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Glycemic index differences of high-fat diets modulate primarily lipid metabolism in murine adipose tissue

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van Schothorst EM, Bunschoten A, Verlinde E, Schrauwen P, Keijer J. Glycemic index differences of high-fat diets modulate primarily lipid metabolism in murine adipose tissue. Physiol Genomics 43: 942–949, 2011. First published June 14, 2011; doi:10.1152/physiolgenomics.00042.2011.—A low vs. high glycemic index of a high-fat (HF) diet (LGI and HGI, respectively) significantly retarded adverse health effects in adult male C57BL/6J mice, as shown recently (Van Schothorst EM, Bunschoten A, Schrauwen P, Mensink RP, Keijer J. FASEB J 23: 1092–1101, 2009). The LGI diet enhanced whole body insulin sensitivity and repressed HF diet-induced body and white adipose tissue (WAT) weight gain, resulting in significantly reduced serum leptin and resistin levels and increased adiponectin levels. We questioned how WAT is modulated and characterized the molecular mechanisms underlying the glycemic index-mediated effects using whole genome microarrays. This showed that the LGI diet mainly exerts its beneficial effects via substrate metabolism, especially fatty acid metabolism. In addition, cell adhesion and cytoskeleton remodeling showed reduced expression, in line with lower WAT mass. An important transcription factor showing enhanced expression is PPAR-γ. Furthermore, serum levels of triglycerides, total cholesterol, and HDL- and LDL-cholesterol were all significantly reduced by LGI diet, and simultaneously muscle insulin sensitivity was significantly increased as analyzed by protein kinase B/Akt phosphorylation. Cumulatively, even though these mice were fed an HF diet, the LGI diet induced significantly favorably changes in metabolism in WAT. These effects suggest a partial overlap with pharmacological approaches by thiazolidinediones to treat insulin resistance and statins for hypercholesterolemia. It is therefore tempting to speculate that such a dietary approach might beneficially support pharmacological treatment of insulin resistance or hypercholesterolemia in humans.

LOW VS. HIGH GLYCEMIC INDEX (LGI and HGI, respectively) diets have previously been shown to result in beneficial effects on health, as expressed by fat mass and measures of insulin resistance, including glucose tolerance tests (GTT), in rodents as well as in humans (3, 16, 22, 23, 30, 31). Although rodents consume the same experimental diet throughout the intervention period, the results obtained in humans indicate major effects, even when only part of the daily consumed diets fulfills the glycemic index (GI) criteria of experimental design. However, some human studies reported a lack of effects (e.g., Ref. 47) or confounding factors (20). The rodent studies have been performed using low-fat (~10% kcal fat) diets, which are healthier than diets with a high-fat (HF) content (11). In contrast to low-fat diets, HF diets induce obesity and insulin resistance in mouse strains sensitive to development of diet-induced obesity, such as C57BL/6J (9). Therefore, we previously studied the effects of differences in GI (LGI vs. HGI) in the context of an HF (30% kcal fat) diet, rather than a low-fat diet, in obesity-prone C57BL/6J adult male mice (43). The dietary HF content of 30 kcal% can be considered as moderate and more human relevant fat content compared with rodent studies using 60 kcal% HF diets (i.e., Refs. 12, 15). We showed that an LGI diet significantly delays the HF-induced increase in fat mass and progression of insulin resistance, while simultaneously levels of white adipose tissue (WAT) secreted peptide hormones (adipokines) were beneficially affected compared with an HGI diet.

While this outcome is clear cut, it is not clear what the underlying mechanisms are. WAT is essential for maintaining a healthy glucose and lipid homeostasis, and dysfunctional WAT results in insulin resistance and dyslipidemia. The primary function of adipose tissue is the storage of lipids. Its capacity to expand is essential to maintain healthy levels of circulating lipids in times of abundant food supply and provides a source of energy in times of need. WAT is an active organ communicating with other tissues by receiving signals and secreting a variety of adipokines and metabolites (48). Daily intake of HF diets results in general in a constant energy overload and will result in metabolic dysfunction, leading to diseases associated with obesity, in particular insulin resistance, which progresses into Type II diabetes. Dysfunctional WAT is considered as one of the initial steps in the progression of diet-induced obesity-related metabolic disturbances (reviewed by e.g., Refs. 18, 46).

