— **Regular Article** —

Effect of Nobiletin on Lipid Metabolism in Rats

Eri Nagata,[a] Ikuyo Ichi,[a] Ryoko Kataoka,[a] Miyuki Matsushima,[a] Noriko Adachi,[a] Yuko Kitamura,[a] Takao Sasaki,[b] and Shosuke Kojo*,[a]

[a]*Department of Food Science and Nutrition, Nara Women's University, Nara 630–8506, Japan and* [b]*Arkray Inc., Kyoto 601–8045, Japan*

(Received August 6, 2010; Accepted September 21, 2010; Published online September 22, 2010)

Nobiletin enhances differentiation and lipolysis of 3T3-L1 adipocytes and improves hyperglycemia and insulin resistance in obese (ob) diabetic ob/ob mice. We investigated the effects of nobiletin on lipid metabolism and accumulation of body fat in rats. The control group was fed a 20% high-fat diet and 1% cholesterol, and the nobiletin group was fed same diet supplemented with 0.1% (w/w) nobiletin. The rats were fed for 4 weeks. Weights of epididymal, perirenal, total white adipose tissues (WAT: mesenteric, perirenal, and epididymal), and the subcutaneous WAT in the nobiletin group were significantly lower than those in the control group. This decrease was brought about by nobiletin without affecting triglyceride (TG) levels in the liver and skeletal muscle. Plasma TG levels tended to be decreased by nobiletin. The size and diameter of WAT adipocytes in the nobiletin group were significantly lower than those in the control group. This decrease may be partly due to lower lipoprotein lipase (a major determinant for the development of obesity) levels in WAT of the nobiletin group than that of the control group. Plasma levels of high density lipoprotein cholesterol and apolipoprotein A-I increased significantly with administration of nobiletin. These results suggested a beneficial effect of nobiletin on lipid metabolism. However, no significant differences were observed between the nobiletin and the control groups in proteins such as ATP-binding cassette transporter A1, and sterol regulatory element-binding protein-1 in the liver, PPARγ and tumor necrosis factor-α (TNF-α) in WAT, and adiponectin and TNF-α in plasma.

Key words —— nobiletin, high density lipoprotein, cholesterol, apolipoprotein A-I, white adipose tissue, triglyceride

INTRODUCTION

Metabolic syndrome is a serious public health concern. It is a multiple risk factor for atherosclerotic cardiovascular disease and is characterized by atherogenic dyslipidemia, glucose intolerance, elevated blood pressure, and proinflammatory states.[1)] However, many food components are believed to retard metabolic syndrome progression. These components also include 4000 types of plant flavonoids, and the physiological effects of these flavonoids on metabolic syndrome progression have been studied.[2)] The *in vitro* anti-metabolic syndrome property that reduces insulin resistance has been reported for the flavonoid puerarin.[3)] In addition, we showed that propolis containing a variety of flavonoids decreased body fat, plasma cholesterol, and plasma triglycerides (TGs) in rats fed a high-fat diet.[4)]

Nobiletin (5,6,7,8,3′,4′-hexamethoxyflavone) is a polymethoxylated flavonoid (structure is shown in Fig. 1) found in *Citrus depressa* (shiikuwasa) of the citrus. Nobiletin is more easily absorbed by Caco-2 cells than other polyhydroxyflavonoids.[5)] It also has beneficial anti-inflammatory,[6–8)] and anti-carcinogenic[7,8)] properties and has shown neuroprotective effects against ischemia[9)] or in model animals of Alzheimer's disease.[10,11)] A metabolite of nobiletin, 3′,4′-demethylnobiletin, has anti-inflammatory and anti-tumor promotional effects.[12,13)] In addition, nobiletin enhances differentiation and lipolysis of 3T3-L1 adipocytes[14)] and improves hyperglycemia and insulin resistance in obese diabetic obese (ob)/ob mice.[15)]

In the present study, we investigated the effects of nobiletin on lipid metabolism and accumulation of body fat in rats fed a high-fat diet. We also exam-

*To whom correspondence should be addressed: Department of Food Science and Nutrition, Nara Women's University, Nara 630–8506, Japan. Tel. & Fax: +81-742-20-3459; E-mail: kojo@cc.nara-wu.ac.jp

