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Urinary F2-Isoprostanes, Obesity, and Weight Gain in the IRAS Cohort

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Abstract

Obesity has been associated with increased F_2-isoprostane (F_2-IsoP) levels cross-sectionally. However, the prospective association may be inverse, based on our earlier finding that elevated urinary F_2-IsoP levels predict lower risk of diabetes. This earlier finding led us to hypothesize that urinary F_2-IsoPs reflect the intensity of oxidative metabolism and as such predict lower risk of both diabetes and weight gain. We examined cross-sectional relationships with obesity and prospective relationships with weight gain using the data from 299 participants of the Insulin Resistance Atherosclerosis Study (IRAS), all of whom were free of diabetes at baseline. Four urinary F_2- IsoPs were assayed in stored baseline urine samples using liquid chromatography with tandem mass spectrometry: iPF(2α)-III, 2,3-dinor-iPF(2α)-III, iPF(2α)-VI, and 8,12-iso-iPF(2α)-VI (F_2-IsoP 1–4, respectively). Baseline F_2-IsoPs were positively associated with baseline measures of obesity; the strongest associations were found with two F_2-IsoPs: odds ratios (95% confidence intervals) for overall and abdominal obesity were 1.74 (1.26–2.40) and 1.63 (1.18–2.24) for F_2-IsoP2 and 1.47 (1.12–1.94) and 1.64 (1.22–2.20) for F_2-IsoP4. F_2-IsoP2 showed the strongest and significant inverse association with weight gain during the 5-year follow-up period: increase in F_2-IsoP2 equal to 1 s.d. was associated with 0.90 kg lower weight gain (P = 0.02) and the odds ratios for relative (≥5%) and absolute (≥5 kg) weight gain were 0.67 (0.47–0.96) and 0.57 (0.37–0.87), respectively. The other three F_2-IsoPs were consistently inversely associated with weight gain, although not significantly, suggesting that different F_2-IsoPs vary in their ability to detect the association with weight gain.

INTRODUCTION

F_2-isoprostanes (F_2-IsoPs) have been studied as markers of oxidative status in multiple chronic conditions, including obesity (1–5). Increased levels of F_2-IsoPs have been associated with BMI (2–5), visceral fat area (6), and waist circumference (7). However, no information on the prospective relationship between F_2-IsoPs and weight gain is available.

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Disclosure

The authors declare no conflict of interest.

Our pilot study within the Insulin Resistance Atherosclerosis Study (IRAS) cohort showed that the baseline levels of urinary 2,3-dinor-5,6-dihydro-iPF(2α)–III (a β-oxidation metabolite of iPF(2α)-III) were positively associated with baseline BMI, but inversely associated with the risk of type 2 diabetes (8). We previously hypothesized that urinary F_2-IsoPs reflect the intensity of metabolism (8), which is a major endogenous source of reactive oxygen species in aerobic organisms (9). From this point of view, the cross-sectional positive associations between obesity and F_2-IsoPs may be considered as adaptation to a positive energy balance through an increase in fat oxidation (10). At the same time, relatively slow fat oxidation—reflected by low urinary F_2-IsoPs—promotes further weight gain and obesity-related deterioration of the glucose homeostasis toward frank diabetes (11–14). Recently, we confirmed the inverse association between F_2-IsoPs and incident type 2 diabetes in a larger case-control study nested in the IRAS cohort (15). This analysis examines the same study population, focusing on the relationship between baseline levels of urinary F_2-IsoPs and baseline indices of obesity and the risk of weight gain during a 5-year follow-up period.

We selected four urinary F_2-IsoPs based on the previous studies (5,16), hereafter referred to as F_2-IsoP1, F_2-IsoP2, F_2- IsoP3, and F_2-IsoP4. Two F_2-IsoPs were selected from the III-series, iPF(2α)-III (F_2-IsoP1) and 2,3-dinor-iPF(2α)-III (F_2-IsoP2). F_2-IsoP1 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 (1,17). F_2-IsoP2 was selected as a β-oxidation metabolite of F_2-IsoP1; because renal tissues may contribute disproportionally to the total production of F_2-IsoP1, F_2-IsoP2 theoretically better reflects the total body production of the parent compound, as β-oxidation occurs predominantly in the extra-renal tissues. In addition, we selected two F_2-IsoPs from the VI-series (iPF(2α)-VI (F_2- IsoP3) and 8,12-iso-iPF(2α)-VI (F_2-IsoP4)) because studies show them as the most abundant in human urine (18,19). Because of their abundance, the VI-series F_2-IsoPs may be more sensitive biomarkers than the III-series.

