Impairment of fat oxidation under high- vs. low-glycemic index diet occurs before the development of an obese phenotype

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EXPOSURE TO HIGH VS. LOW GLYCEMIC INDEX (GI) DIETS UNFAVORABLY AFFECTS FAT Mass AND MARKERS OF INSULIN resistance IN Rodent MODELS (1, 12, 15–17, 21, 23). Metabolic flexibility that can be defined as the capacity to adapt fuel oxidation to fuel availability (3, 7, 13) could also be changed by a high-GI diet, although no consensus exists as to whether diets varying in GI directly affect substrate oxidation or energy expenditure (4, 21). Alternatively, changes in substrate oxidation might be mainly a consequence of high- vs. low-GI diet-induced differences, e.g., in body weight, body fat distribution, or insulin resistance. Therefore, elucidating whether changes in substrate oxidation follow or precede an obese phenotype in high-GI-fed animals could be helpful for the understanding of potentially involved mechanisms.

Most of the previous studies investigating effects of high- vs. low-GI diets on metabolic markers were relatively short term (1, 12, 15, 16, 23), lasting between 3 and up to 13 wk. We are aware of only two previous longer-term studies, both of which reported unfavorable effects of high- vs. low-GI diets on body fat distribution and metabolic markers in rodents (17, 21). However, the first study (18 wk) investigated partial pancreatectomized male Sprague-Dawley rats, which is an animal model that also reflects reduced β-cell mass, and therefore outcomes might be different compared with obesity-prone mouse models. In the other long-term study (48 wk), authors investigated effects of diets differing in GI in male 129SvPas mice (21). These mice are commonly used for gene targeting strategies, but particularly male 129SvPas mice are resistant to age-induced obesity and insulin resistance (2, 5). Although using this mouse model is a clearly interesting approach, allowing investigation of changes in metabolism independent of diet-induced changes in body weight, differences between high- vs. low-GI diet-induced effects might have been underestimated.

Importantly, the investigation of both long- and short-term metabolic effects under comparable conditions, exposing an obesity-prone animal model to high- vs. low-GI diets, has not been reported to date. In the present study, we investigated C57BL/6J mice that are commonly used in metabolic studies due to their susceptibility to diet-induced obesity and hyperglycemia (5, 22). Four groups of male C57BL6/J mice (n = 10 per group) were exposed to long-term (20 wk) or short-term (6 wk) isoenergetic and macronutrient matched diets that only differed in GI. Body composition, molecular factors of lipid metabolism, and markers of metabolic flexibility and insulin sensitivity, as well as the chronological order of their appearance were measured in all four groups of mice.

MATERIAL AND METHODS

Animals. The protocol for all animal experiments was approved by the local governmental animal ethical review board (State of Brandenburg, Germany). Animals were kept in accordance with the NIH guidelines for care and use of laboratory animals. Experiments were performed in adult (16 wk old) male C57BL/6J mice obtained by Charles River. Animals were housed individually at a temperature of 22°C with a 12:12-h light-dark cycle in cages with soft wood bedding. After the experimental periods (20 wk in the mice exposed to the
long-term experiments and 6 wk in the group of mice exposed to the short-term experiments, respectively). Mice in fed state were sedated using ether inhalation and killed by decapitation. Organs were isolated after rapid preparation.

Characterization of experimental diets. Before the main experiments, postprandial glucose and insulin responses to the here used experimental high (100% amylopectin, 0% amylose) vs. low (30% amylopectin, 70% amylose) GI diets were tested in a trained meal test with a separate group of male C57BL/6J mice (n = 16), as detailed previously (11). Both diets contained 65% carbohydrate, 23% protein, and 12% fat. The macronutrient composition of the experimental diets is shown in Table 1.

Study design. Both in long- and short-term intervention, mice received isoenergetic and macronutrient high-carbohydrate diets only differing in amylopectin/amylose content and as such GI. The duration of the respective intervention was 20 wk in the long-term study (n = 10 per group) and 6 wk in the short-term study (n = 10 per group). Food intake rate was recorded weekly until week 18.

