Human Intestinal Fatty Acid Binding Protein 2 Expression Is Associated with Fat Intake and Polymorphisms

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Abstract

The intestinal fatty acid binding protein (FABP2) is involved in lipid metabolism whereby variations in the promoter (haplotypes A/B) and exon 2 (Ala54Thr) are associated with dyslipidemia and insulin resistance. To elucidate which factors determine FABP2 expression in human mucosa, we investigated the association between fat intake, genotypes, biochemical variables, and FABP2 expression. FABP2 gene expression was assessed in duodenal specimens from 100 participants who answered a FFQ and who were genotyped and characterized for traits of metabolic syndrome and further biochemical data. Homozygotes for haplotype A tended to have lower fat intake than B-allele carriers (P = 0.066). Searching for an explanation, we evaluated the orexigenic glucose-dependent insulinotropic polypeptide (GIP) in a subset from the Metabolic Intervention Cohort Kiel. AA homozygotes had lower postprandial GIP concentrations than BB homozygotes. Duodenal FABP2 expression was correlated with (n-3) fatty acid (FA) intake in AA homozygotes (r = 0.49; P = 0.021). It was higher in AA homozygotes than in B-allele carriers after adjustment for (n-3) FA intake (P = 0.049) and was negatively correlated with serum FFA (r = -0.41; P < 0.01). Our data indicate that FABP2 expression depends on (n-3) FA intake and FABP2 genotypes. FABP2 might be involved in regulating food intake and intestinal FA utilization. J. Nutr. 140: 1411–1417, 2010.

Introduction

The intestinal fatty acid binding protein (FABP) is encoded by the FABP2 gene and its expression is confined to the mature enterocytes of the small intestine, whereas only marginal amounts have been identified in the stomach and large intestine in rodents (1). FABP2 gene deletion in mice resulted in increased weight gain and higher circulating triglyceride concentrations compared with wild-type mice (2). Moreover, the redistribution of FABP2 from the apical region to the entire cytoplasm after fat feeding pointed to the involvement of FABP2 in the intracellular binding, transport, and metabolism of fatty acids (3). FABP2 expression increased by 100% after 3 d of feed deprivation and slightly increased after a high-fat diet (4) but remained unaltered by a 7-d fat feeding in mice (5). Decreased expression of FABP2 protein, but not of FABP2 mRNA, was observed in lipid malabsorption syndromes in humans (6).

A polymorphism in the human FABP2 gene, which results in a substitution of alanine (Ala) with threonine (Thr) at codon 54, is associated with dyslipidemia, insulin resistance, and obesity (7–9). The Thr54-allele is linked to the previously described promoter haplotype B (10,11). Accordingly, allele B was associated with a higher BMI in females (12) and with a higher LDL-cholesterol (LDL-C):HDL-cholesterol (HDL-C) ratio in males (11). This haplotype defines 2 promoter variants: haplotype A and B (10,12–14). Interestingly, only participants who were homozygous for both allele B and the Thr54-allele had higher concentrations of postprandial triglycerides and insulin (11).

In this study, we aimed to elucidate which factors determine FABP2 gene expression. For this purpose, we investigated the
following: 1) the local distribution of FABP2 expression along the gastrointestinal tract in humans; 2) the differences in food intake between promoter haplotypes and 3) the basis of such differences by reevaluating the postprandial concentrations of the potential orexigenic glucose-dependent insulinotropic polypeptide (GIP) in a subset from a Metabolic Intervention Cohort Study (MICK); 4) the associations of dietary fat intake, genotypes, and human FABP2 expression; and 5) the effects of FABP2 expression and genotypes on biochemical variables.

Materials and Methods

Participants and study design. One hundred participants were recruited among patients undergoing upper endoscopy for medical reasons (abdominal pain, exclusion of carcinoma, celiac disease, gastrointestinal bleeding, dysphagia, suspicion or control of esophageal varices, reflux, and nausea) in the Gastroenterological Unit of the University Clinic of Kiel. No participant had diabetes mellitus type 1, but 7 had diabetes mellitus type 2 (n (AA) = 3; n (AB) = 3; n (BB) = 1; n (Ala54Ala) = 5; n (Ala54Thr) = 2). Exclusion criteria were: portal hypertension, abnormal coagulation, and other risk factors for bleeding, short bowel syndrome, celiac disease, Crohn’s disease, severe exocrine pancreatic insufficiency, and gastrectomy. Of the 100 participants, 14 underwent dietary data and were excluded. No participant took known PPAR ligands (e.g. lipid modifying drugs of the fibrate family).

