Carbohydrate Quality and Quantity Affect Glucose and Lipid Metabolism during Weight Regain in Healthy Men

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Abstract

In this controlled, parallel-group feeding trial, we examined the impact of carbohydrate (CHO) intake and glycemic index (GI) on glucose and lipid metabolism during refeeding after weight loss. Healthy men (n = 32 total; age: 25.5 ± 3.9 y, BMI: 23.5 ± 2.0 kg/m²) overconsumed diets containing either 50% or 65% CHO for 1 wk (+50% of energy requirements) and then underwent 3 wk of calorie restriction (CR; −50%) followed by 2 wk of overconsuming (refeeding, +50%) the same diet but with either a low or high GI (40 vs. 70 during CR, 41 vs. 74 during refeeding) so that glycemic load (GL; dietary CHO content x GI) differed between groups during all phases. Glucose profiles were assessed by continuous interstitial glucose monitoring, insulin sensitivity (IS) by fasting blood sampling, oral glucose tolerance test (OGTT) and hyperinsulinemic-euglycemic clamp, and liver fat by MRI. Daytime area under the curve-glucose during refeeding was higher with high compared with low GI (P = 0.01) and 65% compared with 50% CHO intake (P = 0.05) and correlated with dietary GL (r = 0.71; P < 0.001). IS increased with CR and decreased again with refeeding in all groups. The decrease in OGTT-derived IS was greater with high- than with low-GI diets (−41% vs. −15%; Pinteraction = 0.01) and correlated with dietary GL during refeeding (r = −0.51; P < 0.01). Serum triglycerides (TGs) and liver fat also improved with CR (−17 ± 38 mg/dL and −1.1 ± 1.3%; P < 0.05 and <0.001) and increased again with refeeding (+48 ± 48 mg/dL and +2.2 ± 1.6%; P < 0.001). After refeeding, serum TGs and liver fat were elevated above baseline values with 65% CHO intake only (+59.9 ± 37.5 mg/dL and +1.1 ± 1.7%, Pinteraction <0.001 and <0.05). In conclusion, a diet low in GI and moderate in CHO content (i.e., low GL) may have health benefits by positively affecting daylong glycemia, IS, and liver fat.

Introduction

Low-fat, high-carbohydrate (CHO) diets have long been considered a cornerstone for preventing chronic diseases such as type 2 diabetes (1, 2). Whereas a high-CHO intake has been shown to beneficially affect insulin sensitivity (IS) in healthy lean adults in some studies (3–6), others found no relation or even a decreased IS with high-CHO diets, especially those rich in fructose and sucrose (5, 7–10). However, the majority of these studies were performed under isocaloric conditions and not in the setting of positive energy balance with a high-CHO intake. A state of positive energy balance during the weight regain phase within a weight cycle is associated with a sudden shift from fat to CHO metabolism (11). During this vulnerable refeeding phase, CHO intake may therefore affect metabolic regulation (i.e., IS).

A high-CHO intake has been shown to result in postprandial spikes in glucose and insulin concentrations and places excessive demand on the β cells (1, 8, 12).

A previous study revealed that the glycemic index (GI) explained a similar amount of variability in glycemic responses as the amount of CHO intake and together they accounted for ~90% of the total variability (13). Accordingly, insulin responses have been shown to be proportional to glycemic load (GL), the product of GI and the amount of CHO (14, 15). Thus, with moderate-CHO intake, choosing low-GI foods rich in dietary fiber may
be favorable in terms of preventing subclinical metabolic derangements. By contrast, the American Diabetes Association stated that the total amount of CHOs in meals and snacks has a more pronounced effect on glycemic response than the source or type (i.e., GI/GL) (16). If so, one might speculate that a high-CHO intake beyond a certain threshold overrides any GI effects (17).

