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Effects of a high-fat, low- versus high-glycemic index diet: retardation of insulin resistance involves adipose tissue modulation

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ABSTRACT Beneficial effects of low glycemic index (GI) diets in rodents have been studied using healthy low-fat diets, while the effects might be different on high-fat diets inducing progression of insulin resistance. We fed C57BL/6J male mice high-fat low/ high-GI (LGI/HGI) diets for 13 wk. Glucose and insulin tolerance and serum substrates, including adipokines, were measured longitudinally. The LGI group showed a significantly higher glucose tolerance from wk 2 onwards, which was supported by lower serum insulin and free fatty acids levels at 8 wk, and a tendency for lower leptin levels, while resistin levels remained similar. At 11 wk, when differences in serum resistin started to increase, differences in serum insulin were diminished. Although food intake was similar throughout the study, body weights and epididymal adipose tissue mass became significantly lower in the LGI group at necropsy. Several serum substrates and adipose tissue leptin mRNA levels, as analyzed by Q-PCR, were, again, significantly lower, whereas adiponectin mRNA levels were higher. Taken together, an LGI high-fat diet maintains higher glucose tolerance and insulin sensitivity via adipose tissue modulation solely because of a difference in the type of carbohydrate, supporting a nutritional approach in the fight against insulin resistance.—Van Schothorst, E. M., Bunschoten, A., Schrauwen, P., Mensink, R. P., Keijer, J. Effects of a high-fat, low versus high glycemic index diet: retardation of insulin resistance involves adipose tissue modulation. FASEB J. 23, 1092–1101 (2009)

Key Words: carbohydrate · adipokines · glucose tolerance

The pandemic of obesity, type II diabetes, and associated cardiovascular diseases is growing worldwide, and prevention is of utmost urgency (1). Although pharmacological intervention will help in the treatment of these diseases, prevention might be achieved by nutritional and lifestyle interventions. Furthermore, a dietary approach might add a small but relevant cumulative effect to treatment of insulin resistance and type II diabetes. This is especially relevant in the light of recent findings that intense glucose-lowering pharmacological therapy increased mortality and did not reduce cardiovascular events in type II diabetes patients, independent of the class of drugs used (2). As one strategy, a reduction of the glycemic index (GI) of the diet was proposed to have beneficial health effects (3). The GI classifies carbohydrates in terms of the acute blood glucose-elevating properties compared to a standard amount of glucose intake and distinguishes a low GI (LGI; a lower rise in blood glucose and insulin levels spread over a longer time) from a high GI (HGI; a high and quick rise and subsequently fall in blood glucose and insulin levels). Detailed reviews of meta-analyses in humans provide evidence for improvement of diabetes-, cardiovascular disease-, and obesity-related parameters (4–6), with the majority of these studies reporting health benefits only for LGI diets. However, inconsistencies also exist, most likely because of differences in human diets, such as the proportion and types of carbohydrates and fats ingested, or the total fiber intake, which has profound effects on postprandial glucose response (7). These can be better controlled in animal studies.

Animal GI studies were primarily performed using healthy or diabetic rats (8–13), although only a few studies focused on mice: either wild-type (10, 14, 15), chemically induced obese (15), or genetically modified knockout mice (16). In wild-type mice, an LGI vs. HGI diet with a low-fat diet content resulted in less body fat (10, 15), which was confirmed and extended to observations of lower plasma insulin and triglycerides levels and absence of liver steatosis (14), suggesting but not proving an increased insulin sensitivity.

Dysfunctional white adipose tissue (WAT) leads to disease (17–19). WAT performs its metabolic functions
in active communication with other organs, for which it secretes and perceives a large number of different signals (20). Among the secreted molecules are the adipokines leptin, adiponectin, and resistin, as well as several cytokines. These adipokines play an important role in diet-induced obesity (21–23) and insulin resistance and/or diabetes (24–28). An LGI vs. HGI diet resulted in reduced plasma plasminogen activator-1 (PAI-1) activity (29) and lower leptin levels (15). However, WAT leptin mRNA expression was found not to be significantly different (15). Furthermore, an LGI diet significantly increased plasma adiponectin levels in rats (10).

