Rose hip exerts antidiabetic effects via a mechanism involving downregulation of the hepatic lipogenic program

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IN THE RECENT DECADES, there has been a dramatic increase in the prevalence of type 2 diabetes worldwide (43). Type 2 diabetes is strongly associated with obesity; ~90% of all patients with type 2 diabetes are overweight or obese. Both type 2 diabetes and obesity are also strongly associated with nonalcoholic fatty liver disease (NAFLD), the most common liver disease worldwide. More than 70% of subjects with type 2 diabetes and/or obesity are affected with NAFLD, compared with 20% in the general adult population (12). Many people are unable to make the lifestyle and dietary changes needed for weight maintenance and prevention of obesity-associated conditions (13). Therefore, the development of new foods helping to reduce energy storage is of importance to public health.

Rose hip (Rh) is the fruit of a plant belonging to the Rosaceae family. The fruit is rich in antioxidants, such as ascorbic acid (36), phenolic compounds (9), and carotenoids (16), and has traditionally been used in health food products in many European countries. Dietary supplementation with either nutrients rich in phenolic compounds or pure phenolic compounds has been reported to lower plasma lipids and reduce adiposity in animal studies (14, 18, 24). It has been shown that administration of an acetone extract from fruit and seeds from Rosa canina prevented body weight gain in mice fed a normal chow diet (30). Furthermore, administration of trans-tiliroside, a constituent of the fruit and seed extract, reduced body weight gain and improved glucose clearance following an intraperitoneal glucose injection.

The aim of this study was to explore the beneficial metabolic effects of Rh in greater detail and to elucidate some of the mechanisms underlying the observed antidiabetic effects. To this end, we used the high-fat-fed C57BL/6J mouse, which is a model for obesity, impaired glucose tolerance, and early type 2 diabetes (39). Long-term metabolic effects were investigated in this mouse model following administration of powdered Rh together with a high-fat diet (HFD) to lean mice (prevention study) as well as to mice in which the obese state had been induced by prior HFD feeding (intervention study).

EXPERIMENTAL PROCEDURES

Prevention study. Female 6- to 8-wk-old C57BL/6J mice, weighing 19.1 ± 0.1 g, were purchased from Taconic (Skensved, Denmark). The animals were maintained in a temperature-controlled room on a 12:12-h light-dark cycle. The study was approved by the Local Animal Ethics Committee (Lund, Sweden), and the principles of laboratory animal care were followed (Institute for Laboratory Animal Research, National Research Council, Guide for the Care and Use of Laboratory Animals, Washington, DC: National Academy Press, 1996). After 1 wk of acclimatization, the mice were randomly divided into two groups (n = 20) and shifted to either a control (ctrl) HFD or a HFD supplemented with Rh powder. The mice were fed the experimental diets ad libitum for 20 wk. Body weights were monitored throughout the study period, and body composition was determined with dual-energy X-ray absorptiometry at the indicated time points using a Lunar PIXImus (5).

Intervention study. Female 6- to 8-wk-old C57BL/6J mice were fed a HFD, n = 14, or a control low-fat diet (LFD), n = 7, for 14 wk (D12309 and D12310, respectively, from Research Diets, New Brunswick, NJ). An intravenous glucose tolerance test (IVGTT) was performed to confirm that mice in the HFD group had decreased glucose tolerance compared with mice in the LFD group. Mice in the HFD group were randomly divided into two groups (n = 7) and fed either of the two diets used in the prevention study for 10 wk. The LFD group was kept for comparison. Body weights and body compositions were monitored as above.

Diet. A commercially available pasteurized lyophilized puréé made out of ground, strained (through a 0.6-mm sieve) Rh, R. Canina...
Table 1. Composition of experimental diets

