Naringenin Prevents Dyslipidemia, Apolipoprotein B Overproduction, and Hyperinsulinemia in LDL Receptor–Null Mice With Diet-Induced Insulin Resistance

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OBJECTIVE—The global epidemic of metabolic syndrome and its complications demands rapid evaluation of new and accessible interventions. Insulin resistance is the central biochemical disturbance in the metabolic syndrome. The citrus-derived flavonoid, naringenin, has lipid-lowering properties and inhibits VLDL secretion from cultured hepatocytes in a manner resembling insulin. We evaluated whether naringenin regulates lipoprotein production and insulin sensitivity in the context of insulin resistance in vivo.

RESEARCH DESIGN AND METHODS—LDL receptor–null (Ldlr−/−) mice fed a high-fat (Western) diet (42% calories from fat and 0.05% cholesterol) become dyslipidemic, insulin and glucose intolerant, and obese. Four groups of mice (standard diet, Western, and Western plus 1% or 3% wt/wt naringenin) were fed ad libitum for 4 weeks. VLDL production and parameters of insulin and glucose tolerance were determined.

RESULTS—We report that naringenin treatment of Ldlr−/− mice fed a Western diet corrected VLDL overproduction, ameliorated hepatic steatosis, and attenuated dyslipidemia without affecting caloric intake or fat absorption. Naringenin 1) increased hepatic fatty acid oxidation through a peroxisome proliferator–activated receptor (PPAR) γ coactivator 1α (PGC-1α)–mediated program; 2) prevented sterol regulatory element–binding protein (SREBP) 1c–mediated lipogenesis in both liver and muscle by reducing fasting hyperinsulinemia; 3) decreased hepatic cholesterol and cholesterol ester synthesis; 4) reduced both VLDL-derived and endogenously synthesized fatty acids, preventing muscle triglyceride accumulation; and 5) improved overall insulin sensitivity and glucose tolerance.

CONCLUSIONS—Thus, naringenin, through its correction of many of the metabolic disturbances linked to insulin resistance, represents a promising therapeutic approach for metabolic syndrome.

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protein kinase B (PKB)-β (AKT2), but not patients with insulin receptor mutations, manifest increased lipogenesis, elevated liver fat content, triglyceride-enriched plasma VLDL, and hypertriglyceridemia (20).

The citrus-derived flavonoid, naringenin, has both lipid-lowering and insulin-like properties. In streptozotocin-induced diabetic rats, a diet supplement of naringenin lowered and insulin-like properties. In streptozotocin-induced diabetic rats, a diet supplement of naringenin 7-O-d-glucoside reduces blood glucose and improves plasma lipids (21). In cholesterol-fed rats, naringenin lowers plasma cholesterol by inhibiting hepatic cholesterol synthesis and esterification (22). In HepG2 hepatoma cells, naringenin, like insulin, inhibits apoB100 secretion resulting from both enhanced intracellular degradation of apoB100 and increased LDL-mediated uptake of mature particles (23, 24). The effect of naringenin does not require insulin receptor activation (7, 9). Furthermore, naringenin potentiates intracellular signaling responses to low insulin doses, suggesting that naringenin sensitizes hepatocytes to insulin (7). Therefore, we hypothesize that naringenin lowers plasma lipids in vivo through inhibition of VLDL-apoB100 secretion and regulates insulin sensitivity in the setting of insulin resistance.

In the present study, we use C57BL/6J Ldlr<sup>−/−</sup> mice fed a Western diet, a model of diet-induced insulin resistance. These mice display many characteristics of the metabolic syndrome including dyslipidemia, obesity, and insulin resistance (25). Addition of naringenin to a high-fat diet corrects a wide range of metabolic disturbances associated with insulin resistance independent of caloric intake or dietary lipid absorption. Naringenin prevents hyperinsulinemia and, subsequently, SRGBP1-stimulated lipogenesis; activates hepatic fatty acid oxidation resulting in prevention of hepatic triglyceride accumulation; and leads to normalization of VLDL overproduction and amelioration of dyslipidemia. Finally, in muscle, naringenin prevents lipid accumulation, leading to improved glucose utilization and increased insulin sensitivity.

**RESEARCH DESIGN AND METHODS**

Male C57BL/6J and Ldlr<sup>−/−</sup> mice on the C57BL/6J (Jackson Laboratory) background were housed in pairs and maintained at 23°C on a 12-h light/dark cycle. Experiments were approved by the animal care committee of the University of Western Ontario. Eight- to 12-week-old mice were fed ad libitum a rodent standard diet (4% of calories from fat, TD8604; Harlan Teklad) or a Western diet containing 1 or 3% naringenin for 4 weeks.

