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The association of fatty acid–binding protein 2 A54T polymorphism with postprandial lipemia depends on promoter variability

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Abstract

Studies on the association of fatty acid–binding protein 2 (FABP2) A54T and promoter polymorphism, and type 2 diabetes mellitus, insulin, and triglyceride levels are controversial. The aim of this study was to investigate the interfering effect of FABP2 A54T and promoter polymorphism on the postprandial response to a mixed meal and an oral glucose load. Seven hundred men from the Metabolic Intervention Cohort Kiel underwent a standard glucose tolerance test and a standardized mixed meal test and were genotyped by use of the Taqman method. When calculated independently from promoter variability, postprandial triglyceride levels were significantly higher and postprandial insulin sensitivity (homeostasis model assessment index) was lower in homozygous carriers of FABP2 T54T compared with carriers of the FABP2 exon wild-type allele (FABP2 A54A and A54T). This confirms previous findings. The effect of the exon T54T genotype on triglyceride levels and insulin sensitivity, however, was dependent on promoter variability. We found a significant increase in postprandial triglyceride levels and a decrease in insulin sensitivity due to T54T only in the presence of the homozygous B genotype at the promoter polymorphism. Similar results were obtained after oral glucose tolerance test. Reporter gene assays indicated a higher responsiveness to peroxisome proliferator-activating receptor-γ (PPAR-γ)/retinoid X receptor (RXR) of FABP2 promoter B vs promoter A. Synergism between a higher inducibility of FABP2 expression and a higher activity of T54 variant may explain higher postprandial triglycerides in case of combined genotype (promoter B + T54). This interference and different linkage disequilibrium between FABP2 exon and promoter polymorphisms may explain the different results obtained in different cohorts.

1. Introduction

Fatty acid–binding proteins (FABPs) belong to a protein family that seems to be involved in the intracellular binding, transport, and metabolism of long-chain fatty acids [1-3]. To date, nine different FABPs have been detected. The nomenclature is based on the tissue by which it is mainly expressed and on the chronological order of detection [2]. The physiologic function of FABPs is not completely understood, and different hypotheses exist regarding its function. One possible role of FABP is to bind intracellular fatty acids and to minimize their toxicity [4]. Furthermore, it has been hypothesized that FABPs serve as cytosolic transporters of fatty acids to the mitochondria and peroxisomes, where they are oxidized to the endoplasmic reticulum for phospholipid synthesis, and to the nucleus, where they modulate gene expression [5-8]. The intestinal FABP, also called FABP2, was found exclusively in the epithelium of the small intestine and this suggests that it is involved in the absorption of dietary fatty acids [3]. FABP2-deficient mice put on more weight and develop hyperinsulinemia; when stratified for gender, elevated plasma triglyceride levels were found in male FABP2-deficient mice [9]. A single nucleotide polymorphism (SNP) of the FABP2 exon results in an amino acid exchange from alanine to threonine in amino acid position 54 [10]. The transfection of this variant to Caco-2 cells resulted in FABP2 having a higher affinity with fatty acids and this leads to an increased

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secretion of chylomicrons [11,12]. In the first report on this exon polymorphism, Baier et al [10] described an association between fasting insulin and insulin resistance in 147 Pima Indians. Further association studies on FABP2 A54T polymorphism with triglycerides and insulin levels or resistance showed controversial results in different cohorts. In obese subjects from Finland who had type 2 diabetes mellitus and familial combined hyperlipidemia, no association was found between exon SNP and fasting insulin levels, resistance, and triglycerides [13-15]. Postprandial lipemic response and insulin response, however, differed between 8 T54T and 7 A54A homozygote subjects recruited from a weight-reduction project [16]. In a South Indian cohort, the T54 genotype was associated with fasting dyslipidemia [17]. In a Japanese cohort, an association between intra-abdominal fat and fasting lipid metabolism was found [18,19], whereas in a Tonga cohort, an association was found only with cholesterol [20]. In a study of 120 White and 103 Afro-American women, a clear difference was shown between the 2 cohorts; in Afro-Americans, no association was found between polymorphism and fat distribution, whereas in the White carriers of the FABP2 T54 allele, the total and subcutaneous adipose tissue were lower than in noncarriers of the mutation [21]. Furthermore in a European cohort of 666 male students, this polymorphism had no influence on fasting and postprandial insulin, glucose, or triglyceride levels [22]. In a Spanish cohort, T54 carriers had lower insulin sensitivity after a saturated fatty acid diet than after a carbohydrate-rich diet or a Mediterranean diet, whereas the A54A homozygotes showed no differences [23].

