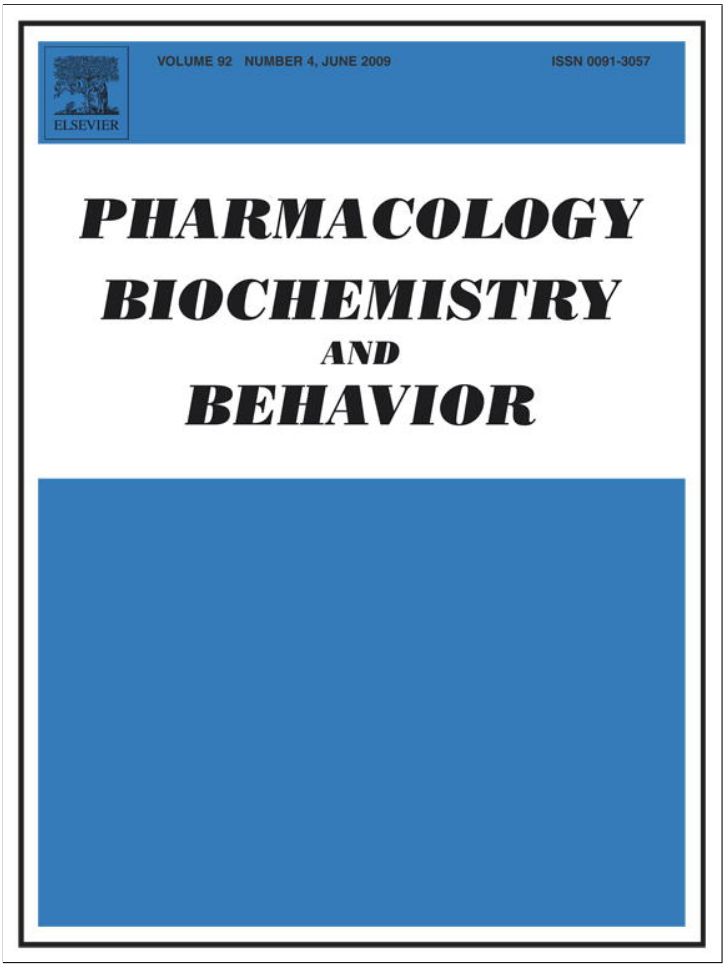

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Link between facultative melanin and tobacco use among African Americans

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ABSTRACT

Nicotine's affinity for melanin-containing tissues may result from its precursor function in melanin synthesis or the irreversible binding of melanin and nicotine. The objective of this study was to investigate a hypothesized association of tobacco use, dependence, and nicotine exposure with melanin pigmentation among African American smokers. A criterion-based sample was employed to collect data from a study of 147 adult African American current smokers. Carbon monoxide, saliva cotinine samples, and skin reflectance measures were obtained from each participant. Questionnaire data on demographic, sociological and behavioral questions related to smoking and skin color were gathered. The three dependent measures were the average number of cigarettes per day (CPD), Fagerström Test of Nicotine Dependence (FTND) score, and cotinine concentration. Analysis of variance, Pearson Correlations, and Multiple Linear Regression were conducted to analyze findings. The mean constitutive melanin reading was 56.3 and 66.5 for facultative melanin. Respondents on average smoked 19 CPD, had a mean FTND of 5.6, and a cotinine concentration of 435 ng/ml. Facultative melanin level was correlated with CPD and cotinine concentration in the bivariate analysis. The multiple linear regression results revealed that facultative melanin was significantly and positively related to CPD, the FTND, and cotinine. The results of this analysis support the hypothesis of a positive association between melanin levels and tobacco use, dependence, and exposure among African American smokers. This analysis may have important implications for research and interventions on tobacco dependence and disease outcomes. Further research on melanin and nicotine among African Americans as well as other population groups is warranted.

1. Introduction

Melanin is the primary determinant of skin color (Freedberg and Fitzpatrick, 1999a,b; Hedin and Larsson, 1978) and has also been found in other parts of the body, including the hair, eyes, heart, lung, liver, brain (Altschule and Hegedus, 1976), and lymphatic system (Wassermann, 1965). Nicotine and the tobacco-specific carcinogens N-nitrosamines N9-nitrosonornic (NNN), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), and benzo(a)pyrene have been shown to accumulate in animal tissues containing melanin (Brittebo and Tjalve, 1980; Brittebo and Tjalve, 1981; Domellof et al., 1987; Iwata et al., 1981; Larsson and Tjalve, 1996; Tjalve and Castonguay, 1983; Waddell and Marlowe, 1980). Nicotine and tobacco-specific toxins may also be sequestered in human melanin-containing tissues, resulting in long term exposure to these substances and substantial cumulative damage among smokers (Hecht, 1999). The enzymatic capacity of the melanocytes (melanin producing cells) may be a factor in the release of or degrading of toxic tobacco compounds (Brittebo and Tjalve, 1980) and in greater susceptibility to carcinogenicity (Castonguay et al., 1983).