In studies using low-fat diets, impaired hepatic fatty acid oxidation was identified as potential cause of effects induced by an HGI vs. LGI diet (14, 37). Another mechanism may involve a direct effect on insulin signaling. Insulin signals are transferred through the insulin receptor via a cascade of protein phosphorylations to either phosphorylation and activation of proteins including protein kinase B (PKB)/Akt or to transcription factors regulating target gene expression involved in fatty acid metabolism (1, 24). PKB activation is crucial for glucose transporter Slc2a4/Glut4 translocation to the cell membrane mediating glucose uptake in many tissues and, as such, for insulin sensitivity (49). For effective translation to humans not only the beneficial effects, but also elucidation of underlying molecular mechanisms, are needed.

Therefore, we analyzed here gene expression profiles in white adipose tissue of mice fed an HF diet, LGI vs. HGI, to elucidate pathways underlying the changes in WAT by GI differences.
MATERIALS AND METHODS

Animals and diets. The study has been reported previously and was approved by the Animal Welfare Committee of Wageningen University, Wageningen, The Netherlands (43). In brief, 110 wild-type C57BL/6J male mice aged 9 wk were fed a semipurified HF (30% kcal fat) LGI diet for 3 wk, followed by placing them into three groups matched in body weight at the start of the experimental intervention: 3 groups (n = 10 each dietary group) was determined using the modified Friedewald formula (6, 8). Only differences we observed in serum glycerol levels. LDL-cholesterol was determined using the modified Friedewald formula (6, 8). Only samples with coefficient of variation <15% between duplicates were included for data analysis.

Protein phosphorylation detection by Western blot analysis. Gas
trocnemius muscle homogenates for Western blot analysis of (phos
dorylated) PKB/Akt were performed as described before (36). Equal amounts of protein were loaded on SDS-PAGE and, after Western blotting, incubated with p-Akt (Ser273) and Akt antibodies (#9271 and #9227, respectively; Cell Signaling Technology, Delfzijl, The Netherlands). After incubation with the appropriate secondary antibodies, specific protein bands were visualized by chemiluminescence and analyzed by Chemidoc XRS system (Bio-Rad).