Importantly, CHO overnutrition has not only been associated with changes in IS and glucose metabolism but also with hypertriglyceridemia and hepatic steatosis (10,18–29). Whereas de novo lipogenesis (DNL) is thought to be a quantitatively unimportant metabolic pathway in nonobese (19) and weight-stable men (23), it can be markedly stimulated when simple CHOs are overfed (19,21,22,27,28). High-GI/GL diets have also been associated with increased TGs (30) and liver fat (31). Although increased liver fat is strongly associated with decreased hepatic IS and impaired peripheral IS (20,24), it is unclear whether a decline in IS is a cause or a consequence of hepatic steatosis (20,24).

We recently showed that refeeding a moderate-CHO diet following caloric restriction (CR) decreased IS in healthy young men (32) and that refeeding a diet with a high GI (HGI) resulted in a greater impairment in IS compared with a high-fiber, low-GI diet (33). The aim of the present study was to examine the effect of a moderate-compared with a high-CHO intake with different GIs on IS and liver fat during the refeeding phase of a weight cycle. We hypothesized that both high-GI and high-CHO intake increase daylong glycemic response, impair IS, and result in elevated liver fat and serum TGs. In addition, we hypothesized that IS is negatively associated with liver fat and serum TGs during refeeding.

**Materials and Methods**

**Subjects.** Thirty-two healthy young men aged 20–37 y (BMI 23.5 ± 2.0 kg/m²) were recruited according to a previous description (33). Those eligible for participation were nonsmokers, weight stable (± 2 kg) within the preceding 12 mo, did not use any medications on a daily basis, had no family history of diabetes, no food allergies, no contraindications for MRI, and did not follow any special diets. Competitive athletes were also excluded from the study. None of the participants had a BMI >30 kg/m².

Data were collected between February 2010 and September 2012 and experimental data of the first 16 participants were previously reported (33). The study was approved by the ethics committee of the Medical Faculty of the Christian-Albrechts-University of Kiel and conducted according to the principles of the Helsinki Declaration. All participants gave written consent after receiving oral and written information.

**Experimental protocol.** The 6-wk controlled, parallel-group, nutritional intervention study comprised 1 wk of overfeeding, 3 wk of CR, and 2 wk of refeeding. An outline of the study protocol is shown in Figure 1. The study protocol was previously thoroughly described (33). Starting from overfeeding, participants were divided into 2 groups (n = 16) differing in macronutrient composition of the diet (50%CHO group: 50% CHO, 35% fat, 15% protein; 65%CHO group: 65% CHO, 20% fat, 15% protein). During CR, the 50%CHO group was assigned to a low GI (LGI) diet, while the 65%CHO group was assigned to a HGI diet. During refeeding, the 50%CHO and 65%CHO groups were further subdivided into 2 groups (n = 8) receiving either high-fiber LGI or lower-fiber HGI foods. The LGI and HGI groups were matched according to age, body weight, percent body fat, glucose tolerance as assessed by oral glucose tolerance test (OGTT), and IS as measured by hyperinsulinemic-euglycemic clamp. In addition, participants were standardized with respect to physical activity (~5000 steps/d). The initial overfeeding period was added to the study protocol to limit minimum body weight.

**Dietary interventions.** During the preintervention period, participants were asked to not change their habitual food intake. Energy intake (EI) was increased by 50% of energy requirements during overfeeding and refeeding (mean EI = 17.0 ± 1.9 MJ/d) and was reduced by 50% during CR (mean EI = 5.7 ± 0.6 MJ/d). Individual caloric requirements were calculated as resting energy expenditure (REE) multiplied by a sedentary physical activity level of 1.4 as previously described (32,33). The dietary intervention has been described in detail elsewhere (33). In brief, all foods and beverages consumed were provided during the intervention and meal intake was supervised by a skilled nutritionist. Seven-day-cycle menus were prepared in the metabolic kitchen by an experienced dietitian and analyzed using PRODI software (Nutri-Science). GI values of individual foods were taken from the 2002 International Table of GI Values of Foods (34). The GI of the diet was calculated according to WHO/FAO guidelines (35) and averaged 41 and 74 in the LGI and HGI diets. The GL of a food was calculated by multiplying the GI by the amount of CHO in grams provided by the food and dividing the total by 100. Dietary GI was the sum of the GLs for all foods consumed in the diet (36). GI ranged from 53 to 192 during CR and from 188 to 614

