Eur J Nutr (2011) 50:81–88
DOI 10.1007/s00394-010-0117-8

ORIGINAL CONTRIBUTION

Dietary naringenin increases hepatic peroxisome proliferators–activated receptor α protein expression and decreases plasma triglyceride and adiposity in rats

Kae Won Cho · Yong Ook Kim · Juan E. Andrade · John R. Burgess · Young-Cheul Kim

Received: 20 January 2010 / Accepted: 7 June 2010 / Published online: 23 June 2010

Abstract
Background Naringenin, a flavonoid present in grapefruit, has recently been shown to exert hypolipidemic and hypocholesterolemic effects, which has a particular importance for protecting against chronic diseases. However, the lipid-lowering potential of naringenin at the concentrations in the dietary range and its underlying mechanisms have yet to be fully elucidated.
Aim The aim of the present study was (1) to investigate the effects of dietary naringenin on plasma and hepatic triglyceride and cholesterol levels and on adipose deposition in rat and (2) to determine the contribution of hepatic peroxisome proliferators–activated receptor α (PPARα) expression to fatty acid oxidation.
Methods Male Long-Evans hooded rats were fed a diet supplemented with naringenin (0.003, 0.006, and 0.012%) for 6 weeks. We analyzed plasma and hepatic lipid contents and determined the protein expression of PPARα, carnitine-palmitoyl transferase 1L (CPT-1), and uncoupling protein 2 (UCP2), all of which are critical genes for fatty acid oxidation.
Results Naringenin supplementation caused a significant reduction in the amount of total triglyceride and cholesterol in plasma and liver. In addition, naringenin supplementation lowered adiposity and triglyceride contents in parametrial adipose tissue. Naringenin-fed animals showed a significant increase in PPARα protein expression in the liver. Furthermore, expression of CPT-1 and UCP2, both of which are known to be regulated by PPARα, was markedly enhanced by naringenin treatment.
Conclusions Our results indicate that the activation of PPARα transcription factor and upregulation of its fatty acid oxidation target genes by dietary naringenin may contribute to the hypolipidemic and anti-adiposity effects in vivo.

Keywords Naringenin · Hypolipidemic effect · Anti-adiposity · PPAR-α · CPT-1 · UCP2 · In vivo

Introduction

It has been recognized that a high intake of fruits and vegetables protects against chronic diseases. Among fruits and vegetables, Joshipura et al. [1] showed the protective relationship between the consumption of citrus fruits or juices and the risk of ischemic stroke. In addition, it has been reported that high consumption of citrus juice or grapefruit improved blood lipid profile in hyperlipidemic humans [2, 3]. Citrus juices, especially orange juice and grapefruit juice, are rich source of flavonoids. Thus, citrus flavonoids have been attributed to the beneficial effects of citrus juice in ameliorating cardiovascular disease risk factors.

Naringenin is one of major citrus flavonoids and is predominantly found in grapefruit and orange [4]. It has been shown to reduce plasma lipids in rodent models [5–8] and, more recently, to reduce atherosclerosis in rabbits [9]. The proposed hypolipidemic effects of naringenin have

Kae Won Cho and Yong Ook Kim have contributed equally.

K. W. Cho · Y. O. Kim · Y.-C. Kim (✉)
Department of Nutrition, University of Massachusetts, Amherst, MA 10003, USA
e-mail: yckim@nutrition.umass.edu

J. E. Andrade · J. R. Burgess
Department of Foods and Nutrition, Purdue University, West Lafayette, IN 47907, USA

been primarily linked to reduced hepatic enzymes, such as 3-hydroxy-3-methyl-glutaryl CoA reductase, acyl-coenzyme A, cholesterol O-acyltransferase (ACAT), peroxisomal acyl-CoA oxidase, cyanide insensitive palmitoyl-CoA oxidase, carnitine palmitoyltransferase (CPT), enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase, and 3-ketoacyl-CoA thiolase in vivo [5, 10]. Huong et al. [10] reported that naringenin up-regulated the gene expression of various enzymes involved in peroxisomal fatty acid oxidation, such as carnitine octanoyltransferase, acyl-CoA oxidase, bifunctional enzyme, and 3-ketoacyl-CoA thiolase in mice. Further, they found that dietary naringenin has been shown to markedly activate the mRNA expression of microsomal cytochrome P-450 IV A1 involved in ω-oxidation of fatty acids in mice. In addition, it has been shown that naringenin decreases the availability of lipids for assembly of apoB-containing lipoproteins [11–13] and decreases the expression and/or activity of genes such as ACAT2 and microsomal triglyceride transfer protein (MTP) in human hepatoma cells (HepG2) [13, 14]. However, the concentrations used in those studies were 20–3,000 mg/100 g diets, which are relatively higher than a dietary dosage, because the average intakes have been estimated as 8.3 and 9.6 mg/d in Finland and Mediterranean diet [15, 16]. Currently, information about the lipid-lowering effects of naringenin at the dietary concentration ranges and molecular mechanisms responsible for these activities in vivo is lacking.

