Thr-encoding Allele Homozygosity at Codon 54 of FABP 2 Gene May be Associated with Impaired Delta 6 Desatruase Activity and Reduced Plasma Arachidonic Acid in Obese Children

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Background: Alanine-for-threonine substitution at codon 54 (A54T polymorphism) in the fatty acid-binding protein 2 gene (FABP2) has been associated with hypertriglyceridemia and insulin resistance. Impairment in the activity of delta 6 and 5 desaturases is also supposed to be a factor predisposing the development of insulin resistance syndrome.

Aim: We investigated the relationship between A54T polymorphism in FABP2 and the impairment of long-chain polyunsaturated fatty acid metabolism in obese children.

Methods: Thirty-two obese children participated. During the study, the children continued their habitual diet, which was documented in a 3-day food record using household measures. Anthropometry was performed, and serum lipid and fatty acid composition in plasma were analyzed. The polymorphism of codon 54 in the FABP2 gene was analyzed.

Results: The allele frequency was 0.66 and 0.34 for Ala54 and Thr54, respectively. There were no significant differences in age, body mass index, fasting serum glucose, insulin or serum lipoproteins among the three polymorphism groups. There were also no significant differences in the intake of energy, the percentage of energy nutrients or in the dietary lipid composition. The content of arachidonic acid (AA) in plasma was lowest in Thr/Thr54 (p  0.05). The indices of delta-6 desaturase (D6D) activity in Thr/Thr54 were significantly lower than in Thr/Ala54 or Ala/Ala54 (p  0.05, p  0.01, respectively).

Conclusions: In obese children, Thr/Thr54 of the FABP2 gene is associated with impaired activation of D6D and reduced AA content. The results in the LCPUFA profile suggest that Thr/Thr54 may predispose the to development of insulin resistance.


Key words: Intestinal fatty acid binding protein, Obesity, Delta-6 desaturase, n-6 long chain polyunsaturated fatty acid, Arachidonic acid

Introduction

Fatty acid binding protein (FABP) plays a role of intracellular fatty acid (FA) transfer and metabolism. Intestinal FABP (I-FABP) encoded by the FABP2 gene is expressed in intestinal enterocytes. The G to A polymorphism of codon 54 of the FABP2 gene, resulting in the substitution of threonine (Thr) for alanine (Ala) in I-FABP, has been reported as showing increased affinity of I-FABP for long-chain FA and increased triglyceride secretion by Caco-2 cells. Among Pima Indians, who are known to have the highest prevalence of type 2 diabetes mellitus, the Thr encoding allele (Thr54) is associated with insulin resistance and enhanced fat oxidation rates. This polymorphism was further associated with postprandial-
al lipemic response\textsuperscript{6, 7}, which may be caused by the altered amount of absorbed FA\textsuperscript{8} and increased trafficking of fatty acids to chylomicron assembly\textsuperscript{9}. In contrast, reports in other human populations did not confirm the role of Thr54 in lipid and glucose metabolism\textsuperscript{10-12}. Moreover, the increased lipid oxidation and postprandial response associated with Thr54 may be due to various confounding in vivo factors, including insulin resistance, fasting triglyceride concentration, and various polymorphisms of apolipoprotein (apo) E, apo CII, lipoprotein lipase, and cholesteryl ester transfer protein\textsuperscript{10, 13-15}. Recent experimental studies in I-FABP-deficient mice and Caco-2 cells overexpressing human I-FABP have suggested that I-FABP is not essential for dietary fat absorption\textsuperscript{16, 17}.

On the other hand, many studies have shown the characteristic long chain polyunsaturated fatty acid (LCPUFA) profile in obesity. Decsi \textit{et al.} reported that linoleic acid (LA) metabolism is activated in obese children\textsuperscript{18}. The data from obese animal experiments reported by Phinney \textit{et al.} showed decreased levels of LA and increased levels of dihomo gamma linolenic acid (DHGLA) and no difference in arachidonic acid (AA) compared with non-obese mice\textsuperscript{19}. These results indicated that increased FA load accompanied with obesity may accelerate beta-oxidation, resulting in increased degradation of AA\textsuperscript{20}. With activated beta-oxidation, obese subjects may need to compensate for the increased demand of AA by activating the n-6 LCPUFA metabolic pathway from LA to AA. Impairment of this compensatory mechanism may be another feature of insulin resistance\textsuperscript{21}. Das also proposed in a recent review that a defect in the activity of D6D and delta-5 desaturase (D5D) may be a factor predisposing the development of insulin resistance syndrome\textsuperscript{22}. We already have data supposing impaired D6D activity in obese children with low levels of serum AA (data not shown).