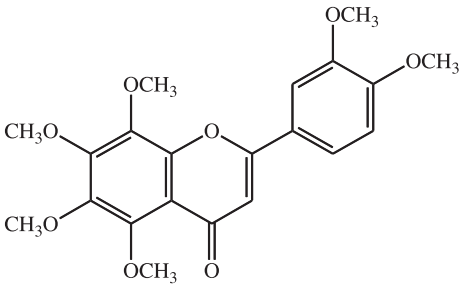

Fig. 1. Nobiletin

ined the effect of nobiletin on the proteins involved in lipid metabolism.

MATERIALS AND METHODS

Animals and Diets —— This study was approved by the Animal Care Committee of Nara Women's University. Three-week-old male rats (SLC: S.D. strain) were obtained from Japan SLC Co. (Shizuoka, Japan). The animals were housed in a room at 24 ± 2°C, with a 12/12 hr light-dark cycle. A standard diet was formulated according to the AIN-93G formula.[16] The composition of this diet is shown in Table 1. The rats were randomly divided into 2 groups of 6 each. The control group was fed a 20% high-fat and 1% cholesterol diet, and the nobiletin group was fed the same diet supplemented with 0.1% (w/w) nobiletin as shown in Table 1. Nobiletin was purified according to a previous study.[17] The rats were fed *ad libitum* for 4 weeks after which they were starved for 6 hr before being sacrificed.

Analytical Methods —— The rats were anesthetized with diethyl ether and sacrificed by collecting the blood from the inferior vena cava using a syringe containing sodium heparin as an anticoagulant. After perfusion of ice-cooled saline through the portal vein, the liver, adipose tissues, and skeletal muscles were dissected out.

Plasma cholesterol, high density lipoprotein (HDL) cholesterol, TGs, free fatty acids (FFA), and glucose were measured using commercially available diagnostic kits (Wako Chem., Osaka, Japan). TG in the liver and skeletal muscle were also measured using commercially available diagnostic kits (Wako Chem.). Cholesterol levels in the liver were measured by gas-liquid chromatography (GC-2014, Shimadzu, Kyoto, Japan) using 5α-cholestane as an internal standard.[18]

Western Blot Analysis —— To determine levels of peroxisome proliferator-activated receptor-γ (PPARγ), 0.2 g of adipose tissue was homogenized in 6 volumes of solubilizing buffer [1% Triton X-100, 100 mM Tris-HCl (pH 7.4), 100 mM sodium orthovanadate, 2.0 mM phenylmethylsulfonyl fluoride, and 0.1 μg/ml aprotinin] on ice. The homogenates were centrifuged at 9000 g for 15 min at 4°C. Analysis of PPARα protein levels in the liver was performed as described previously.[19]

Table 1. Composition of Test Diets

Ingredient	Control	Nobiletin
	g/kg diet	
β-Corn starch	257.5	256.5
Casein	200	200
α-Corn starch	132	132
Sucrose	100	100
Cellulose	50	50
Lard	200	200
Mineral mixture[a]	35	35
Vitamin mixture[a]	10	10
Choline bitartrate	2.5	2.5
L-cystine	3	3
tert-butylhydroquinone	0.014	0.014
Cholesterol	10	10
Nobiletin	0	1.0

a) AIN-93G formulation.

Levels of sterol regulatory element-binding protein-1 (SREBP-1) in nuclear and microsomal fractions of the liver were determined as described previously.[20] To determine lipoprotein lipase (LPL) levels, adipose tissue and skeletal muscle were extracted as described previously.[21] Plasma apolipoprotein A-I (apoA-I) levels were determined as reported previously.[22] Levels of ATP binding cassette transporter A1 (ABCA1) protein were determined as described previously.[23] Levels of tumor necrosis factor-α (TNF-α) in adipose tissue were determined as described previously.[24] Protein concentrations were determined according to the method of Lowry *et al.*[25] using bovine serum albumin (BSA) as the standard.