Nicotine's affinity for melanin-containing tissues may result from its precursor function in melanin synthesis (Larsson and Olsson, 1979; Mizuno et al., 1997) or the irreversible binding of melanin and nicotine (Claffey et al., 2001; Dehn et al., 2001). Studies have attributed smokers' melanosis, or gingival pigmentation, to either of these two processes (Hedin, 1991, 1977; Iwata et al., 1981; Sarswathi et al., 2003; Taybos, 2003). The association between smoking and increased melanin production may also play a role in the pathogenesis of certain pulmonary diseases such as cryptococcosis (Casadevall et al., 2000; Khan, 2006).

As posited by Yerger and Malone (2006), the role of melanin in tissue uptake of nicotine and tobacco-specific carcinogens has potential implications for individuals with high levels of melanin. Given that nicotine has affinity for melanin, high levels of melanin might allow for a greater amount of nicotine to accumulate in melanin-containing tissues. Considering that melanin-containing tissues may represent a reservoir or storage for nicotine, it is plausible that higher melanin concentrations may contribute to higher degree of nicotine dependence and lower quit rates.

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The melanin and nicotine hypothesis may have particular import for African American smokers as they tend to have darker skin color and, therefore, higher concentrations of melanin and are disproportionately burdened by tobacco-related diseases. Studies indicate that though African Americans smoke fewer cigarettes than some other racially classified social groups (RCSGs), they have higher intake of nicotine per cigarette smoked (Benowitz, 1999; Caraballo et al., 1998; Moolchan et al., 2006; Perez-Stable et al., 1998; Wagenknetcht et al., 1990) and report greater difficulty quitting (King et al., 2004). Exploring whether the concentration of melanin contributes to greater retention of nicotine in melanin-containing tissues may also help to explain the higher cotinine levels (Caraballo et al., 1998; Moolchan et al., 2006) and the significantly slower metabolism of nicotine (Benowitz, 1999; Perez-Stable et al., 1998; Wagenknetcht et al., 1990) observed among African Americans.

Historically, skin color has been employed to characterize populations into RCSGs and to stratify opportunity structures and life chances via racism and systematic discrimination against individuals and groups (King, 1997). Thus, the study of skin color and health behavior and outcomes such as tobacco consumption and related diseases necessarily entails both biological and sociological perspectives.

The objective of this study was to investigate the degree of association of tobacco use, dependence, and nicotine exposure with skin pigmentation among African American smokers. The primary hypothesis is that melanin concentration is positively related to the number of cigarettes smoked daily, levels of nicotine dependence, and nicotine exposure.

2. Methods

2.1. Study sample

A criterion based non-probability sample was employed to collect data from African American smokers at three sites (i.e., Harambee United Church of Christ, Bethesda Men's Shelter, and the Children Health Clinic) in inner city Harrisburg, PA. Data were collected during the months of June, July, and August in 2007. Several meetings were organized with representatives of each study site to present the goals and methodology of the study and to discuss recruitment strategies. Each participant was paid a total of $20.00 upon completing all phases of the data collection process.

This research project was approved by the Pennsylvania State University Office of Research Compliance (Institutional Review Board approval #24899). Each participant was required to read and sign two copies of the informed consent form prior to providing any data. All questions regarding consent or procedures were answered by the principal investigator or the research coordinator.

Only those respondents who identified themselves according to the study criteria as current smokers, African American or Black, and were 18 years or older were included in the study. Former smokers were excluded. Specific biomarker exclusion criteria were: for carbon monoxide — pregnancy, breastfeeding, chronic obstructive lung or other pulmonary disease; for saliva — any current use of nicotine replacement therapy or medication affecting salivary functions; and for skin color reflectance — current use of skin lighteners or pregnancy.

To determine smoking status, potential participants were asked to exhale into a carbon monoxide meter. Subjects who met the "smoking" criterion of >10 ppm (Middleton and Morice, 2000; Tonnesen et al., 1993) were enrolled in the study. Due to time constraints, some subjects ($n=25$) did not complete the carbon monoxide test. These individuals were very similar to the CO tested group ($n=125$) with respect to age (41.4 vs 42.5), age of smoking initiation (16.8 vs 17), cigarettes smoked per day (19.8 vs 18.8), cotinine (430 vs 436), and constitutive (67 vs 66.4) and facultative (56.5 vs 56.2) melanin measures. They differed by gender (males, 70% vs 59.5%) and education (non-high school graduates, 25.2% vs 32%). One hundred and fifty participants ($n=150$) were included in the study. In the case of two subjects, misclassification was strongly suspected and they were subsequently excluded as their cotinine levels were <10 ng/ml. Another individual was dropped because of duplication resulting in a final sample of 147.