The rationale for selecting multiple F_2-IsoPs is twofold. First, it remains unknown how closely different urinary F_2-IsoPs reflect mitochondrial oxidative metabolism as opposed to other oxidative processes not related to obesity/diabetes etiology. Second, the strength of the cross-sectional associations with obesity varies for different F_2-IsoP measurements (5). Therefore, it is likely that some F_2-IsoP measurements are more sensitive biomarkers for weight gain risk than others. Acknowledging possible differences in the strength of the associations, we hypothesize on the directions of the associations of interest: urinary F_2-IsoPs will be directly associated with baseline measurements of obesity and inversely associated with the risk of weight gain.

METHODS AND PROCEDURES

This is a secondary data analysis of a prospective study that initially examined the risk of type 2 diabetes within the IRAS sub-cohort (15).

Study population

The IRAS is a multicenter cohort study (20) that recruited a total of 1,625 men and women, 40–69 years of age, from four US 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 non-Hispanic whites, Hispanics, and African Americans. Glucose tolerance was measured precisely at both the baseline and follow-up examinations using an oral glucose tolerance test and the World Health Organization criteria. In the follow-up examination conducted 5 years after recruitment, 80% of the cohort participated. The IRAS protocol was approved by local institutional review committees, and all subjects gave informed consent.

The eligible cohort for this study included a subset of the IRAS cohort (n = 850) who had normal or impaired glucose tolerance at baseline, available baseline urine sample, and participated in the follow-up examination. The original analysis (15) included 140 participants who developed type 2 diabetes during the follow-up period and 177 noncases who did not develop diabetes (representing a 25% random sample of the 710 noncases with available urine sample), for a total of 317 subjects.

Definition of obesity and weight gain

Anthropometric measurements included height and waist circumference (both measured to the nearest 0.5 cm), and weight (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, calculated as weight/height^2 (kg/m^2), was used as an estimate of overall adiposity. Abdominal obesity was estimated based on the waist circumference. Obesity was defined as BMI ≥30 kg/m^2 and abdominal obesity was defined as waist circumference ≥102 cm for men and ≥88 cm for women according to the National Institutes of Health guidelines (21). Relative weight gain was defined as increase in weight at the follow-up ≥5%. Absolute weight gain was defined as increase in weight ≥5 kg.

Urinary F_2-IsoPs

Morning spot urine samples were collected from all participants at the baseline examination and stored at −70 °C. Four isomers of F_2- IsoPs— iPF(2α)-III, 2,3-dinor-iPF(2α)-III, iPF(2α)-VI, and 8,12-iso-iPF(2α)-VI (F_2-IsoPs 1–4)—were quantified by liquid chromatography with tandem mass spectrometry detection on Shimadzu 20A series LC and Applied Biosystems API 4000 QTrap MS/MS instruments as previously described (22). Creatinine was assayed by a fast electrospray ionization–tandem mass spectrometry method as described previously (22). The urine samples were diluted to 0.65 mg/ml creatinine; samples with creatinine levels equal to or below this value were analyzed without dilution. Valid measures of the analytes could not be obtained for four participants due to the strong suppression of the signal by the urine matrix.

Behavioral variables

Smoking status was assessed by self-report at the baseline and follow-up examinations. Percent calories from fat were assessed with a 114-item food frequency questionnaire, which

was modified for the IRAS to incorporate regional and ethnic food habits and supplements. Participation in vigorous physical activity was assessed by asking the participants to choose between the following categories: rarely/never, 1–3 times/month, 1 time/week, 2–4 times/week, and ≥5 times/week.

Statistical analysis

A total of 18 participants were excluded from the analysis for the following reasons: four participants without valid measurements of F_2-IsoPs, six with missing values of weight at the follow-up, six with inconsistent changes (opposite direction) in weight and waist circumference, and two with missing values for participation in vigorous physical activity, leaving 299 participants for the analysis. All subjects in this analysis were free of diabetes at the time of urine collection used for the measurements of F_2-IsoP levels.