RESULTS

Physiological data. Wild-type C57BL/6J male mice fed a semipurified HF, low LGI vs. an HF, HGI diet showed a significant lower body weight, lower serum levels of WAT secreted adipokines like leptin and resistin, as well as significant lower basal and insulin-stimulated serum levels of free fatty acids (FFA), as previously reported (43). Glucose tolerance was significantly increased and insulin resistance decreased in the LGI-fed mice, while food and energy intake were not significantly different. Furthermore, hepatic TG accumulation was significantly reduced (43). Together, this indicated a more insulin-sensitive phenotype for the LGI vs. HGI group at the whole body level (43). In agreement with significant lower body weight (LGI: 29.9 ± 1.2 g vs. HGI: 34.0 ± 1.2 g, P < 0.05) and lower serum leptin levels (LGI: 3.53 ± 0.74 mg/ml vs. HGI: 10.45 ± 1.37 mg/ml, P < 0.0001), also a lower WAT weight [LGI: 579 ± 78 mg epiWAT corresponding to 1.9 ± 0.2% epiWAT (wt/wt%)] vs. HGI: 997 ± 96 mg epiWAT corresponding to 2.9 ± 0.2% epiWAT, all P < 0.01] was observed in the subset of mice analyzed here (Fig. 1). To further support the dietary effects on insulin sensitivity, we investigated the phosphorylation status of PKB/Akt in skeletal muscle. Phosphorylated Akt is significantly induced upon insulin stimulation only in the LGI group (Fig. 2), while total Akt levels remained similar. This strengthens the higher insulin sensitivity in the LGI-fed mice not only for WAT, but also for muscle.
Transcriptome analysis. The role of WAT in the development of insulin resistance and the reduced WAT weights led us to speculate that retardation of whole body insulin insensitivity may be caused by differences in WAT, which, due to the endocrine and metabolic function of WAT, affects other organs like liver and muscle. To strengthen this hypothesis and obtain mechanistic insights in effects imposed by the dietary GI differences, we performed whole genome transcriptomics analysis of epididymal WAT. After normalization, 20,134 transcripts were found to be expressed (48%), of which 1,649 were differentially expressed between LGI and HGI; these comprise 1,390 transcripts based on significance supplemented with 259 based on RF criterion. The majority of significant differentially expressed genes showed an FC of LGI over HGI between 1.2 and 1.5 (Fig. 3), that is, upregulation of gene expression. Remarkably, although these relative small FC are in line with other nutritional studies using wild-type animals (17, 29), they appeared lower than expected based on the physiological effects seen in these mice. We then analyzed differential regulated pathways to assess orchestrated regulation at the pathway level. This revealed insulin regulated fatty acid metabolism as highest ranked pathway based on significance (Table 1). Other pathways that appeared in the top 15 were associated with substrate metabolism, such as TCA cycle and carbohydrate metabolism and regulation, but also cell adhesion and cytoskeleton remodeling appeared (Table 1). Pathway analysis using the subset of genes identified by significance and not by RF criterion revealed an identical ranking order of pathways (data not shown). Detailed examination of the genes constituting the top 15 pathways showed that cytoskeleton remodeling and cell adhesion constitute the same pathway. Likewise, the propionate and butyrate pathways overlap for seven out of nine upregulated transcripts, which in turn overlap with two genes of the TCA cycle and largely overlaps with the pathway containing metabolism of amino acids leucine, valine, and isoleucine. Thus, an LGI modifies WAT gene expression encoding proteins mainly involved in cellular glycolysis, TCA cycle, and fatty acid synthesis on the one hand and cell adhesion and cytoskeleton remodeling on the other.

Changes in expression of cell adhesion and cytoskeleton remodeling are in agreement with the significant differences in the epididymal WAT weight between the LGI and HGI groups, most likely resulting from differences in adipocyte hypertrophy. As expected, this process was downregulated in the LGI-fed mice. Beta-actin and alpha-2 actin (Fig. 4), exemplifying this process, were significantly lower expressed in the LGI-fed mice. In contrast, peroxisome proliferator-activated receptor-gamma (Ppar-γ) showed an increase upon LGI (FC 1.20).
A large number of the target genes encoding the enzymes involved in cellular glycolysis, TCA cycle, and fatty acid synthesis are under the control of the nuclear transcription factor SREBF/SREBP1, which is upregulated by LGI at gene expression level (FC 1.24, Fig. 4). Furthermore, SREBP1 target genes involved in TG synthesis like Scd-1 and Gpam are significantly upregulated, while Fasn shows a tendency to upregulation (Fig. 4). Genes encoding key enzymes involved in fatty acid oxidation like subunits Acaca and Acacb encoding acetyl-coA carboxylase and carnitine palmitoyl transferases present (Cpt1a, Cpt1b, and Cpt2) are nonsignificantly upregulated but are included in the subset on the basis of RF criterion. Cumulatively, this might be indicative for an increased storage of TG in WAT. However, we observed significantly lower epiWAT weights after an LGI vs. HGI intervention (Fig. 1, Ref. 43). Therefore, we focused on a “pathway” not included in the software program, namely differentially expressed genes between classical adipocytes containing a single large lipid droplet and adipocytes containing multiple mitochondria and lipid droplets, the so-called brownish phenotype (38, 45). Several genes are regulated in such a way that the LGI diet favors a more brownish phenotype, including Cidea and Ppargc1alpha (Fig. 4), and TCA genes. The delicate balance between synthesis, deposition, and usage of energy in the form of TG and theoretically the size of adipocytes and its lipid droplets seems therefore marginally changed favoring TG usage and smaller adipocytes containing more but smaller lipid droplets upon LGI vs. HGI intervention.