Peroxisome proliferator activated receptor α (PPARα), which is a member of the nuclear receptor family of ligand-activated transcription factor, controls the transcription of many genes involved in lipid catabolism. Activation of PPARα with PPARα agonists such as fibrates increases fatty acid β-oxidation and thus reduces the levels of circulating and/or cellular lipids [17, 18]. Moreover, addition of a synthetic PPARα ligand effectively dissipated fat accumulation in rats [19]. It has been thus suggested that PPARα activation is a potential molecular mechanism for the hypolipidemic effects of plant-derived polyphenolic compounds such as genistein [20] and catechin [21]. Although Liu et al. [22] have shown a significant activation of PPARα-induced reporter-gene expression using human U-2OS cells co-transfected with the expression vectors of PPARα, it has not been firmly established whether the lipid-lowering activity of dietary naringenin involves PPARα activation and increased lipid oxidation in vivo.

In the present study, we hypothesized that dietary naringenin at low doses achievable in humans exerts hypolipidemic effects by upregulating PPARα that targets fatty acid oxidation genes. Here, we demonstrated that dietary naringenin-induced genes that are involved in hepatic fatty acid catabolism, which resulted in reduction in total triglyceride and cholesterol levels, thus exerting hypolipidemic effects in rats. In addition, naringenin supplementation decreased adiposity by reducing triglyceride contents in adipose tissue.

Materials and methods

Animals, diets, and experimental design

Male Long-Evans hooded rats (Harlan, Indianapolis, IN) obtained at 3 weeks of age (40–50 g) were housed individually in animal cages in a room with controlled temperature (23 to 24 °C) and lightning (12-h light/dark cycle) and fed a commercial non-purified diet (Purina, St. Louis, MO) and water prior to receiving the dietary treatments. Animals were assigned to treatments so that each group was balanced for body weight and fed one of four experimental diets ($n = 6$) for 6 weeks. Semi-purified, powdered diets were prepared for concentrations of naringenin: 0, 0.003, 0.006, and 0.012% of diet. After 7 days of acclimatization, rats were assigned to one of four groups, with six animals per group, and fed semi-purified experimental diets for 6 weeks. The experimental diets contained 16% fat, 45.5% sucrose, and different naringenin concentration (0, 0.003, 0.006, or 0.012%) (Table 1). Rats had ad libitum access to food and water during the study period. Food intake and body weight were measured throughout the experiment. At the end of the experiment, the animals were fasted for 12 h before the intraperitoneal injection of ketamine (90 mg/kg) and xylazine (10 mg/kg) and euthanized by exsanguination. Blood was immediately collected into heparinized tubes for further analysis. Livers and other

Table 1 Composition of the experimental diets (g/100 g diet)

Component	Control	Naringenin (%)		
		0.003	0.006	0.012
Torula yeast	30	30	30	30
Sucrose	44.5	44.5	44.5	44.5
Alphacell	4	4	4	4
D,L-Methionine	0.3	0.3	0.3	0.3
Lard	6	6	6	6
Corn oil	10	10	10	10
Mineral mixture[a]	4	4	4	4
Vitamin mix[b]	1	1	1	1
Sodium selenite	5.0×10^{-5}	5.0×10^{-5}	5.0×10^{-5}	5.0×10^{-5}
Naringenin	0.0	3.0×10^{-3}	6.0×10^{-3}	1.2×10^{-2}
Total	100.0	100.0	100.0	100.0

[a] AIN-76 mineral mixture (Dyets, USA)

[b] AIN-76 vitamin mixture (MP Biomedical, USA)

tissues were perfused with physiological saline, removed, weighed, and frozen in liquid nitrogen and stored at −80 °C until further analysis. All experimental procedures were approved by the Animal Care and Use Committee of our institute and followed the guidelines for the care and use of laboratory animals (#00-078-03).

Plasma and hepatic lipid analyses

Plasma and liver cholesterol were determined using a commercial kit (Wako, Richmond, VA) with some modification of the cholesterol oxidase method of Allain et al. [23]. Plasma and liver triglyceride, plasma-free fatty acids were measured using a kit from Sigma Chemical Co. (Sigma, St. Louis, MO). The hepatic lipids were extracted using the procedure developed by Folch et al. [24]. The dried lipid residues were dissolved in 1 mL of ethanol for cholesterol and triglycerides analysis. Triton X-100 solutions were added to 200 μL of dissolved lipid solution to produce a final concentration of 5 g/L and 3 mmol/L, respectively.