We used the Student's *t*-test and ANOVA *F*-test to assess whether baseline F_2-IsoP levels differed in those who did and did not gain weight (at least 5% or 5 kg) during the follow-up period, demographic, and baseline characteristics of the study population. The Spearman correlation coefficient was used to estimate the correlation between F_2-IsoPs and continuous variables. We examined unadjusted cross-sectional associations between F_2-IsoP measurements and obesity; analysis of abdominal obesity was stratified by gender. Adjusted cross-sectional associations with the continuous measures of waist circumference and BMI were assessed using linear regression models. Logistic regression models were used to assess adjusted associations with the categorical gender-specific definition of abdominal obesity (waist circumference ≥102 cm for men and ≥88 cm for women) and the categorical definition of overall obesity (BMI ≥30) for both genders. The adjustment variables included age (years), gender, and ethnicity/clinic (eight categories). To examine whether the associations differed by gender, we tested interaction terms between F_2-IsoP measurements and gender in these models.

Similarly, we used linear regression models for the continuous outcomes and logistic regression models for categorical outcomes to examine prospective associations with weight gain among individuals free of diabetes at baseline. In linear regression models, weight at the follow-up was regressed against baseline weight and F_2-IsoP levels, adjusting for demographic variables (age, gender, ethnicity/clinic) (Model 1). The fully adjusted models included additional behavioral and metabolic independent predictors (Model 2). Behavioral variables potentially influencing weight change were baseline smoking status (never/former/ever) and change in smoking status (no change/former-current/current-former), baseline percent calories from fat (%), and participation in vigorous physical activity (<1, 2–4, or ≥5 times/week). Metabolic variables potentially influencing weight gain were baseline BMI and diabetes status at follow-up. The final reduced models (Model 3) included the demographic variables and the three variables from Model 2 that were significantly associated with weight change (two indicators of smoking behavior and participation in vigorous physical activity). In addition, the three independent predictors retained in Model 3 were applied to the logistic regression models examining the association with relative (Model 4) and absolute weight gain (Model 5). Statistical analysis was performed using the SAS software package (version 9.2; SAS Institute, Cary, NC).

RESULTS

At baseline, 18.7% of the study population had normal BMI (<25), 45.5% were overweight (25≤ BMI <30), and 35.8% were obese (BMI ≥30). Based on the gender-specific cut-points for abdominal obesity, 39.8% of participants were abdominally obese. The average increase in weight during the follow-up period was 2.2 kg (Table 1). Approximately 30% of participants gained ≥5% of their initial weight and 25% gained ≥5 kg. The absolute and relative weight gain categories largely overlapped: the 76 out of 79 participants with absolute weight gain also had relative weight gain. Moreover, the increment in weight change was similar in both weight gain categories. Baseline weight, BMI, and waist circumference were lower among those with relative weight gain as compared to participants with absolute weight gain. Both weight gain categories had lower proportions of Hispanics, greater proportions of smokers at baseline, and greater proportions of smokers who quit smoking during the follow-up period. Diabetes incidence was greater in both categories of weight gain compared to the entire study population. Age and gender composition did not differ by weight gain category or by baseline level of fat intake (percent calories from fat) or participation in vigorous physical activity.

Analysis of the unadjusted baseline associations between F_2-IsoPs and different study characteristics showed greater F_2-IsoP levels (all four F_2-IsoPs) among females and the category of low participation in vigorous physical activity, and lower F_2-IsoP levels among African Americans (Table 2). In addition, greater F_2-IsoP1 levels were found among current smokers. No correlations were found with age or percent calories from fat. With respect to the outcomes of interest, there was a trend of positive unadjusted cross-sectional associations with BMI (significant for F_2-IsoP2-4) and waist circumference (significant with F_2-IsoP2 and 4 among women and F_2-IsoP2 among men). In contrast to the positive cross-sectional association with measures of obesity, the relative and absolute weight gain measures were associated with lower levels of F_2-IsoP2 (Table 2).

Adjusted cross-sectional associations between baseline F_2-IsoPs and BMI and obesity were positive, with the strongest association found for F_2-IsoP2 (Table 3). Baseline F_2-IsoP levels also were positively associated with waist circumference and abdominal obesity, with the strongest associations found for F_2-IsoP2 and F_2-IsoP4 (Table 3). The linear associations with BMI and waist circumference did not significantly differ by gender, and all interaction terms were nonsignificant.