![FIGURE 1](https://academic.oup.com/jn/article-abstract/143/10/1593/4571600) Outline of the study protocol. CGM, continuous glucose monitoring; HGI, high glycemic index; LGI, low glycemic index; OGTT, oral glucose tolerance test; 50%CHO and 65%CHO, percent energy from carbohydrate.
during refeeding and differed between groups in both phases \((P < 0.001)\). Mean CHO intake during CR was 142 ± 13 g/d (105–160 g/d) in the 50% CHO-LGI group and 218 ± 28 g/d (179–273 g/d) in the 65% CHO-HGI group \((P < 0.001)\). During refeeding, CHO intake averaged 47 ± 49 g/d (424–626 g/d) in the 50% CHO groups and 641 ± 68 g/d (520–754 g/d) in the 65% CHO groups \((P < 0.001)\). Detailed information on the nutrient intake during refeeding is provided in Supplemental Table 1.

During the initial overfeeding period, all participants received a normal mixed diet. To standardize dietary intake during CR and refeeding, 50% of the energy intake was given as a liquid formula diet (InsuLean). The remaining 50% of energy was provided as HGI and LGI mixed meals and snacks.

**Anthropometric measurements and body composition analysis.** Height was measured to the nearest 0.01 cm using a stadiometer, with participants not wearing shoes. Body weight was measured to the nearest 0.05 kg on an electronic scale (Seca 285) with participants in underwear and after voiding. Fat mass and fat-free mass were assessed by air-displacement plethysmography (BOD POD, Cosmed) as described in detail elsewhere \((37)\). Liver fat was determined by MRI (Magnetom Avanto 1.5-T Siemens) along with the 2-point Dixon method with a volume interpolated breathhold examination as previously described \((32)\). Briefly, a T1-weighted gradient-echo sequence with in-phase and out-of-phase imaging was performed using the following variables: repetition time, 10.4 ms; echo time, 4.76 (in-phase) and 7.14 (opposed-phase) ms; flip angle, 10°; matrix, 80 × 128; and field of view, 440 mm. Fat-only and water-only images were calculated from in-phase and opposed-phase images as follows: water only = \(1/2\) \(\text{in phase + opposed phase}\); fat only = \(1/2\) \(\text{in phase-opposed phase}\). Forty adjacent slices were acquired within a 19-s breath-hold to cover the liver with a slice thickness of 5 mm and 1-mm interslice gap. Images were analyzed and processed using ImageJ software \((\text{U.S. NIH})\) to calculate hepatic fat fraction (HFF) images from fat-only and water-only images. A single continuous region of interest was defined \((20.62 \times 20.62)\) in each of 5 adjacent HFF images and was placed in the liver parenchyma, avoiding major blood vessels. The region of interest was placed in the same area for all repeated measurements. The quantity of liver fat was determined as percentage of the total liver core and was averaged for the 5 HFF images. The intra-organ fat percentage was evaluated from 2 liver regions of interest, defined and averaged by one observer. In the 50% CHO group, liver fat could not be measured at the end of CR because of instrument failure. Baseline liver fat measurement failed in one participant.

**Indirect calorimetry.** REE was measured as previously described \((33,38)\). In brief, REE was assessed by indirect calorimetry (ventilated hood system Vmax 29n, SensorMedics; Sensor Medics 130) according to a standard equation \((39)\). Oxygen consumption and carbon dioxide production were continuously measured for 30 min in a fasted state and supine position and only steady-state periods of measurements were selected for calculation.

**Hyperinsulinemic-euglycemic clamp.** During the preintervention periods and at the end of refeeding, IS was assessed by the hyperinsulinemic-euglycemic clamp technique according to deFronzo et al. \((40)\) as previously described \((32,41)\). Whole-body IS was determined during the last 20 min of the hyperinsulinemic glucose clamp as steady-state glucose disposal rate calculated from the mean rate of exogenous glucose infusion corrected for glucose space \([\text{M-value, mg/kg} \times \text{min}]\).

