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Racial differences in urinary F₂-isoprostane levels and the cross-sectional association with BMI

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Abstract

Levels of four urinary F₂-isoprostanes (F₂-IsoPs) were examined in a large sample of the Insulin Resistance Atherosclerosis Study (IRAS) multiethnic cohort: 237 African Americans (AAs), 342 non-Hispanic Whites (NHWs), and 275 Hispanic Whites (HWs). F₂-IsoP isomers – iPF_{2a}-III, 2,3-dinor-iPF_{2a}-III, iPF_{2a}-VI, and 8,12-iso-iPF_{2a}-VI – were measured in 854 urine samples using liquid chromatography with tandem mass spectrometry detection. In AAs, levels of all four F₂-IsoPs were lower compared with NHWs and HWs (p-values < 0.05). When stratified by BMI, this gap was not observed among participants with normal BMI but appeared among overweight and increased among obese participants. Examining the slopes of the associations between BMI and F₂-IsoPs showed no association between these variables among AAs (p-values > 0.2), and positive associations among Caucasians (p-values < 0.05). Taking into account that positive cross-sectional associations between systemic F₂-IsoP levels and BMI have been consistently demonstrated in many study populations, the lack of such an association among AAs reveals a new facet of racial/ethnic differences in obesity-related risk profiles.

Keywords

F₂-isoprostanes; BMI; racial differences; epidemiology

INTRODUCTION

F₂-isoprostanes (F₂-IsoPs) present stable products of non-enzymatic oxidation of arachidonic acid by different types of free radicals, including reactive oxygen species (1). As such, these biomarkers reflect an individual's inner oxidative environment (i.e., oxidative status) (1). Elevated F₂-IsoP levels have been associated with multiple chronic conditions cross-sectionally, which commonly has been interpreted as an etiological link between oxidative stress and the disease in question (1). Specifically, type 2 diabetes (1) and its risk

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factors, such as obesity (2), impaired glucose tolerance (IGT) (2), and insulin resistance (3), have been associated with increased F₂-IsoP levels cross-sectionally.

In contrast, prospective associations between urinary F₂-IsoPs and the risks of type 2 diabetes (4) and weight gain (5, 6) are inverse. We have explained these prospective findings by proposing that levels of urinary F₂-IsoPs reflect a beneficial physiological trait that is related to some compensatory mechanism. This would account for the positive cross-sectional and inverse prospective associations with type 2 diabetes and obesity. One possible candidate for such a physiological trait is the intensity of oxidative metabolism (7), a major endogenous source of reactive oxygen species in aerobic organisms (8). We have hypothesized that the positive association between F₂-IsoPs and obesity (as assessed by body mass index [BMI] and waist circumference) (2) reflects long-term metabolic adaptation to higher levels of adiposity via intensification of metabolism, predominantly fat oxidation (9, 10). Slow fat oxidation promotes further weight gain and obesity-related deterioration of glucose homeostasis toward frank diabetes (9, 10). As an indicator of intensity of fat oxidation, low F₂-IsoP levels would be expected to be inversely associated with type 2 diabetes and weight gain, as demonstrated by prospective studies (5, 6).

Our previous analyses (5, 5) revealed that in the multiethnic Insulin Resistance Atherosclerosis Study (IRAS) cohort, unadjusted urinary F₂-IsoP levels were lower among African-American (AA) participants compared with Caucasians (that is, non-Hispanic Whites [NHWs] and Hispanic Whites [HWs]). This finding is of interest, because AAs have greater rates of type 2 diabetes as compared with Caucasians (11) as well as a different obesity phenotype (12) and different obesity-related risk profiles (13, 14). To follow up on this initial observation, we therefore conducted more detailed analysis of the racial differences in urinary F₂-IsoP levels. We chose four F₂-IsoP isomers. Two F₂-IsoPs were selected from the III-series: iPF₂α-III was selected because it is the first isomer proposed as an index of lipid peroxidation *in vivo* and, therefore, is the most frequently measured isomer (1); and 2,3-dinor-iPF₂α-III was selected as a beta-oxidation metabolite of iPF₂α-III, addressing a theoretical concern that renal tissues may contribute disproportionately to the total production of iPF₂α-III. In addition, we selected two F₂-IsoPs from the VI-series, iPF₂α-VI and 8,12-iso-iPF₂α-VI, because they are most abundant in human urine (15). Because of their abundance, the VI-series F₂-IsoPs may be more sensitive biomarkers than the III-series. Here, we particularly focus on 2,3-dinor-iPF₂α-III, the F₂-IsoP isomer that, as our previous analyses revealed, showed stronger inverse association with the risk of weight gain and type 2 diabetes (4, 5). The results for the other three F₂-IsoP isomers (iPF₂α-III, iPF₂α-VI, and 8,12-iso-iPF₂α-VI) are similar and therefore, are presented in the text but not in the table or figure.