Subjects were instructed on how to expectorate 5 cc into a conical saliva collection tube through a straw. Saliva samples were checked and coded with ID numbers to match questionnaire and reflectometer data. Specimens were frozen until analysis. Salivary levels of cotinine were determined by enzyme immunoassay (EIA) using commercially available kits (Salimetrics LLC, State College, PA). Assays were conducted at the Pennsylvania State University. All samples were tested in duplicate in a single assay batch. Duplicate test values that varied by more than 6% error were subject to repeat testing; the average of the duplicate tests is reported. Intra-assay variation (CV) computed for the mean of 30 replicate tests was less than 5.8%. Inter-assay variation computed on the average duplicates for 12 separate runs was less than 8.2%. The assay had a lower level of sensitivity of .05 ng/ml. Cotinine levels ranged from 27 to 1300 ng/ml.

2.2. Instruments and measures

2.2.1. Questionnaire

The questionnaire consisted of items on sociodemographic variables, attitudes toward "race" and health, smoking behavior, and questions about racial discrimination, and perceptions about skin color and stress.

2.2.2. Reflectometer

The reflectometer used in this project was the Derma Spectrometer (Cortex Technology). It emits light at two defined wavelengths: 568 nm (green) and 655 nm (red); it has a photo detector that measures the absorbed and the reflected light by the skin. A melanin index, called *M*, is computed from the intensity of the absorbed and the reflected light and provides an objective quantification of skin color (Takiwaki et al., 2002). This handheld device has been used to detect melanin content in other research on skin color (Shriver and Parra, 2000). Average *M* values for the forehead (facultative melanin) and the inner arm (constitutive melanin) were used in all the analyses.

2.2.3. Independent variables

Constitutive melanin is genetically determined and not directly affected by sun exposure (Freedberg and Fitzpatrick, 1999a,b, p. 194), whereas facultative melanin includes both the constitutive (genetic) component and melanin which is induced by direct exposure of the skin to ultraviolet radiation (tanning) (Fitzpatrick and Pathak, 1974). There is no difference at the molecular level between constitutive and facultative melanin. The only difference is that facultative has higher melanin concentration. Each type of melanin was estimated separately by using a reflectometer to measure exposed and unexposed skin color (Shriver and Parra, 2000) at each site (i.e., the forehead and the upper under arm).

Chronological age (18–30, 31–45, >45 years), gender (male, female) and education (non-high school graduate, high school graduate, college or more) were the key socio-demographic variables in this analysis.

The Perceived Stress Scale (PSS 10) was employed to measure stress (Cohen and Williamson, 1988). Although previous studies have shown the PSS14 to have a high reliability estimate (Cronbach's $\alpha>.80$) among African American respondents (Manning et al., 2005; Sellers et al., 2003), we employed the PSS10 in this analysis because it had slightly better internal consistency ($\alpha=.78$) than the PSS14 ($\alpha=.71$). Responses were "never", "almost never", "sometimes", "often", or "very often". The PSS10 has been employed in other studies of smoking and stress (Croghan et al., 2006; Naquin and Gilbert, 1996; Siqueira et al., 2000) and was recoded for bivariate analysis to reflect the categories "low" "medium", "medium to high" and "high" levels of stress.

Secondly, to control for the effect of racial discrimination, we modified questions that have previously been used in studies (King et al., 2003; Williams, 1999) of African Americans to create a scale consisting of the following three items ($\alpha = .82$).

"During your lifetime, how often do you think that you have been treated unfairly or badly because you are African American or Black? Would you say, …?"

"During your lifetime, while shopping at a store or when attempting to make a purchase, how often were you ignored as if you were not a serious customer or were followed by store personnel? Would you say,…?"

"During your lifetime, how many times have you been stared at as if you did not belong in a place or situation? Would you say,…?"

The responses to each of these questions were "often", "sometimes", "seldom" or "never". For categorical analysis, the Discrimination Scale was recoded as "high" (1–3), "medium to high" (4–6), "low to medium" (7–9), and "low" (10–12).

Smoking status and behavioral variables asked whether an individual had smoked more than 100 cigarettes in their lifetime and whether they currently smoked everyday or some days, average number of cigarettes smoked per day (CPD), and the age they began smoking cigarettes regularly. Data on menthol cigarettes were collected but were not included in the analyses because there were just 13 non-menthol smokers and only 9 of these smokers provided complete information. "Number of years smoked" was calculated by subtracting the age at which a respondent began smoking from their current age.

