A Thr\(^{94}\)Ala mutation in human liver fatty acid-binding protein contributes to reduced hepatic glycogenolysis and blunted elevation of plasma glucose levels in lipid-exposed subjects

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Weickert MO, v. Loeffelholz C, Roden M, Chandramouli V, Brehm A, Nowotny P, Osterhoff MA, Isken F, Spranger J, Landau BR, Pfeiffer AF, Möhl M. A Thr\(^{94}\)Ala mutation in human liver fatty acid-binding protein contributes to reduced hepatic glycogenolysis and blunted elevation of plasma glucose levels in lipid-exposed subjects. Am J Physiol Endocrinol Metab 293:E1078–E1084, 2007. First published August 21, 2007; doi:10.1152/ajpendo.00337.2007.—Liver fatty acid-binding protein (L-FABP) is a highly conserved key factor in lipid metabolism. Amino acid replacements in L-FABP might alter its function and thereby affect glucose metabolism in lipid-exposed subjects, as indicated by studies in L-FABP knockout mice. Amino acid replacements in L-FABP were investigated in a cohort of 1,453 Caucasian subjects. Endogenous glucose production (EGP), gluconeogenesis, and glycogenolysis were measured in healthy carriers of the only common Thr\(^{94}\)-to-Ala amino acid replacement (Ala/Ala\(^{94}\)) vs. age-, sex-, and BMI-matched wild-type (Thr/Thr\(^{94}\)) controls at baseline and after 320-min lipid/heparin-somatostatin-insulin-glucagon clamps (n = 18). Whole body glucose disposal was further investigated (subset; n = 13) using euglycemic-hyperinsulinemic clamps without and with lipid/heparin infusion. In the entire cohort, the only common Ala/Ala\(^{94}\) mutation was significantly associated with reduced body weight, which is in agreement with a previous report. In lipid-exposed, individually matched subjects there was a genotype vs. lipid-treatment interaction for EGP (P = 0.009) driven mainly by reduced glycolysis in Ala/Ala\(^{94}\) carriers (0.46 ± 0.05 vs. 0.59 ± 0.05 mg·kg\(^{-1}\)·min\(^{-1}\), P = 0.013). The lipid-induced elevation of plasma glucose levels was smaller in Ala/Ala\(^{94}\) carriers with wild types (P < 0.0001). Whole body glucose disposal was not different between lipid-exposed L-FABP genotypes. In summary, the Ala/Ala\(^{94}\)-mutation contributed significantly to reduced glycolysis and less severe hyperglycemia in lipid-exposed humans and was further associated with reduced body weight in a large cohort. Data clearly show that investigation of L-FABP phenotypes in the basal overnight-fasted state yielded incomplete information, and a challenge test was essential to detect phenotypical differences in glucose metabolism between L-FABP genotypes.

endogenous glucose production; gluconeogenesis; insulin resistance; free fatty acids; nutrigenomics; randomized controlled study

LIPID ACCUMULATION AND COMPARTMENTALIZATION in the liver are strongly associated with insulin resistance and are subject to nutritional influences (8, 31). High circulating concentrations of free fatty acids (FFA) induce both hepatic and peripheral insulin resistance (16). The transport and metabolism of FFA are mediated by fatty acid-binding proteins (FABPs), which are highly conserved and abundantly expressed in various tissues, including the liver. It has been suggested that FABPs are important factors for the fine-tuning of metabolic responses but are likely to influence metabolic homeostasis negatively under conditions of excessive caloric and fat intake (20). Supporting this concept, FABP knockout mice show no obvious phenotype under chow-fed conditions but are remarkably protected from fatty liver, insulin resistance, atherosclerosis, type 2 diabetes, and obesity when exposed to a high flux of fatty acids (20, 22) despite circulating concentrations of FFA not being reduced in these animals.

