Intestinal *FABP2* A54T Polymorphism: Association with Insulin Resistance and Obesity in Women

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Abstract

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Objective: To assess the association between the Ala54Thr genetic polymorphism of the fatty acid-binding protein 2 (*FABP2*) gene with insulin resistance and obesity.

Research Methods and Procedures: According to a sampling scheme based on BMI, 33 adult obese women (BMI $\geq$ 30) and 30 adult normal-weight women (BMI $>$ 18.5 and $<$ 25 kg/m^2) were recruited for this study. Women with chronic inflammatory diseases or acute pathology were excluded. Glucose, insulin, leptin, lipids, and tumor necrosis factor α (TNFα) were measured in fasting plasma samples. Insulin resistance was estimated through the homeostasis model assessment for insulin resistance method. The *Ala54Thr* allelic variant was determined by polymerase chain reaction, followed by restriction fragment-length polymorphism analysis.

Results: The *Thr54* allele was more frequent in obese than in nonobese women (47.0% vs. 31.7; $p = 0.08$). Among obese women, higher TNFα concentrations were found when comparing the *Thr54*/*Thr54* genotype (30.0 $\pm$ 7.1 pg/mL) with either the *Ala54*/*Thr54* genotype (21.2 $\pm$ 8.4 pg/mL) or the *Ala54*/*Ala44* genotype (20.1 $\pm$ 7.0 pg/mL) ($p < 0.05$). In addition, higher fasting plasma insulin and leptin levels were found among *Thr54*/*Thr54* homozygotes compared with the other genotypes ($p < 0.05$).

Discussion: Our results suggest that the Ala54Thr polymorphism of the *FABP2* gene is associated with obesity and insulin resistance. The effect of this polymorphism might be mediated by elevated production of TNFα.

Key words: TNFα, BMI, genotype, fatty acids, FABP2

Introduction

Obesity and insulin resistance, both major risk factors for type 2 diabetes, have been shown to cluster within families, suggesting a genetic component for their etiology (1). Both conditions are complex traits influenced by multiple genetic and environmental factors.

Fatty acid-binding protein (FABP)[1] 2 is an intracellular protein expressed only in the intestine (2). The gene for FABP2 is located in the long arm of chromosome 4. The G-to-A polymorphism of codon 54 results in the substitution of threonine (Thr) for alanine (Ala) (3). In vitro experiments have shown that this substitution increases the affinity of FABP2 for long-chain fatty acids and is associated with increased triglyceride transport in human intestinal cells (4,5).

The associations between the *FABP2* Ala54Thr polymorphism and increased fasting insulin concentration, fasting fatty acid oxidation, and reduced glucose uptake during a hyperinsulinemic euglycemic clamp were identified in Pima Indians (3). Furthermore, the linkage analysis of the *FABP2* locus with insulin resistance was also found in a study in

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[1] Nonstandard abbreviations: FABP, fatty acid-binding protein; Thr, threonine; Ala, alanine; TNF, tumor necrosis factor; HOMA, homeostasis model assessment; PCR, polymerase chain reaction; FFA, free fatty acid; PUFA, polyunsaturated fatty acid; AA, arachidonic acid.

Mexican Americans who were of a mixed American-Indian and -European ancestry (6). However, sib-pair analysis failed to detect any linkage of the *FABP2* locus or the Ala54Thr polymorphism with diabetes-related phenotypes in other ethnic groups. In addition, no association was found in Finnish individuals. However, some lipid abnormalities were detected in the Finnish population with the *Thr54* allele in *FABP2* (7).

FABP2 plays a key role in the absorption and intracellular transport of dietary long-chain fatty acids. Carriers of the *Thr54* allele in *FABP2* have a 2-fold greater affinity for the long-chain fatty acids than those with the *Ala54*-containing *FABP2* (8), which supports the role of the *FABP2* Ala54Thr polymorphism in the etiology of metabolic disorders.

On the other hand, high levels of circulating tumor necrosis factor (TNF) α, a cytokine expressed in adipocytes, where it is an important modulator of gene expression, are a putative risk factor for the development of obesity and obesity-related disorders (9). There is evidence that suggests that a higher ω-6-to-ω-3 fatty acid ratio in the diet is associated with increased circulating levels of TNFα (10–12). TNFα affects lipid metabolism and may lead to hypertriglyceridemia by decreasing hepatic lipoprotein lipase activity and by increasing hepatic de novo fatty acid synthesis (13). Circulating levels of TNFα have also been reported to reduce the insulin receptor tyrosine kinase activity and induce the down-regulation of the GLUT4 transporter in adipocytes in a manner that correlates with insulin resistance and type 2 diabetes (14).