Protein samples were electrophoresed on sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) gels. After electrophoresis, the proteins were transferred to BioTrace NT membranes (Pall Gelman Laboratory, Ann Arbor, MI, U.S.A.). After blotting and treatment with blocking buffer containing 0.1% Tween-20 and 5% skimmed milk for 1 hr at room temperature, the membranes were incubated with pertinent primary antibodies and dilution buffer containing 0.1% Tween-20 and 5% BSA overnight at 4°C. The primary antibodies

used were anti-LPL (1 : 500), anti-PPARα (1 : 100), anti-PPARγ (1 : 500), anti-SREBP-1 (1 : 500), anti-apoA-I (1 : 500), and anti-TNF-α (1 : 500, dilution rates are shown in parenthesis). These primary antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, U.S.A.). Anti-ABCA1 (1 : 2000) monoclonal antibody (HJ1) was purchased from Novus Biol. Inc. (Littleton, CO, U.S.A.).

The membranes were incubated with horseradish peroxidase-conjugated secondary antibody, which was anti-rabbit, anti-mouse, or anti-goat immunoglobulin G for 1 hr at room temperature. Chemiluminescence was recorded using a cooled charge coupled device (CCD) camera system (Type AE-6972, ATTO Co. Ltd, Tokyo, Japan) and analyzed using the ATTO Densitograph Software Library, CS Analyzer Ver2.0 (Tokyo, Japan).

ELISA —— Plasma adiponectin levels were determined using the ELISA kit (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan). Plasma TNF-α levels were determined using the Endogen rat ELISA kit (Thermo Fisher Scientific Co., Rockford, IL, U.S.A.).

Histology of Adipose Tissue —— The histology of adipose tissue was determined by staining with oil red O.

Statistical Analysis —— The data were expressed as means ± S.E. Differences between the control and the nobiletin groups were considered significant at $p < 0.05$ using the Student's t-test.

RESULTS

Effect of Nobiletin on White Adipose Tissues (WAT)

After 4 weeks of feeding, no significant difference was observed in body weight between the control and nobiletin groups (Table 2). Daily food consumption did not significantly differ between the 2 groups (data not shown). However, weights of epididymal, perirenal, total WAT (mesenteric, perirenal, and epididymal), and subcutaneous WAT in the nobiletin group were significantly lower than those in the control group (Table 2). The size and diameter of mesenteric WAT in the nobiletin group were significantly lower than those in the control group (Fig. 2).

Effect of Nobiletin on Lipids in the Plasma, the Liver, and Skeletal Muscle

Liver weight of the control group was

Table 2. Effect of Nobiletin on Body Weight, and WAT Weight

	Control	Nobiletin
Body weight at start (g)	110 ± 3.4	111 ± 3.0
Body weight after 4 weeks (g)	305.19 ± 3.50	294.92 ± 9.75
Epididymal WAT weight (g)	4.78 ± 0.29	3.06 ± 0.20*
Mesenteric WAT weight (g)	4.39 ± 0.26	3.62 ± 0.41
Perirenal WAT weight (g)	6.96 ± 0.32	4.17 ± 0.39*
Total WAT weight (g)	16.1 ± 0.41	10.84 ± 0.89*
Subcutaneous WAT weight (g)	18.3 ± 0.59	13.6 ± 0.99*

The nobiletin group was supplemented with 0.1% (w/w) nobiletin for 4 weeks. Values are means ± S.E. for 6 rats per group. Asterisks indicate significant difference from the control group at $p < 0.05$.