The minimally adjusted, continuous measure of weight gain showed an inverse and marginally significant association with baseline levels of F_2-IsoP2 (*P* for β-coefficient was 0.06) (Table 4, Model 1). Importantly, the direction of the associations for the other three F_2-IsoPs (F_2-IsoP1, F_2-IsoP3, and F_2- IsoP4) also was inverse, although the standard errors for the main effect estimate were high, resulting in high *P* values for these associations (Table 4). Adjustment for potential confounders did not change the direction of these associations but decreased the standard error of the main effect estimates (Table 4, Model 2). The final models, minimized to the most predictive adjustment variables, showed results similar to the fully adjusted models (Table 4, Model 3).

The logistic regression models for the two categorical weight gain outcomes showed similar inverse associations with F_2-IsoP levels (Table 4, Models 4, and 5). Consistent with the linear regression results, the inverse associations were more pronounced in the case of F_2-IsoP2 for both weight gain categories. The odds of relative and absolute weight gain were reduced by ~30–40% for a 2.78 ng/mg creatinine increase in F_2-IsoP2. Finally, we tested whether these inverse associations differed by follow-up converter status and found no interaction between the weight gain variables and conversion to type 2 diabetes.

DISCUSSION

This analysis explored the cross-sectional association between baseline urinary F_2-IsoP levels and obesity measures, and the prospective association of baseline F_2-IsoPs and weight gain determined at follow-up. This study produced three main findings. First, our analysis confirms previously published reports (2–7) of a cross-sectional positive association between urinary F_2-IsoP levels and overall and abdominal obesity (Table 3). Second, these cross-sectional associations were strongest for F_2-IsoP2, suggesting that this biomarker is the most sensitive in detecting the link between urinary F_2-IsoPs and obesity. Third, the direction of the prospective association between F_2-IsoPs and weight gain was inverse, with the strongest association again being found for F_2-IsoP2 (Table 4). The finding that increased F_2-IsoP levels predict lower weight gain is consistent with our hypothesis that urinary F_2-IsoPs reflect the intensity of oxidative fat metabolism (8), which is known to protect against development of obesity (11–14). In this context, the positive cross-sectional associations between the F_2-IsoPs and obesity measures can be explained as a compensatory effect of metabolic adaptation to positive energy balance (10).

The unadjusted cross-sectional relationships between F_2-IsoPs and baseline study characteristics (Table 2) produced several expected as well as novel findings. Expected findings include absence of a crude association with future development of diabetes (8,15) and positive associations with female gender (2–5) and current smoking (17). In this study, urinary F_2-IsoPs were assessed at baseline among individuals who were free of diabetes. Although higher levels of F_2-IsoPs are cross-sectionally associated with frank diabetes (3,23,24), the prospective association between urinary F_2-IsoPs and diabetes is inverse (8,15). Therefore, we expected that the baseline levels of F_2-IsoPs among those who develop diabetes later should be lower than among the individuals who stay free from diabetes. However, this difference in the baseline F_2-IsoPs becomes apparent only after adjustment for key factors, such as gender, race, BMI, and baseline impaired glucose tolerance status. We addressed the opposite direction of the cross-sectional and prospective associations previously by developing the hypothesis that urinary F_2-IsoPs may reflect the intensity of fat oxidation and as such, predict lower risk of weight gain and diabetes (8).

The consistent association of urinary F_2-IsoPs with female gender can be explained by the correction for creatinine as suggested by Basu and coauthors (25). Because creatinine excretion is tightly correlated with lean body mass, the levels of urinary creatinine are generally lower among women (26). Therefore, correction for creatinine may artificially increase the levels of urinary F_2-IsoPs per mg creatinine among women. Commonly, urinary levels of biomarkers are corrected for creatinine to account for a measurement error due to

urine diluteness. At the same time, the possible artificial gender differences can be offset by the statistical adjustment for gender. Taking these considerations into account, we followed the common practice by correcting urinary F_2-IsoPs for creatinine and adjusting for gender in the statistical analysis.

Surprisingly, participation in vigorous physical activity was inversely associated with F_2-IsoP levels. This contradicts our hypothesis, given that vigorous activity should promote an increase in resting metabolism and, accordingly, an increase in F_2-IsoP levels. We previously documented the trend of increasing resting F_2-IsoP excretion in an exercise intervention study (27); a positive association of circulating F_2-IsoPs with habitual physical activity has been documented by others (28). One possible explanation for the current study's observed inverse association between vigorous activity and F_2-IsoPs could be confounding by BMI; however, our additional analysis showed that adjustment for BMI lowered the magnitude but did not completely eliminate this inverse association (data not shown). We believe that the observed association of F_2-IsoPs with vigorous physical activity is confounded by unmeasured variables or reflects a measurement error.