2.2.4. Dependent variables

The dependent variables included CPD, Fagerström Tolerance and Nicotine Dependence (FTND) score, and cotinine concentrations. The six-item FTND scale (range of 1–10) was employed to measure degree of nicotine dependence (Heatherton et al., 1989). The FTND included the following questions:

1) How soon after you wake up do you smoke your first cigarette? 2) Do you find it difficult to refrain from smoking in places where it is forbidden? 3) Which cigarette would you hate most to give up? 4) How many cigarettes per day do you smoke? 5) Do you smoke more frequently during the first hours after awakening than during the rest of the day? 6) Do you smoke even if you are so ill that you are in bed most of the day?

2.3. Statistical analysis

For descriptive purposes, and for each variable except gender, data were categorized into three to four levels and a one-way analysis of variance was computed comparing the 3–4 mean values. Histograms were obtained (results not shown). For FTND, the histogram was near-normal with a slight left skewness as a few subjects had very low dependence FTND scores. For CPD, the histogram showed positive right skewness, which was normalized by taking a (natural) log transformation. For cotinine, a square root transformation normalized the positive right skewed data. In all analyses the transformed variables were used, but for descriptive purposes the actual un-transformed means are presented.

To assess linearity, bivariate plots were obtained comparing each independent variable with each of the three response variables(See Fig. 1A— 1F for plots with the melanin variables. Polynomial regression was also used to confirm linearity by computing sequential polynomials which in all cases showed that the second and third degree terms were non-significant ($p > .05$), as illustrated in Freund and Littell (2000).

Multiple Linear Regression (MLR) analysis was employed to examine the relationship between constitutive and facultative melanin and each dependent variable of tobacco use. In the MLR, age and age of smoking initiation were not used because of their high correlation with smoking duration. For the cotinine model, involving the (significant) gender effect, analysis of covariance methods were used to assess and confirm homogeneity of regression. For all multivariate models, standardized residuals were computed and plotted to assess normality of residuals, which were confirmed to be approximately normal. Statistical analysis was performed using the Statistical Packages for the Social Sciences (SPSS-Version 15) and Statistical Analysis System (SAS version 9.1).

Multiple linear regression models included facultative or constitutive melanin, gender, education, smoking duration, PSS10, a Discrimination Scale,and for cotinine, CPD. All predictors were entered into the regression models as continuous variables, except for those with only two categories.

3. Results

As shown in Table 1, the mean age was 42.3 years ± 11.4 and almost half of the respondents were between 31 and 45 years old. Most participants were men (61.6%) and 47.3% were high school graduates. The proportion of homeowners was 6.8% (data not shown) and about 82% had annual family incomes of $30,000 or less (data not shown).

Average reflectometer mean readings of constitutive and facultative melanin were 56.3 nm ± 10.2 (range 34.6–80.7) and 66.5 nm ± 13.6 (range 31.7–103.9), respectively. Almost two-thirds of the respondents had begun smoking before 18 years of age ($\bar{x} = 17$ years ± 5.4). On average, participants had smoked for 25.2 years ± 12.2 and over 90% consumed menthol cigarettes (data not shown). The mean number of CPD was 19 ± 10.6, mean FTND score 5.6 ± 2.3, and the average cotinine level was 435 ng/ml ± 234.

The PSS10 mean score was 18.8 ± 6.0 (range 4–34), and the Discrimination Scale mean was 6.4 ± 2.4 (range 1–12).

Pearson correlations (Table 1) revealed that facultative melanin was positively and significantly related to CPD ($p < .05$) and cotinine ($p < .01$). Constitutive melanin scores were not significantly related to the dependent variables. Age ($p < .01$) was positively and significantly related to cotinine and women had significantly lower concentrations than men ($p < .01$). Age of smoking initiation and smoking duration were significantly associated with each of the dependent variables.

3.1. Multiple linear regression analysis

In separate regression models of CPD (Table 2), constitutive melanin was not statistically significant ($\beta = .15$, $p = .11$), where beta is the standardized regression coefficient. Facultative melanin was a significant predictor of CPD ($\beta = .27$, $p = .01$). Smoking duration was a significant covariate in each model.

Facultative melanin ($\beta = .24$, $p = .02$), but not constitutive melanin ($\beta = .11$, $p = .24$), was significantly associated with FTND score (Table 2). As expected, smoking duration was significantly related to FTND scores in both regression models.

In the models predicting cotinine level (Table 2), facultative melanin ($\beta = .21$, $p = .03$), but not constitutive melanin ($\beta = .14$, $p = .12$), was significantly related to cotinine. The covariate smoking duration was significantly associated with cotinine, but this was not the case when CPD was included as a covariate.

