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Interaction between a peroxisome proliferator-activated receptor γ gene polymorphism and dietary fat intake in relation to body mass

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The peroxisome proliferator-activated receptor γ (PPARγ) is a critical regulator of adipogenesis. *PPARγ*$^{+/-}$ mice are resistant to high-fat diet-induced obesity and thus PPARγ may mediate physiological responses to dietary fat in other mammals. The aim of this study was to determine whether the human *PPARγ* proline to alanine substitution polymorphism (Pro12Ala) modifies the association between dietary fat and adiposity and plasma lipids. Subjects ($n = 2141$) were controls selected for three case–control studies nested within the Nurses' Health Study, a large ongoing prospective cohort study. Associations between intake of total fat, fat subtypes and BMI were different in *PPARγ* 12Ala variant allele-carriers compared with non-carriers. Among homozygous wild-type Pro/Pro individuals, those in the highest quintile of total fat intake, had significantly higher mean body mass index (BMI) compared with those in the lowest quintile (27.3 versus 25.4 kg/m^2, respectively; *P*-trend < 0.0001) whereas among 12Ala variant allele-carriers there was no significant trend observed between dietary fat intake and BMI (*P*-trend $= 0.99$; *P*-interaction $= 0.003$). In contrast, intake of monounsaturated fat was not associated with BMI among homozygous wild-type women but was inversely associated with BMI among 12Ala variant allele-carriers (mean in lowest quintile $= 27.6$ versus mean in highest quintile $= 25.5$ kg/m^2; *P*-trend $= 0.006$; *P*-interaction $= 0.003$). The relationship between dietary fat intake and plasma lipid concentrations also differed according to *PPARγ* genotype. These data suggest that *PPARγ* genotype is an important factor in physiological responses to dietary fat in humans.

INTRODUCTION

Over the last two decades the prevalence of overweight and obesity has increased markedly in the USA and many parts of the world (1). Obesity increases the risk of numerous chronic health conditions including type 2 diabetes, coronary heart disease, hypertension and some cancers (2). Although the recent secular increases in obesity are almost certainly caused by dietary and lifestyle factors, genetic factors likely influence which individuals gain weight under specific environmental conditions. Studies on human pedigrees estimate the genetic influence on body mass to be between 30 and 40%, suggesting a substantial genetic contribution to obesity (3,4). In addition to the independent effects of genetics and diet on body weight, animal studies also support a role for interactions between genes and diet on obesity risk. Strains of rodents have been identified that are differentially sensitive to obesity induced by a high-fat diet (5). Sensitivity to dietary fat-induced obesity appears to be a polygenic phenotype and is influenced by genes mapped to at least six genetic loci in rodents (5). In humans

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there is much debate on the role of dietary fat in weight gain and obesity, but little empirical evidence to support specific common genotypes in gene–diet interactions (6–8).

PPARγ is a member of the steroid hormone receptor superfamily and is a critical transcriptional regulator of adipogenesis. PPARγ is the target of the thiazolidinedione class of antidiabetic drugs that improve insulin sensitivity. *In vivo* ligands for PPARγ are thought to include a variety of unsaturated fatty acids and it has been proposed that PPARγ may be a mediator of physiological responses to lipids (9,10). A reduced-activity variant protein is produced by the proline to alanine substitution in codon 12 of *PPARγ* (Pro12Ala) and the variant 12Ala allele is associated with reduced risk of type 2 diabetes (11–13). Although many lines of research indicate that *PPARγ* has a central role in adipogenesis, direct associations between the Pro12Ala *PPARγ* polymorphism and body mass index (BMI) have been inconsistent in human studies (13).