All participants gave written informed consent to participate in this study, which was approved by the ethics committee of the medical faculty, Christian-Albrechts-University, Kiel (protocol A120/05).

Tissue biopsies. In addition to medically indicated biopsies, 4 duodenal biopsies were taken from each participant after fasting overnight. Ileal and colonic biopsies from 4 participants who underwent gastroscopy and coloscopy were obtained in addition to the duodenal biopsies. Specimens were frozen in liquid nitrogen and stored at −80°C until analysis.

RNA isolation and real-time PCR analysis. Total RNA was isolated, verified, and reverse transcribed as described previously (15) and mRNA was quantified by real-time PCR (Applied Biosystems Prism 7000 system). Results were normalized to β-actin expression (Applied Biosystems). FABP2 primer sequences were as follows: forward primer: 5'-GTTTGAGCATGGTTGGAACCTAGA-3'; reverse primer: 5'-AAG TTCACTCCAAAAATGTCCTTA-3'. The sequence of the fluorescence probe was 5'-GGGATGAAACTATG-3'. For carnitine palmitoyltransferase 2 expression, the TaqMan probe Hs00264677_m1 was used.

The thermal cycling conditions were as follows: 2 min at 50°C, 10 min at 95°C, followed by 40 cycles of amplification for 15 s at 95°C and 1 min at 60°C.

DNA extraction and genotyping. Genomic DNA was purified using the NucleoSpin Blood Isolation kit (Macherey-Nagel). Variations in the promoter (rs2282688) and exon 2 (rs1799883) of the FABP2 gene were typed by TaqMan analysis as described elsewhere (11).

Dietary, anthropometric, and lifestyle variables. The participants’ habitual dietary intake was assessed by the European Prospective Investigation into Cancer and Nutrition FFQ (16). To identify underreporting, the ratio between energy intake and the basal metabolic rate <1.14 was used as fixed cutoff (17). Lifestyle habits, medical history, body weight, height, and waist circumference were assessed during a personal interview. Participants were asked about gastrointestinal symptoms.

Biochemical variables. Blood samples were collected from participants before gastroscopy. Plasma triglycerides, cholesterol, LDL-C, HDL-C, and glucose were assessed enzymatically in lithium-heparin-plasma using a clinical laboratory analyzer (Konelab). Serum insulin was analyzed by RIA (Adaltis) and serum FFA determined by an enzymatic assay (Wako). The homeostasis model assessment for insulin resistance was calculated as [glucose (mmol/L) × insulin (µU/L)]/22.5 (18). The fatty acid (FA) composition of serum cholesterol esters (CE) was measured by GC as previously described (19).

MICK cohort. The design and methodology of the population-based cohort, MICK (n = 1508) have been described elsewhere (11). AA homozygotes (n = 32) were matched on the basis of BMI with BB homozygotes. They underwent an oral metabolic tolerance test (20). GIP was measured in serum over 9 h using an enzyme immunoassay (Phoenix). The area under the curve was calculated by the trapezoidal method.

Cell culture studies. Caco-2 cells (DSMZ) were used between passage 10 and 13 and cultured in minimal essential media (Invitrogen) supplemented with 20% fetal calf serum (Invitrogen) and 1 mmol/L nonessential amino acids (PAA) in a humidified incubator at 37°C in an atmosphere of 5% CO2.

Sequencing of the FABP2 promoter. A 1057-bp fragment upstream from the initiation codon of the FABP2 promoter was sequenced. Sequencing was performed by terminator cycle sequencing using an ABI 3700 capillary DNA sequencer (Applied Biosystems). Sequences of PCR primers (Eurofins MWG Operon) were as follows: 5'GC TTCTTTTCTGACCCCTT3 and 5'CAGAATGCTGCTACACCGC3.

