Smoking habits and parathyroid hormone concentrations in young adults: The CARDIA study

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A B S T R A C T

Conflicting results have been reported concerning a relationship between smoking and serum PTH. Our study objective was to examine whether smoking was associated with serum PTH independent of correlates of PTH among young adults, and explore potential mechanisms.

This was a cross-sectional study of healthy individuals, 24–36 years old, examined during 1992 through 1993 in California, USA (a subset of Coronary Artery Risk Development in Young Adults study). Linear regression was used to obtain adjusted means of PTH according to smoking habit (current, former, never). Biomarkers for calcium metabolism and bone turnover (including serum concentrations of osteocalcin, bone-specific alkaline phosphatase, and 24-hour urinary excretion of calcium) and bone mineral density were similarly compared by smoking.

376 participants were analyzed (171 women, 181 black). Over half reported never smoking. We observed lower PTH in current smokers compared to non-smokers and found no evidence of an interaction by race and sex. PTH was lowest in current smokers, intermediate in former smokers, and highest in never smokers (geometric mean PTH: 23.6, 26.7, 27.4 pg/mL, respectively; P for trend, 0.006) after adjusting for potential confounders including calcium intake. Among the biomarkers, serum osteocalcin concentration and 24-hour urinary excretion of calcium were lowest in current smokers. We observed no smoking-related difference in bone mineral density.

In this community-based sample of young adult men and women, smoking was associated with significantly lower PTH concentration. The mechanism and clinical implication of the finding, however, remains uncertain.

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1. Introduction

Smoking is generally associated with lower concentration of vitamin D, lower bone mineral density and increased risk of fracture in the elderly. Therefore, a compensatory increase in parathyroid hormone (PTH) may be expected (Jorde et al., 2005). However, conflicting results have been reported in the literature on the relation between smoking and PTH. While some studies reported higher PTH among smokers (i.e. expected direction) (Rapuri et al., 2000; Szulc et al., 2002; Ortego-Centeno et al., 1997), others have reported lower PTH concentration among smokers (Mellstrom et al., 1993; Landin-Wilhelmsen et al., 1995; Broetz et al., 1999; Need et al., 2002). One of the largest population-based observational studies documented this unexpected finding cross-sectionally (Jorde et al., 2005), yet the underlying mechanism and clinical implication of the finding remains to be elucidated.

In this study, we investigated a cross-sectional relationship between smoking habits and concentration of PTH using a population-based sample of men and women in the USA. The primary aim was to investigate whether PTH concentration differs among current, former and...
never smokers after accounting for potential confounders. In light of inconsistencies in the literature, we did not have a hypothesis about the direction of the PTH and smoking association. The secondary aim was to explore potential mechanisms using biological markers related to calcium metabolism and bone turnover according to smoking habit.

2. Methods

2.1. Study population

We studied a subgroup of participants of the Coronary Artery Risk Development in Young Adults (CARDIA) study. A detailed description of CARDIA has been published elsewhere (Friedman et al., 1988). In brief, CARDIA is a cohort study of the evolution of cardiovascular risk among young adults, conducted since 1985. The participants for the present study were enrolled in the CARDIA study in the Oakland Clinical Center, California, who were returning for their fourth examination from June 1992 through May 1993 to participate in an ancillary study of bone mineral homeostasis (Bikle et al., 1999). All subjects, aged 24–36 years, were in good health and had a serum creatinine level < 1.6 mg/dL and a serum calcium level ranging from 8.8 to 10.3 mg/dL. Within the year before the examination, all women menstruated regularly, were not taking oral contraceptive agents, and had not been pregnant ≥ 10 weeks or breastfeeding. Subjects with medical conditions or taking medications known to alter calcium homeostasis were excluded from the study (Bikle et al., 1999). The data were collected with written informed consent and with approval of the local Institutional Review Board. Of 402 ancillary study participants, we included those participants with no missing variables directly relevant to the primary aim (i.e. to examine association between smoking and PTH). We excluded those who gave unrealistic estimate of energy intake (800 or 8000 kcal/day in men; 600 or 6000 kcal/day in women), leaving 376 participants for analysis.

