Impairment of fat oxidation under high vs low glycemic index diet occurs prior to the development of an obese phenotype

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Abstract

Exposure to high vs low glycemic index (GI) diets increases fat mass and insulin resistance in obesity prone C57BL/6J mice. However, the longer-term effects and potentially involved mechanisms are largely unknown. We exposed four groups of male C57BL/6J mice (n = 10 per group) to long-term (20 weeks) or short-term (6 weeks) isoenergetic and macronutrient matched diets only differing in starch type and as such GI. Body composition, liver fat, molecular factors of lipid metabolism, and markers of insulin sensitivity and metabolic flexibility were investigated in all four groups of mice. Mice fed the high GI diet showed a rapid-onset (from week five) 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. However, fatty acid oxidation was significantly blunted as early as three weeks after beginning of the high GI intervention, at a time where most measured phenotypic markers including body fat mass were comparable between groups. Thus, long-term high GI feeding resulted in an obese, insulin-resistant, and metabolically inflexible phenotype in obesity prone C57BL/6J mice. Early onset and significantly impaired fatty acid oxidation preceded these changes, thereby indicating a potentially causal involvement.
Introduction

Exposure to high vs low GI diets unfavourably 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 weeks. We are aware of only two previous longer-term studies, both reporting unfavourable effects of high vs low GI diets on body fat distribution and metabolic markers in rodents (17, 21). However, the first study (18 weeks) investigated partial pancreatectomised male Sprague-Dawley rats, which is an animal model also reflecting reduced beta-cell mass and therefore outcomes might be different in comparison to obesity prone mouse models. In the other long-term study (48 weeks) 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 to investigate 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-term 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. We investigated C57BL/6J mice in the present study 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 weeks) or short-term (6 weeks) isoenergetic and macronutrient matched diets only differing 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 ethic 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 weeks old) male C57BL/6J mice obtained by Charles River, Germany. 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 weeks in the mice exposed to the long term experiments, and 6 weeks in the group of mice exposed to the short-term experiments, respectively), mice in fed state were sedated using ether inhalation and sacrificed by decapitation. Organs were isolated after rapid preparation.

Characterisation of experimental diets. Prior to the main experiments, postprandial glucose and insulin responses to the here used experimental high (100% amylopectin, 0% amylose) vs low (30% amylopectin, 70% amylose) glycemic index (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% of 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 amylose/amylopectin composition of starch content and as such GI. The duration of the respective intervention was 20 weeks in the long term study (n = 10 per group) and 6 weeks 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 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.

High vs low GI diets could affect metabolic markers before changes in body composition occur. Therefore, in order to receive comparable information about potential changes in liver fat and molecular markers in tissue samples that cannot be obtained from living animals, we additionally performed a short-term study in a separate group of mice, exposing mice to otherwise identical dietary conditions as performed in the long-term study. Because of the shorter intervention period mice were not exposed to the complete set of partly invasive experimental methods as performed in the long-term intervention.

Body composition. Body composition (fat mass, lean mass and free fluids) was measured every two to four weeks using nuclear magnetic resonance spectroscopy (Mini Spect MQ 10 NMR Analyser Bruker, Karlsruhe, Germany).

Glucose clearance and insulin resistance (GTT) Glucose tolerance tests were performed in the long-term study after 15 weeks of dietary intervention in over-night fasted mice, as detailed previously (10). Plasma was collected before and 10, 30, and 60 min after glucose challenge (2g/kg body weight) and immediately stored at -80°C for measurement of glucose and insulin. Mouse plasma insulin levels were measured by ELISA for rat insulin using a
mouse insulin standard (both from Crystal Chem Inc., Chicago, Illinois, USA), as described (8).

Digestibility of diets. Feces were collected at week 15 and food intake was recorded for further analysis of energy balance. After drying, energy content of diet samples and feces was determined by bomb calorimetry (IKA C5003, IKA Werke, Germany) and digested energy (defined as diet energy intake (kJ/g) minus energy loss via the feces (kJ/g)) was calculated for both groups. Digestibility of the diet (%) was defined as [(digested energy (kJ/g)/diet energy intake (kJ/g)) 100]. Cumulative digested energy was calculated over the experimental period for 9 and 18 weeks, by multiplying diet energy intake with digestibility, as measured over one week for each individually housed mouse. Hydrogen (H₂) breath test as a marker of colonic fermentation was performed in week 14 as described previously (10).

