Urinary F2-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 F2-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 F2-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 F2-isoprostanes showed significant inverse associations with type 2 diabetes risk: the adjusted odds ratios were 0.52 (95% CI 0.39–0.67), 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 F2-isoprostanes are inversely associated with type 2 diabetes risk beyond the traditional risk factors and may be useful in identifying high-risk populations.

F2-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 F2-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 F2-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 F2-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 F2-isoprostanes suggests metabolic adaptation to higher adiposity, reflecting increased fat oxidation (4). This study, expanded to the entire cohort, tested the hypothesis that F2-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 [S1]), 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 F2-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 F2-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(S1 + 1)], family history of diabetes, and waist circumference. Student t and χ² 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 S1), and lower insulin secretion (lower AIR) (P < 0.05). The baseline levels of three of


**F₂-isoprostanes and type 2 diabetes risk**

<table>
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<tr>
<th>F₂-isoprostanes, ng/mg creatinine</th>
<th>Unit, 75th–25th percentile</th>
<th>All subjects (138 case and 714 control subjects)</th>
<th>BMI &lt;30 kg/m² (69 case and 540 control subjects)</th>
<th>BMI ≥30 kg/m² (69 case and 174 control subjects)</th>
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<tbody>
<tr>
<td>iPF₂α-III</td>
<td>0.14</td>
<td>0.52 (0.39–0.67) 0.66 (0.45–0.91) 0.37 (0.23–0.56)</td>
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<tr>
<td>2,3-dinor-iPF₂α-III</td>
<td>2.74</td>
<td>0.56 (0.42–0.73) 0.59 (0.37–0.89) 0.50 (0.34–0.73)</td>
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<tr>
<td>iPF₂α-VI</td>
<td>3.93</td>
<td>0.62 (0.48–0.79) 0.66 (0.45–0.92) 0.59 (0.39–0.84)</td>
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<tr>
<td>8,12-iso-iPF₂α-VI</td>
<td>2.83</td>
<td>0.91 (0.72–1.12) 1.07 (0.77–1.42) 0.76 (0.53–1.05)</td>
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No potential conflicts of interest relevant to this article were reported.

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 F₂-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 manuscript, Dr. Dora Il'yasova takes full responsibility for the work as a whole.

**References**