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Effects of the Mango Components Mangiferin and Quercetin and the Putative Mangiferin Metabolite Norathyriol on the Transactivation of Peroxisome Proliferator-Activated Receptor Isoforms

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Mangos are a source of bioactive compounds with potential health-promoting activity. This study evaluated the abilities of the mango components quercetin and mangiferin and the aglycone derivative of mangiferin, norathyriol, to modulate the transactivation of peroxisome proliferator-activated receptor isoforms (PPARs). PPARs are transcription factors important in many human diseases. Through the use of a gene reporter assay it was shown that quercetin inhibited the activation of all three isoforms of PPARs (PPARγ $IC_{50} = 56.3\ \mu M$; PPARα $IC_{50} = 59.6\ \mu M$; PPARβ $IC_{50} = 76.9\ \mu M$) as did norathyriol (PPARγ $IC_{50} = 153.5\ \mu M$; PPARα $IC_{50} = 92.8\ \mu M$; PPARβ $IC_{50} = 102.4\ \mu M$), whereas mangiferin did not inhibit the transactivation of any isoform. These findings suggest that mango components and metabolites may alter transcription and could contribute to positive health benefits via this or similar mechanisms.

KEYWORDS: Mango; quercetin; mangiferin; PPAR; norathyriol

INTRODUCTION

Epidemiological evidence suggests there are positive health benefits of a diet rich in fruits and vegetables (*1*). Fruits and vegetables contain a variety of phytochemicals with in vitro bioactivity associated with potential health-promoting actions (*2*). Often the precise biomolecular mechanisms involved in the potential health promotion effects such as protection against cardiovascular disease by flavonoids from *Allium* species (*3*) or anticancer effects of glucosinolates and their derivatives from *Brassica* species (*4*) are not fully understood. Although most studies have focused on nutritional bioactives in fruits and vegetables from temperate climates, many of these bioactive compounds are also present in tropical fruit such as mangos (*5*). Flavonols, such as quercetin, and glucosylxanthones, such as mangiferin, are found in both mango and papaya (*6*−*8*). Quercetin is a flavonoid found widely in fruits, vegetables, nuts, tea, and red wine (*9*). It has vasodilator and antihypertensive effects and reduces the vascular remodeling associated with elevated blood pressure in spontaneously hypertensive rats (*10*). Mangiferin is present in a more select group of fruit including mangos and is also present in relatively large amounts in some traditional antidiabetic herbs such as *Anemarrhena asphodeloides* rhizome and *Salacia oblonga* (*11*). Mangiferin, when orally administered to rats or mice, exerts biological antidiabetic, antitumor, hepatoprotective, antioxidant. and immunomodulative activities (*12*−*14*). Given that flavonoid and phenolic compounds isolated from plant products can modulate transcription factors, such as the lipid-regulating peroxisome proliferator-activated receptors (PPARs) (*11, 15, 16*), we investigated the activity of quercetin and mangiferin in relation to this transcriptional target.

As discussed, one potential target for nutritional bioactives is transcription factors. PPARs are ligand-activated transcription factors belonging to the nuclear steroid hormone receptor superfamily (*17*). They were originally named for their stimulation of peroxisome proliferation in rodent liver, but many cellular and systemic roles are now attributed to these receptors (*17, 18*). In keeping with their various developmental and physiological functions, PPARs show broad, but isoform-specific, tissue expression patterns (*17*).

There are three PPAR isoforms, PPARα, PPARβ, and PPARγ. For transcriptional regulation each PPAR isoform requires the formation of a heterodimer with the retinoid X receptor (RXR), which when activated by a ligand can bind to a specific response element in the promoter region of target genes, known as a peroxisome proliferator response element (PPRE), to influence transcription of the target gene (*19, 20*). In a complex process, transcriptional regulation also involves

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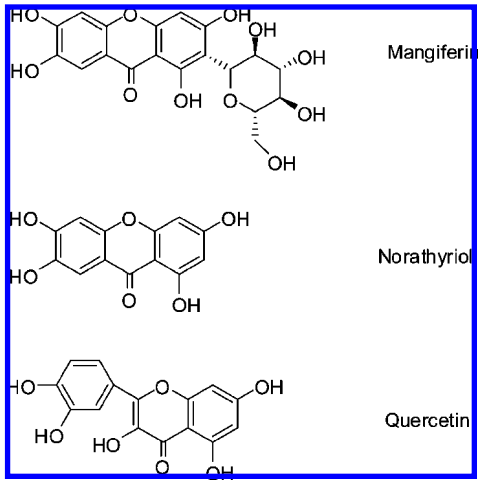

Figure 1. Chemical structures of mangiferin, norathyriol, and quercetin.

the recruitment of cofactors, both positive and negative, termed coactivators and corepressors, which function to modulate transcription, often by changing receptor conformation (*21*).

