### Relationships of Cotinine and Self-Reported Cigarette Smoking With Hemoglobin $\text{HbA}_1c$ in the U.S.

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<th>Citation</th>
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Relationships of Cotinine and Self-Reported Cigarette Smoking With Hemoglobin A₁c in the U.S.

Results from the National Health and Nutrition Examination Survey, 1999–2008

CAROLE CLAIR, MD¹
ASAF BITTON, MD, MPH²
JAMES B. MEIGS, MD, MPH³
NANCY A. RIGOTTI, MD¹

OBJECTIVE—Whether nicotine leads to a persistent increase in blood glucose levels is not clear. Our objective was to assess the relationship between cotinine, a nicotine metabolite, and glycated hemoglobin (HbA₁c), an index of recent glycemia.

RESEARCH DESIGN AND METHODS—We used cross-sectional data from the National Health and Nutrition Examination Survey (NHANES) from 1999 to 2008. We limited our analysis to 17,287 adults without diabetes. We created three cotinine categories: <0.05 ng/mL, 0.05–2.99 ng/mL, and ≥3 ng/mL.

RESULTS—Using self-report, 25% of the sample were current smokers, 24% were former smokers, and 51% were nonsmokers. Smokers had a higher mean HbA₁c (5.36% ± 0.01) compared with never smokers (5.31% ± 0.01) and former smokers (5.31% ± 0.01). In a similar manner, mean HbA₁c was higher among participants with cotinine ≥3 ng/mL (5.35% ± 0.01) and participants with cotinine 0.05–2.99 ng/mL (5.34% ± 0.01) compared with participants with cotinine <0.05 ng/mL (5.29% ± 0.01). In a multivariable-adjusted analysis, we found that both a cotinine ≥3 ng/mL and self-reported smoking were associated with higher HbA₁c compared with a cotinine <0.05 ng/mL or not smoking. People with a cotinine level ≥3 ng/mL had a relative 5% increase in HbA₁c compared with people with a cotinine level <0.05 ng/mL, and smokers had a relative 7% increase in HbA₁c compared with never smokers.

CONCLUSIONS—Our study suggests that cotinine is associated with increased HbA₁c in a representative sample of the U.S. population without diabetes.

Diabetes Care 34:2250–2255, 2011

Cigarette smoking and type 2 diabetes are major public health burdens. Both are risk factors for cardiovascular disease and their co-occurrence has a dramatic impact on the absolute risk of mortality (1). Studies suggest that cigarette smoking is associated with an increased risk of developing type 2 diabetes (2).

The effects of smoking on risk of diabetes are commonly attributed to nicotine.

In the short term, nicotine is known to cause elevations in blood glucose concentration (3). Whether this effect is transient or leads to persistent increase in blood glucose level is not clear. Several studies have shown that smoking is associated with increased levels of glycosylated hemoglobin (HbA₁c) (4–6), which is an objective index of chronic glycemia, but this relationship might be biased if smokers do not accurately report their smoking status. A more accurate assessment of the relationship between tobacco use and HbA₁c would be obtained by using a biologic marker for smoking, such as cotinine, the major proximate metabolite of nicotine. Two studies performed in populations with type 1 or type 2 diabetes have used cotinine (7,8), but to our knowledge, no study performed among people without diabetes has assessed systematically the relationship between HbA₁c and a biologic marker of nicotine exposure.

We used data from the National Health and Nutrition Examination Survey (NHANES) to assess the relationship between cotinine and HbA₁c among individuals with normal glucose metabolism and among people with impaired fasting glycemia in subanalysis. Our hypothesis was that nicotine (using either blood cotinine or self-reported smoking status as a measure of nicotine exposure) is associated with higher HbA₁c in people without diabetes and in people in a prediabetic state.

RESEARCH DESIGN AND METHODS

Study sample

We analyzed data from NHANES, a nationally representative, cross-sectional survey that became a continuous biannual survey in 1999 (9). The continuous NHANES uses a complex stratified, multistage, cluster-sampling design to select a representative sample of the U.S. civilian noninstitutionalized population. We combined five successive waves (1999–2008) of the continuous NHANES for our analysis, producing a total sample of 51,623 people (Fig. 1). Participants answered a household interview and most completed clinical examinations in a mobile examination center. We limited the analysis to people who were both interviewed and examined, not pregnant, and ≥20 years old because the smoking questionnaire we used was administered to adults aged 20 years and older. We further excluded subjects...
with missing data on cotinine (n = 1,589), HbA1c (n = 1,271), education (n = 44), waist circumference (n = 1,290), diabetes status (n = 13), smoking status (n = 32), alcohol consumption (n = 1,967), or physical activity (n = 5). A total of 3,707 subjects (14%) were excluded because of missing values; compared with the nonexcluded participants, they were more likely to be female, black or Hispanic, smokers, and have a lower level of education and were less likely to be physically active. Excluded participants had slightly higher mean HbA1c (5.6 vs. 5.5%, P = 0.001) and lower waist circumference (95.2 vs. 97.1 cm, P < 0.0001), but their mean age and cotinine levels were similar to the nonexcluded participants. We limited our analyses to people without diabetes, excluding another 2,560 individuals. The NHANES protocol was approved by a human subjects review board, and informed consent was obtained from all participants.