**Blood samples, tissue collection, and tissue histology.** See online supplement for details (available at http://diabetes.diabetesjournals.org/cgi/content/full/db09-0634/DC1).

**Plasma and tissue lipids, blood glucose, insulin, and liver enzymes.** Plasma triglycerides and total cholesterol (Roche Diagnostics, Laval, Canada) and plasma nonesterified fatty acids (NEFAs) (Wako Chemicals, Richmond, VA) were determined enzymatically (26). Blood glucose was determined using an Ascensia Elite glucometer (Bayer Healthcare, Toronto, Canada). Plasma insulin and leptin were measured using ultrasensitive mouse-specific enzyme-linked immunosorbent assays (Alpco Diagnostics, Windham, NH). Liver enzymes (aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase) were measured using the kinetic rate method. Tissue lipids and oleate incorporation into triglycerides and cholesterol ester were determined as previously described (26). Homeostatic model assessment for insulin resistance (HOMA-IR) was calculated as a surrogate for insulin resistance, as described previously for mice (27). Plasma cholesterol and triglyceride distributions were evaluated by size exclusion chromatography (26).

**Lipid absorption, triglycerides, and apoB secretion.** Intestinal triglyceride and cholesterol absorption was determined using a modified fecal isotope ratio method, and the intraperitoneal Tyloxapol method was used to measure triglyceride and apoB secretion. See online supplement for details.

**Glucose and insulin tolerance tests and glucose uptake.** Mice were administered glucose (intraperitoneally or gavage) or insulin (intraperitoneally). Blood glucose was measured up to 180 min postinjection. Glucose uptake was determined following intraperitoneal injection of 2-deoxy-D-[1-<sup>3</sup>H]-glucose. See online supplement for details.

**Fatty acid and cholesterol synthesis, fatty acid oxidation, and lipoprotein lipase activity.** Fatty acid and cholesterol synthesis were measured following an intraperitoneal injection of [1-<sup>14</sup>C]-acetate acid. Fatty acid oxidation was determined by conversion of [1-<sup>14</sup>C]-palmitate to [1,2-<sup>14</sup>C]-glycerol. See online supplement for details.

**Energy expenditure.** Energy expenditure (EE) was determined using an indirect open-circuit calorimeter (Oxylet, Panlab, Cornell, Spain). See online supplement for details.

**Cold test.** Whole-body temperature was assessed rectally (Harvard Homeothermic Blanket Control Unit), initially at room temperature, then hourly (6 h) following transfer to a 4°C room.

**Statistical analysis.** Data are presented as means ± SE. Analysis was performed using the Statistical Package for Social Science (SPSS version 14.0). Significant differences between groups were determined by one-way ANOVA. Multiple comparisons were made using a post hoc Tukey’s test, and differences were identified between groups at P < 0.05. Differences between groups for body weight, food consumption, glucose tolerance tests, and insulin tolerance tests were calculated using general linear modeling for repeated measures.

**RESULTS**

**Naringenin does not affect food intake but suppresses diet-induced weight gain.** The metabolic effects of naringenin were initially evaluated in Ldlr<sup>−/−</sup> mice fed a Western diet containing 1 or 3% naringenin for 4 weeks. Western-fed mice gained significantly more weight than standard diet–fed mice. Naringenin dose-dependently attenuated weight gain so that the 3% naringenin group was not different from the standard diet group (Fig. 1A). Among groups, there were no significant differences in caloric intake (Fig. 1B) or liver enzyme levels (data not shown). Intestinal triglyceride and cholesterol absorption were >90 and 40%, respectively, and were unaffected by any diet (Fig. 1C and D). Intestinal total, free, and cholesteryl ester concentrations were unchanged (data not shown). Intestinal triglycerides were elevated fourfold in Western diet–fed mice and dose-dependently decreased by the addition of 1% (~50%) and 3% (~90%) naringenin (Fig. 1E).

**Naringenin attenuates dyslipidemia and corrects VLDL secretion.** The Western diet elevated fasting plasma cholesterol threefold compared with standard diet–fed mice (Fig. 2A). Naringenin at 1 and 3% decreased plasma cholesterol by 17 and 30%, respectively, compared with Western diet–fed mice (Fig. 2A). The Western diet increased peak levels of both VLDL cholesterol and LDL cholesterol relative to standard diet–fed mice, confirming the profile observed previously (25). Addition of 3% naringenin reduced VLDL cholesterol and LDL cholesterol, while HDL cholesterol was unaffected (Fig. 2C).