The PPARs are pivotal regulators of body lipid homeostasis and are proposed as possible therapeutic targets for metabolic disorders (*18*). PPARα is a major regulator of fatty acid metabolism and catabolism within the body, and clinically its ligands are used in the treatment of hypertriglyceridemia (*17, 18*). Other roles for PPARα include anti-inflammatory activity, improvements in insulin sensitivity, and antiatherosclerotic efficacy (*18, 22, 23*). PPARγ is traditionally associated with adipose-related functions and is involved in adipocyte differentiation among other adipocyte specific functions (*17*). The discovery that PPARγ regulates insulin sensitivity led to a whole new class of drugs (e.g., thiazolidinediones) that are now used to correct the hyperglycemia in type 2 diabetic patients (*17, 18*). PPARγ agonists also have utility as anti-inflammatory agents, and there is the suggestion that they may also act as antiatherosclerotic agents (*17, 18*). Of the three isoforms, PPARβ is perhaps more of an enigma, although recent research has revealed it also has a role in energy homeostasis (*17*), and agonists may be of use in the treatment of dyslipidemia, obesity, and insulin resistance (*18*). Furthermore, PPARβ can induce cell differentiation and inhibit cell proliferation in keratinocytes, leading to speculation that it may represent a therapeutic target for skin disorders (*24*). Overall, the PPAR isoforms have important clinical implications and are molecular targets for therapeutic and/or neutraceutical intervention for the prevention of disorders including metabolic syndrome.

There are limited studies on mango components and PPARs, with a report of modest activation of PPARα by mangiferin (*11*). Moreover, there has been no assessment of possible mango bioactive activity across all PPAR isoforms or the activity of potential bioactive derivatives of mango components. In these studies, we assessed the modulation of PPAR isoforms by two major mango components, quercetin and mangiferin. Our studies also assessed the activity of norathyriol, the aglycone derivative of mangiferin, which is formed by human fecal flora in vitro (*25*) and, presumably, in vivo, and which has effects on inflammation, vasorelaxation, and platelet aggregation (*26*). The compounds tested are from molecular families that are characteristic of many fruits and vegetables, so results may also have relevance for better understanding of general health-promoting mechanisms.

MATERIALS AND METHODS

Chemicals. Quercetin and mangiferin were purchased from Sigma Aldrich (Sydney, Australia). Norathyriol was isolated and purified as previously described (*27*). Dulbecco's Modified Eagle's Medium (DMEM) and fetal bovine serum (FBS) were obtained from JRH (Sydney, Australia). Dimethyl sulfoxide (DMSO) and fatty acid free bovine serum albumin (BSA) were purchased from Sigma Aldrich. GW7647 and GW0742 were purchased from Calbiochem (EMD Biosciences, Inc., San Diego, CA). Ciglitazone was purchased from Sapphire Biosciences (Sydney, Australia). Stock solutions of ciglitazone, GW7647, and GW0742 were dissolved in DMSO and stored at −20 °C. Stock solutions for quercetin, mangiferin, and norathyriol were prepared freshly in DMSO on the day of the experiment.

Plasmids. The human PPARγ plasmid pcDNA3.1hPPARγ was obtained from Professor John Prins (School of Medicine, University of Queensland, Australia). The cDNAs for human PPARα and PPARβ, both in the plasmid pcDNA3.1, were provided by A/Professor Jeffrey M. Peters (Department of Veterinary Science, Center for Molecular Toxicology and Carcinogenesis, The Pennsylvania State University, Univeristy, PA). The gene reporter plasmid pTK3XPPREluc (*28, 29*) was kindly provided by Dr. Ron Evans (Howard Hughes Medical Institute, The Salk Institute for Biological Studies, San Diego, CA). pTK3XPPREluc contains three copies of a PPRE cloned upstream of the TK-LUC reporter (*28*).

