Association of the Ala54-thr Polymorphism in the Intestinal Fatty Acid–Binding Protein With 2-h Postchallenge Insulin Levels in the Framingham Offspring Study

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OBJECTIVE — To investigate the association of variants of the intestinal fatty acid–binding protein gene (FABP2) with fasting and postchallenge glucose and insulin levels, HbA_{1c}, and prevalence of type 2 diabetes in a separate sample of men and women.

RESEARCH DESIGN AND METHODS — Subjects were participants in the Framingham Offspring Study, a long-term community-based prospective observational study of risk factors for cardiovascular disease. The study sample consisted of 762 men and 922 women.

RESULTS — In women, carriers of the thr54 allele had significantly higher 2-h postchallenge insulin levels than noncarriers (104.4 ± 73.0 vs. 93.4 ± 61.5 μU/ml; $P = 0.0139$). This relationship remained significant after adjustment for familial relationship, age, BMI, triglycerides, APOE genotype, smoking, alcohol intake, the use of β-blockers, menopausal status, and estrogen therapy. No such significant association was observed in men. In both men and women, there were no statistical associations between the FABP2 polymorphism and BMI, fasting glucose, fasting insulin, 2-h postchallenge glucose levels, HbA_{1c}, and prevalence of type 2 diabetes.

CONCLUSIONS — These results suggest that the FABP2 thr54 allele may have a minor contribution to the insulin resistance syndrome in a white general population.

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Fatty acid metabolism has been linked to insulin resistance since Randle et al. (1) proposed the glucose–fatty acid cycle 38 years ago. The intestinal fatty acid–binding protein (IFABP) is a member of the family of cytoplasmic fatty acid–binding proteins. It is synthesized exclusively in the intestine and is involved with the transport and metabolism of saturated and unsaturated long-chain fatty acids (LCFAs) (2,3). The intestinal fatty acid–binding protein gene (FABP2) located at chromosome 4q28-31 is a candidate gene possibly implicated in insulin resistance and the pathogenesis of type 2 diabetes.

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Abbreviations: ANOVA, analysis of variance; CHD, coronary heart disease; FABP2, fatty acid–binding protein gene; FOS, Framingham Offspring Study; IFABP, intestinal fatty acid–binding protein; LCFA, long-chain fatty acid; TG, triglyceride.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

In 1995, Baier et al. (4) discovered a G → A mutation at codon 54 of FABP2, resulting in an amino acid substitution in IFABP, ala54 (wild-type) → thr54 (mutant-type). This amino acid substitution was first found to be associated with elevated fasting insulin levels, insulin resistance, and a higher mean fat oxidation rate in Pima Indians without type 2 diabetes (4). In vitro studies have found that the mutated thr54 IFABP has greater affinity for LCFAs than the wild-type ala54 IFABP, and it transports LCFAs and secretes triglycerides (TGs) and cholesterol esters to a greater degree than wild-type IFABP (4,5), suggesting that this ala54thr substitution is in fact a functional mutation.

Several studies have found significant associations between the FABP2 gene and type 2 diabetes or phenotypes associated with impaired glucose tolerance (4,6–9). Conversely, other studies have failed to find significant associations between the FABP2 gene and type 2 diabetes or related phenotypes (10–22). Moreover, a study of Keewatin Inuit (Eskimos) found the thr54 allele to be associated with lower 2-h glucose concentrations. This finding in Eskimos is opposite to other studies, which found the thr54 allele to be associated with poorer glucose tolerance (23).

The effect of this mutation in a large, normal white population is unknown. Although there has been no previous evidence that would lead us to expect a sex difference, this is the first study to investigate men and women separately—a possibility afforded by the current study's large sample size. The aim of the present study was to examine the associations of the FABP2 genetic polymorphism with variations in fasting and postchallenge plasma glucose levels, insulin levels, HbA_{1c}, and prevalence of type 2 diabetes in Framingham Offspring Study (FOS) participants. Based on most previous

findings with the ala54thr polymorphism, we believe that the thr54 allele would be associated with a worse glucose and insulin profile compared with the ala54 allele in both men and women.