**Smoking status**
Smoking status was self-reported and was collected during the household interview of adults aged 20 years and older. Participants who reported smoking at least 100 cigarettes in their entire life and reporting that they did not smoke at all at the time of the interview were classified as current smokers. Participants who reported smoking fewer than 100 cigarettes during their lifetime were defined as never smokers. This smoking definition has been widely used (10) and classifies participants who have recently initiated smoking and very light smokers as never smokers.

**Cotinine**
Cotinine is a major metabolite of nicotine that is used as a marker for active smoking and as an index of exposure to secondhand smoke (11). Serum cotinine was measured using an isotope dilution–high performance liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry (12). The detection limits have changed over time in NHANES: in 1999–2000, it was 0.05 ng/mL; subsequently, it was lowered to 0.015 ng/mL. For consistency, we used the higher detection limit (cotinine <0.05 ng/mL). Participants below this threshold were classified as unexposed for all surveys as done in previous studies (13). We created cotinine categories reflecting smoking exposures and used the cut point of 3 ng/mL recently proposed by Benowitz et al. (14) to distinguish cigarette smokers and nonsmokers. We chose to use this new cut point rather than the older one (14 ng/mL) because it is more sensitive and adapted to the current relatively low level of secondhand smoke exposure in the U.S. and it would pick up light or nondaily smokers. Our three categories of cotinine levels were 1) cotinine <0.05 ng/mL, 2) cotinine 0.05–2.99 ng/mL, and 3) cotinine ≥3 ng/mL.

**HbA1c and fasting plasma glucose**
HbA1c was measured using a high performance liquid chromatography system. Fasting plasma glucose was measured in participants who were examined in the morning session after an 8- to 24-h fast (approximately half of the sample examined), using hexokinase enzymatic method.

**Diabetes**
Diabetes was defined based on self-reported data and fasting glucose. People were considered to have diabetes if they had been told by a health professional that they had diabetes, if they reported taking insulin or diabetic pills to lower blood glucose, or if they had a fasting blood glucose ≥126 mg/dL.
Smoking, cotinine, and hemoglobin $A_{1c}$

(15), whether or not they reported having diabetes or being treated for diabetes. NHANES does not differentiate between type 1 and type 2 diabetes, but given the fact that we limited our analyses to participants without diabetes, it was not useful to make a distinction between them. Individuals were defined as having impaired fasting glucose if they had been told by a health professional that they were borderline for diabetes but did not take insulin or diabetic pills or if they had a fasting blood glucose $\geq 100$ mg/dL and $<126$ mg/dL. The normal group consisted of participants who did report not having diabetes and who had a fasting blood glucose $<100$ mg/dL.

Other covariates

Demographic variables and information on alcohol consumption and physical activity were collected during the household interview. Race/ethnicity was categorized as non-Hispanic white, non-Hispanic black, Mexican American, other Hispanic, and other race. Alcohol consumption was categorized as 0, $\leq 1$, 2–3, 4–5, and $\geq 6$ drinks per week. For physical activity, we considered leisure time physical activity and categorized it in no or light, moderate, and vigorous. Weight and height were measured using standardized techniques and equipment during clinical examinations. BMI was calculated as weight in kilograms divided by height in meter squared. Waist circumference was measured at the uppermost lateral border of the ilium.