Recently, we and others have demonstrated several SNPs in the promoter region of FABP2 to be in linkage disequilibrium (LD), resulting in 2 different haplotypes: A and B [24-27]. Although the results of reporter gene assays are not consistent, an influence of these SNPs on promoter activity is conceivable [24-27]. In Pima Indians, the A54T polymorphism is in complete LD with promoter B (deletion variant), which precludes a differential analysis of promoter and exon polymorphism [26]. In the North American cohort described by Damcott et al [25], an LD of $|D'| = 0.9$ was found. Formanack and Baier [26] observed a genotypic concordance of 0.6 in a sample of 48 Whites. Based on the LD analysis, they found that a differentiation between the effect of promoter and exon polymorphisms on Whites might be possible. In this study, therefore, we investigated a White cohort to determine whether, alongside exon polymorphism, promoter polymorphism has an additional effect on the fasting and postprandial metabolism.

2. Research design and methods

2.1. Study subjects

The Metabolic Intervention Cohort Kiel is a population-based cohort in which the traits of the metabolic syndrome, according to Adult Treatment Panel III criteria, were assessed, and postprandial metabolism was characterized. Men aged 45 to 65 years were recruited with the aid of the registrar in the region surrounding and encompassing the city of Kiel, Germany. Patients with a diagnosis of diabetes, maldigestion or malabsorption, liver diseases, renal diseases, intestinal surgical intervention within the last 3 months, thyroid disorders, and hormone therapy were excluded. The volunteers underwent a clinical examination including measurements of pulse rate, blood pressure, weight, height, waist circumference, and hip circumference [28,29]. Fasting plasma levels of triglycerides, cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), insulin, and glucose were assessed. The subjects underwent an oral glucose tolerance test (OGTT) and an oral metabolic tolerance test (OMTT) on different days with a minimum of 2 days between tests. The local ethics committee approved the study design (AZ A106/03 from April 16, 2003). The study was performed according to the Declaration of Helsinki and informed consent was obtained from all subjects.

2.2. Oral glucose tolerance test

An OGTT was performed according to the guidelines of the American Diabetes Association [30]. The blood-sampling period was extended by 4 hours. In brief, after a 12-hour fasting period, a venous fasting blood sample was drawn. Glucose (75 g) dissolved in 250 mL water was ingested within 5 minutes. Before and 0.5, 1, 2, 3, and 4 hours after ingestion, blood samples were taken, cooled on ice, centrifuged, and frozen for later analysis.

2.3. Oral metabolic tolerance test

An OMTT was performed as described elsewhere [31]. In brief, after a 12-hour fasting period and withdrawal of fasting blood sample, a standardized liquid mixed meal containing 51.6 kJ% fat, 29.6 kJ% carbohydrates, and 11.9 kJ% protein, with a total of 4392 kJ, was ingested within 5 minutes. Before and 0.5, 1, 2, 3, 4, and 5 hours after ingestion, blood samples were drawn for the analysis of insulin, glucose, and triglycerides, and 6, 7, 8, and 9 hours after ingestion for the analysis of triglyceride. Blood samples were taken on ice and were centrifuged and frozen for later analysis.

2.4. Plasma and serum samples

Insulin levels were determined by radioimmunoassay (Adaltis, Bologna, Italy) according to the manufacturer's instructions.

Other parameters were measured enzymatically by using a Konelab analyzer (Espoo, Finland) according to the manufacturer's instructions.

2.5. Genotyping

Genomic DNA was isolated from 10 mL of frozen blood samples with a Gigakit DNA extraction kit (Invitec, Berlin,

Germany). The following Taqman probes and polymerase chain reaction (PCR) primers were used.

For promoter site (rs2282688)

PCR forward primer: GGCAATGCTAAACACAATG-CAAAA
PCR reverse primer: TCACAACAGCAATTATCTTG-TAAAGTAAGACT
Taqman probe allele 1: AATCTTATTAACTTTAACTT-TTC
Taqman probe allele 2: TCTTATTAACTTTAGCTTTTC

For exon site (rs1799883)

PCR forward primer: AAGGAAGCTTGCAGCTCAT-GAC
PCR reverse primer: CACCAAGTTCAAAAACAA-CTTCAATG
Taqman probe allele 1: ATCAAGCACTTTTC
Taqman probe allele 2: TCAAGCGCTTTTC

All primers and probes were constructed by Applied Biosystems (Foster City, CA). Taqman analysis was performed as described elsewhere [32]. In brief, genomic DNA was arrayed and dried on 96-well plates. Taqman PCR was performed with Genesis pipetting robots (Tecan, Männedorf, Switzerland), ABI 9700 PCR machines (Applied Biosystems) and ABI 7700 and ABI 7900 fluorometers (Applied Biosystems).