Plasma triglycerides were significantly elevated 3.8-fold in Western diet–fed mice compared with standard diet–fed mice. Naringenin at 1 and 3% decreased plasma triglycerides by 36 and 68%, respectively (Fig. 2B), which was primarily due to a reduction in VLDL-triglycerides (Fig. 2D). In metabolic studies, the Western diet significantly increased triglyceride secretion by 50%, which was completely prevented by 3% naringenin (Fig. 2E). Western diet–fed mice secreted twofold more radiolabeled apoB.
NARINGENIN PREVENTS DYSLIPIDEMIA

FIG. 1. Naringenin prevents diet-induced weight gain. *Lpr*−/− mice (n = 12/group) were fed a standard diet or a high-fat diet alone or supplemented with naringenin (1 or 3%) for 4 weeks. A: Body weight gain. ■, standard diet; ▼, Western diet; ▼, Western diet and 1% naringenin; ♦, Western diet and 3% naringenin. B: Caloric intake expressed as kcal · g body wt−1 · day−1. Intestinal triglyceride (C) and cholesterol absorption (D) were determined in fasted mice using a fecal dual-isotope ratio method. Values are reported as percent absorption calculated from the isotopic ratio determined in feces collected over 48 h (n = 4/group). E: Intestinal triglyceride content determined in mice at the time they were killed. Samples of small intestine were cleaned, lipids extracted, and determined enzymatically. Values are the means ± SE, different letters are statistically different, P < 0.05. ○, standard diet; ▼, Western diet; ▼, Western diet and 1% naringenin; ♦, Western diet and 3% naringenin.

into plasma compared with standard diet–fed mice (Fig. 2F), whereas apoB secretion was reduced significantly by 36% in mice fed 3% naringenin. ApoB48 secretion was reduced similarly (data not shown). Postheparin LPLA increased 3.5-fold in Western diet–fed mice, which was increased a further twofold by 3% naringenin (Fig. 2G). Fasting plasma NEFAs were not different among the dietary groups (Fig. 2H).

Naringenin prevents hepatic triglyceride accumulation. Hepatic total cholesterol and cholesteryl ester concentrations were significantly elevated by 1.7- and 3.5-fold, respectively, in Western diet–fed mice compared with standard diet–fed mice. Total cholesterol and cholesteryl ester were decreased by 50% with 1 and 3% naringenin (Fig. 3A). Hepatic triglyceride levels were significantly elevated 1.9-fold in Western diet–fed mice and were markedly reduced by 1% naringenin (−40%) and by 3% naringenin (−67%) (Fig. 3B and C). The Western diet increased liver *Srebp1c* expression by 3.9-fold compared with standard diet–fed mice. *Srebp1c* was reduced dose dependently by 1% (−35%) and 3% naringenin (−65%) (Fig. 3D). Hepatic fatty acid and cholesteryl synthesis increased twofold and threefold in Western diet–fed mice, which were attenuated by 3% naringenin (Fig. 3E and F). The 1.5-fold increases in hepatic triglyceride and cholesteryl ester synthesis in Western diet–fed mice were also reduced by 3% naringenin (Fig. 3G and H). No diet altered hepatic mRNA expression of *Mttp*, *Acat1/2*, or *Dgat1* or protein expression of FoxO1 (data not shown).

Naringenin increases hepatic fatty acid oxidation. The marked reduction in hepatic triglyceride and VLDL-triglyceride and VLDL-apoB secretion suggested that naringenin also increased hepatic fatty acid oxidation. Peroxisome proliferator–activated receptor (PPAR) γ coactivator 1α (PGC1α) activation can initiate mitochondrial biogenesis (28). Enzymes involved in mitochondrial and peroxisomal fatty acid oxidation, including *Cpt1a* and *Aco*, can be upregulated by PPARα (29). The Western diet decreased liver *Pgc1a* mRNA by 18% compared with standard diet–fed mice. However, 3% naringenin significantly increased *Pgc1a* expression by 30% compared with Western diet–fed mice (Fig. 4A). *Pparα* mRNA (Fig. 4B) and liver weight (data not shown) were unaffected by any diet. Naringenin increased *Cpt1a* expression by 25% compared with the Western diet alone (Fig. 4C). *Aco* expression was not affected in Western diet–fed mice, whereas naringenin significantly increased *Aco* mRNA by 30% (Fig. 4D). Consistent with these changes, 3% naringenin significantly increased mitochondrial DNA content (twofold) and fatty acid oxidation (twofold) compared with Western diet–fed mice (Fig. 4E and F). These data suggest that induction of hepatic fatty acid oxidation by naringenin contributes to the reduction of hepatic triglyceride availability for VLDL secretion.