The PSS10 and the Discrimination Scale were not significant covariates in the MLR models.

In Table 2, the four models whose R^2 is .11 or higher are statistically significant ($p < .05$). For the three facultative melanin models: $F = 3.9$, $df = 7,118$, $p = .001$ (cotinine); $F = 3.0$, $df = 6,114$, $p = .009$ (FTND); and $F = 2.6$, $df = 6,119$, $p = .023$ (CPD). For the significant constitutive-cotinine model, $F = 3.5$, $df = 7,118$, $p = .002$. For all models,

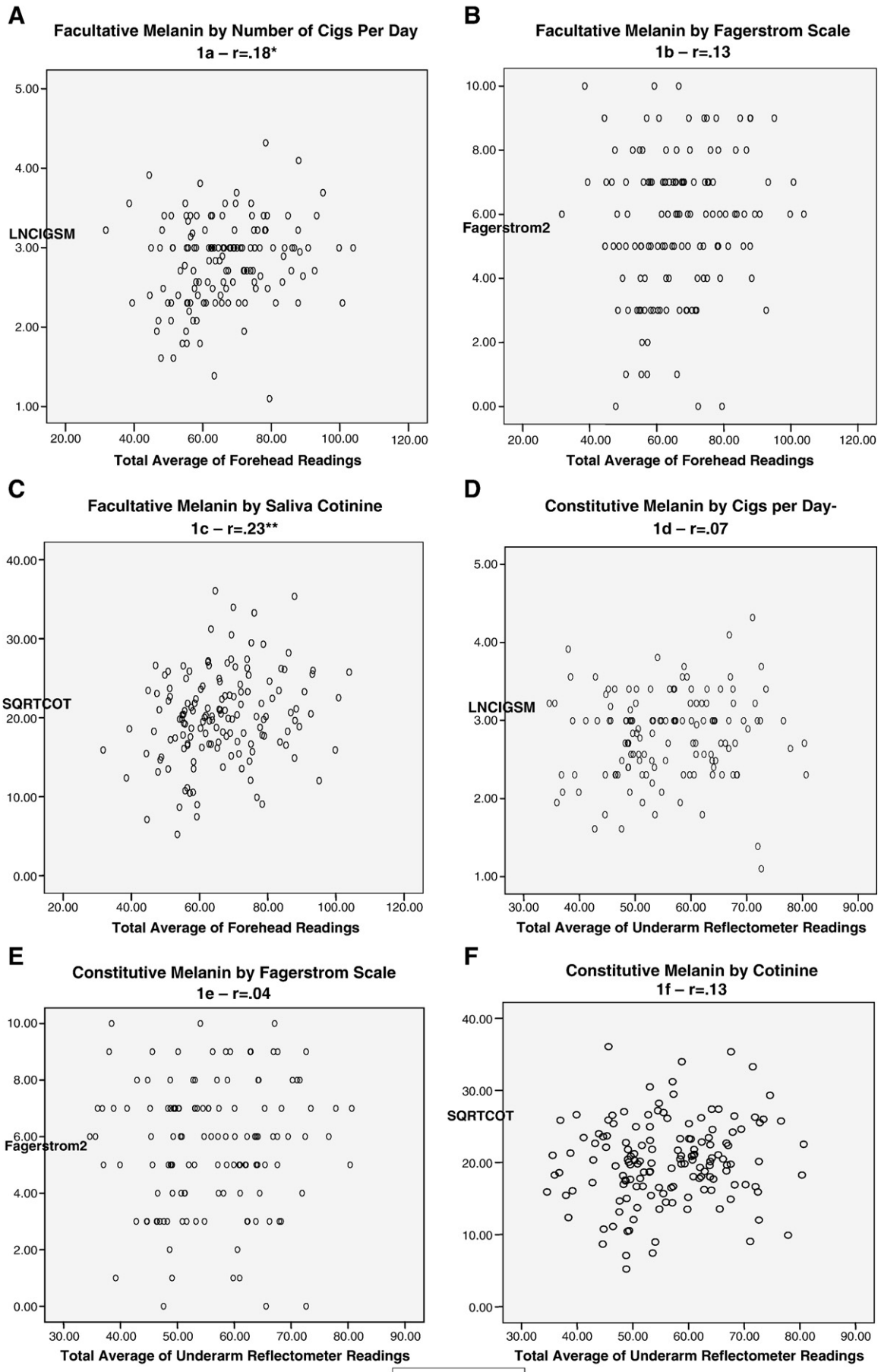

Fig. 1.

Table 1
Frequency and cross-tabulations: descriptive data showing sample frequencies, categorical and overall means, and Pearson correlations (*n*s from 127–147).