Detailed analysis of progression of whole-body insulin resistance as a consequence of the dietary GI has not extensively been performed. Furthermore, the effects of GI in rodents may be different on a diet with a fat content that is more relevant to humans (30, 31) as compared to the rodent low-fat (chow) diets. Thus far, no rodent studies on the effect of GI have been performed using diets that resemble the human diet in fat content and composition. This is of particular relevance because it was shown that fat, and not the caloric intake, is the crucial stimulus for obesity- and diabetes-related parameters in C57BL/6j wild-type mouse (32), which can be modulated by different types of carbohydrates as explained above. We hypothesize that, on a Western high-fat-diet background, an LGI diet delays the progression of insulin resistance compared to an HGI diet. Young-adult wild-type mice were fed (moderate) high-fat diets differing in GI to investigate the causal role of an LGI diet in delaying the high-fat diet-induced insulin resistance. The outcome of this study might contribute to improved nutritional advice for those who are at risk for developing obesity, impaired glucose tolerance, or even diabetes.

### MATERIALS AND METHODS

#### Animal study and diets

Male, wild-type C57BL/6jOlaHsd mice (Harlan, Horst, The Netherlands) 9 wk of age were individually housed in macron cages. Housing was maintained constant at 21°C and 45% humidity, and lights were switched on from 6 AM to 6 PM. The experimental protocol was approved by the Animal Welfare Committee of Wageningen University, Wageningen, The Netherlands. Food and water were supplied weekly ad libitum, and remaining food was weighed to calculate food intake. Body weight was measured weekly.

The semisynthetic diets (Research Diet Services B.V., Wijk bij Duurstede, The Netherlands) resembled a Western human diet (Table 1) and differed only in the type of starch; the LGI diet consisted of ~60% amylose and 40% amylopectin (C*Amylo Gel 03003, Cerestar Benelux, Bergen op Zoom, The Netherlands), whereas the HGI diet consisted of 100% amylopectin (C*Gel 04201). Vitamin and minerals were according to the AIN93G diet. Gross energy content was determined in feed using adiabatic bomb calorimetry (model IKA calorimeter C7000; IKA Werke GmbH & Co. KG, Staufen, Germany) according to ISO Standard 9831. Analyses were carried out in duplicate.

All 110 mice started the 3 wk acclimation period on the LGI diet. At the start of the intervention, 10 animals were sacrificed (t=0 group), and the remaining 100 mice were stratified on body weight (BW) in 2 groups. One group continued on the LGI diet (mean BWt=0 25.62±0.25 g), whereas the other group was switched to an HGI diet (mean BWt=0 25.68±0.23 g) for a period of 13–14 wk. Of both groups, 10 animals were used for glucose tolerance tests (GTTs), 30 for insulin tolerance tests (ITTs), and 10 others for blood sampling only, of which the latter are further referred to as nonchallenged (Fig. 1).

#### Single test meal

In wk 3 of the acclimation period, a single test meal (n=10) after an overnight fast was used to determine the in vivo GI of

### TABLE 1. Diet composition

<table>
<thead>
<tr>
<th>Component</th>
<th>RGI (glucose)</th>
<th>LGI (amylose)</th>
<th>HGI (amylopectin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid casein</td>
<td>220.0</td>
<td>220.0</td>
<td>220.0</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Dextrose monohydrate</td>
<td>534.5</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Amylose (C*Amylo Gel 03003)</td>
<td>0.0</td>
<td>534.5</td>
<td>0.0</td>
</tr>
<tr>
<td>Amylopectin (C*Gel 04201)</td>
<td>0.0</td>
<td>0.0</td>
<td>534.5</td>
</tr>
<tr>
<td>Cellulose (Arbocel B800)</td>
<td>50.0</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Lard</td>
<td>101.5</td>
<td>101.5</td>
<td>101.5</td>
</tr>
<tr>
<td>Corn oil</td>
<td>43.5</td>
<td>43.5</td>
<td>43.5</td>
</tr>
<tr>
<td>Mineral premix AIN-93G</td>
<td>35.0</td>
<td>35.0</td>
<td>35.0</td>
</tr>
<tr>
<td>Vitamin premix AIN-93</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Total (g)</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Calculated energy density (kcal/kg)</td>
<td>4375</td>
<td>4375</td>
<td>4375</td>
</tr>
<tr>
<td>Measured energy density (kcal/kg)*</td>
<td>n.d.</td>
<td>4718 ± 45</td>
<td>4671 ± 33</td>
</tr>
<tr>
<td>Carbohydrates (kcal%)</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Fat (kcal%)</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Protein (kcal%)</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