2.6. Assessment of promoter activity

Because FABP2 expression is regulated by peroxisome proliferator-activating receptor (PPAR) [33], we investigated PPAR-γ–induced activity of FABP2 promoter alleles A and B. To exclude cell-specific endogenous FABP2 expression, nonintestinal HeLa cells, which normally do not express FABP2, were used for assessment of allele-specific, PPAR-dependent promoter activity.

2.7. FABP2 promoter luciferase constructs

The dual luciferase system was used (Promega, Mannheim, Germany). Cloning procedures were performed with Gateway Technology (Invitrogen, Karlsuhe, Germany). An 836-base-pair fragment upstream from initiation codon of FABP2, encompassing all 6 promoter polymorphisms, was amplified with *Pfu* polymerase (Invitrogen) from the genomic DNA of subjects homozygous for the promoter haplotype A or B. Amplified products were inserted into pENTR/D-TOPO vector and sequenced. The resulting plasmids pENTR-FABP2-Prom-*att*L contained *att*L sites allowing a recombination reaction with *att*R sites. *att*R sites were inserted into an *Eco*RV blunt-end site of the reporter plasmid (pGL4.10[luc2]) encoding firefly luciferase. Subsequent recombination, catalyzed by LR Clonase, of the *att*L-containing vector pENTr-FABP2-Prom-*att*L with *att*R-containing pGL4.10[luc2] vector resulted in the final pGL4.10[luc2]-FABP2-Prom(A) and pGL4.10[luc2]-FABP2-Prom(B) reporter constructs. The empty pGL4.10[luc2] vector served as a negative control.

2.8. Cell culture

HeLa cells were provided by the German National Resource Centre for Biological Material (Braunschweig, Germany) and were used between passages 5 and 30. Cells were cultured in modified Eagle's medium (Invitrogen) supplemented with 10% fetal calf serum (Invitrogen) and 1 mmol/L nonessential amino acids (PAA, Cölbe, Germany) in a humidified incubator at 37°C under an atmosphere of 5% carbon dioxide.

2.9. Transfection and luciferase reporter assay

Transient transfections were performed with FuGene6 (Roche, Mannheim, Germany) according to the manufacturer's instructions. HeLa cells (4×10^3) were plated on 96-well plates. Cells were cotransfected with 30 ng pGL4.10[luc2]-FABP2-Prom(A/B) or pGL4.10[luc2], 33 ng of the expression vectors pDest40-PPARγ [34] and pDest40-retinoid X receptor α (RXRα) (kindly provided by Dr Weitzel, University Hospital Hamburg-Eppendorf, Hamburg, Germany), or 66 ng empty pDest40 vector and 3 ng pGL4.74[hRluc/TK] vector encoding *Renilla* luciferase as internal control. The culture medium was changed 24 hours after transfection with serum-free medium containing the PPAR-γ and RXR-α ligands rosiglitazone (2 μmol/L, ALEXIS Biochemicals, Grünberg, Germany) and 9-*cis*-retinoic acid (1 μmol/L, Sigma, Deisenhofen, Germany) or dimethyl sulfoxide (Sigma) as control. Luciferase activities were measured 48 hours after transfection by Dual-Luciferase Reporter Assay System (Promega).

2.10. Data analysis

SPSS software package (SPSS for Windows, version 14.0.0, LEAD Technologies, Chicago, IL) was used for statistical analysis. Allele frequencies were estimated by the maximum likelihood approach. Deviation from Hardy-Weinberg equilibrium was tested by using Pearson χ^2 test. The Kruskal-Wallis test was used to test for associations between genotypes and metabolic features. Wilcoxon rank sum test (Mann-Whitney U test) was used to compare the phenotypes of one genotype against the other(s). Both are nonparametric tests and were chosen because of nonparametric distribution of parameters. Results are presented as mean ± SEM. Cumulating parameters were subsumed as the area under the postprandial curve by using the following formula:

$$\frac{yt_0 + yt_1}{2} \times (t_1 - t_0) + \frac{yt_1 + yt_2}{2} \times (t_2 - t_1) + \ldots \frac{yt_{n-1} + yt_n}{2} \times (t_n - t_{n-1})$$

For a pairwise description of linkage (or gametic) disequilibrium (LD), we estimated the pairwise haplotype frequencies and subsequently calculated the commonly used measures D' and r^2 according to Devlin and Risch [35].

3. Results

3.1. Frequencies

Genomic DNA from 700 unrelated men was analyzed. The genotype frequency of the FABP2 A54T polymorphism (rs1799883) was as follows: A54A, 0.514 (n = 360); A54T, 0.410 (n = 287); T54T, 0.076 (n = 53). The genotype frequency of FABP2 promoter variability (rs228268) was as follows: AA, 0.279 (n = 195); AB, 0.511 (n = 358); BB, 0.210 (n = 147). No deviations from Hardy-Weinberg equilibrium were obtained.