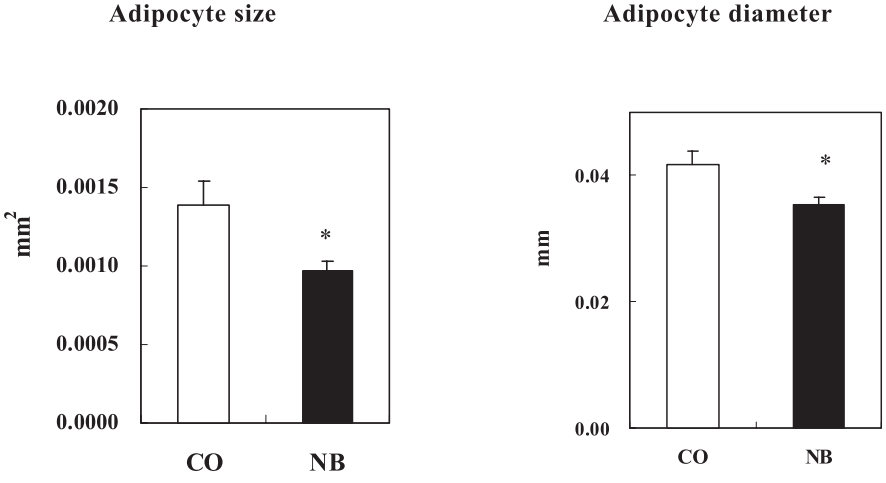

Fig. 2. Effect of Nobiletin on Adipocyte Size (mm^2) and Diameter (mm)

The nobiletin group was supplemented with 0.1% (w/w) nobiletin for 4 weeks. Adipose tissue was stained with oil red O. The nobiletin group was designated as NB and the control group was designated as CO. Values are means ± S.E. for 6 rats per group. Asterisks demonstrate significant difference from the control group at $p < 0.05$.

Table 3. Effect of Nobiletin on the Plasma, the Liver, and Muscle

	Control	Nobiletin
Plasma		
Cholesterol (mg/dl)	91.2 ± 7.7	117.3 ± 11.5 ($p = 0.089$)
TG (mg/dl)	155 ± 23.1	104 ± 13.8 ($p = 0.084$)
HDL cholesterol (mg/dl)	33.4 ± 1.7	65.2 ± 6.2*
Glucose (mg/dl)	199 ± 9.4	189 ± 9.1
FFA (mEq/l)	0.58 ± 0.05	0.53 ± 0.05
Liver		
Cholesterol (mg/g)	78.6 ± 18.1	49.2 ± 6.5
TG (mg/g)	50.9 ± 7.9	49.3 ± 3.5
Muscle		
TG (mg/g)	8.55 ± 1.7	6.39 ± 0.9

The nobiletin group was supplemented with 0.1% (w/w) nobiletin for 4 weeks. Values are means ± S.E. for 6 rats per group. Asterisk indicates significant difference from the control group at $p < 0.05$.

18.3 ± 0.75 g, which was not significantly different from that of the nobiletin group (16.9 ± 0.80 g). In the nobiletin group, plasma cholesterol levels tended to be higher and plasma TG levels tended to be lower than those in the control group (Table 3). The nobiletin group showed significantly increased plasma HDL cholesterol levels compared to the control group. No significant difference was observed in liver cholesterol content between the nobiletin and control groups. Plasma glucose and FFA levels were not significantly different between the 2 groups (Table 3). In the liver and muscle, no significant difference was observed in TG levels between the nobiletin and control groups (Table 3).

Effect of Nobiletin on Proteins Involved in Lipid Metabolism

Plasma apoA-I levels in the nobiletin group were significantly higher than those in the control group (Fig. 3). Plasma adiponectin levels were not different between the control and nobiletin groups (data not shown). ABCA1 protein levels in the liver of the nobiletin group were not significantly different from those of the control group (Fig. 4). PPARα protein levels in the liver of the nobiletin group were significantly lower than those of the control group (Fig. 5), while no difference was observed in PPARγ levels in adipose tissue between these groups (data not shown). The nobiletin group showed significantly lower LPL levels in adipose tissue compared to the control group (Fig. 6). The muscle LPL protein level of the nobiletin group was also significantly decreased compared to that in the control

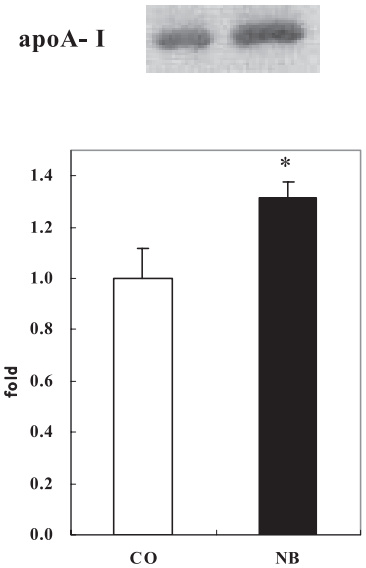

Fig. 3. Effect of Nobiletin on apoA-I Levels in Plasma

The nobiletin group was supplemented with 0.1% (w/w) nobiletin for 4 weeks. Values are normalized relative to the control group. The nobiletin group was designated as NB and the control group was designated as CO. Values are means ± S.E. for 6 rats per group. Asterisks indicate significant difference from the control group at $p < 0.05$.