A selected subset of genes, from different significantly regulated pathways, was analyzed by real-time quantitative PCR (Q-PCR), confirming fold changes observed by microarray data analysis (data not shown).

**Lipid metabolism.** Based upon the observations that several WAT lipid metabolism pathways were differentially altered,

![Image of a bar graph showing numbers of significant differentially regulated genes in murine WAT by an HF, LGI vs. HGI diet. Significant differential expression is shown for downregulation and upregulation by LGI vs. HGI. Observed absolute fold changes are subdivided into 3 groups: 1.07 ≤ |FC| ≤ 1.2 (white), 1.2 < |FC| < 1.5 (gray), and |FC| ≥ 1.5 (black), and the number of transcripts per group is shown.](image)

**Table 1. Top 15 differentially regulated pathways by LGI vs. HGI dietary intervention**

<table>
<thead>
<tr>
<th>Map/Process</th>
<th>General Function</th>
<th>Cell Process</th>
<th>P Value</th>
<th>Objects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin regulation of fatty acid metabolism</td>
<td>regulation of lipid metabolism</td>
<td>response to hormone stimulus</td>
<td>3.15E-06</td>
<td>15 (10/5)</td>
</tr>
<tr>
<td>Regulation of lipid metabolism via LXR, NF-Y, and SREBP</td>
<td>regulation of lipid metabolism</td>
<td>transcription</td>
<td>9.32E-06</td>
<td>13 (8/2)</td>
</tr>
<tr>
<td>Propionate metabolism</td>
<td>carbohydrates metabolism</td>
<td>transcription</td>
<td>1.67E-05</td>
<td>11 (9/0)</td>
</tr>
<tr>
<td>Ligand-dependent transcription of retinoid-target genes</td>
<td>transcription factors</td>
<td>transcription</td>
<td>7.80E-05</td>
<td>17 (7/10)</td>
</tr>
<tr>
<td>Regulation of fatty acid synthase activity in hepatocytes</td>
<td>regulation of lipid metabolism</td>
<td>transcription</td>
<td>1.17E-04</td>
<td>8 (3/0)</td>
</tr>
<tr>
<td>Integrin-mediated cell adhesion and migration</td>
<td>regulatory processes/cell adhesion</td>
<td>cell adhesion</td>
<td>2.07E-04</td>
<td>15 (5/10)</td>
</tr>
<tr>
<td>RXR-dependent regulation of lipid metabolism via PPAR, RAR, and VDR</td>
<td>regulation of lipid metabolism</td>
<td>transcription</td>
<td>2.45E-04</td>
<td>10 (5/0)</td>
</tr>
<tr>
<td>Cytoskeleton remodeling</td>
<td>cytoskeleton remodeling</td>
<td>transcription</td>
<td>5.39E-04</td>
<td>23 (10/13)</td>
</tr>
<tr>
<td>Butanoate metabolism</td>
<td>carbohydrates metabolism</td>
<td>transcription</td>
<td>6.28E-04</td>
<td>10 (9/1)</td>
</tr>
<tr>
<td>TCA</td>
<td>aminoacid metabolism</td>
<td>transcription, G protein-signaling pathway</td>
<td>7.68E-04</td>
<td>8 (6/0)</td>
</tr>
<tr>
<td>ChREBP regulation pathway</td>
<td>transcription factors</td>
<td>G protein-signaling pathway</td>
<td>1.50E-03</td>
<td>8 (3/5)</td>
</tr>
<tr>
<td>Kappa-type opioid receptor activation of ERK</td>
<td>protein function/G proteins/GPCR</td>
<td>response to extracellular stimulus, G protein-signaling pathway</td>
<td>1.60E-03</td>
<td>8 (0/8)</td>
</tr>
<tr>
<td>Apoptosis and survival, HTR1A signaling</td>
<td>protein function/G proteins/GPCR</td>
<td>apoptosis, G protein-signaling pathway</td>
<td>1.92E-03</td>
<td>12 (5/8)</td>
</tr>
<tr>
<td>Dopamine D2 receptor</td>
<td>G proteins/GPCR regulatory processes</td>
<td>G protein-signaling pathway</td>
<td>2.01E-03</td>
<td>11 (4/7)</td>
</tr>
<tr>
<td>Leucine, isoleucine, and valine metabolism</td>
<td>amino acid metabolism</td>
<td>cell process</td>
<td>2.22E-03</td>
<td>9 (7/0)</td>
</tr>
</tbody>
</table>

