FABP2 Genotype Is Associated With Insulin Sensitivity in Older Women

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This study determined whether sequence variations in genes related to glucose and insulin metabolism are associated with insulin sensitivity in postmenopausal women after accounting for habitual physical activity levels, body composition, and hormone-replacement therapy (HRT). Eighteen sedentary, 19 physically active, and 23 athletic postmenopausal white women underwent a frequently sampled intravenous glucose tolerance test to determine insulin sensitivity (S_I) and dual-energy x-ray absorptiometry to determine body composition. After accounting for the effects of body composition, habitual physical activity levels, and HRT status, S_I was 26% lower in subjects with the Thr54 fatty acid–binding protein 2 (FABP2) allele compared with Ala54 homozygotes (4.3 ± 0.5 *v* 5.8 ± 0.6 $\mu U \times 10^{-4}$/min/mL; $P < .05$). Angiotensin-converting enzyme genotype was not significantly associated with S_I. There were no significant associations between Gln27Glu β_2-adrenergic receptor or Pro12Ala peroxisome proliferator-activated receptor γ variants and glucose or insulin kinetic parameters. It was concluded that FABP2 genotype influences insulin sensitivity independent of body composition, habitual physical activity levels, and HRT status in postmenopausal white women.

A NUMBER OF common polymorphic genetic variants have been implicated in type 2 diabetes and insulin resistance, although the results of previous studies are not completely consistent.[1] However, a number of other nongenetic factors also substantially affect insulin resistance, including body composition, habitual physical activity levels, and, in postmenopausal women, hormone-replacement therapy (HRT). Numerous studies have established the association between obesity/overweight and insulin resistance.[2] Some, but not all, of the previous studies assessing the association between polymorphisms in candidate genes and insulin resistance have addressed potential interactions with body composition. We and others have demonstrated that habitual physical activity levels also dramatically alter insulin sensitivity.[3,4] However, to our knowledge only 1 study has attempted to examine potential interactions of candidate genes with habitual physical activity levels.[5] We hypothesized that insulin sensitivity in postmenopausal women would be independently associated with common genetic polymorphisms, after accounting for the potential interaction effects of body composition, habitual physical activity levels, and HRT status. The polymorphisms selected for study were chosen because they are relatively common variants and they have all been shown by some investigators to be associated with type 2 diabetes or insulin resistance.[1]

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METHODS

Sixty unrelated healthy postmenopausal white women were recruited for this study via advertisements and media announcements. Women were classified as postmenopausal by self-reported lack of menses for more than 2 years and elevated follicle-stimulating and luteinizing hormone levels. All subjects were nondiabetic based on fasting plasma glucose levels. Eighteen of the women were sedentary and had not participated in regular exercise for more than 2 years (Table 1). Nineteen women participated in regular aerobic exercise but were not training for endurance-based competitive events and were classified as physically active. Twenty-three women were competitive distance runners who regularly placed in regional, national, and international competitions. Approximately half of the women in each group were taking HRT. HRT usage, dosages, and intake patterns were similar in all physical activity groups. The physical activity level and HRT status of all subjects had been constant for more than 2 years before the study. All subjects provided written informed consent, and the study was approved by the Institutional Review Board of the University of Pittsburgh.

Subjects with known cardiovascular (CV) disease and those taking medications affecting glucose metabolism were excluded from the study. All women were screened for CV disease with a physical examination, resting electrocardiogram (ECG), and maximal exercise test.[6] Only women whose exercise tests were stopped because of exhaustion with no evidence of significant ECG changes or CV decompensation were included in the study. Body composition was determined by dual-energy x-ray absorptiometry (DPX-L; Lunar Radiation, Madison, WI). Body composition measurements were performed after an overnight fast with the subject lying comfortably, wearing standard hospital-issue clothing. Body mass index (BMI) was calculated as a function of body mass and stature (kg/m^2). Blood samples were drawn on 2 separate days, and standard methods were used to assess plasma lipoprotein-lipid levels.[7]

Subjects underwent an insulin-assisted frequently sampled intravenous glucose tolerance test (FSIVGTT) according to the methods of Bergman et al.[8,9] Subjects ingested 250 to 300 g of carbohydrates daily for 3 days before the FSIVGTT and fasted for ~12 hours before the test. The physically active and athletic women underwent the FSIVGTT 15 to 24 hours after their last exercise session. Blood samples for glucose and insulin were drawn at 28 standard time points for 3 hours after the glucose injection of 300 mg/kg glucose in solution.