Lever-type FABP (L-FABP) accounts for up to 5% of the cytoplasmatic protein in hepatocytes (3) and is a key regulator of hepatic lipid metabolism by influencing the uptake, transport, mitochondrial oxidation, and esterification of fatty acids (1). L-FABP knockout mice that are exposed to high FFA concentrations by prolonged 48-h fasting show about 10-fold reduced liver fat (21), which might affect hepatic insulin sensitivity (31). Lower insulin concentrations in fed animals have been further reported (21), indicating improved glucose metabolism. Obviously, in humans, knockout models are not available. However, amino acid variations in L-FABP could be functionally relevant and thus exert a phenotype, particularly in lipid-exposed subjects. This is supported by observational studies showing associations of the common threonine (Thr)\(^{94}\)-to-alanine (Ala) amino acid replacement in L-FABP with reduced body weight (7) and protection against high apolipoprotein B levels in Ala/Ala\(^{94}\) carriers that consume a high-fat, Western diet (24).

To date, potential contributions of amino acid variants in L-FABP to hepatic and peripheral glucose metabolism have not been reported. Thus, we first investigated all known polymorphisms leading to amino acid replacements in L-FABP. Only one of these coding polymorphisms was found in the subjects investigated, resulting in a Thr\(^{94}\)-to-Ala amino acid
replaced with a minor allele frequency of 36%. We then performed a randomized, single-blind, controlled intervention with nine healthy subjects carrying the Ala/Ala\textsuperscript{44} mutation vs. nine sex-, age-, and body mass index (BMI)-matched wild-type (Thr/Thr\textsuperscript{44}) controls to assess endogenous glucose production (EGP), gluconeogenesis, and glycogenolysis at baseline and during 320-min lipid/heparin-insulin-glucagon-somatostatin clamps. Potential differences in lipid-induced peripheral insulin resistance between L-FABP genotypes were investigated in a substudy.

**MATERIALS AND METHODS**

The Clinicaltrials.gov Identifier number for this study is NCT00277342.

**Investigation of polymorphisms in L-FABP.** All known polymorphisms leading to an amino acid replacement in L-FABP (Thr\textsuperscript{94}, Ala\textsuperscript{94}, and Leu\textsuperscript{42}) were first genotyped in 93 subjects. In these subjects, only the Thr\textsuperscript{94} variant was present and subsequently investigated in 1,453 participants of our cohort of metabolically characterized volunteers.

DNA of all participants was extracted from whole blood using Magnasep magnetic beads (Agowa, Berlin, Germany). The region around the polymorphisms was amplified by PCR (Thr\textsuperscript{94} Ala: upper primer 5’-ACACGCTCAGAGCACCACCA, lower primer 5’-GA-CAGTTGTT-CAGTTGGAAG; Thr\textsuperscript{94} Ala and Leu\textsuperscript{42} Val: upper primer 5’-GTCATTGTCTCCAGCTCA). Amplificates were controlled by restriction enzyme digestion. The single nucleotide polymorphism (SNP) diagnostic was performed by primer elongation using SnuPE (NCT00277342).

**Participants of the intervention trial.** The experimental protocol was approved by the local ethics committee, and all subjects gave written, informed consent. Healthy carriers of the homozygous Ala/Ala\textsuperscript{44} mutation with normal fasting glucose and normal glucose tolerance were recruited from 1,453 participants of our cohort of metabolically characterized volunteers. Exclusion criteria were impaired glucose metabolism as indicated by an oral glucose tolerance test, menstrual irregularities, a history of smoking, or a medication with cortisone. For nine of the subjects willing to participate, age-, sex-, and BMI-matched wild-type (Thr/Thr\textsuperscript{94}) controls were selected (Table 1). Subjects were invited in random order according to their availability for participation in the study. The participants of the study and the researchers that performed the laboratory analyses were not aware of the L-FABP genotype. Fertile female subjects were studied in the early follicular phase of the menstrual cycle. Subjects were instructed to maintain normal physical activity for 3 days before all study days.

**Experimental design.** The design of the intervention studies is shown in Fig. 1, A and B. Subjects arrived at the metabolic unit in the morning after a 10-h overnight fast. To reduce variance, all subjects consumed standardized liquid meals at 10 PM on the evening before all study days (2 portions, Biosorp; Pfrimmer Nutricia, Erlangen, Germany). No other meals and only tap water were allowed in the 12 h before the studies. After arrival, two intravenous catheters were inserted into contralateral forearm veins. The arm at which blood samples were drawn was placed into a heating box (65°C) throughout the studies.