The association of F_2-IsoPs with race is intriguing. African Americans had lower levels of F_2-IsoPs, suggesting lower levels of fat oxidation (Table 2). Several *in vitro* studies demonstrated lower rates of fatty acid oxidation in homogenates from rectus abdominus among African Americans (29,30). The *in vivo* studies with indirect calorimetry also showed lower rates of fat oxidation among African Americans compared to whites (31–33). These phenotypic studies corroborate the genetic findings on human uncoupling protein 3 (*UCP3* (34)). Expressed in skeletal muscle, *UCP3* encodes a mitochondrial transmembrane carrier protein that participates in thermogenesis by uncoupling mitochondrial respiration from oxidative phosphorylation, leading to increased fat oxidation (35). Several polymorphisms and mutations in *UCP3* that are associated with lower rates of fat oxidation were found among African Americans but not in whites (34). Together, these findings support our assumption that lower levels of urinary F_2-IsoPs may reflect slower rates of fat oxidation among African Americans.

The major strength of our analysis is its truly observational nature without any targeted interventions. In addition, the diverse metabolic and racial/ethnic profiles of the study population (Table 1) suggest that the study's results are generalizable to other populations. The major limitation pertains to the selection of the sub-cohort, which was focused on examining the relationships between F_2-IsoPs and incident diabetes and, therefore, resulted in oversampling converters to diabetes. We found no indication, however, that this oversampling actually influenced the direction and/or magnitude of the observed associations.

This analysis, to the best of our knowledge, presents the first data on the prospective association between urinary F_2-IsoPs and weight gain. The inverse association between F_2-IsoP2 and weight gain challenges the developing scientific consensus that increased levels of F_2-IsoPs portend the harmful consequences of obesity (3–5). At the same time, the wide variation in the effect estimates observed for different F_2-IsoPs suggests that specific F_2-IsoP measurements reflect different processes related to the production of reactive oxygen

species. These aspects of the underlying biology of F_2-IsoPs remain unknown and require further investigation.

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Table 1

Characteristics of the study population

Characteristics[a]	All (*n* = 299)	Relative weight gain ≥5% (*n* = 91)	Absolute weight gain ≥5 kg (*n* = 79)
Age (years)	55.2 (8.1)	54.2 (8.5)	53.9 (8.4)
Sex, female (%)	57.2	61.5	58.2
Ethnicity (%)			
Non-Hispanic white	41.1	48.4	49.4
African American	26.8	28.6	32.9
Hispanic	32.1	23.1	17.7
Weight at baseline (kg)	82.9 (17.9)	82.6 (17.3)	86.9 (19.6)
Weight at follow-up (kg)	85.1 (19.2)	91.3 (18.8)	96.3 (20.1)
Weight change (follow-up—baseline, kg)	2.2 (6.0)	8.6 (5.2)	9.4 (4.1)
BMI at baseline (kg/m^2)	29.7 (6.1)	29.6 (6.1)	30.6 (6.6)
Waist circumference at baseline (cm)	93.0 (13.2)	92.3 (13.7)	95.4 (14.9)
Smoking status at baseline (%)			
Never	47.8	42.9	36.7
Past	37.8	37.4	40.5
Current	14.4	19.8	22.8
Change in smoking status at follow-up (%)			
No change	92.6	87.9	84.8
Past/never-current	1.0	0.0	0.0
Current-past	6.4	12.1	15.2
Percent calories from fat at baseline	34.6 (7.4)	34.5 (7.9)	34.3 (7.7)
Participation in vigorous activity at baseline (%)			
<1/week	46.5	46.2	44.3
1–4/week	44.2	45.1	44.3
≥5/week	9.4	8.8	11.4
Diabetes status at follow-up (%)	44.8	46.2	50.6

[a] Mean (s.d.) presented for continuous variables; % presented for categorical characteristics.

