Short Report

Effects of four-week high-fructose diet on gene expression in skeletal muscle of healthy men

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Abstract

Aims. – A high-fructose diet (HFrD) may play a role in the obesity and metabolic disorders epidemic. In rodents, HFrD leads to insulin resistance and ectopic lipid deposition. In healthy humans, a four-week HFrD alters lipid homoeostasis, but does not affect insulin sensitivity or intramyocellular lipids (IMCL). The aim of this study was to investigate whether fructose may induce early molecular changes in skeletal muscle prior to the development of whole-body insulin resistance.

Methods. – Muscle biopsies were taken from five healthy men who had participated in a previous four-week HFrD study, during which insulin sensitivity (hyperinsulinaemic euglycaemic clamp), and intrahepatocellular lipids and IMCL were assessed before and after HFrD. The mRNA concentrations of 16 genes involved in lipid and carbohydrate metabolism were quantified before and after HFrD by real-time quantitative PCR.

Results. – HFrD significantly (P < 0.05) increased stearoyl-CoA desaturase-1 (SCD-1) (+50%). Glucose transporter-4 (GLUT-4) decreased by 27% and acetyl-CoA carboxylase-2 decreased by 48%. A trend toward decreased peroxisomal proliferator-activated receptor-γ coactivator-1α (PGC-1α) was observed (−26%, P = 0.06). All other genes showed no significant changes.

Conclusion. – HFrD led to alterations of SCD-1, GLUT-4 and PGC-1α, which may be early markers of insulin resistance.

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Résumé

Effets d’un régime riche en fructose durant quatre semaines sur l’expression génique dans le muscle squelettech chez l’homme..

Objectifs. – Une alimentation riche en fructose pourrait jouer un rôle dans l’augmentation de la prévalence de l’obésité et autres troubles métaboliques. Chez l’être humain, un régime riche en fructose (HFrD) d’une durée de quatre semaines perturbe l’homéostasie lipidique, cela sans toutefois induire une résistance à l’insuline ou une accumulation de lipides intramyocellulaires (IMCL). Le but de cette étude était d’examiner si le fructose modifie l’expression génique dans le muscle squelettique, avant qu’une résistance à l’insuline ne soit observable au niveau macroscopique.

Méthodes. – Des biopsies musculaires ont été réalisées chez cinq volontaires sains de sexe masculin, ayant précédemment participé à une étude impliquant un HFrD durant quatre semaines. Lors de cette étude, leur sensibilité à l’insuline (clamp hyperinsulinémique euglycémique) et leur concentration d’IMCL ont été mesurées. Les concentrations de mRNA de 16 gènes impliqués dans le métabolisme des lipides et glucides ont été mesurées également avant et après HFrD.

Résultats. – Le HFrD a augmenté de manière significative (P < 0.05) le stearoyl-CoA desaturase-1 (SCD-1) (+50%). Le transporteur au glucose-4 (GLUT-4) a diminué de 27% et l’acetyl-CoA carboxylase-2 de 48%. Une tendance à une baisse du peroxysomal-proliferator activated receptor-γ coactivator 1α (PGC-1α) a été observée (−26%, P = 0.06). Les autres gènes sont demeurés inchangés.

Conclusion. – Le fructose modifie l’expression génique de SCD-1 et GLUT-4, ce qui pourrait constituer des marqueurs précoces de la résistance à l’insuline.

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Keywords: Fructose; Gene expression; Skeletal muscle; Insulin resistance

Mots clés : Fructose ; Expression génique ; Muscle squelettique ; Résistance à l’insuline

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Over the past decades, fructose consumption has dramatically increased and several authors have suggested that fructose may play a role in the onset of metabolic disorders [1,2]. In rodents, a high-fructose diet (HFrD) induces accumulation of intrahepatic (IHCL) and intramyocellular (IMCL) lipids, together with hepatic and muscle insulin resistance (IR) [3]. In healthy humans, we recently showed that HFrD increased fasting plasma triglycerides (TG), very low-density lipoprotein (VLDL)-TG, leptin and glucose [4,5]. Despite a sustained increase in VLDL-TG secretion, HFrD did not affect ectopic lipids or insulin sensitivity (hepatic/muscle). One possible explanation is that the deleterious effects of HFrD may not be detectable at the whole-body level, but that subtle molecular changes may be occurring in peripheral tissues. To evaluate this, we measured the changes in expression of selected genes involved in lipid, carbohydrate and energy metabolism in muscle biopsies taken from five subjects who had participated in a four-week HFrD study [5].