PPARγ null mice generally do not survive due to a placental dysfunction. However, a *PPARγ* knockout mouse that survived past birth had a remarkable absence of both brown and white adipose tissue. Animal studies on *PPARγ* heterozygous null mice demonstrate that mice lacking one copy of the *PPARγ* gene are similar to their wild-type littermates under standard dietary conditions while those given a high-fat diet gain significantly less weight than their corresponding wildtype controls (14). Similarly, reduction in PPARγ activity with PPARγ-specific antagonist treatment confers resistance to high-fat diet-induced weight gain in mice (15). Here we analyzed in humans whether the *PPARγ* Pro12Ala polymorphism interacts with dietary factors and BMI, plasma high-density lipoprotein (HDL) and plasma total cholesterol in humans. Our study population consisted of a subsample of the Nurses' Health Study, a large ongoing prospective cohort study.

RESULTS

Dietary information obtained from 2141 study women in 1980, 1984, 1986 and 1990 was used to calculate an average of dietary fat intake for each individual and this was examined in relation to BMI reported in 1992. Among 2141 study women there were 1637 Pro/Pro, 469 Pro/Ala and 35 Ala/Ala individuals at the *PPARγ* Pro12Ala position and genotype frequencies were in Hardy–Weinberg equilibrium ($P > 0.99$). Pro/Pro homozygous women and women carrying the 12Ala allele (Ala/Ala and Pro/Ala genotypes) were similar with regard to BMI, weight gain since age 18, alcohol intake, smoking habits, menopause status and PMH use and level of physical activity (Table 1). Among the 2141 participants, the 10th and 90th percentiles of energy derived from fat were 28.2 and 40.8% for total fat; 9.8 and 15.2% for saturated fat; 10.5 and 15.9% for monounsaturated fat; 4.7 and 7.6% for polyunsaturated fat; and 1.2 and 2.5% for trans unsaturated fat, respectively. The 10th and 90th percentiles for the ratio of polyunsaturated to saturated (P:S) fat were 1.9 and 3.4, respectively. Intake of total fat, intake of each fat subtype and the P:S ratio were found to be similar in Pro12Ala variant allele-carriers and Pro/Pro homozygotes (data not shown).

The association between *PPARγ* Pro12Ala genotype and BMI according to dietary fat intake was investigated. Within the lowest quintile of total fat intake, the BMI of *PPARγ*12Ala variant allele carriers was higher than BMI of non-carriers (26.7 versus 25.4 kg/m^2 for variant allele carriers and non-carrier, respectively, $P = 0.005$). Moreover, a positive trend between increasing intake of total fat and BMI was observed in Pro/Pro homozygotes but not in *PPARγ* 12Ala variant-allele carriers (P-trend <0.0001 and 0.99 for Pro/Pro homozygotes and 12Ala allele-carriers, respectively; P-interaction 0.003). Intake of saturated fat was directly associated with increased BMI among individuals of both genotype classes (Table 2), whereas intake of monounsaturated fat was inversely associated with BMI in Ala12 allele-carriers but not in Pro/Pro homozygotes (Table 2; P-interaction 0.003; P-trend 0.28 and 0.006 for Pro/Pro homozygotes and 12Ala allele-carriers, respectively). P:S ratio was directly associated with lower BMI among Pro/Pro but not among *PPARγ* 12Ala allele-carriers (Table 2; P-trend 0.002 and 0.24 for Pro/Pro homozygotes and 12Ala allele-carriers, respectively). No trend for intake of polyunsaturated fat and current BMI was observed for either genotype (Table 2; P-trend 0.21 and 0.33 for Pro/Pro homozygotes and 12Ala allele-carriers, respectively). When control subjects from each case–control study were analyzed independently, the trends were almost identical to the combined trends, but the interactions did not reach statistical significance due to the smaller sample sizes (data not shown).

PPARγ Pro12Ala genotype modified the association between total dietary fat intake and risk of obesity (BMI of ≥30 kg/m^2).