RESEARCH DESIGN AND METHODS

Population subjects

Subjects were participants in the FOS, a long-term community-based prospective observational study of risk factors for cardiovascular disease in which participants are the offspring of the subjects of the original Framingham Heart Study cohort, and their spouses. The details of the design of the FOS have been reported elsewhere (24). Starting in 1971, a total of 5,124 subjects were enrolled (25). From January 1991 to September 1993, during the fifth cycle of the FOS, 2,784 consecutive participants had standardized medical history and physical examinations. Plasma samples were drawn for glucose and insulin after an overnight fast. HbA_{1c} (percentage of total Hb), a time-integrated measure of glycemia over the preceding 2–3 months, was used to assess glycemic control. A 75-g oral glucose tolerance test was administered according to World Health Organization standards (26) to subjects not known to have diabetes, and glucose and insulin levels were measured 2 h after the oral challenge. Subjects were known to have diabetes if the fasting plasma glucose level was >7.8 mmol/l at any two prior examinations or if the subject reported current or past hypoglycemic drug therapy. Subjects were classified as having type 2 diabetes glucose tolerance according to World Health Organization criteria (26). Data on smoking, alcohol, height, and weight were obtained on these subjects as previously described (25).

FABP2 genotypes and diabetes status were available for 848 men and 978 women. Of these, 79 men (9.3%) and 48 women (4.9%) were classified as having type 2 diabetes. In the final sample for analysis, those with and without type 2 diabetes were included; however, those taking lipid-lowering medications at examination five were excluded (84 men and 56 women). FABP2 genotypes, insulin and glucose measurements, and other risk variables were available for 1,684 subjects (762 men and 922 women). Almost all subjects were white.

Laboratory methods

Genomic DNA was isolated from peripheral blood leukocytes by standard methods (27). Genotyping for the FABP2 polymorphism was performed using the Perkin-Elmer/Applied Biosystems 7,700 Sequence Detection System and Taqman reagents as previously described (28).

Plasma was isolated from venous blood drawn in EDTA-containing tubes after a 12-h fast. Plasma total cholesterol, HDL cholesterol, and TG levels were measured as previously described (29). The equation of Friedewald et al. (30) was used to estimate LDL cholesterol concentrations.

Fasting plasma glucose was measured with a hexokinase reagent kit (A-gent glucose test; Abbott, South Pasadena, CA). HbA_{1c} was measured by high-performance liquid chromatography after overnight dialysis against normal saline to remove the labile fraction (31). Fasting insulin was measured in plasma as total immunoreactive insulin (Coat-A-Count Insulin; Diagnostic Products, Los Angeles, CA) and calibrated to serum levels for reporting purposes. The intra-assay and interassay reproducibility of these measurements has been reported elsewhere (32).

Statistical analyses

Female and male participants were compared by using χ^2 tests for categorical measures and two-sample *t* tests for continuous measures. Allele frequency of the thr54 allele was estimated with the chromosome-counting method, and a χ^2 test was used to compare it in men and women. Subjects with the GG genotype (ala54) were compared with subjects with either the GA (heterozygotes, ala54/thr54) or AA genotype (thr54) to increase the statistical power. The relationship between carriers and noncarriers of the FABP2 thr54 allele and insulin and glucose measures was evaluated by analysis of covariance techniques, which accounted for the familial relationships among the study participants (mostly siblings and cousins). A repeated-measures approach was used that assumed an exchangeable correlation structure among all members of a family, using PROC MIXED in SAS. Because this approach does not accurately represent the true correlation structure within these pedigrees, we also used a measured-genotype approach (33) as implemented in SOLAR, a variance component analysis computer package for quantitative traits measured in pedigrees of arbitrary size (34). The measured-genotype approach fully accounts for the different types of relationships within a pedigree in performing an analysis of variance (ANOVA) on the defined genotypes. In these analyses, we adjusted for familial relationship, age, BMI, plasma TGs, APOE genotype, smoking, alcohol consumption, β-blockers, and menopausal status and hormone-replacement therapy in women. APOE genotypes were added to the model with E2/E2 and E2/E3 in one group, E3/E4 and E4/E4 in a second group, and E3/E3 as the reference group. Four subjects with the very rare APOE E2/E4 genotype were excluded. For a single outcome, there is no need to adjust for multiple comparisons because we are comparing two groups (thr54 carriers versus noncarriers). Because this is the first study to report results separately in men and women and all of the ANOVAs were planned, we report our results without adjustment for multiple testing across outcomes. Because we report *P* values, people can use their own judgment as to what is significant and what is not.