The top 15 pathways obtained using MetaCore (GeneGO) are sorted based on significance. For each pathway, the function, process, its P value, and the number of unique regulated genes/objects (within parentheses the number of significantly up- and downregulated genes) and total number of genes are shown. LGI, low glycemic index; HGI, high glycemic index.
we analyzed a subset of serum lipid metabolites. Previously, we showed that basal fasting serum FFA levels were significantly lower in the LGI-fed mice supportive for a more insulin-sensitive phenotype in WAT at the tissue level (43). Basal fasting serum TG and glycerol levels were decreased in these mice, although nonsignificantly (Fig. 5, A and B). Total cholesterol and LDL- and HDL-cholesterol levels were all significantly lower in the LGI mice (Fig. 5, C–E), suggesting hepatic differences between LGI- and HGI-fed mice and supportive for a more insulin-sensitive phenotype at the whole body level. Moreover, the serum cholesterol levels in the LGI-fed mice are nonsignificantly different from \( t = 0 \) group, thus abolishing the HF diet-induced increase in serum cholesterol.

**DISCUSSION**

Physiological changes following a LGI vs. HGI HF dietary intervention in mice, as performed here, shows that WAT gene expression is significantly changed in pathways relating to insulin sensitivity and its regulation of fatty acid metabolism. Furthermore, the PKB/Akt phosphorylation status of muscle supports a more insulin-sensitive phenotype in LGI-fed mice. Previously, we showed a decreased level of intrahepatic fat, suggesting beneficial effects in liver as well (43). This was accompanied with significantly decreased lipid serum levels of total cholesterol and LDL- and HDL-cholesterol in LGI mice. This suggests that besides WAT, muscle and liver also play an important role in the differential whole body responses to diets of different GI. It is tempting to speculate that previous findings with respect to differential effects of GI diets on substrate use (higher fat oxidation in low fat, low vs. high GI mice) and hepatic gene expression patterns (14, 37) are due to accelerated WAT dysfunction on an HGI compared with LGI and that the reduced WAT insulin sensitivity in HGI vs. LGI fed mice might be a first step in the cascade of development of insulin resistance (14). As shown, epiWAT weight was significantly lower in LGI vs. HGI-fed mice. Based on earlier observations using HF diets with 30% fat, it can be expected that other WAT depots are similarly affected (44), This suggests that body weight gain is mainly caused by an increase in WAT, especially given the fact that increased WAT weight, but not lean mass, was observed after HF feeding (e.g., Ref. 5), or a GI-based intervention (14).

Recently, a significant positive correlation was shown between omental adipocyte cell size and fatty liver in metabolically healthy, severely obese (26), suggesting, but not proving, that insulin sensitivity and insulin regulation in the omental adipocytes are of major impact. This is supported by the notion that treatment of insulin resistance with TZD ligands of PPAR-\( \gamma \) results in smaller adipocytes and hence results in increased insulin sensitivity (10, 33, 34), partly due to a shift in adipokine secretion (39). Furthermore, PPAR-\( \gamma \) activation induces multilocularization of adipocytes, and it is suggested that this leads to favorable conditions for mobilization of lipids from adipocytes with enhanced lipolytic and lipogenic activities (19). In fact, our gene expression patterns support this view, and although the gene expression changes are quite small, the long-term increase of expression of these genes might be underlying the biological effects observed after 13 wk. Moreover,
changes in adipocyte size directly correlate with alterations in cytoskeletal proteins.

