

Nobiletin, a citrus flavonoid isolated from tangerines, selectively inhibits class A scavenger receptor-mediated metabolism of acetylated LDL by mouse macrophages

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Abstract

Flavonoids are a class of chemically related polyphenols that are nearly ubiquitous in nature. Of the more-than 4000 flavonoids thus identified, citrus fruit-derived flavonoids are suggested to have an inverse association with the occurrence of coronary heart disease via their ability to reduce plasma cholesterol concentrations. Our current studies examined whether citrus flavonoids possess an additional antiatherogenic effect by modulating macrophage metabolism of the specific class A scavenger receptor (SR-A) ligand, acetylated LDL (acLDL). In this study, both acLDL-metabolism and SR-A expression by cultured murine J774A.1 macrophages was examined following 24 h pretreatment (100 μ M) with the flavonoids: naringenin (from grapefruit), hesperetin (from oranges), and tangeretin and nobiletin (from tangerines). Of these flavonoids, only nobiletin inhibited (50–72%) acLDL metabolism as measured by both cellular cholesterol ester mass and [³H]oleate incorporation into cholesterol esters. This nobiletin-mediated effect was specific for SR-A and not a global effect on lipoprotein metabolism by the macrophage, as all four citrus flavonoids significantly reduce the metabolism of beta-VLDL, which is primarily taken up by macrophages via the LDL receptor. Nevertheless, nobiletin did not affect SR-A protein expression, as measured by Western blot analysis, nor was cell surface expression of SR-A affected as measured by 4 °C binding studies using [¹²⁵I]acLDL. In conclusion, our findings suggest that in addition to reducing plasma cholesterol concentrations, nobiletin may prevent atherosclerosis at the level of the vascular wall by inhibiting macrophage foam-cell formation.

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1. Introduction

Flavonoids are a class of chemically related polyphenols of plant origin that are nearly ubiquitous in nature and that also exhibit a broad spectrum of pharmacological properties [1–3]. Of the more-than 4000 naturally occurring

flavonoids thus identified, citrus fruit-derived flavonoids and their metabolites have been shown to impart important protective biological action including anticancer [1–3], anti-inflammatory [2,3] and antiatherogenic [1,3] activities. Flavonoids are polyphenolic compounds having a basic 15-carbon skeleton consisting of two benzene rings joined by a linear three-carbon chain, and can be represented as C6–C3–C6. Flavonoids from citrus fruits have a benzo-gamma-pyrone derivative at the C3 position, and these particular flavonoids belong largely to two classes named flavanones and flavones. The most prevalent flavanones are

Abbreviations: SR-A, class A scavenger receptor; LDL, low-density lipoprotein; acLDL, acetylated LDL; PMFs, polymethoxylated flavones

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hesperetin from oranges and naringenin from grapefruit; both found in the fruit tissue and peel largely as their glycosides, hesperidin and naringin. Relatively common in citrus are also two polymethoxylated flavones (PMFs), tangeretin and nobiletin, both present in tangerines sweet orange peel (*Citrus sinensis*) and in bitter orange peel (*Citrus aurantium*) [4].

Strong *in vivo* [5] and *in vitro* [6,7] evidence now exists to indicate that citrus flavonoids could reduce the occurrence of cardiovascular disease through their ability to reduce hepatic production of cholesterol containing lipoproteins [6], and hence reduce total plasma cholesterol concentrations. Cardiovascular disease resulting from atherosclerosis is the leading cause of morbidity and mortality in Westernized countries. Atherosclerotic lesions form *de novo* from a focal accumulation of lipoproteins, monocyte-derived macrophages and lymphocytes within the wall of arterial blood vessels. Within this space, lymphocyte signaling promotes the accumulation of cholesterol by intimal-associated macrophages. The source of this cholesterol is lipoprotein particles that pass from the blood into the vessel wall and are subsequently modified by oxidation. Over time, this process culminates in the formation of large structurally unstable lesions that are prone to rupture, thrombus formation, occlusion of the affected vessel and the death of tissue distal to the blockage.