Western blot analysis

Total proteins were extracted by homogenizing 300 mg of liver tissue in 1 mL of lysis buffer (250 mM sucrose, pH 7.4, 50 mmol/L HEPES, 100 mmol/L NaPPi, 100 mmol/L NaF_2, 10 mmol/L EDTA, 1% Triton-×100, 10 mmol/L activated $NaVO_4$) containing complete mini EDTA-free protease inhibitor cocktail (Roche Diagnostics, Indianapolis, IN), followed by centrifugation for 30 min at 10,000×*g*, 4 °C. The resulting supernatant was assayed for total protein concentration, and same amounts of protein were loaded to the SDS–PAGE gels and transferred onto PVDF membranes (Amersham Pharmacia Biotech, Piscataway, NJ). Ponceau S (Sigma, St. Louis, MO) staining of the membranes was performed to assess the equivalence of sample loading and gel transfer as previously described [25, 26]. After blocking in Tris-buffered saline containing 5% milk, 1.0% BSA, and 0.1% Tween-20 for 1 h at RT, the membranes were immunoblotted with antibodies for PPARα (1:100) (Santa Cruz Biotechnology, Santa Cruz, CA), CPT-1 (1:200) (Alpha Diagnostic International, San Antonio, TX), UCP2 or actin (Santa Cruz Biotechnology; Santa Cruz, CA), then with the secondary antibody conjugated to horseradish peroxidase (1:2000). The visualization of the proteins was performed with the enhanced ECL detection kit (Amersham Pharmacia Biotech, Piscataway, NJ) according to manufacturer's instruction. The relative expression of PPARα, CPT-1, and UCP2 proteins was normalized to the actin control using the ImageJ software (available through NIH, http://rsb.info.nih.gov/ij/download.html).

Statistical analysis

All data were analyzed by one-way analysis of variance (ANOVA) and post hoc comparisons (Fisher's Least Significant Difference) using the SAS software (SAS Institute, Cary, NC, USA). Differences were considered significant at $p < 0.05$. Data are presented as means ± SEM.

Results

Effects of naringenin supplementation on food intake, weight gain, and organ weight

Naringenin did not affect the average food intake during the 6-week experiment period (data not shown). Although the mean body weights of the naringenin-supplemented groups relative to the control group tended to increase, they were not significantly different. No significant differences were observed in liver weights across all the groups after 6 weeks of treatment (data not shown).

Naringenin decreases adipose triglyceride (TAG)

The weight of parametrial fat pad, consisting of epididymal and perirenal, was decreased in naringenin-fed rats. Compared to the control group, naringenin treatment decreased adipose tissue weight by 24.6, 14.9, and 41.1% at dietary naringenin concentrations of 0.003, 0.006, and 0.012%, respectively (Fig. 1). The difference in the parametrial adipose tissue weight at 0.012% naringenin treatment and control was significant ($p < 0.05$), although the differences in other groups did not reach the significance. Since triglyceride is the major components in adipose tissue, we measured adipose tissue triglyceride amounts. Consistent with the changes in parametrial fat pad weights, naringenin supplementation at highest dose significantly decreased TAG amounts in adipose tissue (Fig. 1).

Naringenin decreases plasma triglyceride and cholesterol level

Naringenin supplementation resulted in a significant lowering of the blood triglyceride levels compared to that of the control group (Table 2). Although naringenin dose-dependently lowered plasma triacylglycerol concentrations (6, 42 and 55% of control at 0.003, 0.006, and 0.012%, respectively), the difference among groups did not reach the statistical significance. The plasma total and free cholesterol concentrations were also significantly lower in the 0.006% naringenin treatment compared to the control group. However, plasma-free fatty acids concentrations were not different among groups (Table 2).

