Effects of an Ala54Thr polymorphism in the intestinal fatty acid-binding protein on responses to dietary fat in humans

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Abstract A polymorphism in *FABP2* that results in an alanine-to-threonine substitution at amino acid 54 of the intestinal fatty acid-binding protein (IFABP) is associated with insulin resistance in Pima Indians. In vitro, the threonine form (Thr54) has a higher binding affinity for long-chain fatty acids than does the alanine form (Ala54). We tested whether this polymorphism affected metabolic responses to dietary fat, in vivo. Eighteen healthy Pima Indians, half homozygous for the Thr54 form of IFABP and half homozygous for the Ala54 form, were studied. The groups were matched for sex, age, and body mass index. Plasma triglyceride, nonesterified fatty acid (NEFA), glucose, and insulin responses were measured after a mixed meal (35% of daily energy requirements, 50 g of fat) and after a high fat challenge (1362 kcal, 129 g of fat). NEFA concentrations were ~15% higher after the mixed meal and peaked earlier and were ~20% higher at 7 h in response to the high fat test meal in Thr54 homozygotes compared with Ala54 homozygotes. Insulin responses to the test meals tended to be higher in Thr54 homozygotes, but glucose and triglyceride responses were not different. The results of this study suggest that the Thr54 form of IFABP is associated with higher and prolonged NEFA responses to dietary fat in vivo. Higher NEFA concentrations may contribute to insulin resistance and hyperinsulinemia in individuals with this allele.—Pratley, R. E., L. Baier, D. A. Pan, A. D. Salbe, L. Storlien, E. Ravussin, and C. Bogardus. **Effects of an Ala54Thr polymorphism in the intestinal fatty acid-binding protein on responses to dietary fat in humans.** ***J. Lipid Res.*** **2000.** 41: **2002–2008.**

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Type 2 diabetes mellitus is common in the Pima Indians of Arizona (1). Obesity, insulin resistance, and defects in insulin secretion and higher nonesterified fatty acid (NEFA) concentrations are risk factors for developing diabetes in this population (2, 3) and, as in other populations, the disease is strongly familial, suggesting a significant genetic basis (1).

Preliminary studies of the genetics of diabetes and its metabolic precursors in the Pimas suggested a linkage between insulin action, determined during a hyperinsulinemic-euglycemic glucose clamp, and polymorphic DNA markers on chromosome 4q in a region containing *FABP2*, the gene encoding the intestinal fatty acid-binding protein (IFABP) (4). Subsequent analysis of *FABP2* revealed a nucleotide substitution (G→A in codon 54 of exon 2) resulting in an alanine-to-threonine substitution at amino acid 54 of the protein (5). Pima Indians with the Thr54 allele had higher fasting plasma NEFA concentrations and increased rates of lipid oxidation in vivo (5, 6). In addition, those with the Thr54 allele had higher insulin responses during oral glucose tolerance and mixed meal tests and were insulin resistant compared with those who were homozygous for the Ala54 allele (5). In other populations, the Thr54 allele was associated with increased insulin concentrations and insulin resistance in some studies (7, 8), but not in others (9–14). Ethnic differences may partly account for these disparate findings.

Human IFABP is a 15-kDa intracellular protein expressed exclusively in the columnar absorptive epithelial cells of the small intestine. IFABP is believed to bind and transport long-chain fatty acids in the cytoplasm of intestinal cells. The protein contains a single ligand-binding site that noncovalently binds saturated and unsaturated long-chain fatty acids with high affinity. The Ala54→Thr54 substitution occurs in a region of the molecule involved in fatty acid binding and in vitro studies using titration microcalorimetry demonstrated that the Thr54 form of

Abbreviations: Ala54-IFABP, intestinal fatty acid-binding protein with alanine at codon 54; ANOVA, analysis of variance; BMI, body mass index; *FABP2*, gene encoding intestinal fatty acid-binding protein; IFABP, intestinal fatty acid-binding protein; NEFA, nonesterified fatty acid; Thr54-IFABP, intestinal fatty acid-binding protein with threonine at codon 54.

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IFABP has a 2-fold higher affinity for long-chain fatty acids (oleate and arachidonate) than does the Ala54 form (5). Furthermore, Caco-2 cells expressing the Thr54 form transported long-chain fatty acids and secreted triglycerides at significantly higher rates than those expressing the Ala54 form (15).