RESULTS — The demographic, genotypic, and biochemical characteristics of study subjects according to sex are listed in Table 1. The frequency of the FABP2 mutant thr54 allele was similar in men and women (27.1 and 26.9%, respectively), and the genotypic frequencies were in Hardy-Weinberg equilibrium. Compared with women, men had significantly higher BMI, TGs, LDL cholesterol, fasting glucose, and fasting insulin levels. Women had significantly higher total and HDL cholesterol levels than men. There were no statistical differences in age, 2-h postchallenge glucose levels, 2-h postchallenge insulin levels, or HbA_{1c} between men and women. Men consumed significantly more alcohol per week than women. Of the women studied, 67.4% were postmenopausal, and 19.7% were on estrogen therapy.

Table 2 shows the characteristics of study subjects according to FABP2 group using the measured-genotype approach (33) as implemented in SOLAR. In men and women, there were no differences in age, alcohol consumption, or smoking between carriers and noncarriers of the FABP2 thr54 allele. In men, there were no

Table 1—*Demographic, genotypic, and biochemical characteristics of participants according to sex*

	Men	Women	*P*
n	762	922	—
Ala54 homozygotes (%)	396 (52.8)	484 (52.4)	—
Thr54 carriers (%)	366 (47.2)	438 (47.6)	0.8330
Age (years)	56.3 ± 10.0	55.4 ± 9.8	0.0514
BMI (kg/m^2)	28.3 ± 4.2	26.7 ± 5.5	0.0001
Fasting glucose (mmol/l)	5.82 ± 1.88	5.42 ± 1.47	0.0001
Fasting insulin (μU/ml)	32.7 ± 18.9	30.0 ± 28.4	0.0311
2-h glucose (mmol/l)	6.19 ± 2.82	6.19 ± 2.25	0.9913
2-h insulin (μU/ml)	98.1 ± 81.9	98.4 ± 67.4	0.9412
HbA_{1c} (%)	5.52 ± 1.20	5.43 ± 0.88	0.0834
TGs (mmol/l)	1.71 ± 1.11	1.53 ± 1.24	0.0001
Total cholesterol (mmol/l)	5.18 ± 0.85	5.36 ± 0.96	0.0001
LDL cholesterol (mmol/l)	3.29 ± 0.76	3.20 ± 0.87	0.0376
HDL cholesterol (mmol/l)	1.14 ± 0.31	1.47 ± 0.41	0.0001
Alcohol (ounces/week)	3.6 ± 4.6	1.7 ± 2.4	0.0001
Cigarettes/day	4.3 ± 10.7	3.7 ± 9.1	0.1882
Postmenopausal (%)	—	67.4	—
On estrogen Rx (%)*	—	19.7	—

Data are *n* (%) or means ± SD, unless otherwise stated. *Includes hormonal replacement therapy and the use of oral contraceptives.

statistically significant differences in BMI, TGs, cholesterol, fasting glucose, fasting insulin, 2-h glucose, 2-h insulin levels, or HbA_{1c} between carriers and noncarriers of the thr54 allele.