Naringenin improves glucose utilization and insulin sensitivity. Fasting plasma insulin was significantly elevated 2.2-fold by the Western diet and completely prevented by 3% naringenin (Fig. 5A). Plasma insulin correlated with islet size, as significant hyperplasia was only identified in Western diet–fed mice (Fig. 5B). Despite hyperinsulinemia, mild hyperglycemia was observed in Western diet–fed mice, whereas naringenin at 3% normalized plasma glucose (Fig. 5C). HOMA-IR was significantly elevated 2.6-fold in Western diet–fed compared with standard diet–fed mice. HOMA-IR was completely normalized by 3% naringenin (Fig. 5D). The Western diet impaired glucose tolerance relative to standard diet–fed mice, whereas 3% naringenin completely normalized glucose
FIG. 2. Plasma lipid metabolism and apoB secretion in naringenin-treated mice. Ldlr−/− mice (n = 12/group) were fed a standard diet or a high-fat diet alone or supplemented with naringenin (1 or 3%) for 4 weeks. A and B: Plasma cholesterol and triglyceride concentrations. □, standard diet; ■, Western diet; △, Western diet and 1% naringenin; ▽, Western diet and 3% naringenin. C and D: Plasma was subjected to fast protein liquid chromatography analysis and cholesterol and triglycerides were measured in the eluted fractions. △, standard diet; ▽, Western diet; ▽, Western diet and 1% naringenin; ●, Western diet and 3% naringenin. E: Mice were injected with tyloxapol and plasma triglycerides were measured at 0, 30, 60, and 120 min (n = 5–6/time point/group). F: Mice were injected with tyloxapol and [35S]methionine. Plasma was obtained at 60 and 120 min (n = 5–6/time point/group), and VLDL/IDL was isolated by ultracentrifugation. The rate of apoB secretion was measured as the difference in radiolabel in apoB and apoB48 between 60 and 120 min. G: Lipase activity was measured in plasma from mice (n = 6/group) obtained 30 min postinjection intraperitoneally with heparin. LPLA was determined as the difference between total and hepatic lipase activity. H: NEFA concentrations in plasma from fasted mice. Values are the means ± SE, different letters are statistically different, P < 0.05.
FIG. 3. Liver lipid metabolism and gene expression in naringenin-treated mice. Ldlr−/− mice (n = 12/group) were fed a standard diet or a high-fat diet alone or supplemented with naringenin (1 or 3%) for 4 weeks. A: Cholesterol concentrations in liver extracts. B: Oil Red O and hematoxylin-stained sections of liver. Representative photomicrographs. Scale bar = 100 μm. C: Triglyceride concentrations in liver extracts. D: Expression of Srebp1c relative to Gapdh in liver by qRT-PCR. E and F: Hepatic synthesis of fatty acid and cholesterol in liver (n = 5–7/group) obtained 60 min postinjection intraperitoneally with [14C]acetic acid. G and H: Hepatic synthesis of triglyceride and cholesteryl ester in liver homogenates using [14C]oleoyl-CoA (n = 6/group). □, standard diet; ■, Western diet; ◊, Western diet and 1% naringenin; □, Western diet and 3% naringenin. Values are means ± SE, different letters are statistically different, P < 0.05. (A high-quality digital representation of this figure is available in the online issue.)
utilization (Fig. 5F). Normalization by naringenin was maintained following correction for fasting glucose concentrations (Fig. 5F). Western diet–fed mice also showed a blunted response to exogenous insulin, resulting in a greater area under the curve compared with standard diet–fed mice. Naringenin completely normalized insulin sensitivity (Fig. 5G–I).