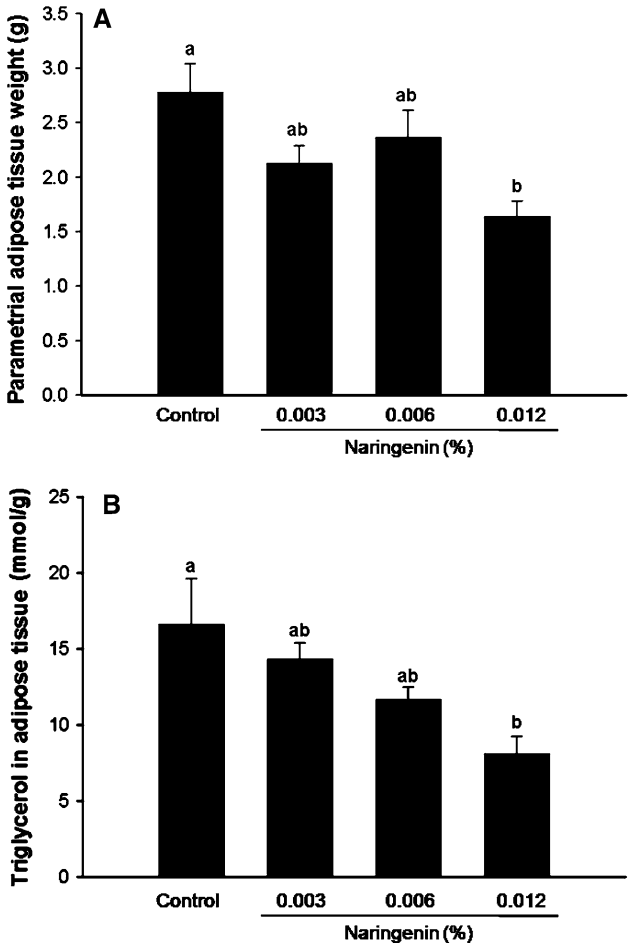

Fig. 1 Effects of naringenin on parametrial adipose tissue weight (**a**) and *triglyceride* of adipose tissue (**b**) in rats. Values are expressed as the mean ± SEM. ($n = 6$). The means not sharing a common letter are significantly different between groups ($p < 0.05$)

Naringenin lowers hepatic triglyceride and cholesterol levels

Hepatic triglyceride levels were decreased in rats fed a naringenin-supplemented diet relative to those of animals fed a control diet (Table 3). However, only 0.012% naringenin decreased hepatic triglyceride concentrations with a statistical significance ($p < 0.05$). Cholesterol concentrations in liver were also decreased in naringenin-fed rats compared to the control group, but only 0.006% naringenin resulted in a significant reduction of the hepatic cholesterol levels compared to that of the control group ($p < 0.05$).

Naringenin increases hepatic PPARα, CPT-1, and UCP2 protein expression

To examine whether the decrease in the plasma and hepatic triglyceride levels is due to increased hepatic fatty acid oxidation, we analyzed the expression of PPARα, a key transcriptional regulator of lipid metabolism and fatty acid β-oxidation, and 0.012% naringenin significantly induced hepatic PPARα protein expression as shown in Fig. 2. Quantitative analysis showed that PPARα protein abundance was increased by 25, 40, and 50% in 0.003, 0.006, and 0.012% naringenin-fed groups relative to the control group, respectively. We further assessed whether activation of PPARα by naringenin treatment leads to increased expression of PPARα-regulated genes such as CPT-1 and UCP2 in the liver [27–29]. Consistent with increased PPARα protein expression, naringenin dose-dependently increased CPT-1 and UCP2 protein amounts (112, 110, and 130% increase for CPT-1, and 113, 125, and 127% increase for UCP2 in 0.003, 0.006, and 0.012% naringenin group, respectively). The protein levels of PPARα, CPT1, and UCP2 in 0.012% naringenin were significantly higher than those of the control group (Fig. 2).

Discussion

In the present study, we show that dietary levels of naringenin at concentrations equivalent to 1–4 cups of grapefruit juice significantly lowered both blood and hepatic lipid levels in rats. This hypolipidemic effect of dietary naringenin at low doses achievable in humans could partially explain the hypolipidemic effects of grapefruit intake observed in hyperlipidemic humans [2] and the inverse association between intake of citrus fruit and cerebrovascular disease in several observational studies [1, 30]. Citrus flavonoids have been reported to exhibit beneficial properties against the onset of several chronic diseases. Among naturally occurring citrus flavonoids,

Table 2 Effects of dietary naringenin on plasma lipids in rats (values are means ± standard error of the mean, $n = 6$)

Groups	Control	Naringenin (%)		
		0.003	0.006	0.012
Triglyceride (mg/dL)	86.46 ± 11.62[a]	50.97 ± 8.17[b]	49.95 ± 9.34[b]	38.83 ± 7.90[b]
Total cholesterol (mg/dL)	78.59 ± 3.30[a]	71.49 ± 1.96[ab]	67.77 ± 3.35[b]	73.04 ± 2.63[ab]
Free cholesterol (mg/dL)	20.17 ± 1.75[a]	17.76 ± 1.10[ab]	14.46 ± 1.41[b]	17.19 ± 1.33[ab]
Non-esterified fatty acid (μEq/L)	756 ± 127[a]	692 ± 100[a]	741 ± 151[a]	774 ± 132[a]

Values bearing different letters in the same row are significantly different at $p < 0.05$

Table 3 Effects of dietary naringenin on hepatic lipids in rats (values are means ± standard error of the mean, $n = 6$)

Values bearing different letters in the same row are significantly different at $p < 0.05$

Groups	Control	Naringenin (%)		
		0.003	0.006	0.012
Triglyceride (μmol/g)	5.67 ± 0.82^{a}	4.99 ± 0.37^{ab}	4.92 ± 0.83^{ab}	4.62 ± 0.37^{b}
Cholesterol (μmol/g)	6.55 ± 0.49^{a}	6.33 ± 0.40^{ab}	6.12 ± 0.30^{b}	6.23 ± 0.24^{ab}
Liver weight (g/100 g B.W.)	3.00 ± 0.23^{a}	2.78 ± 0.31^{a}	2.88 ± 0.12^{a}	2.91 ± 0.21^{a}