Table 1. Descriptive characteristics of study population according to PPARγ genotype

	Pro/Pro	Pro/Ala and Ala/Ala	*P*-value
n	1637	504	
Age (year)	56.4	55.9	0.22[a]
BMI 1992 (kg/m^2)	26.4	26.7	0.31[a]
Weight gained since age 18 (kg)	13.1	13.5	0.52[a]
Alcohol intake, *n* (%)			
Non-drinker	612 (38.6)	191 (39.3)	0.25[b]
<5 g/day	497 (31.3)	169 (34.8)	
5–10 g/day	157 (9.9)	46 (9.5)	
>10 g/day	320 (20.2)	80 (16.5)	
Smoking, *n* (%)			
Never smoker	741 (45.4)	235 (46.8)	0.56[b]
Past smoker	703 (43.1)	206 (41.0)	
Current smoker			
1–14 cigarettes/day	69 (4.2)	29 (5.8)	
15–24 cigarettes/day	73 (4.5)	20 (4.0)	
>24 cigarettes/day	46 (2.8)	12 (2.4)	
Menopausal status, *n* (%)			
Premenopausal	236 (14.4)	82 (16.0)	0.13[b]
Postmenopausal			
No PMH use	577 (35.2)	198 (39.3)	
Current PMH user	496 (30.3)	139 (27.6)	
Past PMH user	328 (20.0)	85 (16.9)	
Activity, met *n* (%)			
<1.5 met h/week	167 (10.4)	41 (8.3)	0.45[b]
1.5–5.9 met h/week	451 (28.2)	130 (26.3)	
6.0–11.9 met h/week	320 (20.0)	103 (20.8)	
12–20.9 met h/week	262 (16.4)	92 (18.6)	
≥21 met h/week	400 (25.0)	129 (26.1)	

[a]*P*-value for Wilcoxon rank sum test.
[b]*P*-value for χ^2-test.

Table 2. Adjusted associations of PPARγ genotype and BMI by quintile of dietary fat[a]

Variable	Quintile					*P* for trend[b]	*P* for interaction[c]
	1	2	3	4	5		
Total fat[d]							
Intake (% of total energy)	27.6	31.9	34.5	37.1	41.4		
Pro/Pro (*n*)	330	341	322	317	327		
Pro/Ala + Ala/Ala (*n*)	98	88	106	111	101		
Mean BMI (kg/m²)							
Pro/Pro	25.4	25.8	26.2	27.1	27.3	<0.0001	
Pro/Pro + Ala/Ala	26.7	26.4	26.3	26.4	26.9	0.99	0.003
Saturated fat[d]							
Intake (% of total energy)	9.6	11.4	12.5	13.6	15.6		
Pro/Pro (*n*)	330	327	338	325	317		
Pro/Ala + Ala/Ala (*n*)	98	102	90	104	110		
Mean BMI (kg/m²)							
Pro/Pro	25.3	25.7	26.4	27.2	27.2	0.004	
Pro/Ala + Ala/Ala (*n*)	26	26.7	26	26.8	27.1	0.002	0.08
Monounsaturated fat[d]							
Intake (% of total energy)	10.2	12.0	13.2	14.4	16.3		
Pro/Pro (*n*)	331	339	322	316	329		
Pro/Ala + Ala/Ala (*n*)	97	90	105	113	99		
Mean BMI (kg/m²)							
Pro/Pro	26.4	26	26.4	26.5	26.6	0.28	
Pro/Ala + Ala/Ala	27.6	27.1	26	26.5	25.5	0.006	0.003
Polyunsaturated fat[d]							
Intake (% of total energy)	4.6	5.4	6.0	6.6	7.9		
Pro/Pro (*n*)	334	319	343	317	324		
Pro/Ala + Ala/Ala (*n*)	93	110	85	112	104		
Mean BMI (kg/m²)							
Pro/Pro	26.3	26.4	26.6	26.5	26.1	0.21	
Pro/Ala + Ala/Ala	26.4	27.4	26.2	26.4	26.1	0.33	0.17
Trans unsaturated fat[d]							
Intake (% of total energy)	1.2	1.6	1.8	2.1	2.5		
Pro/Pro (*n*)	344	323	322	330	318		
Pro/Ala + Ala/Ala (*n*)	84	106	105	99	110		
Mean BMI (kg/m²)							
Pro/Pro	25.7	26.1	26.6	26.5	27	0.05	
Pro/Ala + Ala/Ala	26.6	27.1	26.2	26.5	26.3	0.58	0.46
Polyunsaturated to saturated fat ratio[d]							
Mean ratio	0.36	0.45	0.5	0.57	0.73		
Pro/Pro (*n*)	329	329	316	334	329		
Pro/Ala + Ala/Ala (*n*)	98	100	112	95	99		
Mean BMI (kg/m²)							
Pro/Pro	26.6	26.4	27.1	26.3	25.4	0.002	
Pro/Ala + Ala/Ala	26.7	26.9	26.7	26.5	25.8	0.24	0.72

