Codon 54 Polymorphism of the Fatty Acid Binding Protein 2 Gene Is Associated With Increased Fat Oxidation and Hyperinsulinemia, But Not With Intestinal Fatty Acid Absorption in Korean Men

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The alanine to threonine substitution at codon 54 (Ala54Thr) of the fatty acid binding protein 2 (FABP2) gene has been reported to be associated with increased fat oxidation and insulin resistance in several populations. It has been hypothesized that Ala54Thr substitution results in enhanced intestinal uptake of fatty acids and thereby an impairment of insulin action, but this hypothesis has not been proven in vivo. We studied the association between the Ala54Thr polymorphism of the FABP2 gene and intestinal ^{3}H-oleic acid absorption, as well as basal insulin level, basal metabolic rate, and fat oxidation rate in 96 healthy young Korean men. Among our subjects, the allele frequency of the Ala54Thr substitution was 0.34. Subjects with Thr54-encoding allele were found to have a higher mean fasting plasma insulin concentration and a higher basal fat oxidation rate compared with the subjects who were homozygous for the Ala54-encoding allele. However, there was no significant difference in basal metabolic rate or ^{3}H-oleic acid absorption according to the FABP2 gene polymorphism. These results suggest that the Ala54Thr substitution in the FABP2 gene is associated with increased fat oxidation and hyperinsulinemia in normal Korean men, but these effects are not mediated by an increase in the intestinal fatty acid absorption.

OBESITY AND INSULIN resistance, the major risk factors for type 2 diabetes mellitus, have been shown to cluster within families,[1,2] suggesting a genetic background for these conditions. A number of candidate genes have been suggested, but the major gene determining obesity and insulin resistance has not yet been uncovered. The human intestinal fatty acid binding protein 2 (FABP2) locus has been proposed to be a major candidate gene in determining insulin resistance.[3-5] Sibling pair linkage analysis in Pima Indians has indicated a major gene associated with insulin resistance in a region on chromosome 4q near the FABP2 gene.[3] In addition, a significant association between the FABP2 gene locus and 2-hour postglucose challenge insulin levels has been shown in Mexican-Americans.[4] More recently, a substitution of alanine to threonine at codon 54 (Ala54Thr) of the FABP2 gene was reported to be associated with insulin resistance in the Pima Indians,[5] as well as in Mexican Americans[4] and Japanese.[6] However, studies performed in European populations, such as English, Welsh, and Finnish,[7-10] did not show an association of this gene to insulin resistance.

The mechanism of association between the FABP2 gene polymorphism and insulin resistance or body fuel metabolism is currently unknown. FABP2 expression is limited to the columnar absorptive epithelial cells of the small intestine.[11,12] This suggests that FABP2 should have a role in the absorption and intracellular transport of dietary long-chain fatty acids.[13] Baier et al[5] showed that the Thr54-containing protein had a 2-fold greater affinity for long-chain fatty acids than the Ala54-containing protein. In addition, they also showed that Caco-2 cells expressing Thr54 FABP2 protein transport long-chain fatty acids and secrete triglycerides to a greater degree than Caco-2 cells expressing Ala54 FABP2.[14] From this, it was hypothesized that subjects with Thr54 allele have higher intestinal absorption of fatty acids resulting in higher fat oxidation rate and insulin resistance. In fact, the Ala54Thr polymorphism has been associated with increased lipid oxidation rate in Pima Indians.[5] However, it has not been studied whether there is a real difference in the intestinal absorption of fatty acids in vivo.

In this study, we have investigated the possible association between the FABP2 gene polymorphism and fatty acid absorption, as well as insulin level and energy metabolism in healthy young Korean men.

SUBJECTS AND METHODS

Subjects

We recruited 96 healthy young male volunteers among medical students attending the Soonchunhyang University and doctors working at the Soonchunhyang University Hospital. None of the subjects were first degree relatives. No one was taking any medications or had a history of diabetes mellitus or other metabolic diseases. The study protocol was approved by the Institutional Review Board, and all subjects gave written informed consent.

Anthropometric Measurements

Blood pressure was measured using a sphygmomanometer after at least a 5-minute rest on the morning of the experiments. Two readings were taken from the right arm, and the average was used for the analysis. Height and weight of the subjects were measured with light clothes without shoes. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Waist circumference was measured at the umbilicus level and hip circumference at the maximum hip girth. Waist-to-hip ratio was calculated from these measurements. Lean body mass was determined by body composition analysis using dual energy x-ray absorptiometry (QDR 2000; Hologic, Waltham, MA).