Thus, the primary cellular event that drives early atherosclerotic lesion formation is the unregulated accumulation of cholesterol ester by intimal-associated macrophages. Internalization of lipoproteins by macrophages is a complex process that begins when lipoproteins interact with specific cell surface receptors and these lipoprotein–receptor complexes become absorbed by the cell via clathrin-coated vesicles [8–10]. Cell surface receptors that mediate lipoprotein uptake differ in their specificity for lipoprotein particles. The low-density lipoprotein (LDL) receptor binds LDL [10] while class A scavenger receptors (SR-A) preferentially bind chemically modified LDL (e.g. oxidized LDL). Macrophages express both of these receptors. However, unlike with native LDL, incubation of macrophages with modified LDL results in unregulated cholesterol accumulation and “foam cell” formation [11]. Thus, SR-A-mediated internalization of modified LDL is considered a key atherogenic process.

In addition to the noted cholesterol lowering potential imparted to citrus flavonoids, we wanted to examine whether citrus flavanones and PMFs have other beneficial effects at the level of the vascular wall by affecting the process of macrophage-derived foam cell formation. As a marker for SR-A mediated cholesterol accumulation by macrophages, we examined the ability of four citrus flavonoids to modulate the metabolism of acetylated LDL (acLDL), a modified lipoprotein that will induce macrophage foam cell formation [12,13] and that has previously been shown to bind exclusively to SR-A expressed on cultured macrophages [14].

2. Materials and methods

2.1. Chemicals

Dulbecco's modified Eagle's medium (DMEM) with L-glutamine and high glucose, DMEM with 25 mM HEPES but without sodium bicarbonate, and heat-inactivated fetal bovine serum (FBS) were purchased from GibcoBRL (Grand Island, NY). Penicillin, streptomycin, hesperetin and naringenin were purchased from Sigma (St. Louis, MO). The purified tangeretin and nobiletin were obtained as described previously [15]. Stock solutions of each flavonoid were made in dimethyl sulfoxide (DMSO). Na[¹²⁵I] and [³H]oleate were obtained from Amersham (Piscataway, NJ).

2.2. Lipoprotein isolation, acetylation, and radioiodination

LDL ($d = 1019$ – 1063 g/L) was isolated by sequential ultracentrifugation [16] of EDTA-anticoagulated plasma obtained from healthy normolipidemic volunteers. LDL was dialyzed against saline containing 1 mM EDTA (pH 7.4). acLDL was prepared by chemical modification of the LDL with acetic anhydride as described by Basu et al. [17] and confirmed by comparing the relative electrophoretic mobility of acLDL to native LDL on a 1% agarose gel. Beta-VLDL ($d = 1006$ – 1019 g/L) was isolated by sequential ultracentrifugation of EDTA-anticoagulated plasma obtained from 12 h fasted male New Zealand White rabbits (Myrtles, TN) maintained on a cholesterol-enriched diet (1% (w/w) cholesterol; Purina test diet 5731-6, Richmond, IN). Lipoprotein preparations were sterilized by passage through 0.22 μ m filters and stored at 4 °C. Lipoprotein samples were analyzed for protein content as described by Markwell et al. [18] and for cholesterol using a commercially available kit (WAKO). acLDL was radiolabeled using an indirect labeling method with Na[¹²⁵I] using IODO-GEN7 pre-coated tubes (Pierce Chemical Co., Rockford, IL) following manufacture's instructions; in this method, the scavenging wash step was omitted and the radioiodination reaction was terminated by passing the sample over a desalting column (Bio-gel P6DG, Pierce Chemical Co.).

2.3. Cell culture

J774A.1 cells, a murine macrophage cell line, was obtained from American Type Culture Collection (Manassas, VA) and were maintained in DMEM containing penicillin (10 U/mL), streptomycin (10 mg/L) and 10% FBS.