Whether the in vitro differences in binding affinity between the Ala54 and Thr54 forms of IFABP are physiologically significant is not known, nor is it understood why Thr54-IFABP is associated with insulin resistance. The increased binding affinity of Thr54-IFABP could alter the rate of transport of fatty acids across the intestine epithelium and their subsequent appearance in plasma in response to a mixed meal. Higher NEFA concentrations could directly lead to insulin resistance in individuals with the Thr54 form of IFABP via the competitive glucose/fatty acid cycle or via feedback inhibition on the pathways of glucose disposal (16). Agren et al. (17) reported that Finnish subjects homozygous for the Thr54 allele had higher triglyceride responses in response to an oral fat-loading test than a control group homozygous for the Ala54 allele, but NEFA responses were similar in the two groups. It is also possible that differences in the affinity of the two forms of IFABP for certain long-chain fatty acids could alter the distribution of long-chain fatty acids in cell membrane phospholipids. Decreased concentrations of unsaturated fatty acids in skeletal muscle membrane phospholipids have been associated with insulin resistance in vivo (18, 19).

This study examines whether the in vitro differences in long-chain fatty acid binding affinity between the Thr54 and Ala54 forms of IFABP affect the metabolism and disposition of fatty acids in vivo in Pima Indians. Metabolic responses to dietary fat were measured on two separate occasions: after a mixed meal, containing 40% of calories as fat, and after a high fat challenge (85% of calories from fat) in a group of individuals homozygous for the Thr54 allele and a group of homozygous Ala54 controls matched for age, sex, and degree of obesity. In addition, we examined whether the polymorphism affects the fatty acid composition of membrane phospholipids in skeletal muscle and fat in these individuals.

MATERIALS AND METHODS

Subjects

Healthy Pima Indians were recruited from the Gila River Indian Community from among a group of individuals genotyped for the *FABP2* Ala54Thr polymorphism (5). Individuals homozygous for the Thr54 *FABP2* allele were matched for sex, age (±5 years), and body mass index (BMI) (±3 kg/m^2) to a group of subjects homozygous for the Ala54 allele. All subjects were in good health, as determined by a medical history, physical examination, and routine laboratory studies and all were weight stable (±2 kg) for at least 3 months prior to study. Smokers, those known to have type 2 diabetes, and those taking medications were excluded, as were individuals with hyperlipidemia (total cholesterol concentration >300 mg/dl, fasting plasma triglyceride concentration >250 mg/dl). This study was approved by the National Institutes of Health (NIH, Bethesda, MD) Institutional Review Board, and written, informed consent was obtained from all volunteers.

Subjects were admitted to the NIH Clinical Research Unit in Phoenix, Arizona and placed on a standard weight-maintaining diet. Caloric requirements for this diet were calculated according to the following formula: kcal/24 h = patient weight (kg) × 9.5 + 1,745 kcal (women) or +1,973 kcal (men). The diet contained 40% of total calories as fat, 40% as carbohydrate, and 20% as protein and was provided as a 14-day rotating menu. Body density was determined by hydrodensitometry corrected for residual lung volume, which was simultaneously measured by the helium dilution technique (Warren E. Collins, Braintree, MA) (20). Percent body fat was estimated from body density (21) and fat-free mass was calculated as the difference between total body mass and fat mass. Body fat distribution was indexed as the ratio between the waist circumference (measured while supine) and the thigh circumference (measured while standing). After at least 3 days on the weight-maintaining diet, a 3-h, 75-g oral glucose tolerance test was performed. Metabolic responses to dietary fat were assessed after at least 5 days on the standard diet.

Metabolic responses to a mixed meal

Studies were performed after a 12-h overnight fast. At 07:00 h an intravenous catheter was placed in an antecubital vein for blood sampling. After resting quietly in the supine position for 30 min, two blood samples (10 ml each) were obtained for baseline measurement of total triglycerides, NEFA, glucose, and insulin. At 08:00 h subjects consumed a standard test meal containing 35% of their calculated 24-h energy requirements distributed as 40% of total calories from fat, 40% from carbohydrate, and 20% from protein. On average, Thr54 homozygotes consumed 1,148 kcal (51 g of fat) and Ala54 homozygotes 1,125 kcal (50 g of fat). All subjects finished the meal within 15 min. Subjects fasted thereafter, but were allowed access *ad libitum* to water and noncaffeinated, noncaloric beverages. Every 2 h until 20:00 h, and then every 3 h thereafter, blood samples were collected as described above.