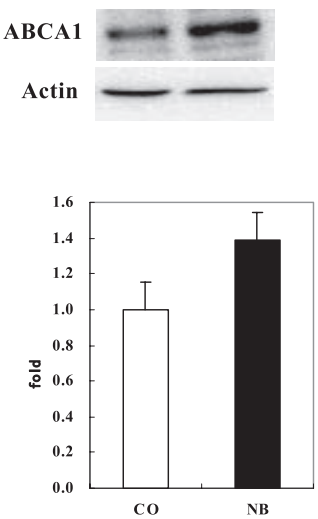

Fig. 4. Effect of Nobiletin on ABCA1 Protein Levels in the Liver

The nobiletin group was supplemented with 0.1% (w/w) nobiletin for 4 weeks. Values are normalized relative to the control group. The nobiletin group was designated as NB and the control group was designated as CO. Values are means ± S.E. for 6 rats per group.

group (data not shown). No significant difference was observed in SREBP-1 levels either in the microsomal or nuclear fractions of the liver between these 2 groups (data not shown). No significant difference was observed in the TNF-α level of plasma or adipose tissue between the nobiletin and control groups (data not shown).

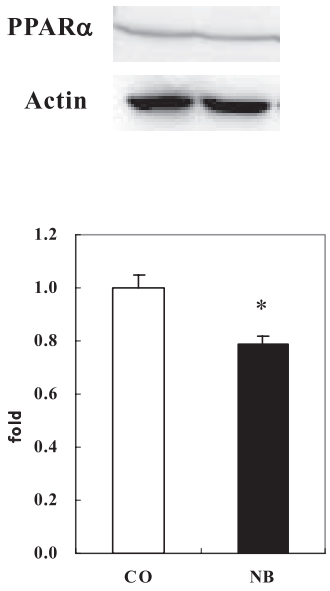

Fig. 5. Effect of Nobiletin on PPARα Protein Level in the Liver

The nobiletin group was supplemented with 0.1% nobiletin (w/w) for 4 weeks. Values are normalized relative to the control group. The nobiletin group was designated as NB and the control group was designated as CO. Values are means ± S.E. for 6 rats per group. Asterisks demonstrate significant difference from the control group at $p < 0.05$.

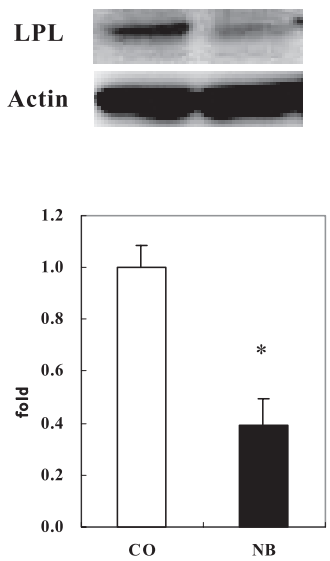

Fig. 6. Effect of Nobiletin on LPL Levels in Adipose Tissue

The nobiletin group was supplemented with 0.1% (w/w) nobiletin for 4 weeks. Values are normalized relative to the control group. The nobiletin group was designated as NB and the control group was designated as CO. Values are means ± S.E. for 6 rats per group. Asterisks indicate significant difference from the control group at $p < 0.05$.

DISCUSSION

Weight of WAT and the size and diameter of WAT adipocytes in the nobiletin group were significantly lower than those in the control group. This decrease was brought about by nobiletin without affecting TG levels in the liver and skeletal muscle. The decrease may be partly due to lower LPL (a major determinant for the development of obesity[26]) levels in WAT of the nobiletin group than that of the control group.

