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

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Andersson U, Henriksson E, Ström K, Alenfall J, Göransson O, Holm C. Rose hip exerts antidiabetic effects via a mechanism involving downregulation of the hepatic lipogenic program. *Am J Physiol Endocrinol Metab* 300: E111–E121, 2011. First published October 19, 2010; doi:10.1152/ajpendo.00268.2010.—The aim of this study was to investigate the metabolic effects of a dietary supplement of powdered rose hip to C57BL/6J mice fed a high-fat diet (HFD). Two different study protocols were used; rose hip was fed together with HFD to lean mice for 20 wk (prevention study) and to obese mice for 10 wk (intervention study). Parameters related to obesity and glucose tolerance were monitored, and livers were examined for lipids and expression of genes and proteins related to lipid metabolism and gluconeogenesis. A supplement of rose hip was capable of both preventing and reversing the increase in body weight and body fat mass imposed by a HFD in the C57BL/6J mouse. Oral and intravenous glucose tolerance tests together with lower basal levels of insulin and glucose showed improved glucose tolerance in mice fed a supplement of rose hip compared with control mice. Hepatic lipid accumulation was reduced in mice fed rose hip compared with control, and the expression of lipogenic proteins was downregulated, whereas AMP-activated protein kinase and other proteins involved in fatty acid oxidation were unaltered. Rose hip intake lowered total plasma cholesterol as well as the low-density lipoprotein-to-high-density lipoprotein ratio via a mechanism not involving altered gene expression of sterol regulatory element-binding protein 2 or 3-hydroxymethylglutaryl-CoA reductase. Taken together, these data show that a dietary supplement of rose hip prevents the development of a diabetic state in the C57BL/6J mouse and that downregulation of the hepatic lipogenic program appears to be at least one mechanism underlying the antidiabetic effect of rose hip.

glucose tolerance; cholesterol; polyphenol

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.

Diets. A commercially available pasteurized lyophilized purée made out of ground, strained (through a 0.6-mm sieve) Rh, *R. Canina*

(Norfoods Sweden, Malmö, Sweden), was thoroughly mixed in a HFD (D12309; Research Diets). The carbohydrate and dietary fiber content in the Rh powder was compensated for with sucrose (Danisco sugar) and microcrystalline cellulose (Avicel PH 101; FMC BioPolymer, Brussels, Belgium) in the ctrl diet (Table 1). Six batches of each diet were prepared during the 20-wk study period. The diets were stored at -20 °C in single portion packs. New portions of food were provided to the mice daily. Food intake was monitored 5 days a week by weighing the leftover food in each cage. The cages were carefully inspected for spillage. Food intake was measured on a per cage basis.

Glucose tolerance tests. Oral glucose tolerance tests (OGTT) and IVGTT were performed in 12-h-fasted anesthetized mice as previously described (3). In brief, glucose was given either as intragastric gavage (0.75 mg in 0.5 ml; OGTT) or injected in a tail vein (1 g/kg; IVGTT). Blood samples were drawn by orbital puncture. After immediate centrifugation, plasma was collected and stored at -20°C until glucose and insulin were analyzed.

Assays of plasma samples. Plasma was collected from 12-h-fasted anesthetized mice and prepared as above. Glucose, total cholesterol, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol, triacylglycerol (TAG) (Infinity; Thermo Electron, Melbourne, Australia), nonesterified fatty acids (NEFA) (NEFA C; Wako Chemicals, Neuss, Germany), and alanine aminotransferase (ALT) (DiscretePak ALT; Catachem, Bridgeport, CT) were measured enzymatically. Insulin and adiponectin were measured radioimmunochemically (Linco Research, St. Charles, MO). Serum amyloid-A (SAA) was measured with ELISA (Tridelta Development, Wicklow, Ireland). IL-6 was measured using a Meso Scale Discovery (MSD) assay and analyzed on a SECTOR 2400 instrument (MSD, Gaithersburg, MD). Plasminogen activator inhibitor 1 (PAI-1), tumor necrosis factor (TNF)-α, and monocyte chemotactic protein 1 (MCP-1) were analyzed using Luminex technology (LX200; Luminex, Austin, TX). SAA, IL-6, PAI-1, TNF-α, and MCP-1 were analyzed in an aliquot of plasma that had been snap-frozen and stored at -80 °C.

Isolation of islets and insulin secretion assay. Islets were isolated and subjected to an in vitro insulin secretion assay using 3.3, 8.6, or 16.7 mmol/l glucose as previously described (3).

Lipid extraction from liver. Livers were collected, snap-frozen in liquid nitrogen, and stored at -80°C until analysis. Frozen livers were grounded in a mortar before analysis. Lipids were extracted with hexane-isopropanol (3:2), dried under nitrogen gas, and then redissolved in 100–500 μl isopropanol with 1% Triton X for cholesterol and TAG analysis (Infinity; Thermo Electron).