Metabolic responses to a high fat load

In an attempt to enhance potential differences between groups, metabolic responses to a high fat load were measured in a subset of the subjects (n = 12, six Thr54 homozygotes and their matches) on a second admission. After a 12-h overnight fast, an intravenous catheter was placed in an antecubital vein for blood sampling. Two blood samples (10 ml each) were obtained for baseline measurement of total triglycerides, NEFA, glucose, and insulin. Subjects then consumed a standard test meal that contained 1,362 calories (85% of calories from fat, 129 g of fat) prepared by mixing 350 g of heavy cream (39.5% fat), 2 tablespoons of chocolate syrup, 1 tablespoon of granulated sugar, and 1 tablespoon of nonfat dry milk. Subjects drank the high fat shake within 15 min and then fasted for the duration of the study but were allowed beverages as described above. Blood samples were obtained every hour for 10 h.

Tissue biopsies

Percutaneous needle biopsies of the vastus lateralis muscle were performed under local anesthesia (1% lidocaine) after a 12-h overnight fast as previously described (10). Muscle samples were blotted dry, quick frozen in liquid nitrogen, and stored at −70°C until analysis. Percutaneous needle biopsies of the abdominal subcutaneous adipose tissue were also performed under 1% lidocaine local anesthesia. Adipose tissue samples were immediately washed in normal saline, quick frozen in liquid nitrogen, and stored at −70°C until analysis.

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Analytical techniques

All blood samples were kept on ice and centrifuged at 4°C within several minutes of collection. Plasma glucose concentrations were measured by the glucose oxidase method (Beckman Instruments, Fullerton, CA). All other samples were frozen and stored at −20°C until assayed. Plasma insulin concentrations were measured by radioimmunoassay, using an automated instrument (Concept 4; ICN Biochemicals, Costa Mesa, CA). Triglycerides were measured by an automated enzymatic method (EXPRESS 550; Ciba-Corning, Norwood, MA) and NEFA were measured by a colorimetric assay (Wako Chemicals USA, Richmond, VA).

The fatty acid components of skeletal muscle and adipose tissue membranes were extracted and derivatized as previously described (22). Briefly, tissue was homogenized in chloroform–methanol 2:1 (v/v) and total lipid extracts were prepared. Phospholipids were separated by solid-phase extraction on silica cartridges (Waters, Milford, MA), transmethylated, and then separated and quantified by gas chromatography (22).

Statistical analyses

All statistical analyses were performed using the procedures of the SAS Institute (Cary, NC). Plasma insulin, triglyceride, and NEFA concentrations were not normally distributed, and hence the data were log transformed prior to parametric analyses. The effects of the meal and subsequent fast were tested by repeated measures analysis of variance (ANOVA). Data are expressed as means ± SEM (geometric means and 95% confidence levels for insulin, NEFA, and triglyceride concentrations) and *P* values ≤0.05 were considered significant.

RESULTS

Subjects

Nine subjects (five males and four females) homozygous for the Thr54 *FABP2* polymorphism were matched to nine subjects homozygous for the Ala54 polymorphism for sex, age, and BMI. The characteristics of the groups are summarized in **Table 1**. By design, there were no significant differences in age, height, weight, BMI, or percent body fat between the two groups. Fasting plasma glucose concentrations were <110 mg/dl in all subjects. There were no significant differences between the groups in fasting or 2-h plasma glucose or insulin concentrations during the oral glucose tolerance test.

TABLE 1. Subject characteristics

	Ala54	Thr54	*P*
Sex (M/F)	5/4	5/4	
Age (years)	36 ± 3	32 ± 3	0.39
Height (cm)	168 ± 3	170 ± 3	0.63
Weight (kg)	99 ± 6	105 ± 7	0.52
BMI (kg/m^2)	35 ± 3	37 ± 3	0.70
Body fat (%)	37 ± 4	34 ± 4	0.61
Fat mass (kg)	33 ± 5	37 ± 6	0.64
Fat-free mass (kg)	62 ± 3	67 ± 3	0.23
Waist/thigh	1.83 ± 0.05	1.71 ± 0.05	0.09
Fasting glucose (mg/dl)	88 ± 5	86 ± 2	0.87
2-Hour glucose (mg/dl)	106 ± 13	130 ± 11	0.20
Fasting insulin (μU/ml)	50 ± 8	40 ± 8	0.29
2-Hour insulin (μU/ml)	107 ± 23	162 ± 88	0.74

Values represent means ± SEM. *P* values are for unpaired *t*-tests. Insulin concentrations were $\log_{10}$ transformed prior to performing parametric analyses.