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Oral Glucose Tolerance Test

A 75-g oral glucose tolerance test (OGTT) was performed after an overnight fast of 12 to 14 hours. Subjects ingested 75 g of glucose, and blood samples were taken at 0, 30, 60, 90, and 120 minutes. Plasma samples were stored at −20°C for later analysis of glucose and insulin.

Indirect Calorimetry

Basal metabolic rate (BMR) was measured by indirect calorimetry (Deltatrac metabolic monitor; SensorMedics, Anaheim, CA) after an overnight fast. A clear plastic ventilated hood was placed over each subject's head. Room air was drawn through the hood at a measured rate of flow, and a constant fraction of expired air was withdrawn and analyzed for oxygen and carbon dioxide content. Continuous, integrated calorimetric measurements were made every 1 minute for 60 minutes, of which the first 20 minutes were discarded and the mean value of the last 40 minutes was used for calculations. Protein oxidation rate was estimated from the urinary nitrogen excretion rate. The nonprotein respiratory quotient was then calculated, and the substrate oxidation rate was determined as described by Ferrannini.[15]

^{3}H-Oleic Acid Absorption Test

After an overnight fast, 40 uCi (diluted in 25 mL of corn oil) of ^{3}H-oleic acid (NEN, Boston, MA) was administered orally. Blood samples were collected at 0, 2, 4, 6, and 8 hours. Serum was separated and treated with tissue solubilizer (Soluene 350; Packard, Downers Grove, IL) for 3 hours at 60°C. Radioactivity of ^{3}H in serum samples was counted by a liquid scintillation counter (Tri-Carb 1500, Packard).

Determination of the FABP2 Gene Polymorphism

Genomic DNA was extracted from peripheral blood leukocytes using a commercial kit (Promega, Madison, WI) according to the manufacturer's instructions. The exon 2 of FABP2 gene was amplified by polymerase chain reaction (PCR) with the forward primer 5′-ACAGGTGTTAATATAGTGAAAAG-3′ and the reverse primer 5′-TACCCTGAGTTCAGTTCCGTC-3′ (product size, 180 bp). PCR amplification was conducted in a 20-μL volume containing 200 ng of genomic DNA, 5 pmol of each primer, 0.25 U of Taq DNA polymerase (Takara, Kyoto, Japan), 200 mmol/L deoxynucleotide triphosphates (dNTP), 10 mmol/L Tris-HCl (pH 9.0), 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$. Amplifications were performed for 30 cycles in a thermal cycler (Gene Amp PCR System, MJ Research, Watertown, MA) and each cycle consisted of denaturation at 95°C for 1 minute, annealing at 55°C for 1 minute, and extension at 72°C for 1 minute with final extension at 72°C for 5 minutes. Amplified PCR products of 180 bp were digested with the addition of 5 U HhaI restriction enzyme (Promega, Madison, WI) in 10 mmol/L Tris-HCl (pH 7.4), 50 mmol/L NaCl, 1 mmol/L dithiothreitol, and 10 mmol/l $MgCl_2$. After an incubation at 60°C for 2 hours, the digested samples were separated by electrophoresis through 4% agarose gel (FMC Bioproducts, Rockland, ME) and visualized by staining with ethidium bromide. PCR products of the samples with the normal sequence (Ala54) were cleaved into 99-bp and 81-bp, whereas the Ala54Thr substitution abolished the HhaI restriction site, yielding only a 180-bp product.

Analytical Methods

Plasma glucose was measured using a glucose oxidase method on an autoanalyzer (Beckman, Palo Alto, CA). Serum total cholesterol concentration was measured by sulfo-phospho-vanillin method, triglyceride by an enzymatic colorimetric method, and high-density lipoprotein (HDL) cholesterol by heparin-manganese precipitation method. Plasma-free fatty acids were measured using an enzymatic assay kit (Eiken, Osaka, Japan). Plasma immunoreactive insulin (Dainabot, Tokyo, Japan) and leptin (Linco, St Louis, MO) levels were measured in duplicate using immunoradiometric assay. The cross-reactivity of the insulin assay with proinsulin was less than 0.1%.