The role of cytoskeleton alterations is known to play an important role in adipocyte differentiation. It involves drastic cell shape alterations that are accompanied by changes in expression of cytoskeletal and extracellular matrix proteins, including decreases in actin and tubulin levels (reviewed by Ref. 41). Alternatively, upon a decrease in WAT mass in overweight/obese subjects following a weight loss program, proteomics analysis of WAT showed also differential effects of the cytoskeletal protein tubulin 5 as well (2). Moreover, microtubule disruption substantially inhibited insulin-stimulated GLUT4 translocation, suggesting that transport of GLUT4 storage vesicles to the plasma membrane takes place along microtubules and that this transport is obligatory for insulin-stimulated GLUT4 translocation in adipocytes (4). Insulin increases actin polymerization near the plasma membrane and disruption of this process inhibited GLUT4 exocytosis, linking alterations in the cytoskeleton to insulin sensitivity (21). Indeed, we see decreased actin and beta2-microglobulin gene expression levels in WAT and simultaneously increased WAT and whole body insulin sensitivity in LGI-fed mice. This is further supported by the gene expression profile for genes involved in classification of type of adipocytes: classical adi-
pocytes containing one lipid droplet versus a brownish phenotype in which adipocytes contain multiple smaller lipid droplets. Therefore, we hypothesize that alterations in mitochondrial content and/or lipid droplets may be underlying the phenotypic differences observed, but this needs to be elaborated on in future studies.

Of notice, the dietary intervention is comprised solely of a difference in the type, and not the amount, of dietary starch as carbohydrate, while the biological pathways differentially regulated are mainly linked to fatty acid metabolism. The latter is not completely unexpected, as we analyze here the effects in WAT, the main organ for lipid accumulation in times of surplus energy provided by the HF diets. However, while Isken et al. (14) reported a lack of changes in serum FFA and cholesterol levels in low fat low vs. high GI-fed mice, we observed significant reduced serum levels of FFA (43) in line with findings in impaired glucose tolerance subjects consuming LGI food (50). Furthermore, we observed significant lower serum levels of total, HDL-, and LDL-cholesterol in LGI vs. HGI mice, showing a similar, although reduced, effect compared with dietary treatment with phytotherapies/standals (25). Besides genetic differences between mice and human linked to lipid metabolism, e.g., a functional CETP gene has not yet been identified in mice (13), the differences in serum levels of cholesterol might partly be explained by the higher animal fat content, and thus the cholesterol content, of our diets, with beneficial effects of the LGI diet. Indeed, in humans consuming an average dietary fat content of 30–40%, significant lower LDL-cholesterol levels were observed accompanied with a tendency for lower total cholesterol levels in the LGI vs. HGI group after 10 wk (40), while a single LGI meal/day for 3 wk showed no effects on serum cholesterol levels (28). Similarly, the GI of diets did not result in serum cholesterol differences in obese men when analyzed during 1 wk integrated with an exercise protocol (42). Reduction of serum cholesterol levels is a major aim in treatment of obesity, diabetes Type II, metabolic syndrome, and cardiovascular disease, and it is therefore tempting to speculate that a pharmacological approach using statins or a dietary approach using phytotherapies/standals might be enhanced by dietary treatment using low GI diets, even on an HF diet.

In conclusion, we show that on an HF diet background, an LGI vs. HGI diet mainly exerts its beneficial effects in WAT on substrate metabolism, especially insulin signaling of fatty acid metabolism. In addition, cell adhesion and cytoskeleton remodeling showed reduced expression in line with lower WAT mass and lower serum lipid markers, which cumulatively indicate beneficial health effects of LGI.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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