Transient Transfections and Transactivation Assays. Cos-7 cells were maintained and plated in high-glucose DMEM supplemented with 10% FBS, L-glutamine (4 mM), penicillin G (100 units/mL), and streptomycin sulfate (100 μg/mL). Cos-7 cells are widely used for PPAR transactivation studies (*30*).

Cells were maintained at 37 °C in a humidified 5% CO_2/95% air incubator. For reporter assays, Cos-7 cells were transfected at 70% confluency. Prior to reaching confluence, cells were trypsinized with a 0.05% trypsin/0.53 mM EDTA solution and resuspended in fresh growth medium before plating onto a new growth surface. The cells were plated at 1.2×10^4 cells/well into a 96-well plate and were allowed to adhere for 24 h. All transient transfections used LipofectAMINE 2000 reagent (0.8 μL/well) (Promega, Sydney, Australia) and were performed in serum- and antibiotic-free media as described previously (*31*) and according to the manufacturer's directions. Each transfection consisted of the transfection control plasmid pSV-b-Gal (200 ng), the gene reporter plasmid pTK3XPPREluc (250 ng), and 100 ng of PPARγ, PPARα, or PPARβ, respectively. Five hours after transfection, the medium was replaced with serum- and phenol red-free complete medium supplemented with 1.5% BSA and the appropriate chemical compound and PPAR ligand. All chemical stock solutions were in DMSO, and DMSO was added to all control wells. The highest concentration of DMSO added to the cell cultures was 0.65% v/v, which was used for all experimental and control cultures. After a further 19 h, the cells were either lysed with 1× luciferase lysis buffer (Promega) for PPRE-luciferase activity or else lysed with 1× reporter lysis buffer (50 μL) (Promega) for β-galactosidase (β-gal) assay. PPRE-driven reporter luciferase activity was measured using the Bright-Glo Luciferase Assay System (Promega) in a NOVOstar or FLUORstar fluorescence microplate reader (BMG Labtechnologies, Offenburg, Germany).

***β*-Galactosidase Activity.** Cos-7 cells transiently transfected as above were also assayed for β-gal activity as previously described (*29*). Following lysis in 1× reporter lysis buffer, 2× β-gal assay buffer (50 μL; 200 mM sodium phosphate buffer, pH 7.3, 2 mM $MgCl_2$, 100 mM β-mercaptoethanol, 1.33 mg/mL *o*-nitrophenyl β-galactopyranoside) was added, and the plates were incubated for 120 min at 37 °C prior to the reading of absorbance (415 nm) using a Bio-Rad model 550 microplate reader (Bio-Rad Laboratories, Reagents Park, Australia).

Statistical Analysis. Prism V4.03 software (GraphPad Software Inc., San Diego, CA) was used for statistical analysis, for the generation of dose−response curves, and for IC_{50} values. Significance was determined using one-way analysis of variance with Tukey's test for all pairwise multiple comparisons for normally distributed data of equal variance.

