Urinary F_2-Isoprostanes as a Biomarker of Reduced Risk of Type 2 Diabetes

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OBJECTIVE—We have previously reported evidence of an inverse association between a urinary F_2-isoprostane and type 2 diabetes risk in a pilot case-control study nested within the Insulin Resistance Atherosclerosis Study (IRAS). Here, we report the results from the study extended to the entire IRAS cohort.

RESEARCH DESIGN AND METHODS—This prospective study included 138 incident type 2 diabetes case and 714 noncase subjects. Four F_2-isoprostanes (iPF2α-III; 2,3-dinor-iPF2α-III; iPF2α-VI; and 8,12-iso-iPF2α-VI) were assayed in baseline urine samples using liquid chromatography–tandem mass spectrometry.

RESULTS—Three F_2-isoprostanes showed significant inverse associations with type 2 diabetes risk: the adjusted odds ratios were 0.52 (95% CI 0.39–0.67), 0.56 (0.42–0.73), 0.62 (0.48–0.79), and 0.91 (0.72–1.12) for iPF2α-III; 2,3-dinor-iPF2α-III; iPF2α-VI; and 8,12-iso-iPF2α-VI, respectively.

CONCLUSIONS—Our findings indicate that urinary F_2-isoprostanes are inversely associated with type 2 diabetes risk beyond the traditional risk factors and may be useful in identifying high-risk populations.

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F_2-isoprostanes are formed during nonenzymatic oxidation of arachidonic acid by free radicals, including reactive oxygen species, and their systemic levels are a well-studied index of in vivo oxidative status (1). Type 2 diabetes (1) and its risk factors, such as obesity (2), impaired glucose tolerance (IGT) (2), and insulin resistance (3), have been associated with increased F_2-isoprostane levels cross-sectionally. To study their prospective association, we previously conducted a pilot case-control study nested in the Insulin Resistance Atherosclerosis Study (IRAS) cohort (26 case and 26 control subjects). Contrary to cross-sectional associations, baseline levels of F_2-isoprostanes (quantified as 2,3-dinor-5,6-dihydro-iPF2α-III) were inversely associated with type 2 diabetes incidence, with an odds ratio (OR) of 0.32 (95% CI 0.12–0.81) (4). We postulated that interindividual differences in F_2-isoprostanes reflect a variability of the intensity of oxidative metabolism, specifically fat oxidation (4), because glucose uptake accounts for only a minor proportion of peripheral oxygen consumption (5). We also hypothesized that the concentration of F_2-isoprostanes suggests metabolic adaptation to higher adiposity, reflecting increased fat oxidation (4). This study, expanded to the entire cohort, tested the hypothesis that F_2-isoprostane levels are inversely associated with type 2 diabetes risk.

RESEARCH DESIGN AND METHODS

The IRAS is a multicenter cohort study (6) that recruited a total of 1,625 people, 40–69 years of age, from four U.S. communities in 1992–1994, with approximately equal numbers of persons with normal glucose tolerance (NGT), IGT, and type 2 diabetes, as well as equal numbers of non-Hispanic whites, Hispanics, and African Americans. The IRAS protocol was approved by local institutional review committees, and all subjects gave informed consent. Glucose tolerance was measured at the baseline and follow-up examinations through use of an oral glucose tolerance test and the World Health Organization criteria (7). Of 901 participants with NGT or IGT at baseline, 145 IRAS participants had converted to type 2 diabetes at follow-up. A baseline urine sample was available for 138 case and 714 noncase subjects.

Insulin sensitivity (insulin sensitivity index [S_I]), acute insulin response (AIR), height, and weight were determined as previously described (8). Morning spot urine samples collected at the baseline examination were stored at −70°C. Four F_2-isoprostane isomers (iPF2α-III; 2,3-dinor-iPF2α-III; iPF2α-VI; and 8,12-iso-iPF2α-VI) were quantified by liquid chromatography with tandem mass spectrometry detection, and creatinine was assayed as previously described (9). We also measured urinary allantoin, an oxidative modification of urate, in all case subjects (n = 138) and in a subset of noncase subjects (n = 182) (10).

Adjusted ORs (95% CI) for the associations between F_2-isoprostane isomers and incident type 2 diabetes were calculated from logistic regression models. The minimally adjusted models included demographic variables (age, sex, and a combined variable, ethnicity/clinic), baseline IGT status, and BMI. The addition of the following risk factors did not influence the association estimates obtained from the minimally adjusted model: AIR, insulin sensitivity [$\log(S_I + 1)$], family history of diabetes, and waist circumference. Student t and χ^2 tests were used to assess differences in the distribution of demographic and baseline variables by case/noncase status.