Values are g/kg diet, unless stated otherwise. Reference GI (RGI) diet was used only for a single test meal. *Measured by bomb calorimetry; mean ± sd of 2 samples. n.d. = not determined.
these two GI diets vs. a reference (RGI) diet containing dextrose monohydrate as carbohydrate fraction. Animals receiving the single RGI dietary test meal were all grouped in the $t_0$ group. Blood glucose levels were measured using a blood glucose device (FreeStyle, Abbot, Amersfoort, The Netherlands) after a tail incision ($t_0$ min) at 8:00 AM. After an additional 2 h fasting, a weighed amount of food (2.03±0.05 g) was supplied for exactly 15 min, reweighed, and food intake was calculated. Blood glucose levels were measured at time points 15 (end of food intake), 30, 45, 60, 90, and 120 min.

**GTTs and ITTs**

The GTTs and ITTs were performed in wk 0, 2, 5, 8, and 11. Animals were fasted for 5 h from 8:00 AM onwards before the test. At $t_0$ min, fasting blood glucose levels were measured after a tail incision. A glucose (2 g/kg BW) or insulin (0.75 U/kg BW; Humuline® Regular, Eli Lilly, Houten, The Netherlands) solution was injected into the intraperitoneal cavity, followed by blood glucose measurements at time points 15, 30, 45, 60, 90, and 120 min.

**Blood sampling**

In wk 1, 2, 5, 8, and 11, nonchallenged mice were fasted for 5 h from 8:00 AM onwards, followed by anesthesia using isoflurane. Blood was drawn by orbital puncture and collected in serum collection tubes (Greiner Bio-One, Frickenhausen, Germany). Fasting blood glucose levels were measured directly, and serum was collected after centrifugation following the manufacturer’s protocol and stored in aliquots at $-80°C$ for further analyses.

**Necropsy**

Mice were fasted for 2 h in wk 13 (GTT and ITT mice) and wk 14 (nonchallenged mice) from 8:00 AM onwards and anesthetized using isoflurane. Blood was sampled after eye extraction, glucose levels were measured, and serum was collected. Mice were killed using cervical dislocation. Organs were isolated, weighted, and immediately frozen in liquid nitrogen and stored at $-80°C$ for subsequent analyses. Throughout the manuscript we denote the time point of necropsy as wk 13.

Of the ITT mice, 10 mice received 30 min before necropsy an insulin injection (0.75 U/kg BW) to analyze acute insulin effects, which were compared and analyzed with ITT mice not receiving an insulin injection.

**Serum parameters**

Serum free fatty acid (FFA) levels were measured using the NEFA C kit (Wako Chemicals, GmbH, Neuss, Germany), and volumes were scaled down to analyze the samples with a microplate reader (BioTec Synergy HT, Bad Friedrichshall, Germany). Serum samples (5 μl) were measured in duplicate and averaged, and FFA concentrations were calculated using a standard curve of oleic acid (supplied by the kit). Hepatic triglyceride (TG) content was analyzed as described using a 2% liver homogenate (33).

Serum levels of leptin, resistin, insulin, monocyte chemoattractant protein-1 (MCP-1), interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α), and PAI-1 were measured using the mouse serum adipokine Lincoplex Kit (Linco Research, Nuclilab, Ede, The Netherlands). Sera were diluted 5X in HPE buffer (Sanquin, Amsterdam, The Netherlands), debris was removed by spinning the sample using SpinX columns (Corning, Schiphol-Rijk, The Netherlands), and possible inhibition of the immunological Ig fraction in serum was circumvented by an incubation at gentle shaking for 1 h at room temperature in 96-well Reacti-Bind™ protein L-coated plates (Pierce, Rockford, IL, USA) before adipokine assaying. The assays were conducted according to the manufacturer’s protocol and measured using the Luminex X100 system with Starstation software (Applied Cytometry Systems, Dinnington, Sheffield, UK). All individual samples were analyzed in duplicate and averaged when the difference between the 2 measurements was $\leq 5\%$. 