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Ctrl, g/kg</th>
<th>Rh, g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>157.3</td>
<td>155.5</td>
</tr>
<tr>
<td>dl-Methionine</td>
<td>1.4</td>
<td>1.3</td>
</tr>
<tr>
<td>Maltodextrin</td>
<td>117.3</td>
<td>114.3</td>
</tr>
<tr>
<td>Sucrose</td>
<td>311.2</td>
<td>315.6</td>
</tr>
<tr>
<td>Lard</td>
<td>255.1</td>
<td>238.1</td>
</tr>
<tr>
<td>Cellulose</td>
<td>112.0</td>
<td></td>
</tr>
<tr>
<td>Mineral mix</td>
<td>27.5</td>
<td>26.9</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>7.2</td>
<td>7.1</td>
</tr>
<tr>
<td>Potassium citrate</td>
<td>2.7</td>
<td>2.7</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>6.9</td>
<td>6.7</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>1.4</td>
<td>1.3</td>
</tr>
<tr>
<td>Rose hip powder</td>
<td></td>
<td>330.4</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>195</td>
<td>195</td>
</tr>
<tr>
<td>Sucrose</td>
<td>103</td>
<td>103</td>
</tr>
<tr>
<td>Total fiber</td>
<td>112</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>1,000</td>
</tr>
<tr>
<td>Total protein</td>
<td>159</td>
<td>157</td>
</tr>
<tr>
<td>Total carbohydrates</td>
<td>429</td>
<td>426</td>
</tr>
<tr>
<td>Sucrose</td>
<td>311</td>
<td>319</td>
</tr>
<tr>
<td>Total fat</td>
<td>255</td>
<td>238</td>
</tr>
</tbody>
</table>

ctrl, Control; Rh, rose hip.
Statistical analysis. Data are presented as means ± SE. To compare body fat content, glucose, insulin, total cholesterol, TAG, and NEFA during the prevention study as well as body weights during the intervention study, two-way ANOVA with Bonferroni posttest was used. For protein and gene expressions, the nonparametric Mann-Whitney test was used. For all other comparisons, Student’s *t*-test was used. The statistical analyses were performed using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA). A *P* value of <0.05 was considered significant.

RESULTS

**Rh exerts an antiobesity effect.** The Rh group had a markedly suppressed body weight gain during the prevention study compared with the ctrl group (7.1 ± 0.4 vs. 17.1 ± 1.1g, *P* < 0.001). Already after 2 wk, a significant difference in body weight was detected between the two groups (24.8 ± 0.5 vs. 22.4 ± 0.2 g, *P* < 0.01), and at the end of the study this difference was more pronounced (36.1 ± 1.0 vs. 24.9 ± 0.2 g, *P* < 0.001) (Fig. 1A). The body fat content was significantly decreased in the Rh group at week 5 compared with the ctrl group, and this difference was increased at the end of the study (Table 2). The mean energy intake during the prevention study did not differ between the two groups (50.1 ± 0.67 vs. 50.6 ± 1.04 kJ/day and mouse). At the start of the intervention study, the HFD group had significantly higher body weight compared with the LFD group (33.8 ± 1.4 vs. 24.5 ± 0.6 g, *P* < 0.001). During the 10-wk study, the ctrl group kept gaining body weight while the Rh group immediately started to lose weight. After 3 wk, the body weight of mice in the Rh group was significantly lower compared with the ctrl mice (29.2 ± 0.8 vs. 37.9 ± 1.8 g, *P* < 0.01), and at the end of the study this difference was even more pronounced (28.6 ± 1.2 vs. 38.5 ± 1.3 g, *P* < 0.001) (Fig. 1B). Furthermore, the Rh group had a significantly lower body fat content compared with ctrl at the end of the 10-wk period (Table 3). In contrast to the prevention study, a lower mean energy intake during the intervention study was observed in the Rh group compared with the ctrl group (45.9 ± 2.1 vs. 50.9 ± 2.4 kJ/day and mouse, *P* < 0.05).

In view of the pronounced antiobesity effects of Rh, the expression of a selection of key metabolic genes and proteins was analyzed in WAT. The Rh group had a significantly higher expression of the lipogenic enzyme ACC, both at the gene and the protein level (Fig. 2, A and B, and Table 4). FAS, however, was not differentially expressed between the groups (Fig. 2C and Supplemental data). The lipogenic gene aP2 was significantly upregulated in the Rh group compared with the ctrl group (Table 4). On the contrary, the lipogenic genes DGAT2 and SCD-2 were significantly decreased in the Rh group compared with ctrl (Table 4). Otherwise, the lipogenic program did not appear to be altered between the two groups (Supplemental data). The expression of genes and proteins involved in fatty acid oxidation was not altered between the Rh (Supplemental data) and the ctrl group, but mRNA levels of PGC1α, a key regulator of energy metabolism, were almost threefold higher in the Rh group compared with the ctrl group (Table 4).