Table 2

Relationship between baseline urinary F_2-isoprostanes (ng/mg creatinine) and demographic, metabolic, and behavioral characteristics

		F_2-IsoP1 (iPF(2α)-III)	F_2-IsoP2 (2,3-dinor-iPF(2α)-III)	F_2-IsoP3 (iPF(2α)-VI)	F_2-IsoP4 (8,12-iso-iPF(2α)-VI)
Categorical demographic and baseline characteristics, mean (s.d.)					
Gender	Females (*n* = 171)	0.23 (0.14)	4. 62 (2.68)	6.86 (3.68)	4.81 (3.25)
	Males (*n* = 128)	0.16 (0.12)	2.97 (2.23)	4.12 (2.08)	3.63 (2.16)
	P value[a]	<0.001	<0.001	<0.001	0.0002
Race/Ethnicity	Non-Hispanic white (*n* = 123)	0.21 (0.16)	3.72 (2.15)	5.45 (3.34)	4.52 (3.28)
	African American (*n* = 80)	0.14 (0.09)	3.12 (1.91)	4.49 (2.58)	3.15(1.80)
	Hispanic (*n* = 96)	0.24 (0.14)	4.83 (3.34)	6.99 (3.60)	4.99 (2.86)
	P value (AA vs. NHW)[b]	0.001	0.1	0.04	0.001
	P value (Hisp vs. NHW)[b]	0.1	0.002	0.001	0.2
	P value (Hisp vs. AA)[b]	<0.0001	<0.0001	<0.0001	<0.0001
Smoking status	Never (*n* = 143)	0.19 (0.14)	3.99 (2.49)	5.89 (3.66)	4.36 (3.21)
	Past (*n* = 113)	0.18 (0.14)	3.62 (2.31)	5.09 (2.96)	4.04 (2.55)
	Current (*n* = 43)	0.27 (0.15)	4.45 (3.63)	6.58 (3.23)	4.84 (2.61)
	P value(past vs. never)[b]	0.7	0.3	0.06	0.4
	P value(current vs. never)[b]	0.001	0.3	0.2	0.3
Participation in vigorous physical activity	<1/week (*n* = 139)	0.23 (0.16)	4.46 (2.97)	6.33 (3.54)	4.85 (3.30)
	2–4/week (*n* = 132)	0.18 (0.11)	3.40 (2.21)	5.19 (3.25)	3.88 (2.50)
	≥5/week (*n* = 28)	0.18 (0.17)	3.16 (1.84)	4.84 (2.60)	3.64 (1.89)
	P value (2–4 vs. <1)[b]	0.002	0.0002	0.006	0.006
	P value (≥5 vs. <1)[b]	0.1	0.01	0.03	0.04
Diabetes status at follow-up	Yes (*n* = 134)	0.19 (0.12)	3.93 (2.79)	5.61 (3.04)	4.12 (2.52)
	No (*n* = 165)	0.21 (0.16)	3.91 (2.49)	5.75 (3.65)	4.45 (3.16)
	P value[a]	0.2	0.9	0.7	0.3
Relative weight gain	Weight gain ≥5% (*n* = 91)	0.19 (0.13)	3.52 (1.93)	5.23 (2.34)	3.96 (2.18)
	Weight gain <5% (*n* = 208)	0.20 (0.15)	4.09 (2.86)	5.89 (3.73)	4.46 (3.15)
	P value[a]	0.6	0.04	0.1	0.1

		F_2-IsoP1 (iPF(2α)-III)	F_2-IsoP2 (2,3-dinor-iPF(2α)-III)	F_2-IsoP3 (iPF(2α)-VI)	F_2-IsoP4 (8,12-iso-iPF(2α)-VI)
Absolute weight gain	Weight gain ≥5 kg (*n* = 79)	0.19 (0.13)	3.34 (1.79)	5.09 (2.25)	3.87 (2.13)
	Weight gain <5 kg (*n* = 220)	0.20 (0.14)	4.12 (2.84)	5.90 (3.68)	4.46 (3.11)
	P value[a]	0.5	0.01	0.02	0.06
Continuous demographic and baseline characteristics. Spearman correlation coefficients (*P* value)					
Age		0.04 (0.5)	−0.02 (0.7)	0.001 (1.0)	−0.12 (0.04)
BMI		0.09 (0.1)	0.26 (<0.0001)	0.16 (0.01)	0.15 (0.01)
Waist circumference					
Males		0.02 (0.8)	0.18 (0.04)	0.01 (0.9)	0.03 (0.7)
Females		0.09 (0.2)	0.26 (0.001)	0.14 (0.06)	0.21 (0.01)
Percent calories from fat		0.12 (0.04)	0.09 (0.1)	0.08 (0.2)	0.11 (0.06)
Weight change (kg)		−0.37 (0.5)	−0.10 (0.1)	−0.04 (0.5)	−0.04 (0.5)

[a] Student's *t*-test;

[b] ANOVA *F*-test with *P* values.