**OGTT.** OGTTs were performed in the preintervention period and following CR and refeeding. Blood samples for glucose and insulin determinations were collected in the fasted state at 30, 90, 120, and 180 min after ingestion of 75 g glucose. Tests were performed at the same time in the morning across phases to eliminate diurnal influences. Incremental AUC (iAUC), ignoring the area below baseline, and total AUC (tAUC), including the areas falling below baseline, for glucose and insulin were calculated by using the trapezoidal rule \((42)\). Missing data were due to not being able to collect blood samples.

**Continuous glucose monitoring.** In a subgroup of participants \((n = 8\) in the 50% CHO group and \(n = 16\) in the 65% CHO group), subcutaneous interstitial fluid glucose concentrations were measured by means of the FreeStyle Navigator continuous glucose monitoring (CGM) device (Abbott Diabetes Care) at the end of the refeeding period as described elsewhere in detail \((33)\). Difficulties in the self-calibration of glucose sensors occurred frequently at the beginning of the study and led to a loss of data in the 50% CHO group. Those difficulties were overcome by improved instruction of the participants and help from trained trial staff. In short, a CGMS monitor was worn for 5 consecutive days, data were downloaded, and glucose profiles were analyzed. Interstitial glucose iAUC during the day \((0800–0700\ h)\), i.e., daytime glycemia, and tAUC during the night \((2400–0700\ h)\), i.e., nighttime glycemia, were calculated using the trapezoidal rule \((42)\).

**Blood sampling and analytic procedures.** Plasma glucose was measured immediately upon sampling during hyperinsulinemic-euglycemic clamp and OGTT using a glucose oxidase method \((\text{BIOSENI C C-Line, EKF-diagnostic})\). Serum insulin was determined by electrochemiluminescence immunoassay \((\text{Elecys, Roche Diagnostics})\). Serum adiponectin was analyzed using an RIA \((\text{Millipore})\). Serum TGs were determined by a colorimetric method on a Vitros 5.1 FS analyzer \((\text{Johnson & Johnson, Ortho-Clinical Diagnostics})\).

**Calculation and statistical analyses.** Measured body weight changes were adjusted for predicted weight changes using a Web-based body weight simulation model in which body weight, age, height, physical activity level, REE, percent energy from CHO, sodium intake in mg/d, percent body fat, and EI in kcal/d were entered as input variables \((43)\). Overall glucose-stimulated insulin secretion during OGTT was calculated as tAUC-insulin/tAUC-glucose \((40)\). The Matsuda IS index \((\text{Matsudais})\) was used as an index of whole-body IS \((44)\). An estimate of fasting IS was obtained by HOMA-IR \((45)\).

Results are expressed as means ± SDs. Data normality was tested by the Kolmogorov-Smirnov test. Differences in adjusted body weight changes between the 50% CHO-LGI and the 65% CHO-HGI groups at the end of CR were tested with independent samples t tests. One-way ANOVA was conducted to test for differences in outcome variables between the 4 intervention groups at baseline and following CR as well as for differences in adjusted body weight changes after refeeding. Bonferroni post hoc tests were performed to examine which groups differ if overall analysis was substantial. Between-group differences in the area under the interstitial glucose curve during the day and during the night at the end of refeeding were tested by a 2-way ANOVA with CHO intake and GI as independent variables. Changes between refeeding compared with CR and baseline were tested by a 2-way repeated-measures ANOVA to compare the significance of time as within-subject variable and CHO intake and GI as between-subject variables and possible interactions between those variables \((\text{time} \times \text{CHO}, \text{time} \times \text{GI}, \text{time} \times \text{CHO} \times \text{GI})\). If baseline or CR values differed substantially or tended to be substantially different between groups, they were included as covariates and a repeated-measures ANCOVA was performed. Substantial main effects were followed up by pairwise comparisons with Bonferroni adjustment. Substantial interactions were followed up by splitting the file by group and conducting separate paired-samples t tests. Pearson correlation coefficients were calculated to examine relations between CHO intake and variables of IS and liver fat. Stepwise linear regression analysis was performed to determine independent predictors of the area under the interstitial glucose curve during the day. All statistical analyses were performed by using SPSS 17.0. Differences were considered substantial if \(P \leq 0.05\).