In women, carriers of the thr54 allele had significantly higher 2-h insulin levels (104.0 ± 73.0 vs. 93.4 ± 61.5 μU/ml; $P = 0.0139$), total cholesterol (5.42 ± 0.93 vs. 5.30 ± 0.98 mmol/l; $P = 0.0418$), and LDL cholesterol levels (3.26 ± 0.85 vs. 3.15 ± 0.89 mmol/l; $P = 0.0308$) than noncarriers after adjustment for familial relationship, age, BMI, TGs, APOE genotype, smoking, alcohol intake, the use of β-blockers, menopausal status, and estrogen therapy. In addition, female carriers of the thr54 allele had a nonsignificant trend of higher fasting insulin levels than noncarriers (31.4 ± 37.4 vs. 28.8 ± 16.4 μU/ml; $P = 0.0708$). Also, in women there were no statistical differences in BMI, fasting glucose levels, 2-h glucose levels, or HbA_{1c} between carriers and noncarriers of the thr54 allele.

In both men and women, there were no significant interactions between APOE genotype and the ala54thr polymorphism with HbA_{1c} and fasting and 2-h insulin and glucose levels (data not shown).

Type 2 diabetes was present in 79 men (9.3%) and 48 women (4.9%). Of the 403 men who were thr54 carriers, 40 (9.9%) had type 2 diabetes, whereas only 39 (8.8%) of 445 subjects who were not carriers of the thr54 allele had type 2 diabetes. The FABP2 polymorphism was not associated with type 2 diabetes in a logistic regression model adjusting for age, BMI, smoking, alcohol, β-blockers, and systolic blood pressure ($P = 0.7625$).

In women, 22 (4.7%) of 465 thr54 carriers had type 2 diabetes, and 26 (5.1%) of 513 subjects who were not carriers of the thr54 allele had diabetes. As observed in men, this association was not significant after adjustment for covariates including menopausal status and hormone-replacement therapy ($P = 0.8339$).

CONCLUSIONS — The ala54thr polymorphism at the FABP2 gene was investigated as a possible genetic factor determining variance in glucose and insulin levels. This is the first study that aimed to examine men and women separately. Previous studies involving the FABP2 polymorphism have combined results from men and women, with the exception of Yamada et al. (8), who studied only Japanese men. We found that in women, carriers of the thr54 allele had significantly higher 2-h postchallenge plasma insulin levels than noncarriers. In men, we did not find any significant associations with the FABP2 polymorphism.

The ala54thr polymorphism appears to have different effects in men and women. In women, the thr54 allele was associated with significantly elevated 2-h insulin levels. In addition, female carriers of the thr54 allele had a nonsignificant trend to have higher fasting insulin levels ($P = 0.0708$) than noncarriers. In men, this relationship was not significant. There was also a significant association between the thr54 allele and higher total and LDL cholesterol levels in women. An analysis of the ala54thr polymorphism and plasma lipids in a larger sample of the FOS participants is presented elsewhere (35). Other studies have found that coronary heart disease (CHD) risk factors have stronger associations in women than men. In prior Framingham Heart Study data, HbA_{1c} was significantly related to prevalent CHD in women but not men (36). A potential CHD risk factor, an FABP2 variant adversely affecting lipid levels, might interact with sex to accentuate levels of another CHD risk factor: insulin. This may contribute further to the adverse CHD profile that seems to eliminate the female CHD advantage in women with type 2 diabetes or insulin resistance.

In addition, the FABP2 knockout in mice also had some sex-specific associations (37). The IFABP deficiency caused a dramatic elevation (1.4- to 4-fold of $FABP^{+/+}$) in plasma insulin levels, but not in plasma glucose levels, independent of sex or dietary fat status. However, on a low-fat diet the male knockout ($FABP^{-/-}$) mice were consistently heavier (110%) than wild-type ($FABP^{+/+}$) mice. Females on the low-fat diet had no differences in weight. When the male mice were switched to a high-fat diet, the $FABP^{-/-}$ mice gained more weight (130% of $FABP^{+/+}$), but the female $FABP^{-/-}$ mice gained less weight (70% of $FABP^{+/+}$). Plasma TG concentrations in male $FABP^{-/-}$ mice were also higher (130–150% of $FABP^{+/+}$) on both the low-fat and high-fat diets than in female mice, which showed no difference. Vassileva et al. (37) conclude that IFABP feeds information about dietary lipid status into mechanisms that universally control energy utilization, energy storage, and eventually body weight. Because male and female mice responded differently to IFABP deficiency, sex hormones may influence this lipid-sensing mechanism.