From −10 min, Somatostatin (3 mg; Somatostatin Deltaselect, Dreieich, Germany) was infused to inhibit endogenous hormone secretion (Fig. 1A), followed by the replacement of insulin (Insumin Rapid; Aventis, Bad Soden, Germany) and glucagon (Glucagen; Novo Nordisk, Mainz, Germany) within the low fasting range (Fig. 2, C and D). Plasma glucose concentrations are known to decrease during the first 2–3 h of somatostatin clamps (2, 19, 26). Therefore, to avoid hypoglycemic symptoms, a 2% enriched (99% [6,6-\textsuperscript{2}H\textsubscript{2}]glucose; Eurisop-Top, Saarbrücken, Germany), 20% glucose infusion (Glucose-20-pfrimmer; Baxter, Unterschießheim, Germany) was administered when blood glucose was ≤4.4 mmol/l and immediately reduced/stopped when it was ≥4.4 mmol/l. Plasma FFA concentrations were raised from 0 min to +320 min by a constant lipid infusion (20% Deltalipid LCT; Deltaselect, Pfullingen, Germany) (Fig. 1B). Plasma glucose concentrations were allowed to rise freely after the start of the lipid/heparin infusion.

A subset of the same subjects (7 wild types, 6 Ala/Ala carriers) was willing to participate again and was investigated on separate days for the assessment of whole body glucose disposal upon lipid exposure (Fig. 1B). Two-stepped euglycemic-hyperinsulinemic clamps were performed (euglycemic-hyperinsulinemic clamps from baseline until steady state, followed by an additional lipid/heparin infusion for an additional 320 min), with 40 mU·m\textsuperscript{-2}·min\textsuperscript{-1} human insulin (Actrapid; Novo Nordisk, Bagsvaerd, Denmark), a variable infusion of 20% glucose (Serag Wiessner, Naila, Germany), and a constant infusion of a lipid solution (20% Deltalipid LCT; Deltaselect, Pfullingen, Germany) combined with heparin (Heparin-Natrium-25000-ratiopharm; Merckle, Blaubeuren, Germany) (Fig. 1A). Plasma glucose concentrations were allowed to rise freely after the start of the lipid/heparin infusion.

Table 1. Description of the age-, sex-, and BMI-matched participants enrolled in the intervention study

<table>
<thead>
<tr>
<th>L-FABP</th>
<th>Wild Types (n = 9)</th>
<th>Ala/Ala\textsuperscript{44} Carriers (n = 9)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>52.9 ± 4.5</td>
<td>51.9 ± 4.9</td>
<td>0.64</td>
</tr>
<tr>
<td>BMI, kg/m\textsuperscript{2}</td>
<td>23.7 ± 1.0</td>
<td>23.9 ± 1.3</td>
<td>0.77</td>
</tr>
<tr>
<td>WHR</td>
<td>0.85 ± 0.04</td>
<td>0.88 ± 0.03</td>
<td>0.35</td>
</tr>
<tr>
<td>Plasma FFA, mmol/l</td>
<td>0.4 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>0.12</td>
</tr>
<tr>
<td>Plasma cholesterol, mmol/l</td>
<td>5.0 ± 0.3</td>
<td>5.2 ± 0.3</td>
<td>0.28</td>
</tr>
<tr>
<td>Plasma triacylglycerol, mmol/l</td>
<td>0.9 ± 0.1</td>
<td>1.2 ± 0.2</td>
<td>0.31</td>
</tr>
<tr>
<td>Plasma apoipoprotein B, g/l</td>
<td>0.92 ± 0.08</td>
<td>0.95 ± 0.09</td>
<td>0.77</td>
</tr>
<tr>
<td>Serum insulin, pmol/l</td>
<td>29.6 ± 3.1</td>
<td>36.1 ± 4.3</td>
<td>0.17</td>
</tr>
<tr>
<td>Plasma glucose, mmol/l</td>
<td>5.0 ± 0.1</td>
<td>4.8 ± 0.1</td>
<td>0.27</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.1 ± 0.1</td>
<td>1.3 ± 0.2</td>
<td>0.23</td>
</tr>
<tr>
<td>Serum C-peptide, mmol/l</td>
<td>0.7 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.54</td>
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<tr>
<td>Hb A\textsubscript{1c}, %</td>
<td>5.5 ± 0.1</td>
<td>5.4 ± 0.1</td>
<td>0.67</td>
</tr>
<tr>
<td>(\gamma)-Glutamyltransferase, IU/l</td>
<td>18.8 ± 1.8</td>
<td>20.2 ± 2.8</td>
<td>0.56</td>
</tr>
<tr>
<td>Alanin aminotransferase, IU/l</td>
<td>15.8 ± 4.0</td>
<td>13.9 ± 1.7</td>
<td>0.54</td>
</tr>
<tr>
<td>Aspartate aminotransferase, IU/l</td>
<td>18.9 ± 1.5</td>
<td>19.2 ± 1.4</td>
<td>0.83</td>
</tr>
</tbody>
</table>