The combined genotype frequencies of promoter and exon polymorphism were as follows: (promoter) AA/(exon) A54A, 0.253 (n = 177); AA/A54T, 0.023 (n = 16); AA/

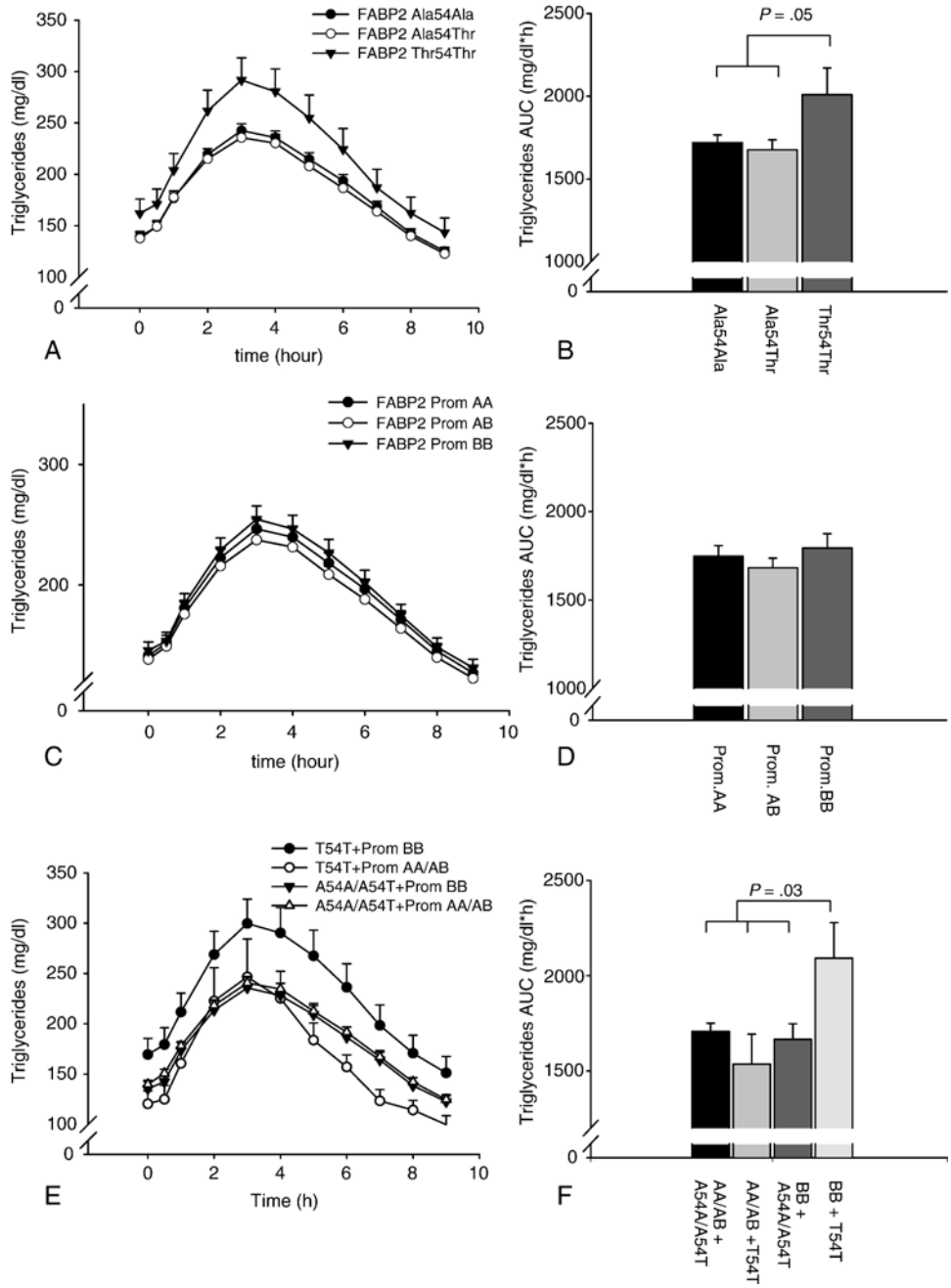

Fig. 1. Serum triglycerides (mg/dL ± SEM) after OMTT. A, postprandial triglyceride curve depending on FABP2 Ala54Thr polymorphism. A54A, —●— (n = 360); A54T, —○— (n = 287); T54T, —▼— (n = 53). B, AUC of postprandial triglycerides depending on FABP2 Ala54Thr polymorphism. A54A, ■; A54T, □; T54T, ▩. C, Postprandial triglyceride curve depending on FABP2 promoter polymorphism. AA, —●— (n = 195); AB, —○— (n = 358); BB, —▼— (n = 147). D, AUC of postprandial triglycerides depending on FABP2 promoter polymorphism. AA, ■; AB, □; BB, ▩. E, Postprandial triglyceride curve depending on FABP2 Ala54Thr polymorphism coupled with FABP2 promoter polymorphism. T54T + promoter BB, —●— (n = 45); T54T + promoter AA/AB, —○— (n = 8); A54A/A54T promoter BB, —▼— (n = 102); T54T + promoter BB, —△— (n = 45). F, AUC of postprandial triglycerides depending on FABP2 Ala54Thr polymorphism coupled with FABP2 promoter polymorphism. Promoter AA/AB + A54A/A54T, ; promoter AA/AB + T54T, □; promoter BB + A54A/A54T, ▩; promoter BB + T54T, □. Conversion of triglycerides, mg/dL to mmol/L with factor 0.013.

Table 1
Postprandial parameters of study subjects by genotype combination of FABP2 exon and promoter polymorphism

	Ala54Ala promoter			Ala54Thr promoter			Thr54Thr promoter		
	AA (n = 177)	AB (n = 151)	BB (n = 32)	AA (n = 16)	AB (n = 201)	BB (n = 70)	AA (n = 2)	AB (n = 6)	BB (n = 45)
Adult Treatment Panel III criteria									
Waist circumference (cm)	100.44 ± 0.91	99.25 ± 0.97	98.34 ± 2.00	101.94 ± 2.56	99.97 ± 0.92	100.73 ± 1.50	98.5 ± 7.50	99.33 ± 3.93	100.62 ± 1.65
Blood pressure (mm Hg)									
Systolic	129.42 ± 1.42	129.63 ± 1.49	130.72 ± 2.43	125.63 ± 3.76	128.85 ± 1.29	131.29 ± 2.23	130.00 ± 20.00	116.67 ± 3.33	129.00 ± 2.20