[a]Estimates of BMI by quintile of dietary fat and PPARγ Pro12Ala genotype were obtained from multivariate linear regression models including the following covariates: age (5-year categories), total energy intake (quintiles), energy derived from protein (quintiles), cigarette smoking (never, past and current smoking of 1–14, 15–24 and 25 or more cigarettes per day), menopausal status and post-menopausal hormone (PMH) therapy (premenopausal, postmenopausal without PMH use, postmenopausal current PMH user and postmenopausal past PMH user), physical activity (<1.5, 1.5–5.9, 6.0–11.9, 12–20.9 and ≥21 metabolic units/week), alcohol consumption (non-drinker, <5, 5–10 and >10 g/day). Models with types of fat included all four types of fat in the model. Models with P : S ratio also adjusted for energy derived from total fat.

[b]*P*-value for genotype-specific trend was assessed from the multivariate model described above considering each genotype class singly. Quintiles of fat were treated as an ordinal variable.

[c]*P*-value for interaction was derived from the Wald test statistic of the interaction term included in the model.

[d]Quintile-specific means for fat intake and P : S ratio are shown.

Among Pro/Pro homozygotes, the highest quintile of total dietary fat was associated with increased risk of obesity compared to the lowest quintile of total dietary fat intake (Fig. 1; OR = 3.4; 95% confidence interval, 2.1–5.4; <0.0001). In contrast, among variant allele-carriers no trend between total dietary fat intake and obesity was observed (Fig. 1; *P*-trend = 0.85; *P*-interaction = 0.006).

We also examined the interaction between *PPARγ* Pro12Ala polymorphism and intake of total dietary fat in relation to plasma lipids. Plasma HDL and total plasma cholesterol were assayed for individuals from the breast cancer case–control study ($n = 656$). Among Pro/Pro homozygotes, intake of total fat was inversely correlated with plasma HDL (58.3 versus 61.8 mg/dl for highest quintile compared to lowest quintile, respectively; *P*-trend = 0.11) and inversely correlated with plasma cholesterol (213 versus 230 mg/dl for highest quintile compared to lowest quintile, respectively; *P*-trend = 0.02; Table 3). In contrast intake of total fat was directly correlated