**Results**

**Baseline characteristics and changes in body weight during the intervention**

There were no substantial differences in anthropometric and body composition variables between groups at baseline (Table 1).

Compared with baseline, body weight was reduced after CR \((-4.2 ± 0.9\ kg; P < 0.001)\) and increased again with refeeding.
(+3.5 ± 1.2 kg; P < 0.001). Adjusted weight changes did not differ between groups (data not shown).

### Continuous glucose monitoring

Both GI (P = 0.01) and CHO (P = 0.05) intake, but not their interaction, affected daytime glycemia at the end of refeeding and (Fig. 2). The daytime interstitial iAUC-glucose was substantially higher in participants consuming the HGI compared with the LGI diet as well as with the 65%CHO compared with the 50%CHO diet. The nighttime glycemia did not differ between groups.

The interstitial iAUC-glucose correlated with energy intake (r = 0.42; P < 0.05), CHO intake (r = 0.45; P < 0.05), and GL (r = 0.71; P < 0.001) and tended to correlate with total (r = –0.34; P = 0.10) and soluble (r = –0.39; P = 0.08) fiber intake during refeeding. Stepwise regression analysis selected only GL as a substantial predictor of daytime glycemia, with R² = 0.51 (P < 0.001).

### Variables of IS

**CR compared with baseline.** Compared with baseline, CR resulted in an improved IS and a concomitant increase in glucose-stimulated insulin secretion; however, serum adiponectin concentrations substantially decreased with CR (Table 2). All changes occurred independently of dietary CHO content and GI. Incremental AUC-insulin was not altered by CR.

**Refeeding compared with CR.** Compared with CR, refeeding increased serum concentrations of adiponectin and insulin, HOMA-IR, and glucose-stimulated insulin secretion and decreased iAUC-glucose and Matsuda ISI (Table 3). Larger increases in serum adiponectin (ΔHGI = 6.09 ± 3.05 μg/L, ΔLGI = 3.89 ± 2.31 μg/L; both P < 0.001) and a greater decline in Matsuda ISI (ΔHGI = –5.90 ± 4.92, P < 0.001; ΔLGI = –1.45 ± 2.43, P < 0.05) were observed in the HGI compared with the LGI diet groups. The time × GI interaction for Matsuda ISI remained substantial after adjustment for CR differences (P-interaction < 0.05). In addition, a correlation was observed between the decrease in Matsuda ISI and the GL of the refeeding diet (r = –0.51; P < 0.01). No time × CHO or time × GI interactions were present. Plasma glucose and iAUC-insulin were not altered by refeeding.

**Refeeding compared with baseline.** Compared with baseline, plasma glucose decreased at the end of refeeding in all diet groups and serum adiponectin increased above baseline concentrations in the HGI group only (ΔHGI = 2.89 ± 2.52 μg/L, P < 0.001; ΔLGI = 0.20 ± 2.96 μg/L, P = 0.79) (Table 4). Serum insulin, HOMA-IR, iAUC-glucose, and insulin, Matsuda ISI, and M-value were unchanged compared with baseline values after the CR-refeeding cycle. No time × CHO or time × CHO × GI effects were found.

### Liver fat and serum TGs

**CR compared with baseline.** Compared with baseline, serum TGs and liver fat substantially decreased after CR (Table 1). No time × CHO/GI interactions were present.

**Refeeding compared with CR.** Compared with CR, serum TGs and liver fat increased with refeeding (Table 3). The increase in serum TGs was greater in the 65%CHO groups compared with the 50%CHO diet groups (Δ65%CHO = 73.06 ± 45.27 mg/dL, P < 0.001; Δ50%CHO = 16.87 ± 6.94 mg/dL, P < 0.05). No time × GI or time × CHO × GI interaction was found. Both serum TGs and liver fat were positively correlated with available CHO intake during refeeding (Fig. 3). The correlation between liver fat and CHO intake remained marginally substantial after the exclusion of the 2 participants with higher liver fat (r = 0.35; P = 0.07).