Table 1. *Composition of experimental diets*

Ingredient	Ctrl, g/kg	Rh, g/kg
Casein	157.3	155.5
DL-Methionine	1.4	1.3
Maltodextrin	117.3	114.3
Sucrose	311.2	115.6
Lard	255.1	238.1
Cellulose	112.0	
Mineral mix	27.5	26.9
Sodium bicarbonate	7.2	7.1
Potassium citrate	2.7	2.7
Vitamin mix	6.9	6.7
Choline bitartrate	1.4	1.3
Rose hip powder		330.4
Carbohydrates		195
Sucrose		103
Total fiber		112
Total		1,000
Total protein	159	157
Total carbohydrates	429	426
Sucrose	311	219
Total fat	255	238

ctrl, Control; Rh, rose hip.

Tissue lysis and western blot analysis. Total liver lysates were prepared by homogenization of ground tissue in lysis buffer containing 50 mM Tris·HCl, pH 7.5, 1 mM EGTA, 1 mM EDTA, 1% (wt/vol) Nonidet P-40 (NP-40), 1 mM Na_3VO_4, 50 mM NaF, 5 mM $Na_4P_2O_7$, 0.27 M sucrose, 1 mM dithiothreitol, and complete protease inhibitor (1 tablet/. 50 ml) and centrifugation at 13,000 g for 10 min at 4°C. For preparation of nuclear fractions, tissue was homogenized in lysis buffer without NP-40. Following centrifugation at 100 g for 5 min to remove unbroken cells, nuclei were collected at 750 g for 10 min. Periovarian white adipose tissue (WAT) was collected, snap-frozen in liquid nitrogen, and stored at -80° C until analysis. WAT lysates were prepared by homogenization in the same buffer as above, but without NP-40, using a knife-homogenizer, followed by centrifugation at 5,000 g for 5 min at 4°C for removal of fat. NP-40 was added to a final concentration of 1%, and the lysates were kept on ice for 15 min before centrifugation at 13,000 g for 10 min at 4°C.

Lysates or nuclear fractions were subjected to SDS-PAGE and Western blot analysis as described previously (4). Primary antibodies against the following proteins were used: sterol regulatory element-binding protein 1 (SREBP-1), fatty acid synthase (FAS) (Santa Cruz Biotechnology, Santa Cruz, CA), cAMP response element-binding protein (CREB) (Upstate Biotechnologies, Upstate, NY), transducer of regulated CREB activity-2 (TORC2) (Calbiochem, San Diego, CA), acetyl-CoA carboxylase (ACC), phosphorylated (p) ACC S79, AMP-activated protein kinase (AMPK), pAMPK T172, serine/threonine kinase 11 (LKB1, also called STK11) (AbCam, Cambridge, MA), pLKB1 S431 (Cell Signaling Technology, Danvers, MA), glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Sigma, St. Louis, MO), and LIPIN1 (Novus Biological, Littleton, CO).

In vitro kinase assay. Liver lysates (25 μg) were assayed for AMPK activity using AMPKα_1 and -α_2 isoform-specific antibodies (kind gift from Grahame Hardie, Dundee University), as described (4). One unit of activity was defined as incorporation of 1 nmol of ^{32}P/min into the substrate.

RNA preparation and real-time quantitative PCR. Total RNA was isolated from $\sim$10 mg of ground liver samples or $\sim$200 mg of WAT (homogenized using a glass/teflon homogenizer) using either the RNeasy Mini Kit (liver) or the RNeasy Lipid Tissue Mini Kit (WAT) (Qiagen, Hilden, Germany). Total RNA (1 μg) was treated with DNase I (DNase I amplification grade; Invitrogen, Carlsbad, CA) and then reversely transcribed using random hexamers (Amersham Biosciences, Piscataway, NJ) and SuperScript II RNaseH reverse transcriptase (Invitrogen). The cDNA was used in quantitative PCR reactions using TaqMan chemistry cluster of differentiation 36 (CD36), liver X receptor (LXR), peroxisome proliferator-activated receptor γ coactivator α (PGC-1α), pregnane X receptor, stearoyl-CoA desaturase-1 (SCD-1), SCD-2, TATA box-binding protein (TBP) or SYBR green chemistry (GAPDH), hypoxanthine ribosyltransferase 1 (HPRT1), ribosomal protein S29 (RPS29), peroxisome proliferator-activated receptor α (PPARα), PPARγ2, fatty acid-binding protein 4 (FABP 4, also known as aP2), ACC1, diacylglycerol acyltransferase 1 (DGAT1), DGAT2, FAS, fructose-1,6-bisphosphate (FBP), glucose-6-phosphatase (G-6-*P*ase), phospho*enol*pyruvate carboxykinase (PEPCK), carnitine palmitoyl transferase 1A (CPT-1A), CPT-1B, uncoupling protein 1 (UCP1), UCP2, apolipoprotein E, 3-hydroxymethylglutaryl-CoA reductase (HMGCR), SREBP-1c, and SREBP-2 with an ABI 7900 system (Applied Biosystems, Foster City, CA). SYBR green primers were designed using the software Primer Express 1.5 (Applied Biosystems), and the sequences or Assay Ids (TaqMan) are presented as supplemental data (Supplemental data for this article may be found on the *American Journal of Physiology: Endocrinology and Metabolism* website). Relative abundance of mRNA was calculated after normalization by geometric averaging of three internal control genes (HPRT1, RPS29, and GAPDH) for liver samples and two internal control genes (RPS29 and TBP) for WAT samples (37).