Statistical analysis

For all analyses, we accounted for the sampling design and used sample weights that account for unequal probabilities of selection and include adjustment for noncoverage and nonresponse. In univariate analysis, we calculated age-adjusted mean $HbA_{1c}$ according to cotinine and smoking status. To adjust for age, we used the direct method to the year 2000 census population projections using the age-groups 20–29, 30–39, 40–49, 50–59, 60–69, and 70 years and older. We built different linear regression models to assess the relationship between both cotinine and self-reported smoking categories and $HbA_{1c}$, the continuously distributed dependent variable. For each cotinine/smoking measure, we built two models. In the first model, we adjusted for age only. In the second model, we adjusted for age, sex, education, race/ethnicity, waist, alcohol consumption, and physical activity. Additional to the demographic variables, we chose to adjust for waist because it has been shown that smokers tend to have higher abdominal fat compared with never smokers (16) and abdominal fat is a good predictor of insulin resistance and, hence, higher $HbA_{1c}$ (17). Moreover, it has been shown that smokers tend to have higher alcohol consumption and less physical activity compared with never smokers (18). Because these factors are also associated with diabetes and insulin resistance, we thought it was important to adjust for them in our analyses. We performed test for trend across categories of cotinine and across categories of self-reported smoking. These analyses were performed among participants without diabetes or impaired fasting glucose. In secondary analyses, we replicated these analyses among people with impaired fasting glucose. We also built logistic regression models with cotinine/self-reported smoking predicting high $HbA_{1c}$ ($\geq$90th percentile). The cutoffs for high $HbA_{1c}$ were 5.8 mg/dL for people without diabetes or impaired fasting glucose and 6.1 mg/dL for people with impaired fasting glucose. Again, we ran age- and multivariable-adjusted models. Finally, we explored if there was effect modification by age or ethnicity and performed stratified analyses for those two variables.

Statistical tests were two-sided and $P < 0.05$ was considered statistically significant, based on a Bonferroni correction for multiple testing of two different smoking exposure measures. We used SAS software version 9.2 (SAS Institute Inc., Cary, NC) and SUDAAN software version 10.0 (RTI, Research Triangle Park, NC) for our analysis.

RESULTS—After excluding people with diabetes, the final sample consisted of 17,287 people—14,096 without diabetes or impaired fasting glucose and 3,191 with impaired fasting glucose (Fig. 1).

Using self-report to identify smoking status, the sample included 4,073 smokers (25%), 4,377 former smokers (24%), and 8,837 never smokers (51%). Using cotinine levels, the sample included 4,991 participants with cotinine levels $\geq$3 ng/mL (30%), 4,946 participants with cotinine levels between 0.05 and 2.99 ng/mL (28%), and 7,350 participants with cotinine levels $<0.05$ ng/mL (42%). The majority of participants who reported themselves as smokers had cotinine levels $\geq$3 ng/mL (96%). Only 3% had cotinine levels between 0.05 and 2.99 ng/mL and $<1$% had cotinine levels $<0.05$ ng/mL. However, among those who self-reported themselves as nonsmokers (either never smokers or former smokers), 9% had cotinine levels $\geq$3 ng/mL and 36% had cotinine levels between 0.05 and 2.9 ng/mL.

Characteristics of participants are shown in Table 1. Self-reported smokers and participants with a cotinine level $\geq$3 ng/mL were more likely to be younger, male, have lower education, consume more alcohol, and have low level of physical activity compared with self-reported nonsmokers or participants with a cotinine level $<0.05$ ng/mL.

Mean age-adjusted $HbA_{1c}$ according to cotinine and self-reported smoking status are shown in Table 2. Among people without diabetes or impaired fasting glucose, mean age-adjusted $HbA_{1c}$ was slightly higher in participants with cotinine $\geq$3 ng/mL and participants with cotinine 0.05–2.99 ng/mL compared with participants with cotinine $<0.05$ ng/mL, with a significant trend across nicotine exposure categories suggesting a dose-response phenomenon. When we repeated the analysis among people with impaired fasting glucose, we also found that $HbA_{1c}$ increased with nicotine exposure, but the trend was not significant. We did the same comparison according to self-reported smoking status and found similar results, with smokers having a higher mean $HbA_{1c}$ compared with never smokers and former smokers.

In the linear regression models (Table 3), we found that cotinine $\geq$3 ng/mL and smoking were associated with higher $HbA_{1c}$ compared with cotinine $<0.05$ ng/mL or not smoking. People with a cotinine level $\geq$3 ng/mL had a 5% increase in $HbA_{1c}$ compared with people with a cotinine level $<0.05$ ng/mL, and smokers had a 7% increase in $HbA_{1c}$ compared with never smokers in multivariable-adjusted analyses. For trend across cotinine categories or self-reported smoking categories was significant and suggested a dose-response relationship. Subanalyses among people with impaired fasting glucose were similar. The stratified analyses for sex and ethnicity suggested an effect modification by sex (among males, smokers had an 11% increase in $HbA_{1c}$ compared with never smokers) and ethnicity (among non-Hispanic whites, smokers had an 8% increase in $HbA_{1c}$ compared with never smokers).

In the logistic regression models, among people without diabetes or impaired fasting glucose, we found in multivariable-adjusted analyses that participants with cotinine $\geq$3 ng/mL had 31% increased odds of elevated $HbA_{1c}$ ($HbA_{1c} \geq$5.8%) compared with participants with cotinine
levels <0.05 ng/mL. Smokers had 37% increased odds of elevated HbA1c compared with nonsmokers (Supplementary Table 1).