In this study, we investigated the effect of I-FABP gene polymorphism on the n-6 LCPUFA metabolic pathway, especially on D6D activity and plasma AA content, in obese children, and its possible role in the development of insulin resistance.

**Subjects and Methods**

Informed consent for this study was obtained from each child and all parents. This study was performed according to the Declaration of Helsinki 1964. Thirty-two children (22 boys and 10 girls, 12.0 \( \pm \) 3.0 years old (mean \( \pm \) SD), relative body weight, 77.8 \( \pm \) 22.7\%) were recruited from our out-patient clinic. During the study, the children continued their habitual diet, which was documented in a 3-day food record.

Blood samples were taken from the antecubital vein after overnight fasting. The polymorphism of codon 54 (Ala or Thr) in the FABP 2 gene was analyzed by the method of Baier \textit{et al.}\textsuperscript{3}. PCR products (274bp) in exon 2 of the FABP 2 gene were digested with \textit{Hha I}, and the digested fragments were detected by 3% agarose electrophoresis.

Serum insulin levels (IRI) were determined by the liquid phase method using a commercial kit (Insulin RIABEAD Kit, Dainabott). Serum leptin was determined using a Human Leptin RIA Kit (LINCO Research, Inc.). Measurements of plasma total cholesterol, triglyceride, and HDL-cholesterol concentrations were performed with a Hitachi 7450 automated analyzer using commercial kits. LDL-cholesterol concentration was calculated according to Friedewald \textit{et al.}\textsuperscript{23}.

Fatty acid analysis was performed by gas chromatography. The ratios of n-6 series 20:4/18:2 express the activity of the n-6 LCPUFA metabolic pathway from LA to AA. The ratio of (18:3 + 20:3)/18:2 represents D6D activity. Fatty acid results were expressed as percentages of fatty acids detected with a chain length between 12 and 24 carbon atoms (%w/w).

**Statistical Analyses**

Results were evaluated with the SAS statistical package (Statview 5, SAS Institute Inc, Cary NC, USA). The mean values were compared by Kruskal-Wallis test among three polymorphism groups. \( p \) values less than 0.05 were considered statistically significant.

**Results**

**Characteristics of the Subjects (Table 1)**

There were 15 children homozygous for the Ala54, 5 children homozygous for Thr54 and 12 children heterozygous (Ala/Thr54). The allele frequency was 0.66 and 0.34 for Ala and Thr, respectively. There were no significant differences in age, body mass index (BMI), fasting serum glucose, insulin or serum lipoproteins among the three polymorphism groups (Table 1). No significant difference was shown in the daily intake of energy, percentage of energy nutrients or the dietary lipid composition.

**Plasma FA Profile Classified by FABP 2 gene Polymorphism at Codon 54 (Table 2)**

There was no significant difference in the level of linoleic acid (LA); however, the level of AA was lowest in Thr/Thr54 \( (p=0.0342) \) among the three groups. The indices of n-6 LCPUFA pathway and D6D activity were significantly lower among the three groups \( (p=0.0448, p=0.0091) \).
Discussion

In this study, the content of AA in plasma and the D6D activity index were significantly lower in obese children homozygous for Thr54 of the FABP 2 gene than those homozygous for Ala54, although no difference in the level of total fatty acid, fasting glucose or insulin was demonstrated among the groups. These results suggested that the reduced amount of AA in obese children was caused by impaired n-6 LCPUFA metabolism with reduced activation of D6D, and that the polymorphism in the FABP 2 gene was closely related with the regulation of D6D activity.

It has been described that insulin sensitivity was associated with AA content and desaturase activity\(^22\), and AA facilitates glucose uptake mediated by insulin action\(^20\). In this study, serum insulin and glucose levels were not different among FABP 2 gene polymorphism groups. However, the indices of activity in n-6 LCPUFA and D6D were reduced in children homozygous for Thr54, suggesting that their AA demand was not compensated (Table 2). There seemed to be a discrepancy in serum levels between insulin, and glucose and fatty acid compositions in the early phase of obesity\(^27\). Their low AA content may affect insulin-mediated glucose uptake, and may cause insulin resistance. This possible mechanism is compatible with the recent review by Das, who proposed that a defect in the activity of delta 6 and delta 5 desaturases is a factor predisposing the development of insulin resistance syndrome\(^22\). Gasperikova et al. also suggested that the impairment of D6D activation with a reduced level of AA was a feature of insulin resistance in the study of hereditary hypertriglyceridemic rats, an animal model of insulin resistance\(^11\).