We hypothesized that the Ala54Thr polymorphism of the *FABP2* gene elevates the risk of insulin resistance by influencing lipid metabolism, mediated by elevated circulating inflammatory cytokines, particularly TNFα.

Research Methods and Procedures

Subjects

We recruited 63 unrelated Chilean premenopausal women (with regular menses and no hormone replacement therapy) of European ancestry, selected from public and private medical records (age range: 20 to 50 years), with an extreme sampling procedure: 33 were obese (BMI $\geq$ 30 kg/m^2), and 30 were normal weight (BMI between 18.5 and 24.9 kg/m^2). None of the subjects was taking any medications or had a history of diabetes or other metabolic diseases. The study protocol was approved by the Institutional Review Board at the Institute of Nutrition and Food Technology (University of Chile), and all subjects gave written, informed consent.

Anthropometric Measurements

Blood pressure was measured using a sphygmomanometer after at least a 5-minute rest. Two readings were taken from the right arm, and the average was used. Height and weight were measured while subjects wore light clothing, without shoes. BMI was calculated as weight in kilograms divided by height in meters squared. Waist circumference at the umbilicus level and hip circumference at the maximum hip girth were tabulated.

Biochemical Measurements

Blood samples were taken in the morning between 7:00 and 9:00 AM after an overnight fast. A standard 75-gram oral glucose-tolerance test was performed in each woman; after the test, subjects were classified as normal, glucose intolerant, and diabetic on the basis of World Health Organization criteria. Serum levels of glucose were measured by glucose oxidase technique, and serum levels of insulin were determined by means of radioimmunoassay (RIA Diagnostic Corporation, Los Angeles, CA). Serum leptin levels were analyzed using a specific radioimmunoassay kit (Linco Research, St. Charles, MO), and circulating levels of TNFα were determined using an enzyme-linked immunosorbent assay (Roche Diagnostics, Mannheim, Germany). The lipid profile (total cholesterol, high-density lipoprotein-cholesterol, and low-density lipoprotein-cholesterol) was determined with enzymatic colorimetric methods using commercial kits (Boehringer Mannheim, Mannheim, Germany). Insulin resistance was assessed by the relationship between fasting glucose and insulin concentrations and analyzed by the homeostasis model assessment (HOMA) (15).

Determination of the Polymorphism of the FABP2 *Gene*

Genomic DNA was extracted from leukocytes by the phenol/chloroform method followed by proteinase K (Life Technologies/Gibco-BRL, Cleveland, OH). A total of 100 ng of genomic DNA was used to amplify a specific *FABP2* gene sequence by polymerase chain reaction (PCR) in a volume of 25 μL containing 0.5 U of Taq DNA polymerase (Invitrogen, Carlsbad, CA), 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, and 100 μM deoxyribonucleotide triphosphates using the following primers: 5′-AC AGG TGT TAA TAT AGT GAA AAG-3′ and 5′-TA CCC TGA GTT CAG TTC CGT C-3′. After 35 cycles of 1 minute at 94 °C, 1 minute at 58 °C, and 1 minute at 72 °C, aliquots (7 μL) of PCR products were analyzed on 2% agarose gels (Invitrogen). The amplified PCR product (180 bp) was digested with 2 U of HhaI (Invitrogen) in 10 mM Tris-HCl (pH 7.9), 50 mM NaCl, 10 mM $MgCl_2$, and 1 mM dithiothreitol. After an incubation at 37 °C for 3 hours, the digested samples were separated by electrophoresis through 3% agarose gel and visualized by staining with ethidium bromide. PCR products having an intact HhaI site were cleaved into 99- and 81-bp fragments, whereas the *Ala54Thr* substitution abolished the restriction site.

Statistical Methods

Continuous variables are expressed as means $\pm$ SD. Differences among study groups were assessed through

Table 1. Metabolic characteristics of the women participating in the study

Metabolic variables	Normal ($n = 30$)	Obese ($n = 33$)	p value*
Fasting glycemia (mM)	5.3 ± 0.4	5.5 ± 0.8	0.10
Fasting insulin (pM)	70.8 ± 31.2	164.6 ± 102.0	<0.001
HOMA index	2.4 ± 1.2	5.7 ± 3.8	<0.001
Triglycerides (mM)	1.0 ± 0.5	1.6 ± 1.0	0.002
Total cholesterol (mM)	4.7 ± 0.7	5.3 ± 0.9	0.008
High-density lipoprotein cholesterol (mM)	1.0 ± 0.3	0.9 ± 0.3	0.17
Leptin (ng/mL)	18.0 ± 9.5	30.7 ± 8.8	<0.001
TNFα (pg/mL)	19.1 ± 7.6	22.5 ± 8.4	0.048

* Student's t test.