**Rh improves glucose homeostasis but has no effect on β-cell function.** Plasma parameters measured showed no differences before the start of the prevention study. At the end of the prevention study, the Rh group had significantly lower plasma levels of glucose and insulin compared with the ctrl group (Table 2). An OGTT performed after 4 wk of dietary treatment did not reveal any differences in glucose clearance and insulin response between the groups (data not shown). An OGTT performed at week 18 showed a significantly lower insulin response in the Rh group compared with the ctrl group, although no differences were observed with regard to glucose clearance (Fig. 3, A and B). In contrast to the results obtained in the OGTT, an IVGTT showed no significant differences between the groups (Fig. 3, C and D). Islets were isolated from five individual mice from each dietary group for batch incubations. No significant differences in either basal or glucose-stimulated insulin secretion, expressed as insulin release per total insulin content, were found between the two groups (data not shown).

Before the start of the intervention study, both groups had increased basal glucose and insulin levels compared with the LFD ctrl (Table 3), and an IVGTT confirmed that the HFD group was more glucose intolerant than the LFD group (data not shown). At the end of the intervention, plasma glucose levels were lower and insulin levels trended lower in the Rh group compared with HFD ctrl (Table 3). The IVGTT performed at week 8 showed no statistically significant differences between the two groups (Fig. 3, E and F).

**Rh prevents hepatic fat accumulation and alters hepatic gene and protein expression patterns.** In the prevention study, mice fed a supplement of Rh had significantly lower levels of TAG and cholesterol in the liver compared with ctrl mice (Fig. 4, A and B) as well as lower levels of plasma ALT, a
Table 2. Plasma parameters at indicated weeks in the prevention study in the ctrl and Rh group

<table>
<thead>
<tr>
<th>Week</th>
<th>Glucose, mmol/l</th>
<th>Insulin, pmol/l</th>
<th>TAG, mmol/l</th>
<th>Cholesterol, mmol/l</th>
<th>HDL cholesterol, mmol/l</th>
<th>LDL/HDL ratio</th>
<th>PAI-1, pg/ml</th>
<th>Body fat content, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.4 ± 0.2</td>
<td>55 ± 2</td>
<td>3.8 ± 0.7</td>
<td>38 ± 0.1</td>
<td>0.6 ± 0.01</td>
<td>0.9 ± 0.04</td>
<td>0.6 ± 0.04</td>
<td>12 ± 0.8</td>
</tr>
<tr>
<td>12</td>
<td>5.2 ± 0.2</td>
<td>170 ± 41</td>
<td>10.0 ± 0.04</td>
<td>98 ± 2</td>
<td>0.6 ± 0.01</td>
<td>0.9 ± 0.04</td>
<td>0.6 ± 0.04</td>
<td>10 ± 0.7</td>
</tr>
<tr>
<td>20</td>
<td>9.4 ± 0.5</td>
<td>351 ± 99</td>
<td>1.1 ± 0.02</td>
<td>91 ± 15</td>
<td>0.8 ± 0.04</td>
<td>0.8 ± 0.04</td>
<td>0.8 ± 0.04</td>
<td>8 ± 0.6</td>
</tr>
<tr>
<td>12</td>
<td>13 ± 0.05</td>
<td>177 ± 362</td>
<td>1.2 ± 0.05</td>
<td>97 ± 13</td>
<td>0.62 ± 0.03</td>
<td>0.8 ± 0.03</td>
<td>0.8 ± 0.03</td>
<td>6 ± 0.4</td>
</tr>
<tr>
<td>20</td>
<td>1.5 ± 0.04</td>
<td>1.1 ± 0.1</td>
<td>0.8 ± 0.03</td>
<td>0.8 ± 0.03</td>
<td>0.63 ± 0.03</td>
<td>0.8 ± 0.03</td>
<td>0.8 ± 0.03</td>
<td>5 ± 0.3</td>
</tr>
</tbody>
</table>

Table 3. Plasma glucose, insulin, total cholesterol, and body fat content in the intervention study in the ctrl and Rh group