The relation between FABP 2 gene polymorphism and FA composition \textit{in vivo} has been previously investigated. A polymorphism at codon 54 did not affect serum fatty acid composition in obese adult Finns\(^2\), and in healthy Pima Indians there were no significant differences between Thr54 homozygotes and Ala54 homozygotes in the amount of any LCPUA measured in either skeletal muscle or adipose tissue membrane phospholipids\(^28\). These results seem inconsistent with our findings; however, our study was in children. The discrepancy between these studies and our results may be related to the different study subjects. The former study was conducted on obese subjects, aged 24-56 years. In the latter study, Pima Indians may have other genetic predispositions to develop insulin resistance. In oral fat-loading tests in adults, the postprandial response of 14-18-carbon FAs in chylomicron and VLDL triacylglycerols was significantly elevated in Thr54 homozygotes, whereas the amount of 20- and 22-carbon PU-
FAs was not significantly different. In the high-fat meal test, plasma insulin level was significantly higher immediately after the meal in Thr54 subjects, although there were no significant differences in either glucose or triglyceride responses. Furthermore, the type of dietary fat affects insulin sensitivity in healthy human subjects with the Thr54 allele. These results suggest that FABP 2 gene polymorphism plays a role in the regulation of insulin sensitivity in some conditions. Further studies are necessary to clarify the role of FABP 2 gene polymorphism in FA profile, D6D activation and insulin sensitivity, as a genetic predisposition to diabetes.

In summary, our study showed that the homozygous Thr54 genotype of the FABP 2 gene is associated with impaired activation of D6D and reduced AA content in obese children. Thr/Thr54 may predispose the development of insulin resistance when children become obese.