2.2. Measurement

Demographic information was obtained using standardized questionnaires. Weight was measured on a balance beam scale. Height was measured using a vertically mounted metal centimeter ruler. Body mass index (BMI) was computed as weight (kg) divided by square of height (m). At examination, fasting blood including creatinine, phosphate and calcium (collected between 8:00 and 11:00 AM) and 24-hour urine samples were obtained for analysis using routine laboratory procedures and an automated chemistry analyzer (Bikle et al., 1999).

PTH and other biomarkers were measured on stored samples 3 years after study completion (Bikle et al., 1999; Ettinger et al., 1997). PTH was measured using a 2-site immunoradiometric assay that detects only intact PTH (i.e. biologically active 84-amino-acid peptide) (Nussbaum et al., 1987). Serum 25-hydroxyvitamin D (25OHD) was measured by RIAs in the Calcitropic Hormone Reference Laboratory, University of California, San Francisco. Serum 1,25-dihydroxyvitamin D (1,25(OH)₂D) was measured by a competitive protein-binding assay using the vitamin D receptor present in calf thymus cytosol (Bikle et al., 1999). Osteocalcin was measured using a mid-molecule-specific RIA for human osteocalcin (Taylor et al., 1988). Bone-specific alkaline phosphatase (BAP) was measured spectrophotometrically after heat inactivation (Farley et al., 1981). Free pyridinoline cross-links in urine were measured using an ELISA developed by Metra Biosystems (Seyedin et al., 1993). The results of this method were compared with that of direct assessment after HPLC purification (Black et al., 1988) for 20 subjects in each race and gender group to verify the comparability of this method with that using HPLC (r > 0.9 for all groups) before adopting this method for use in assessing all subjects (Bikle et al., 1999). 24-hour urinary excretion of pyridinoline was standardized for urinary excretion of creatinine (U-PyIDcr) for analysis.

2.3. Assessment of lifestyle information

The usual diet was assessed by a diet history interview (CARDIA dietary history) in which food models and measuring cups and spoons were used to estimate portion size. The previous month was used as a frame of reference for estimating the usual intake. Intake of dietary calories and calcium were estimated according to the nutrient data base developed by the Nutrition Coordinating Center at the University of Minnesota. The estimate of calcium intake included supplemental sources. Validity and reliability of the CARDIA dietary history has been published elsewhere (Liu et al., 1994). Sun exposure time was assessed by query for weekday and weekend separately and then calculated as hours per week. The CARDIA physical activity history questionnaire was used to quantify physical activity levels, and scored accordingly (physical activity score). The amount of time per week spent in leisure, occupational, and household physical activity over the previous 12 months was assessed. We estimated total physical activity expressed in exercise units as a product of intensity and frequency. The validity and reliability of the questionnaire was published elsewhere (Jacobs et al., 1989).

Additionally, we examined bone mineral density (BMD) at various sites according to smoking habit. Dual-energy x-ray absorptiometry measurement of BMD for the whole body, total hip, left and right total arms, and lumbar spine was obtained using a Hologic QDR 2000 densitometer (Hologic, Inc.) in the array scanning mode, separately for each area of the body (total hip, lumbar spine, and whole body). The total arm was assessed using the whole-body scan (Fujyoshi et al., 2013). In vivo precision for BMD, based on repeated scans of 20 volunteers done 1 to 6 weeks apart and expressed as a coefficient of variation, was 0.5% for whole-body BMD.