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**Figure 1.** Schematic representation of the study design. All mice started the 3 wk acclimation period on LGI diet (solid line). Asterisk (*) indicates single test meals. Ten mice were sacrificed at the start of the intervention ($t_0$ group, $n=10$). Simultaneously, half of the remaining mice were switched to HGI diet (dotted line). Each dietary group was subsequently subdivided into 3 subgroups, which underwent in a 3 wk time interval only blood collection (nonchallenged animals, $n=10$; B), GTTs ($n=10$; G), or ITTs ($n=30$; I). Just 30 min before necropsy, a subset of ITT mice received an insulin injection (arrow, $n=10$) to measure acute insulin function. Dagger (†) indicates necropsy.
RNA isolation from WAT was performed as described (34). Briefly, after grinding WAT, RNA was extracted using Trizol (Invitrogen, Breda, The Netherlands) and washed, pelleted, and dissolved in RNase-free water and purified using the RNeasy MinElute Cleanup Kit (Qiagen, Venlo, The Netherlands). RNA was quantified using the Nanodrop ND-1000 spectrophotometer (Isogen Life Sciences, Maarssen, The Netherlands) and qualified using capillary zone electrophoresis (Experion, Bio-Rad, Veenendaal, The Netherlands).

Real-time quantitative polymerase chain reaction (Q-PCR)

Differential gene expression of resistin, leptin, and adiponectin was assessed using real-time Q-PCR as described (35). Briefly, PCR amplification to obtain relative levels of gene expression using 2 reference genes (ribosomal protein S15 and hypoxanthine phosphoribosyltransferase 1) was performed in duplicate and averaged. Primer sequences are shown in Supplemental Table 1. The expression of the genes of interest was normalized against the reference genes using the GeNorm normalization factor (36).

Statistical analyses

All data are expressed as means ± se. Statistical analyses were performed using Prism software (GraphPad Software, San Diego, CA, USA). Incremental area under the curve (iAUC) for GTTs and ITTs was analyzed using Prism software. Insulin resistance index was estimated by homeostasis model assessment (HOMA) using the program HOMA2-IR version 2.2 (37) using fasting glucose (mM) and insulin levels. Measurements at single time points between 2 groups were analyzed by Student’s t test (Q-PCR results), and those between 3 groups were analyzed using 1-way ANOVA (test meal, adipokines at section). Time course longitudinal analyses were analyzed by repeated-measurements (mixed model) ANOVA. Bonferroni post-tests were used in case ANOVA showed significance. P < 0.05 was considered statistically significant.

RESULTS

To investigate the causal role of an LGI diet in delaying the progression of insulin resistance in comparison to an HGI diet, we fed young-adult wild-type mice moderate high-fat diets differing in GI (Table 1). The dietary fat content of the diets is based on a Western diet (30 kcal% fat), which induces insulin resistance in mice to a mild extent. The only difference between the diets is the type of starch, thereby not influencing the quantity of macro- and micronutrients and the energy content of the diet. Bomb calorimetry analysis of both diets, which overestimates the amount of energy a body can absorb, was performed in duplicate to compare measured energy density with calculated energy density. The measured energy density closely approximated calculated energy density (Table 1), and the latter was used in the conversion of food intake to caloric intake.

Test meal

To analyze the difference in GI between the LGI vs. HGI diet, a subset of mice received 1 wk before the start of the intervention a single test meal after an overnight fast, and blood glucose responses were assessed and compared to an RGI diet with only glucose as carbohydrate, being otherwise identical (Table 1). Food intake was similar between the 3 groups (data not shown). The GI was significantly lower for the LGI vs. HGI diet, the latter resembling the RGI diet (Supplemental Fig. 1). Furthermore, the peak time point of plasma glucose levels for each dietary group shifted from 75 min with the LGI diet to 60 min with the HGI and 45 min with RGI diet. Altogether, the composition of the GI diets resulted in a significant GI difference between the 2 diets.

Intervention study

During the 13 wk intervention study, several measurements were performed periodically, in addition to the weekly assessment of body weight and food intake, as shown schematically in Fig. 1; subgroups are described in more detail in the Animal Study and Diets section of Materials and Methods.

The first observable differences between the 2 GI groups in body weight appeared at wk 9 (Fig. 2A) and became significantly different at wk 13: the HGI mice gained on average 7.3 ± 0.4 g, whereas the LGI mice...
had a significantly lower weight increase of 5.8 ± 0.2 g. Food intake did not differ between the 2 GI groups throughout the study (Fig. 2B), resulting in an average total energy intake of 1350 ± 20 kcal for the HGI mice and 1355 ± 20 kcal for the LGI mice.