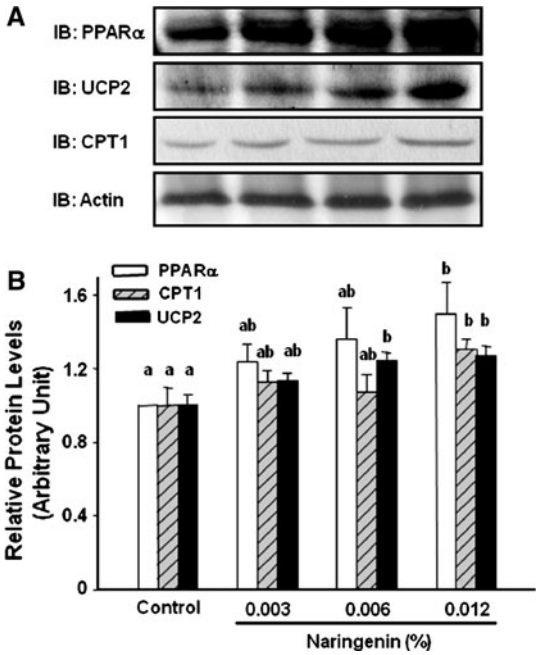

Fig. 2 Effect of naringenin treatment on liver protein expression of PPARα, UCP2, and CPT-1. Expression of protein levels were analyzed by Western blot analysis as described in "Method" section (*top panel,* **a**). The expression of actin was monitored to demonstrate equal loading and transfer of the samples. *Bar graph* represents ImageJ analysis of the immunoblots of each protein, which was normalized to the expression of actin control (**b**). Data are the mean ± SEM. ($n = 6$). The means not sharing a common letter are significantly different between groups ($p < 0.05$)

naringenin has been pharmacologically evaluated as a potential hypolipidemic agent [31]. Wood [32] has shown that 0.6% naringenin lowered hepatic neutral and polar lipids in rats. Lee et al. [7] reported that 0.1% naringenin lowered the plasma and the hepatic cholesterol contents in rats. The dietary levels at 0.02 and 0.05% naringenin have also been shown decrease plasma total cholesterol levels as well as hepatic TAG and cholesterol biosynthesis in animal models [5, 33, 34]. However, the dietary level of naringenin which have been employed in previous studies (0.02 to 1%) is higher than that of this study [7, 32, 34]. Our results showed that both blood and hepatic lipid levels were lowered by naringenin supplementation even at the lowest concentration of 0.003%.

In reference to actual amount of natural naringenin in citrus juice, Mouly et al. [35] reported that 113–481 mg/L of naringenin is present in grapefruit juice. Since one medium-sized grapefruit juice only has ~100 mg naringenin, one cup (250 mL) of grapefruit juice per day would be equivalent to ~1.5 mg/day/kg body weight, considering an average body weight of 70 kg. Thus, the dietary levels at 0.003, 0.006, and 0.012% naringenin supplementation employed in the current study would be equivalent to 1, 2, and 4 cups of grapefruit juice in humans. Our results showed that both blood and hepatic lipid levels were lowered by naringenin supplementation even at the lowest concentration of 0.003%. The mechanisms underlying the triglyceride-lowering effect of naringenin are not fully elucidated. Previous reports have speculated that one mode of naringenin's lipid-lowering action is through the inhibition of HMG-CoA reductase or ACAT activity [5, 7, 34]. Although inhibitors of HMG-CoA reductase lower the plasma TAG by inhibiting the hepatic TAG secretion [36], it is unclear how inhibition of HMG-CoA reductase activity could contribute to the lowering effects of hepatic TAG levels. It was reported that atorvastatin, a HMG-CoA reductase inhibitor drug, does not affect mRNA levels of fatty acid biosynthetic enzyme [37]. Modulation of hepatic fatty acid oxidation could be a mechanism to regulate serum and hepatic lipid levels by modifying the availability of fatty acid for *triglyceride* synthesis [38]. It has shown that activation of nuclear-receptor PPARα with PPARα agonists, such as fibrates, not only reduces plasma TAG but also greatly decreases fatty liver by inducing hepatic mitochondrial and peroxisomal fatty acid oxidation in both humans and animals [38–41]. In the present study, we demonstrated that naringenin feeding in rats induced the up-regulation of hepatic PPARα protein expression (Fig. 2).