<table>
<thead>
<tr>
<th>Week</th>
<th>Glucose, mmol/l</th>
<th>Insulin, pmol/l</th>
<th>Cholesterol, mmol/l</th>
<th>Body fat content, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>9.9 ± 1.5</td>
<td>9.2 ± 1.1</td>
<td>353 ± 177</td>
<td>36.1 ± 3.6</td>
</tr>
<tr>
<td>10</td>
<td>10.9 ± 1.2</td>
<td>5.7 ± 0.8</td>
<td>362 ± 151</td>
<td>38.2 ± 4.1</td>
</tr>
<tr>
<td>12</td>
<td>6.6 ± 0.2</td>
<td>6.7 ± 0.1</td>
<td>79 ± 11</td>
<td>12.0 ± 0.7</td>
</tr>
<tr>
<td>20</td>
<td>0.6 ± 0.04</td>
<td>2.8 ± 0.1</td>
<td>108 ± 13</td>
<td>17.8 ± 1.1</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 10. HFD, high-fat diet.
groups (Supplemental material). Similar to the prevention study, the levels of total cholesterol were lower at the end of the intervention study in the Rh group compared with the ctrl (Table 3).

Rh lowers PAI-1 but does not have any effect on SAA and adiponectin. In the prevention study, the plasma levels of PAI-1, a prothrombotic serpin, were significantly lower in the Rh group compared with ctrl. SAA, a marker of systemic inflammation, was low with no difference between the groups. Also, the levels of adiponectin, an adipokine with anti-inflammatory properties, did not differ between the two groups (Table 2). TNF-α, MCP-1, and IL-6 levels were below the detection limit.

DISCUSSION

Here we used two different study protocols to show that a dietary supplement of Rh prevents the weight gain induced by high-fat feeding and promotes weight loss in obese HFD-fed C57BL/6J mice. Measurements of body fat mass confirmed that the effects on body weight in both studies reflected effects on adiposity. The effects on adiposity imposed by Rh may in part be due to reduced energy intake, as observed in the intervention study, suggesting that ingestion of Rh increases satiety, an effect that could be mediated via the high fiber content of Rh. Reduced energy intake was, however, not observed in the prevention study, in agreement with data by Ninomiya et al. (30) showing that administration of an aqueous extract from the fruit and seeds of *R. canina* to ddY mice inhibited weight gain without affecting energy intake. Thus reduced energy intake cannot be the only mechanism underlying the antiobesity effects of Rh. Rh intake may affect body weight also via inhibition of intestinal lipid absorption, in agreement with the observation that some polyphenols, especially tea catechins, have inhibitory effects on digestive enzymes (20, 29) and thereby impair intestinal lipid and carbohydrate absorption. In fact, the fecal lipid content in the Rh group tended to be higher than in the ctrl group, although the difference, based on three measurements, did not attain statistical significance (data not shown). The pronounced antiobesity effect of Rh was accompanied by altered expression of a few metabolic genes/proteins in WAT, whereas most genes/proteins analyzed exhibited unaltered expression. With regard to lipogenesis, DGAT2 and SCD-2 were downregulated, whereas ACC and aP2 were upregulated. These changes in the lipogenic program are difficult to interpret, since there is no coordinated up- or downregulation. It could be speculated that

Table 4. Gene expression analysis in periovarial WAT using real-time quantitative PCR

<table>
<thead>
<tr>
<th>Gene/mRNA</th>
<th>ctrl Mean</th>
<th>ctrl SE</th>
<th>Rh Mean</th>
<th>Rh SE</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>aP2/FABP4</td>
<td>1.02</td>
<td>0.04</td>
<td>1.16</td>
<td>0.07</td>
<td>0.038</td>
</tr>
<tr>
<td>ACC1</td>
<td>0.23</td>
<td>0.03</td>
<td>0.47</td>
<td>0.04</td>
<td>0.0095</td>
</tr>
<tr>
<td>DGAT2</td>
<td>0.92</td>
<td>0.07</td>
<td>0.44</td>
<td>0.10</td>
<td>0.0087</td>
</tr>
<tr>
<td>SCD2</td>
<td>0.95</td>
<td>0.08</td>
<td>0.33</td>
<td>0.08</td>
<td>0.0043</td>
</tr>
<tr>
<td>PGC1α</td>
<td>0.40</td>
<td>0.03</td>
<td>1.11</td>
<td>0.12</td>
<td>0.0043</td>
</tr>
</tbody>
</table>