Statistical Analysis

Data are expressed as means ± SE. Variables that showed skewed distribution, such as plasma insulin and serum triglyceride levels, were log-transformed before analyses and then back-transformed to their natural units for presentation. The significance of differences between groups was assessed using the Student's unpaired *t* test and *P* values less than .05 (2-tailed) were considered to be statistically significant. Because the metabolic rates correlated with the lean body mass, analysis of covariance was used to adjust for lean body mass.

RESULTS

Of the 96 subjects, 43 (44.8%) were Ala/Ala homozygotes, 12 (12.5%) were Thr/Thr homozygotes, and 41 (42.7%) were heterozygous for the codon 54 of FABP2 polymorphism. The frequency of Thr54 allele was 0.34.

Characteristics of the subjects according to the FABP2 gene Ala54Thr polymorphism are shown in Table 1. Because only a small number of subjects were homozygous for the Thr54 allele, data were analyzed after combining the subjects with Thr/Thr homozygote (TT) and Ala/Thr heterozygote (AT). BMI, waist-to-hip ratio, fasting plasma levels of glucose, free fatty acids, and triglycerides were similar between the groups. However, the subjects having Thr54 allele showed higher basal plasma insulin levels (76.7 ± 7.1 *v* 50.4 ± 6.7 pmol/L, $P < .05$) than Ala/Ala homozygotes (AA). All of the subjects showed normal glucose tolerance, and there was no significant difference in glucose and insulin responses after oral glucose load between the 2 groups.

Basal energy expenditure (1,531 ± 37 kcal/d in AT/TT *v* 1,454 ± 47 kcal/d in AA) and basal metabolic rate adjusted for lean body mass (30.6 ± 0.8 *v* 28.2 ± 0.8 kcal/kg/d) were similar between the 2 groups. However, percent fat oxidation was higher in subjects with Thr54 allele than those with Ala/

Table 1. Characteristics of the Subjects According to FABP2 Gene Codon 54 Polymorphism

	AA	AT/TT	*P* Value
No.	43	53 (41/12)	
Age (yr)	24.4 ± 2.6	25.5 ± 3.0	NS
BMI (kg/m^2)	22.4 ± 2.7	22.8 ± 2.6	NS
Waist-to-hip ratio	0.83 ± 0.04	0.84 ± 0.05	NS
Systolic BP (mm Hg)	119 ± 4	120 ± 5	NS
Diastolic BP (mm Hg)	81 ± 3	82 ± 4	NS
Total cholesterol (mmol/L)	4.5 ± 0.8	4.6 ± 0.8	NS
Triglycerides (mmol/L)	1.4 ± 0.8	1.7 ± 1.3	NS
HDL cholesterol (mmol/L)	1.1 ± 0.2	1.0 ± 0.2	NS
LDL cholesterol (mmol/L)	2.8 ± 0.8	2.7 ± 0.7	NS
FFA (μmol/L)	468 ± 44	502 ± 51	NS
Fasting glucose (mmol/L)	5.2 ± 0.1	5.4 ± 0.1	NS
Fasting insulin (pmol/L)	50.4 ± 6.7	76.7 ± 7.1	.013
2-hour glucose (mmol/L)	5.6 ± 0.2	5.9 ± 0.2	NS
2-hour insulin (pmol/L)	152 ± 22	203 ± 24	NS
Leptin (ng/mL)	2.8 ± 0.3	3.5 ± 0.4	NS

NOTE. Values are means ± SE.

Abbreviations: AA, Ala/Ala; AT, Ala/Thr; TT, Thr/Thr; BMI, body mass index; BP, blood pressure; FFA, free fatty acids, NS, not significant.

Ala homozygotes (34.4 ± 4.7 *v* 21.9% ± 3.6%, $P < .05$), and percent carbohydrate oxidation tended to be lower (45.8 ± 4.3 *v* 54.8% ± 4.6%, $P = .16$).

After the ingestion of ^{3}H-labeled oleic acid, peak serum ^{3}H-activity was reached after 6 hours in both groups. There was no significant difference in peak serum ^{3}H-activity (315 ± 29 cpm/mL in AT/TT *v* 381 ± 48 cpm/mL in AA) or area under the curve (1,936 ± 146 cpm · h/mL in AT/TT *v* 2,114 ± 234 cpm · h/mL in AA) according to the FABP2 polymorphism.