**Refeeding compared with baseline.** Compared with baseline values, serum TGs and liver fat differed by CHO intake at the end of refeeding (Table 4). Serum TGs (Δ65%CHO = +59.9 ± 37.5 mg/dL, P = 0.001; Δ50%CHO = +3.1 ± 24.7 mg/dL, P = 0.62) and liver fat (Δ65%CHO = +1.1 ± 1.7%, P < 0.05; Δ50%CHO = +0.3 ± 1.1%, P = 0.27) remained substantially elevated...
above baseline values in the 65\%CHO diet group only. The CHO effect on liver fat remained substantial after the exclusion of the 2 participants with higher liver fat (Fig. 3) ($P$-interaction $< 0.05$). No time x GI or time x CHO x GI effect was observed.

**Relation between variables of IS and liver fat**

At the end of refeeding, liver fat was positively correlated with serum TGs ($r = 0.62$), serum insulin ($r = 0.72$), HOMA-IR ($r = 0.69$), iAUC-insulin ($r = 0.83$), and glucose-induced insulin secretion ($r = 0.84$) (all $P < 0.001$) and negatively correlated with Matsuda$_{ISI}$ ($r = -0.47; P = 0.01$) and M-value ($r = -0.41; P < 0.05$). Similar correlations were observed for serum TGs.

**Discussion**

This study has examined the impact of CHO intake and GI on IS and liver fat during the refeeding phase of a weight cycle in healthy men. Daytime glycemia during refeeding was increased by HGI vs. LGI and 65\%CHO vs. 50\%CHO diets. IS increased with CR and decreased toward baseline values with refeeding.
Impact of CHO intake and GI on daylong glycaemia. CGM revealed higher glycaemia during the day with both HGI compared with LGI and 65%CHO compared with 50%CHO content of the refeeding diet (Fig. 2). Accordingly, GL, which is the product of GI and CHO intake, showed the strongest correlation with interstitial AUC glucose throughout the day. This is in accordance with a previous study showing that GL explained ~60% of the variability in postmeal glucose responses (15). As opposed to previous studies, we could not find a leveling off in glycaemia above a certain threshold of dietary GI (14,17). The strong association between GL and daytime glycaemia even at +50% energy surplus underscores dietary GI as a good surrogate measure of postprandial glycaemia in healthy individuals (15). Because already minor elevations in postprandial glucose together with related hyperinsulinemia have been linked to the development of cardiovascular disease and type 2 diabetes (46) as well as to metabolic adaptations such as increased β-cell size and mass (1), consuming foods low in GI and CHO may be beneficial for the prevention of chronic metabolic diseases.

Impact of CHO intake and GI on IS and liver fat during CR. Fasting and OGTT-derived IS as well as liver fat and serum TGs improved after CR independently of diet composition (Tab. 2). The effect of negative energy balance may thus have overridden any effect of diet composition and quality. This is in agreement with previous studies suggesting that the effect of GI and/or GL of the diet on glucose metabolism and IS is overridden by the strong effect of weight loss per se (47,48). A ketogenic diet with extreme CHO restriction was more effective at reducing liver and plasma TG than was CR in patients with nonalcoholic fatty liver disease (NAFLD) (49). However, these findings may not apply to healthy individuals with normal CHO intake.

Impact of CHO intake and GI on IS during refeeding. IS increased with CR and decreased toward baseline values with refeeding in all diet groups. The refeeding period was thus characterized by a state of “relative insulin resistance.” The decrease in OGTT-derived IS was greater with the HGI than with LGI diets and was correlated with the GL of the refeeding diet. Similarly, OGTT-derived IS during weight maintenance after weight loss has been shown to be highest with the lowest dietary GL (50). The beneficial effect of a low-GL diet on glucose metabolism is not only supported by our CGM data but also by a recent meta-analysis of prospective cohort studies showing a dose-response relation between dietary GL and risk of developing type 2 diabetes in healthy adults (51). At the end of refeeding, IS did not differ from baseline values regardless of dietary GI and CHO content. This is in agreement with previous data suggesting that in the state of energy balance, neither GI (30) nor CHO intake (5,9) adversely affects IS in healthy euglycemic individuals.