Differences in hormone levels may explain some of the varied results in men

Table 2—*Characteristics of FOS subjects according to FABP2 allele*

	ala54 Homozygotes	thr54 Carriers	*P**
Men			
n	396	366	—
Age (years)	56.1 ± 10.1	56.5 ± 10.0	0.6076
BMI (kg/m^2)	28.6 ± 4.3	28.0 ± 4.1	0.1850
TGs (mmol/l)	1.77 ± 1.26	1.66 ± 0.93	0.3658
Total cholesterol (mmol/l)	5.17 ± 0.86	5.20 ± 0.84	0.4481
LDL cholesterol (mmol/l)	3.26 ± 0.77	3.32 ± 0.76	0.2407
HDL cholesterol (mmol/l)	1.13 ± 0.32	1.14 ± 0.28	0.4790
Fasting glucose (mmol/l)	5.77 ± 1.67	5.87 ± 2.07	0.1198
Fasting insulin (μU/ml)	33.3 ± 16.5	32.0 ± 21.3	0.8110
2-h glucose (mmol/l)	6.21 ± 2.47	6.16 ± 3.14	0.7449
2-h insulin (μU/ml)	104.4 ± 86.6	91.3 ± 76.1	0.1141
HbA_{1c} (%)	5.53 ± 1.17	5.52 ± 1.25	0.8843
Women			
n	484	438	—
Age (years)	54.9 ± 9.9	55.9 ± 9.8	0.1200
BMI (kg/m^2)	26.9 ± 5.6	26.4 ± 5.3	0.2392
Triglycerides (mmol/l)	1.54 ± 1.49	1.52 ± 0.89	0.8211
Total cholesterol (mmol/l)	5.30 ± 0.98	5.42 ± 0.93	0.0418†
LDL cholesterol (mmol/l)	3.15 ± 0.89	3.26 ± 0.85	0.0308†
HDL cholesterol (mmol/l)	1.45 ± 0.40	1.48 ± 0.42	0.3763
Fasting glucose (mmol/l)	5.43 ± 1.57	5.40 ± 1.36	0.7331
Fasting insulin (μU/ml)	28.8 ± 16.4	31.4 ± 37.4	0.0708
2-h glucose (mmol/l)	6.06 ± 2.02	6.33 ± 2.48	0.2578
2-h insulin (μU/ml)	93.4 ± 61.5	104.0 ± 73.0	0.0139†
HbA_{1c} (%)	5.45 ± 0.91	5.41 ± 0.85	0.5167

Data are *n* or means ± SD, unless otherwise stated. *After adjustment for familial relationship, age, BMI, TGs, APOE genotype, smoking, alcohol intake, and use of β-blockers (and menopausal status and estrogen therapy in women); †$P < 0.05$.

and women. Fatty acid–binding protein concentrations in members of the cytoplasmic fatty acid–binding protein family are higher in female rats than in male rats, although this result has not yet been demonstrated with the IFABP (38–40). If a higher concentration of the IFABP exists in female humans, this may lead to even more enhanced absorption of fatty acids in those with the thr54 allele and resulting impairment of insulin action.

The FABP2 polymorphism may be more important in determining glucose homeostasis in some populations than others. The relationship between the thr54 allele and impaired glucose tolerance has been inconsistent in different ethnic populations. In a combined population of Pima Indian men and women, those with the thr54 allele, compared with ala54 homozygotes, had significantly higher mean 2-h insulin levels, fasting insulin levels, fat oxidation rate, and greater insulin resistance, as determined by the hyperinsulinemic-euglycemic clamp (4). Moreover, Mitchell et al. (7) found significant linkage between 2-h insulin levels and the FABP2 locus in a combined group of Mexican-American men and women. They found that 32% of the phenotypic variance in 2-h insulin levels could be attributed to the FABP2 or a tightly linked gene (7). In the study of only Japanese men, it was found that thr54 homozygotes had significantly higher fasting insulin levels, 2-h insulin levels, and greater insulin resistance than those with the ala54 allele (8). However, studies in combined populations of Japanese men and women have failed to confirm these results (13, 20,21,41). In addition, there have been no significant associations found between the FABP2 polymorphism and impaired glucose tolerance in Finnish or African-American populations (15,16,19,22,42).