1. Research design and methods

Skeletal muscle biopsies were obtained from five healthy, non-smoking, Caucasian men, who had participated in a primary clinical study [5]. During the initial two weeks, they were instructed to consume an isocaloric diet, with a minimal amount of artificially sucrose-sweetened drinks and food (< 20 g/day). Thereafter, they were switched to an HFrD consisting of the same isoenergetic diet, with additional 1.5 g fructose/kg body weight/day for four weeks (18% excess energy requirement). Insulin sensitivity (two-step euglycaemic hyperinsulinaemic clamp; insulin infusion rates: 0.3 and 1.0 mU/kg/min, 90 min

The effects of fructose on hormones and substrate concentrations have been reported in detail in a previous publication [5]. However, as muscle biopsies were obtained from only five of the seven subjects included in the initial study, only the metabolic parameters of these five are shown in this section. Fructose caused significant ($P < 0.05$) increases in fasting plasma concentrations of TG (0.63 ± 0.08 vs. 0.98 ± 0.08 mmol/L, +55%), VLDL-TG (0.37 ± 0.06 vs. 0.78 ± 0.05 mmol/L, +110%), glucose (4.9 ± 0.1 vs. 5.1 ± 0.1 mmol/L, +4%) and leptin (2.0 ± 0.2 vs. 3.2 ± 0.6 ng/mL, +58%), with no changes in body weight (68 ± 4 vs. 68 ± 4 kg), IHCL (6.1 ± 1.2 vs. 6.3 ± 1.4 mmol/kg), IMCL (1.4 ± 0.2 vs. 1.5 ± 0.2 mmol/kg), and insulin-mediated glucose disposal at both low (3.5 ± 0.1 vs. 3.7 ± 0.2 mg/kg/min) and high (5.6 ± 0.7 vs. 6.2 ± 0.8 mg/kg/min) insulin concentrations.

Table 1
Gene expression in skeletal muscle from healthy subjects before and after four weeks of a high-fructose diet at the end of a three-hour euglycaemic hyperinsulinaemic clamp

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Gene symbol</th>
<th>Baseline</th>
<th>High fructose</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid metabolism</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatty acid translocase</td>
<td>FAT/CD36</td>
<td>21 ± 3</td>
<td>19 ± 4</td>
<td>1.0</td>
</tr>
<tr>
<td>Lipoprotein lipase</td>
<td>LPL</td>
<td>88 ± 29</td>
<td>71 ± 9</td>
<td>0.7</td>
</tr>
<tr>
<td>Cytosolic fatty acid-binding protein-3</td>
<td>FABP-3</td>
<td>49 ± 13</td>
<td>46 ± 20</td>
<td>0.6</td>
</tr>
<tr>
<td>Acyl-CoA synthetase long-chain-5</td>
<td>ACSL-5</td>
<td>3.8 ± 0.8</td>
<td>3.7 ± 0.6</td>
<td>0.5</td>
</tr>
<tr>
<td>Stearoyl-CoA desaturase-1</td>
<td>SCD-1</td>
<td>0.04 ± 0.01</td>
<td>0.08 ± 0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>Diacylglycerol O-acyltransferase</td>
<td>DGAT</td>
<td>1.6 ± 0.1</td>
<td>1.5 ± 0.2</td>
<td>0.6</td>
</tr>
<tr>
<td>Adipose differentiation-related protein</td>
<td>ADP</td>
<td>1.0 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.6</td>
</tr>
<tr>
<td>Sterol regulatory element-binding protein</td>
<td>SREBP-1c</td>
<td>49 ± 2</td>
<td>53 ± 7</td>
<td>0.7</td>
</tr>
<tr>
<td>Carnitine palmitoyl-CoA transferase-1</td>
<td>CPT-I</td>
<td>193 ± 28</td>
<td>155 ± 22</td>
<td>0.14</td>
</tr>
<tr>
<td>Acetyl-CoA carboxylase-2</td>
<td>ACC-2</td>
<td>23 ± 4</td>
<td>12 ± 1</td>
<td>0.02</td>
</tr>
<tr>
<td>Malonyl-CoA decarboxylase</td>
<td>MCD</td>
<td>1.9 ± 0.2</td>
<td>1.2 ± 0.2</td>
<td>0.11</td>
</tr>
<tr>
<td>Glucose metabolism</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose transporter, type 4</td>
<td>GLUT-4</td>
<td>26 ± 3</td>
<td>19 ± 3</td>
<td>0.02</td>
</tr>
<tr>
<td>Hexokinase-2</td>
<td>HK-2</td>
<td>98 ± 17</td>
<td>157 ± 25</td>
<td>0.14</td>
</tr>
<tr>
<td>Pyruvate dehydrogenase kinase-4</td>
<td>PDK-4</td>
<td>575 ± 136</td>
<td>460 ± 217</td>
<td>0.4</td>
</tr>
<tr>
<td>Energy metabolism</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncoupling protein-3</td>
<td>UCP-3</td>
<td>511 ± 86</td>
<td>393 ± 69</td>
<td>0.14</td>
</tr>
<tr>
<td>Peroxisomal proliferator-activated receptor-γ coactivator-1α</td>
<td>PGC-1α</td>
<td>195 ± 26</td>
<td>144 ± 16</td>
<td>0.06</td>
</tr>
</tbody>
</table>

$^a$ Data are expressed as a percentage ratio referring to expression of hypoxanthine phosphoribosyltransferase (HPRT). Values are means ± SEM, $n = 5$.