2.4. Statistical analysis

We used linear regression models to obtain adjusted PTH concentrations according to smoking habits (Current, Former, and Never). Due to skewed distributions, we log-transformed the following variables in our statistical models: PTH, 25OHD, BAP, U-PyIDcr and calcium intake in density. When any of these variables was used as a dependent variable, we back-transformed the results to present the adjusted geometric means for ease of interpretation. For the main analyses, we constructed the following models: Model 1 adjusted for age (years), race (Black, White), sex, and education (up to 15 years, 16 years or greater). Model 2 adjusted additionally for calcium intake in density (mg/1000 kcal/day, supplements included, log-transformed), serum concentrations of 25OHD (ng/ml, log-transformed), creatinine (mg/dl), and season (Jan–Mar, Apr–Jun, July–Sep, and Oct–Dec) of blood draw. Model 3 further adjusted for physical activity score and sun-exposure time (hours/week). Model 4 further adjusted for body mass index (kg/m²).

For each sex and race/ethnicity group, we observed that PTH concentrations tended to be high in never smokers, and lower in current smokers, then we tested interaction by sex or race/ethnicity on the association of smoking and PTH by inserting a product term in the models. All the interaction terms were non-significant throughout the models. Therefore, we presented sex- and race/ethnicity combined results. We tested linear trend of PTH across the smoking habits by using an ordinal variable (0 = never, 1 = former, and 2 = current smoker). As sensitivity analyses, we repeated the main analyses replacing calcium density in the models with either a) calcium density excluding supplemental source (i.e. dietary source of calcium only) or, b) absolute amount of calcium intake (mg/day) or, c) serum calcium concentration (mg/dl).

For the secondary aim, we estimated the adjusted means of the following biological markers: serum phosphate [mmol/L], serum 1,25(OH)₂D [mmol/L], 24-hour urinary calcium [mmol/day], fractional excretion of calcium (FEca, also known as calcium/creatinine clearance
We analyzed 376 participants (171 women, 181 black, age 24–36 years). Demographics of the participants according to smoking habit for each sex and race/ethnicity are given in Table 1. In all sex-, race/ethnicity- groups, more than half of the participants (56–72%) reported never smoking. Over a quarter of black men (25.8%) and black women (26.1%) reported current smoking, whereas 10.7% of White men and 15.7% of White women did so. Across the sex and race/ethnicity strata, current smokers tended to have greater sun-exposure hours and higher BMI (with exception for calcium intake in white women and BMI for black men), and tended to have fewer education years. We observed no clear patterns by smoking habit for serum concentrations of calcium, 25OHD and creatinine across the strata.