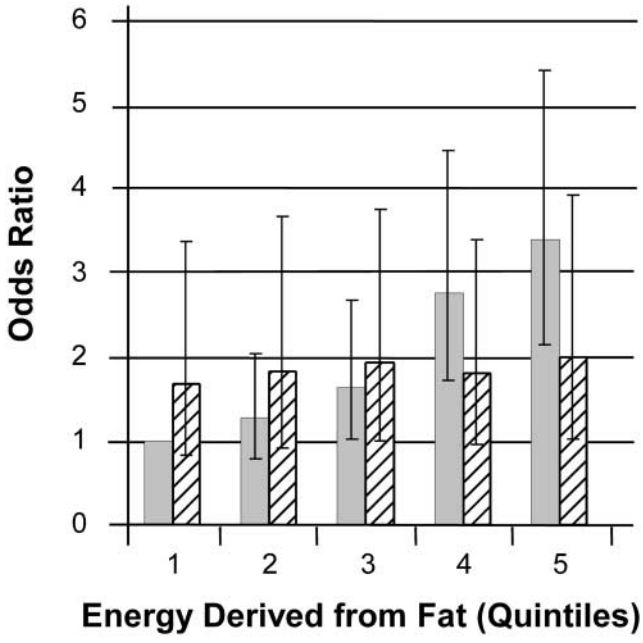

Figure 1. Risk of obesity associated with total dietary fat and *PPARγ* Pro12Ala genotype. Estimates of obesity risk by intake of total fat for 12Ala variant allele-carriers (hatched bars) and non-carriers (shaded bars) were derived from multivariate logistic regression models including the following covariates: age (5-year categories), total energy intake (quintiles), energy derived from protein (quintiles), cigarette smoking (never, past and current smoking of 1–14, 15–24 and 25 or more cigarettes per day), menopausal status and post-menopausal hormone (PMH) therapy (premenopausal, postmenopausal without PMH use, postmenopausal current PMH user and postmenopausal past PMH user), physical activity (<1.5, 1.5–5.9, 6.0–11.9, 12–20.9 and ≥21 metabolic units/week), alcohol consumption (non-drinker, <5, 5–10 and >10 g/day). Obesity was defined as having a BMI ≥ 30 kg/m^2 and compared with individuals who were neither overweight nor obese (BMI < 25 kg/m^2). *P*-trend among variant allele-carriers = 0.85. *P*-trend among non-carriers <0.0001.

with plasma HDL among 12Ala variant allele-carriers (64.8 versus 58.1 mg/dl for highest quintile compared with lowest quintile, respectively; *P*-trend = 0.0008; *P*-interaction = 0.01; Table 3). The trend between intake of total fat and plasma cholesterol among 12Ala variant allele-carriers was not statistically different from zero but was significantly different from the inverse trend observed in Pro/Pro homozygotes (*P*-interaction = 0.05). The multivariate estimates and interactions did not change appreciably when BMI was included in the model, suggesting that the association between *PPARγ* Pro12Ala polymorphism and plasma lipids is not mediated solely through effects on body mass (Table 3).

DISCUSSION

Consistent with numerous prior reports we observed no direct association between *PPARγ* Pro12Ala genotype and BMI in the Nurses' Health Study (13). However, we did observe that the relationship between total dietary fat with BMI differed according to *PPARγ* genotype with the relationships being attenuated in carriers of the reduced-activity 12Ala allele. This is consistent with mouse models in which reducing PPARγ activity, either genetically or pharmacologically, results in animals with resistance to diet-induced obesity (14–16). This resistance is specific to high-fat diet-induced obesity and is not observed with high-carbohydrate diet-induced obesity (14). Fatty acids, particularly unsaturated fatty acids, are ligands for PPARγ (10). Thus the relation of fatty acid intake to obesity may depend on *PPARγ* transcriptional response.

In humans, four studies in addition to our own have investigated the interaction between *PPARγ* Pro12Ala polymorphism and diet with respect to body weight and weight change. Luan *et al.* (17) reported an inverse association between P:S ratio with BMI and plasma insulin among 12Ala allele-carriers but not among Pro/Pro homozygotes whereas they observed no interaction between *PPARγ* Pro12Ala genotype with total dietary fat intake in relation to BMI. More recently, Robitaille *et al.* (18) reported observations similar to our own in a French Canadian population; total dietary fat intake was positively associated with BMI among homozygous wild-type Pro/Pro individuals but not among 12Ala variant allele carriers. The current study and that described by Robitaille *et al.* (18) did not confirm the interaction between *PPARγ* Pro12Ala genotype and P:S ratio on BMI reported by Luan *et al.* (17). The discrepancy between these studies may reflect the difficulty of accurate assessment of intake of specific types of dietary fat rather than a discrepancy in biological relevance.