2.4. Cholesterol esterification assay

The incorporation of [³H]oleic acid into cholesterol esters was used as a measure of macrophage-mediated uptake of lipoproteins. Lipoproteins were added to the J774A.1 macrophages and incubated for 5 h in DMEM plus antibi-

otics but no serum. Prior to the addition of lipoproteins, macrophages were treated with working solutions of each of the flavonoids for 24 h. In addition, each well of cells received 0.9 μCi [^3H]oleic acid complexed with fatty acid-free bovine serum albumin (BSA) in a molar ratio of 5:1. The cholesterol esters were analyzed as described previously [19].

2.5. Cellular cholesterol mass assay

For determination of cholesterol mass, adherent macrophages were treated with the various flavonoids for 24 h (control cells received a working solution of DMSO without flavonoids), washed with serum-free DMEM, and incubated for an additional 16 h with the indicated lipoproteins and flavonoids in DMEM supplemented with 5% lipoprotein-deficient serum. Cells were washed with ice-cold Tris buffer (pH 7.4) and lipid was extracted as described above using hexane-isopropanol. Cell proteins were solubilized in 0.5 mL 0.1N NaOH for 16 h at room temperature and protein content was determined as described above. Free and esterified cholesterol content in the lipid extracts were determined as described previously [20]. Briefly, the extracted lipid was solubilized in Triton X-100 containing chloroform and converted to an aqueous suspension. Aliquots were transferred to 96-well microtiter plates and free and esterified cholesterol content was determined (WAKO). Results are expressed as micrograms of cholesterol/milligram of cell protein.

2.6. Detection of SR-A protein

For determination of SR-A protein expression, J774A.1 macrophages were incubated with or without the various flavonoids for 24 h. Incubation medium was removed, cell lysates were prepared in reducing Laemmli buffer, proteins were resolved by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and proteins were transferred to polyvinylidene difluoride. Blots were probed sequentially with an anti-SR-A polyclonal antiserum (Mac 5.2) and anti-beta-actin antibody followed by incubation with species-specific horseradish peroxidase-coupled secondary antibodies. Blots were incubated with a chemiluminescence substrate (Supersignal; Pierce, Rockford, IL) and luminescent bands were detected using a Kodak Image Station 440.

2.7. [^{125}I]acLDL specific binding to macrophages

To quantify lipoprotein binding, J774A.1 macrophages were cultured in 12-well plates in the presence of DMEM plus antibiotics and serum. When the cells were 75% confluent, flavonoids were added and the cell incubated for an additional 24 h. Prior to beginning the binding study, the cell media was replaced with ice-cold DMEM supplemented with 0.5% BSA and 25 mM HEPES; pH 7.4. Following an addi-

tional 0.5 h at 4 °C, [^{125}I]acLDL (0.25–80 mg/L) was added to the cells in the absence or presence of a 20-fold excess of unlabeled acLDL, and the cells were then incubated for an additional 2.5 h at 4 °C. The cells were then washed once with ice-cold buffer A (154 mM NaCl, 42 mM Tris (hydroxymethyl) aminomethane hydrochloride, 8 mM Tris (hydroxymethyl) aminomethane, 0.2% BSA, pH 7.4) and twice with BSA-free buffer A. Cellular protein was solubilized for 16 h at room temperature in 0.5 mL 0.1N NaOH. Radioactivity in the protein extract was determined using a ClinGamma 1272 gamma counter (Wallac Oy, Turku, Finland); cell protein was determined using the Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA) with BSA used to generate a standard curve. The amount of specific binding was calculated by subtracting the amount of [^{125}I]acLDL bound in the presence of a 20-fold excess of unlabeled acLDL from the total amount of [^{125}I]acLDL bound. The results are expressed as nanograms of acLDL bound per milligram cell protein.

2.8. Statistical analysis

Data analysis was performed using SigmaStat 2.03 software (SPSS Inc.). For each parameter, the mean and standard error of mean (S.E.M.) were calculated. Differences between control and experimental groups were evaluated using a one-way ANOVA with all pair wise multiple comparison procedures conducted using the Tukey test. Values with $P < 0.05$ were considered statistically significant.