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. 1*A*). 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
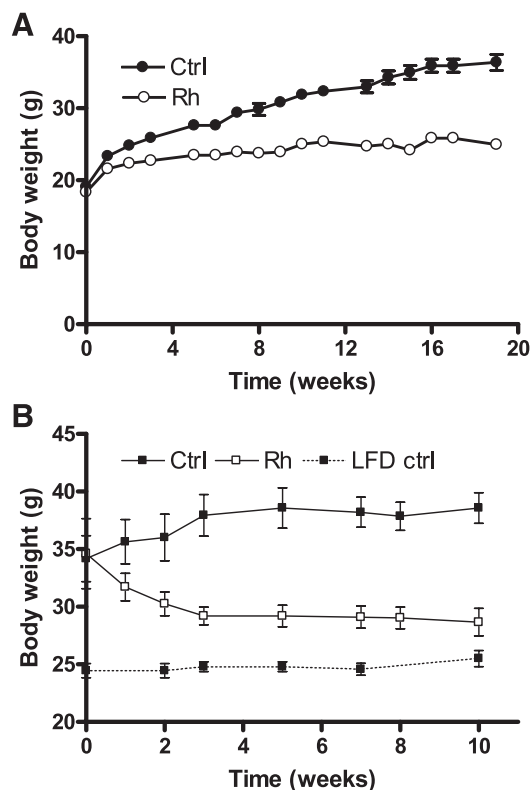

Fig. 1. Dietary supplementation of rose hip (Rh) suppresses body weight gain. *A*: body weight in the control (ctrl) and Rh group during the prevention study. After 2 wk, a significant difference in body weight was detected between the two groups ($P < 0.01$), $n = 20$. *B*: body weight during the intervention study in the ctrl, Rh, and low-fat diet (LFD) ctrl group. At *week 5*, the group fed Rh had a significantly lower body weight compared with ctrl, $n = 10$. Data are means ± SE.

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. 1*B*). 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. 2*C* 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*

	Week	ctrl	Rh	P
Glucose, mmol/l	0	4.4 ± 0.2	4.4 ± 0.3	NS
	12	5.2 ± 0.2	4.4 ± 0.2	NS
	20	9.4 ± 0.5	5.5 ± 0.3	<0.001
Insulin, pmol/l	0	55 ± 2	58 ± 2	NS
	12	170 ± 41	99 ± 17	NS
	20	351 ± 99	91 ± 15	<0.001
TAG, mmol/l	0	1.0 ± 0.04	1.1 ± 0.02	NS
	12	1.3 ± 0.05	1.2 ± 0.05	NS
	20	1.5 ± 0.04	1.4 ± 0.04	NS
Cholesterol, mmol/l	0	3.8 ± 0.07	3.9 ± 0.07	NS
	12	4.8 ± 0.1	3.8 ± 0.08	<0.001
	20	6.6 ± 0.3	4.9 ± 0.1	<0.001
NEFA, mmol/l	0	0.6 ± 0.01	0.6 ± 0.01	NS
	12	0.9 ± 0.04	0.8 ± 0.04	NS
	20	0.8 ± 0.06	0.8 ± 0.03	NS
HDL cholesterol, mmol/l	20	1.2 ± 0.07	0.9 ± 0.04	<0.01
LDL cholesterol, mmol/l	20	1.0 ± 0.06	0.7 ± 0.04	<0.001
LDL/HDL ratio	20	0.8 ± 0.03	0.7 ± 0.03	<0.01
SAA, μg/ml	20	6.6 ± 0.82	4.8 ± 0.63	NS
Adiponectin, mg/l	20	17.8 ± 0.36	16.2 ± 1.0	NS
PAI-1, pg/ml	20	2,508 ± 591	876 ± 263	<0.05
Body fat content, %	0	12.8 ± 0.6	13.9 ± 0.7	NS
	5	29.0 ± 2.5	22.2 ± 0.8	<0.05
	12	36.8 ± 1.4	20.4 ± 1.2	<0.001
	20	43.3 ± 1.7	18.4 ± 1.1	<0.001