Student's t test and Kruskal-Wallis test. χ^2 statistics and odds ratios were calculated to compare allele and genotype frequencies between obese and normal-weight women. Hardy-Weinberg equilibrium was evaluated through an exact method. All statistical analyses were performed with the STATA 7.0 package (2001; Stata Corp., College Station, TX).

Results

Thirty-three obese women (BMI 37.2 ± 5.6 kg/m^2) and 30 nonobese women (BMI 22.5 ± 0.28 kg/m^2) participated in the study. There were no significant differences in mean age between groups (36.4 ± 1.2 in normal-weight women and 38.3 ± 8.3 in the obese group).

Summary statistics for metabolic variables of both groups are reported in Table 1. Mean plasma glucose levels were similar in both groups, but insulin was significantly higher in obese women. This fact agrees with significantly higher HOMA and triglyceride levels in obese women than in nonobese women. As expected, leptin levels were higher in the obese group. Likewise, TNFα and total cholesterol showed higher levels in the obese group compared with the control group.

Table 2 shows *FABP2* genotype frequencies in obese and normal-weight women. Genotype frequencies were concordant with Hardy-Weinberg expectations in normal-weight (p value = 0.40) and obese (p value = 0.49) women. The frequency of the *Thr54* allele was higher in the obese group compared with the normal-weight group (47.0% vs. 31.7%, $p = 0.08$). The odds ratio for the association between *Thr54* carriers and obesity status was estimated as 3.13 (95% confidence interval: 0.95; 10.57).

Table 2. FABP-2 genotype frequencies in normal-weight and obese adult women

	Normal *N* (%)	Obese *N* (%)	Odds ratio (95% CI)
Ala54/Ala54	15 (50)	8 (24.2)	Reference
Ala54/Thr	11 (36.7)	19 (57.6)	3.24 (1.04 to 10.07)
Thr54/Thr54	4 (13.3)	6 (18.2)	2.81 (0.61 to 12.97)
Total	30 (100)	33 (100)	

Table 3 shows the distribution of selected metabolic variables in relation to the three genotypes of *FABP2*. High plasma levels of fasting insulin, TNFα, and leptin were detected in *Thr*/*Thr* homozygote genotypes compared with the other genotypes (*Ala*/*Thr* or *Ala*/*Ala* genotypes) ($p < 0.05$) (Table 3). Importantly, there were no significant differences in BMI across genotypes.

Discussion

The results of the present study are consistent with an association between the Ala54Thr polymorphism of intestinal *FABP2* and the presence of insulin resistance and obesity. We have found a significant association between the homozygous *Thr* genotype and elevated levels of both serum TNFα and fasting insulin in obese women. Importantly, these associations were found in obese women with similar BMI values, which supports an effect of the polymorphism that is independent of its putative effect elevating the risk for obesity. To the best of our knowledge, this is the first report of an association between the Ala54Thr mutation of the *FABP2* gene and a phenotype characterized by elevated levels of an inflammatory cytokine (TNFα), obesity, and insulin resistance. These interesting findings would need to be confirmed in independent studies involving a larger number of subjects.

The frequency of the *Thr54* allele variant was estimated as 0.40 in our group of Chilean women. Slightly lower

Table 3. Summary statistics for metabolic variables according to *FABP2* genotype in obese adult women

	$FABP_2$		
	Ala54/Ala54 **(*n* = 8)**	***Ala54/Thr54*** **(*n* = 19)**	***Thr54/Thr54*** **(*n* = 6)**
Fasting insulin (pM)	161.0 ± 72.9	161.2 ± 125.7	180.0 ± 44.4*
HOMA	5.7 ± 2.0	5.8 ± 4.8	5.8 ± 1.8
Triglyceride (mM)	1.4 ± 0.5	1.8 ± 1.2	1.5 ± 0.7
TNFα (pg/mL)	20.1 ± 7.0	21.2 ± 8.4	30.0 ± 7.1*
Leptin (ng/mL)	31.6 ± 9.7	27.5 ± 7.4	39.4 ± 8.0*
BMI (kg/m^2)	37.2 ± 4.3	37.2 ± 6.4	35.4 ± 4.4
Waist-to-hip ratio	0.85 ± 0.06	0.86 ± 0.06	0.88 ± 0.02
Waist (cm)	102.3 ± 10.3	105.1 ± 14.3	103.9 ± 7.1