Metabolic responses to the mixed meal

There were no significant differences between Thr54 and Ala54 homozygotes in baseline fasting plasma concentrations of glucose (87 ± 2 vs. 88 ± 3 mg/dl), insulin [18 (9, 40) vs. 20 (15, 27) μU/ml], triglycerides [71 (55, 92) vs. 71 (53, 96) mg/dl], or NEFA [0.37 (0.30, 0.45) vs. 0.34 (0.28, 0.40) mM] on the morning of the mixed meal test. The metabolic responses to the mixed meal are depicted in **Fig. 1**. Plasma glucose, insulin, and triglyceride concentrations increased significantly after the meal and then returned to baseline, whereas NEFA concentrations were suppressed immediately after feeding and subsequently increased above baseline as subjects fasted. Plasma insulin concentrations tended to be higher shortly after the mixed meal in Thr54 homozygotes compared with Ala54 homozygotes [2-h insulin, 90 (40, 209) vs. 70 (45, 111) μU/ml], but this difference was not significant and during the latter hours of the study, insulin concentrations were similar in the two groups. NEFA concentrations tended to be higher in Thr54 subjects throughout the study ($P = 0.07$, repeated measures ANOVA). There were no significant differences between groups in plasma glucose or triglyceride concentrations during the study.

Metabolic responses to the high fat meal

On the morning of the high fat test meal, there were no significant group differences between the six Thr54 homozygotes and their matched controls with respect to fasting plasma concentrations of glucose (91 ± 10 vs. 96 ± 1 mg/dl), insulin [17 (9, 32) vs. 15 (9, 27) μU/ml], triglycerides [69 (49, 100) vs. 71 (45, 114) mg/dl], or NEFA [0.38 (0.30, 0.49) vs. 0.41 (0.32, 0.53) mM]. Metabolic responses to the high fat feeding are depicted in **Fig. 2**. There was no significant change in plasma glucose concentration after the high fat meal, whereas plasma concentrations of insulin, triglycerides, and NEFAs increased. There were no significant differences between the Thr54 and Ala54 homozygotes in either the glucose or the triglyceride responses to the high fat test meal. Plasma insulin concentrations were significantly higher immediately after the meal in Thr54 subjects than in the comparison group [1-h insulin, 58 (41, 81) vs. 21 (8, 55) μU/ml, $P = 0.04$, $P = 0.06$ for genotype × time interaction, repeated measures ANOVA), but were similar thereafter. NEFA concentrations were initially similar in the two groups, but peaked earlier (7 vs. 8 h) and were significantly higher at 7 h in Thr54 compared with Ala54 homozygotes [0.98 (0.80, 1.20) vs. 0.70 (0.51, 0.97) mM, $P = 0.05$].

Skeletal muscle and adipose tissue membrane phospholipid fatty acid content

There were no significant differences between Thr54 homozygotes and Ala54 homozygotes in the amount of any of the long-chain fatty acids measured in either skeletal muscle or adipose tissue membrane phospholipids (**Table 2**). The average degree of unsaturation (the unsaturation