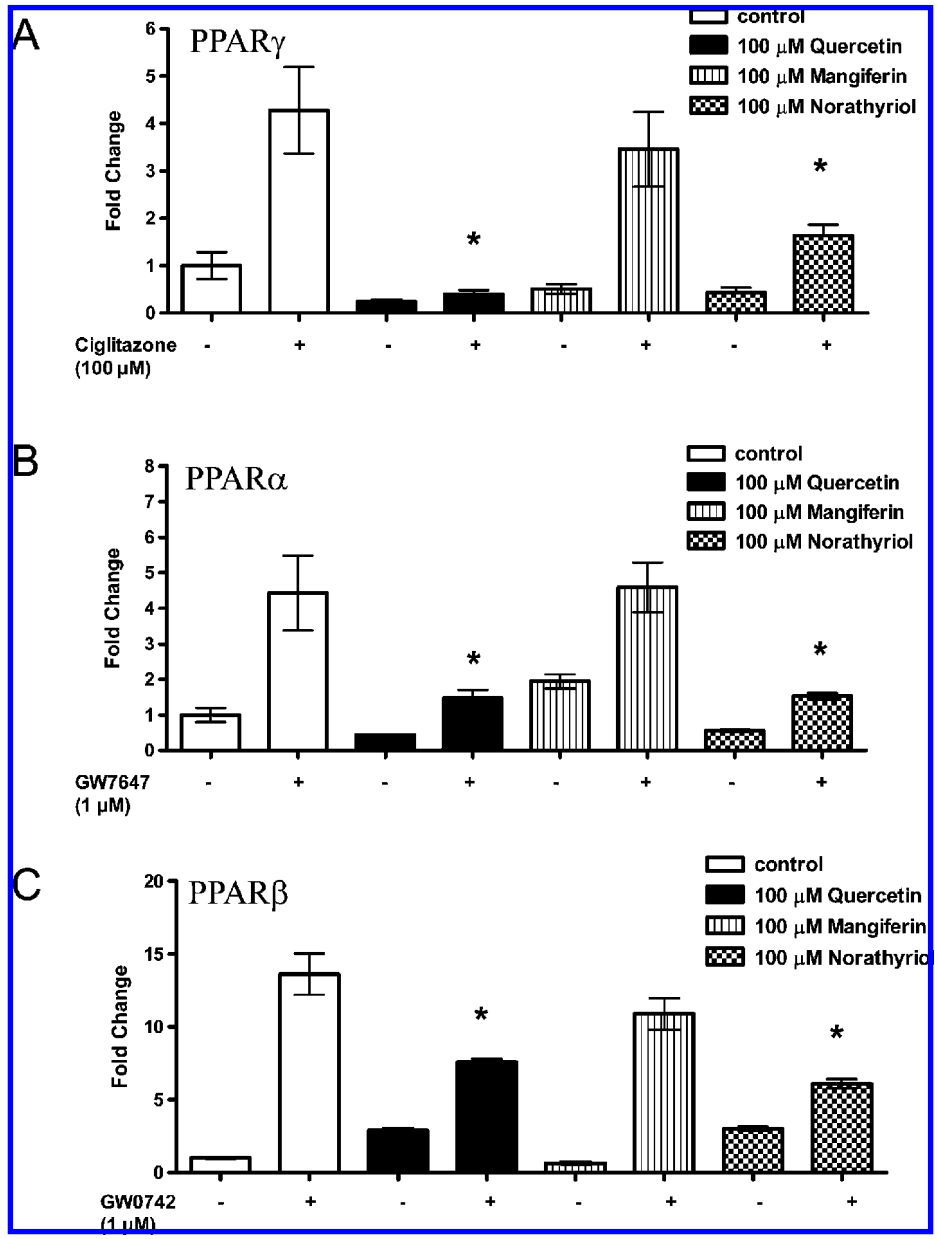

Figure 2. Quercetin, mangiferin, and norathyriol effects on basal and ligand-activated transactivation of (**A**) PPARγ, (**B**) PPARα, and (**C**) PPARβ in Cos-7 cells. Luciferase values for PPAR transactivation were normalized to β-gal, and fold change is given relative to untreated controls and presented as mean $\pm$ SEM from three separate experiments performed in triplicate. $*$ denotes significance at $P < 0.05$ compared to (**A**) 100 μM ciglitazone, (**B**) 1 μM GW7607, or (**C**) 1 μM GW0742.