3. Results

3.1. Determination of the working concentrations of each flavonoid

Preliminary experiments (data not shown) were first conducted using a range of concentrations of each flavonoid (100 nmol/L, 1, 10 and 100 $\mu\text{mol/L}$). In all experiments where an effect was noted, the concentration of 100 $\mu\text{mol/L}$ gave the greatest effect and so this concentration was used in all repeated experiments. We recently showed that the combined content of tangeretin metabolic products in fasting serum from hamsters fed a 1% tangeretin diet was in the range of 20 $\mu\text{mol/L}$ [5] and that higher circulating concentrations of PMFs could be achieved postprandially. Based on this preliminary finding, we feel that the concentrations of flavonoids, particularly those of the PMFs, which were used in these studies, are in the range predicted to be physiologically relevant.

3.2. Effect of flavonoid pretreatment on the metabolism of acLDL by macrophage

Using incorporation of [^3H]oleic acid into cholesterol esters as a measure of macrophage-mediated lipoprotein

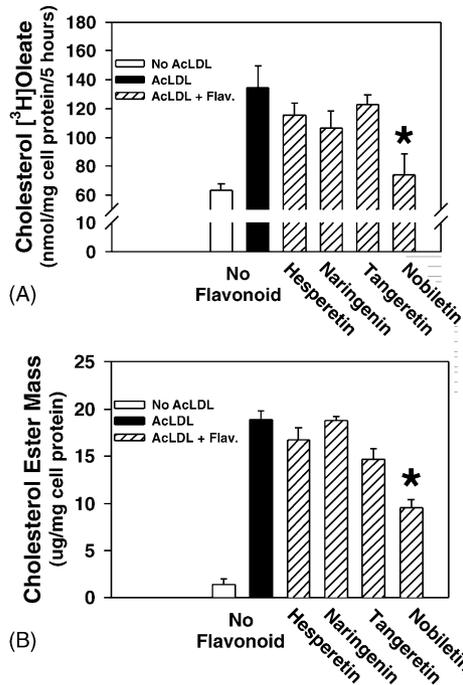


Fig. 1. (A) Only incubation of J774A.1 macrophages with nobiletin significantly reduced the uptake of acLDL as measured by incorporation of [³H]oleate into cellular cholesterol esters; $N = 3$ experiments, with each experiment run in triplicate, $*P < 0.001$. (B) Only incubation of J774A.1 macrophages with nobiletin significantly reduced the uptake of acLDL as measured by determining the cellular mass of esterified cholesterol; $N = 3$ experiments, with each experiment run in triplicate, $*P < 0.001$.

metabolism, we found that compared to cells not treated with flavonoids, only nobiletin pretreatment significantly reduced (72%) acLDL-induced cholesterol ester deposition in J774A.1 macrophages (Fig. 1A). As a second marker of cholesterol loading induced by the incubation of cells with acLDL, we also measured the amount of cholesterol ester mass in cells of control versus flavonoid-treated macrophages. As with the [³H]oleic acid incorporation studies, only nobiletin significantly reduced (50%) cellular cholesterol ester mass (Fig. 1B).

3.3. Effect of flavonoid pretreatment on the metabolism of beta-VLDL by macrophage

To examine whether the nobiletin effect was specific for SR-A, or whether it represented a more global effect on the capacity of macrophages to metabolize lipoproteins in general, we examined the ability of macrophages to metabolize beta-VLDL, a ligand for the LDL receptor, in the absence or presence of flavonoids. Compared to cells not exposed to flavonoids, treatment of J774A.1 macrophages with all four citrus flavonoids caused a significant reduction (40–60%) in cholesterol ester accumulations measured by both [³H]oleic acid incorporation and by cholesterol ester mass in cells incubated with beta-VLDL (Fig. 2A and B).