Diastolic	80.98 ± 0.81	80.07 (0.93)	80.91 ± 1.90	77.19 ± 2.04	79.84 ± 0.76	81.74 ± 1.19	80.00 ± 10.00	71.67 ± 3.07	82.11 ± 1.74
Fasting triglycerides (mg/dL)	142.66 ± 6.58	135.82 ± 7.67	144.48 ± 14.74	128.14 ± 16.40	140.82 ± 7.60	130.97 ± 8.57	126.85 ± 33.05	118.18 ± 15.33	169.35 ± 16.16*
HDL (mg/dL)	53.33 ± 1.09	53.17 ± 0.98	51.47 ± 2.19	55.28 ± 5.25	54.70 ± 1.16	52.68 ± 1.70	46.73 ± 4.30	59.23 ± 7.18	49.39 ± 2.27
Fasting glucose (mmol/L)	5.70 ± 0.09	5.81 ± 0.07	5.72 ± 0.09	5.99 ± 0.20	5.82 ± 0.06	5.78 ± 0.08	5.84 ± 0.86	5.48 ± 0.20	6.01 ± 0.17
OMTT									
Triglycerides									
AUC (mg/dL per hour)	1759.70 ± 63.90	1673.77 ± 78.02	1740.39 ± 148.11	1645.10 ± 226.20	1693.63 ± 78.03	1631.86 ± 98.34	1553.96 ± 372.96	1529.33 ± 193.19	2092.36 ± 186.38*
Maximum (mg/dL)	274.46 ± 9.36	258.89 ± 10.86	273.01 ± 21.03	255.60 ± 33.23	265.07 ± 10.27	251.10 ± 14.70	264.10 ± 94.50	258.07 ± 41.33	329.49 ± 25.85*
Increase (mg/dL)	130.30 ± 5.36	121.44 ± 5.10	128.53 ± 10.78	119.49 ± 19.38	122.38 ± 4.84	120.13 ± 8.05	137.25 ± 61.45	139.89 ± 36.76	160.14 ± 11.71**
Insulin									
AUC (μU/mL per hour)	195.74 ± 9.65	207.22 ± 14.81	219.82 ± 32.52	189.87 ± 33.49	201.04 ± 14.03	217.33 ± 24.20	193.14 ± 19.80	162.64 ± 26.70	220.34 ± 15.67*
Increase (μU/mL)	67.07 ± 4.73	78.50 ± 8.69	65.41 ± 10.01	53.38 ± 10.06	68.27 ± 7.13	67.14 ± 7.80	62.73 ± 3.93	57.18 ± 10.37	82.00 ± 11.85*
HOMA (AUC) (μU/mL [mmol/L] per 22.5 hours)	56.68 ± 3.67	61.73 ± 5.47	63.03 ± 12.03	59.28 ± 13.25	58.39 ± 5.43	64.70 ± 9.85	55.54 ± 5.30	43.53 ± 6.88	65.56 ± 6.59*
OGTT									
Insulin (AUC) (μU/mL per hour)	171.11 ± 7.85	172.98 ± 11.55	169.06 ± 19.12	151.56 ± 32.48	172.24 ± 11.14	186.73 ± 20.21	172.24 ± 31.09	116.60 ± 10.56	191.92 ± 17.80
HOMA (AUC) (μU/mL [mmol/L] per 22.5 hours)	62.56 ± 3.70	63.95 ± 2.21	58.74 ± 8.50	53.65 ± 11.25	62.64 ± 4.70	72.86 ± 10.34	59.34 ± 9.53	37.40 ± 3.37	79.11 ± 10.51*