Table 3

Cross–sectional association between baseline urinary F_2-isoprostanes and obesity adjusted for age, gender, and race/ethnicity

	Overall obesity		Abdominal obesity	
	BMI (kg/m^2) *n* = 299	Obese (vs. nonobese) 107/192	Waist circumference (cm) *n* = 299	Abdominally obese (vs. no abdominal obesity) 119/180
F_2-isoprostanes (ng/mg creatinine)	**Regression coefficient (s.e.)[a] *P* value**	**OR (95% CI)[a]**	**Regression coefficient (s.e.)[a] *P* value**	**OR (95% CI)[a]**
F_2-IsoP1 (iPF(2a)-III)	0.66 (0.38) 0.09	1.18 (0.91, 1.54)	1.18 (0.81) 0.15	1.12 (0.86, 1.46)
F_2-IsoP2 (2,3-dinor-iPF(2a)-III)	1.63 (0.38) <0.0001	1.74 (1.26, 2.40)	2.97 (0.80) 0.0002	1.63 (1.18, 2.24)
F_2-IsoP3 (iPF(2a)-VI, ng/mg)	1.08 (0.40) 0.01	1.41 (1.01, 1.95)	1.57 (0.84) 0.06	1.35 (0.97, 1.87)
F_2-IsoP4 (8,12-iso-iPF(2a)-VI)	1.30 (0.37) 0.0004	1.47 (1.12, 1.94)	2.31 (0.77) 0.003	1.64 (1.22, 2.20)

CI, confidence interval; OR, odds ratio.

[a]Linear regression coefficients were scaled to 1 s.d., ORs were scaled to the difference between 75th and 25th percentile.

Table 4

Association between urinary F_2-isoprostanes and weight gain

	Weight (kg) (n = 299) Logistic regression			Relative weight gain (≥5% vs. <5%) (91 cases/208 noncases)	Absolute weight gain (≥5 kg vs. <5 kg) (79 cases/220 noncases)
	Linear regression coefficient (s.e.)[a] *P* values			OR (95% CI)[a]	
F_2-isoprostanes (ng/mg creatinine)	**Model 1[b]**	**Model 2[b]**	**Model 3[b]**	**Model 4[b]**	**Model 5[b]**
F_2-IsoP1 (iPF(2a)-III)	0.12 (0.39) 0.7	−0.05 (0.40) 0.9	−0.11 (0.39) 0.8	0.86 (0.63,1.18)	0.84 (0.60,1.18)
F_2-IsoP2 (2,3-dinor- iPF(2a)-III)	−0.74 (0.39) 0.06	−0.85 (0.40) 0.04	−0.90 (0.39) 0.02	0.67 (0.47,0.96)	0.57 (0.37,0.87)
F_2-IsoP3 (iPF(2a)-VI, ng/mg)	−0.16 (0.41) 0.7	−0.21 (0.41) 0.6	−0.27 (0.40) 0.5	0.68 (0.46,1.02)	0.64 (0.41,1.01)
F_2-IsoP4 (8,12-iso- iPF(2a)-VI)	−0.53 (0.38) 0.2	−0.58 (0.38) 0.1	−0.64 (0.37) 0.09	0.75 (0.54, 1.02)	0.71 (0.51 1.00)

CI, confidence interval; OR, odds ratio.

[a] Linear regression coefficients were scaled to 1 s.d., ORs were scaled to the difference between 75th and 25th percentile.

[b] The associations with F_2-IsoPs were adjusted for the following variables: Model 1, baseline weight, age, gender, ethnicity/clinic; Model 2, baseline weight, age, gender, ethnicity/clinic, baseline BMI, baseline smoking status, change in smoking status, percent fat from diet, participation in vigorous activity, diabetes status at follow-up; Models 3–5, baseline weight, age, gender, ethnicity/clinic, baseline smoking status, change in smoking status, and participation in vigorous physical activity.