Whereas CR increased and refeeding decreased IS, fasting adiponectin changed inversely, i.e., decreased with CR and increased with refeeding (Tables 2 and 3). On the one hand, this is consistent with our previous findings (32,33) as well as with recent studies reporting elevated adiponectin after 5 d and 2 wk of overfeeding (+50% energy) in healthy men (52,53). On the other hand, it contrasts with the positive association between IS

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**TABLE 3. Indices of glucose and lipid metabolism after CR (T1) and refeeding (T2) in men who consumed diets differing in GI and CHO content.**

<table>
<thead>
<tr>
<th></th>
<th>T1</th>
<th>T1 x CHO</th>
<th>T2</th>
<th>T2 x CHO</th>
<th>T1 x GI</th>
<th>T2 x GI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum glucose, mmol/L</td>
<td>4.17±1.20</td>
<td>15.9±6.1</td>
<td>3.56±1.0</td>
<td>14.9±5.8</td>
<td>3.94±1.0</td>
<td>16.7±7.1</td>
</tr>
<tr>
<td>Serum insulin, mU/L</td>
<td>7.48±2.3</td>
<td>24.7±10.2</td>
<td>6.5±2.0</td>
<td>23.8±10.0</td>
<td>7.1±2.0</td>
<td>25.0±10.0</td>
</tr>
<tr>
<td>Liver fat, %</td>
<td>15.7±4.9</td>
<td>20.8±6.2</td>
<td>14.8±4.5</td>
<td>20.0±5.8</td>
<td>15.3±4.0</td>
<td>20.5±5.0</td>
</tr>
</tbody>
</table>

*p* values for main effects: GI (30%) or CHO (60%) and interaction with CHO (60%).

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1 Values are means ± SDs; *n* = 7–8/group. Different symbols (*, #) indicate differences between groups at T1 as assessed by ANOVA, followed by Bonferroni tests, *P* < 0.05. Means without a common superscript letter differ between groups at T1 based on post hoc.

2 Two-way repeated-measures ANOVA (or ANCOVA if group differences at T1) to test for main effect of time (T), CHO intake (CHO), and GI as well as for interactions between T, CHO, and GI with Bonferroni adjustments for multiple comparisons.

3 Liver fat measurements at the end of CR (T1) were performed in the 65%CHO diet groups only.
TABLE 4

| LGI | HGI | P  | 50%CHO  | 65%CHO  | 50%CHO  | 65%CHO  | T0   | T2   | T0   | T2   | T0  | T2  | T0   | T2   | T0  | T2  | T0   | T2   | T0  | T2  | T0   | T2   | T0  | T2  | T0   | T2   |
|-----|-----|----|---------|---------|---------|---------|-------|------|------|------|------|-----|-----|------|------|-----|-----|------|------|-----|-----|------|------|-----|-----|------|------|
|     |     |    | 65%CHO  |         | T       |         |       |      |      |      |      |     |     |      |      |     |     |      |      |     |     |      |      |     |     |      |      |

Values are means ± SD. CHO, carbohydrate; GI, glycemic index; HGI, high glycemic index; LGI, low glycemic index; T, CHO intake was positively correlated with both serum TGs and liver fat at the end of refeeding (Fig. 3). A dose-response relation between excess CHO intake and hepatic DNL in healthy individuals was previously shown (19,57). Because metabolic adaptation and disease progression are a continuum, already minor changes within the physiological range can be detrimental and lifestyle advice may be useful at an early stage (58). Although the present results do not allow us to draw conclusions regarding their applicability to patients, e.g., with type 2 diabetes or NAFLD, a CHO-restricted diet has also been shown to decrease the risk of metabolic syndrome and the severity of NAFLD histopathology in patients with NAFLD (59,60).