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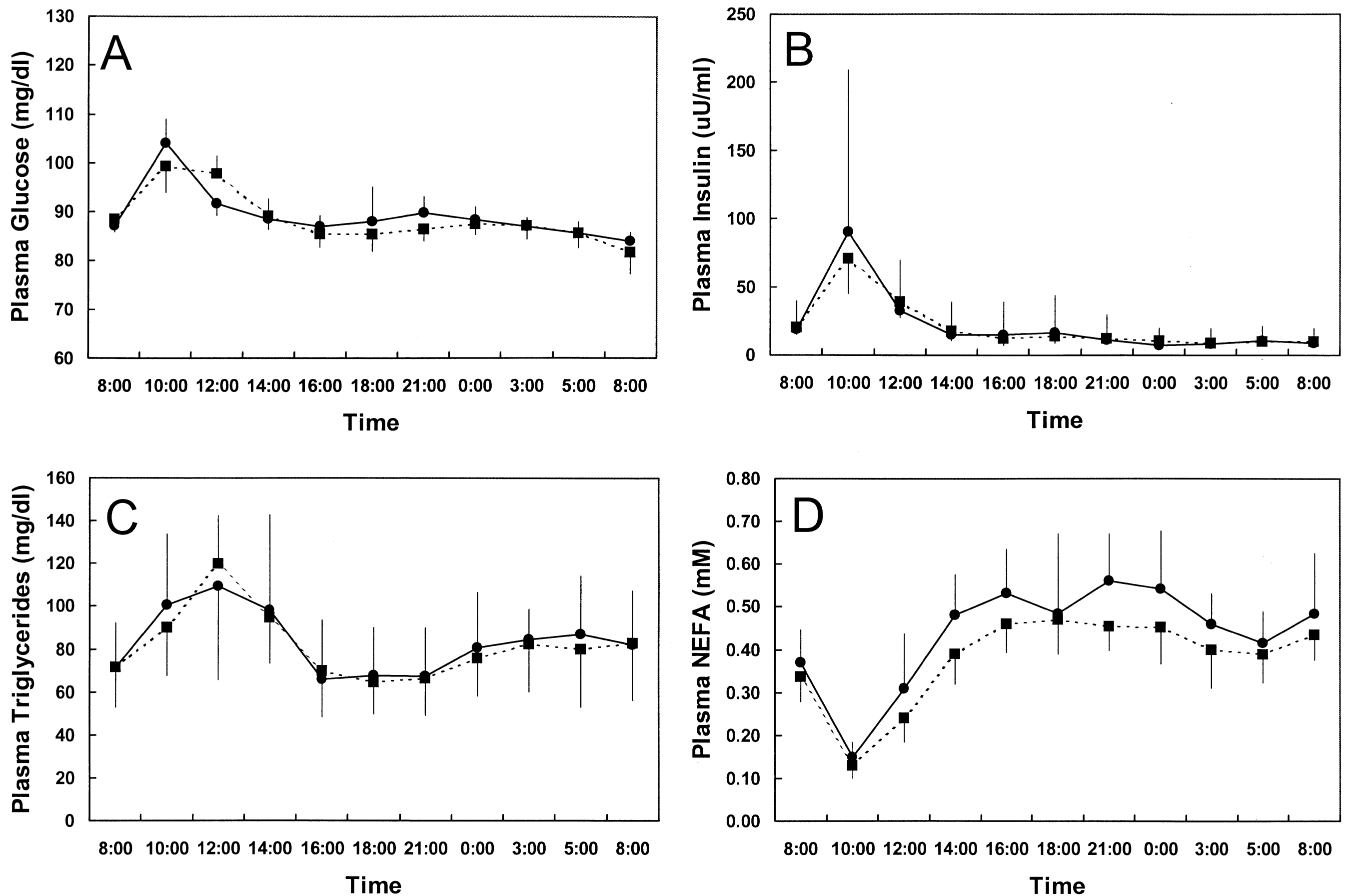

Fig. 1. Metabolic responses to mixed meal in nine Pima Indians homozygous for the Thr54 form of IFABP (solid circles, solid lines) and nine matched Pima controls homozygous for the Ala54 of IFABP (solid squares, dotted lines). (A) Plasma glucose responses (mean ± SEM, mg/dl). (B) Plasma insulin responses (geometric mean ± 95% CI, μU/ml). (C) Plasma triglyceride responses (geometric mean ± 95% CI, mg/dl). (D) Plasma NEFA responses (geometric mean ± 95% CI, mM).

index) and the percentage of long-chain polyunsaturated fatty acids with ≥20 carbon units (C_{20-22} polyunsaturated fatty acids) in skeletal muscle and fat also did not differ between the groups.