METHODS AND PROCEDURES

Study population

The IRAS is a multicenter cohort study that recruited a total of 1625 men and women, 40 to 69 years of age, from four U.S. communities from 1992 to 1994. The study recruited approximately equal numbers of persons with normal glucose tolerance, impaired glucose tolerance, and type 2 diabetes, as well as equal numbers of NHWs, HWs, and AAs. This

study included participants free from diabetes. Glucose tolerance was measured precisely at the baseline and 5-year follow-up examinations using an oral glucose tolerance test and World Health Organization criteria (16). A 75-gram glucose load (Orange-dex; Custom Laboratories, Baltimore, MD) was administered over a period of <10 minutes. Blood was collected at 0 and 2 hours. Normal glucose tolerance (NGT) was defined as fasting glucose and 2-hour glucose < 140 mg/dl. Impaired glucose tolerance (IGT) was defined as fasting glucose < 140 mg/dl and 2-hour glucose 140 and <200 mg/dl.

The IRAS protocol was approved by local institutional review committees, and all subjects gave informed consent. The analytical cohort included a subset of the IRAS cohort (n=854) with normal or impaired glucose tolerance at baseline and for whom baseline urine samples were available for measurement of F₂-IsoP levels.

Urinary F₂-isoprostanes

We specifically chose to use urine for F₂-IsoP analysis, although plasma samples were available for the IRAS participants. Our choice was based on our and others' findings that urinary levels of F₂-IsoP are stable in urine even at room temperature and their levels are not sensitive to freeze/thaw cycles (17-19).

Morning spot urine samples were collected from participants at the baseline examination and stored at -70°C. F₂-IsoPs were quantified by liquid chromatography with tandem mass spectrometry detection (LC-MS/MS) on Shimadzu 20A series LC and Applied Biosystems API 4000 QTrap MS/MS instruments as previously described (20). Four quantified F₂-IsoP isomers included iPF₂α-III, 2,3-dinor-iPF₂α-III, iPF₂α-VI, and 8,12-iso-iPF₂α-VI. Urinary F₂-IsoP levels were corrected for creatinine excretion to adjust for urine diluteness. Creatinine was assayed by a fast electrospray ionization–tandem mass spectrometry method (20).

Demographic and anthropometric characteristics

Age, race, and ethnicity were self-reported. Height and waist circumference were measured to the nearest 0.5 cm. Weight was measured to the nearest 0.1 kg. All measures were obtained in duplicate following a standardized protocol, and averages were used in the analysis. BMI was calculated as weight/height² (kg/m²).

Statistical analysis

Student t-tests and χ^2 -tests were used to assess differences in the distribution of demographic and baseline variables by race/ethnicity. Full analysis by three racial/ethnic groups was conducted for 2,3-dinor-iPF₂α-III as the most sensitive isomer to detect associations with weight changes and type 2 diabetes risk. General linear models were used to calculate mean levels of F₂-IsoPs and slopes for the correlation between F₂-IsoPs and BMI, adjusted for age (years, continuous) and gender (female/male, indicator variable). Adjustment for age and gender was critical to offset possible confounding, because creatinine levels differ by age and gender (24).

BMI was used as a continuous variable in calculations of the slopes and was categorized (normal < 25; 25 – overweight < 30; and obese ≥ 30) in calculations of adjusted mean F₂-IsoP levels. Race/ethnicity was categorized as AAs, NHWs, and HWs for calculating mean F₂-IsoPs levels and slopes. Taking into account that NHWs and HWs showed similar trends in the relationship between F₂-IsoPs and BMI, the test for interaction categorized racial groups as Caucasians (this group included NHWs and HWs) and AAs. Further analysis for three other isomers (iPF₂α-III, iPF₂α-VI, and 8,12-iso-iPF₂α-VI) was condensed to two racial groups. All effects were fixed. Statistical analysis was performed using the SAS software package (version 9.2; SAS Institute, Cary, NC).

RESULTS

Overall, demographic characteristics were similar in AAs and Caucasians, although NHWs were slightly older (Table 1). Unadjusted levels of 2,3-dinor-iPF₂α-III were lower in AAs compared with NHWs and HWs (Table 1). AAs had similar BMIs compared with HWs but greater BMIs as compared with NHWs (Table 1). Prevalence of impaired glucose tolerance was similar in AAs and HWs and lower in NHWs, whereas rates of conversion to diabetes during the 5-year follow-up were similar for all three groups, approximately 16%. The lowest levels of 2,3-dinor-iPF₂α-III were observed among AA participants (p-values for comparisons with NHWs and HWs were < 0.05). These racial differences in 2,3-dinor-iPF₂α-III levels persisted after adjustment for age and gender, becoming increasingly apparent at higher levels of BMI (Fig. 1). As seen in Figure 1, 2,3-dinor-iPF₂α-III levels were similar in AAs, NHWs and HWs among the participants with normal BMI, but among the overweight and obese participants, AAs had lower levels of 2,3-dinor-iPF₂α-III. The slopes presented in Figure 1 (Panel B) show that 2,3-dinor-iPF₂α-III levels increase with BMI among NHWs and HWs but stay at the same level among AAs. Formal testing for the race-BMI interaction confirmed racial differences in the association between 2,3-dinor-iPF₂α-III and BMI (Fig. 1, Panel B).