Measurements of body weight, body composition, digestibility of diets, glucose clearance following a glucose load, (glucose tolerance test; GTT), markers of glucose and lipid oxidation, liver fat contents, and molecular markers related to hepatic lipid metabolism were performed at timed intervals and are detailed below.

Frozen liver tissue were additionally investigated in a separate group of 16-wk-old C57BL/6J male mice (n = 6) fed a standard rodent chow diet (Altromin 1324, Altromin, Lage, Germany).

Energy expenditure. Total energy expenditure (TEE) was estimated in all groups of mice after dietary intervention periods of 3 wk (short-term study) and 19 wk (long-term study, respectively). Indirect calorimetry was performed in individual mice, using an open respiratory system (gas analyzers: Magnos 16 and Uras 14, Hartmann & Braun, Woburn, MA) was applied by oral gavage in a volume of 50 μl/20 g body wt. Breath samples were obtained in duplicates at baseline and 10, 20, 30, 40, 50, 60, 80, 100, 110, and 120 min after glucose (U-13C6, 98%, MW 181.6 g/mol; Cambridge Isotope Laboratory, Woburn, MA) was applied by oral gavage in a volume of 50 μl/20 g body wt. Breath samples were obtained in duplicates at baseline and 10, 30, 40, 50, 60, 80, 100, 110, and 120 min after glucose oxidation administration. For the collection of 13CO2 samples, animals were placed individually at each time point into 140-ml syringes for 30 s. This time interval had been tested before the experiments to be sufficient for the detection of CO2 concentration and 13C enrichment in mice (data not shown). The syringes were equipped with a wave to collect representative breath samples into evacuated 10-ml tubes (Extainer, Labco, High Wycombe, UK) for storage and measurement. Breath 13CO2 enrichments were analyzed by isotope ratio mass spectrometry (BreathMAT, Thermo Scientific, Bremen, Germany) and were expressed as δ13C in the conventional delta per mill notation as described (19). The delta over baseline values (DOB) were expressed by calculating the difference between enrichment of each breath sample and the baseline value.

GI. Glycemic index.
esternified fatty acid (NEFA) were measured using commercial kits (glucose HK 125; triacylglycerols, total cholesterol: ABX Pentra, Montpellier, France; Wako, Germany) by using an autoanalyzer (Cobas Mira S, Hoffmann La Roche, Switzerland).

RNA extraction and real-time RT-PCR. Hepatic, adipose, and muscle tissue samples were submerged in nitrogen and immediately stored at −80°C until further preparation.

Total RNA was extracted from tissue of animals killed in the non-fasted state after dietary intervention periods of 6 wk (short-term study) and 20 wk (long-term study), respectively, as detailed previously (10). All samples were measured in triplicates, and nontemplate controls were used to confirm specificity. The quantity of target and the housekeeping gene hypoxanthine-guanine phosphoribosyltransferase (Hprt) were calculated according to a standard curve. Expression of stearyl regulatory element binding protein 1c (Srebp-1c), acetyl-CoA carboxylase (Acc), fatty acid synthase (Fas), stearoyl-CoA desaturase1 (Scd-1), diacylglycerol acyltransferase-2 (Dgat-2), L-carnitine palmitoyl transferase1 (Cpt-1), and long-chain acyl-CoA dehydrogenase (Lcad) was analyzed in hepatic, muscle, and epididymal white adipose tissue in all groups of mice. The oligonucleotide specific primers were: Hprt (forward 5'-CACCT-CACCGCTCTGTGATTA-3', reverse 5'-AGGAAGTCCTTCGAGCCA-3'); Srebp-1c (forward 5'-GGAGGAGTCTATCCCCCCATGGGGA-3', reverse 5'-GAAACGTGTCAAGAAGTGCAGG-3'); Acc (forward 5'-CACGTTTCA-GAACGCCCACTACG-3', reverse 5'-TAACGTTCTGGTCTGCTGAG-3'); Fas (forward 5'-AGAGCCGTGGATAGCCGCGGATAT-3', reverse 5'-GGTAGAATCCATAGAGGCCACGCTT-3'); Scd-1 (forward 5'-GCCCAACCATCGCACAAGAGTCT-3', reverse 5'-AGGACCCGTGCCCAGCCTG-3'); Dgat-2 (forward 5'-CCGCCACAGGATACGGCAGGATT-3', reverse 5'-GGAGGTTGGTGGTCTTGCTGAG-3'); Cpt-1 (forward 5'-CCTGCCATCTGCCCTCCCCATTCTG-3', reverse 5'-CCGATCTGCTCTTTATGTCGCC3'; and Lcad (forward 5'-GGTTTGCATTGAAATATCCTGGG-3', reverse 5'-CAGTAAAGCTTGGGAAATCGG-3').