Semiquantitative in vivo analysis of glucose and fatty acid oxidation. In order to examine the oxidation of injected or ingested substances, ¹³CO₂ breath tests were applied as non invasive methods and performed in all groups of mice (n = 10 per group), after dietary intervention periods of 3 weeks (short-term study) and 12 weeks (long-term study), respectively. Examinations were started at 11:00 a.m. after a four hour fasting period. For the analysis of glucose oxidation, 10 µmol/kg of ¹³C-labeled glucose (U-¹³C₆, 98%, MW 181.6 g/mol; Cambridge Isotope Lab., Inc., Woburn, MA, USA) was applied by oral gavage in a volume of 50 µl/20 g body weight. Breath samples were obtained in duplicates at baseline and 10, 20, 30, 40, 50, 60, 80, 100, 110, and 120 min after ¹³C-glucose oxidation administration. For the collection of ¹³CO₂ samples, animals were placed individually at each time point into 140 ml syringes for 30 sec. This time interval had been tested prior to 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 (Exetainer, Labco Ltd., High Wycombe, U.K.) for storage and measurement. Breath $^{13}$CO$_2$ enrichments were analysed by isotope ratio mass spectrometry (BreathMAT. Thermo Scientific Corp., Bremen, Germany) and were expressed as $\delta^{13}$C 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.

Three days after collection of $^{13}$CO$_2$ breath tests (week 12) a similar procedure was used to analyse oxidation of $^{13}$C-labeled potassium palmitate (1-$^{13}$C, 99%, MW 294.5 g/mol, Cambridge Isotope Lab., Inc. Andover, MA, USA). $^{13}$C-labeled potassium palmitate was emulsified by vortexing at 60°C in 5% lecithine (Emultop, Cargill Texturizing Solutions Deutschland GmbH & Co. KG, Hamburg, Germany) dissolved in sterilized water and 60 µmol/kg were applied by oral gavage in a volume of 50 µl/20 g body weight. Breath samples were taken at baseline and 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, 240, 270, and 300 min postprandially. Baseline $^{13}$C abundances in breath CO$_2$ were in accordance to abundances obtained after consumption of C$_3$ plant-based experimental diet low in natural $^{13}$C (20).

Palmitate oxidation was additionally investigated in a separate group of 16 weeks 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 weeks (short-term study) and 19 weeks (long-term study), respectively. Indirect calorimetry was performed in individual mice, using an open respirometric system (gas analysers: Magnos 16 and Uras 14, Hartmann & Braun, Germany). Mice were unrestrained and had free access to chow after a 5 h-fasting period. Oxygen consumption and CO2 production were determined every 6 min over a 22-h period. Fasted RQ was calculated from the mean of the last three hours of the fasting period. As a marker for
metabolic flexibility postprandial RQ was calculated from the mean of a four hours period after starting free access to the respective experimental diets with low and high GI.

Liver histology and analysis of hepatic triacylglycerols. Histology from the middle lobe of the liver was performed 20 weeks after the dietary long-term intervention, using standard procedure for hematoxilin eosin (HE) staining. Hepatic triacylglycerols were measured in all four groups of mice, as described previously (10).

Plasma analyses. Animals were investigated after a 3 h fasting period in week 8. Blood was obtained from the retro-orbital sinus during anaesthesia using Isoflurane® (Baxter, Unterschleissheim, Germany). Plasma triacylglycerols, plasma total cholesterol and not esterified fatty acid (NEFA) were measured using commercial kits (glucose HK 125; triacylglycerols, total cholesterol: ABX Pentra, Montpellier France, Nfac: 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 sacrificed in the non fasted state after dietary intervention periods of 6 weeks (short-term study) and 20 weeks (long-term study), respectively, as detailed previously (10). All samples were measured in triplicates, and non template 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 steoryl regulatory element binding protein1c (Srebp-1c), acetyl-CoA carboxylase (Acc), fatty acid synthase (Fas), stearoyl-CoA desaturase1 (Scd-1), diacylglycerol acetyltransferase-2 (Dgat-2), L-carnitine palmitoyl transferase1 (Cpt-1), and
long chain acyl-CoA dehydrogenase (Lcad) was analysed in hepatic, muscle and epididymal white adipose tissue in all groups of mice. The oligonucleotide specific primers were:

