% CI = –20.15–29.18, $p > .05$). Similar patterns were observed at T2 and T3 (results available by request). We also examined the distributions of cotinine by self-reported smoke exposure. Those distributions indicate a general progression toward higher levels of cotinine with increased smoke exposure. However, even in girls reporting primary smoke exposure in the day prior to and/or of the cotinine assay, there were samples where cotinine was below the LOD.

Optimum Cut-Points for Distinguishing Smoke Exposure

Results from estimated ROC curves are provided in Table 2. Those findings indicate excellent differentiation of primary from secondary smoke-only and no smoke exposure ($AUC > 0.900$), but fair to poor differentiation of secondary smoke-only from no smoke exposure ($AUC < 0.800$). In distinguishing primary from no smoke exposure, cut-points were fairly stable across time, ranging from 3.01 ng/ml at T1 to 1.45 ng/ml at T3. Similarly, in distinguishing primary exposure from secondary smoke-only, cut-points ranged between 1.05 ng/ml to 1.15 ng/ml. Sensitivity and specificity were calculated using 3.0 ng/ml to distinguish primary smoke exposure, with results indicating a sensitivity of 1 and a specificity of 0.92. In contrast, sensitivity in distinguishing secondary smoke-only using 1.15ng/ml as a threshold was 0.31, and specificity was 0.94.

The effects of estrogen exposure due to contraceptives in menarcheal girls was also examined (see Table 2). When differentiating primary from no smoke exposure and primary from secondary smoke-only, the optimal cut-point for girls using progesterone-based hormone contraceptives was 2.14 ng/ml (primary vs. no smoke exposure) and 2.19 ng/ml (primary vs. secondary smoke-only), higher than for girls using estrogen-based hormone contraceptives. Similarly, the use of progesterone-based hormone contraceptives led to a higher optimal cut-point than for menarcheal girls using no hormone contraceptives.

Discussion

Our overall goal was to examine the validity of smoking reports in adolescent girls using salivary cotinine as a biomarker. Prior cotinine literature has focused more on adults and of the few studies in adolescents, secondary exposure has rarely been addressed. An additional gap in the literature was that various cutoffs for cotinine concentrations were used and factors in adolescence that may impact validity were often not addressed (e.g., pubertal development, use of hormone contraceptives). An underlying rationale for conducting such analyses was to obtain a more inclusive variable for future studies that would reflect not only primary smoke exposure but also secondary exposure as factor impacting health outcomes. This is the first study, to our knowledge, that has examined the optimal cut-point for salivary cotinine in adolescents when hormone exposure is taken into account. Adolescence represents a time in the lifespan where smoking uptake and exposure is very different from adults and where metabolism of nicotine can be at different rates (Jarvis et al., 2008; Kandel et al., 2006; Rubinstein, Shiffman, Rait, & Benowitz, 2012). Further, longitudinal data

allowed us to replicate the relations between type of smoke exposure and salivary cotinine across a two year period, with general consistency found across three timepoints. Our relatively large sample of adolescent girls strengthened our ability to examine the impact of progesterone-only and estrogen based hormone contraceptives on optimal cut-points. Rubinstein and colleagues previously examined estrogen-containing contraceptive users and nicotine metabolism and found no differences (Rubinstein et al., 2012). However, they indicate analyses may have limited the power to detect differences, as the sample was small for users (n=19) and adherence rates for use of contraceptives may have been low. Based on our findings, use of hormone contraception and type of contraception used are important to consider in studies of adolescent girls, smoking, and cotinine. Of note, Hukkanen and colleagues report that the pharmacokinetics of nicotine and cotinine are no different between the luteal and follicular phases in healthy adults (Hukkanen, Gourlay, Kenkare, & Benowitz, 2005). We found no such studies in the adolescent population; based on Hukkanen's findings and because we conducted lab visits in post-menarcheal girls in the follicular phase of their menstrual cycle, we feel confident that there was less of a chance for un-accounted for variance in cotinine thresholds resulting from changing estrogen exposure based on phase in the menstrual cycle in this sample.

Our overall findings indicate that adolescent girls have a great deal of exposure to smoke. A very relevant point is that 36–46% of girls who were not primary smokers reported secondary smoke exposure, with a decrease in secondary exposure as girls got older. Although mean levels of cotinine concentrations in those exposed to secondary smoke-only were no different than those reporting no exposure, ranges of salivary cotinine levels did differ. Importantly, we do not know what concentration of salivary cotinine represents a health risk for other systems outcomes (e.g., cardiovascular health, bone accrual). It may be that not accounting for secondary exposure, either because girls exposed to second-hand smoke have low levels of cotinine or because self-reported measures are not used, may result in underestimating the impact of nicotine on health outcomes.