Mean values are arbitrary units. Relative mRNA quantities are normalized by geometric averaging of two internal control genes (ribosomal protein S29 and TATA box-binding protein). Differences between the groups are analyzed using a nonparametric Mann-Whitney *U*-test, *n* = 5–6 in each group. WAT, white adipose tissue; aP2/FABP4, fatty acid-binding protein 4; ACC1, acetyl-CoA carboxylase 1; DGAT2, diacylglycerol acyltransferase 2; SCD2, stearoyl-CoA desaturase-2; PGC1α, peroxisome proliferator-activated receptor-γ coactivator 1α.
the alterations observed serve to prevent the intracellular levels of fatty acids in the adipocyte to decrease below a critical level. In addition to the alterations in the lipogenic program, the gene expression of PGC1α/H9251 was found to be increased threefold without an accompanying upregulation of PPARγ/H9251 and UCP1. Again, this effect of Rh on gene expression is difficult to interpret but warrants further investigations, since it may indicate that Rh exerts its antiobesity effect via a PGC1α-dependent mechanism.

The antiobesity effect of Rh is accompanied by improved glucose tolerance. This was observed in both study modes but was most pronounced in the prevention study, where Rh intake in fact prevented the development of a diabetic state. The improved glucose tolerance required long-term intake of Rh to be fully manifested, suggesting that it is not only a consequence of the reduced adiposity, which was observed already after 2–3 wk. One possibility is involvement of gut-related factors, such as incretins, as supported by the observation, in the prevention study, that improvement in glucose tolerance was more pronounced in the OGTT than in the IVGTT.

Islet function was assessed as basal and glucose-stimulated insulin secretion with no differences between the Rh and ctrl groups. Although more extensive studies, including the use of other secretagogues, would be needed to completely rule out the possibility that dietary intake of Rh alters islet function, available data suggest that this is not a major mechanism.

Fig. 3. Rh intake improves glucose tolerance. A–D: results of the prevention study. Plasma glucose and insulin after an oral glucose tolerance test (OGTT; A and B) and after an iv glucose tolerance test (IVGTT; C and D) performed at week 18 in the ctrl (○) and Rh (□) group, n = 9 for each test. E and F: results of the intervention study. Plasma glucose and insulin after an IVGTT performed at week 8 in the LFD ctrl (dotted curve), high-fat diet (HFD) ctrl (■), and Rh (□) group, n = 10. Data are means ± SE. Insets show the area under the curve (AUC). NS, not significant. ***P < 0.001.
underlying the improvement in glucose tolerance. Alterations in incretins or other signals affecting insulin secretion, however, are potential mechanisms that were not explored in this study.

Insulin sensitivity was not directly assessed in this study; however, the improvement in glucose tolerance together with the lowering of basal plasma insulin and glucose levels indicates that insulin sensitivity is increased by dietary Rh intake. The large reduction in hepatic fat content in the Rh group is one probable mechanism whereby insulin sensitivity and thus glucose tolerance could be improved. In agreement with the reduction in hepatic fat content, several lipogenic proteins, such as SREBP-1, ACC, FAS, and LIPIN1, were found to be downregulated in the Rh group. Moreover, the mRNA levels for several additional lipogenic genes, i.e., CD36, SCD-1, DGAT2, PPARγ and aP2, were downregulated. The expression of genes and proteins involved in fatty acid oxidation, i.e., CPT-1, PPARα and PGC-1α, was, on the other hand, not altered by Rh intake. The mechanism whereby Rh induces a coordinated downregulation of genes and proteins involved in lipogenesis without affecting those involved in fat oxidation is not known. It has been shown that polyphenols, as the antidiabetic drug metformin, can activate AMPK and thereby increase fatty acid oxidation, decrease lipogenesis, and inhibit glucose production (1, 17, 40–42). Resveratrol, for instance, was recently shown to activate AMPK and increase the expression of SIRT1, PGC1α and adiponectin, whereas SREBP-1 was downregulated (1). In our study, we found no evidence for increased AMPK activity in response to Rh intake, suggesting that Rh acts via an AMPK-independent mechanism. Very recently, Kim et al. (21) showed that a polyphenol-rich extract of amla decreased SREBP-1 expression without affecting PPARα, which would also suggest a mechanism of action independent of activation of AMPK. In the study by Ninomiya et al. (30), PPARα expression in the liver was increased 24 h after a single oral administration of trans-tiliroside, a polyphenolic constituent of Rh that exerts antiobesity effects on its own. AMPK was not investigated, but the observed increase in PPARα is compatible with an AMPK-dependent mechanism. The reason for the discrepancy between our study and that by Ninomiya et al. is not known, but, first, they tested a specific bioactive compound of Rh rather than a whole powder and, second, the increase in PPARα was observed after 24 h. We cannot exclude that AMPK was activated early in our studies.