RESULTS

—As expected, case subjects were older and had weaker glucose homeostatic control (higher levels of fasting and postload glucose), greater adiposity (greater BMI), lower insulin sensitivity (lower S_I), and lower insulin secretion (lower AIR) ($P < 0.05$). The baseline levels of three of

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Table 1—*Association between F_2-isoprostanes and incident type 2 diabetes in the IRAS cohort*

		OR (95% CI)*		
F_2-isoprostanes, ng/mg creatinine	Unit, 75th–25th percentile	All subjects (138 case and 714 control subjects)	BMI <30 kg/m^2 (69 case and 540 control subjects)	BMI ≥30 kg/m^2 (69 case and 174 control subjects)
iPF2α-III	0.14	0.52 (0.39–0.67)	0.66 (0.45–0.91)	0.37 (0.23–0.56)
2,3-dinor-iPF2α-III	2.74	0.56 (0.42–0.73)	0.59 (0.37–0.89)	0.50 (0.34–0.73)
iPF2α-VI	3.93	0.62 (0.48–0.79)	0.66 (0.45–0.92)	0.59 (0.39–0.84)
8,12-iso-iPF2α-VI	2.83	0.91 (0.72–1.12)	1.07 (0.77–1.42)	0.76 (0.53–1.05)

*ORs are adjusted for age, sex, race/ethnicity/clinic categories, baseline BMI, and IGT status.

four F_2-isoprostanes were significantly lower among case subjects: the *P* values for the case/noncase comparisons were <0.001, 0.06, 0.003, and 0.9 for iPF2α-III; 2,3-dinor-iPF2α-III; iPF2α-VI; and 8,12-iso-iPF2α-VI, respectively. These three urinary F_2-isoprostane isomers showed inverse associations with incident type 2 diabetes, whereas 8,12-iso-iPF2α-VI showed no association (Table 1). Associations were not modified by sex ($P > 0.30$ for the interaction terms). There was a trend toward a somewhat stronger inverse association among obese subjects for three F_2-isoprostanes (iPF2α-III; 2,3-dinor-iPF2α-III; and iPF2α-VI): the ORs ranged between 0.59 and 0.66 among nonobese subjects and between 0.37 and 0.59 among obese subjects. The association between 8,12-iso-iPF2α-VI and type 2 diabetes risk changed from the null among nonobese subjects to a weak inverse association (though marginally significant) among obese subjects. Testing for statistical interaction between urinary F_2-isoprostanes and BMI showed no interaction for three F_2-isoprostanes: the *P* values for interaction terms were ≥0.3 for iPF2α-III; 2,3-dinor-iPF2α-III; and iPF2α-VI. The interaction term between 8,12-iso-iPF2α-VI and BMI was borderline significant ($P = 0.05$). Allantoin was inversely associated with risk of type 2 diabetes: the OR for the 75th–25th percentile difference of allantoin distribution was 0.80 (95% CI 0.58–1.01) (not shown).

CONCLUSIONS—The central question of this study concerns the relationship between urinary F_2-isoprostanes and the risk of type 2 diabetes. Based on our pilot data (4), we hypothesized that greater F_2-isoprostane levels present a protective factor as reflecting metabolic adaptation to higher adiposity. We further hypothesized that inverse association of interest will be stronger among the obese because metabolic adaptation in obesity is likely to be more important to preservation of metabolic health. In accord with our main hypothesis, the risk of type 2 diabetes was reduced at the higher levels of three F_2-isoprostanes by approximately 40–50% (Table 1), even after adjustment for the major type 2 diabetes risk factors. Largely similar associations for the three F_2-isoprostanes in contrast with no association with 8,12-iso-iPF2α-VI suggest that different urinary F_2-isoprostanes may vary in their sensitivity for predicting type 2 diabetes risk. Our secondary hypothesis about stronger association among obese subjects is only weakly supported by our results.

These results require confirmation by measurement of F_2-isoprostanes in 24-h urine collections and by measurement of other oxidative status markers. However, allantoin, an oxidative modification of urate, measured in a subset of IRAS, was consistently inversely associated with risk of type 2 diabetes. Thus, the hypothesis of metabolic adaptation has been extended to another index of oxidative status not directly related to lipid oxidation.

Our findings suggest that urinary F2-isoprostanes are a biomarker of reduced risk of type 2 diabetes. The proposed relationships between F_2-isoprostanes and the individual ability to use fat as fuel, however, remain an assumption; therefore, more detailed studies are needed to identify physiological determinants of urinary F_2-isoprostanes to explain the observed associations.

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D.I. researched data, wrote the manuscript, and contributed to data analysis. I.S., K.B., H.Z., S.P.Y., and D.S.M. developed the F_2-isoprostane assay. F.W. contributed to data analysis. R.B.D. and L.E.W. contributed to data analysis and discussion and reviewed and edited the manuscript.

As the corresponding author and guarantor of this article, D.I. takes full responsibility for the work as a whole.

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