Statistical analysis. Quantitative data are presented as means ± SE. Data were analyzed using two-tailed Student’s t-test for unpaired samples or ANOVA for repeated measurement to analyze time course longitudinal changes in body weight (SPSS 14, SPSS, Chicago, IL). For characterization of the high- vs. low-GI test meals before the study, the incremental area under the plasma glucose curve was calculated over 120 min after feeding the respective test meals to a separate group of trained mice. P < 0.05 was considered significant.

RESULTS

GI and fermentation of the experimental diets. After a 5-day training period, six of eight animals in each group consumed the entire test meals. AUCglucose over 120 min was significantly higher in animals fed the high- vs. low-GI diet (P = 0.036; Fig. 1A). The observed 44% reduction in AUCinsulin in low-GI-fed animals failed to reach significance level (P = 0.09; Fig. 1B). Colonic fermentation of both diets was estimated by using hydrogen breath tests at week 14 (Fig. 1C). Hydrogen exhalation in low-GI-fed animals was significantly increased compared with high-GI-fed mice (P = 0.03).

Increased weight gain and body fat with high- vs. low-GI diet. Figure 2A shows changes in body weight during the 20-wk experimental period. The influence of the glycemic index on time course of increases in body weight was statistically significant (time × diet, P = 0.015), with an increase of body weight under the high-GI diet. The body fat content in the high- vs. low-GI-fed animals was markedly and significantly increased as early as from week 5 of dietary intervention (Fig. 2B). Lean mass was higher in low-GI-fed animals during weeks 3–7, but no difference in lean mass between low- and high-GI-fed animals was observed in the later phases of the experiments (weeks 9–16; Fig. 2C).

Decreased glucose clearance with high- vs. low-GI diets during GTT. GTTs were performed in overnight fasted animals in the long-term intervention. AUCglucose was markedly and significantly lower in mice exposed to 15 wk of low- vs. high-GI dietary intervention (3,714 ± 548 vs. 6,350 ± 364 mmol·min⁻¹·P = 0.002), although some overestimation of the here observed differences cannot be excluded, given that the administered glucose challenge was calculated according to body weight rather than to lean mass.
There was no difference in AUC_{insulin} (13,597 ± 1,054 vs. 14,382 ± 1,541 pmol/l·min; \( P = 0.68 \)). Significant differences at single time points for plasma glucose and insulin responses during GTT are shown in Fig. 2, \( D \) and \( E \). Compared with high-GI-fed mice, the change in glucose clearance under low-GI diet indicates improved insulin sensitivity, whereas increased insulin levels after 120 min in high-GI-fed mice might also reflect delayed insulin secretion.