The statistical analysis for the other three F₂-IsoP isomers – iPF₂α-III, iPF₂α-VI, and 8,12-iso-iPF₂α-VI – in two racial groups showed similar results. Their urinary levels were lower among AAs as compared to Caucasians (all p-values were < 0.05). No correlation between BMI and these three F₂-IsoP isomers was observed among AAs: estimates of slope were –0.0023 (p=0.2), 0.0233 (p=0.5), and –0.0091 (p=0.7) for iPF₂α-III, iPF₂α-VI, and 8,12-iso-iPF₂α-VI, respectively. In contrast, among Caucasians BMI correlated with the three F₂-IsoP isomers: estimated slopes were 0.0031 (p=0.02), 0.0879 (p=0.003), and 0.1155 (p<0.0001) for iPF₂α-III, iPF₂α-VI, and 8,12-iso-iPF₂α-VI, respectively. Formal test for the race-BMI interaction showed statistically significant interactions for iPF₂α-III (p=0.02) and 8,12-iso-iPF₂α-VI (p=0.001) and a border-line significant interaction for iPF₂α-VI (p=0.1).

DISCUSSION

Our more detailed analysis confirmed that overall levels of F₂-IsoPs are lower among AAs as compared with Caucasians. However, it is particularly important to note the pattern of differences detected. Our results suggest that among AAs there is no cross-sectional

association between F₂-IsoP levels and BMI, which has been observed in other study populations (2, 21, 22). Thus, the racial/ethnic gap appeared among overweight participants and increased among the obese due to the positive association between F₂-IsoPs and BMI among Caucasians and the lack of such an association among AAs (Fig. 1).

The observed racial/ethnic differences in F₂-IsoP levels can be interpreted through our proposed concept that F₂-IsoP levels reflect intensity of oxidative fat metabolism (7). Other studies have found that AAs have lower fat oxidation (23). According to this concept, the lack of a positive cross-sectional association between F₂-IsoP levels and BMI, a commonly used index of adiposity, among AAs may indicate weaker long-term adaptation to increased adiposity (9, 10) among AAs, a trend proposed on the basis of a cross-sectional study by Weyer and colleagues (23). Ultimately, the hypothesis that AAs tend to have weaker long-term adaptation to increased adiposity has to be confirmed by prospective data and direct measurement of adiposity.

Our study of urinary F₂-IsoP levels in the multiethnic IRAS cohort showed lower levels of these biomarkers among AAs as compared with Caucasians, with the gap increasing at higher BMI. Although the physiological meaning of these differences awaits elucidation by more detailed studies, there is already evidence to indicate that long-term metabolic adaptation related to greater adiposity (namely, increased fat oxidation) is weaker in African Americans as a group.

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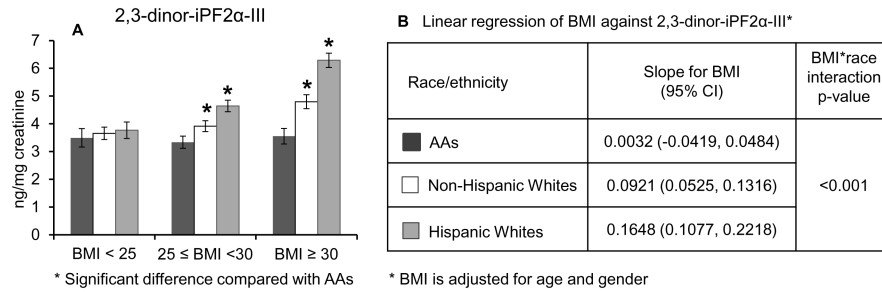


Figure 1.

Association between 2,3-dinor-iPF2 α -III and BMI by race/ethnicity adjusted for age and gender. (A) Age- and gender-adjusted mean (standard errors) of urinary 2,3-dinor-iPF(2a)-III levels by BMI categories among AAs (50/115/71), NHWs (110/143/88), and HWs (64/127/84); mean values are compared with AAs within each BMI category. (B) Slopes for the regression of age- and gender-adjusted BMI against 2,3-dinor-iPF(2a)-III; p-value is presented for the interaction term between race and BMI.

Table 1

Characteristics of IRAS racial/ethnic groups

Characteristics	Means (SD) or percent (counts)		
	African American (n=237)	Caucasian	
		Non-Hispanic White (n=342)	Hispanic (n= 275)
Age, years	54.1 (8.3)	55.5 (8.5)*	53.7 (7.9)
Gender (female), %	59.5 (141)	52.3 (179)	62.7 (172)
BMI, kg/m ²	29.0 (5.6)	27.7 (5.4)*	28.9 (6.0)
Abdominal obesity by waist circumference, % ^a	31.9 (75)	27.6 (94)	33.5 (92)
Impaired glucose tolerance	34.2 (81)	30.4 (104)*	33.9 (91)
2,3-dinor-iPF(2a)-III, ng/mg creatinine	3.61 (2.1)	4.08 (2.30)*	5.18 (3.24)*

^a Abdominal obesity is defined as waist circumference \geq 102 cm for men and \geq 88 cm for women.

* P-value for comparison with African-American participants <0.05