Values are means ± SE for 9 subjects in each group. L-FABP, liver fatty acid-binding protein; Ala, alanine; BMI, body mass index; WHR, waist-to-hip ratio; HOMA-IR, homeostasis model assessment for insulin resistance, calculated as [fasting insulin (mU/l) × fasting glucose (mmol/l)/22.5]. Baseline parameters (−150 min) after a standardized liquid meal in the evening before the first and a 10-h overnight fast.

A subset of the same subjects (7 wild types, 6 Ala/Ala carriers) was willing to participate again and was investigated on separate days for the assessment of whole body glucose disposal upon lipid exposure (Fig. 1B). Two-stepped euglycemic-hyperinsulinemic clamps were performed (euglycemic-hyperinsulinemic clamps from baseline until steady state, followed by an additional lipid/heparin infusion for an additional 320 min), with 40 mU·m\textsuperscript{-2}·min\textsuperscript{-1} human insulin (Actrapid; Novo Nordisk, Bagsvaerd, Denmark), a variable infusion of 20% glucose (Serag Wiessner, Naila, Germany), and a constant infusion of a lipid solution (20% Deltalipid LCT; Deltaselect, Pfullingen, Germany) combined with heparin (Heparin-Natrium-25000-ratiopharm; Merckle, Blaubeuren, Germany) (Fig. 1A). Plasma glucose concentrations were allowed to rise freely after the start of the lipid/heparin infusion.
gluconeogenesis. This was followed by free access to 0.5% \textsuperscript{2}H\textsubscript{2}O in tap water throughout the study to maintain isotopic steady state (Fig. IA) (18). Body water was assumed to be 50% of body weight in women and 60% in men (18). Enrichments of \textsuperscript{2}H in the hydrogens bound to carbon 2 (C2) and carbon 5 (C5) of blood glucose were measured as previously detailed (9, 18, 27). \textsuperscript{3}H enrichment in plasma water was measured by an exchange with acetone as described by Yang et al. (33).

Calculations. Rates of EGP were determined from the tracer infusion rate of \textsuperscript{d-}[6,6-\textsuperscript{2}H\textsubscript{2}]glucose and its enrichment to the hydrogens bound to carbon 6 divided by the mean percent enrichment of \textsuperscript{d-}[6,6-\textsuperscript{2}H\textsubscript{2}]glucose. Because tracer-to-trace ratios were constant after 300 min, steady-state equations were appropriate for calculation of EGP. The percent contribution of gluconeogenesis to EGP was set to the ratio of \textsuperscript{2}H enrichment at C5 to that at C2. The percent contribution of glycogenolysis was calculated as \(\left[1 - \left(\text{C2}/\text{C5}\right)\right] \times 100\), and absolute contributions of gluconeogenesis and glycogenolysis were calculated by multiplying their percent contributions by the rate of EGP (29). Metabolic clearance rate of glucose (expressed in \text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) was calculated from the rate of disappearance of glucose divided by the mean glucose concentration over the respective time period (23). Whole body glucose disposal (\(M\) value) in the substudy was calculated from the glucose infusion rate (14). Homeostasis model assessment for insulin resistance (HOMA-IR) was calculated as \(\left(\text{fasting insulin (mU/l)} \times \text{fasting glucose (mmol/l)}\right)/22.5\).