Despite the fact that the LGI mice had an equal energy intake, their body weights were reduced, and at necropsy absolute and relative epididymal WAT weight was significantly decreased compared to the HGI mice; this same trend holds for visceral and subcutaneous WAT (Table 2). Other organs investigated, such as heart, and lungs as nonmetabolic tissue, were not different (Table 2), as was absolute liver weight (Table 2). Thus, the body composition is altered because of a nutritional GI difference. Furthermore, hepatic lipid content was significantly increased in the HGI group (Table 2), supporting previously reported data (14).

We next investigated whether the difference in adipose tissue mass is reflected in secreted serum adipokines. Of these, the inflammation markers MCP-1, TNF-α, and IL-6 showed nondetectable levels. An insulin challenge (see below) changed serum adipokine levels rapidly (data not shown), and we therefore focus on the data of the non-insulin-stimulated 40 mice/dietary group. Leptin was significantly lower in the LGI group (Fig. 3A), whereas PAI-1 serum levels were not different (Fig. 3B). Furthermore, fasting serum FFA and insulin levels were significantly lower in the LGI group (Fig. 3C), whereas ITTs in 3 wk interval periods. The GTTs showed glucose tolerance remained significantly increased at 5 and 8 wk (data not shown). Furthermore, the ITTs in 3 wk interval periods. The GTTs showed glucose tolerance remained significantly increased at 5 and 8 wk (data not shown). Furthermore, the ITTs in 3 wk interval periods. The GTTs showed glucose tolerance remained significantly increased at 5 and 8 wk (data not shown). Furthermore, the ITTs in 3 wk interval periods. The GTTs showed glucose tolerance remained significantly increased at 5 and 8 wk (data not shown). Furthermore, the ITTs in 3 wk interval periods. The GTTs showed glucose tolerance remained significantly increased at 5 and 8 wk (data not shown).

**Time course of appearance of insulin resistance**

To gain insight into the development and delay in progression of insulin resistance, we performed longitudinal analyses in subgroups. Whole-body glucose tolerance and insulin sensitivity were tested using GTTs and ITTs in 3 wk interval periods. The GTTs showed significantly increased glucose tolerance for the LGI group vs. HGI group from wk 2 onwards (Fig. 5), while body weights were similar (Fig. 2A). When IACG data were adjusted for the difference at t = 0, the LGI group glucose tolerance remained significantly increased at 5 and 8 wk (data not shown). Furthermore, the ITTs in wk 5 and 8 also showed small but significant decreases in blood glucose levels in the LGI group (Supplemental Fig. 2).

FFA levels were lower from wk 2 onwards in the

### TABLE 2. Body and tissue weight

<table>
<thead>
<tr>
<th>Measure</th>
<th>SI unit</th>
<th>t = 0</th>
<th>LGI</th>
<th>HGI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight</td>
<td>g</td>
<td>25.75 ± 0.50</td>
<td>31.20 ± 0.40*</td>
<td>32.93 ± 0.50*</td>
</tr>
<tr>
<td>WAT, epididymal</td>
<td>mg</td>
<td>317 ± 40</td>
<td>593 ± 32†</td>
<td>878 ± 55*</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>1.2 ± 0.1</td>
<td>1.9 ± 0.1‡</td>
<td>2.6 ± 0.1*</td>
</tr>
<tr>
<td>WAT, visceral</td>
<td>mg</td>
<td>129 ± 12</td>
<td>271 ± 29*</td>
<td>298 ± 18†</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>0.5 ± 0.0</td>
<td>0.8 ± 0.1</td>
<td>0.9 ± 0.0*</td>
</tr>
<tr>
<td>WAT, subcutaneous</td>
<td>mg</td>
<td>136 ± 43</td>
<td>103 ± 20</td>
<td>145 ± 15</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>0.5 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>0.4 ± 0.0</td>
</tr>
<tr>
<td>Liver</td>
<td>mg</td>
<td>104 ± 33</td>
<td>1169 ± 17†</td>
<td>1155 ± 21†</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>4.1 ± 0.1</td>
<td>3.8 ± 0.1‡</td>
<td>3.5 ± 0.0*</td>
</tr>
<tr>
<td>Hepatic triglyceride content</td>
<td>g/100 g tissue</td>
<td>2.7 ± 0.4</td>
<td>4.3 ± 0.9‡</td>
<td>9.7 ± 2.1†</td>
</tr>
<tr>
<td>Heart</td>
<td>mg</td>
<td>179 ± 9</td>
<td>171 ± 3</td>
<td>214 ± 33</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>0.7 ± 0.0</td>
<td>0.6 ± 0.0</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>Lung</td>
<td>mg</td>
<td>166 ± 9</td>
<td>150 ± 4</td>
<td>160 ± 24</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>0.6 ± 0.0</td>
<td>0.5 ± 0.0</td>
<td>0.5 ± 0.1</td>
</tr>
</tbody>
</table>