As a consequence, the expression of the genes coding for the rate-limiting enzyme of mitochondrial fatty acid oxidation, CPT-1 and UCP2, both of which are known to be regulated by PPARα [29, 41] was markedly increased by naringenin supplementation (Fig. 2). These effects were associated with the decrease in the plasma TAG levels as well as in the hepatic TAG content (Tables 2 and 3). Our finding is in line with the recent cell culture study that naringenin activates PPARα-induced reporter-gene expression in human U-2OS osteosarcoma cell line [22]. Consistent with our results, Huong et al. [10] reported that 1% naringenin feeding increased the expression of mRNAs

associated with hepatic fatty acid oxidation and decreased plasma lipids in mice. However, they did not analyze the level of PPARα gene expression. Recently, Mulvihill et al. [42] have shown that 1 or 3% naringenin increased microsomal and peroxisomal fatty acid oxidation through an activation of factor PPARγ co-activator 1α transcription program with PPARα mRNA level unaffected in LDL receptor-null [(Ldlr(−/−)] mice fed a high-fat diet. No significant difference in PPARα gene expression in this study is in contrast to our finding, and the different results may due, in part, to the use of a different genetic model of dyslipidemic animals, a Western diet with high levels of naringenin, and/or regulation of PPARα by naringenin at protein levels. Nevertheless, both studies are in agreement with the effectiveness of dietary naringenin in increasing hepatic fatty acid oxidation that can account for the serum lipid-lowering in vivo.

In addition to a principal role in lipid metabolism, activation of PPARα has been associated with reduced adiposity and increased insulin sensitivity. For example, fenofibrate, a synthetic PPARα ligand, has been shown to reduce adipose tissue mass in both genetic and diet-induced animal models [19, 40]. A plant-derived bioactive compound, genistein, not only increased PPARα activity and its target genes involved in fatty acid oxidation [20, 21] but also reduced adiposity in rats fed high-fat diet [43, 44].

However, there are no available data that naringenin at a dietary level comparable to human consumption might induce anti-adiposity effects. In the present study, we demonstrated that dietary naringenin resulted in reduction of adiposity of parametrial fat pads at physiological concentrations. Consistent with this change, the amount of TAG in the adipose tissue was significantly decreased in naringenin-fed rats (Fig. 1). Since there was no difference in food intake, these effects were independent of caloric intake. Even though parametrial adipose tissue weights in naringenin-fed rats were decreased, naringenin treatment did not cause a decrease in body weight. The effects of naringenin on both food intake and body weight are consistent with the reports in mice and rats [10, 45]. However, it remains to be examined whether reduced TAG level and adiposity would lead to weight loss, since other parameters such as the length of the study, a species difference, and/or concentrations of naringenin could have an effect. It is thus reasonable to speculate that naringenin may induce smaller fat cells but not necessarily decrease adipocyte numbers. Recent studies [46, 47] have reported that naringenin is able to activate PPAR-γ, a nuclear receptor for thiazolidinediones (TZDs), an insulin-sensitizing class of anti-diabetic drugs. One of the adipose tissue-specific effects of TZDs is the stimulation of adipocyte differentiation, resulting in an increased population of small, but more insulin-sensitive adipocytes [48]. It is certainly our limitation in this study to establish the relationship between reduced adipose tissue mass and phenotypic characteristics.

In summary, dietary naringenin exerts hypolipidemic and anti-adiposity effects in vivo at physiologically relevant concentrations. These effects can be explained in part by upregulation of hepatic PPARα and its target genes and by decreasing TAG level in adipose tissue. Together, our results indicate that decreased adipose TAG amounts by naringenin treatment may increase the release of TAG, which is in turn diverted into hepatic fatty acid oxidation, resulting in lowered plasma level of TAG. However, we could not exclude the possibility that naringenin may also upregulate fatty acid oxidation in the adipose tissue itself. Future studies should address this to clarify a potential mechanism by which dietary naringenin elicits its beneficial effects in vivo.

Acknowledgments This research was in part supported by Grants (S00000005000000) from the University of Massachusetts, Amherst.