PPARγ is expressed predominantly in the adipose tissue and is associated with adipocyte differentiation.[27,28] Nobiletin induced a significant increase in PPARγ2 mRNA levels and differentiation of 3T3-L1 adipocytes[14] and ST-13 preadipocytes.[29] Nobiletin increased PPARγ mRNA levels in WAT of obese diabetic ob/ob mice.[15] In the present study, PPARγ protein levels were not affected by nobiletin, suggesting that PPARγ was not involved with decrease in WAT weight. Along with the difference in animal species, this variance may be explained by difference in nobiletin dose administered. Nobiletin was administered at approximately 80 mg/kg in the present study, whereas at 200 mg/kg in a previous study.[15] We selected the dose of 80 mg/kg for rats because this dose was the upper limit that did not elevate plasma level of aspartate aminotransferase (a marker of liver damage) levels.

Nobiletin enhanced adiponectin secretion in ST-13 preadipocytes,[29] and administration of a high dose of nobiletin to obese diabetic ob/ob mice caused an increase in plasma adiponectin levels without decreasing TNF-α mRNA levels in WAT.[15] Although WAT weight and adipocyte size were significantly decreased by nobiletin in this study, the decrease was not accompanied by an increase in plasma adiponectin levels and decrease in TNF-α levels of adipose tissue and plasma. These results showed that a low dose of nobiletin did not affect the production of these adipocytokines and inflammation in adipocyte.

Plasma levels of HDL cholesterol and apoA-I increased significantly with administration of nobiletin. These results suggest a beneficial effect of nobiletin on lipid metabolism, because HDL plays an important role in hyperlipidemia treatment as the carrier of reverse cholesterol transport. This increase in HDL cholesterol appeared to contribute to higher plasma cholesterol levels in the nobiletin group than in the control group. Although ABCA1 plays a key role in the formation of HDL in the liver,[30] nobiletin did not increase the levels of this protein in the liver. The mechanism by which nobiletin increases HDL remains to be explored.

Levels of liver SREBP-1, which regulates the transcription of genes involved in fatty acid syn-

thesis,[31] were not changed by nobiletin and those of PPARα, which promotes fatty acid oxidation,[32] were decreased by nobiletin. The role played by the liver in the effect that nobiletin has on WAT is unclear at present. This is different from the case of propolis, which exhibits hypolipidemic effects in rats fed 0.5% propolis through a decrease in levels of PPARγ in WAT and SREBP-1 in the liver as well as an increase in PPARα levels in the liver.[4]

In conclusion, nobiletin (0.1% in diet) administered to rats significantly reduced WAT weight and adipocyte size, partly by lowering LPL. In addition, nobiletin significantly increased HDL cholesterol and apoA-I levels. In spite of these clear effects, no significant differences were observed between the nobiletin and control groups in proteins such as ABCA1, SREBP-1, and PPARα in the liver, PPARγ and TNF-α in WAT, and adiponectin and TNF-α in plasma.

Acknowledgements This work was supported by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology, Japan and by Nara women's University Intramural Grant for Project Research (2009).