**Hprt:**
- **up5'-** CAGTCCCAGCGTGTGATTA-3', **lo5'-** AGCAAGTCTTTACGTCTGTC-3',

**Srebp-1c:**
- **up5'-** GGAGATGCTATCTCCATGGCA-3', **lo5'-** GAAACGTGTCAAGAAGTGCAGG-3',

**Acc:**
- **up5'-** CAGTTCAGAAGGCGACTACG-3', **lo5'-** TAGCGTTTGTTGTCTCTGCTGAG-3',

**Fas:**
- **up5'-** AGGAGGTGGTGATAGCCGGTAT-3', **lo5'-** GGTAATCCATAGAGCCAGGGT-3',

**Scd-1:**
- **up5'-** GCCCACATGCTCAAGAGATCT-3', **lo5'-** AGGACGGATGTCTTCTCCAGG-3',

**Dgat-2:**
- **up5'-** CCAAGAAAGGTGGCAGGAGAT-3', **lo5'-** GCAGGTTGTGTGTCTTCACCA-3',

**Cpt-1:**
- **up5'-** CCTGCATTCCCTCCCATTG-3', **lo5'-** CCCATGTCCTTTGTAATGCG-3',

**Lcad:**
- **up5'-** GCTTCCATGGCAAAATACTGG-3', **lo5'-** CACGTAAGCCTTTGCAATCGG-3',

**Statistical analysis.** Quantitative data are presented as means ± SEM. Data were analysed using two-tailed Student’s t test for unpaired samples or ANOVA for repeated measurement to analyse time course longitudinal changes in body weight (SPSS 14, Chicago, USA). For characterisation of the high vs low GI test meals prior to the study, the incremental area under the plasma glucose curve was calculated over a 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 five days training period, six of eight animals in each group consumed the entire test meals. AUC_{glucose} 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 AUC_{insulin} 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 as compared to high GI fed mice (P = 0.03).

Increased weight gain and body fat with high vs low GI diet. Fig. 2A shows changes in body weight during the 20 weeks experimental period. The influence of the glycemic index on time course of increases in body weight was statistically significant (time x 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 five 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. AUC_{glucose} was markedly and significantly lower in mice exposed to 15 weeks of low vs high GI dietary intervention (3714 ± 548 vs. 6350 ± 364 mmol/l·min, 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} (13597 ± 1054 vs. 14382 ± 1541 pmol/l·min, P = 0.68).

Significant differences at single time points for plasma glucose and insulin responses during GTT are shown in Fig. 2D and Fig. 2E. Compared to 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, Tab 1). Food intake was multiplied with these values. Tab 2 shows no significant difference in digestible energy intake during a one week period. Fecal excretion (feces weight in grams) and feces energy contents were significantly lower in high vs low GI diet fed animals (Tab 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 inter-individual 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 weeks on experimental diets was not significantly increased in high vs 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 utilisation with low GI diet.** After 19 weeks 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/d, 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 hours 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 weeks) 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 (Fig. 4A, measured after 12 weeks of dietary intervention), as compared both to long-term low GI diet and to short-term high GI exposure (Fig. 4A, C). Delta over baseline (DOB) of glucose-derived $^{13}$CO$_2$ 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 (Fig. 4C, measured after 4 weeks on experimental diets).

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 $^{13}$C-labelled 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 exposure. DOB of palmitate-derived $^{13}$CO$_2$ in low GI fed animals declined to baseline after 210 min, whereas there was a significantly less pronounced but sustained increase of $^{13}$CO$_2$ 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 chow fed animals that had been additionally performed for further comparison.

Therefore, the here observed significant delay in early postprandial fat oxidation (AUC$_{120\text{min}}$, short-term study: $363 \pm 67$ vs $921 \pm 61$ DOB ($\%$)$\cdot$min, $P < 0.001$; long-term study: $460 \pm 86$ vs $887 \pm 66$ DOB ($\%$)$\cdot$min, $P = 0.021$) appeared to be primarily caused by the high GI diet, rather than being a consequence of diet induced changes in body composition. Significant differences in palmitate oxidation at single time points are shown in Fig. 4B and 4D.

Fat accumulation and changes in gene expression of hepatic enzymes of fat metabolism. After 20 weeks of dietary intervention marked microvesicular and macrovesicular steatosis was present in the histology of high vs low GI fed mice (Fig. 5A and 5B). Triacylglycerol accumulation in hepatic tissue after long-term intervention was nearly threefold increased in high vs low GI fed mice (Fig. 5D, $P = 0.006$). 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 (Fig. 5D; $P = 0.009$ and $P = 0.003$, respectively). 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 weeks 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 as compared to 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. 5D, F), while 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 weeks of dietary exposure, while NEFA (0.85 ± 0.04 vs 0.77 ± 0.03, P = 0.14) and cholesterol (2.58 ± 0.29 vs 2.33 ± 0.21, P = 0.48) levels were not significantly different between groups.

**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. Further, 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 co-workers (17), with high GI fed rodents being unable to further increase insulin secretion after a glucose load. Further, 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 in order to detect potential changes in metabolic flexibility. The here observed differences in RQ between high vs low GI fed animals may have resulted both from changes in fat and glucose utilisation, 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 weeks) 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 five hours fasting period in order to measure metabolic flexibility. Moreover, the her chosen design should more accurately reflect feeding behaviour in humans with prolonged (e.g. overnight) fasting periods followed by a 16 – 18 hours postprandial state over the day, as
compared to 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 three weeks 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 changed 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 have been directly oxidised 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 over night feeding period.