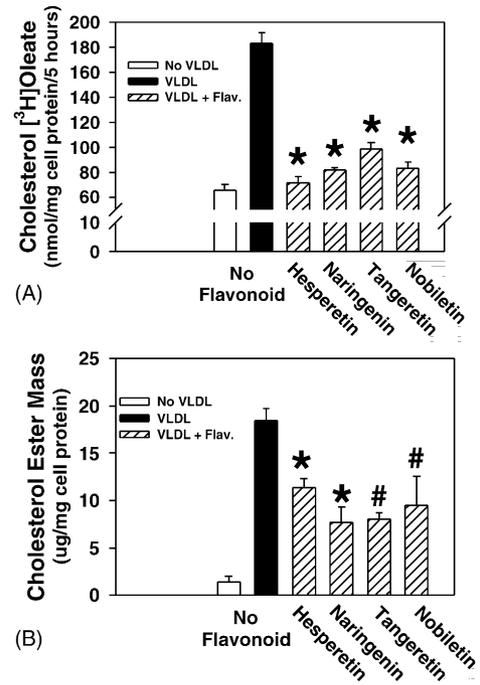


Fig. 2. (A) All four flavonoids each significantly reduced the uptake of beta-VLDL by J774A.1 macrophages as measured by incorporation of [³H]oleate into cellular cholesterol esters; $N = 3$ experiments, with each experiment run in triplicate, $*P < 0.001$. (B) All four flavonoids each significantly reduced the uptake of beta-VLDL as measured by determining the cellular mass of esterified cholesterol; $N = 3$ experiments, with each experiment run in triplicate, $*P < 0.001$, $#P = 0.004$.

3.4. Effect of nobiletin pretreatment on the expression of SR-A by macrophages

One possible explanation for selective decrease in the metabolism of acLDL following nobiletin pretreatment is decreased SR-A expression. To assess this possibility, total cell lysates were prepared from macrophages treated with and without nobiletin and the resulting cellular protein resolved by SDS-PAGE. SR-A was detected by immunoblotting using the SR-A-specific polyclonal antisera Mac 5.2. As shown in Fig. 3A and B, treatment of cells with nobiletin did not alter the relative abundance of immune-detectable SR-A present in J774 cell lysates. The amount of beta-actin present in each cell extract was used to normalize the abundance of SR-A (Fig. 3B). Thus, nobiletin treatment decreases the uptake of acLDL by a mechanism that does not involve decreased expression of SR-A.

3.5. Effect of nobiletin pretreatment on the cell surface expression of SR-A

In light of the Western blot results, a second potential explanation for the decreased uptake of acLDL after nobiletin treatment would be a decrease in the number of acLDL binding sites at the cell surface. To assess this possibility, binding studies were conducted at 4 °C (to prevent ligand internal-