Values are means ± SE, $n = 20$ mice for all parameters except high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol ($n = 16$–20), serum amyloid A (SAA, $n = 9$–11), adiponectin ($n = 12$–14), and plasminogen activator inhibitor (PAI)-1 ($n = 11$–13). TAG, triacylglycerol; NEFA, nonesterified fatty acid; NS, not significant.

marker of hepatic damage (Fig. 4*C*). No differences in the activity of hepatic AMPKα_1 or -α_2, important regulators of cellular energy homeostasis, or in the expression of the upstream kinase LKB1 were detected between the two groups (Fig. 5). However, the group fed Rh had lower total and phosphorylated protein levels of the lipogenic enzymes ACC (Fig. 6*A*), a downstream target of AMPK, and of FAS (Fig. 6*B*). The pACC-to-ACC ratio was not altered between the ctrl and Rh group (0.76 ± 0.16 vs. 0.64 ± 0.13), indicating that ACC expression rather than ACC phosphorylation is decreased. The protein level of the lipogenic transcription factor SREBP-1 was also lower in the group fed Rh (Fig. 6*B*), whereas the expression of CPT-1, a key enzyme in fatty acid oxidation, did not differ between the groups (data not shown). The mRNA levels of PPARγ2 and its target aP2, both of which have been implicated in hepatic lipogenesis, were significantly lower in the Rh group compared with ctrl (Fig. 7*A*). Also, the mRNA levels of the lipogenic genes CD36, SCD-1, and DGAT2 (Fig. 7*B*) were lowered in the Rh group, whereas DGAT1 mRNA levels were significantly upregulated in the

Rh group compared with ctrl (1.10 ± 0.09 vs. 0.87 ± 0.03, $P = 0.015$). CREB, a regulator of gluconeogenesis, and LIPIN1, a phosphatidic acid phosphatase, were expressed at a lower level in the Rh-fed mice compared with ctrl (Fig. 8). Protein expression of TORC2, a coactivator of CREB, was almost completely blunted in the mice fed Rh compared with ctrl (Fig. 8). PGC-1α was not differentially expressed between the two groups (Supplemental data). No differences in mRNA levels of G-6-*P*ase, FBP, and PEPCK, enzymes regulating gluconeogenesis, were detected (Supplemental data).

Rh lowers plasma cholesterol. Total plasma cholesterol levels were significantly lower in the Rh group already after 12 wk in the prevention study. HDL and LDL cholesterol were analyzed at the end of the prevention study, and both were significantly lower in the Rh group compared with ctrl; however, the LDL-to-HDL ratio was lower in the Rh group. No differences were seen in TAG and NEFA levels (Table 2). Hepatic mRNA levels of HMGCR and SREBP-2, regulators of cholesterol synthesis, did not differ between the

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

	Week	HFD ctrl	Rh	LFD ctrl	P (HFD ctrl vs. Rh)
Glucose, mmol/l	0	9.9 ± 1.5	10.9 ± 1.2	6.6 ± 0.2	NS
	10	9.2 ± 1.1	5.7 ± 0.8	6.7 ± 0.1	<0.05
Insulin, pmol/l	0	353 ± 177	362 ± 151	79 ± 11	NS
	10	279 ± 89	135 ± 17	108 ± 13	NS
Cholesterol, mmol/l	0	4.5 ± 0.7	4.4 ± 0.5	2.5 ± 0.2	NS
	10	5.0 ± 0.5	3.1 ± 0.2	2.8 ± 0.1	<0.01
Body fat content, %	0	36.1 ± 3.6	38.2 ± 4.1	12.0 ± 0.7	NS
	10	42.6 ± 3.1	28.8 ± 4.2	17.8 ± 1.1	<0.05