Results are described after OMTT and OGTT. Statistical analysis was performed by Mann-Whitney *U* test, FABP2 T54T promoter BB combination vs other genotypes.

* $P < .05$.

** $P < .01$.

T54T, 0.003 (n = 2); AB/A54A, 0.216 (n = 151); AB/A54T, 0.287 (n = 201); AB/T54T, 0.009 (n = 6); BB/A54A, 0.046 (n = 32); BB/A54T, 0.100 (n = 70); BB/T54T, 0.064 (n = 45). No deviations from Hardy-Weinberg equilibrium were obtained.

3.2. Linkage disequilibrium analysis

Both polymorphisms, FABP2 A54T and promoter A/B, showed intermediate levels of gametic LD: $D' = 0.85$, $r^2 = 0.31$. The genotypic concordance between both SNPs is equal to 0.60.

3.3. Marginal analysis of exon and promoter polymorphisms

3.3.1. FABP2 A54T exon polymorphism

No significant difference was found among the 3 genotypic groups for weight, body mass index (BMI), waist circumference, cholesterol, LDL/HDL ratio, fasting and postprandial insulin, and glucose levels. However, there was a significant association with postprandial triglyceride increases ($P < .05$) (Kruskal-Wallis test), with the highest triglyceride response in the FABP2 T54T group. FABP2 T54T homozygotes had a higher atherogenic index (LDL/HDL ratio) when compared with FABP2 A54A/A54T carriers (3.21 ± 0.16 vs 2.86 ± 0.04; $P < .05$; Wilcoxon rank sum test), higher postprandial triglycerides (area under the curve [AUC]) (2008.31 ± 161.95 mg/dL per hour [22.69 ± 1.83 mmol/L per hour] vs 1701.57 ± 37.59 mg/dL per hour [19.23 ± 0.43 mmol/L per hour]; $P = .05$) (Fig. 1A and B), and higher postprandial triglyceride increases (156.98 ± 10.82 mg/dL [1.77 ± 0.12 mmol/L] vs 124.35 ± 2.65 mg/dL [1.41 ± 0.03 mmol/L]; $P < .05$) after an OMTT. Accordingly, we found higher postprandial insulin levels (AUC) in homozygote carriers (T54T) than in A54A carriers after an OMTT (212.63 ± 13.80 μIU/mL per hour [1476.71 ± 95.84 pmol/L per hour] vs 203.22 ± 6.90 μIU/mL per hour [1419.36 ± 47.92 pmol/L per hour]; $P < .05$), as well as higher insulin resistance (homeostasis model assessment [HOMA] index) (62.63 ± 5.71 vs 59.54 ± 2.64 μIU/mL [mmol/L] per 22.5 hours; $P < .05$). After an OGTT, insulin and HOMA tended to be higher in homozygote carriers of FABP2 T54T ($P = .14$ and $P = .12$, respectively).

3.3.2. FABP2 promoter polymorphism

No significant association was found between the promoter polymorphisms and weight, BMI, waist circumference, LDL/HDL ratio, and cholesterol, fasting and postprandial triglyceride (Fig. 1C and D), insulin, and glucose levels (Kruskal-Wallis test). Homozygote B carriers of the FABP2 promoter (FABP2 promoter BB) had a higher LDL/HDL ratio compared with carriers of FABP2 promoter A (3.08 ± 0.085 vs 2.84 ± 0.416; $P < .05$). Postprandial parameters did not differ significantly between these groups. Postprandial insulin sensitivity (HOMA) was not significantly different in promoter BB homozygotes compared with carriers of promoter A (65.13 ± 5.56 vs 58.52 ± 2.74 μIU/mL [mmol/L] per 22.5 hours; $P = .26$).