References

1. Joshipura KJ, Ascherio A, Manson JE et al (1999) Fruit and vegetable intake in relation to risk of ischemic stroke. JAMA 282:1233–1239
2. Gorinstein S, Caspi A, Libman I et al (2006) Red grapefruit positively influences serum triglyceride level in patients suffering from coronary atherosclerosis: studies in vitro and in humans. J Agric Food Chem 54:1887–1892
3. Kurowska EM, Spence JD, Jordan J et al (2000) HDL-cholesterol-raising effect of orange juice in subjects with hypercholesterolemia. Am J Clin Nutr 72:1095–1100
4. Erlund I (2004) Review of the flavonoids quercetin, hesperetin, and naringenin. Dietary sources, bioactivities, bioavailability, and epidemiology. Nutr Res 24:851–874
5. Bok SH, Shin YW, Bae KH et al (2000) Effects of naringin and lovastatin on plasma and hepatic lipids in high-fat and high-cholesterol fed rats. Nutr Res 20:1007–1015
6. Choi MS, Do KM, Park YS et al (2001) Effect of naringin supplementation on cholesterol metabolism and antioxidant status in rats fed high cholesterol with different levels of vitamin E. Ann Nutr Metab 45:193–201
7. Lee SH, Park YB, Bae KH et al (1999) Cholesterol-Lowering Activity of Naringenin via Inhibition of 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase and Acyl Coenzyme A: Cholesterol Acyltransferase in Rats. Ann Nutr Metab 43:173–180
8. Santos KFR, Oliveira TT, Nagem TJ et al (1999) Hypolipidaemic effects of naringenin, rutin, nicotinic acid and their associations. Pharmacol Res 40:493–496
9. Lee CH, Jeong TS, Choi YK et al (2001) Anti-atherogenic effect of citrus flavonoids, naringin and naringenin, associated with hepatic ACAT and aortic VCAM-1 and MCP-1 in high cholesterol-fed rabbits. Biochem Bioph Res Co 284:681–688
10. Huong DTT, Takahashi Y, Ide T (2006) Activity and mRNA levels of enzymes involved in hepatic fatty acid oxidation in mice fed citrus flavonoids. Nutrition 22:546–552
11. Borradaile NM, de Dreu LE, Barrett PH, Behrsin CD, Huff MW (2003) Hepatocyte ApoB-containing lipoprotein secretion is decreased by the grapefruit flavonoid, naringenin, via inhibition