Variables	Sample frequency%	Cigarettes per day	Fagerstrom (FTND)	Cotinine level
		$\bar{x}$ = 19.0	$\bar{x}$ = 5.6	$\bar{x}$ = 435
		SD = 10.6	SD = 2.3	SD = 234
Constitutive melanin reading	$\bar{x}$ = 56.3, SD = 10.2	r = .07	r = .04	r = .13
34.6–50.6	33.3	18.1	5.5	393
50.7–60.9	33.3	18.6	5.7	446
61.0–80.7	33.3	20.3	5.8	467
Facultative melanin reading	$\bar{x}$ = 66.5, SD = 13.6	r = .18*	r = .13	r = .23**
31.7–58.6	33.3	17.2	5.2	352**
58.7–71.1	33.3	18.7	5.8	481
71.2–103.9	33.3	21.2	5.9	473
Age	$\bar{x}$ = 42.3, SD = 11.4	r = .10	r = .11	r = .25**
18–30	12.7	18.3	4.8	298**
31–45	48.6	19.0	5.8	421
>45	38.7	19.1	5.7	496
Gender		r = −.06	r = −.01	r = −.25**
Male	61.6	18.7	5.7	484**
Female	38.4	19.6	5.6	357
Education		r = −.13	r = −.13	r = −.04
<High school	26.0	22.7*	6.2	443
High school grad	47.3	17.1	5.6	424
Some college or graduate	26.7	18.7	5.4	449
Age of smoking initiation	$\bar{x}$ = 17.0, SD = 5.4	r = −.20*	r = −.20*	r = −.19*
Before 18 years	65.5	20.6*	5.9*	453
18 years or older	34.5	15.9	5.1	390
Smoking duration (years)	$\bar{x}$ = 25.2, SD = 12.2	r = .17*	r = .20*	r = .33***
1–10	14.5	16.0**	4.6	293***
11–20	21.7	16.2	5.5	391
21–30	25.4	22.1	6.0	399
>30	38.4	19.4	5.8	522
Perceived stress scale (10)	$\bar{x}$ = 18.8, SD = 6.0	r = −.08	r = .11	r = −.06
Low(<13)	12.2	16.9	4.8	521
Medium(13–19)	42.5	19.8	5.6	418
Medium to high (20–25)	33.8	18.9	5.9	412
High (>25)	11.5	19.4	5.7	438
Racial discrimination scale	$\bar{x}$ = 6.4, SD = 2.4	r = −.01	r = .03	r = .07
High (1–3)	13.8	18.2	5.6	434
Medium to high (4–6)	44.1	18.8	5.5	426
Low to medium (7–9)	31.7	19.9	5.7	440
Low (10–12)	10.3	18.2	6.3	470

* Indicates significance in the one-way ANOVA and Pearson correlations (*r*), *p<.05; **p<.01; ***p<.001.

collinearity was nearly non-existent as the largest variance inflation factor was 1.4, considerably less than 10, a value often considered a threshold for concern (Myers, 1986).

It is noteworthy that if the independent variables are entered into a forward selection algorithm to assess how the standardized regression coefficients (partial correlations) between the melanin and dependent variable change with variable entry, the order for the facultative/cotinine model is: .22 (.23, smoking duration), .23 (.24, Perceived Discrimination Scale), .23 (.24, PSS10), .21 (.21, gender), .20 (.20, CPD), and .21 (.21, education). In short, the relationship between cotinine and facultative melanin barely changes, implying that the confounders do not appreciably alter the positive association. This is also true for the other response variables (data not shown).

4. Discussion

To our knowledge, this is the first study to have examined the association between skin pigmentation and tobacco use behavior and exposure. The results of this study reveal a positive association between facultative melanin levels and tobacco use, dependence, and

Table 2
Multiple linear regression model of melanin and tobacco use: standardized regression coefficients and associated *p*_values.

Independent variables	Cigarettes per day*		FTND scale		Cotinine*	
	R^2 = .08	R^2 = .11	R^2 = .10	R^2 = .14	R^2 = .17	R^2 = .19
Facultative melanin reading		.27 (.01)		.24 (.02)		.21 (.03)
Constitutive melanin reading	.15 (.11)		.11 (.24)		.14 (.12)	
Gender	−.01 (.89)	.08 (.42)	.07 (.49)	.15 (.14)	−.13 (.17)	−.06 (.56)
Smoking duration (years)	.21 (.03)	.24 (.01)	.28 (.01)	.31 (.00)	.33 (.00)	.35 (.00)
Education	−.14 (.13)	−.15 (.09)	−.11 (.22)	−.12 (.17)	.00 (.97)	−.01 (.93)
Perceived stress scale	.09 (.33)	.08 (.37)	.15 (.11)	.15 (.12)	.10 (.28)	.09 (.30)
Perceived discrimination scale	.01 (.92)	.02 (.87)	.05 (.63)	.05 (.61)	.15 (.11)	.15 (.10)
Cigarettes per day*					.06 (.46)	.04 (.68)