**Energy digestion of high- vs. low-GI diets.** For analysis of digestibility, fecal excretion and food uptake were recorded at week 15 for further analysis of digested energy. Energy contents of the respective diets were measured using a calorimeter and were comparable between groups (high GI 17.4 kJ/g, low GI 17.5 kJ/g; Table 1). Food intake was multiplied with these values. Table 2 shows no significant difference in digestible energy intake during a 1-wk period. Fecal excretion (feces weight in grams) and feces energy contents were significantly lower in high- vs. low-GI diet fed animals (Table 2). Accordingly, digestibility of the diets expressed as digested diet energy was significantly higher in the high-GI-fed animals (Table 2). Although feces energy loss was significantly different between groups, there was no difference in energy intake likely explained by the relatively high interindividual variations in food intake. The high digestibility of both diets (>90%) further reduced the influence of feces energy loss to whole energy intake. For comparison, digestible energy of a standard rodent chow (Altromin1324) was 74% (energy density of 17.1 kJ/g).

Long-term cumulative energy digestion over 9 and 18 wk on experimental diets was not significantly increased in high- vs.
Table 2. Energy digestion during 1 wk (exemplary week 15) and cumulative energy digestion during 9 and 18 wk of intervention

<table>
<thead>
<tr>
<th>Balance in week 15</th>
<th>Low GI</th>
<th>High GI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food intake, g</td>
<td>23.0 ± 0.55</td>
<td>22.4 ± 0.59</td>
<td>0.49</td>
</tr>
<tr>
<td>Energy intake, kJ</td>
<td>398.9 ± 9.5</td>
<td>389.0 ± 10.2</td>
<td>0.49</td>
</tr>
<tr>
<td>Feces weight, g</td>
<td>2.7 ± 0.2</td>
<td>1.7 ± 0.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Feces energy, kJ/g</td>
<td>13.8 ± 0.17</td>
<td>10.6 ± 0.11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Feces energy loss, kJ</td>
<td>36.8 ± 2.1</td>
<td>18.4 ± 0.82</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Digestible energy intake, kJ</td>
<td>362.1 ± 7.9</td>
<td>370.7 ± 10.0</td>
<td>0.51</td>
</tr>
<tr>
<td>Digestible energy, %</td>
<td>90.8 ± 0.4</td>
<td>95.3 ± 0.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Balance over 9 wk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food intake, g</td>
<td>178.4 ± 3.4</td>
<td>176.5 ± 2.7</td>
<td>0.66</td>
</tr>
<tr>
<td>Net energy intake, kJ</td>
<td>2,831.5 ± 54.0</td>
<td>2,920.4 ± 44.5</td>
<td>0.22</td>
</tr>
<tr>
<td>Balance over 18 wk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food intake, g</td>
<td>359.9 ± 6.28</td>
<td>356.9 ± 6.56</td>
<td>0.75</td>
</tr>
<tr>
<td>Net energy intake, kJ</td>
<td>5,710.1 ± 99.4</td>
<td>5,904.8 ± 108.4</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Values are means ± SE.

low-GI-fed animals (P = 0.2) but still might have contributed to the above-described increase in body fat content.

Improved metabolic flexibility and accelerated glucose and lipid utilization with low-GI diet. After 19 wk of intervention, indirect calorimetry was performed during a 5-h fasting period, after free access to experimental diets (Fig. 3A). Total energy expenditure was not significantly different between long-term high- vs. low-GI-exposed mice (51.5 ± 0.8 vs. 52.7 ± 1.1 kJ/day; P = 0.43). There was also no difference between dietary interventions regarding RQ, as measured in the fasted state (Fig. 3B). In the postprandial state (following a 5-h fasting period), both low- and high-GI-fed animals showed an increased RQ, as expected and consistent with switching from fat oxidation to augmented carbohydrate oxidation. However, postprandially measured RQ was significantly lower in high- vs. low-GI-fed animals (P = 0.003; Fig. 3B), indicating a blunted switch to carbohydrate oxidation in long-term high-GI-fed mice.

Investigating RQ in the group of mice exposed to short-term high- vs. low-GI diets revealed a different picture (Fig. 3C). In the fasted state, short-term (3 wk) low-GI-fed mice showed significantly higher RQ, indicating increased glucose oxidation in these animals (P < 0.001; Fig. 3D), whereas differences in RQ measured postprandially were less pronounced between dietary interventions, with RQ tending to be increased in high-GI-fed animals (Fig. 3D).