FABP2 promoter-reporter constructs. The Dual-Luciferase Reporter Assay System was used (Promega). Genomic DNA was used to amplify a FABP2 promoter segment for −1066 to −1 relative to the transcription start site: AA/−834CC, AA/−834TT, BB/−834CG, and BB/−834TTT. The following primers containing the restriction site were used: FABP2-KpnI-F: 5’CTGGTTACCCCTTCCTCTGAGCCCTTAG 3’, FABP2-Xhali-R: 5’CAGCTCAAAGTATCTTTGATCAGCC3’. The fragments were ligated (Rapid Ligation kit; Fermentas) with KpnI Bgl II double-digested pGL4.10 reporter vector (Promega) and transformed into competent DH5α cells. Recombinant plasmid DNA was purified (Fermentas) and confirmed by sequencing.

Transfection and luciferase reporter assay. The transfection and reporter assay has been described in detail elsewhere (21) with minor modifications: 24 h prior to transfection, 4 x 10³ Caco-2 cells were seeded on 96-well plates and luciferase activities were measured 96 h after transfection.

Statistical analysis. B-allele (AB+BB) carriers and Thr-54 carriers (Ala54Thr+Thr54Thr), respectively, were combined for statistical analysis, because they were considered to be the dominant allele. The Mann-Whitney U-test was used to test for associations between genotypes and metabolic features. Spearman correlation coefficient was chosen for correlation analyses. Chi-square tests were employed to test for Hardy-Weinberg equilibrium. We calculated the pairwise haplotype frequencies and subsequently measured the commonly used measures D' and r² (Pearson correlation coefficient) (22).

To investigate the association of dietary fat intake and FABP2 expression, a linear regression analysis was performed with FABP2 expression as the dependent variable. The probability plot of the standardized residuals is close to normal. The β-coefficients for the regression of the various types of fat with FABP2 expression were tested by multiple linear regression models using the General Linear Model procedure whereby the fat intake was nested within genotypes, each in an individual model. All models were controlled for gender, age, BMI, and total energy intake. To test for no interaction between the FABP2 genotypes and dietary fat intake (continuous) on FABP2 expression, interaction terms were included in the models. Heterogeneity of allelic effects due to gender and environmental factors were assessed by corresponding interaction terms in a linear regression model. Men and women were analyzed conjointly because no heterogeneity of allelic effect by sex was detected. The results are presented as median (1st, 3rd
quartile) and GIP data are presented as mean ± SEM. For all tests, \( P \)-values < 0.05 were considered significant. Statistical analyses were performed with StatgraphicsPlus (Machintosh, version 4.1).

**Results**

**FABP2 genotype frequencies.** The genotype frequency of FABP2 promoter variability (rs2282688) was: AA, 0.27 \( (n = 23) \); AB, 0.52 \( (n = 45) \); and BB, 0.21 \( (n = 18) \). The genotype frequency of the FABP2 Ala54Thr polymorphism (rs1799883) was: Ala54Ala, 0.64 \( (n = 55) \); Ala54Thr, 0.26 \( (n = 22) \); and Thr54Thr, 0.10 \( (n = 9) \). No deviations from Hardy-Weinberg equilibrium were observed. The polymorphisms FABP2 Ala54Thr and haplotype A/B showed intermediate concentrations of gametic linkage disequilibrium: \( D' = 0.95, r^2 = 0.34 \). The genotypic concordance between both single nucleotide polymorphisms (SNP) equaled 0.65.

**FABP2 gene expression in duodenum, ileum, and colon.** Due to the lack of FABP2 expression in humans, we analyzed duodenal, ileum, and colon biopsy samples. FABP2 expression was intermediate in the duodenum \( (0.56 \text{ AU}) \), highest in the ileum \( (0.83 \text{ AU}) \), and close to zero in the colon \( (0.04 \text{ AU}) \).

**FABP2 promoter haplotypes are associated with differing eating behaviors.** Habitual food intake was investigated, because the Ala54Thr polymorphism was associated with food intake in a Japanese cohort \( (23) \). We validated the FFQ by analyzing the FA composition of serum CE. The estimates of FA intake in a Japanese cohort \( (23) \). We validated the FFQ by analyzing the FA composition of serum CE. The estimates of FA intake from the FFQ were positively correlated with CE SFA \( (r = 0.29; P = 0.031) \) and PUFA \( (r = 0.28; P = 0.042) \) but not with CE monounsaturated FA \( (\text{MUFA}) \) \( (r = 0.15; P = 0.164) \).