Data shown as means ± se. Statistical significance was analyzed using 1-way ANOVA and Bonferroni post hoc tests. *P < 0.001, †P < 0.05 vs. t = 0 group (n=10). ‡P < 0.001, §P < 0.05 vs. HGI (n=50). Relative weight (%) = organ weight (g)/BW (g). *Determined in 10 mice/dietary group.
nonchallenged LGI group; differences became significant in wk 8, lost significance in wk 11, but reappeared significantly thereafter at necropsy (Fig. 4G). It is known that a high-fat diet induces initially a reduction in FFA levels (e.g., ref. 38), and it is therefore interesting to note that the FFA levels in the HGI group remained similar, whereas those in the LGI group indeed decreased in time. Adipokine profiling showed that leptin and resistin gradually increased less in the LGI vs. the HGI group (leptin from wk 8 onwards, resistin from wk 11 onwards), reaching significance for both adipokines at necropsy, whereas PAI-1 levels were not different (Fig. 4A–C). Last, fasting insulin levels and HOMA-IR index reached significantly lower levels in wk 8, the difference diminished in wk 11, but at necropsy significantly lower levels were again observed (Fig. 4D, F). At necropsy, as discussed above, all significant differences observed between the nonchallenged subgroups were also observed in the GTT and ITT subgroups (Figs. 3, 4). Taken together, this shows higher insulin sensitivity in the LGI compared to HGI group.

The lower serum adipokine levels in the LGI group...
were investigated further by epididymal WAT gene expression analysis in the nonchallenged mice; adiponectin gene expression analysis was included to enhance our understanding of WAT physiology and its gene expression changes. Leptin mRNA levels were significantly lower in the LGI group, whereas resistin and adiponectin mRNA levels were nonsignificantly higher (Fig. 6A–C). Gene expression and serum protein level correlations were significant for leptin (Fig. 6D), but not for resistin (Fig. 6E).

Finally, insulin sensitivity was also investigated in a subset of animals, using a single insulin injection 30 min before necropsy. As glucose levels dropped on average 4.2 mM in both dietary groups, lower glucose levels were observed in the LGI group (Fig. 5).

**Figure 5.** GTTs performed in a 3 wk time interval between HGI (triangles) and LGI (squares) diets, shown as means ± se \( (n=7–10) \). *P < 0.05, **P < 0.01, ***P < 0.001; repeated measurements mixed-model ANOVA and Bonferroni post hoc tests.

**Figure 6.** Q-PCR and correlation graphs of adipokines. A–C) Gene expression results in epididymal WAT, as measured by Q-PCR (LGI group, white bars; HGI group, black bars); leptin (A), resistin (B), adiponectin (C). Data were normalized using the mean of reference genes Rps15 and Hprt1 and shown as means ± se \( (n=8–10) \). *P < 0.05; Student’s t test. D, E) Correlation graphs and analyses for leptin (D) and resistin (E) between epididymal WAT mRNA levels and serum protein levels \( (n=8) \) in the HGI (triangles) vs. LGI (squares) dietary groups.
levels in the LGI group were preserved (4.8±2.8 vs. 5.5±1.2 mM in LGI and HGI groups, respectively). More important, FFA levels were significantly altered, especially in the LGI group: FFA levels dropped 39%, although this was only 25% in the HGI group (Fig. 3G). This may suggest a higher suppression of WAT lipolysis in the LGI group, implicating a better physiological response of adipose tissue to insulin.

DISCUSSION

We show that an LGI diet on a high-fat-diet background retards progression of insulin resistance, compared to an HGI diet. We base this not only on serum parameters such as insulin, glucose, and FFAs, but also on whole-body physiology, hepatic lipid accumulation, the acute response to insulin injection, adipose tissue gene expression, and longitudinal adipokine profiling. This retardation is solely caused by a difference in the type of carbohydrate of the diet, supporting the belief that a nutritional approach based on GI might provide a valuable added tool in the fight against insulin resistance.