Oxidation-kinetic profiles of orally administrated glucose further supported more pronounced disturbance of glucose oxidation after long-term high-GI diet (measured after 12 wk of dietary intervention; Fig. 4A) compared with both long-term low-GI diet and short-term high-GI exposure (Fig. 4, A and C). Delta over baseline (DOB) of glucose-derived 13CO2 was significantly higher in low-GI-fed mice after 10 and 20 min (P = 0.012 and P = 0.017, respectively). In contrast, no differences in the glucose oxidation profiles were observed after short-term high- vs. low-GI exposure (measured after 4 wk on experimental diets; Fig. 4C). As stated above, body composition in high- vs. low-GI-fed animals was not measured at week 4 but was comparable at week 3 and significantly different only from week 5, with differences between groups further increasing under prolonged dietary intervention (Fig. 2B). These data indicate that the blunted switch to carbohydrate oxidation under long-term high-GI diet was more likely to have been secondary to changes in body composition and fat mass rather than representing a direct effect of the high-GI diet on markers of glucose oxidation.

In contrast to markers of glucose oxidation, the palmitate oxidation profile indicated that lipid oxidation was affected even after a short-term high- vs. low-GI dietary intervention. Oral administration of 13C-labeled palmitate led to a blunted lipid oxidation in high- vs. low-GI-fed animals, which was comparable both after short-term (Fig. 4B) and long-term (Fig. 4D) dietary intervention. DOB of palmitate-derived 13CO2 in low-GI-fed animals declined to baseline after 210 min, whereas there was a significantly less pronounced but sustained increase of 13CO2 in high-GI-fed mice, indicating delayed fat oxidation. This pattern in low-GI-fed mice was very similar to the palmitate oxidation profile as observed in standard group-fed animals that had been additionally performed for further comparison.

Therefore, the here-observed significant delay in early postprandial fat oxidation [AUC120 min, short-term study: 363 ± 67 vs. 921 ± 61 DOB (%)/min, P < 0.001; long-term study: 460 ± 86 vs. 887 ± 66 DOB (%)/min, P = 0.021] appeared to be primarily caused by the high-GI diet rather than by being a consequence of diet-induced changes in body composition. Significant differences in palmitate oxidation at single time points are shown in Fig. 4, B and D.

Fat accumulation and changes in gene expression of hepatic enzymes of fat metabolism. After 20 wk of dietary intervention, marked microvesicular and macrovesicular steatosis was present in the histology of high- vs. low-GI-fed mice (Fig. 5, A and B). Triacylglycerol accumulation in hepatic tissue after long-term intervention was nearly threefold increased in high- vs. low-GI-fed mice (P = 0.006; Fig. 5D). Quantitative PCR was performed to investigate gene expression of key enzymes in fat metabolism. Expression of important enzymes in regulation of de novo lipogenesis such as Acc and Fas was significantly increased in high- vs. low-GI-fed mice (P = 0.009 and P = 0.003, respectively, Fig. 5D). Scd-1 was also significantly increased with high-GI diet (P = 0.024). In agreement with these findings, also the gene expression of Srebp-1c was significantly increased in high-GI-fed mice (P = 0.004). Gene expression of Cpt-1 or Lcad was not significantly different between dietary groups (Fig. 5D).

After 6-wk short-term intervention, liver triacylglycerol content was already significantly increased in high- vs. low-GI-fed mice (P = 0.003; Fig. 5E). The gene expression pattern of the metabolic key enzymes tended to a similar direction compared with longer-term dietary exposure. A significant difference after short-term intervention was, however, measured only in Scd-1 mRNA expression (P = 0.008; Fig. 5F).