DISCUSSION

An Ala54→Thr54 substitution in the human IFABP is associated with insulin resistance, hyperinsulinemia, increased fasting plasma NEFA concentrations, and increased rates of lipid oxidation in vivo in Pima Indians (5, 6). In vitro, the Thr54 form of the protein demonstrates increased binding affinity for long-chain fatty acids (5). When expressed in Caco-2 cells, a widely used model of the intestinal epithelium, the Thr54 form enhances NEFA transport across a polarized monolayer and basolateral triglyceride secretion compared with the Ala54 form (15). On the basis of these results, it was predicted that both NEFA and triglyceride responses to a dietary fat challenge would be higher in individuals homozygous for the Thr54 allele than in persons homozygous for the Ala54 allele. We, therefore, sought to determine whether there were any differences in the in vivo metabolism and disposition of fatty acids in individuals homozygous for the two forms of IFABP. We found that NEFA tended to be higher after a mixed meal in Pima Indians homozygous for theThr54 form compared with matched Pima control subjects homozygous for the Ala54 form and peaked earlier and higher in response to a high fat test meal in Thr54 homozygotes compared with their matched controls. These differences were small, however, and no effect of the polymorphism was noted on triglyceride responses to either challenge.

We have previously reported that fasting plasma NEFA concentrations were ~13% higher in individuals with the Thr54 allele than in individuals who were homozygous for the Ala54 form of IFABP (6). Similarly, it was reported that fasting plasma NEFA concentrations tended to be higher in obese Finnish subjects with the Thr54-IFABP allele than in those with the Ala54 allele (23). In the present study, in response to a mixed meal challenge, plasma NEFA concentrations were ~15% higher, on average, in individuals homozygous for the Thr54 allele than in sex-matched, comparably obese Ala54 controls. This difference approached statistical significance ($P = 0.07$) and would likely have been significant had the sample size been larger, as it was in our prior study (6). In the present study,

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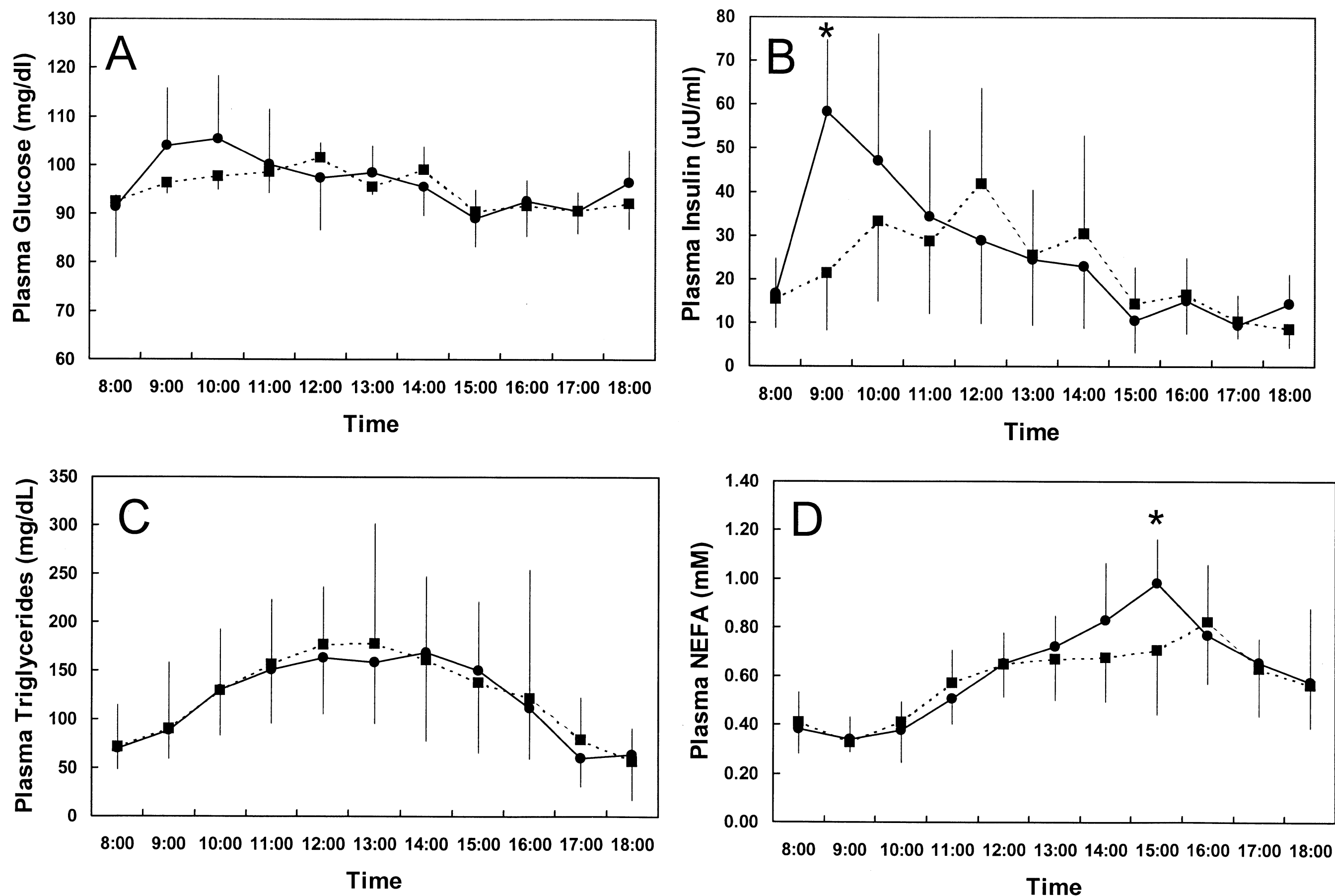