Statistical analyses. Results are presented as means \pm SE. The genotypes of the entire cohort were compared by Kruskal-Wallis test (when comparing 3 categories) or Mann-Whitney test (when comparing two categories). The age-adjusted comparison was performed using analysis of covariances. For this purpose, BMI was 1/square root transformed to achieve normal distribution regarding the Kolmogorov-Smirnoff test.

The age-, sex-, and BMI-matched subjects (Ala/Ala carriers vs. wild types) were compared using two-tailed Student’s \(t\)-test for paired analysis or two-way-ANOVA with the Huynh-Feldt epsilon procedure as correction factor. Results from the substudy were compared using two-tailed Student’s \(t\)-test for unpaired samples. Total area under the curve (AUC) was calculated using the trapezoid method. Statistical significance was defined as \(P < 0.05\). Calculations were performed using SPSS version 12.0 (SPSS, Chicago, IL).

RESULTS

Characteristics of the L-FABP genotype in the entire cohort. Only the A277-to-G polymorphism substituting Thr\textsuperscript{94} by Ala was detected in 93 subjects and subsequently investigated in the entire cohort (\(n = 1,453\)). Its minor allele frequency was 36% [41% wild-type Thr/Thr\textsuperscript{94} carriers, 46% heterozygous subjects (Thr/Ala\textsuperscript{94}), and 13% homozygous Ala/Ala\textsuperscript{94} carriers (Ala/Ala\textsuperscript{94})]. The genotype distribution was not significantly different from the Hardy-Weinberg equilibrium. In the overnight-fasted state there were no significant differences in plasma cholesterol (\(P = 0.08\), plasma triacylglycerols (\(P = 0.78\)), Hb A\textsubscript{1c} (\(P = 0.31\)), and HOMA-IR (\(P = 0.58\)) with respect to the genotypes.

There was, however, a significant difference in BMI (Thr/ Thr wild types 29.5 \pm 0.3, Thr/Ala 28.6 \pm 0.2, Ala/Ala\textsuperscript{94} carriers 28.4 \pm 0.4 kg/m\(^2\), \(P\) for comparing 3 categories = 0.04). This was explained by the difference in BMI between wild types (Thr/Thr) and Ala carriers (Thr/Ala or Ala/Ala). BMI of Ala carriers (28.6 \pm 0.2 kg/m\(^2\)) was significantly lower than the BMI of wild types (\(P = 0.012\)). Because of the significant difference in age (51.5 \pm 0.6, 52.3 \pm 0.5, 54.9 \pm 1.0 yr, \(P\) for comparing 3 categories = 0.01), we additionally...
performed an age-adjusted comparison of BMI (P for comparing the 3 genotypes = 0.01, P for comparing wild types vs. Ala carriers = 0.003), which confirmed the lower BMI in Ala carriers compared with the wild types.

Characteristics of the subjects enrolled into the intervention studies. The matched subjects of the intervention trial (n = 18) did not differ in baseline parameters (Table 1).

EGP, gluconeogenesis, and glycogenolysis without lipid challenge (baseline study). Before the pancreatic clamp was started in 10-h overnight-fasted subjects (Fig. 1A), there were no significant differences in rates of EGP (wild types 1.92 ± 0.08 vs. Ala/Ala94 carriers 1.99 ± 0.12 mg·kg⁻¹·min⁻¹, P = 0.62), gluconeogenesis (0.96 ± 0.04 vs. 0.98 ± 0.04 mg·kg⁻¹·min⁻¹, P = 0.69), and glycogenolysis (0.96 ± 0.09 vs. 1.00 ± 0.10 mg·kg⁻¹·min⁻¹, P = 0.75) between L-FABP genotypes.