Table 1. Subject Characteristics of the 3 Habitual Physical Activity Level Groups

	Sedentary (n = 18)	Physically Active (n = 19)	Endurance Athletes (n = 23)
Age (yr)	64 ± 1	63 ± 1	65 ± 1
Weight (kg)	59 ± 2	64 ± 2*	56 ± 2
Body fat (%)	37 ± 1	37 ± 1	25 ± 2*
HRT (%)	50%	45%	46%
PA history (yr)	—	12 ± 2	15 ± 1
PA (h/wk)	—	5 ± 1	6 ± 1
Vo_{2max} (mL/kg/min)	24 ± 1	26 ± 1	38 ± 1*

NOTE. Values are presented as means ± SE.
Abbreviation: PA, physical activity.
*$P < .05$ compared with all other groups.

Insulin (0.02 U/kg Humulin-Regular; Eli Lilly Inc, Indianapolis, IN) was injected 20 minutes after the glucose injection to augment the insulin response. Plasma samples for insulin were stored at −70°C. Insulin was measured by radioimmunoassay, and glucose was measured by the glucose oxidase method (YSI Glucose Analyzer; YSI, Yellow Springs, OH). The glucose and insulin data were analyzed using the Bergman Minimal Model (MINMOD) program (R.N. Bergman, 1989) to determine insulin sensitivity index (S_I).[10] Intravenous glucose tolerance (K_G) was calculated as the slope of the regression relating the logarithm of glucose concentration to time between 10 and 19 minutes after the glucose injection.[10] The acute insulin response to glucose (AIR_G) was calculated as the mean plasma insulin concentration during the first 10 minutes after glucose injection minus the basal plasma insulin level.[11] The associations of habitual physical activity and HRT status with glucose and insulin metabolism kinetic parameters in these women have been published previously.[8] In that study, glucose and insulin metabolism kinetic parameters were found not to differ between sedentary and physically active women[8]; therefore, these groups were combined for statistical analyses in the present study.

Subjects had peripheral blood sampled and DNA isolated using standard procedures.[12] DNA was typed at the following loci using standard methods: Ala54Thr fatty acid–binding protein 2 (FABP2),[13] the intron 16 insertion/deletion angiotensin-converting enzyme (ACE),[14] Gln27Glu β_2-adrenergic receptor (β_2AR).[15] Pro12Ala peroxisome proliferator-activated receptor γ (PPARγ),[16] Trp64Arg β_3-adrenergic receptor (β_3AR),[17] and Gly972Arg insulin receptor substrate1 (IRS-1)[18] polymorphisms. These variants were chosen for study because they are relatively common and have previously been found to be associated with type 2 diabetes or insulin resistance.[1]

Data are reported as means ± SE. Data were analyzed using Statview (Abacus Concepts, Berkeley, CA). Glucose and insulin metabolism kinetic parameters were compared among genotype groups using analysis of covariance covarying for physical activity status, HRT status, and body composition. In these analyses, physical activity was based on the 3 habitual physical activity level groups, HRT status as on or not on HRT, and body composition as percent body fat. An α level of .05 was accepted for statistical significance.

RESULTS

Thr54Ala and Thr54Thr genotypes were the least frequent at the FABP2 locus in these women (Table 2), and all glucose and insulin metabolic parameters were similar in these genotype groups; therefore, they were combined for statistical analyses. After accounting for the associations with body composition, habitual physical activity levels, and HRT status, S_I was 26% lower in FABP2 Thr54Ala and Thr54Thr than in Ala54Ala genotype women ($P < .05$; Table 3). AIR_G was somewhat higher in FABP2 Thr54Ala and Thr54Thr than in Ala54Ala genotype women, but this difference was not significant ($P = .28$). Fasting glucose, fasting insulin, and K_G levels did not differ among FABP2 genotype groups. Plasma triglyceride (TG) levels were somewhat higher in FABP2 Thr54Thr and Thr54Ala than in Ala54Ala genotype women (125 ± 11 *v* 105 ± 8 mg/dL; $P = .17$) after controlling for habitual physical activity levels.