Intramyocellular lipids are associated with insulin resistance in vivo (30). Western diet–fed mice showed accumulation of both triglyceride and cholesterol ester in muscle, whereas 3% naringenin completely prevented lipid deposition (Fig. 6A and B). In contrast to liver, there was no change in Pgc1α or Cpt1B expression or fatty acid oxidation (Fig. 6C–E). Furthermore, naringenin had no effect on uncoupled oxidation as both Ucp1 and Ucp3 mRNA were unchanged (Fig. 6F and G). However, both Srebp1c and fatty acid synthesis were significantly increased in Western diet–fed compared with standard diet–fed mice, and both were decreased by naringenin (Fig. 6H and I). Western diet–fed mice had impaired deoxyglucose uptake in muscle compared with standard diet–fed mice, whereas 3% naringenin significantly improved deoxyglucose uptake (Fig. 6J). These data suggest that naringenin decreases lipid accumulation in muscle, which may prevent peripheral insulin resistance.

**Naringenin decreases obesity.** Western diet–fed mice gained significantly more total and visceral adipose tissue compared with standard diet–fed mice (Fig. 7A and B); however, lean body mass was unchanged (data not shown). Naringenin dose-dependently attenuated adiposity so that 3% naringenin–fed mice were similar to standard diet–fed mice (Fig. 7A and B). Adipocytes in both epididymal fat and intrascapular brown adipose tissue (BAT) of Western diet–fed mice shifted toward a hypertrophic phenotype compared with standard diet–fed mice and 3% naringenin–treated mice (Fig. 7C and D); however, macrophage infiltration into epididymal adipose stores was not observed with any diet (Fig. 7D). Plasma leptin levels correlated well with adipose tissue mass (Fig. 7E).

Energy expenditure decreased with the Western diet compared with the standard diet. However, consistent with increased fatty acid oxidation, enhanced glucose uptake, and decreased lipogenesis, energy expenditure was significantly higher in mice fed 3% naringenin and was not different from standard diet (Fig. 7F). The respiratory quotient was 0.90 for standard diet–fed animals and 0.79 for Western diet–fed mice, whereas an intermediate value of 0.83 was observed with naringenin treatment, although differences were not significant. Intrascapular BAT is responsible for induction of thermogenesis; therefore, expression of Ucp1 and mtDNA were determined. Ucp1 mRNA was similar among the dietary groups (data not shown). While the Western diet modestly increased mtDNA, no further increase was observed in naringenin–treated mice (data not shown). Heat production was evaluated by a cold-challenge test. The ability of naringenin–treated mice to maintain body temperature at 4°C did not differ from standard diet– or Western diet–fed mice (data

**FIG. 4.** Hepatic fatty acid oxidation and gene expression in naringenin-treated mice. Ldlr−/− mice (n = 12/group) were fed a standard diet or a high-fat diet alone or supplemented with naringenin (1 or 3%) for 4 weeks. A–D: Expression of Pgc1α (A), Ppara (B), Cpt1α (C), and Aco (D) relative to Gapdh in livers by qRT-PCR. E: Fatty acid oxidation in livers (n = 4–6/group) as determined as [1H]palmitate conversion to [1H]2O. F: mtDNA copy number in liver (n = 10/group). Values are means ± SE, different letters are statistically different, P < 0.05.
FIG. 5. Glucose utilization and insulin sensitivity in naringenin-treated mice. LDLR−/− mice (n = 12/group) were fed a standard diet or a high-fat diet alone or supplemented with naringenin (1 or 3%) for 4 weeks. A: Plasma insulin concentrations. B: Hematoxylin-eosin–stained sections of pancreas. Quantifications were performed on 100 islets/group. Scale bar = 100 μm. C: Blood glucose concentration. D: HOMA-IR. E: Glucose tolerance test was performed by intraperitoneal injection of 15% glucose (1 g/kg body wt) into mice and blood glucose was measured at 0, 15, 30, and 60 min postinjection (n = 7–8/group). ○, standard diet; ▼, Western diet; ◆, Western diet and 3% naringenin. F: Incremental area under the curve. G: Insulin tolerance test was performed by intraperitoneal injection of insulin (0.5 IU/kg body wt) into mice, and blood glucose was measured at 0, 15, 30, 45, and 60 min postinjection (n = 6/group). ○, standard diet; ▼, Western diet; ◆, Western diet and 3% naringenin. H: Insulin tolerance test area under the curve. J: Insulin tolerance test corrected for fasting glucose. ○, standard diet; ▼, Western diet; ◆, Western diet and 1% naringenin; ▼, Western diet and 3% naringenin. Values are means ± SE, different letters are statistically different, P < 0.05. (A high-quality digital representation of this figure is available in the online issue.)
not shown), collectively suggesting that naringenin does not stimulate adaptive thermogenesis.