CONCLUSIONS—In the current study, we used recent data representative of the U.S. population to show that both cotinine and self-reported smoking are associated with an increase in HbA1c in a population without diabetes and in people with impaired fasting glucose. Our analysis showed a relative increase in HbA1c of 5% for people with cotinine levels ≥3 ng/mL compared with people with cotinine levels <0.05 ng/mL. This would correspond to an absolute increase in HbA1c of 0.3%, for example, for someone with an HbA1c of 6.0%. Our findings support the hypothesis that smoking, and more specifically nicotine, leads to a persistent increase in blood glucose levels; however, it does not prove causality because of its cross-sectional design. Studies have shown that even a small increase in HbA1c concentrations has an impact on cardiovascular disease risk and mortality and that at a population level, an absolute reduction of just 0.1% HbA1c might reduce total mortality by 5–10% (19).

According to our data, 9% of people who reported being nonsmokers (never or former smokers) had cotinine levels ≥3 ng/mL, and 36% had cotinine levels between 0.05 and 2.9 ng/mL. This can be a consequence of either inaccurate reporting, use of nicotine replacement therapy, or exposure to secondhand smoke. Self-reported smoking has been evaluated as valid in NHANES in a study that took into account use of nicotine replacement therapy and exposure to secondhand smoke (20). Therefore, in our analyses,
because <0.2% of the nonsmokers used nicotine replacement therapy, we can consider the group with cotinine between 0.05 and 2.9 ng/mL as exposed to second-hand smoke. In this group, we observed a small increase in HbA1c levels. Our hand smoke. In this group, we observed a

AIC: Association of HbA1c with cotinine levels and with self-reported smoking status

<table>
<thead>
<tr>
<th>Cotinine categories</th>
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<th>Multivariable adjusted*</th>
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<td>People without diabetes or IFG</td>
<td>&lt;0.0001</td>
<td>0.0002</td>
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<td>&lt;0.05 ng/mL (Ref)</td>
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<td></td>
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<tr>
<td>0.05–2.99 ng/mL</td>
<td>0.05 ± 0.01</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>≥3 ng/mL</td>
<td>0.06 ± 0.01</td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td>People with IFG</td>
<td>0.027</td>
<td>0.008</td>
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<tr>
<td>&lt;0.05 ng/mL (Ref)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.05–2.99 ng/mL</td>
<td>0.02 ± 0.02</td>
<td>−0.00 ± 0.02</td>
</tr>
<tr>
<td>≥3 ng/mL</td>
<td>0.04 ± 0.02</td>
<td>0.05 ± 0.02</td>
</tr>
<tr>
<td>Self-reported smoking status</td>
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<td></td>
</tr>
<tr>
<td>People without diabetes or IFG</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Nonsmokers (Ref)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Former smokers</td>
<td>−0.00 ± 0.01</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>Smokers</td>
<td>0.05 ± 0.01</td>
<td>0.07 ± 0.01</td>
</tr>
<tr>
<td>People with IFG</td>
<td>0.36</td>
<td>0.004</td>
</tr>
<tr>
<td>Nonsmokers (Ref)</td>
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<td></td>
</tr>
<tr>
<td>Former smokers</td>
<td>−0.04 ± 0.02</td>
<td>0.00 ± 0.02</td>
</tr>
<tr>
<td>Smokers</td>
<td>0.03 ± 0.02</td>
<td>0.06 ± 0.02</td>
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*p for trend across cotinine categories or self-reported smoking status; a Bonferroni-corrected P < 0.025 was considered statistically significant. IFG, impaired fasting glucose. Ref, reference group. *Adjusted for age (continuous), sex, education, race/ethnicity, waist (continuous), alcohol consumption (0, 1–2, 3–4, 5–6 drinks per week), and physical activity (none or light, moderate, vigorous).

In conclusion, our study suggests that smoking is associated with an increase in HbA1c in a representative sample of the U.S. population with normal glucose metabolism as well as in people with impaired fasting glucose. The dose-response phenomenon suggests that there might be a linear relationship between cotinine and HbA1c. These results support, but do not prove, the hypothesis that smoking, and more specifically nicotine, leads to a persistent increase in blood glucose levels.

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C.C. led analyses of data and statistical analyses and wrote the manuscript. A.B. assisted with interpretation of data and reviewed the manuscript. J.B.M. and N.A.R. interpreted data and wrote and reviewed the manuscript.

Parts of this study were presented in abstract form at the 33rd Annual Meeting of the Society of General Internal Medicine, Minneapolis, Minnesota, 28 April–1 May 2010.

**References**