REFERENCES

1) Grundy, S. M. (2008) Metabolic syndrome pandemic. *Arterioscler. Thromb. Vasc. Biol.*, **28**, 629–636.
2) Middleton, E., Jr., Kandaswami, C. and Theoharides, T. C. (2000) The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. *Pharmacol. Rev.*, **52**, 673–751.
3) Xu, M. E., Xiao, S. Z., Sun, Y. H., Zheng, X. X., Ou-Yang, Y. and Guan, C. (2005) The study of anti-metabolic syndrome effect of puerarin in vitro. *Life Sci.*, **77**, 3183–3196.
4) Ichi, I., Hori, H., Takashima, Y., Adachi, N., Kataoka, R., Okihara, K., Hashimoto, K. and Kojo, S. (2009) The beneficial effect of propolis on fat accumulation and lipid metabolism in rats fed a high-fat diet. *J. Food Sci.*, **74**, H127–H131.
5) Murakami, A., Kuwahara, S., Takahashi, Y., Ito, C., Furukawa, H., Ju-Ichi, M. and Koshimizu, K. (2001) In vitro absorption and metabolism of nobiletin, a chemopreventive polymethoxyflavonoid in citrus fruits. *Biosci. Biotechnol. Biochem.*, **65**, 194–197.
6) Akachi, T., Ahiina, Y., Ohishi, Y., Kawaguchi, T., Kawagishi, H., Morita, T., Mori, M. and Sugiyama, K. (2010) Hepatoprotective effects of flavonoids from Shekwasha (*Citrus depressa*) against D-galactosamine-induced liver injury in rats. *J. Nutr. Sci. Vitaminol.* (Tokyo), **56**, 60–67.
7) Murakami, A., Nakamura, Y., Torikai, K., Tanaka, T., Koshiba, T., Koshimizu, K., Kuwahara, S., Takahashi, Y., Ogawa, K., Yano, M., Tokuda, H., Nishino, H., Mimaki, Y., Sashida, Y., Kitanaka, S. and Ohigashi, H. (2000) Inhibitory effect of citrus nobiletin on phorbol ester-induced skin inflammation, oxidative stress, and tumor promotion in mice. *Cancer Res.*, **60**, 5059–5066.
8) Miyamoto, S., Yasui, Y., Ohigashi, H., Tanaka, T. and Murakami, A. (2010) Dietary flavonoids suppress azoxymethane-induced colonic preneoplastic lesions in male C57BL/KsJ-db/db mice. *Chem. Biol. Interact.*, **183**, 276–283.
9) Yamamoto, Y., Shioda, N., Han, F., Moriguchi, S., Nakajima, A., Yokosuka, A., Mimaki, Y., Sashida, Y., Yamakumi, T., Ohizumi, Y. and Fukunaga, K. (2009) Nobiletin improves brain ischemia-induced learning and memory deficits through stimulation of CaMKII and CREB phosphorylation. *Brain Res.*, **1295**, 218–229.
10) Matsuzaki, K., Yamakuni, T., Hashimoto, M., Haque, A. M., Shido, O., Mimaki, Y., Sashida, Y. and Ohizumi, Y. (2006) Nobiletin restoring β-amyloid-impaired CREB phosphorylation rescues memory deteriolation in Alzheimer's disease model rats. *Neurosci. Lett.*, **400**, 230–234.
11) Onozuka, H., Nakajima, A., Matsuzaki, K., Shin, R.-W., Ogino, K., Saigusa, D., Tetsu, N., Yokosuka, A., Sashida, Y., Mimaki, Y., Yamakuni, T. and Ohizumi, Y. (2008) Nobiletin, a citrus flavonoid, improves memory impairmant and Aβ pathology in a transgenic mouse model of Alzheimer's disease. *J. Pharmacol. Exp. Ther.*, **326**, 739–744.
12) Li, S., Sang, S., Pan, M. H., Lai, C. S., Lo, C. Y., Yang, C. S. and Ho, C. T. (2007) Anti-inflammatory property of the urinary metabolites of nobiletin in mouse. *Bioorg. Med. Chem. Lett.*, **17**, 5177–5181.
13) Lai, C.-S., Li, S., Chai, C.-Y., Lo, C.-Y., Dushenkov, S., Ho, C.-T., Pan, M.-H. and Wang, Y.-J. (2008) Anti-inflammatory and antitumor promotional effects of a novel urinary metabolite, 3′,4′-didemethylnobiletin, derived from nobiletin. *Carcinogenesis*, **29**, 2415–2424.
14) Saito, T., Abe, D. and Sekiya, K. (2007) Nobiletin enhances differentiation and lipolysis of 3T3-L1 adipocytes. *Biochem. Biophys. Res. Comm.*, **357**, 371–376.
15) Lee, Y.-S., Cha, B. Y., Saito, K., Yamakawa, H.,

Choi, S. S., Yamaguchi, K., Yonezawa, T., Teruya, T., Nagai, K. and Woo, J.-T. (2010) Nobiletin improves hyperglycemia and insulin resistance in obese diabetic ob/ob mice. *Biochem. Pharmacol.*, **79**, 1674–1683.