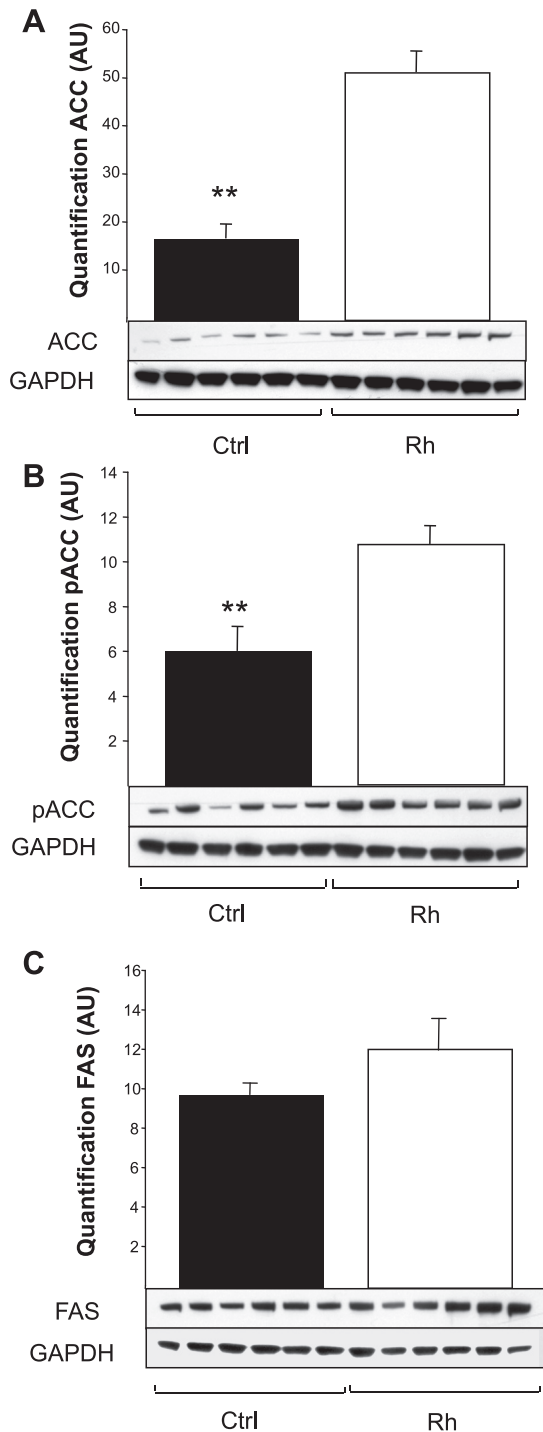Values are means ± SE; $n = 10$. HFD, high-fat diet.

Fig. 2. Increased expression of acetyl-CoA carboxylase (ACC) in adipose tissue following Rh intake. Adipose tissue lysates from mice of the prevention study were analyzed by Western blot using antibodies against ACC (*A*) phosphorylated (p) ACC S79 (*B*) and Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a loading control. Total ACC and pACC S79 were quantified and are expressed as means ± SE. *C*: lysates were also analyzed by Western blot using an antibody against fatty acid synthase (FAS). GAPDH was used as a loading control. FAS was quantified and is expressed as means ± SE; $n = 6$. **$P < 0.01$.

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 group fed Rh 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*

Gene/mRNA	ctrl		Rh		
	Mean	SE	Mean	SE	*P* Value
aP2/FABP4	1.02	0.04	1.16	0.07	0.038
ACC1	0.23	0.03	0.47	0.04	0.0095
DGAT2	0.92	0.07	0.44	0.10	0.0087
SCD2	0.95	0.08	0.33	0.08	0.0043
PGC1α	0.40	0.03	1.11	0.12	0.0043

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 acetyltransferase 2; SCD2, stearoyl-CoA desaturase-2; PGC1α, peroxisome proliferator-activated receptor-γ coactivator 1α.