Based on the results of our findings, it appears that lower thresholds are more appropriate for distinguishing primary smoke exposure from secondary/no smoke exposure among adolescent girls more generally. Specifically, thresholds below 3.0 ng/ml appear to be most appropriate for identifying whether an adolescent girl is smoking or not. This is different from previous studies indicating the use of 3.85 ng/ml or even higher in identifying primary smoke exposure (Benowitz et al., 2009; Dolcini et al., 2003; Jarvis et al., 2008; Kandel et al., 2006; McNeill et al., 1987). Using cotinine levels to determine whether adolescent girls are exposed to secondary smoke-only is much more challenging. No reliable cut-point for cotinine levels with secondary smoke-only was found in this sample.

Important information regarding critical cotinine cutoffs accounting for estrogen exposure due to hormone contraceptive use was also found. Optimal cut-points for salivary cotinine varied for post-menarcheal girls depending if they used no hormone contraceptives, use a progesterone only contraceptive or use an estrogen based hormone contraceptive. These findings appear to reflect the impact of estrogen on metabolism of nicotine – something that has historically not been accounted for in the literature linking smoking to adolescent health outcomes. The pattern of these findings are consistent with work done in a variety of other

studies of adolescents and adults, which consistently demonstrate that estrogen accelerates the metabolism of nicotine (Benowitz et al., 2006; Berlin et al., 2007; Higashi et al., 2007; Rubinstein et al., 2012; Soldin, Makambi, Soldin, & O'Mara, 2011). While an in-depth discussion of possible biochemical pathways linking estrogen and nicotine metabolism is beyond the scope of this paper, these results are consistent with previous studies implicating the hepatic enzyme CYP2A6, which is known to determine rates of nicotine metabolism and is activated by higher rates of estradiol (Benowitz et al., 2006; Higashi et al., 2007). Of particular import to this study, estradiol may be lower in adolescents (compared to adult women) since it often takes 1–2 years to have regular ovulatory cycles (Diaz, Laufer, & Breech, 2006; Rosenfield, 2013; Treloar, Boynton, Behn, & Brown, 1967). Estradiol levels may also be impacted by nicotine, at least among adult menopausal women, where estradiol is lower in active and passive smokers compared to non-smoking, 18–45 year old women (Soldin et al., 2011). Future research is needed to examine this further in adolescents and to uncover other potential mechanisms linking estrogen and nicotine metabolism.

Our findings should be interpreted within the context of some limitations. First, although one intention was to consider the influence of pubertal stage (i.e., Tanner criteria by physical exam), we were limited by distributions across the five stages within our sample. In our age 11 cohort, only 4% of the total sub-sample of girls were Tanner I or II for breast development and 82% were Tanner IV or V. Thus, using age at menarche (pre vs. post) was more compatible with our data. Even with that, no premenarcheal girls reported smoking cigarettes, limiting our ability to consider endogenous estrogen exposure as a moderator of cotinine thresholds for primary smoke exposure. In a recent study of nicotine metabolism, Rubinstein and colleagues examined self-reported puberty and found no association with pubertal development (Rubinstein et al., 2012). However, we believe that there likely was little variability in pubertal development in their study, based on the age of enrollees. As one would expect, fewer girls in earlier Tanner stages would likely be smokers because they are younger; the average age of smoking experimentation is 14 years (Everett et al., 1999), nearly 1.5 years after the average age of menarche in American samples (Chumlea et al., 2003). Second, our sample only includes girls and therefore cannot be generalized to both sexes. Sex differences are noted in nicotine metabolism in adults (Benowitz et al., 2006; Kandel, Hu, Schaffran, Udry, & Benowitz, 2007) but not adolescents (Rubinstein et al., 2012). Sex differences are also noted in nicotine and cotinine clearance in adults (Benowitz et al., 2006) and in prevalence of smoking, where more adolescent boys smoke than adolescent girls (Center for Disease Control & Prevention, 2010). Third, the use of cotinine to reflect nicotine intake is not perfect based on individual differences in metabolism (Benowitz, 1996) but it is the most frequently used biomarker. Our study is further limited by the availability of salivary cotinine only and a lower LOD of 0.8 ng/ml; this work should be replicated with other measures of cotinine (e.g., serum) and lower LOD levels. Related to secondary smoke exposure, questions about exposure to family members and friends did not specify a timeframe, which may have resulted in girls reporting exposure to secondhand smoke that did not occur within the timeframe necessary to be detected by salivary cotinine. Likewise, self-report of either primary or secondary smoke-only can lead to bias and under-reporting of smoke exposure.