**Impact of CHO intake and GI on liver fat during refeeding.** Compared with CR, refeeding increased serum TGs and liver fat in all diet groups, with a greater rise in serum TGs with 65% CHO compared with 50% CHO intake. After refeeding, serum TGs and liver fat were elevated above baseline values with the 65% CHO diet only (Table 3). When CHO intake exceeds storage and oxidation capacities, extra CHO energy is converted to fat by DNL (18,57). Newly synthesized fatty acids from excess CHO are incorporated into TGs and transferred into the bloodstream; any imbalance among TG synthesis, secretion, and clearance may lead to lipid accumulation in the liver and/or elevated serum TGs (18,20,27,29). Comparable with our findings, 4 d to 3 wk of overfeeding with simple CHO increased fasting serum TGs (18,21,22,27) and liver fat (21,22,28) in healthy participants. Lipogenic enzymes are stimulated by transcription factors that are upregulated by insulin as well as glucose (26). We have shown that the CHO intake was positively correlated with both serum TGs and liver fat at the end of refeeding (Fig. 3). A dose-response relation between excess CHO intake and hepatic DNL in healthy individuals was previously shown (19,57). Because metabolic adaptation and disease progression are a continuum, already minor changes within the physiological range can be detrimental and lifestyle advice may be useful at an early stage (58). Although the present results do not allow us to draw conclusions regarding their applicability to patients, e.g., with type 2 diabetes or NAFLD, a CHO-restricted diet has also been shown to decrease the risk of metabolic syndrome and the severity of NAFLD histopathology in patients with NAFLD (59,60).

**Association between liver fat and IS during refeeding.** Inter-individual variation within the physiological range of hepatic fat content and serum TGs was related to fasting and OGTT- and clamp-derived IS as well as to glucose-induced insulin secretion at the end of the refeeding phase. Previous studies have shown that liver fat is strongly associated with hepatic and peripheral IS (20,24–26), but it remains unclear whether insulin resistance causes liver fat or whether the accumulation of fat in the liver is the primary event leading to hepatic and later on peripheral insulin resistance (20,24,26). A higher glucose-induced insulin secretion may have contributed to DNL and TG accumulation in the liver, which in turn may have impaired IS. Accordingly, the majority of previous studies suggest that liver insulin resistance is secondary to TG accumulation in the liver (10,20,26).

The mechanisms of a HGI and 65% CHO diet underlying decreased IS and increased liver fat may not only be related to increased insulin secretion but also to a lower metabolic flexibility (i.e., the ability to adapt fuel oxidation to fuel availability) due to lower products of fiber fermentation (i.e., SCFA) that may also exert an effect on incretin hormones like glucagon-like peptide 1 [for a review see (61)].

**Study limitations.** Group sizes were small and inter-individual variability in outcome variables was high. This might have limited the power of our findings. It would have been advantageous to use an intra-individual crossover design with individuals serving as their own controls and to perform meal
tests to study postprandial insulinemia. Because GL has been shown to account for 46% of the variability in insulinemia (15), postprandial insulin responses were presumably elevated in our participants. Although the CHO concentrations of the 65% and 50% CHO diets did not differ very much, CHO intake was 33% greater in the 65% CHO group (P < 0.001). Finally, the increase in dietary CHO content at constant protein intake was inevitably accompanied by a decrease in fat intake (P < 0.001). It has been shown, however, that there is little effect of fat energy content on metabolism during energy excess or deficit (19).

In conclusion, we found: 1) an increased daytime glycemia with HGI and 65% CHO intake; 2) a greater decline in OGTT-derived IS with the HGI diet compared with CR; and 3) a detrimental effect of 65% CHO diets on liver fat and fasting serum TGs during refeeding. A diet low in GI and moderate in CHO content (i.e., low GL) may therefore have health benefits by positively affecting daylong glycemia, IS, and liver fat.

Acknowledgments

M.J.M. and A.B.-W. designed the research; M.L., J.E., B.E., W.B., M.J., and A.B.-W. conducted research; M.L., D.P., M.J.M., and A.B.-W. were responsible for discussion of data; M.L. analyzed data; M.L., M.J.M., and A.B.-W. wrote the paper; and M.L. and A.B.-W. had primary responsibility for final content. All authors read and approved the final manuscript.

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