**Table 1**

| TABLE 1 Association of FABP2 gene variants with anthropometric data, biochemical variables, and reported daily nutrient intakes

<table>
<thead>
<tr>
<th></th>
<th>All participants</th>
<th>Promoter haplotypes</th>
<th>Exon polymorphism Ala54Thr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( n )</td>
<td>AA</td>
<td>AB+BB</td>
</tr>
<tr>
<td>Men/women</td>
<td>52/24</td>
<td>23</td>
<td>63</td>
</tr>
<tr>
<td>Age, y</td>
<td>54 (42, 64)</td>
<td>58 (36, 63)</td>
<td>53 (43, 68)</td>
</tr>
<tr>
<td>BMI, kg/m(^2)</td>
<td>24.9 (23.1, 28.3)</td>
<td>24.4 (22.0, 27.5)</td>
<td>25.5 (23.5, 28.4)</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>90.1 (77.8, 100.3)</td>
<td>88.0 (75.1, 107.5)</td>
<td>91.0 (78.1, 99.0)</td>
</tr>
<tr>
<td>Serum FFA, mmol/l</td>
<td>0.54 (0.39, 0.86)</td>
<td>0.51 (0.29, 0.90)</td>
<td>0.55 (0.39, 0.72)</td>
</tr>
<tr>
<td>Plasma triglycerides, mmol/l</td>
<td>1.17 (0.85, 1.59)</td>
<td>1.29 (0.83, 1.68)</td>
<td>1.17 (0.86, 1.57)</td>
</tr>
<tr>
<td>Plasma cholesterol, mmol/l</td>
<td>4.78 (3.99, 5.58)</td>
<td>4.67 (3.86, 4.99)</td>
<td>4.82 (4.16, 5.60)</td>
</tr>
<tr>
<td>Plasma LDL-C, mmol/l</td>
<td>3.02 (2.37, 3.51)</td>
<td>2.65 (1.98, 3.11)</td>
<td>3.14 (2.41, 3.71)</td>
</tr>
<tr>
<td>Plasma HDL-C, mmol/l</td>
<td>1.25 (1.00, 1.65)</td>
<td>1.59 (1.16, 2.00)</td>
<td>1.21 (0.96, 1.45)</td>
</tr>
<tr>
<td>Plasma glucose, mmol/l</td>
<td>5.35 (4.84, 5.93)</td>
<td>5.52 (4.71, 5.71)</td>
<td>5.27 (4.85, 6.03)</td>
</tr>
<tr>
<td>Serum insulin, mUI/l</td>
<td>86.8 (62.5, 111.8)</td>
<td>88.9 (61.1, 111.1)</td>
<td>86.8 (62.5, 113.2)</td>
</tr>
<tr>
<td>Homeostasis model assessment</td>
<td>2.9 (2.0, 4.3)</td>
<td>2.4 (2.0, 4.4)</td>
<td>2.9 (2.0, 4.2)</td>
</tr>
</tbody>
</table>

1 Values are the estimated median \((1\text{st}, 3\text{rd}\text{ quartile})\), \( n = 86 \). P-values for differences between genotypes.

Ala54 homozygotes had a lower \((n-3)\) FA intake than Thr54-allele carriers \( (P = 0.023) \) and tended to have a lower PUFA intake \( (P = 0.052) \) \( (\text{Table 1}) \). Fat intake tended to be less \((27\%)\) in haplotype AA compared with B-allele carriers \( (P = 0.066) \). The consumption of SFA, MUFA, and \((n-3)\) FA tended to be lower in AA homozygotes than in AB+BB participants \( (\text{Table 1}) \). Dietary fat intake did not differ when expressed as a percentage of energy intakes.

When searching for an explanation for the lower fat intake in AA homozygotes \( (\text{Table 1}) \), postprandial GIP concentrations, a potentially orexigenic gut-derived hormone \( (24,25) \), in a subset of the MICK were reevaluated and AA and BB homozygotes were compared. AA homozygotes had lower postprandial GIP concentrations than BB homozygotes following a mixed meal \( (\text{Fig. 1}) \). Based on the area under the curve, the postprandial GIP concentration tended to be lower in AA \( (22.7 ± 2.6 \mu\text{g/L}) \) than in BB homozygotes \( (27.9 ± 2.6 \mu\text{g/L}) \) \( (P = 0.061) \), whereas concentrations in Ala54- \((23.4 ± 1.5 \mu\text{g/L}) \) and Thr54-homozygotes \( (25.4 ± 2.1 \mu\text{g/L}) \) did not differ \( (P = 0.880) \).