In conclusion, the findings from this study extend previous work in nicotine metabolism and salivary cotinine among adolescent girls by providing empirical guidance about the use of cotinine thresholds to determine nicotine exposure. Further, our findings demonstrate the impact of menarche and hormone contraception use should be considered when interpreting salivary cotinine levels among adolescent girls.

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Table 1.

Descriptive characteristics of study sample at Time 1 in 262 adolescent girls

Variable	Full Sample M(SD) or %(n)	Non-Smokers	Secondary Smoke-Only	Primary Smoke
Time 1 (0 months)	N = 262	n = 87	n = 111	n = 64
Age (years)	14.35 (2.16)	13.28 (2.07)	14.13 (2.05)	16.09 (1.17)
% Minority status	38% (100)	31% (27)	45% (50)	36% (23)
% Menarcheal	80% (209)	66% (57)	79% (88)	100% (64)
% Progesterone Contraception	8% (20)	3% (3)	6% (7)	16% (10)
% Estrogen Contraception	18% (47)	5% (4)	17% (19)	36% (23)
Saliva cotinine ng/ml	38.76 (94.31)	0.13 (0.51)	4.65 (32.19)	144.30 (135.63)
Family members smoke	61% (161)	0% (0)	90% (100)	83% (53)
Friends smoke	45% (118)	0% (0)	48% (53)	91% (58)
Time 2 (12 months)	N = 216	n = 80	n = 95	n = 41
Age	15.34 (2.14)	14.03 (2.01)	15.26 (2.06)	16.93 (1.27)
% Menarcheal	90% (194)	80% (64)	49% (47)	100% (41)
% Progesterone Contraception	5% (10)	1% (1)	7% (7)	2% (1)
% Estrogen Contraception	16% (35)	10% (8)	16% (15)	27% (11)
Salivary cotinine	38.87 (94.09)	0.44 (0.66)	4.28 (18.57)	178.64 (129.52)
Family members smoke	54% (117)	0% (0)	83% (79)	83% (34)
Friends smoke	44% (95)	0% (0)	56% (53)	93% (38)
Time 3 (24 months)	N=211	n = 82	n = 77	n = 52
Age	16.36 (2.16)	15.10 (2.02)	16.12 (1.91)	17.96 (1.22)
% Menarcheal	97% (205)	96% (79)	96% (74)	100% (52)
% Progesterone Contraception	9% (18)	5% (4)	10% (8)	12% (6)
% Estrogen Contraception	19% (40)	13% (11)	22% (17)	23% (12)
Salivary cotinine	49.11 (116.25)	0.43 (2.42)	3.01 (8.88)	188.17 (166.48)
Family members smoke	52% (110)	0% (0)	77% (59)	88% (46)
Friends smoke	46% (97)	0% (0)	61% (47)	92% (48)

Note: Non-smokers comprise of girls who did not report any primary or secondary smoke exposure; the “secondary smoke-only” group is comprised of girls who reported friends and/or family members who smoked, but did not report primary smoke exposure; the “primary-smoke” group consists of girls who reported both primary smoke exposure (i.e., active smoking) and also secondary smoke exposure.

Table 2.

Ideal salivary cotinine (COT) cut-point and area under the curve (AUC) statistics to distinguish primary smoke exposure, secondary smoke-only, and no smoke exposure.

	Primary vs. No Smoke			Primary vs. Secondary Smoke-Only			Secondary Smoke-Only vs. No Smoke		
	COT (ng/ml)	AUC	N	COT (ng/ml)	AUC	N	COT (ng/ml)	AUC	N
T1 full sample	3.01	1.000	151	1.05	0.987	176	2.70	0.667	197
T2 full sample	3.45	0.986	119	1.06	0.973	141	1.54	0.600	168
T3 full sample	1.45	0.999	132	1.15	0.992	129	1.28	0.715	157
T1 pre-menarche	---	---	---	---	---	---	2.88	0.600	53
T1 menarche	2.80	1.00	121	1.05	0.984	153	2.57	0.682	144
T1 progesterone OCP	2.14	1.000	13	2.19	1.000	17	1.22	0.571	10
T1 estrogen OCP	1.32	1.000	28	1.08	0.972	43	5.75	0.763	23
T1 no OCP	1.69	1.000	80	1.04	0.985	93	1.66	0.667	111

Note: oral contraceptive pill (OCP)