3.4. Interaction analysis of exon and promoter polymorphisms

Only homozygous B carriers at the FABP2 promoter who were also homozygous for the T allele at FABP2 A54T had significantly higher postprandial triglyceride levels (AUC), higher triglycerides, and individual triglyceride maximum compared with the other genotype combinations ($P < .05$) (Table 1 and Fig. 1E and F) (Promoter BB/T54T [n = 45] vs all other genotype combination [n = 655]). The genotypic combination FABP2 promoter BB/T54T was also associated with elevated postprandial insulin levels (AUC) and insulin increase compared with the other genotypes ($P < .05$). Accordingly, postprandial HOMA was higher after the mixed meal in homozygote FABP2 BB/T54T compared with other genotypes ($P < .05$). Furthermore, postprandial HOMA after an OGTT was higher in homozygous FABP2 BB/T54T carriers compared with other genotypes ($P < .05$).

3.5. Promoter activity

As described recently, a comparison of basal FABP2 promoter activity in intestinal Caco-2 cells revealed activity in promoter B up to 4-fold higher than in promoter A [24-27]. To investigate the PPAR-γ–induced activity of FABP2 promoter alleles, the promoter constructs were cotransfected with PPAR-γ and RXR in HeLa cells. In this FABP2-nonexpressing cell line, we observed similar activity in promoter A and B. Interestingly, FABP2

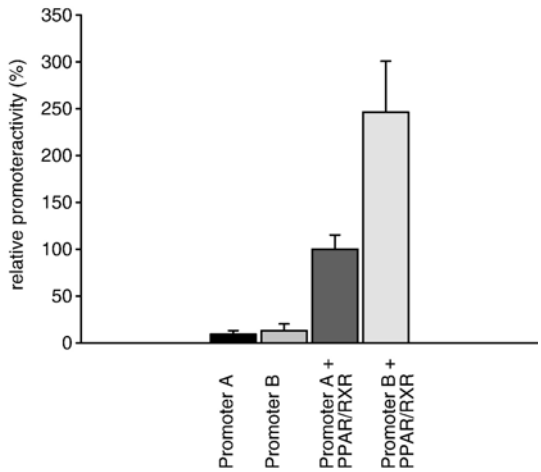

Fig. 2. In vitro analysis of FABP2 promoter activity. FABP2 promoter constructs A and B were cotransfected with PPAR-γ/RXR-α expression vectors in HeLa cells in the presence of rosiglitazone and 9-*cis*-retinoic acid in culture medium. Firefly luciferase activities were normalized to *Renilla* luciferase, and activities of the empty control vector pGL4.10[luc2] were substracted from promoter constructs. The resulting relative promoter activities are reported in relation to haplotype A obtained after cotransfection with PPAR-γ and RXR-α constructs. Data are given as mean ± SEM from 4 independent experiments. Luciferase activity was measured in duplicate.

promoter B is 2.5-fold more inducible by PPAR-γ than promoter A (Fig. 2).

4. Discussion

In this study, we demonstrated that the association of the FABP2 exon polymorphism A54T with postprandial triglycerides and postprandial insulin sensitivity depends on the combination with the homozygosity for FABP2 promoter B. Only the combination of FABP2 T54T with the homozygosity of promoter variant B caused higher postprandial plasma triglycerides and lower insulin sensitivity when compared with all other genotypic combinations. The lower insulin sensitivity was seen after a fat-containing mixed meal as well as after an OGTT.

The physiologic role of FABP2 has not yet been completely elucidated. FABP2 seems to be involved in intracellular protection against free fatty acids and in the intracellular transport to the organelles and the nucleus, as well as to chylomicron formation [5-8]. Female FABP2 knockout mice gained less weight in response to a high-fat diet, whereas male mice had elevated triglycerides and weighed more regardless of the dietary fat content [9].

The polymorphism in exon 2 of the FABP2 gene results in an amino acid exchange from alanine to threonine. This exchange was associated with a higher binding affinity to fatty acids in vitro [10,11]. Several studies showed an association between the T54 variant with elevated postprandial triglycerides and free fatty acids [16,36,37]. Chronically elevated free fatty acids result in an accumulation of intracellular fat in muscle and adipocytes and, consecutively, in insulin resistance, which is followed by hyperglycemia and type 2 diabetes mellitus [31,37-39]. Thus, the association of the T54 coding variant with insulin resistance [10,19,40,41] may be explained by increased postprandial triglycerides and free fatty acids [16,36]. In the cohort examined, we were able to confirm that T54T homozygotes, when calculated irrespectively of promoter combination, have high postprandial triglyceride and low postprandial insulin sensitivity (Fig. 1A and B), whereas the heterozygotes A54T did not differ significantly from the A54A homozygotes.

The promoter of the FABP2 gene shows a common variability of 6 polymorphisms, which are in complete LD, and result in 2 allele haplotypes [24-26]. An association of these alleles with BMI and cholesterol was demonstrated in women in a non-Hispanic subcohort [25]. In this study, we were able to show for the first time an association between the promoter polymorphism and the cholesterol metabolism (LDL/HDL ratio) in men.