**Table 2**

<table>
<thead>
<tr>
<th>Dietary fat intake (g/d)</th>
<th>SFA</th>
<th>MUFA</th>
<th>PUFA</th>
<th>((n-3)) FA</th>
<th>((n-6)) FA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein, g/d</td>
<td>82.2</td>
<td>38.4</td>
<td>16.8</td>
<td>2.6</td>
<td>13.8</td>
</tr>
<tr>
<td>Fat, g/d</td>
<td>96.8</td>
<td>37.4</td>
<td>32.6</td>
<td>2.0</td>
<td>13.0</td>
</tr>
<tr>
<td>Carbohydrate, g/d</td>
<td>254.5</td>
<td>37.4</td>
<td>32.6</td>
<td>2.0</td>
<td>13.0</td>
</tr>
<tr>
<td>Energy, kcal/d</td>
<td>2939</td>
<td>374.5</td>
<td>32.6</td>
<td>2.0</td>
<td>13.0</td>
</tr>
<tr>
<td>Fat, % of energy</td>
<td>32.6</td>
<td>37.4</td>
<td>32.6</td>
<td>2.0</td>
<td>13.0</td>
</tr>
</tbody>
</table>

**Effects of dietary long-chain FA on FABP2 gene expression.** FABP expression has been shown to be under the control of ingested fat in animal studies. Because data in humans are lacking, we investigated whether dietary fat ingestion affects FABP2 expression. Because FABP2 binds long-chain FA \( (\text{LCFA}) \) with high affinity, we assumed there would be a correlation between the intake of LCFA and FABP2 expression, but this was not the case \( (r = -0.13; P = 0.221) \).

**Effects of dietary \((n-3)\) FA and genotypes on FABP2 gene expression.** We searched for other potential modulators and classified the ingested FA according to their degree of saturation.
We found a significant correlation between (n-3) FA intake and FABP2 only in AA homozygotes ($r = 0.49$; $P = 0.021$).

In agreement with the correlation results, (n-3) FA intake was significantly associated with gene expression only in AA homozygotes after controlling for confounders (sex, age, BMI, total energy intake); a 1-g/d increase in (n-3) FA intake was associated with an increase in FABP2 expression by 0.612 AU ($P < 0.05$) (Table 2). Accordingly, an interaction effect was observed between (n-3) FA ingestion and FABP2 promoter haplotypes ($P = 0.012$) in determining FABP2 expression. There were no significant associations for the Ala54Thr polymorphism (Table 2).

**Effects of dietary factors and promoter haplotype on FABP2 gene expression.** Reporter-gene studies showed a higher transcriptional activity for haplotype A than for haplotype B (11) and therefore we tested whether these differences also hold true in vivo. Transcriptional activity did not differ between haplotype A [1.75 (0.77 to 2.18) AU] and B [1.16 (0.70 to 1.67) AU] ($P = 0.09$). As mentioned before, (n-3) FA intake increased the FABP2 expression (Table 2); when adjusted for (n-3) FA intake, the association between promoter haplotypes and their corresponding FABP2 expression became significant ($P = 0.049$) (Fig. 2A). Differences remained significant after controlling for BMI, age, and sex (data not shown).

**Effects of dietary factors and the Ala54Thr polymorphism on FABP2 gene expression.** FABP2 expression differed between Ala54Ala [1.44 (0.86–2.18) AU] and Thr54-allele carriers [0.94 (0.67–1.33) AU] ($P = 0.010$). The effect of Ala54Thr polymorphism became more pronounced after adjusting for (n-3) FA intake ($P = 0.002$) (Fig. 2B). Differences remained significant after controlling for BMI, age, and sex (data not shown).