*CPD was normalized by taking a (natural) log transformation. Cotinine was normalized by a square root transformation. The transformed variables were used in all MLR analyses.

exposure among African American smokers. Constitutive melanin was not found to be a statistically significant predictor in the MLR models. The facultative measure was a stronger predictor of total melanin content in the body than the constitutive measurement. This is important because our data were collected during the summer months when there was higher ultraviolet radiation exposure and probably increased melanin levels due to tanning. Our analysis of the effect of tanning in MLR models indicated a statistically significant association with the number of cigarettes per day and the FTND score. The interpretation of this finding is somewhat unclear as tanning was not significantly related to cotinine.

As expected, duration of smoking was strongly related to each of the smoking outcome measures, indicating that the longer one smokes, the higher the CPD, FTND, and cotinine levels. Other studies have found this association, as well (Al-Delaimy et al., 2002; Setty et al., 2007). The small number of non-menthol smokers ($n = 13$) was insufficient to compare to menthol smokers. However, the predominant use of mentholated cigarettes among African American smokers (Gardiner, 2004) requires that future studies include more non-menthol smokers to assess menthol's role in the relationship between melanin and smoking outcomes.

CPD was not a significant covariate in the cotinine regression models, which included either facultative or constitutive melanin. This is an interesting finding as the mean number of CPD ($\bar{x} = 19$) was fairly high for African American smokers, although perhaps less so for African American smokers in the lower social echelons or African American men (1998). Previously described ceiling effects of higher numbers of CPD on cotinine might explain this phenomenon (Heatherton et al., 1989). When controlling for CPD, African American smokers have significantly higher serum levels of cotinine than do non-Hispanic White and Mexican American smokers (Caraballo et al., 1998; Clark et al., 1996; Perez-Stable et al., 1998; Wagenknetcht et al., 1990). Generally, cotinine level is directly proportional to the quantity of absorbed nicotine (Hill et al., 1983). However, CPD has not been shown to be consistently related to cotinine in some studies with African Americans (Kandel et al., 2007; Mustonen et al., 2005). Also the FTND, which includes CPD as an item, may have less validity among some ethnic populations or RCSGs (Johnson et al., 2008; Schroeder and Moolchan, 2007).

As a product of human evolution, the primary benefit of melanin is protection of the skin from harmful ultraviolet radiation (Jablonski and Chaplin, 2000). The positive association between facultative melanin and nicotine dependence as well as exposure is theoretically intriguing. For example, if nicotine binds with the melanin in human tissues containing melanin, then a darker pigmented individual may sequester more nicotine in the body than a lighter pigmented individual. This sequestering or storage of nicotine in melanin-containing tissues might reduce nicotine clearance, thereby increasing the amount of time one is exposed to nicotine. It is interesting to note that cotinine, which is less pharmacologically active than nicotine (Buccafusco et al., 2007), does not appear to be sequestered in melanin-containing tissues (Szuts et al., 1978).

Greater retention of nicotine in melanin-containing tissues may contribute to higher exposure to nicotine and degree of nicotine dependence, which then might result in lower tobacco cessation rates. Although no proven mechanism exists at present, we conjecture that nicotine-bound melanin provides a reservoir that allows for slow egress of relatively small concentrations of unmetabolized nicotine to migrate from skin and other melanin-containing tissues through the bloodstream to brain nicotine receptors at concentrations that might not desensitize those nicotine receptors (Brody et al., 2006; Buccafusco et al., 2007).

A corollary to this theory is that darker pigmented individuals may be predisposed to greater exposure to nicotine and tobacco-specific toxins, and susceptibility to tobacco-related carcinogens, especially if these toxicants are being slowly released from the reservoir of melanin-containing tissues. Thus, the melanin and nicotine hypothesis may have particular import for African American smokers, who tend to have higher levels of melanin and are disproportionately impacted by the tobacco-related health consequences of dependence, morbidity and mortality (2007, 1998).

Moreover the historical existence of a color-infused social class structure among African Americans, which was not internally generated but evolved from social and cultural subjugation, would suggest that smokers who generally predominate in the lower social classes are also more likely to be of darker skin color (Franklin, 1994; Frazier, 1957; Jordan, 1968) and may have more difficulty quitting due to social and structural barriers.