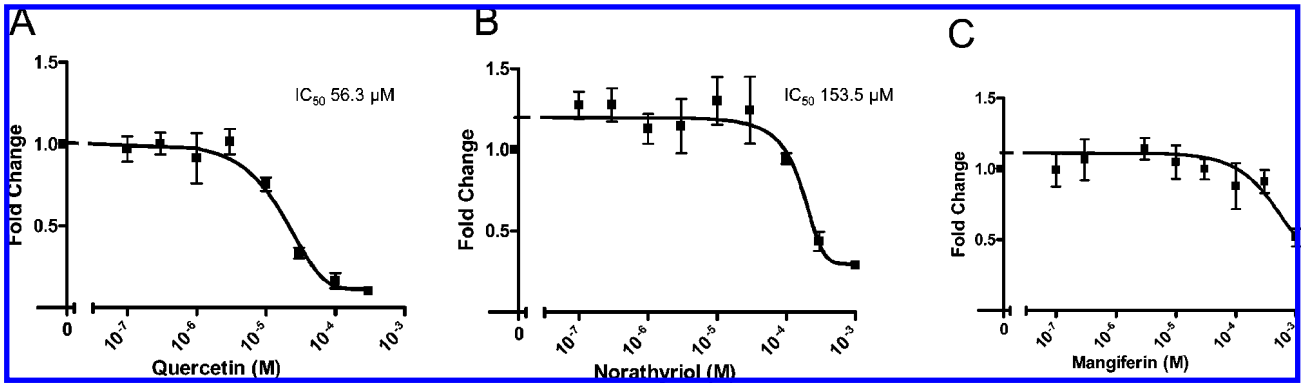

Figure 3. Dose dependency of the effect of (**A**) quercetin, (**B**) norathyriol, and (**C**) mangiferin on ciglitazone (100 μM) activation of human PPARγ in Cos-7 cells. Luciferase values for PPAR transactivation were normalized to β-gal, and fold change is given relative to untreated controls and presented as mean $\pm$ SD ($n = 3$). Results are representative of two independent experiments.

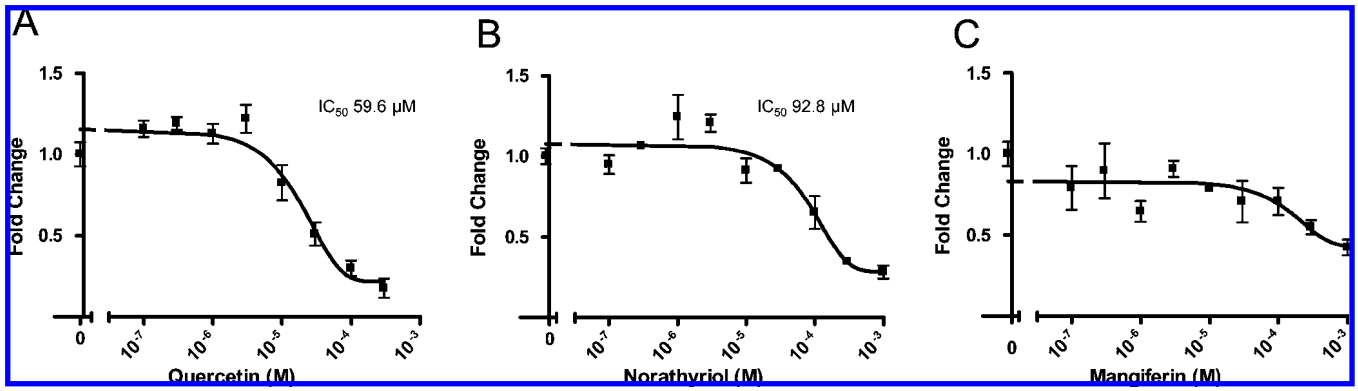

Figure 4. Dose dependency of the effect of (**A**) quercetin, (**B**) norathyriol, and (**C**) mangiferin on GW7647 (1 μM) activation of human PPARα in Cos-7 cells. Luciferase values for PPAR transactivation were normalized to β-gal, and fold change is given relative to untreated controls and presented as mean $\pm$ SD ($n = 3$). Results are representative of two independent experiments.