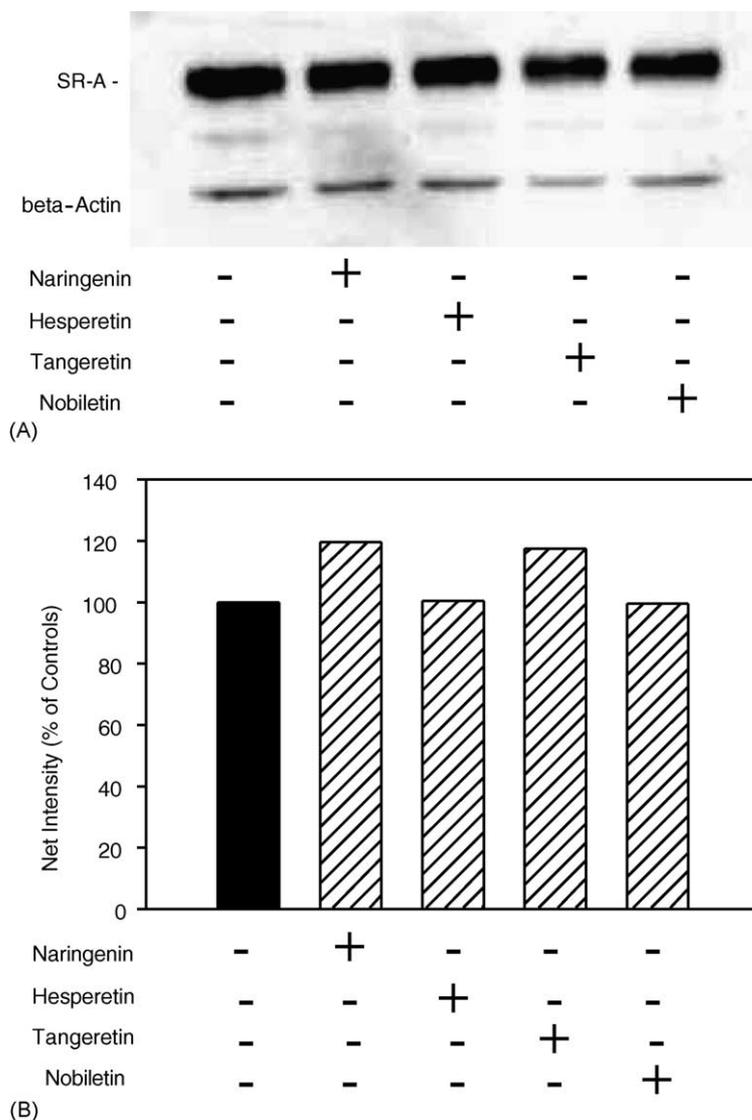


Fig. 3. Treatment of J774A.1 macrophages with nobiletin (100 $\mu\text{mol/L}$) did not alter the relative abundance of immune-detectable SR-A (A and B). SR-A was detected by immunoblotting using the SR-A-specific polyclonal antisera Mac 5.2. The amount of beta-actin present in each cell extract was used to normalize the abundance of SR-A (B). Representative Western blot is shown in (A), with the corresponding net intensity (% of control) graph shown in (B); these blots were repeated a total of three times, with each blot giving similar net intensity (% of control) results.

ization) using [^{125}I]acLDL and J774A.1 macrophages incubated in the absence or presence of nobiletin (100 $\mu\text{mol/L}$). Nobiletin treatment did not enhance the specific binding of acLDL by macrophages at 4 $^{\circ}\text{C}$ (Fig. 4). Thus, nobiletin treatment would appear to decrease the uptake of acLDL by a mechanism that does not involve a decrease in the number of acLDL binding sites at the level of the plasma membrane.

4. Discussion

Citrus flavonoids are hypothesized to reduce the occurrence of coronary heart disease through their ability to reduce plasma cholesterol concentrations [6,21,22]. In addition to lowering plasma cholesterol, we wanted to examine

whether citrus flavonoids could have a beneficial effect at the level of the vascular wall. The primary cellular event that drives early atherosclerotic lesion formation is the unregulated cholesterol ester accumulation leading to macrophage-derived foam cell formation. One of the key cellular receptors responsible for this process is SR-A, which mediates internalization of modified LDL. As a marker for SR-A mediated cholesterol accumulation by macrophages, we examined the ability of four citrus flavonoids to modulate the metabolism of acLDL, a known ligand for SR-A. We found that of the four citrus flavonoids examined, only nobiletin inhibited SR-A mediated metabolism of acLDL, and this occurred without affecting total SR-A protein levels or expression of the receptor on the plasma membrane. These results suggest that nobiletin may prevent atherosclerosis at two levels, firstly by reducing the circulating concentrations of VLDL and LDL in

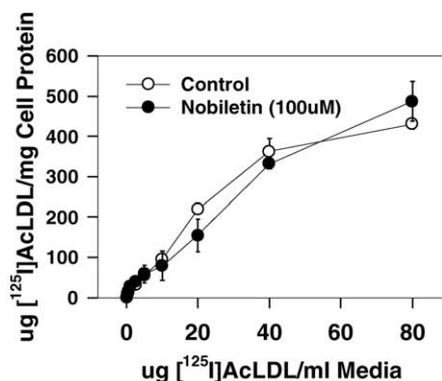


Fig. 4. Nobiletin treatment did not alter the specific binding of acLDL by macrophages; experiments were conducted at 4 °C (to prevent ligand internalization) using [¹²⁵I]acLDL and J774A.1 macrophages incubated in the absence or presence of nobiletin (100 μmol/L). *N* = 3 experiments, with each experiment run in duplicate.

the blood, and secondly by directly inhibiting macrophage-derived foam-cell formation at the site of lesion development within the vessel wall.