DISCUSSION

In the present study, we observed that Ala54Thr substitution of the FABP2 gene was associated with a higher basal insulin level and a higher fat oxidation rate in normal Korean men. This is consistent with the results in Pima Indians,[5] which showed Thr54 allele was associated with hyperinsulinemia, insulin resistance, and a higher rate of fat oxidation. In contrast, no significant association was reported in Finnish subjects between the FABP2 gene polymorphism and fasting insulin levels, basal metabolic rate, insulin sensitivity, or lipid oxidation.[8,9] The reason for such a discrepancy is not clear, but either the effects of the Thr54 allele of FABP2 gene may be population-specific or gene(s) closely linked to the FABP2 gene locus may be linked with insulin action.[4,7]

Although the exact role of FABP2 in the human body is not yet established, limited expression in the intestinal epithelial cells suggests that it has a major role in the absorption and intracellular transport of dietary long-chain fatty acids.[13] Baier et al[5] hypothesized that Ala54Thr substitution of the FABP2 gene may result in enhanced intestinal uptake of fatty acids. In the current study, however, we could not observe any difference in ^{3}H-oleic acid absorption according to the FAPB2 genotype. Thus, our results do not support the idea that Ala54Thr substitution of the FABP2 gene contributes to insulin resistance through enhancement of intestinal uptake of fatty acids. Rather, it is suggested that other gene(s) closely linked to the FABP2 locus are responsible for the apparent association between the FABP2 gene polymorphism and insulin resistance or lipid oxidation rate. It is also possible that another unidentified function of the FABP2 is associated with insulin resistance. Prows et al[16] reported that ^{3}H-oleic acid esterification was increased in fibroblasts transfected with FABP2 gene, but ^{3}H-oleic acid uptake was not changed. This suggested that FABP2 may have a role in the mobilization and trafficking of intracellular fatty acids, rather than uptake of extracellular fatty acids. In line with this, several studies reported that FABP2 gene polymorphism was associated with plasma triglyceride levels,[10,17,18] and there was a strong correlation between triglyceride and insulin responses,[18] suggesting that altered postprandial lipemia may also modify insulin action. On the other hand, Yamada et al[6] reported that the subjects homozygous for the Thr54 allele had greater measurements of ultrasound intraabdominal fat thickness and suggested that fatty acids released from intraabdominal adipose tissue might be involved in the insulin resistance associated with the Thr54 allele.

One limitation of this study is that we examined the absorption of only 1 kind of fatty acid and did not measure the postprandial plasma lipoprotein response. Theoretically, FABP2 may function differently in the absorption of different fatty acids. In fact, it has been shown that intestinal absorption of elaidic acid differs markedly from its isomer oleic acid.[19] However, Baier et al[5,14] showed that Thr54-containing protein has similarly increased binding affinity and transport for both saturated and unsaturated long-chain fatty acids. It is also possible that intestinal absorption of fatty acids after a high-fat meal is different from that measured by ^{3}H-oleic acid absorption test, because dietary fat is usually ingested as a bolus with a high concentration of long-chain fatty acids. In fact, Agren et al[18] showed that the increase of plasma triglyceride concentration after the fat test meal was greater in subjects homozygous for the Thr54 allele. However, previous studies have shown that absorption of ^{3}H-labeled oleic acid is independent of the carrier fat.[20,21]

Because of the possibility that the FABP2 gene polymorphism may affect fat metabolism, previous studies attempted to link this polymorphism to obesity. The presence of Thr54 variant was associated with increased BMI and percent body fat in aboriginal Canadians,[17] but not in the Pima Indians,[5] Japanese,[6] or Finns.[8] In the present study, we also could not find any difference in BMI or waist-to-hip ratio according to the FABP2 gene polymorphism, suggesting that this is not a risk marker of obesity. In fact, the frequency of Thr54 allele in the FABP2 gene was 0.34 in our subjects, which is similar to that of Pima Indians (0.29), Caucasians (0.31), and Japanese (0.34).[5,6] Considering the wide difference in the prevalence of obesity among these populations,[22,23] FABP2 gene polymorphism alone may not be a major factor determining obesity and insulin resistance. However, we cannot rule out the possibility that this polymorphism play a role in the genesis of obesity when combined with other gene(s). In agreement with this concept, Lei et al[24] reported that the FABP2 gene polymorphism was not associated with type 2 diabetes or severe obesity, but the joint effect with insulin receptor substrate-1 gene polymorphism may be associated with an increase in BMI in African Americans.

In conclusion, our data suggest that the Ala54Thr substitution in the FABP2 gene is associated with hyperinsulinemia and increased fat oxidation in normal Korean men, but these effects are not mediated by the changes in intestinal absorption of fatty acids.

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