A third study was a 3-year longitudinal trial of individuals with impaired glucose tolerance randomized to an exercise and diet group, which included dietary advice aimed at lowering intake of total fat, versus a control group. Although the sample size was small, individuals with the Ala/Ala genotype ($n = 6$) on the intervention arm lost more weight than Pro/Pro and Pro/Ala individuals (19). In a fourth study the weight loss and physiological changes resulting from a hypoenergetic diet were assessed. Although weight lost in variant allele-carriers was similar to that in Pro/Pro non-carriers, 12Ala variant allele carriers had more carbohydrate oxidation, less fat oxidation and a greater increase in insulin sensitivity compared with Pro/Pro wild-type individuals, consistent with metabolic differences in response to diet among individuals with different genotypes (20).

Kubota *et al.* (14) first reported resistance to dietary fat-induced obesity and insulin resistance in PPARγ-deficient (*PPARγ*$^{+/-}$) mice. Their results suggest that *PPARγ*$^{+/-}$ mice are resistant to dietary-fat induced obesity due to both decreased food intake and increased energy expenditure. Although *PPARγ*$^{+/-}$ mice given a high-fat diet had lower fat mass than wild-type mice, they had higher levels of leptin, the appetite-suppressing hormone produced by adipocytes. The leptin promoter has a functional peroxisome proliferator response element, indicating that the transcription factor *PPARγ* has a direct role in modulating leptin expression (21). Higher leptin levels have also been reported in humans carrying the reduced-activity 12Ala allele of *PPARγ*, suggesting that modulation of leptin levels is an important biological function of *PPARγ* and may mediate the relation of dietary fat intake, *PPARγ* polymorphism and BMI (22,23).

Here we observed that *PPARγ* interacts with dietary fat to determine BMI. It is important to note that interpretation

Table 3. Adjusted association of *PPARγ* genotype and serum lipids by quintile of total dietary fat

Variable	Quintile					*P*-trend[a]	*P*-interaction[b]
	1	2	3	4	5		
Total fat[c]							
Intake (% of total energy)	28.3	31.8	34.3	36.8	40.4		
Pro/Pro (*n*)	95	112	107	93	101		
Pro/Ala + Ala/Ala (*n*)	28	22	27	31	31		
HDL (mg/dl)							
Multivariate estimate[d]							
Pro/Pro	61.8	61.5	60.1	59.8	58.3	0.11	
Pro/Ala + Ala/Ala	58.1	60.5	54.7	63.2	64.8	0.0008	0.01
Multivariate estimate[e]							
Pro/Pro	60.8	61.2	60.1	60.3	59.6	0.54	
Pro/Ala + Ala/Ala	59.0	59.3	53.6	65.2	64.0	0.004	0.05
Cholesterol (mg/dl)							
Multivariate estimate[d]							
Pro/Pro	230	215	216	220	213	0.02	
Pro/Ala + Ala/Ala	220	211	211	221	229	0.23	0.05
Multivariate estimate[e]							
Pro/Pro	230	215	215	220	214	0.05	
Pro/Ala + Ala/Ala	220	213	212	216	226	0.45	0.11

[a]*P*-value for genotype-specific trend was assessed from the multivariate model described above considering each genotype class singly. Quintiles of fat were treated as an ordinal variable.
[b]*P*-value for interaction was derived from the Wald test statistic of the interaction term included in the model.
[c]Quintile-specific medians for intake of total dietary fat are shown.
[d]Estimates of plasma HDL and total cholesterol by quintile of dietary fat and *PPARγ* Pro12Ala genotype were obtained from multivariate linear regression models including the following covariates: age (5-year categories), total caloric intake (quintiles), energy derived from protein (quintiles), cigarette smoking (never, past and current smoking of 1–14, 15–24 and 25 or more cigarettes per day), menopausal status and PMH therapy (premenopausal, postmenopausal without PMH use, postmenopausal current PMH user and postmenopausal past PMH user), physical activity (<1.5, 1.5–5.9, 6.0–11.9, 12–20.9 and ≥21 metabolic units/week), alcohol consumption (non-drinker, <5, 5–10 and >10 g/day) and laboratory batch.
[e]These estimates were obtained from linear regression models containing all the covariates listed above and BMI (in deciles).