Table 2. Frequency Distributions of the Different Genotypes in the Study Sample

	Entire Population (n = 60)	Sedentary (n = 18)	Physically Active (n = 19)	Athletes (n = 23)
FABP2				
Thr54Thr	0.09	0.06	0.11	0.09
Thr54Ala	0.30	0.30	0.33	0.26
Ala54Ala	0.61	0.64	0.56	0.65
ACE				
II	0.22	0.18	0.21	0.22
ID	0.52	0.47	0.58	0.52
DD	0.26	0.35	0.21	0.26
β_2AR				
Gln27Gln	0.36	0.41	0.37	0.26
Gln27Glu	0.54	0.41	0.47	0.70
Glu27Glu	0.10	0.18	0.16	0.04
PPARγ				
Pro12Pro	0.73	0.53	0.89	0.71
Pro12Ala	0.27	0.47	0.11	0.29

Abbreviation: β_2AR, β_2-adrenergic receptor.

Although the S_I values for the ACE II, ID, and DD genotype women were 4.2 ± 0.6, 5.2 ± 0.6, and 6.0 ± 0.8 $\mu U \times 10^{-4}$/min/mL, respectively, these differences were not statistically significant ($P = .31$). Fasting glucose, fasting insulin, K_G, and AIR_G also did not differ among ACE genotype groups.

There were no significant associations of either β_2AR or PPARγ genotypes with glucose and insulin metabolism kinetic

Table 3. Glucose and Insulin Metabolism Kinetic Parameters as a Function of FABP2 Genotype

	FABP2 Genotype	
	Thr54Thr + Thr54Ala (n = 23)	Ala54Ala (n = 37)
BMI (kg/m^2)	23.1 ± 0.6	23.5 ± 0.4
Fasting glucose (mg/dL)	86 ± 2	85 ± 1
Fasting insulin (μU/mL)	4 ± 1	4 ± 1
S_I ($\mu U \times 10^{-4}$/min/mL)	4.3 ± 0.5	5.8 ± 0.6*
AIR_G (pmol/L)	232 ± 37	184 ± 27
K_G (%/min)	0.8 ± 0.1	0.7 ± 0.1
Total cholesterol (mg/dL)	197 ± 8	205 ± 6
LDL cholesterol (mg/dL)	116 ± 9	127 ± 6
HDL cholesterol (mg/dL)	61 ± 4	61 ± 2
HDL_2 cholesterol (mg/dL)	3.7 ± 0.4	3.6 ± 0.2
HDL_3 cholesterol (mg/dL)	57 ± 4	57 ± 2
TG (mg/dL)	125 ± 11	105 ± 8

NOTE. Values are presented as means ± SE.
*$P < .05$ for the same variable between the 2 FABP2 genotype groups.

parameters in these women. The distributions of the β_3AR and IRS-1 genotypes afforded inadequate statistical power to address their associations with glucose and insulin metabolism kinetics parameters.

DISCUSSION

A number of putative diabetes candidate genes have been identified in the last decade,[1] and some of these loci have been shown, although not consistently, to be associated with type 2 diabetes or insulin resistance.[1] Many of these studies have not accounted for the potentially confounding effect of body composition. We are aware of only 1 study that accounted for the independent effect of habitual physical activity levels on insulin sensitivity, and we could find none that accounted for HRT use in older women. Our primary finding was that FABP2 genotype was associated with insulin sensitivity and that this relationship was independent of body composition, habitual physical activity levels, and HRT status. In terms of potential clinical significance, the 26% difference in insulin sensitivity between the FABP2 genotype groups is similar in magnitude to the difference seen between individuals with low glucose tolerance and those with normal glucose tolerance, as reported by Bergman et al.[9]

To our knowledge, 5 previous studies have found a significant association between FABP2 genotype and type 2 diabetes/insulin sensitivity. Yamada et al[19] found that Japanese Thr54 homozygous men had higher basal and 2-hour glucose challenge insulin levels than heterozygotes or Ala54 homozygotes. However, these men also had higher levels of abdominal fat, and this difference was not accounted for in the analyses. In Pima Indians, Baier et al[13] found that those with at least 1 Thr54 allele at the FABP2 locus had higher fasting insulins, higher insulin responses to glucose and a mixed meal, and lower insulin-stimulated glucose uptake than Ala54 homozygotes after correcting for body mass index. Two other studies[20,21] reported significant linkage at the FABP2 locus with fasting or 2-hour glucose challenge insulin levels. Boullu-Sanchis et al[5] found that the Thr54 allele at the FABP2 locus was associated with type 2 diabetes in Guadeloupe.[5] The present study extends these previous results by finding that insulin sensitivity was significantly lower, in both clinical and statistical terms, in women with at least one Thr54 FABP2 allele compared with Ala54 homozygotes at this locus after accounting for body composition, habitual physical activity levels, and HRT status.