Pancreatic clamp study: peripheral concentrations (somatostatin-insulin-glucagon clamp). After start of the lipid/heparin infusions, FFA increased from +30 min (P < 0.001) and rose further within the range seen in uncontrolled diabetes from 240 min (>1.5 mmol/l), with no difference between groups (P = 0.86; Fig. 2A). After the start of somatostatin infusion, C-peptide concentrations decreased, indicating inhibition of insulin secretion with no difference between L-FABP genotypes (P = 0.64; Fig. 2B). Insulin and glucagon were replaced within the low fasting range, with no difference between groups (molar insulin/glucagon ratio between L-FABP genotypes, P = 0.42; Fig. 2, C and D). In the overnight-fasted state, growth hormone levels tended to be higher in Ala/Ala94 carriers but were not significantly different between L-FABP genotypes (P = 0.185; Fig. 2E), with high variance very likely to be explained by the known pulsatile secretion pattern of this hormone. Upon somatostatin infusion, growth hormone levels were completely suppressed in all subjects. There was no difference in growth hormone concentrations between L-FABP genotypes (P = 0.007) and glycogenolysis (P = 0.015) in Ala/Ala94 carriers vs. wild-type subjects. Gluconeogenesis increased with no difference between genotypes (P = 0.65). Values are means ± SE for 9 age-, sex-, and body mass index (BMI)-matched alanine (Ala)/Ala94 carriers (●) and wild-type subjects (○) in each group. F: lipid-induced changes in rates of EGP, gluconeogenesis (open bars), and glycogenolysis (gray bars). Data are expressed as fold difference in lipid-exposed subjects relative to the overnight-fasted state. After 320-min lipid/heparin infusion in the somatostatin-insulin-glucagon clamp there was a significant reduction in EGP (P = 0.007) and glycogenolysis (P = 0.015) in Ala/Ala94 carriers vs. wild-type subjects. Gluconeogenesis increased with no difference between genotypes (P = 0.65). Values are means ± SE for 9 age-, sex-, and BMI-matched subjects in each group. *P < 0.05; **P < 0.01. G: associated changes in plasma glucose concentrations at baseline (−150 to −10 min) and during lipid/heparin infusion under somatostatin-insulin-glucagon clamp conditions (0 to +320 min; P for genotype vs. lipid-treatment interaction <0.0001). Values are means ± SE for 9 age-, sex-, and BMI-matched Ala/Ala94 carriers (●) and wild-type subjects (○) in each group. ***P < 0.0001.
interaction (P/H11005) genotype per se (P = 0.52), and 2.21 ± 0.16 vs. 2.22 ± 0.16, P = 0.45).

EGP, gluconeogenesis, and glycogenolysis in lipid-exposed subjects (somatostatin-insulin-glucagon clamp). When genotype (Ala/Ala94 carriers vs. wild types) and treatment (basal state vs. lipid infusion) were included in one model, two-way ANOVA showed a significant effect of the lipid treatment (P = 0.005) and a genotype-vs.-treatment interaction (P = 0.009) but no effect of the genotype per se (P = 0.78), indicating that lipid exposure was necessary to detect differences in EGP between L-FABP genotypes. During lipid infusion, EGP significantly decreased in Ala/Ala94 carriers (from 1.99 ± 0.12 to 1.69 ± 0.14 mg·kg⁻¹·min⁻¹, P = 0.002), and this decrease was attenuated and not statistically significant in wild types (from 1.92 ± 0.08 to 1.82 ± 0.09 mg·kg⁻¹·min⁻¹, P = 0.087). Relative to baseline, changes in EGP after 320-min lipid/heparin infusion were significantly different between genotypes (P = 0.007; Fig. 2F). Glucose infusion rates required to prevent a somatostatin-induced decrease of plasma glucose during the first hours of the somatostatin clamps (maximal rates 1.38 ± 0.24 vs. 1.01 ± 0.19 mg·kg⁻¹·min⁻¹ over 5 min, P = 0.18; time of maximal rates 80 ± 32 vs. 75 ± 2 min, P = 0.64) and metabolic clearance rates of glucose (1.54 ± 0.20 vs. 1.40 ± 0.21 ml·kg⁻¹·min⁻¹, P = 0.59) were not significantly different between Ala/Ala94 carriers and wild types.