Fig. 4. Rh intake prevents hepatic fat accumulation. Hepatic triacylglycerol (TAG, A) and cholesterol (B) content and plasma alanine aminotransferase (ALT, C) levels in the ctrl and Rh group at the end of the prevention study. Data are means ± SE, n = 12. ***P < 0.001.

Fig. 5. AMP-activated protein kinase (AMPK) activity and expression in the liver is unaltered following Rh intake. Hepatic AMPKα1 and -α2 activity in the ctrl group and Rh group in the prevention study, analyzed using isofrom specific antibodies and the Amara peptide as a substrate. Results are presented as means ± SE. Liver lysates were also subjected to Western blot analysis using antibodies against pAMPK T172, AMPK, serine/threonine kinase 11 (LKB1), and GAPDH.
since analysis of gene and protein expression was only performed on the end-point samples. With the exception of DGAT2, for which information regarding transcrip
tive regulation is limited, all of the genes/proteins found regulated are controlled by SREBP-1 or PPARα. These transcription factors are in turn controlled by LXR (6, 22, 31, 35), raising the possibility that polyphenols and/or other bioactive compounds in Rh act as LXR antagonists to accomplish the gene and protein expression changes observed. However, other mechanisms are most likely also involved. The discrepancy between regulation at the gene and protein level observed, for instance, ACC and FAS (Fig. 6 and Supplemental material), suggests that control of posttranscriptional events is involved.

In contrast to DGAT2, hepatic DGAT1 was found to be upregulated in response to dietary Rh intake. DGAT2 is the most prominent DGAT isoform in the liver. It is physically linked to SCD-1 and appears to mediate TAG synthesis linked to de novo fatty acid synthesis, in contrast to DGAT1, which is involved in TAG synthesis from dietary fatty acids. Although increased expression of either isoform has been associated with NAFLD, only suppression of DGAT2 reverses diet-induced hepatic steatosis and insulin resistance. Thus the DGAT changes observed in response to Rh intake may in fact be in agreement with improved hepatic insulin signaling and insulin sensitivity (7).

Increased hepatic insulin sensitivity following Rh administration is also suggested by the decreased fasting levels of glucose, since these two parameters have been shown to

Fig. 6. Decreased hepatic expression of lipogenic proteins following Rh intake.
A: liver lysates from mice of the prevention study were analyzed by Western blotting using antibodies against pACC S79 and ACC. GAPDH was used as a loading control. Total ACC was quantified and is expressed as means ± SE. pACC, 125 kDa SREBP, and sterol regulatory element-binding protein (SREBP). FAS, 125 kDa SREBP, and 68 kDa SREBP were quantified and are expressed as means ± SE; n = 8. AU, arbitrary units. Values given are means ± SE. *P < 0.05 and **P < 0.01.

Fig. 7. Decreased mRNA levels of genes implicated in hepatic lipogenesis following Rh intake. A: Hepatic mRNA levels, analyzed with real-time quantitative PCR of peroxi
some proliferator-activated receptor (PPAR)-γ2 and fatty acid-binding protein 4 (aP2, A) and cluster of differentiation 36 (CD36), stearoyl-CoA desaturase-1 (SCD-1), and diacylglycerol acyltransferase 1 (DGAT2) (B) in mice fed Rh compared with ctrl, n = 6. Values given are means ± SE. *P < 0.05 and **P < 0.01.
correlate. Excessive glucose output from the liver is a major cause of fasting hyperglycemia and can also contribute to elevated postprandial hyperglycemia (8, 25, 27). Gluconeogenesis is a major contributor to hepatic glucose output (32). CREB, together with its coactivator TORC2, positively regulate gluconeogenesis (10, 11, 15, 23). Overexpression of hepatic TORC2 results in fasting hyperglycemia (23), and it was recently shown that liver-specific knockdown of TORC2, using a small-interfering RNA approach, corrects hyperglycemia in rodent models of type 2 diabetes (34). In the present study, the expression of TORC2 was found to be almost completely blunted and accompanied by reduced expression of CREB in mice fed Rh. Surprisingly, however, no differences in the expression of key gluconeogenic enzymes were found. The reason for this is not known. It is possible that the dramatically reduced TORC2 levels are counteracted by other changes, thus rendering gluconeogenesis unchanged. Another possibility is that the increased gluconeogenesis imposed by the relatively long fasting period (12 h) that the mice were subjected to before excision of the livers has concealed any differences between the groups. In addition to gluconeogenic enzymes, TORC2 has been reported to increase the expression of LIPIN1 in a CREB-dependent manner (33). Thus the reduction in LIPIN1 levels in the Rh group may be accounted for by both reduced TORC2 and reduced SREBP-1 (19). Hepatic LIPIN1 expression is higher in mouse models with diet-induced obesity and insulin resistance (33). Moreover, high expression of LIPIN1 has been shown to increase intracellular DAG and perturb insulin signaling in part via a protein kinase Cε-mediated pathway, whereas a reduction in LIPIN1 expression improves hepatic insulin sensitivity and normalizes hyperglycemia in diabetic db/db mice (33). Whereas decreased hepatic TAG levels do not necessarily lead to improved hepatic insulin sensitivity, decreased levels of DAG most certainly do. Thus decreased expression of LIPIN1 imposed by dietary Rh administration may indicate that Rh supplementation improves hepatic insulin sensitivity.