Strong *in vivo* [5] and *in vitro* [6,7,21,22] evidence has now demonstrated that the principal citrus flavanones, hesperetin and naringenin, could play a role in diminishing the occurrence of coronary heart disease by reducing hypercholesterolemia, and that this effect was largely related to their ability to inhibit hepatic production of apolipoprotein B-containing lipoproteins such as VLDL and LDL. The citrus PMFs tangeretin and nobiletin have been shown to have an even stronger hepatic apolipoprotein B-lowering potential *in vitro* [7,22] and their hypocholesterolemic effect *in vivo* was also greater than that observed for the hesperetin and naringenin glucosides, hesperidin and naringin [5]. In cultured J774A.1 macrophages, our current studies indicate that all four citrus flavonoid compounds significantly inhibited cellular uptake of beta-VLDL, which is internalized mainly via the LDL receptor. However, only one of these flavonoids,

nobiletin, suppressed macrophage-mediated metabolism of acLDL, which is internalized predominately via the SR-A. These results indicate that not only does nobiletin share the same properties regarding internalization of lipoprotein via the LDL receptor as the other citrus flavonoids tested, nobiletin possesses additional properties that allow it to regulate ligand-mediated internalization through at least one additional receptor, SR-A.

Mechanistically, the inhibition of LDL receptor-mediated beta-VLDL internalization observed in J774A.1 cells pre-incubated with all four citrus flavonoids could be due to reduced activity of acyl CoA:cholesterol acyltransferase (ACAT). ACAT inhibitors have been shown to down-regulate the LDL receptor expressed by cultured macrophages [23] and consistently, both hesperetin and naringenin have been shown to lower the *in vitro* activity of ACAT enzymes, including macrophage-expressed ACAT1 [6]. Although the effect of citrus PMFs on ACAT activity has not been investigated, it is possible that both compounds also suppress this enzyme. A reduction in ACAT activity is a reasonable explanation to account for the general effect of all four citrus flavonoids on beta-VLDL metabolism. However, the ability of all four flavonoids to reduce ACAT activity should have also reduced [³H]oleate incorporation into cholesterol esters induced by incubating macrophages with acLDL. This assumption is based on the concept that the cellular fate of the cholesterol esters contained within acLDL is the same as the cholesterol esters derived from beta-VLDL, since both sources are expected to enter the same pool of cholesterol destined for re-esterification by cellular ACAT.

Although there exist only minor differences in the structure between tangeretin and nobiletin (see Fig. 5), recent studies conducted by our group indicate that incubation of PMFs with various cultured cells results in formation of their partly demethoxylated or partly hydroxylated derivatives [5]. After the 24 h incubation with macrophages, nobiletin and tangeretin were most likely converted into their respective

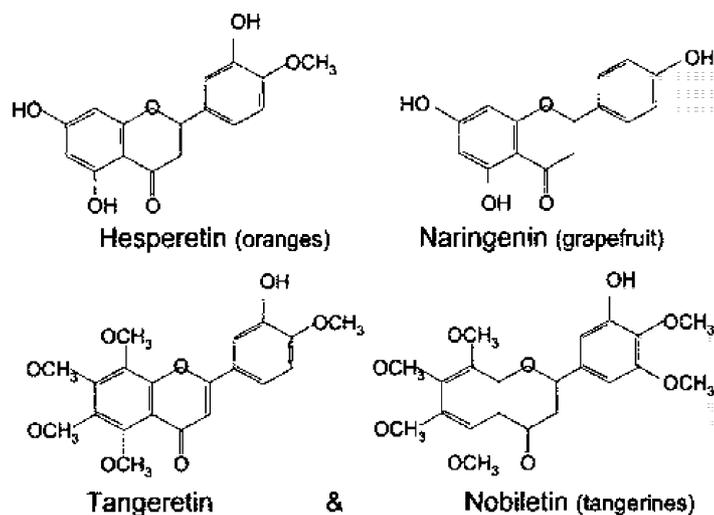


Fig. 5. Structure of the four citrus flavonoids examined.

catabolic derivatives giving rise to new biologically active compounds. In the case of the nobiletin derivatives, these metabolites may have had a greater pharmacological effect compared to the parental compound. Thus, a significant difference in the structure of the metabolites of nobiletin versus tangeretin is a reasonable explanation for the observed difference in biological effects and hence is worthy of future experimental examination.