Considering the sample size and design, appropriate caution must be exercised in interpreting the results of this study as the findings may not be generalizable to the population of African American or darker skin cigarette smokers. We suspect that some selection bias may have occurred as lower income smokers were more likely to volunteer to participate because of the $20 compensation. Data were collected only during the summer months and in the Northeast region of the country. Facultative melanin readings could vary if taken during other times of the year or in other regions of the country (Jablonski and Chaplin, 2000). The association of skin color with social class distinctions (Frazier, 1957; Goldsmith et al., 2006; Keith and Herring, 1991) may have precluded more variation in melanin readings. Any possible overestimation of CPD may be related to variations in amount smoked per cigarette, the number of inhalations and total puff volume per cigarette, and the practice of conserving cigarettes by not smoking an entire cigarette at a time. We also note that the levels of nicotine may also be affected by factors such as diet and water consumption. Funding constraints precluded an analysis of trans-3-hydroxycotinine concentrations which could have provided additional information on nicotine metabolism.

Notwithstanding the above, the current study has some important strengths. First, it included both self-reported and biomarker data on smoking from a population of hard-to-reach smokers, especially Black males, in an inner-city community. Second, skin pigmentation has both a biological and a sociological character and both concepts were measured in this study. Constitutive melanin and facultative melanin represent the underlying biological determinants of skin color. However, how a society determines the distribution of resources and life chances based on beliefs, values, and perceptions regarding skin color also affects health behaviors, including tobacco use and related outcomes (King, 1997; Williams, 1999). African Americans who experience racism and discrimination based on skin color likely internalize certain beliefs creating psychological stress and cognitive dissonance, which could also affect health behaviors such as smoking (Clark et al., 1999; Klonoff and Landrine, 2000; Wyatt et al., 2003). Although neither the discrimination nor the stress variables were significant predictors in this study, these indicators have been found to be significantly associated in other studies of tobacco use (Borrell et al., 2007; Fernander et al., 2005; Harrell et al., 2003; Landrine and Klonoff, 2000; Manning et al., 2005) and therefore further investigation is needed. More importantly, controlling for these factors avoids a strictly biological deterministic perspective linking RCSGs and smoking. Furthermore, it provides a stronger basis for assessing any hypothesized biological association between nicotine and melanin by differentiating between the sociological significance and the biological nature of skin color with respect to tobacco use, dependence, and exposure.

Our results suggest a relationship between melanin in the skin, tobacco use, nicotine dependence and exposure among established and dependent smokers. As previously mentioned, the factors responsible for this association remain unknown. Notwithstanding this, we offer a few speculative observations and relevant questions. First, both the complex factors governing the reinforcement efficacy or dependence-producing potential of nicotine and the specific nature of nicotine's interactions with melanin have not been established. For

example, does the sequestering of nicotine within melanin-containing tissues reduce nicotine metabolism by liver enzyme system CYP2A6? Is nicotine which has been sequestered in melanin-containing tissues still able to cross the blood–brain barrier to reach nicotine receptors but at a delayed or slower rate? Reduced nicotine metabolism in the context of circulating nicotine bound to melanin could possibly prolong the exposure to brain nicotine receptors responsible for reinforcement. This consideration is complicated by the fact that nicotine receptors tend to desensitize upon prolonged exposure. Alternatively, by providing a reservoir for constant nicotine exposure, high melanin concentrations might contribute to reduced urges to smoke, leading to a lower CPD. Another unanswered question is whether this reservoir of sequestered nicotine provides a constant or intermittent diffusion of nicotine from melanin-containing tissues into the bloodstream and/or lymphatic system (in other words, what cleaves the melanin-nicotine bond?). Further, does the relationship between melanin and nicotine differ based on early tobacco use vs later use in the trajectory of smoking, when neuroadaptions such as tolerance and craving are established? Similarly, it is unknown to what degree the activation of tobacco-related procarcinogens (e.g., nitrosamines, benzopyrenes) occurs within melanocytes or is influenced by melanin. Does menthol adhere to melanin? Our study was unable to address this issue and we have not found any literature suggesting an adherence of menthol to melanin but this line of inquiry should be further pursued.

While our findings are based on a sample of current smokers, the suspected effect of nicotine on darker individuals may also apply to non-smokers. For example, environmental tobacco smoke has also been shown to influence gingival pigmentation (Hanioka et al., 2005) and studies have strongly demonstrated its negative health consequences for non-smokers (2007; Glantz and Parmley, 1991; Taylor et al., 1992). Speculatively, our findings may have particular implications for populations residing closer to equatorial zones where exposure to the sun is more extensive and/or where global climate change is occurring, suggesting the universal and malleable character of skin color as a phenotypic attribute as opposed to an immutable genotypic trait (King, 1997).

In summary, though interesting, the results of this study should be viewed as preliminary. Further research on the link between melanin and nicotine among African Americans, as well as other population groups, is warranted.

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