Both glycogenolysis and gluconeogenesis contribute to EGP. The contribution of glycogenolysis to EGP after 320-min lipid/heparin infusion was significantly lower in Ala/Ala94 carriers vs. wild types (0.46 ± 0.05 vs. 0.59 ± 0.05 mg·kg⁻¹·min⁻¹, P = 0.013). Compared with the baseline, glycogenolysis was reduced by 54% in Ala/Ala94 carriers (P < 0.001) and by 38% in wild types (P < 0.001), and these reductions were significantly different between genotypes (P = 0.015) (Fig. 2F).

For the contribution of gluconeogenesis to EGP, there was an effect of lipid treatment (P = 0.003) but neither an effect of genotype per se (P = 0.83) nor a genotype-vs.-treatment interaction (P = 0.78). Gluconeogenesis increased by 29% in wild types (P = 0.003) and by 25% in Ala/Ala94 carriers (P = 0.015) during lipid treatment, with no difference between the genotypes (absolute rates P = 0.97, baseline changes P = 0.65; Fig. 2F). These results indicate that lipid/heparin infusions affected EGP and contribution of glycogenolysis dependent on the L-FABP genotype, whereas gluconeogenesis increased independently of the genotype.

Whole body glucose disposal in overnight-fasted and lipid-exposed subjects (substudy). Steady-state conditions were reached after 150 ± 7 min. Thereafter, lipid/heparin infusions were added for an additional 320 min (Fig. 1B). M values decreased in lipid-exposed subjects (6.93 ± 0.59 vs. 5.67 ± 0.75 mg·kg⁻¹·min⁻¹, P = 0.020; n = 13), reflecting lipid-induced peripheral insulin resistance. When comparing age-, sex-, and BMI-matched L-FABP genotypes (4 of the matched pairs from the main study), M values were not significantly different between L-FABP genotypes in the overnight-fasted state and even tended to be higher in wild types (7.04 ± 0.65 vs. 4.98 ± 0.64 mg·kg⁻¹·min⁻¹, P = 0.19), which was also in agreement with the estimated insulin resistance in nine matched pairs, as calculated with HOMA-IR (Table 1). After lipid exposure, there was not any difference in M values between L-FABP wild types and Ala/Ala94 carriers (3.89 ± 1.14 vs. 4.74 ± 0.62 mg·kg⁻¹·min⁻¹, P = 0.85). There was no difference in apolipoprotein B concentrations between lipid-exposed L-FABP genotypes (0.76 ± 0.07 vs. 0.75 ± 0.09 g/l, P = 0.92).

CONCLUSIONS

Markers of glucose metabolism are improved in fed L-FABP knockout mice, and these animals are resistant to high-fat-, Western diet-induced obesity and fatty liver (21). In contrast, absence of L-FABP exerts no obvious phenotype in mice in the overnight-fasted state (22). In humans, the in vivo significance of L-FABP is largely unknown. The present study shows that the common Thr132-to-Ala amino acid replacement in L-FABP contributed significantly to reduced hepatic glycogenolysis and less severe hyperglycemia in lipid-exposed humans and was further associated with reduced body weight in a large cohort. The observed phenotype shows obvious conformity with the phenotype observed in L-FABP knockout mice, indicating that the Ala94 replacement in L-FABP is likely to be physiologically relevant.