### Table 2. Major LCP fatty acid composition classified with 1-FABP allele in obese children

<table>
<thead>
<tr>
<th></th>
<th>Ala/Ala n=15</th>
<th>Thr/Ala n=12</th>
<th>Thr/Thr n=5</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14:0</td>
<td>1.14 ± 0.28</td>
<td>1.22 ± 0.43</td>
<td>0.99 ± 0.37</td>
<td>ns</td>
</tr>
<tr>
<td>C16:0</td>
<td>25.4 ± 2.6</td>
<td>25.1 ± 2.1</td>
<td>24.8 ± 1.6</td>
<td>ns</td>
</tr>
<tr>
<td>C16:1n-7</td>
<td>3.00 ± 0.60</td>
<td>2.70 ± 1.10</td>
<td>2.50 ± 0.40</td>
<td>ns</td>
</tr>
<tr>
<td>C18:0</td>
<td>7.89 ± 0.68</td>
<td>7.67 ± 0.86</td>
<td>7.16 ± 0.55</td>
<td>ns</td>
</tr>
<tr>
<td>C18:1n-9</td>
<td>21.5 ± 1.60</td>
<td>21.5 ± 2.40</td>
<td>22.9 ± 2.40</td>
<td>ns</td>
</tr>
<tr>
<td>C18:2n-6</td>
<td>25.9 ± 3.40</td>
<td>27.5 ± 3.60</td>
<td>27.8 ± 3.10</td>
<td>ns</td>
</tr>
<tr>
<td>C18:3n-6</td>
<td>0.53 ± 0.20</td>
<td>0.48 ± 0.18</td>
<td>0.35 ± 0.13</td>
<td>ns</td>
</tr>
<tr>
<td>C18:3n-3</td>
<td>0.42 ± 0.10</td>
<td>0.42 ± 0.11</td>
<td>0.62 ± 0.26</td>
<td>ns</td>
</tr>
<tr>
<td>C20:0</td>
<td>0.24 ± 0.05</td>
<td>0.21 ± 0.04</td>
<td>0.22 ± 0.05</td>
<td>ns</td>
</tr>
<tr>
<td>C20:1n-9</td>
<td>0.16 ± 0.03</td>
<td>0.17 ± 0.05</td>
<td>0.21 ± 0.05</td>
<td>ns</td>
</tr>
<tr>
<td>C20:2n-6</td>
<td>0.30 ± 0.06</td>
<td>0.27 ± 0.05</td>
<td>0.30 ± 0.05</td>
<td>ns</td>
</tr>
<tr>
<td>C20:3n-9</td>
<td>0.21 ± 0.04</td>
<td>0.19 ± 0.04</td>
<td>0.17 ± 0.04</td>
<td>ns</td>
</tr>
<tr>
<td>C20:3n-6</td>
<td>1.49 ± 0.35</td>
<td>1.26 ± 0.34</td>
<td>1.14 ± 0.34</td>
<td>ns</td>
</tr>
<tr>
<td>C20:4n-6</td>
<td>5.24 ± 0.87</td>
<td>5.24 ± 0.86</td>
<td>4.15 ± 0.94</td>
<td>0.0342 *</td>
</tr>
<tr>
<td>C20:5n-3</td>
<td>1.35 ± 0.60</td>
<td>1.08 ± 0.43</td>
<td>1.33 ± 1.03</td>
<td>ns</td>
</tr>
<tr>
<td>C22:0</td>
<td>0.48 ± 0.09</td>
<td>0.45 ± 0.08</td>
<td>0.48 ± 0.06</td>
<td>ns</td>
</tr>
<tr>
<td>C22:4n-6</td>
<td>0.19 ± 0.06</td>
<td>0.17 ± 0.04</td>
<td>0.06 ± 0.12</td>
<td>ns</td>
</tr>
<tr>
<td>C22:5n-3</td>
<td>0.41 ± 0.10</td>
<td>0.39 ± 0.05</td>
<td>0.44 ± 0.20</td>
<td>ns</td>
</tr>
<tr>
<td>C22:6n-3</td>
<td>3.25 ± 0.66</td>
<td>3.05 ± 0.63</td>
<td>3.18 ± 1.38</td>
<td>ns</td>
</tr>
<tr>
<td>C24:0</td>
<td>0.37 ± 0.12</td>
<td>0.30 ± 0.05</td>
<td>0.29 ± 0.04</td>
<td>ns</td>
</tr>
<tr>
<td>C24:1n-9</td>
<td>0.88 ± 0.20</td>
<td>0.80 ± 0.20</td>
<td>0.87 ± 0.20</td>
<td>ns</td>
</tr>
<tr>
<td>SFA (%)</td>
<td>35.5 ± 2.4</td>
<td>34.9 ± 2.2</td>
<td>35.5 ± 2.4</td>
<td>ns</td>
</tr>
<tr>
<td>MUFA (%)</td>
<td>25.1 ± 1.9</td>
<td>25.7 ± 3.0</td>
<td>26.4 ± 2.2</td>
<td>ns</td>
</tr>
<tr>
<td>n-3PUFA (%)</td>
<td>5.55 ± 1.2</td>
<td>5.50 ± 1.0</td>
<td>5.92 ± 2.7</td>
<td>ns</td>
</tr>
<tr>
<td>n-6PUFA (%)</td>
<td>33.2 ± 3.8</td>
<td>34.5 ± 4.2</td>
<td>33.3 ± 3.5</td>
<td>ns</td>
</tr>
<tr>
<td>PUFA (%)</td>
<td>38.9 ± 3.5</td>
<td>39.1 ± 4.5</td>
<td>39.2 ± 2.7</td>
<td>ns</td>
</tr>
<tr>
<td>C20:4/C18:2</td>
<td>0.21 ± 0.04</td>
<td>0.19 ± 0.03</td>
<td>0.15 ± 0.04</td>
<td>0.0448</td>
</tr>
<tr>
<td>(C18:3 + C20:3)/C18:2</td>
<td>0.08 ± 0.02</td>
<td>0.07 ± 0.02</td>
<td>0.05 ± 0.02</td>
<td>0.0091</td>
</tr>
</tbody>
</table>

Kruskal-Wallis test mean ± SD %w/w

**Acknowledgements**

This research was supported by Health and Labour Sciences Research Grants: Comprehensive Research on Cardiovascular Diseases, #17160501 in Japan.

We are grateful to Dr. Manabu T. Nakamura (Department of Human Nutrition, University of Illinois at Urbana-Champaign) for advice in preparing this manuscript.

None of the authors had any financial or personal conflicts of interest.

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196

Okada et al.


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