Changes in fat metabolism seemed to affect gene expression levels only in hepatic tissue (Fig. 5, D and F), whereas gene expression profiles in white adipose or muscle tissues were comparable between dietary groups, both in the short-term and in the long-term experiments (data not shown). Changes in hepatic fat metabolism were also reflected by increased plasma triacylglycerol levels (1.60 ± 0.16 vs. 1.16 ± 0.09; P = 0.027) in the high- vs. low-GI-fed animals after 8 wk of dietary exposure, whereas NEFA (0.85 ± 0.04 vs. 0.77 ± 0.03; P = 0.004; Fig. 5D).
DISCUSSION

It is unknown whether potential changes in markers of metabolic flexibility and molecular markers of fat metabolism are a consequence of high-GI diet-induced adiposity or, alternatively, might precede changes in body fat distribution and liver fat, thereby indicating a potentially causal involvement. Furthermore, long- vs. short-term effects of diets varying in GI on body composition, markers of insulin sensitivity, and expression of key enzymes of fat metabolism in obesity-prone mouse models have not been reported to date.

Herein, we show that long-term exposure to high- vs. low-GI diets leads to an obese phenotype in obesity-prone C57BL/6J mice. This phenotype included a moderate but significant increase in body weight, together with a striking increase in fat mass and liver fat contents, as well as more pronounced estimated insulin resistance. The here-observed reduced glucose clearance in high-GI-fed mice might have been induced by a disrupted islet-cell architecture, as described by Ludwig and coworkers (17), with high-GI-fed rodents being unable to further increase insulin secretion after a glucose load. Furthermore, the known defect of mitochondrial metabolism in C57BL/6 mice might have additionally affected insulin secretion (6), thus further contributing to impaired glucose intolerance and the here-observed high glucose levels in challenged animals.

Changes in markers of metabolic flexibility after high- vs. low-GI feeding could contribute to explain these findings. In the present study, we measured RQ under re-feeding conditions to detect potential changes in metabolic flexibility. The here-observed differences in RQ between high- vs. low-GI-fed animals may have resulted from changes in both fat and glucose utilization, with the delayed increase in RQ in the long-term high-GI-fed animals reflecting a blunted reduction of fat oxidation after re-feeding, probably due to reduced insulin sensitivity in white adipose tissue and thus ongoing lipolysis. This may have also resulted in reduced glucose oxidation in long-term high-GI-fed mice since overall energy expenditure was not different between the groups, whereas short-term (3 wk) differences in RQ between high- vs. low-GI-fed animals were less pronounced. These data indicated that short-term high-GI feeding, in the absence of major changes in body fat...
composition at this stage, had no major impact on glucose oxidation in the postprandial state.

At the first glance, these findings appeared to be in contrast to a recent report from others showing opposite changes in RQ in high- vs. low-fed 129SvPas mice (21). However, differences in the respective design of the studies are likely to explain varying results. In the present study, changes in RQ in the postprandial state were measured following a prior 5-h fasting period to measure metabolic flexibility. Moreover, the chosen design should more accurately reflect feeding behavior in humans with prolonged (e.g., overnight) fasting periods followed by a 16- to 18-h postprandial state over the day compared with dietary habits of mice that are unlikely to be in a longer-term fasted state if food availability is unrestricted (14).

The investigation of oxidation-kinetic profiles after orally administrated glucose further supported significant disturbance of glucose oxidation after long-term, but not after short-term, high- vs. low-GI exposure. However, and importantly, in contrast to markers of glucose oxidation, the palmitate oxidation profile indicated that lipid oxidation was markedly and significantly affected even after a short-term high- vs. low-GI dietary intervention as early as 3 wk after the intervention had been started. At that stage, most measured phenotypic markers including body fat mass were comparable between groups. Therefore, the here-observed significant delay in early postprandial fat oxidation appeared to be primarily caused by the high-GI diet rather than reflecting a diet-induced change in body composition.

The exact mechanisms by which palmitate oxidation was reduced in high-GI-fed mice remains to be clarified in more detail. The rapid increase of palmitate oxidation under both the low-GI and the standard chow diets suggests that most of palmitate may not have been re-esterified after uptake into enterocytes but rather may have been directly oxidized in splanchnic tissue, e.g., the intestine and the liver. Consequently, the flattened oxidation kinetic in high-GI-fed mice could be explained, e.g., by delayed intestinal transition time and thus delayed intestinal resorption. Alternatively, fat oxidation may also have been inhibited under high-GI conditions by the here-shown prolonged hyperinsulinemia after the overnight feeding period.