Body composition is known to have an independent effect on insulin sensitivity, with overweight individuals having lower insulin sensitivity and a higher risk of developing type 2 diabetes than their lean counterparts. Previous candidate gene studies of putative type 2 diabetes or insulin resistance loci often did not account for this independent effect of body composition on insulin sensitivity or diabetes risk or used less precise measures of body composition, such as body mass index or waist-hip ratio. In the present study, there was a wide range of body composition, with the sedentary and physically active women averaging 37% body fat and the athletes averaging 25% body fat. On an individual basis, body fat in these women ranged from 13.3% to 45.7%. We previously found insulin sensitivity in these women to be significantly and inversely related to percent body fat.[8] In the present study, insulin sensitivity remained statistically significantly related to FABP2 genotype after accounting for the wide range of body compositions evident in these women.

We and others have demonstrated the marked effects of exercise training on insulin sensitivity in a wide range of individuals.[3,4] To our knowledge, only 1 previous study attempted to account for the independent effect of habitual physical activity levels on the relationship between FABP2 genotype and insulin sensitivity.[5] The methods used to quantify habitual physical activity levels in this previous study are unclear, and it appears that insulin sensitivity was not related to habitual physical activity levels. In the women in the present study, there was a wide range of $V_{O_{2max}}$ values among individuals (1.0 to 2.6 L/min; 18 to 49 mL/kg/min).[8] There also were wide ranges in habitual physical activity levels in these women (0 to 30 years of regular physical activity; 0 to 12 h/wk physical activity; 0 to 45 miles/wk running).[8] We have previously found $V_{O_{2max}}$ to be significantly related to insulin sensitivity in these women.[8] However, after accounting for this wide range of habitual physical activity levels, the association between FABP2 and insulin sensitivity remained significant.

The FABP2 gene is expressed in villus epithelial cells of the small intestine and functions to bind saturated and unsaturated long-chain fatty acids. The FABP2 locus is proposed as a putative diabetes/insulin resistance gene because of the known relationship between abnormal fatty acid metabolism and insulin resistance. When expressed in cultured cells, the variant allele protein binds long-chain fatty acids twice as efficiently as the wild allele protein.[13] These findings led to the hypothesis that the mutant allele results in increased fatty acid uptake in the small intestine, which in turn leads to increased plasma lipid levels, increased fat oxidation (which has been demonstrated previously[13]), and insulin resistance. It might appear surprising that fasting plasma TG levels were not significantly different between FABP2 genotype groups in the present study. However, others have demonstrated that postprandial, but not fasting, TG levels are associated with the codon 54 FABP2 polymorphism.[22,23]

These results do not necessarily imply that variations at the other putative diabetes loci investigated in this study (PPARγ, β_2AR, β_3AR, ACE, and IRS-1) do not associate with insulin sensitivity because this study had limited statistical power to detect an association at these loci. PPARγ, β_3AR, ACE, and IRS-1 genotypes have previously been shown to affect glucose and insulin metabolism in larger populations.[1] Insulin sensitivity differed by ~30% between our ACE II and DD genotype women. However, this difference did not approach statistical significance, indicating large variations in insulin sensitivity within ACE genotype groups, which can be overcome only by much larger sample sizes.[24] However, the results indicate that even if these polymorphisms prove to be significant markers of insulin sensitivity or diabetes risk in future studies, in the present study FABP2 genotype had a marked and statistically significant association with insulin sensitivity despite the same total sample size.

Thus, we conclude that FABP2 genotype is strongly associated with insulin sensitivity in postmenopausal women. Furthermore, this association is independent of the known associations between insulin sensitivity and habitual physical activity levels, body composition, and HRT status.

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