Effects of high vs low GI diets on molecular markers of fat metabolism have been investigated only in 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 intervention 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 six weeks 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, while 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 upon nutritional challenges (18) such as a high GI diet.
Limitations of the present study need to be mentioned. (i) 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. (ii)
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. (iii) 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. (iv) We did not
measure physical activity in the present study. A recent study reported a non-significant 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.
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 three weeks 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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Duality of interest

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### Table 1: Macronutrient composition of experimental diets

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<tr>
<th>Diet (g/kg)</th>
<th>Low GI</th>
<th>High GI</th>
</tr>
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<tbody>
<tr>
<td>Casein</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Starch 1: 100 % amylopectin</td>
<td></td>
<td>542</td>
</tr>
<tr>
<td>Starch 2: 30 % amylopectin</td>
<td>542</td>
<td></td>
</tr>
<tr>
<td></td>
<td>70 % amylose</td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>85</td>
<td>85</td>
</tr>
<tr>
<td>Gelatine</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Vitamin mixture</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

**Measured Diet energy content (kJ/g)**

<table>
<thead>
<tr>
<th></th>
<th>Low GI</th>
<th>High GI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>17.5</td>
<td>17.4</td>
</tr>
</tbody>
</table>
Table 2: Energy digestion during one week (exemplary week 15) and cumulative energy digestion during 9 and 18 weeks of intervention.

<table>
<thead>
<tr>
<th></th>
<th>low glycaemic index</th>
<th>high glycaemic index</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Balance in week 15:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<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 diet energy (%)</td>
<td>90.8 ± 0.4</td>
<td>95.3 ± 0.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>Balance over 9 weeks:</strong></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>2831.5 ± 54.0</td>
<td>2920.4 ± 44.5</td>
<td>0.22</td>
</tr>
<tr>
<td><strong>Balance over 18 weeks:</strong></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>5710.1 ± 99.4</td>
<td>5904.8 ± 108.4</td>
<td>0.20</td>
</tr>
</tbody>
</table>
Figure 1
AUC of plasma glucose (A) and insulin levels (B) over 120 min, following ingestion of 500 mg of experimental diets with low vs high GI. (C) Colonic fermentation estimated by hydrogen breath test. * indicates $P$-value < 0.05.

Figure 2
Change in body composition and glucose tolerance during long-term (20 weeks) dietary intervention (n = 10 per group). (A) Changes in body weight under low vs high GI diet. Longitudinal changes in body fat mass (B) and lean mass (C) as measured by NMR. (D, E) Glucose tolerance test performed at week 15 by i.p. glucose injection. (D) shows plasma glucose clearance after glucose load in fasted state in the high vs low GI group. (E) shows changes in plasma insulin in the same experiment. * indicates $P$-value < 0.05, ** indicates $P$-value < 0.01.

Figure 3
(A) Respiratory quotient (RQ) during light and dark phase (grey section) in indirect calorimetry during the long-term study (after 19 weeks), and (C) during the short-term intervention (after 3 weeks). Mice had free access to low vs high GI diets after a prior five hours fasting period (shaded area). Differences in fasted and postprandially measured RQ values expressed as AUC are presented for high vs low GI groups both after long-term (B) and short-term (D) dietary intervention. ** indicates $P$-value < 0.01.

Figure 4
$^{13}$CO$_2$ breath tests in mice fed with low vs high GI diet, following a four hours fasting period. Glucose oxidation was estimated in breath tests after oral load of $^{13}$C-labeled glucose after 12 weeks (A) vs 4 weeks (C) of dietary intervention. (B) shows kinetic profiles of palmitate
oxidation after orally administrated $^{13}$C-labeled palmitate after 12 weeks (D) vs 3 weeks on experimental diets. Palmitate oxidation profile in standard chow fed mice is additionally shown in the background (D). V-PDB: Vienna – Pee Dee Belemnite limestone carbonate.

* indicates $P$-value < 0.05, ** indicates $P$-value < 0.01, *** indicates $P$-value < 0.001.

**Figure 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 index diets. Typical hematoxilin and eosin stained liver tissues after 20 weeks on low (A) vs high (B) GI diet, original magnification x 100. Hepatic triacylglycerol contents after 20 weeks (C) vs 6 weeks 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 glycemic index diet was set to 1.0. * indicates $P$-value < 0.05, ** indicates $P$-value < 0.01
**Fig. 1**

**A**

![Graph A showing AUC of glucose](image)

**B**

![Graph B showing AUC of insulin](image)

**C**

![Graph C showing H2 Exhalation](image)
Fig. 5  long-term intervention

A  

low GI  

B  

high GI  

C  

hepatic triacylglycerol/protein (mg/mg)  

**  

low GI  high GI  

D  

relative expression (arbitrary units)  

**  

low GI  high GI  

E  short-term intervention  

F  

hepatic triacylglycerol/protein (mg/mg)  

**  

low GI  high GI  

relative expression (arbitrary units)  

**  

low GI  high GI  

Sreb1/Scd1/Degat2/Cpt1/Lcad  

Acc/Lcad