During the progression of insulin resistance development, we observed several interesting aspects. First, after 5 wk intervention, we observed no differences between the 2 dietary groups, except by GTTs that show significantly increased glucose tolerance in the LGI group. It remains to be seen whether known (i.e., visfatin or adiponectin; ref. 20) or unknown serum parameters can be used as an easily accessible serum biomarker correlating with the observed difference in glucose sensitivity.

Second, after 8 wk, most parameters except resistin are lower in the LGI group, and another 3 wk later, resistin also shows lower levels in the LGI group, which indicates that resistin has a slower response in time.

Third, at wk 11 these parameters are all, except insulin, lower in the LGI group, while at the same time the GTT and ITT groups suggest a reduced difference correlating with the fasting serum insulin levels. Reduced differences were also observed in other GI studies to some extent in rats (10) and clearly in humans (39). Of note, our observations of diminished differences are based on several distinct analyses in subgroups (GTTs, ITTs, and nonchallenged mice), which were treated somewhat differently, thus supporting the biological relevance. We therefore hypothesize that the apparent reduced differences may be the result of the animals adopting a new metabolic set point that may involve differences in adipose mass and function. However, after 13 wk at necropsy, acute insulin effects—improved antilipolytic effect of insulin in the LGI group—and all serum parameters analyzed suggest a higher insulin-sensitive phenotype in the LGI group, which is supported by the lower degree of insulin resistance as estimated by the HOMA index. It may be speculated that extension of the GI intervention study might again lead to clear differences in glucose tolerance and insulin sensitivity. Indeed, others showed after a 25 wk murine GI intervention a significantly lower percentage body fat and nonfasted plasma insulin and TG levels in the LGI group, while body weights and blood glucose levels were similar. However, physiological GTTs or ITTs were not performed (14).

Supporting the idea of an adipose-specific set point is the fact that at necropsy indistinguishable blood glucose levels between the dietary groups were observed, even after an acute insulin challenge. This suggests that insulin-stimulated glucose uptake primarily in muscle (40) and hepatic inhibition of gluconeogenesis appear to be metabolically unchanged. The latter is reduced by elevated FFA levels (41), as observed in the HGI group. This clearly warrants further longitudinal investigations on effects of GI on metabolic regulation of WAT, muscle, and liver tissue simultaneously.

Serum protein levels for leptin correlate with adipose tissue gene expression. However, the decreased resistin serum levels do not correlate with the observed slight increase in WAT resistin mRNA levels in the LGI group. In agreement, others observed that murine serum resistin levels, but not WAT resistin mRNA expression levels, are correlated with body weight (42), which suggests resistin serum levels as being more physiologically relevant.

Physiologically relevant reduced serum leptin levels, as observed in the LGI group, reflect lower adipose tissue weight (21), correlating with a healthier phenotype. Likewise, the LGI group showed lower serum resistin levels, implying a healthier phenotype, although this seems regulated mainly outside adipose tissue and may involve the liver. Lower leptin levels were also observed after 7 or 10 wk intervention in the LGI groups of rodents (10, 15) and after 8 d in humans (43) but disagree with findings in rats after 3 and 12 wk (44). Increased WAT adiponectin mRNA levels correlate positively with elevated serum levels both in low-fat- and high-fat-fed mice (45), which suggests that the LGI group most likely will have beneficially higher adiponectin serum levels.

The detailed analyses of metabolic parameters in 3 subgroups showed high similarity between these subgroups for whole-body physiology, that is, FFA levels, adipokine profiling, insulin levels, and in general blood glucose levels. Of interest, the significant lower levels of resistin in the nonchallenged LGI group at necropsy, which was not observed in the other 2 metabolically stressed subgroups, might indicate that metabolic tests like GTTs and ITTs performed during a nutritional intervention may have an impact on physiological performance and outcome, especially in those nutritional studies where small physiological changes are expected.

The differences in the composition of diets, in particular the content and type of macronutrient, will affect the outcome of GI studies. Most, if not all, rodent GI studies are based on diets having a low fat content (<15 kcal% fat) resembling the fat content of chow (9, 10, 14, 15, 46, 47), whereas human GI studies vary from <6 kcal% of fat (29) to a high-fat diet containing 40...
improve evaluation of human studies and may provide insight into the likely adaptations leading to comparative investigation of time points 8 and 11 wk will evaluated the high-fat-diet-induced insulin resistance. Com-

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