### Table 1

| Table 1 | Demographics of participants according to smoking habit. |
|---------|--------------------------------------------------------|
|          | Black men (N = 93) | Black women (N = 88) | White men (N = 112) | White women (N = 83) |
| No. | Current | Former | Never | Current | Former | Never | Current | Former | Never |
|     | 24      | 9      | 60    | 23      | 16      | 49    | 12      | 19      | 81    |
| Age, years | 30.8 | 32.0 | 30.4 | 30.4 | 31.1 | 31.1 | 31.1 | 30.8 | 31.4 |
| Education, years | 14 | 13 | 15 | 13 | 14 | 14 | 13 | 15 | 16 |
| Body mass index, kg/m² | 25.0 | 24.9 | 27.0 | 29.2 | 26.9 | 28.6 | 26.9 | 26.1 | 25.3 |
| Energy intake, kcal/day | 2784 | 2461 | 2061 | 2560 | 2492 | 2378 | 2538 | 2484 | 2378 |
| Calcium intake, mg/day | 410 | 290 | 410 | 370 | 380 | 400 | 430 | 440 | 440 |
| Energy intake, kcal/day | 2.64 | 3.64 | 4.15 | 2.19 | 2.49 | 2.66 | 4.66 | 5.64 | 5.02 |
| Calcium intake, mg/day | 9.5 | 9.5 | 9.6 | 9.3 | 9.4 | 9.3 | 9.7 | 9.5 | 9.6 |
| Ca intake, mg/dL | 3.24 | 3.53 | 3.27 | 2.71 | 2.18 | 2.38 | 18.1 | 17.8 | 17.0 |
| Ca intake, mg/dL | 0.99 | 1.04 | 1.03 | 0.79 | 0.75 | 0.80 | 0.89 | 0.92 | 0.95 |
| Biomarkers related to calcium-metabolism | 3.13 | 2.96 | 3.18 | 3.38 | 3.27 | 3.34 | 2.78 | 3.07 | 2.89 |
| Phosphate (serum), mg/dL | 46.1 | 45.9 | 43.8 | 45.3 | 49.0 | 43.1 | 40.3 | 38.0 | 39.8 |
| 24-hour urinary Ca, mg/dL | 2.64 | 4.56 | 4.15 | 2.19 | 2.49 | 2.66 | 4.66 | 5.64 | 5.02 |
| Fraction of secretion of calcium (× 100) | 0.74 | 0.86 | 0.85 | 0.57 | 0.58 | 0.71 | 1.07 | 1.18 | 1.06 |
| Bone turnover marker | 11.7 | 12.5 | 14.6 | 9.8 | 10.8 | 9.6 | 13.3 | 14.8 | 14.9 |
| Osteocalcin (serum), pg/mL | 9.2 | 7.6 | 9.8 | 7.2 | 8.2 | 8.4 | 9.0 | 9.9 | 8.5 |
| Bone mineral density, g/cm² | 29.8 | 26.7 | 27.4 | 24.9 | 0.095 | 0.082 | 0.051 | 0.005 | 0.005 |
| Whole body | 1.27 | 1.32 | 1.30 | 1.17 | 1.15 | 1.16 | 1.16 | 1.19 | 1.18 |
| Lumbar spine | 1.48 | 1.20 | 1.11 | 1.16 | 1.08 | 1.13 | 1.02 | 1.08 | 1.02 |
| Total hip | 1.16 | 1.22 | 1.19 | 1.05 | 0.98 | 1.04 | 1.05 | 1.06 | 1.03 |
| Arms (average of right and left) | 0.98 | 1.02 | 1.02 | 0.81 | 0.79 | 0.81 | 0.94 | 0.95 | 0.95 |
| Parathyroid hormone, pg/mL | 24.4 | 22.2 | 25.7 | 30.5 | 37.8 | 38.4 | 26.0 | 25.4 | 26.9 |

Abbreviations: 25OHD, 25-hydroxyvitamin D; 1,25(OH)₂D, 1,25-dihydroxyvitamin D; 24-hour urinary Ca, 24-hour urinary excretion of calcium; BAP: bone-specific alkaline phosphatase; U-PyDcr: urinary excretion of pyridinium corrected with urinary excretion of creatine.

* Calcium intake included supplemental source.
significant trend persisted across the models. Excluding supplemental source of calcium intake did not change the overall results (data not shown). Replacing energy-adjusted calcium intake with either absolute amount of calcium intake (mg/day) or serum calcium concentration (mg/dL) in the models yielded similar results (data not shown). In subgroup analysis restricted to current smokers (n = 72), PTH concentration was not associated with number of cigarettes smoked per day (data not shown). Since the participants were not directly queried on duration and amount of cigarette smoking in the past, we did not test a dose-response relationship using pack-years of smoking. Since smoking is often associated with lower BMI, and serum PTH is associated with higher BMI (Kontogeorgos et al., 2015), there was a possibility of residual confounding by BMI on the observed association between smoking and PTH. To address this possibility, we have repeated the analyses stratified by median BMI (25.10 kg/m²) using Model 3. Results were consistent with the main one with no statistical evidence of interaction by BMI (Table 3).

Table 3 summarized results of markers related to calcium metabolism and bone turnover according to smoking habit. We observed significantly lower 24-hour urinary calcium excretion in current smokers compared to non-current smokers, with a similar pattern for FEna. Among bone turnover makers, osteocalcin was lower in current than non-current smokers with a significant linear trend. Serum concentrations of phosphate, 1,25(OH)₂D, BAP, and U-PyDcr had no clear difference among the smoking groups.

We observed no significant difference in any BMD site across smoking categories after adjustment for sex and race with or without other covariates added (data not shown).