Many reports indicate that insulin resistance is a state of low-grade systemic inflammation. The levels of SAA, a marker of systemic inflammation, and adiponectin, an adipokine with anti-inflammatory properties, did not differ between the Rh group and the ctrl group, suggesting that decreased systemic inflammation is not a major mechanism whereby Rh improves glucose tolerance. PAI-1, on the other hand, was found to be reduced following Rh treatment. PAI-1 is produced by many cells but predominantly hepatocytes, adipocytes, and endothelial cells. In insulin-resistant individuals, PAI-1 levels are found elevated in adipocytes, which thereby constitute the major site of PAI-1 production (2, 26, 28). Insulin negatively regulates PAI-1 via the metabolic phosphatidylinositol 3-kinase pathway (38), and the decreased PAI-1 levels following Rh intake most likely reflect improved adipocyte insulin sensitivity due to decreased adipose mass.

In both the prevention and the intervention study, total plasma levels of cholesterol were found to be reduced in the Rh group compared with ctrl, whereas no changes were found with regard to plasma TAG and NEFA. In the prevention study, the reduction in total plasma cholesterol was shown to involve a reduction in both HDL cholesterol and LDL cholesterol. The reduction in LDL cholesterol, however, was more pronounced, resulting in a decrease in the LDL-to-HDL ratio in the Rh group. Also, hepatic levels of cholesterol were investigated and found to be lower in the Rh group. The mechanism whereby dietary intake of Rh promotes reduction in plasma and hepatic levels of cholesterol remains to be elucidated. The expression of SREBP-2 and HMGCR was not altered, indicating that cholesterol biosynthesis is not affected. One possibility is that the large fiber content of Rh promotes increased fecal excretion of cholesterol. This, as well as possible effects on bile acid synthesis, secretion, and reabsorption, need to be investigated.

The bioactive component(s) of Rh exerting the beneficial metabolic effects remain to be determined. As mentioned above, trans-tiliroside is a polyphenolic constituent that has already been demonstrated to exert antiobesity effects on its own but the presence of additional metabolically active components in Rh powder is anticipated. Future studies should also focus on establishing dose dependency of the observed effects to define a more moderate dose of Rh to be applied in human...
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studies, and on a full elucidation of the molecular mechanisms underlying observed effects.

In conclusion, we show that high doses of Rh are capable of both preventing and reversing the increase in body weight and decrease in glucose tolerance imposed by a HFD regimen in the C57BL/6J mouse model. Hepatic lipids are reduced by $>50\%$ following Rh intake as a result of downregulation of the hepatic lipogenic program, and this may represent a major mechanism underlying the improvement in insulin sensitivity and glucose tolerance. In addition to weight reduction and improvements in insulin sensitivity and glucose tolerance, Rh intake also, via as yet unknown mechanisms, lowers plasma cholesterol levels. In view of the pronounced effects observed, the results hold great promise for the use of more moderate doses of Rh to improve glucose tolerance and lower body weight in humans.

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DISCLOSURES

No conflicts of interest are declared by the authors.

REFERENCES