Effects of high- vs. low-GI diets on molecular markers of fat metabolism have been investigated only in a few previous studies (12, 23, 24). After long-term high- vs. low-GI exposure, the expression of important enzymes in regulation of de novo lipogenesis such as Acc and Fas was significantly elevated. Scd-1, which is an enzyme regulating triacylglycerol synthesis, was also increased with the high-GI diet. In agreement with these findings, gene expression of Srebp-1c, which is a well known transcription factor in hepatic fat metabolism that also directly regulates Scd-1 and Fas (9), was increased in high-GI-fed mice. Even after the short-term dietary interven-
tion changes in gene expression patterns tended to a similar direction, with significantly higher Scd-1 expression and markedly increased liver triacylglycerol contents as early as 6 wk of high-GI exposure. Apart from high-GI-induced hyperinsulinemia, increased de novo lipogenesis in these animals might be a contributing factor to long-term accumulation of body and liver fat, given that metabolites such as malonyl-CoA are known to decrease mitochondrial fatty acid oxidation (8).

Importantly, changes in fat metabolism seemed to affect gene expression levels of the here-measured metabolic key enzymes only in hepatic tissue, whereas no significant differences were measured in white adipose or muscle tissues in fed mice, neither in the short-term nor in the long-term experiments. These data support the hypothesis that the liver might be particularly prone to early metabolic changes on nutritional challenges (18) such as a high-GI diet.

Limitations of the present study need to be mentioned. First, hepatic lipid oxidation was not measured directly in the present study, and the oxidation of a mixture of ingested lipids might partly differ from the oxidation of isolated palmitate, as used in the experimental setting. Second, although not statistically different between groups, differences in energy absorption of the respective experimental diets might have contributed to the observed more obese phenotype in high-GI-fed animals. Third, metabolic responses to nutritional challenges might considerably differ between humans and rodents (25). Thus long-term controlled studies are needed also in humans to investigate whether effects of high- vs. low-GI diets on metabolic markers are comparable to the here-observed effects in an obesity-prone mouse model. Fourth, we did not measure physical activity in the present study. A recent study reported a nonsignificant 15% decrease in physical activity under high-GI diet in male 129SvPas mice (21). However, energy expenditure was identical between groups, both in the mentioned study (21) and in the present study, indicating that major diet-induced differences in physical activity were unlikely.

**Fig. 5. Changes in liver histology, hepatic triacylglycerol content, and gene expression of key enzymes of liver fat metabolism after intervention with high- vs. low-GI diets.**

Typical hematoxilin and eosin-stained liver tissues after 20 wk on low (A) vs. high (B) GI diet, with original magnification of ×100. Hepatic triacylglycerol contents after 20 wk (C) vs. 6 wk dietary intervention (E). Real-time PCR analysis of enzymes of lipid metabolism in liver after long-term (D) and short-term (F) experiment. Results were normalized to internal control Hprt, and the intervention group fed low-GI diet was set to 1.0. *P value of <0.05, **P value of <0.01
In conclusion, mice fed the high-GI diet showed a rapid-onset marked increase in body fat mass and liver fat, a gene expression profile in liver consistent with elevated lipogenesis and, after long-term exposure, significantly reduced glucose clearance following a glucose load. The long-term high-GI diet also led to a delayed switch to both carbohydrate and fat oxidation in the postprandial state, indicating reduced metabolic flexibility. In contrast, no difference in carbohydrate oxidation was observed after short-term high-vs. low-GI exposure, whereas palmitate oxidation was significantly blunted as early as 3 wk after beginning of the high-GI intervention at a time where most phenotypic markers, including body fat mass, were comparable between groups. Thus early onset and significant impairment of fatty acid oxidation might be potentially causally involved in the development of the observed metabolically obese phenotype in high-GI-fed mice.

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