of cross-sectional analysis of diet and body mass are controversial in part because individuals who reduce their fat intake may also reduce energy intake, may be more physically active or may be consciously attempting to reduce their weight. It is unlikely, however, that any of the above confounding factors would be related to *PPARγ* genotype. The marked similarity between the current observations and those observed by Robitaille *et al.* (18) and the relation we observe to plasma lipids supports the hypothesis that *PPARγ* genotype modulates physiological responses to dietary fat, specifically. Our study is substantially larger than the previous report of this association, involving subjects from across the USA, and is the first to report an interaction between *PPARγ* genotype, intake of monounsaturated fat and BMI. This observation is important because it reinforces the biological plausibility of the interaction; biochemical studies have demonstrated that unsaturated fats are specific ligands for *PPARγ* (10). Furthermore, to our knowledge this is the first report of a statistically significant interaction between intake of dietary fats, *PPARγ* genotype, and plasma lipids. This observation suggests that dietary counseling aimed at optimizing plasma total and HDL-cholesterol levels may need to take into account *PPARγ* genotype. Thus, uniform dietary recommendations may not be appropriate for all individuals. Given the increasing incidence of obesity, identification of individuals who are genetically more likely to respond to particular dietary changes may be important for successful intervention.

MATERIALS AND METHODS

Study population

The Nurses' Health Study began in 1976 with the recruitment of 121 700 married, female, registered nurses between the ages of 30 and 55 from 11 US states. The participants have been followed with biennial questionnaires updating exposure and disease status. Between 1989 and 1990, blood samples were collected from 32 826 volunteers from the original study. Samples for three case–control studies nested within the Nurses' Health Study blood cohort were selected to identify genetic and plasma biomarkers of breast cancer, type 2 diabetes and endometrial cancer. The breast cancer case–control study consists of 725 incident cases diagnosed after blood collection through 1996 and controls matched 1 : 1 with cases for the following variables: age, menopause status, postmenopausal hormone use, blood draw time of day, month of blood return and fasting status at blood draw. An additional control was matched for each postmenopausal breast cancer case not taking postmenopausal hormones, resulting in a total of 953 controls in this study. The diabetes case–control study consists of 387 incident cases of diabetes diagnosed after blood collection through 1996 and 774 controls matched to cases for the following variables: age, month of blood draw, year of blood draw and fasting status at blood draw. For each case one of the two controls was also matched according to body mass index (±1 unit). The

endometrial case–control study consists of 222 cases and 666 controls matched 1:3 with cases for the following variables: age, menopause status, postmenopausal hormone use, blood draw time of day, month of blood return and fasting status at blood draw. Control subjects from these three nested case-control studies with *PPARγ* Pro12Ala genotype and dietary information were included in the present study of 2141 women.

Exposures and endpoints

All subjects were genotyped for the codon 12 proline to alanine polymorphism in the *PPARγ* gene (11). DNA of breast cancer case–control study subjects was genotyped by a mutagenic polymerase chain reaction (PCR)/restriction fragment length polymorphism (RFLP) method as described (11). PCR amplification using a perfect match upstream primer (5′TTGACTCA-TGGGTGTATT3′) and a downstream mismatched primer [5′GATATGTTTGCAGACAGTG-TATCAGTGAAGGAATCG-CTTTC**C**G3′ (mismatched base is bold and underlined)] was used to create a *BstU*I restriction site in the PCR product only when the variant Ala allele is present. DNA of diabetes case–control study subjects was genotyped by Pyrosequencing (Pyrosequencing, Uppsala, Sweden) using the following primers: PCR primers, 5′BIOTIN-TTCACAAATT-CTGTTAC-TTCA3′ and 5′TTGTGATATGTTTGCAGACA3′; sequencing primer, 5′ATCAGTGAAGGAATCGCTTTCT3′. DNA of endometrial case–control study subjects was genotyped by Taqman technology (Applied Biosystems, Foster City, CA, USA) using the PCR primers 5′TGCAGACAGTGTATCA-GTGAAGGA3′ and 5′TTATGGGTGAAACTCTGGGAG-ATT3′ and probe primers 5′TTCTG(G/C)GTCAATAGG3′. Genotype frequencies determined by RFLP, pyrosequencing and Taqman were not materially different (data not shown).