In the non-lipid-exposed, overnight-fasted state, commonly used markers of glucose metabolism such as fasting glucose, fasting insulin, or HOMA-IR were not different between L-FABP genotypes. In contrast, plasma glucose concentrations markedly increased in wild types vs. Ala/Ala94 carriers when FFA concentrations were raised about threefold above fasting levels, which is within the range seen in obese subjects postprandially after a high-fat meal (13). This was explained mainly by higher rates of glycogenolysis in wild types, whereas lipid-induced increases in gluconeogenesis were comparable between L-FABP genotypes. Although more pronounced hyperglycemia per se should be expected to inhibit hepatic glucose production (11), lipid challenge appeared to interfere with this inhibitory effect, suggesting more severe FFA-induced impairment of hepatic autoregulation in L-FABP wild types.

It has recently been reported that plasma glucose can rise in lipid-exposed subjects, despite unaltered or even decreased rates of EGP (26, 29), due to lipid-induced reduction of whole body glucose disposal (16, 25). Because L-FABP is expressed mainly in hepatocytes and enterocytes (3), differences in whole body glucose disposal between matched L-FABP genotypes after intravenous administration of lipids were unlikely. However, to exclude this possibility, a substudy was performed with the same participants on separate study days, which showed lipid-induced reduction of whole body glucose disposal in all subjects, as expected (16), but no differences between L-FABP genotypes. This is again in agreement with the phenotype described in lipid-exposed L-FABP knockout mice (22).

We can further confirm the previously reported association of the Ala94 allele with reduced body weight (7) in a larger cohort. Because lipid-induced increases in plasma glucose under nonclamp conditions are accompanied by increased insulin secretion, and hyperglycemic-hyperinsulinemic conditions have been shown to inhibit fatty acid oxidation (28), this could be a contributing factor to the observed differences in body weight between L-FABP genotypes.

The rationale for choosing the specific experimental setup in the present study needs to be discussed. In vitro, FFA increase gluconeogenesis through the activation of key gluconeogenic
factors (32). However, in vivo, lipid-induced changes in gluconeogenesis were not generally associated with changes in EGP (5, 26, 29), which can be partly explained by hepatic autoregulation with a matched decrease of glycogenolysis (12). Therefore, in addition to determining EGP, in the present study we also measured the contribution of gluconeogenesis and glycogenolysis to EGP. Generally, clamp studies cannot completely reflect the complex metabolic situation observed in the postprandial state, e.g., after the intake of a high-fat meal. When using hyperinsulinemic clamps, elevation of FFA concentrations is known to impair insulin-mediated suppression of EGP (4). However, hyperinsulinemic conditions might obscure effects of FFA particularly on glycogenolysis, which is known to be sensitive even to very small increases in insulin concentrations (10, 15). In studies not using clamp conditions, another problem arises in that FFA potently increase insulin secretion (4). To circumvent these potential problems we used an infusion of somatostatin and the replacement of insulin and glucagon at low fasting doses, which allowed plasma glucose concentrations to increase freely in response to lipid infusion (6, 26) and was furthermore likely to reduce potential interference of higher insulin doses, particularly with glycogenolysis.

The experimental conditions for matched Ala/Ala94 carriers and wild types were identical in the present study. Potential differences in somatostatin responsiveness between L-FABP genotypes were unlikely, as indicated by near-complete somatostatin-induced suppression of C-peptide and growth hormone concentrations in all subjects, with no difference between L-FABP genotypes. However, the chosen experimental design might have been advantageous for a proof of principle, and we cannot exclude that the metabolic effects of systemic vs. portal lipid administration might be distinct. In addition, we investigated healthy persons with normal glucose metabolism, and potential effects of the Ala/Ala94 variant in patients with high blood lipids, diabetes, and obesity are as yet unknown. Another important limitation was the small number of participants in the intervention studies. However, phenotypic differences between lipid-exposed L-FABP genotypes in the present study were pronounced, statistically significant, and are in agreement with the phenotype observed in L-FABP knockout mice (22).

In conclusion, the common Ala/Ala94 amino acid variant in L-FABP contributed significantly to decreased hepatic glycogenolysis and less severe hyperglycemia in lipid-challenged humans. Investigation of L-FABP genotypes in the basal, overnight-fasted state yielded only incomplete information. It can be hypothesized that L-FABP may not play a significant role in a normal diet but may contribute to disturbed glucose metabolism in a high-fat diet.

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