16) Reeves, P. G., Nielsen, F. H. and Fahey, G. C., Jr. (1993) AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J. Nutr.*, **123**, 1939–1951.
17) Kawaii, S., Tomono, Y., Katase, E., Ogawa, K. and Yano, M. (1999) HL-60 differentiating activity and flavonoid content of the readily extractable fraction prepared from citrus juices. *J. Agric. Food Chem.*, **47**, 128–135.
18) Ikeda, I., Tanaka, K., Sugano, M., Vahouny, G. V. and Gallo, L. L. (1988) Discrimination between cholesterol and sitosterol for absorption in rats. *J. Lipid Res.*, **29**, 1583–1591.
19) Vidal-Puig, A., Jimenez-Liñan, M., Lowell, B. B., Hamann, A., Hu, E., Spiegelman, B., Flier, J. S. and Moller, D. E. (1966) Regulation of PPAR gamma gene expression by nutrition and obesity in rodents. *J. Clin. Invest.*, **97**, 2553–2561.
20) Botolin, D. and Jump, D. B. (2003) Selective proteolytic processing of rat hepatic sterol regulatory element binding protein-1 (SREBP-1) and SREBP-2 during postnatal development. *J. Biol. Chem.*, **278**, 6959–6962.
21) Garcia, E., Lacasa, M., Agli, B., Giudicelli, Y. and Lacasa, D. (1997) Modulation of rat preadipocyte adipose conversion by androgenic status: involvement of C/EBPs transcription factors. *J. Endocrinol.*, **161**, 89–97.
22) Strauss, J. G., Frank, S., Kratky, D., Hämmerle, G., Hrzenjak, A., Knipping, G., von Eckardstein, A., Kostner, G. M. and Zechner, R. (2001) Adenovirus-mediated rescue of lipoprotein lipase-deficient mice. Lipolysis of triglyceride-rich lipoproteins is essential for high density lipoprotein maturation in mice. *J. Biol. Chem.*, **276**, 36083–36090.
23) Lee, J., Timmins, J. M., Mulya, A., Smith, T. L., Zhu, Y., Rubin, E. M., Chisholm, J. W., Colvin, P. L. and Parks, J. S. (2005) HDLs in apoA-I transgenic Abca1 knockout mice are remodeled normally in plasma but are hypercatabolized by the kidney. *J. Lipid Res.*, **46**, 2233–2245.
24) Liu, R. H., Mizuta, M., Kurose, T. and Matsukura, S. (2002) Early events involved in the development of insulin resistance in Zucker fatty rat. *Int. J. Obes. Relat. Metab. Disord.*, **26**, 318–326.
25) Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**, 265–275.
26) Voshol, P. J., Rensen, P. C. N., van Dijk, K. W., Romijn, J. A. and Hevekes, L. M. (2009) Effect of plasma triglyceride metabolism on lipid metabolism on lipid storage in adipose tissue: Studies using genetically engineered mouse models. *Biochim. Biophys. Acta*, **1791**, 479–485.
27) Takano, H. and Komuro, I. (2009) Peroxisome proliferator-activated receptor γ and cardiovascular diseases. *Circ. Res.*, **73**, 214–220.
28) White, U. A. and Stephens, J. M. (2010) Transcriptional factors that promote formation of white adipose tissue. *Mol. Cell. Endocrinol.*, **318**, 10–14.
29) Kunimasa, K., Kuranuki, S., Matsuura, N., Iwasaki, N., Ikeda, M., Ito, A., Sashida, Y., Mimaki, Y., Yano, M., Sato, M., Igarashi, Y. and Oikawa, T. (2009) Identification of nobiletin, a polymethoxyflavonoid, as an enhancer of adiponectin secretion. *Bioorg. Med. Chem. Lett.*, **19**, 2062–2064.
30) Oram, J. F. and Vaughan, A. M. (2006) ATP-binding cassette cholesterol transporters and cardiovascular disease. *Circ. Res.*, **99**, 1031–1043.
31) Shimano, H. (2000) Sterol regulatory element-binding protein-1 as a dominant transcription factor for gene regulation of lipogenic enzymes in the liver. *Trends Cardiovasc. Med.*, **10**, 275–278.
32) Ferre, P. (2004) The biology of peroxisome proliferator-activated receptors. Relationship with lipid metabolism and insulin sensitivity. *Diabetes*, **53** (Suppl. 1), S43–S50.