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).
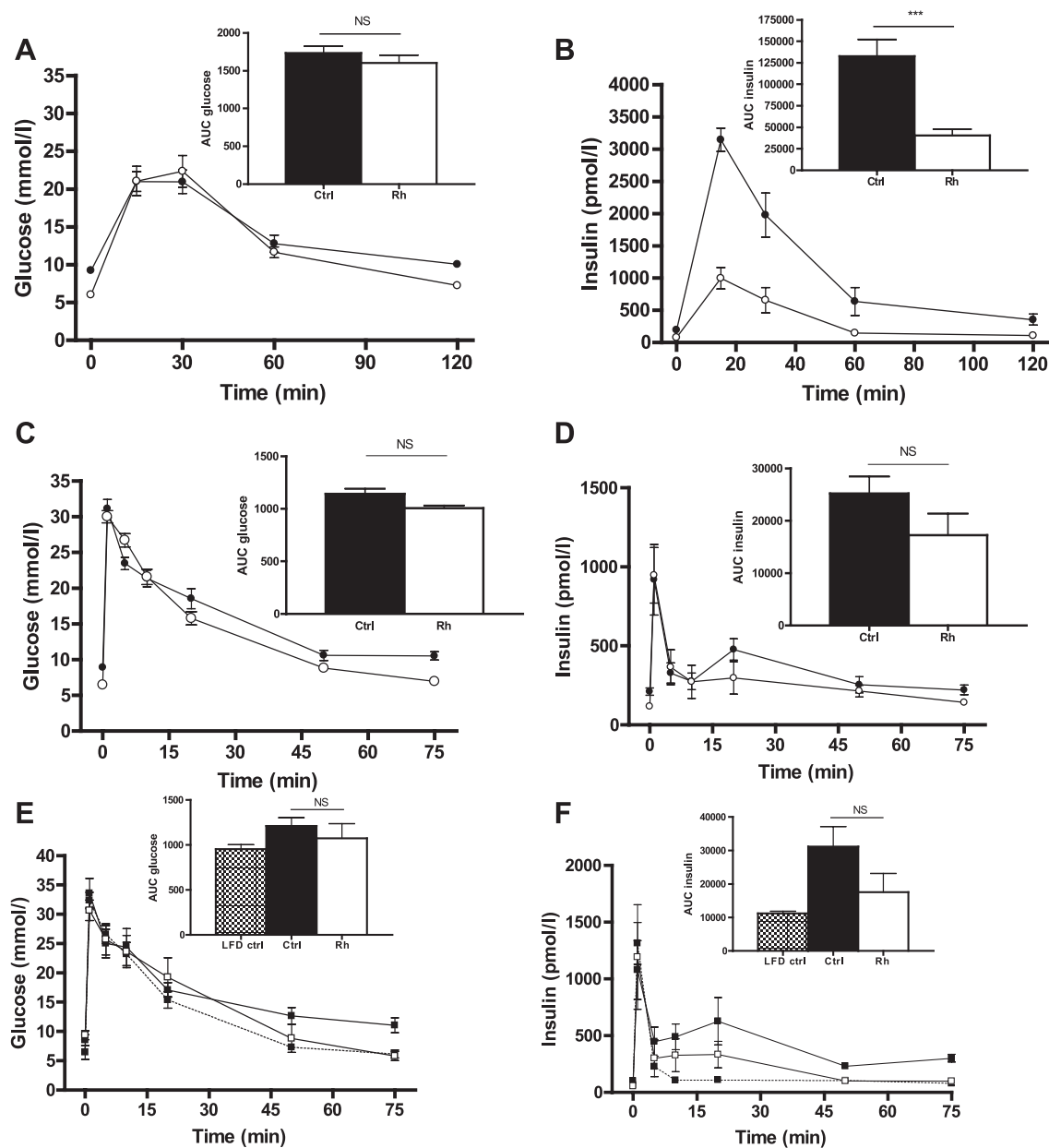
Fig. 3. Rh intake improves glucose tolerance. *A–D*: results of the prevention study. Plasma glucose and insulin after an oral glucose tolerance test (OGTT; *A* and *B*) and after an iv glucose tolerance test (IVGTT; *C* and *D*) performed at *week 18* in the ctrl (●) and Rh (○) group, $n = 9$ for each 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. ***$P < 0.001$.

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 was found to be increased threefold without an accompanying upregulation of PPARα 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
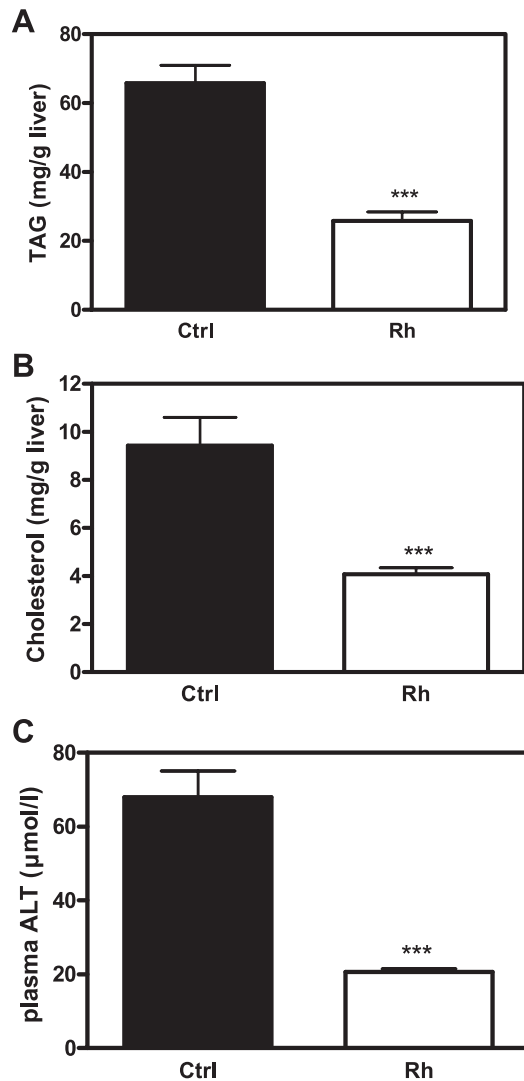
Fig. 4. Rh intake prevents hepatic fat accumulation. Hepatic triacylglycerol (TAG, *A*) and cholesterol (*B*) content and plasma alanine aminotransferase (ALT, *C*) levels in the ctrl and Rh group at the end of the prevention study. Data are means ± SE, $n = 12$. ***$P < 0.001$.