4. Discussion

In this generally healthy sample of black and white, young adult men and women, we observed that PTH concentration was lowest in current smokers, followed by former smokers, and highest in never smokers. This relationship appeared to be independent of factors that affect PTH concentration, such as dietary calcium intake and serum 25OHD vitamin D concentration (Steingrimsdottir et al., 2005).

Smoking is generally associated with reduced bone density, and reduced concentrations of vitamin D among elderly populations. Therefore, a compensatory increase in PTH may be expected in smokers (Jorde et al., 2005). However, studies on PTH concentration comparing smoker/non-smoker are conflicting. Some studies showed lower

### Table 3

| Marker | Current | Former | Never | Trend | P for interaction by median BMI |
|--------|---------|--------|-------|-------|---------------------------------|
| BMI < median* | No. | 31 | 36 | 121 | 21.4 | 25.5 | 25.8 | 0.042 | 0.765 |
| | adjusted PTH¹ | pg/mL | 25.9 | 26.9 | 29.1 | 0.071 |
| BMI ≥ median* | No. | 41 | 28 | 119 | 25.9 | 26.9 | 29.1 | 0.071 |
| | adjusted PTH¹ | pg/mL | 25.9 | 26.9 | 29.1 | 0.071 |

*Median BMI was 25.10 kg/m². Participants were fairly evenly distributed by median BMI across the group of smoking habits, sex, and race/ethnicity. Percentage (%) of the participants with BMI < median for each group was as follows: never (50.4), former (56.3), and current (43.1) smoker, men (48.3), women (52.1), black (40.3), white (59.0).

Values were geometric means adjusted for the same set of covariates as Model 3 in the text as follows: age (years), race (Black, White), sex, education (up to 15 years, 16 years or greater), calcium intake in energy density (mg/1000 kcal/day, log-transformed), serum level of 25OHD (ng/mL, log-transformed), serum level of creatinine (mg/dL), season (Jan–Mar, Apr–Jun, July–Sep, and Oct–Dec), physical activity score and sun-exposure time (hours/week).

To obtain P-values, smoking status was treated as an ordinal variable (0 = never smoker, 1 = former smoker, 2 = current smoker). P for interaction by median BMI was obtained by inserting a product term (smoking habit x median BMI) in the model.

(Jorde et al., 2005; Mellstrom et al., 1993; Landin-Wilhelmsen et al., 1995; Brot et al., 1999; Need et al., 2002), but others reported higher PTH concentrations in smokers (Rapuri et al., 2000; Szulc et al., 2002; Ortego-Centeno et al., 1997). One of the largest population studies on this question examined 7896 men and women in Norway and found lower PTH concentrations in smokers compared to non-smokers regardless of age, sex, BMI, and serum creatinine concentration (the fifth Tromsø study) (Jorde et al., 2005). Our finding of lower PTH in smokers is consistent with the Tromsø study, and the observed relationship in our study was robust to adjustment for other factors associated with PTH including sun-exposure time, serum 25OHD concentrations.

Studies reporting higher PTH in current smokers, the opposite finding to ours, appear to have serious limitations in generalizing their results. In a study of healthy men in Spain, Ortego-Centeno and colleagues reported higher PTH in smokers (n = 15) than non-smokers (n = 17). However, the difference was not statistically significant (P-value not provided) and the sample size was small (Ortego-Centeno et al., 1997). Szulc and colleagues reported statistically significantly higher PTH concentration in French current smokers (n = 35) compared to never smokers (n = 93) (Szulc et al., 2002). However, this comparison was based on subgroup of individuals who weighed <75 kg. Including all their participants (719 men, aged 51–85 years), the results appeared to be consistent with our findings, although not statistically significant (Szulc et al., 2002): In a study of 489 elderly women (65–77 years) in the USA, Rapuri and colleagues showed higher PTH in heavy smokers than non-smokers. However, the difference was not statistically significant, nor was it adjusted for serum 25OHDL and other possible confounders (Rapuri et al., 2000).