Fig. 2. Metabolic responses to a high fat challenge in six Pima Indians homozygous for the Thr54 form of IFABP (solid circles, solid lines) and six matched Pima controls homozygous for the Ala54 of IFABP (solid squares, dotted lines). (A) Plasma glucose responses (mean ± SEM, mg/dl). (B) Plasma insulin responses (geometric mean ± 95% CI, μU/ml). (C) Plasma triglyceride responses (geometric mean ± 95% CI, mg/dl). (D) Plasma NEFA responses (geometric mean ± 95% CI, mM). $^{*}P < 0.05$.

the largest differences between groups occurred later in the course of the study, after subjects had been fasting for several hours, rather than immediately after the meal. It is conceivable that such a pattern of response could occur if NEFA were bound with greater affinity to Thr54-IFABP and consequently released from the protein over a longer period of time. Alternatively, the higher free fatty acid concentrations during the later part of the test could reflect adipose tissue insulin resistance, hence less insulin-mediated suppression of lipolysis, in the Thr54 homozygotes.

In an effort to enhance potential differences between the groups, metabolic responses to a high fat load were measured in a subset of subjects. In response to the high fat challenge, NEFA concentrations peaked earlier (~7 h) and ~20% higher in Thr54 homozygotes than in Ala54 controls. The results of the present and previous studies

TABLE 2. Skeletal muscle and adipose tissue membrane phospholipid fatty acid composition and derived indices

	Skeletal Muscle			Adipose Tissue		
Fatty Acid	Ala54	Thr54	*P*	Ala54	Thr54	*P*
16:0	11.7 ± 0.6	12.3 ± 0.7	0.53	20.8 ± 0.5	21.8 ± 0.4	0.14
18:0	10.2 ± 0.4	9.6 ± 0.7	0.46	2.8 ± 0.4	2.9 ± 0.4	0.84
16:1	1.6 ± 0.4	1.7 ± 0.6	0.91	7.5 ± 0.5	7.9 ± 0.7	0.65
18:1n−9	12.2 ± 0.6	11.5 ± 1.0	0.58	49.2 ± 1.3	46.3 ± 0.6	0.06
18:1n−7	2.6 ± 0.2	3.0 ± 0.2	0.50	2.8 ± 0.6	3.9 ± 0.4	0.13
18:2	35.9 ± 0.9	35.3 ± 1.0	0.67	15.3 ± 0.8	15.1 ± 0.7	0.90
20:3n−6	2.5 ± 0.1	2.5 ± 0.2	0.79	0.2 ± 0.1	0.3 ± 0.0	0.15
20:4	19.3 ± 0.7	20.0 ± 0.7	0.52	0.5 ± 0.1	0.7 ± 0.1	0.10
Unsaturation index	190 ± 3	193 ± 3	0.57	46 ± 2	49 ± 1	0.20
C20–22 polyunsaturated	25.6 ± 0.6	26.3 ± 0.7	0.50	0.9 ± 0.2	1.3 ± 0.1	0.12

Values represent means ± SEM. *P* values for unpaired *t*-tests.

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suggest that plasma NEFA concentrations peak earlier and higher in response to a high fat meal and remain elevated in the late postprandial and postabsorptive state in Pima Indians homozygous for the Thr54 allele.

In addition to enhancing NEFA transport, the Thr54 form of IFABP increases secretion of triglycerides ~2-fold above the Ala54 form when expressed in Caco-2 cells (15). Despite these in vitro differences, the polymorphism had no apparent effects on triglyceride metabolism in vivo in Pimas. Fasting plasma triglyceride concentrations were similar in both groups, as were triglyceride responses to both the mixed meal and the high fat challenge.