$^b$ Significantly different vs. baseline ($P < 0.05$; by Wilcoxon matched-pairs signed-ranks test).
Gene expression is shown in Table 1. Fructose significantly ($P<0.05$) increased stearoyl-CoA desaturase-1 (SCD-1) (+50%). HFrD also significantly decreased glucose transporter-4 (GLUT-4) (−27%) and acetyl-CoA carboxylase-2 (ACC-2) (−48%). A trend toward a decreased expression of peroxisomal proliferator-activated receptor-γ coactivator-1α (PGC-1α) was observed (−26%, $P=0.06$). Other investigated genes showed no significant changes.

3. Discussion

As reported in our primary study, the five subjects from whom muscle biopsies were obtained after four weeks of HFrD had increased fasting plasma VLDL-TG, glucose and leptin, but neither IR nor ectopic lipid deposition was observed [5]. Given the well-documented association between HFrD and IR in rodents [3], we suspected that major alterations following HFrD may not yet be detectable at the whole-body level after a four-week period, but that subtle molecular changes may be occurring in skeletal muscle. To evaluate this, we monitored the expression of 16 genes in skeletal muscle coding for key proteins involved in lipid, carbohydrate and energy metabolism.

Although most monitored genes remained unchanged, the expression of four relevant genes was altered by HFrD. We first observed a two-fold increase in the expression of SCD-1, a major lipogenic enzyme and key controller in the process of lipid partitioning [7]. High SCD-1 expression in mouse liver is associated with hepatic steatosis and IR [8], while treatment with SCD-1 antisense oligonucleotides, leading to an 80% reduction of SCD-1 in the rat liver, reverses a high-fat diet-induced hepatic IR [9]. In skeletal muscle, SCD-1 expression is increased in morbidly obese humans, and is associated with low rates of fatty-acid oxidation and increased monounsaturated fatty-acid concentrations [10]. These observations suggest that increased expression of SCD-1 may result in abnormal lipid partitioning, thus favouring ectopic lipid deposition. In our study, we observed a sustained increase in plasma TG and VLDL-TG, with no significant changes in IMCL after four weeks [5]. However, the rise in SCD-1 may reflect early fructose-induced molecular changes that may, in the long term, favour lipid deposition in skeletal muscle and lead to IR [11].

In addition, HFrD decreased insulin-stimulated GLUT-4 expression by 27%. GLUT-4 expression is induced by insulin, and the effect is blunted in non-diabetic obese individuals and in type 2 diabetic patients [6]. Although whole-body glucose uptake was not altered by HFrD, the reduction in GLUT-4 expression may also represent a first step toward IR.

We also observed a trend towards a decreased expression of PGC-1α, a major regulator of mitochondrial biogenesis and thermogenesis. Mitochondrial dysfunction is commonly observed in IR subjects [12]. Moreover, expression of PGC-1α and genes under its control is down-regulated in type 2 diabetes [13]. A decreased PGC-1α expression may, therefore, indicate a fructose-induced alteration of mitochondrial function that may eventually contribute to IR in the long term. Moreover, there was a trend towards a decrease in carnitine palmitoyl-transferase-I (CPT-I) and malonyl-CoA decarboxylase (MCD), which may be related to the observed decrease in lipid oxidation after fructose [5]. The latter point, however, remains speculative, as the trend towards a decrease in MCD was not mirrored by an increase in ACC-2 expression, as is usually observed in conditions associated with inhibition of lipid oxidation.

Subjects were studied under isocaloric conditions and after fructose supplementation, which suggests an increase in both total calories and the relative contribution of simple carbohydrates to total energy expenditure. However, the fact that our study did not include appropriate control groups with matched calorie/carbohydrate intake is a weakness that has to be acknowledged. Nevertheless, fructose had no detectable effects on body weight and body composition, which suggests that it was at least partly compensated for by a reduction in food intake. The increase in leptin observed after fructose [5] may have been instrumental in this compensation. Furthermore, such a reduction would not have been properly identified as the study was carried out on an outpatient basis with no accurate monitoring of food intake. Based on these considerations, we believe it unlikely that the effects observed were due to an increase in total energy intake. It remains nonetheless possible that the high simple-carbohydrate intake, rather than fructose per se, was responsible for these effects. Further studies comparing higher intakes of fructose versus glucose may help to answer these questions.

We conclude that a four-week HFrD increased the expression of SCD-1, and decreased the expression of GLUT-4, ACC-2 and PGC-1α in skeletal muscle. Although other metabolic genes remained unchanged, these alterations may represent early molecular markers for dietary fructose-induced IR in skeletal muscle.

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