Potential mechanisms have been proposed to explain lower PTH in smokers such as direct toxic effect of smoking on the parathyroid cells, enhanced degradation or suppressed secretion of PTH by smoking (Jorde et al., 2005; Brot et al., 1999; Need et al., 2002). Among those, Need and colleagues hypothesized that direct impairment of osteoblast function by smoking was likely to result in a slight increase in serum ionized calcium, leading to suppression of PTH (Need et al., 2002). Our finding of lower osteocalcin (bone formation marker) in current smokers than non-current smokers is consistent with this hypothesis and consistent with another study on healthy perimenopausal women (Brot et al., 1999) although the other bone formation marker, BAP, did not show a significant pattern in our study and we did not have variables to estimate serum ionized calcium. We also observed lower 24-hour urinary calcium excretion in current smokers than non-smokers, which seems consistent with the fifth Tromsø study reporting a lower but non-significant 24-hour urinary calcium excretion in smokers (n = 54) compared to non-smokers (n = 151) based on the small subgroup who underwent biochemical assessments (Jorde et al., 2005). The lower calcium excretion in current smokers could be due to lower osteoclast function in smokers and a response to low PTH levels. These actions allow for the maintenance calcium balance between formation and reabsorption from bone. Understanding the mechanism and clinical implication of the finding will require further study.

In comparing BMD at various sites, we observed no significant difference according to smoking habit in the study sample. Although smoking is a known risk factor for osteoporosis and reduced BMD in the elderly, it may require longer exposure time and/or a larger sample size for adverse effect of smoking on BMD to be evident given the relatively young age (24–36 years) of our study participants, the age range at which bone mass reaches its peak (Davies et al., 2005). Alternate methods of modeling exposure to smoking such as use of pack-year might have led to different findings (Calleurs et al., 2013). However, we did not model exposure to smoking using pack-year given limitations in the method for querying smoking history in CARDIA.

A few studies have explicitly reported PTH concentration among former smokers relative to those in current and never smokers. The fifth Tromsø study reported similar concentration between former and never smokers, higher than current smokers. However, they took into...
account only those who quit smoking for one year or longer, not accounting for recent quitters. In a study of 405 postmenopausal women, PTH concentrations were lowest in current, intermediate in former, and highest in never smokers (Need et al., 2002), which is consistent with our finding. The association between smoking and PTH may persist for a while, when quitting, then decline over time. To better clarify the relationship in future, a large-scale longitudinal study with repeated assessment for smoking and PTH is warranted. Such study should be able to examine not only whether the association persists longitudinally, but also dose-response of smoking and length of smoking cessation (former smokers) in relation to PTH.

Limitations need to be considered in interpreting our findings. First, the study was cross-sectional in design, making it difficult to prove temporal relationships between exposure (smoking) and outcome (PTH) although we believe it is uncommon for individuals to quit or not to initiate smoking due to high PTH concentration. Second, given the observational nature of the study, we cannot refute the possibility of residual or unmeasured confounding on the results. Third, our data were collected and measured in the 1990s. Some measurements used in the study may differ from the ones more recently used. Strengths of our study include availability of important factors associated with PTH enabling us to control for potential confounders including serum 25OHD and creatinine, calcium intake, body mass index and physical activity. Likewise, various markers of calcium metabolism and bone turnover allowed us to explore potential mechanism of the findings. Another strength is enrollment of black and white men and women aged 24 to 36 years, making the results likely generalizable to healthy young adults.

5. Conclusions

In summary, we found significantly lower concentration of PTH in current smokers, followed by former smokers, compared to never smokers in a population-based sample of young adult men and women. The relation was not explained by determinants of PTH such as dietary calcium intake and serum vitamin D. Additionally, we observed low serum osteocalcin and low 24-hour urinary calcium excretion in current smokers but no difference in BMD according to smoking habits. The clinical implication and potential mechanism of the findings, however, remain unclear, warranting further research.

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Disclosure statement

The authors have nothing to disclose.

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