**Naringenin improves dyslipidemia, hepatic steatosis, and insulin sensitivity in wild-type mice.** To confirm that the effect of naringenin was not restricted to Ldlr<sup>−/−</sup> mice, we performed similar studies in wild-type C57BL/6J mice fed the same diets for 30 weeks to establish insulin resistance. The Western diet increased plasma triglycerides and cholesterol compared with the standard diet, whereas 3% naringenin significantly improved plasma lipids (Fig. 8A–C). Naringenin prevented the significant increase in liver triglycerides and cholesterol observed in Western diet–fed mice (Fig. 8D–F). The hyperglycemia and hyperinsulinemia that developed in Western diet–fed mice (Fig. 8G–J) were normalized by 3% naringenin. Glucose tolerance tests revealed that 3% naringenin corrected impaired glucose utilization and insulin sensitivity observed in Western diet–fed mice (Fig. 8K and L). The Western diet increased weight gain and adipose tissue accumulation, which was significantly attenuated by naringenin (Fig. 8K and L).

**DISCUSSION**

Mice lacking the LDLR, when fed a Western-style diet, display many features of insulin resistance including VLDL overproduction, dyslipidemia, and obesity. Furthermore, hepatic lipids increase and fasting glucose and insulin become elevated, resulting from impaired glucose tolerance and reduced insulin sensitivity (25). The major findings of this study are that addition of naringenin to a high-fat diet 1) decreased plasma lipids, 2) reduced overproduction of total triglycerides and hepatic apoB, 3) decreased liver triglycerides and cholesterol, 4) inhibited stimulation of hepatic lipogenesis and prevented hepatic steatosis, 5) increased hepatic β-oxidation, 6) normalized blood glucose and plasma insulin, and 7) restored glucose tolerance. These results demonstrate a novel treatment to correct the metabolic abnormalities associated with diet-induced insulin resistance.

VLDL secretion is increased in the metabolic syndrome and contributes to the dyslipidemia associated with insulin resistance (31). Although regulation of hepatic VLDL se-
cretion is complex, an important stimulus is lipid availability (6). In mouse models of diet-induced insulin resistance, the concept of selective hepatic insulin resistance has emerged (32). Hepatic lipogenesis remains sensitive to insulin. The hyperinsulinemia observed in Western diet–fed mice greatly increases hepatic SREBP1c, drives lipogenesis (15), and increases the contribution of de novo fatty acids to VLDL-triglycerides (33). In this study, naringenin prevents hyperinsulinemia, leading to a reduction in hepatic SREBP1c and hepatic lipogenesis in the fasted state. The reduction in hepatic triglyceride availability contributes to the significant decrease in VLDL-triglycerides and VLDL-apoB secretion and attenuation of dyslipidemia. These findings are consistent with recent studies in mice demonstrating that blocking the ability of insulin to activate hepatic SREBP1c–induced lipogenesis due to a complete absence (LIRKO) or very low expression of (L1B6Ldlr/H11002/H11002) hepatic insulin receptors greatly diminished VLDL-triglyceride secretion (18,19). In some mouse models, however, hepatic apoB secretion is not tightly correlated with plasma leptin levels (32).

**FIG. 7.** Naringenin-treated mice are resistant to diet-induced obesity. Ldlr−/− mice (n = 12/group) were fed a standard diet or a high-fat diet alone or supplemented with naringenin (1 or 3%) for 4 weeks. A: Total adiposity was determined as the weight of all adipose stores per gram body weight. B: Visceral adiposity was determined by dissection of mesenteric, epidymidal, and intraperitoneal pads from mice. C: Adipocyte size was determined by measuring the diameter of adipocytes from hematoxylin-eosin–stained sections of epidymidal adipose (n = 100 adipocytes/group). D: Representative photomicrographs of epidymidal white adipose tissue stained with MAC-1 antibody and intrascapular brown adipose tissue stained with hematoxylin-eosin. Scale bar = 100 μm. E: Plasma leptin concentrations. F: Energy expenditure was determined by changes in the O2 consumption and CO2 production via indirect calorimetry and area under the curve calculated (n = 6/group). ○, standard diet; ▲, Western diet; ◇, Western diet and 3% naringenin; □, standard diet; ■, Western diet; □, Western diet and 1% naringenin; ■□, Western diet and 3% naringenin. Values are means ± SE, different letters are statistically different, P < 0.05. (A high-quality digital representation of this figure is available in the online issue.)
linked to SREBP1c-stimulated triglyceride availability. For instance, in ob/ob mice, increased hepatic Srebp1c and de novo lipogenesis were not associated with increased VLDL-apoB production (34). Liver-specific overexpression of DGAT1/2 greatly increased Srebp1c, Fas, and triglyceride accumulation; however, VLDL secretion was unchanged (35). Availability of newly synthesized cholesterol and cholesteryl ester, which have been shown to influence VLDL formation and secretion (36,37), were unaffected in ob/ob mice and hepatic DGAT1/2–overexpressing mice (34,35). In cultured HepG2 cells, naringenin inhibited ACAT2 expression and cholesterol esterification, leading to inhibition of apoB secretion (37). In the present study, naringenin also prevented the increase in hepatic cholesterol, and although Acat2 mRNA was unaffected, cholesteryl ester synthesis was significantly reduced. This demonstrates that naringenin may not only limit triglyceride availability for VLDL production but also cholesteryl ester.