of MTP-mediated microsomal triglyceride accumulation. Biochemistry 42:1283–1291
12. Borradaile NM, de Dreu LE, Huff MW (2003) Inhibition of net HepG2 cell apolipoprotein B secretion by the citrus flavonoid naringenin involves activation of phosphatidylinositol 3-kinase, independent of insulin receptor substrate-1 phosphorylation. Diabetes 52:2554–2561
13. Wilcox LJ, Borradaile NM, de Dreu LE, Huff MW (2001) Secretion of hepatocyte apoB is inhibited by the flavonoids, naringenin and hesperetin, via reduced activity and expression of ACAT2 and MTP. J Lipid Res 42:725–734
14. Allister EM, Borradaile NM, Edwards JY, Huff MW (2005) Inhibition of microsomal triglyceride transfer protein expression and apolipoprotein B100 secretion by the citrus flavonoid naringenin and by insulin involves activation of the mitogen-activated protein kinase pathway in hepatocytes. Diabetes 54:1676–1683
15. Kumpulainen J, Lehtonen M, Mattila P (1999) Trolox equivalent antioxidant capacity of average flavonoids intake in Finland. In: Kumpulainen JT, Salonen JT (ed) Natural antioxidants and anticarcinogens in nutrition, health and disease, Cambridge, pp 141–150
16. Vasilopoulou E, Georga K, Joergensen MB, Naska A, Trichopoulou A (2005) The antioxidant properties of greek foods and the flavonoid content of the mediterranean menu. Curr Med Chem 5:33–45
17. Foxworthy PS, Eacho PI (1991) Effect of the peroxisome proliferator LY171883 on triglyceride accumulation in rats fed a fat-free diet. Biochem Pharmacol 42:1487–1491
18. Staels B, Dallongeville J, Auwerx J, Schoonjans K et al (1998) Mechanism of action of fibrates on lipid and lipoprotein metabolism. Circulation 98:2088–2093
19. Lee HJ, Choi SS, Park MK, An YJ et al (2002) Fenofibrate lowers abdominal and skeletal adiposity and improves insulin sensitivity in OLETF rats. Biochem Bioph Res Co 296:293–299
20. Kim S, Shin HJ, Kim SY, Kim JH et al (2004) Genistein enhances expression of genes involved in fatty acid catabolism through activation of PPARα. Mol Cell Endocrinol 220:51–58
21. Murase T, Nagasawa A, Suzuki J, Hase T, Tokimitsu I (2002) Beneficial effects of tea catechins on diet-induced obesity: stimulation of lipid catabolism in the liver. Int J Obes Relat Metab Disord 26:1459–1464
22. Liu Li, Song S, Kun Z, Zhi-Qiang N et al (2008) Naringenin and hesperetin, two flavonoids derived from *Citrus aurantium* up-regulate transcription of adiponectin. Phytother Res 22:1400–1403
23. Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC (1974) Enzymatic determination of total serum cholesterol. Clin Chem 20:470–475
24. Folch J, Lees M, Sloane Stanley GH (1957) A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 226:497–509
25. Hsueh CT, Wu YC, Schwartz GK (2001) UCN-01 suppresses E2F–1 mediated by ubiquitin-proteasome-dependent degradation. Clin Cancer Res 7:669–674
26. Song J, Lu Y, Pang S, Chiu R (2004) An internal control for immunoblot analysis using the blotted membrane. Anal Biochem 331:201–203
27. Brady PS, Marine KA, Brady LJ, Ramsay RR (1989) Co-ordinate induction of hepatic mitochondrial and peroxisomal carnitine acyltransferase synthesis by diet and drugs. Biochem J 260:93–100
28. Kelly LJ, Vicario PP, Thompson GM, Candelore MR et al (1998) Peroxisome proliferator-activated receptors γ and α mediate in vivo regulation of uncoupling protein (UCP-1, UCP-2, UCP-3) gene expression. Endocrinology 139:4920–4927
29. Mascaro C, Acosta E, Ortiz JA, Marrero PF et al (1998) Control of human muscle-type carnitine palmitoyltransferase I gene transcription by peroxisome proliferator-activated receptor. J Biol Chem 273:8560–8563
30. Knekt P, Kumpulainen J, Jarvinen R, Rissanen H et al (2002) Flavonoid intake and risk of chronic diseases. Am J Clin Nutr 76:560–568
31. Wilcox LJ, Borradaile NM, Huff MW (1999) Antiatherogenic properties of naringenin, a citrus flavonoid. Cardiovasc Drug Rev 17:160–178
32. Wood N (2004) Hepatolipidemic effects of naringenin in high cornstarch-versus high coconut oil-fed rats. J Med Food 7:315–319
33. Kim S, Kim H, Lee M, Jeon S et al (2006) Naringin time-dependently lowers hepatic cholesterol biosynthesis and plasma cholesterol in rats fed high-fat and high-cholesterol diet. J Med Food 9:582–586
34. Lee MK, Moon SS, Lee SE et al (2003) Naringenin 7-O-cetyl ether as inhibitor of HMG-CoA reductase and modulator of plasma and hepatic lipids in high cholesterol-fed rats. Bioorgan Med Chem 11:393–398
35. Mouly PP, Arzouyan CR, Gaydou EM, Estienne JM (1994) Differentiation of citrus juices by factorial discriminant analysis using liquid chromatography of flavanone glycosides. J Agr Food Chem 42:70–79
36. Kasim SE, LeBoeuf RC, Khilnani S, Tallapaka L et al (1992) Mechanisms of triglyceride-lowering effect of an HMG-CoA reductase inhibitor in a hypertriglyceridemic animal model, the Zucker obese rat. J Lipid Res 33:1–7
37. Roglans N, Sanguino E, Peris C, Alegret M et al (2002) Atorvastatin treatment induced peroxisome proliferator-activated receptor α expression and decreased plasma nonesterified fatty acids and liver triglyceride in fructose-fed rats. J Pharmalcol Exp Ther 302:232–239
38. Neve BP, Fruchart JC, Staels B (2000) Role of the peroxisome proliferator-activated receptors (PPAR) in atherosclerosis. Biochem Pharmacol 60:1245–1250
39. Chitturi S, Farrell GC (2001) Etiopathogenesis of Nonalcoholic Steatohepatitis. Semin Liver Dis 21:027–042
40. Guerre-Millo M, Gervois P, Raspe E, Madsen L et al (2000) Peroxisome proliferator-activated receptor alpha activators improve insulin sensitivity and reduce adiposity. J Biol Chem 275:16638–16642
41. Keller J, Collet P, Bianchi A, Huin C et al (2000) Implications of peroxisome proliferator-activated receptors (PPARs) in development cell life status and disease. Int J Dev Biol 44:429–442
42. Mulvihill EE, Allister EM, Sutherland BG, Telford DE et al (2009) Naringenin prevents dyslipidemia, apolipoprotein B overproduction, and hyperinsulinemia in LDL receptor-null mice with diet-induced insulin resistance. Diabetes 58:2198–2210
43. Kim S, Sohn I, Lee YS, Lee YS (2005) Hepatic gene expression profiles are altered by genistein supplementation in mice with diet-induced obesity. J Nutr 135:33–41
44. Naaz A, Yellayi S, Zakroczymski MA, Bunick D et al (2003) The soy isoflavone genistein decreases adipose deposition in mice. Endocrinology 144:3315–3320
45. Kannappan S, Anuradha CV (2009) Naringenin enhances insulin-stimulated tyrosine phosphorylation and improves the cellular actions of insulin in a dietary model of metabolic syndrome. Eur J Nutr. doi: 10.1007/s00394-009-0054-6
46. Kawada T, Takahashi N, Fushiki T (2001) Biochemical and physiological characteristics of fat cell. J Nutr Sci Vitaminol (Tokyo) 47:1–12

47. Morikawa K, Nonaka M, Mochizuki H, Handa K et al (2008) Naringenin and hesperetin induce growth arrest, apoptosis, and cytoplasmic fat deposit in human preadipocytes. J Agric Food Chem 56:11030–11037

48. Okuno A, Tamemoto H, Tobe K, Ueki K et al (1998) Troglitazone increases the number of small adipocytes without the change of white adipose tissue mass in obese Zucker rats. J Clin Invest 101:1354–1361