* Kruskal-Wallis Test $p < 0.05$.

frequency figures have been reported for other populations, such as nondiabetic Pima Indians (0.30), Koreans (0.34), Japanese (0.35), Swedish (0.30), and white individuals from the U.S. (0.32) (5,6,16–18,). The *FABP2* gene has been proposed as a candidate gene for diabetes and insulin resistance because the protein it encodes is involved in fatty acid absorption and metabolism (19). Previous studies have found a significant association between *FABP2* genotype and occurrence of type 2 diabetes or decreased insulin sensitivity (3). In Japanese men, the *Thr54Thr* genotype has been associated with higher insulin levels at baseline and 2 hours after a glucose challenge (7). Similar results have been found in the presence of the *Thr54* allele in fasting samples after correcting for BMI in Pima Indians (3). Other studies have reported significant associations between the *FABP2* locus and increased prevalence of insulin resistance in some populations (3,6,16,18).

In addition to the association of *FABP2* with insulin resistance and diabetes, it has been proposed that gain-of-function mutations of *FABP2* could result in postprandial lipid abnormalities (20). The Ala54Thr polymorphism, which results in a higher affinity of *FABP2* for long-chain fatty acids, has been associated with increased total body fat oxidation and a small elevation of plasma free fatty acid (FFA) levels; however, these results have been inconsistent. An association with higher postprandial triglyceride levels and lipoprotein extrusion has also been observed (21–23). We hypothesize that the Ala54Thr polymorphism affects the differential absorption of ω-3 and ω-6 polyunsaturated fatty acids (PUFAs), and that this, in turn, may have an impact on the production of inflammatory cytokines (24,25). PUFAs are powerful modulators of both *PPARγ2* expression (26–28) and the release of inflammatory cytokines (29,30). The balance of ω-6 (linoleic) and ω-3 (linolenic) series PUFAs [and their long-chain derivatives arachidonic acid (AA), eicosopentanoic acid, and docosohexanoic acid] is critical to the direction of these effects (29,30). PUFA ω-6/ω-3 imbalance may act through AA and its metabolites as ligands to peroxisome proliferator-activated receptor γ 2 to stimulate adipocyte differentiation (26,27,31,32). On the other hand, ω-6 PUFAs and their AA-derived eicosanoid products tend to promote inflammatory responses and block the beneficial anti-inflammatory actions of ω-3 PUFAs (28). A high ω-6-to-ω-3 ratio in circulating phospholipids and/or in stored lipids, therefore, may potentiate the release and action of pro-inflammatory mediators implicated in impairing insulin sensitivity (28). For example, elevated levels of TNFα have been shown to elevate the risk of insulin resistance by impairing β cell function and glucose homeostasis (33–35). The hypothesis of a relationship among *FABP2* Ala54Thr polymorphism, elevated inflammatory cytokines, and insulin resistance is supported by our observation of elevated TNFα and fasting insulin in the homozygous *Thr*/*Thr* genotype.

We hypothesize that the *Thr*/*Thr* homozygous genotype confers some degree of susceptibility to obesity, associated with an influence of the genotype on parameters related to lipid metabolism, insulin resistance, and inflammation components. The potential role of this genetic variant could be associated with an increased influx of FFA into circulation. As is well known, elevated FFA increases the accumulation of triglycerides in the liver (related with compensatory hyperinsulinemia, this effect is associated with high levels of fasting insulin) and in the adipocyte (related with imbalance of lipoprotein lipase activity and overproduction of adipokines such as leptin and TNF). The elevation of fasting insulin, TNFα, and leptin levels in *Thr54* carriers is a phenomenon that was not observed among normal-weight women (data not shown). This observation may indicate the

existence of complex unmeasured gene-gene or gene-environment interactions that may enhance metabolic abnormalities in obese adult women.

In summary, we propose the Ala54Thr polymorphism of *FABP2* gene may be associated with obesity and insulin resistance. These data suggest that the *FABP2* polymorphism plays an important role in the pathogenesis of at least some cases of obesity and insulin resistance. Our data strongly suggest that it could be mediated, at least in part, by the elevation of inflammatory cytokines. The differential absorption of fatty acids in the presence of the *Thr* allele is a potential mechanism involved and is a current area of research in our laboratories.

Acknowledgments

This work was supported by Grant Fondecyt 1020703 to Dr. Cecilia Albala. We would like to thank Dr. Teresa Palma for her help with blood sampling procedures.

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