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,
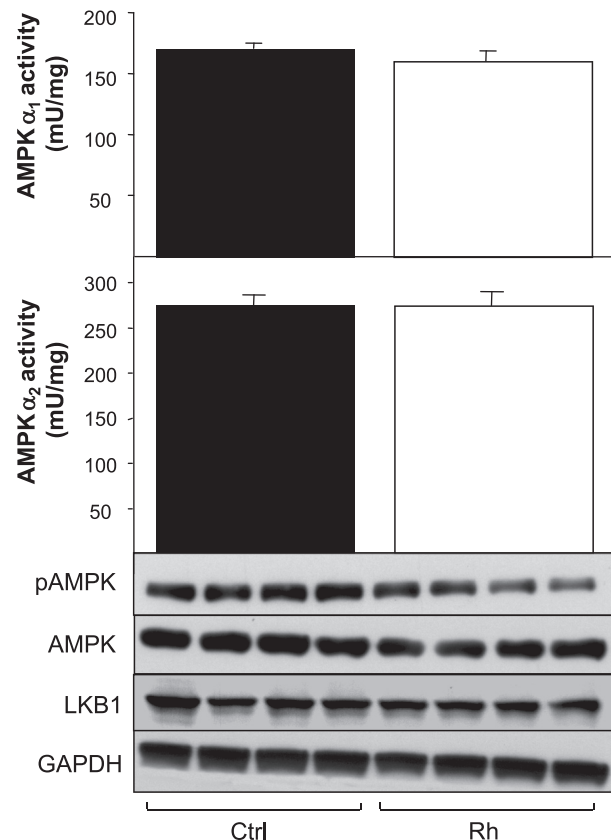
underlying the improvement in glucose tolerance. Alterations in incretins or other signals affecting insulin secretion, however, are potential mechanisms that were not explored in this study.

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

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 isoform 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.
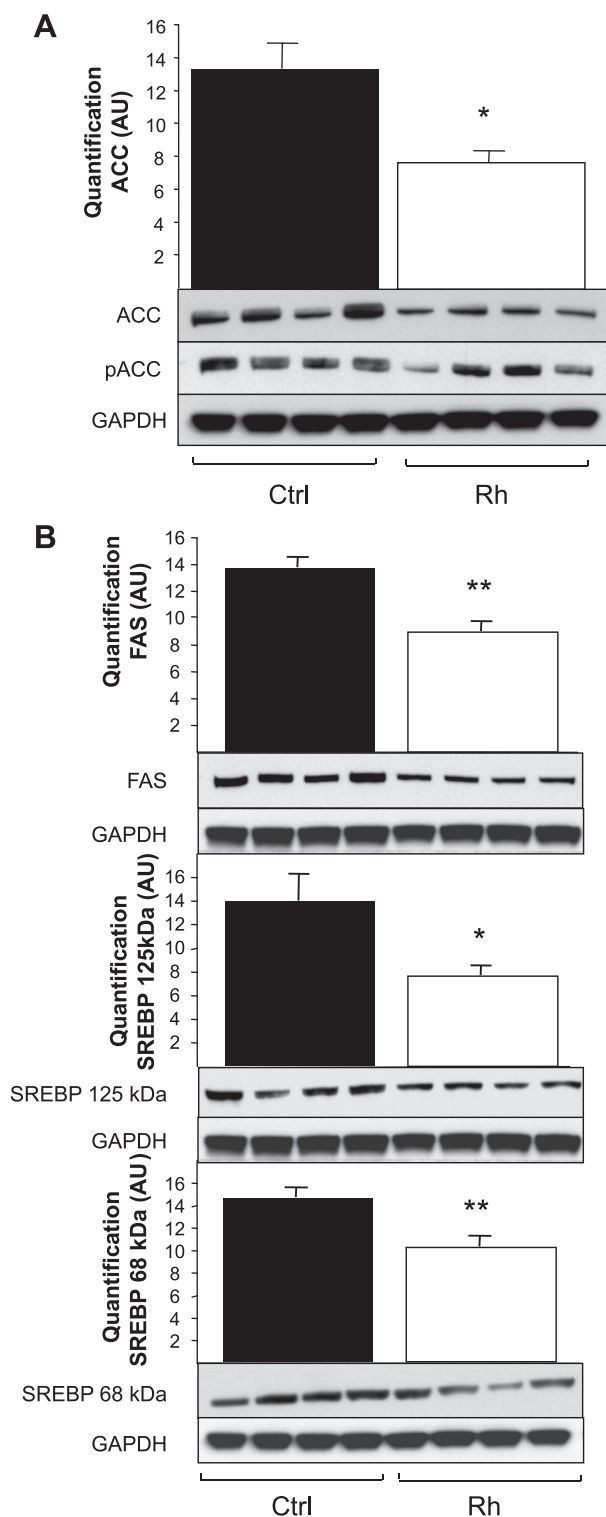
Fig. 6. Decreased hepatic expression of lipogenic proteins following Rh intake. A: liver lysates from mice of the prevention study were analyzed by Western blotting using antibodies against pACC S79 and ACC. GAPDH was used as a loading control. Total ACC was quantified and is expressed as means ± SE. B: lysates were also analyzed by Western blot using antibodies against FAS 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.
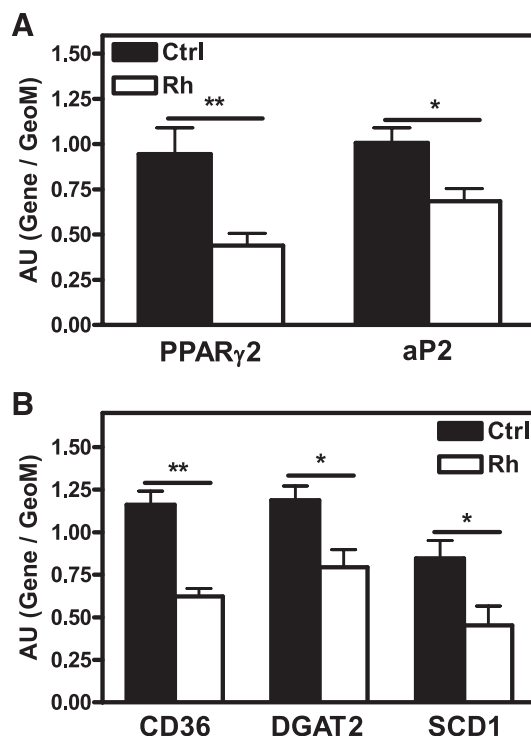