The contribution of increased intestinal triglycerides in Western diet–fed mice to the overproduction of triglycerides into plasma cannot be discounted. In insulin-resistant hamsters, de novo intestinal lipogenesis results in overproduction of intestinally derived lipoproteins (38). Prevention of intestinal triglyceride accumulation by naringenin may result in reduced secretion of intestinally derived lipoproteins and contribute to the normalization of triglyceride secretion into plasma. Whether the mechanism in the intestine is similar to that observed in liver requires further investigation.

Under normal conditions, insulin targets apoB for intracellular degradation in cultured hepatocytes, leading to acute reduction in VLDL secretion (7,39,40). Whether increased intracellular apoB degradation is a direct effect of insulin or is a consequence of reduced apoB lipidation is not well understood. In insulin-resistant hamsters, apoB degradation is compromised in hepatocytes obtained ex vivo (16). Hyperinsulinemic LIRKO mice overproduce cholesterol-rich apoB, even in the absence of insulin-stimu-
lated SREBP1c expression (18), suggesting that insulin-mediated degradation of apoB is an important determinant of VLDL secretion. In contrast, in hyperinsulinemic L1db/Ldlr−/− mice with very few hepatic insulin receptors, apoB production was markedly diminished compared with Ldlr−/− controls, implying that the loss of SREBP1c-stimulated triglyceride synthesis was the primary determinant for reduced apoB secretion (19). Pulse-chase experiments in HepG2 cells revealed that naringenin, like insulin, reduces apoB secretion through rapid intracellular apoB degradation (7). Enhanced apoB degradation by naringenin was observed in the presence or absence of oleate-stimulated triglyceride synthesis and occurred independent of insulin receptor activation (7,9). In the present study, increased hepatic apoB degradation may contribute to the ability of naringenin to decrease VLDL-apoB secretion in Western diet–fed mice. However, direct experimental evidence requires further investigation.

The Western diet significantly increased LPLA compared with the standard diet, suggesting that a lipolytic defect did not contribute to the hypertriglyceridemia. Naringenin increased LPLA over twofold compared with Western diet–fed mice. The mechanism for this is unclear, as LPL mRNA was not different between Western diet–fed or naringenin-fed animals in liver or muscle (data not shown). Nevertheless, increased LPLA likely contributed to the normalization of plasma triglycerides in naringenin-treated mice.

In mice with diet-induced insulin resistance, SREBP1c-stimulated lipogenesis contributes to hepatic triglyceride accumulation. Reduced rates of hepatic fatty acid oxidation may also be involved. Key regulators of hepatic β-oxidation, including CPT1α, are controlled by a complex of transcription factors and coactivators, including PGC1α and PPARG. PGC1α regulates many metabolic pathways in liver, including gluconeogenesis, mitochondrial expansion, and fatty acid oxidation (28). Fasted Pgc1α−/− mice accumulated significant amounts of neutral lipid within the liver due to decreased hepatic fatty acid oxidation and enhanced SREBP1c-stimulated triglyceride synthesis (41). Hepatic-specific overexpression of Cpt1α in fat-fed rats stimulates fatty acid oxidation and corrects liver triglyceride accumulation (42). Here, we demonstrate that the Western diet decreased hepatic Cpt1α and Pgc1α mRNA and reduced fatty acid oxidation. Naringenin significantly increased hepatic Pgc1α mRNA, which coincided with increased mitochondrial DNA and enhanced fatty acid oxidation. Furthermore, hepatic expression of PPARG-responsive genes, Cpt1α and Aco, were significantly increased.