Our results in Pima Indians differ from those reported by Agren et al. (17), who observed, in Finnish men and women, no differences in NEFA responses, but significantly higher triglyceride responses to an oral fat challenge in subjects homozygous for the Thr54 allele compared with those homozygous for the Ala54 allele. It is possible that the differences between these studies are due to ethnic variation. It is known, for example, that Pimas tend to have low plasma triglyceride concentrations relative to their degree of obesity. It is also possible that Agren et al. (17) demonstrated a difference in triglyceride response between Thr54 and Ala54 homozygotes because the distribution of men and women was not strictly balanced, as it was in the present study. Finally, it is possible that differences between the two studies occurred because of the relatively small numbers of subjects available, which limits the power of these studies to discern modest differences in metabolic responses to the oral fat load between Thr54 and Ala54 homozygotes.

In this, as in a previous study (5), Pimas with the Thr54 *FABP2* allele tended to have higher insulin responses to a mixed meal. In the present set of studies, the difference between groups was most marked after the high fat feeding. Higher insulin responses in individuals with the Thr54 allele may be, in part, a compensatory response to the insulin resistance we have previously documented in these individuals (5). Alternatively, it is possible that the higher NEFA concentrations in Thr54 homozygotes directly enhance pancreatic beta cell insulin secretory responses (24). Higher insulin concentrations have also been reported in Japanese men with the Thr54 allele (7) and a possible linkage between the *FABP2* locus and 2-h insulin concentrations was reported in Mexican Americans (8). One report also suggested that the Thr54 allele is associated with diabetes and aspects of the insulin resistance metabolic syndrome in a migrant Asian Indian population (9). Other groups have failed to find an association of the Thr54 *FABP2* polymorphism with insulin concentrations or insulin action, however (10–14).

In the present study, we examined whether the polymorphism in IFABP affected the distribution of long-chain fatty acids in skeletal muscle and adipocyte plasma membrane phospholipids. In Pima Indians (19), and in other populations (18), fasting plasma insulin concentrations and insulin action correlate with the degree of unsaturation in skeletal muscle membrane phospholipids. We, therefore, hypothesized that the higher binding affinity of the Thr54 form of IFABP would, over the long term, result in the selective incorporation of saturated fatty acids into membrane phospholipids and through this mechanism lead to insulin resistance and hyperinsulinemia. However, there were no apparent differences in the amounts or distribution of saturated or unsaturated fatty acids in skeletal muscle or adipose tissue membrane phospholipids in individuals homozygous for the Thr54 and Ala54 forms of *FABP2*. Similarly, Vidgren et al. (23) did not find any effect of this polymorphism on the proportions of long-chain fatty acids in serum triglycerides, cholesteryl esters, or phospholipids. Thus, it is unlikely that the association of the Thr54 isoform of IFABP with insulin resistance and hyperinsulinemia is mediated by changes in the proportions of saturated and unsaturated fatty acids in skeletal muscle or adipocyte membrane phospholipids.

Although the results of this study suggest that individuals homozygous for the Thr54 form of IFABP have higher and more prolonged plasma NEFA responses to dietary fat than individuals homozygous for the Ala54 form, the differences between groups were small and considerably less than might have been expected from in vitro studies (5, 15). Nevertheless, the differences in NEFA observed in the present study are consistent with those of previous reports (6, 23). While it has been speculated that higher plasma NEFA concentrations could explain the insulin resistance and increased insulin secretory responses observed in individuals with the Thr54 allele, it is unknown whether such small differences in NEFA are physiologically relevant. Type 2 diabetes and insulin resistance are likely to have a complex genetic basis, and the phenotypic effect of the *FABP2* Thr54 allele may only be a modifier contributing to interindividual differences. Such an effect may be more apparent in the Pima Indians, who are genetically more homogeneous than other populations in which the *FABP2* polymorphism was studied. Alternatively, it is possible that the *FABP2* Ala54Thr polymorphism itself does not cause insulin resistance, but is associated with the phenotype because it is in linkage disequilibrium with a nearby unidentified disease gene in the Pimas. This linkage may or may not be present in other populations and may explain why an association between *FABP2* and insulin resistance is not seen in all groups.

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