In a cohort of 714 nondiabetic Hispanic and non-Hispanic white individuals living in the geographically isolated San Luis Valley, CO, an LD of $|D'| = 0.9$ between FABP2 promoter B allele and T54 allele was found [25]. In Pima Indians, a perfect genotypic concordance (1.0) was seen between the T54 allele and the promoter B allele [26]. Genetic analysis of 48 Whites with no further specific characteristics showed a genotypic concordance of 0.6 [26]. In our population-based cohort from Kiel, Germany, we found an allelic concordance, or LD, of $r^2 = 0.31$ ($|D'| = 0.85$), whereas the genotypic concordance was also equal to 0.60. Because the frequency of the T54 allele varies considerably between 0.14 in Oji-Cree Indians [42] and 0.4 in Japanese subjects [43], a high variability in genotypic and allelic concordance between the FABP2 promoter polymorphism and the exon polymorphism may be assumed. This may be the reason for conflicting findings in different association studies: in some studies, associations have been shown between the A54T polymorphism and dyslipidemia [15-17,20], whereas in other studies this association has not been confirmed [22,43]. Similarly, contradictory associations have been shown with insulin plasma levels and with insulin sensitivity (association with insulin sensitivity: [10,19,40,41], no association: [13-15,22,43].

We found significantly higher fasting and postprandial triglycerides in homozygosity for T54 and promoter B compared with other genotypic combinations. The postprandial triglyceride increase was faster and the triglyceride maximum was higher compared with the other combinations. Postprandial insulin plasma levels after the mixed meal and postprandial HOMA after the mixed meal and the OGTT were also higher. To enable a stratification of cohorts by genotype combinations, a sufficiently high sample size is required. There has been only one study up to now in which postprandial triglyceride and insulin levels were assessed in such a comprehensive cohort [22]. In this study, 666 students from 11 countries in Europe were investigated. The promoter variability was not studied. Varying levels of LD in Whites may explain why we found T54T homozygosity to have a significant effect on triglyceride levels and insulin sensitivity in a German cohort, whereas no difference could be seen in the trans-European cohort. Compatible with this suggestion, Pratley et al [36] also found clear differences in postprandial free fatty acids between 9 homozygous T54 carriers and 9 homozygous A54 carriers in Pima Indians in which the T54 allele is completely coupled with promoter B [26].

The combination of homozygous promoter B and exon T54 polymorphisms associated with the highest postprandial triglyceride levels and lowest insulin sensitivity compared with other genotype combinations still remains hypothetical. Reporter gene assays in Caco-2 cells showed a lower basal activity in promoter B than promoter A [25,26]. We were able to confirm these findings in unstimulated Caco-2 cells [27]. In contrast, the activity of promoter B was higher than that of promoter A in INT407 cells [24]. For differentiation, INT407 cells were incubated with bezafibrate, a PPAR ligand. Therefore, conflicting results could be due to the different potential of variants A and B in response to PPAR ligands. Indeed, a cotransfection in HeLa cells that normally do not express FABP2 confirmed this hypothesis: when FABP2 promoter constructs were cotransfected with

PPAR-γ and RXR-α, the promoter activity of promoter B was more inducible than that of promoter A (Fig. 2). This implies that promoter B, which is more frequently coupled with the T54 genotype, may be regulated more strongly than promoter A. This hypothesis is supported by the findings of Marin et al [23] that fat modification of the diet resulted in an alteration of insulin sensitivity only in T54T homozygotes but not in A54A homozygotes. Besides differing levels of LD between exon and promoter polymorphisms, the different response of promoter A and promoter B may explain the lacking association in the trans-European cohort with a sample size similar to our study: Differences in dietary habits (Mediterranean diet in the south and more saturated fat in the north) could have resulted in different FABP2 promoter activities, and thus may have diminished differences in postprandial triglycerides and insulin sensitivity between the coupled genotypes.

In conclusion, the presented study shows that the association between A54T polymorphism and postprandial triglycerides, and insulin sensitivity depends on the coupling with the promoter polymorphism. Only in cases homozygous for T54 and promoter B are postprandial triglycerides increased and postprandial insulin sensitivity is decreased. Differences in LD and genotypic concordance in different cohorts and the interfering effect of the promoter polymorphism may explain the conflicting results of different association studies.

The data presented on promoter activity suggest that promoter B has a higher response to environmental factors. Thus, the hypothesis of gene-environment interaction that has been postulated, based on different findings on A54 polymorphism [23], is supported by the interference of exon and promoter polymorphisms and differences in the responses of promoter A and B variant to PPAR ligands described here.

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