Since only nobiletin could reduce cellular accumulation of acLDL-derived cholesterol, our first thoughts were that nobiletin was acting through a reduction in total SR-A protein expression and/or through a reduction in the expression of SR-A at the cell surface. However, in response to these two possible mechanisms, our data demonstrated that nobiletin's effect was not associated with reduced SR-A protein expression, as measured by Western blot analysis of whole cell lysate, or with altered cell surface SR-A expression, as determined by [¹²⁵I]acLDL binding at 4 °C. All of these findings point to a potential role of nobiletin in inhibiting the process of SR-A mediated acLDL internalization.

We and others have shown that inhibition of two well known second-messenger signaling pathways involving phosphatidylinositol-3-OH kinase [24] and the inhibitory/other subclass of guanine nucleotide binding proteins (G_{i/o}-proteins) [19], decreases SR-A mediated internalization of acLDL. In an earlier publication, we have showed that Pertussis toxin, a highly specific inhibitor of intracellular signaling via G_{i/o}-proteins, prevents SR-A mediated uptake of acLDL by macrophages [19]. Furthermore, our work also showed that the Pertussis toxin-mediated inhibition did not involve changes in SR-A mRNA and protein expression, or changes in cell surface SR-A expression [19], which is analogous to our finding with nobiletin. Ongoing work in our laboratory has not been able to physically link G_{i/o}-subunits to the cytoplasmic tail of SR-A (unpublished observations). Whether the actions of nobiletin directly involve the suppression of G_{i/o}-protein-signaling has not been determined, but should be the focus of future studies.

In support of the potential of nobiletin to alter the expression of specific cellular enzymes important for unique signaling pathways, Murakami et al. [25] have shown that nobiletin at a concentration of 100 μmol/L significantly suppressed the expression of cyclooxygenase (COX)-2 and inducible NO synthase proteins and prostaglandin E2 release in mouse macrophage RAW 264.7 cells as measured by Western blotting. Similar to the work of Murakami et al., Lin et al. [26] have found that nobiletin in a concentration-dependent manner (max. 64 μmol/L), blocked the production of COX-2 but not COX-1 in human synovial cells as measured by Western blotting. Furthermore, Lin et al. [26] also showed that nobiletin at concentrations of 32 μmol/L significantly decreased the lipopolysaccharide-induced production of prostaglandin E2 measured by Western blotting and the gene expression of the pro-inflammatory cytokines IL-1 alpha, IL-1 beta, TNF-alpha and IL-6 in mouse J774A.1 macrophages.

Another surprising finding in our study was the fact that the impaired metabolism of acLDL by J774A.1 macrophages was observed for nobiletin but not for the structurally related PMF tangeretin (see Fig. 5). Kurowska and Manthey [22] have shown that similar concentrations of both PMFs were required to reduce HepG2 cell medium content of apolipoprotein B-containing lipoproteins, suggesting a similar functional mechanism as it relates to lipoprotein production by the liver. On the other hand, Manthey et al. [27] reported that nobiletin tended to be more biologically active than tangeretin with regards to its anti-inflammatory potential. Thus, the uniqueness of nobiletin's actions appears to be tissue specific.

In conclusion, our data suggest that nobiletin, a biologically active flavonoid from citrus fruit, reduces acLDL-mediated accumulation of cholesterol esters in cultured macrophages via a mechanism that is specific for this flavone and is not related to the inhibition of SR-A protein expression or expression of this receptor on the cell surface. This observation, if confirmed in vivo, might have important clinical implications in the prevention and treatment of atherosclerosis. Further studies, as alluded to above, are also needed to better understand nobiletin's action in macrophages.

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