Plasma total cholesterol and HDL were determined as previously described (24–26). Within-individual coefficients of variation among redundant samples were 3.2 and 5.0% for total cholesterol and HDL, respectively.

A semiquantitative food-frequency questionnaire was administered to all participants of the Nurses' Health Study in 1980, 1984, 1986 and 1990. Nutrient intake was computed from the reported consumption frequency and the known nutrient content for a particular food. Reproducibility and validity of this method has been described elsewhere (27). Correlation between values obtained from these food-frequency questionnaires and weekly dietary records ranged between 0.46 and 0.68 for dietary fat. Further, the correlation between dietary polyunsaturated fat intake estimated by food-frequency questionnaire and intake estimated from sampling subcutaneous fat was 0.50 (28). Nutrient density for total fat, saturated fat, monounsaturated fat, polyunsaturated fat and trans unsaturated fat was calculated as energy derived from dietary fat divided by total dietary energy. Using information from four questionnaires, average nutrient density was used as a cumulative measure of nutrient intake. For subjects missing one or more of the administered food-frequency questionnaires, average nutrient density was computed from the available data. BMI was calculated as weight in kilograms (obtained in 1992) divided by height in meters (obtained in 1976) squared. Validation of self-reported anthropometric measures has been previously addressed in a subset of individuals. Self-reports of weight and height were highly correlated with measurements and reports from other sources (correlation coefficients of 0.96 and 0.94 for weight and height, respectively) (29,30).

Statistical analysis

All statistical analyses were performed using the Statistical Analysis Software package (SAS Institute Inc., Cary, NC, USA). Associations of energy derived from fat and *PPARγ* genotype with BMI, HDL and total plasma cholesterol were evaluated using linear regression models adjusting for total calorie intake (quintiles of cumulative average), energy derived from protein (quintiles of cumulative average), age (5-year categories), smoking at blood draw (1–14, 15–24 and ≥ 25 cigarettes/day, never smoker and past smoker), menopause status and post-menopausal hormone (PMH) therapy at blood draw (pre-menopausal, post-menopausal no PMH use, post-menopausal current PMH user, post-menopausal past PMH user), physical activity at blood draw (<1.5, 1.5–5.9, 6.0–11.9, 12.0–20.9 and ≥ 21 metabolic units/week) and alcohol consumption at blood draw (non-drinker, 0–4.9, 5–10 and >10 g alcohol/day). To minimize the influence of potential outlying values and to allow for non-linear associations dietary fat intake was treated as categorical (quintiles). Nearly identical associations were observed when dietary fat intake was treated as continuous (data not shown). The association between energy derived from fat and *PPARγ* genotype with obesity was evaluated using unconditional logistic regression adjusting for the same covariates listed above. Obese individuals, defined as having a BMI of $\geq 30\,kg/m^2$, were compared to individuals who were normal weight or underweight, defined as having a BMI < 25. Estimates for continuous outcomes were derived for each of five strata of dietary fat intake (quintiles), stratified by *PPARγ* genotype, from multivariate linear regression models. The interaction between *PPARγ* genotype and dietary fat was assessed using a cross-product term between genotype and quintiles of dietary fat (treated as an ordinal variable); statistical significance was calculated using a Wald test for the interaction.

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