In fat-fed Ldlr−/− mice, the PPARG agonist fenofibrate increased hepatic expression of Aco and Lpl, while Srebp1c, Fas, and Dgat2 were reduced (43). In contrast to a classic PPARG agonist, naringenin did not increase hepatic Ppara expression or liver weight. Naringenin, the glucoside form of naringenin, decreases liver triglyceride and hepatic fatty acid synthesis in db/db mice, leading to decreased plasma lipids (44). Treatment of fat-fed ICR rats with 1% naringenin increased the hepatic activities of Cpt1α and Aco and increased peroxisomal fatty acid oxidation (45). These data indicate that naringenin activates PGC1α and PPARG target genes to shift the metabolic program in the liver, resulting in a diminished triglyceride burden compared with Western diet–fed mice. Furthermore, naringenin-stimulated fatty acid oxidation likely contributed to the decreased availability of triglyceride for VLDL secretion.

Ldlr−/− mice fed a Western diet become dyslipidemic and hyperinsulinemic, with a metabolic profile characterized by reduced glucose utilization and impaired insulin sensitivity (25). Intramyocellular triglyceride accumulation has been associated with peripheral insulin resistance in animals and humans (46,47). Accumulation of fatty acid derivatives in muscle, derived from both plasma NEFAs and triglyceride-rich lipoproteins, inactivates insulin receptor signaling (47). Naringenin prevented the marked accumulation of triglycerides and cholesterol in muscle induced by the Western diet. Naringenin also normalized insulin sensitivity and pancreatic islet morphology, leading to significantly improved glucose tolerance, including enhanced glucose uptake in muscle. Insulin has been shown to increase Srebp1c and Fas in cultured myotubes (48). In quadriceps of Western diet–fed mice, we demonstrate that Srebp1c mRNA and fatty acid synthesis were significantly increased, indicating that hyperinsulinemia stimulated endogenous lipid synthesis, which contributed to muscle lipid accumulation and diminished insulin sensitivity. Naringenin attenuated SREBP1c-induced fatty acid synthesis in muscle, similar to the effect observed in liver. In contrast to liver, naringenin did not increase Pgc1α expression, nor did it stimulate fatty acid oxidation. This suggests that naringenin may not act directly in muscle, and the reduction in muscle lipid accumulation and improved glucose utilization is instead due to reduced uptake of lipoprotein-derived lipid, a consequence of decreased VLDL secretion, as well as reduced de novo lipogenesis, secondary to normalization of hyperinsulinemia.

Obesity is a component of the metabolic syndrome. Naringenin treatment resulted in almost complete resistance to diet-induced obesity without affecting lean body mass. Increased energy expenditure resulted in enhanced hepatic fatty acid oxidation and/or increased peripheral glucose oxidation, leading to normalized weight gain. Increased energy expenditure prevented visceral adipocyte hypertrophy and normalized the size of visceral fat pads. Naringenin had no effect on lipid metabolism in BAT, indicating the prevention of obesity was unrelated to adaptive thermogenesis.

Treatment of fat-fed C57BL/6J mice with another polyphenolic compound, resveratrol, increased fatty acid oxidation in gastrocnemius muscle and BAT through mitochondrial expansion via activation of SIRT1 and deacetylation of PGC1α (49). In contrast to naringenin, resveratrol had little effect in the liver. Resveratrol improved insulin sensitivity and glucose tolerance; however, compared with naringenin, plasma cholesterol was only modestly reduced and plasma triglycerides were unaffected (49,50). These data suggest that flavonoids have diverse and distinctive tissue-specific effects likely based on structural differences and pharmacokinetic behavior.

The protective effect of naringenin was not restricted to mice with Ldlr deficiency. In wild-type mice, we found that naringenin significantly reduced plasma and hepatic lipids, normalized glucose tolerance and insulin sensitivity, and prevented obesity compared with Western diet–fed mice. Collectively, these findings demonstrate that naringenin has marked lipid- and lipoprotein-lowering potential. Naringenin normalizes hepatic VLDL production, glucose tolerance, and insulin sensitivity and prevents hepatic steatosis and obesity associated with a high-fat diet. The ability of naringenin to modulate metabolic pathways...
linked to the metabolic syndrome suggests that these molecules represent valuable tools in the search for regulators of insulin signaling, lipid homeostasis, and energy balance.

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