In addition, there was no significant difference between ala54 homozygotes and thr54 homozygotes in fasting insulin levels or the response of plasma insulin to an oral fat load in a Finnish population or in participants of the European Atherosclerosis Study (43,44). However, there was a strong correlation in the Finnish study between TG and insulin responses in thr54 homozygotes, suggesting an association between postprandial triglyceridemia and insulin action in these subjects (43). In a group of Pima Indians, plasma 1-h insulin levels and nonesterified fatty acid levels were higher after a high-fat test meal in thr54 homozygotes than in ala54 homozygotes (45). There is a multiplicity of genes that are involved in glucose metabolism.

These genes may show significant associations with morbidity in some populations, and in others these contributions may be masked by other genes. Because there have been strong associations with this polymorphism in Pima Indians and Mexican-Americans, this suggests that this polymorphism is a more important determinant of insulin levels in populations of Mexican and American Indian descent—two groups sharing a similar pool of genes.

The impact of the thr54 polymorphism in certain populations may vary due to genetics, environment, lifestyle, and diet. Differences in the environment can interact with functional differences in IFABP to produce phenotypic differences. Differences in the fatty acid composition of diets might explain the differences seen in some populations. For example, Hegele et al. (23) found that Canadian Inuit homozygous for the thr54 allele had significantly lower 2-h glucose levels after an oral glucose tolerance test than those with the ala54 allele. These authors suggest as a possible explanation for these findings that Eskimos have large dietary intakes of ω-3 fatty acids, which have been shown to improve insulin sensitivity (46). They suggest that the type of fat and duration of intake might interact with the FABP2 polymorphism to produce variation in response to dietary components (23). The thr54 mutation has been found to increase the transport of LCFAs in the enterocyte (5). It therefore can be hypothesized that if one carries the thr54 allele and consumes a diet high in ω-3 fatty acids, then more ω-3 fatty acids will be transported into the body, and hence improve insulin sensitivity. The opposite may be true, however, for those consuming other types of fatty acids.

The IFABP is located only in the en-

terocyte and hence it regulates postprandial blood lipid levels more than fasting levels. It therefore is not surprising that 2-h postglucose challenge insulin levels would be most affected by the FABP2 polymorphism. There is a plausible mechanism for the ala54thr mutation to lead to insulin resistance syndrome phenotypes. In 1963, Randle et al. (1) proposed that fatty acids and glucose compete as oxidative fuel sources in muscle, so that increased concentrations of fatty acids inhibit glucose uptake in muscle and result in insulin resistance. On the basis of this glucose–fatty acid cycle and the findings of the present study, it is hypothesized that the thr54 mutation, especially in women, may lead to excessive absorption of fatty acids, which leads skeletal muscles to preferentially use fatty acids for fuel rather than glucose, hence resulting in insulin impairment. Also, to further support this hypothesis, in vivo studies have found that subjects with the thr54 mutation, compared with ala54, have a higher mean fasting fat oxidation rate (4,14). The increase in fat oxidation rate is consistent with the thr54 mutation leading to an increase in fatty acid absorption. An increase in fat oxidation rates is also known to inhibit insulin action (47).

In the present study, we did not find an association between the FABP2 polymorphism and type 2 diabetes, and this result may be due to the small number of cases in this young cohort. We cannot rule out that we found the relationship with 2-h insulin in women by chance because men had the same quantitative difference (though not significant) in 2-h insulin levels between carriers and noncarriers but in the opposite direction, and there was no trend for a higher rate of type 2 diabetes in women thr54 carriers. More studies that look at men and women separately are needed to confirm these results.

In summary, this study investigated the FABP2 polymorphism in the FOS population and found that the thr54 allele was significantly associated with higher 2-h postglucose challenge plasma insulin levels in women. These results suggest that the thr54 allele may have a minor contribution to the insulin resistance syndrome in white individuals.

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