Functional studies of a novel FABP2 promoter SNP in linkage with the Thr54-allele. Because gene expression was unexpectedly more strongly associated with the Ala54Thr polymorphism than the promoter haplotypes, we searched for other regulatory promoter SNP that were in complete linkage with the Ala54Thr polymorphism. We found only 1 SNP at position −834C > T (rs10034661) that was in complete linkage disequilibrium with the Ala54Thr polymorphism (Supplemental Fig. 1) (26).

To study the functional influence of this polymorphism, activities of different haplotypes [−834C > T] combined with promoter A and B, respectively were determined by promoter-reporter assays. There were no differences among investigated promoter constructs −834CC/AA, −834TT/BB, −834CC/AA, and −834TT/BB (CC is linked to Ala54Ala and TT to Thr54Thr; AA and BB correspond to known haplotypes A and B) ($P = 0.141$) (Supplemental Fig. 2).

**Effects of FABP2 gene expression and FABP2 gene variants on biochemical variables.** FABP2 is involved in intestinal FA metabolism whereby FABP2 variants affect biochemical variables. Therefore, we examined the association between FABP2 expression and clinical variables, both in the entire study group as well as considering potential genotype differences.

Only serum FFA concentrations were inversely correlated to FABP2 expression in all participants ($r = −0.41; P < 0.001$) as well as among genotype subgroups (B-allele carriers: $r = −0.47, P < 0.01$; Ala54Ala: $r = −0.34, P = 0.012$; Thr54-allele carriers: $r = −0.31, P = 0.069$). These associations remained significant after controlling for BMI, age, sex, and total energy intake (data not shown). Because FABP2 promotes mitochondrial FA oxidation (27), the association between FABP2 and CPT2 expression was examined and a positive correlation was found ($r = 0.53; P < 0.001$).

There were lower LDL-C ($P = 0.031$) and higher HDL-C concentrations ($P = 0.012$) in plasma in AA homozygotes compared with B-allele carriers (Table 1). Ala54Ala homozygotes had higher plasma HDL-C ($P = 0.034$), lower plasma LDL-C ($P = 0.049$), and lower serum FFA ($P = 0.008$) concentrations than Thr54-allele carriers (Table 1). Differences remained significant after controlling for confounders (data not shown). No other significant differences or associations were found (Table 1).

![FIGURE 1](https://academic.oup.com/jn/article-abstract/140/8/1411/4600359/Downloaded_from.pdf)
FABP2 binds LCFA with high affinity, which is in turn known to modulate the transcriptional activity of their target genes; this points to a potential association of FABP2 expression with LCFA intake. Yet, no such association was found for LCFA, whereas we did find an association with (n-3) FA intake in AA homozygotes. This association is unexpected, because FABP2 promoter B was more readily induced by PPAR ligands than haplotype A in vitro (11). Yet, multiple regulatory elements in the FABP2 promoter (21,28) might account for the distinct transcriptional modulation of haplotype A by dietary (n-3) FA only (Table 2). Based on a different responsiveness of the involved transcription factors to these lipid ligands, this complex interplay might explain why (n-3) FA intake was more strongly associated with FABP2 expression than LCFA intake. Based on FABP2-diet interaction studies (9), the association of the Ala54Thr polymorphism with insulin sensitivity depends on the type of fat consumed. Our study, which assessed habitual dietary intake, suggests the importance of dietary (n-3) FA in regulating FABP2 expression in AA homozygotes.

Reporter-gene studies showed a higher basal activity for haplotype A than for haplotype B (11) and that transcription factors act differently in distinct haplotypes, leading to a higher expression in haplotype A (21,28). Our study is in accordance with these in vitro results, particularly after adjusting for (n-3) FA intake (Fig. 2A), which affected FABP2 expression in our study (Table 2).

Because the Ala54Thr polymorphism was more strongly associated with gene expression than promoter haplotypes (Fig. 2B), other promoter SNP linked to the Ala54Thr polymorphism may account for these differences. Although the SNP rs834C > T (rs10034661) is completely linked to the Ala54Thr polymorphism, reporter assays using FABP2 promoter constructs (Supplemental Fig. 2) did not yield evidence that this SNP is responsible for the lower gene expression in Thr54-allele carriers (Fig. 2B).

**Discussion**

**FABP2 gene expression in duodenum, ileum, and colon.** FABP2 expression in humans seems to resemble the distribution in rodents (1), with the highest values in the ileum, moderate in the duodenum, and hardly existent expression in the colon.