Fig. 7. Decreased mRNA levels of genes implicated in hepatic lipogenesis following Rh intake. Hepatic mRNA levels, analyzed with real-time quantitative PCR of peroxisome proliferator-activated receptor (PPAR)-γ2 and fatty acid-binding protein 4 (aP2, A) and cluster of differentiation 36 (CD36), stearoyl-CoA 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.

since analysis of gene and protein expression was only performed on the end-point samples. With the exception of DGAT2, for which information regarding transcriptional regulation is limited, all of the genes/proteins found regulated are controlled by SREBP-1 or PPARγ. 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
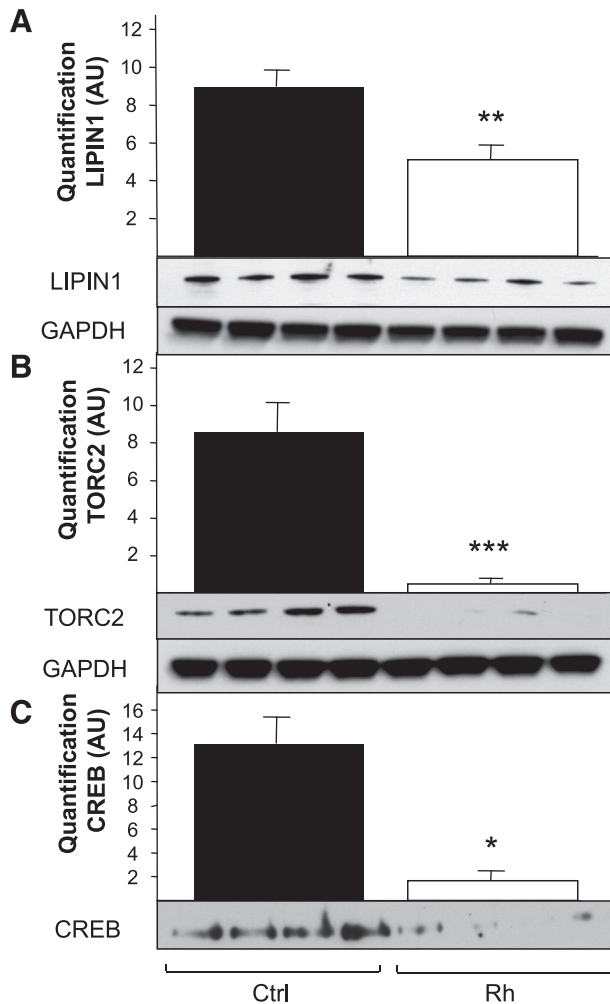
Fig. 8. Decreased hepatic expression of LIPIN1 (*A*) transducer of regulated cAMP response element-binding protein (CREB) activity-2 (TORC2; *B*) and CREB (*C*) following Rh intake. Liver lysates or nuclear fractions (CREB) from mice in the prevention study were analyzed by Western blot using antibodies against LIPIN1, TORC2, and CREB. GAPDH was used as a loading control for the blots for LIPIN1 and TORC2. LIPIN1 ($n = 8$), TORC2 ($n = 8$), and CREB ($n = 4$) were quantified and are expressed as means ± SE, $n = 8$. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$, significant decrease in total protein compared with control.

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

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 knock down 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 the dramatically reduced TORC2 levels are counteracted by other changes, thus rendering gluconeogenesis unchanged. Another possibility is

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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