**FABP2 promoter haplotypes are associated with differing eating behaviors.** The Thr54-allele of the Ala54Thr polymorphism was associated with a higher food intake in Japanese (23). Similarly, our results indicated that promoter haplotypes, linked with the Ala54Thr polymorphism, affect eating behavior. FABP2 is involved in the intestinal utilization of FA (27); thus, a higher FABP2 expression in AA homozygotes (Fig. 2A) may reduce intestinally derived FA more efficiently, which in turn reduce the secretion of the orexigenic gut-hormone GIP (24), as indicated in the MICK-subgroup (Fig. 1). This might subsequently reduce total energy intake. Higher postprandial GIP concentrations in BB homozygotes, however, might also be a consequence rather than a stimulus for higher fat intake, because the release of GIP depends on dietary fat intake (25). In contrast to promoter haplotypes, the Ala54Thr polymorphism was less associated with postprandial GIP response and energy intake (Table 1). This may be due to the lower activity of the FABP2 Ala54-variant which, according to our hypothesis (Fig. 3), should result in increases of GIP and energy intake countercurrent to the expected decreases based on linkage with the promoter haplotypes.

**Effects of dietary factors and genotypes on FABP2 gene expression.** Data on the impact of fat on FABP2 expression in animal studies remain inconsistent (4,5). FABP2 binds LCFA...
Effects of FABP2 gene expression and FABP2 gene variants on biochemical variables. The inverse correlation between FABP2 expression and serum FFA in our study may be explained by the involvement of FABP2 in intestinal FA utilization (3); overexpression of FABP2 may have stimulated mitochondrial \( \beta \)-oxidation and intestinal FA utilization, which may reduce FA availability in the circulation (27). The strong association of the expression of FABP2 with CPT2 underlines this assumption.

In this context, higher serum FFA in Thr54-allele carriers (Table 1) might result from the diminished FABP2 expression in those participants (Fig. 2B). Elevated FFA may in turn lead to higher LDL-C via the induction of hepatic VLDL synthesis. Moreover, the higher binding affinity for the Thr54-allele to LCFA (7) enhanced the absorption of fat and cholesterol (8) and may account for higher LDL-C and postprandial triglycerides in Thr54-carriers (8,11). Again, higher postprandial triglycerides contribute to triglyceride-eriched HDL particles in Thr54-carriers (29) and consequently may reduce HDL-C in these participants (Table 1). In fact, a recent meta-analysis demonstrated a strong association of the Thr54-allele with higher concentrations of total- and LDL-C as well as lower concentrations of HDL-C (30).

There are inconsistent reports regarding the association of gender with FABP2 genotypes (12,14,31). In our study, however, no such heterogeneity was detected. Some limitations of our study should be noted. Our findings were obtained in a clinical setting, which may lead to heterogeneity. Moreover, we did not assess the physical activity of study participants. Therefore, we may have over- or underestimated the associations with FABP2 expression because of differences in lifestyle and physical activity. FFQ data generally underestimate absolute nutrient intake. Yet, according to the FA composition in CE, the FFQ was a reliable tool for assessing dietary habits in our study. The semiquantitative nature of FFQ to assess energy and particular nutrients, however, may explain why certain nutrients, e.g. (n-3) FA, have shown greater differences between genotypes than did energy intake.

In summary, our study indicated that FABP2 expression depends on genotypes and (n-3) FA intake. Additionally, haplotype A has been associated with lower postprandial GIP concentrations, which may then result in lower food intake and body weight (Fig. 3). Increased intestinal \( \beta \)-oxidation due to higher FABP2 expression in people with the common alleles of the promoter and exon polymorphism might reduce serum FFA and by that improve insulin sensitivity and prevent dyslipidemia, as observed in this and other studies (9,11). Notably, the deletion of FABP2 in mice is associated with higher serum triglycerides (2), which pointed to FABP2 being involved in the modulation of intracellular lipid homeostasis. This is in contrast to the assumption that a gain-of-function of the Thr54-allele would explain higher triglycerides. On the basis of our findings, we suggest an alternative model to explain the FABP2 function and gene-nutrient interaction.

Acknowledgments

Literature Cited