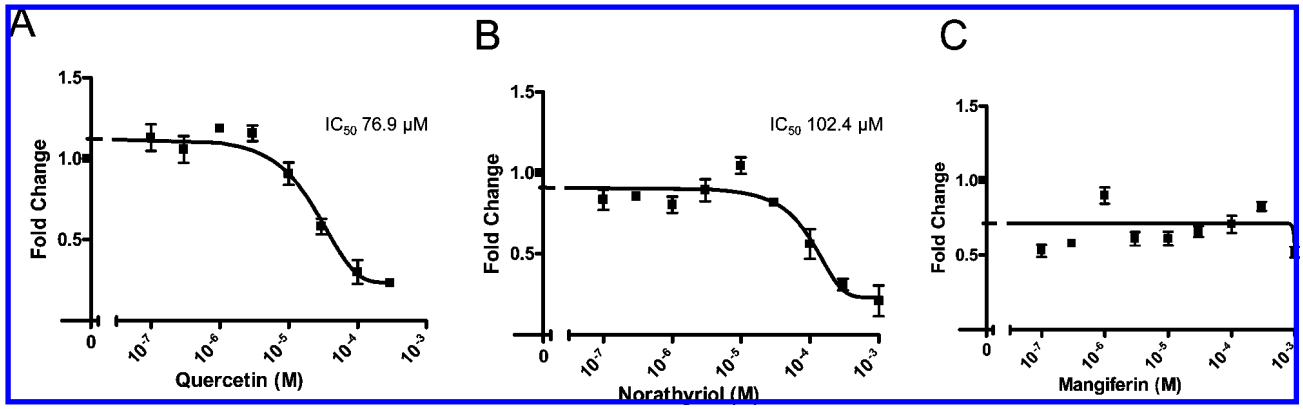

Figure 5. Dose dependency of the effect of (**A**) quercetin, (**B**) norathyriol, and (**C**) mangiferin on GW0742 (1 μM) activation of human PPARβ in Cos-7 cells. Luciferase values for PPAR transactivation were normalized to β-gal, and fold change is given relative to untreated controls and is presented as mean $\pm$ SD ($n = 3$). Results are representative of two independent experiments.

RESULTS AND DISCUSSION

The mango flavonoid and xanthone constituents quercetin and mangiferin together with the mangiferin aglycone norathyriol (**Figure 1**) were tested for bioactivity against the three human PPAR isoforms. Given the significance of PPARs in human health, we sought to characterize whether these compounds had agonist and/or antagonist activities for PPARα, PPARβ, or PPARγ.

We initially examined the ability of mangiferin, quercetin, and norathyriol to activate the three isoforms of PPAR. As can be seen in **Figure 2**, each specific ligand (ciglitazone for PPARγ (*29, 32*), GW7647 for PPARα (*29, 33*), and GW0742 for PPARβ (*29, 34*) activates their respective PPAR isoform as evident by an increase in the fold change in relative luciferase activity. Neither quercetin, mangiferin, nor norathyriol significantly activated PPARα, PPARβ, or PPARγ. We further characterized the effect of these compounds on the transactivation of PPAR isoforms activated by their specific ligands. Quercetin (100 μM) and norathyriol (100 μM) significantly inhibited the transactivation of ciglitazone-activated PPARγ (**Figure 2A**), GW7607-activated PPARα (**Figure 2B**), and GW0742-activated PPARβ (**Figure 2C**), respectively. However, mangiferin showed no effect on the transactivation of ligand-activated PPARs (**Figure 2**).

Given the effect of these compounds on the transactivation of PPARs, we further characterized the potency of each compound in inhibiting specific ligand-activated PPAR transactivation. In agreement with **Figure 2**, mangiferin did not inhibit the transactivation of any activated PPARs (**Figures 3C**, **4C**, and **5C**), and because the IC_{50} values calculated for mangiferin were greater than the highest concentration tested (>1 mM), these values are not considered to be an accurate assessment. Ciglitazone-activated PPARγ was inhibited by both quercetin and norathyriol with IC_{50} values of 56.3 and 153.5 μM, respectively (**Figure 3A,B**). Likewise, quercetin inhibited PPARα activation with an IC_{50} of 59.6 μM (**Figure 4A**) and PPARβ with an IC_{50} of 76.9 μM (**Figure 5A**). Norathyriol inhibited PPARα with an IC_{50} of 92.8 μM (**Figure 4A**) and PPARβ with an IC_{50} of 102.4 μM (**Figure 5B**).

The health benefit of fruits and vegetables is increasingly appreciated with more research directed toward the identification of the bioactive molecules in specific fruits and vegetables. The mechanism of bioactives clearly extends beyond general antioxidant activity to the modulation of complex cellular signaling pathways including regulation of transcription factors. Indeed, genistein, an isoflavone isolated from soy, is a modulator of many transcriptional pathways including NF-κB, Akt, estrogen, and androgen signaling pathways (*35*), and resveratrol, a polyphenolic compound found in red wine, is a modulator of NF-κB- and AP-1-mediated pathways (*36*). By comparison to more temperate crops, the characterization of nutritional bioactives in tropical fruits is still in its infancy. In this study we investigated three potential mango bioactives, quercetin, mangiferin. and the putative mangiferin metabolite norathyriol, for their activity toward the human PPAR isoforms. There has been no previous detailed comparison of the potency of these compounds toward all PPAR isoforms in one study.

Our initial work focused on determining whether quercetin, mangiferin. and norathyriol were capable of activation of each PPAR isoform. It was clear at the concentration assessed (100 μM) that all three agents exhibited no ability to activate PPARγ or PPARβ, although mangiferin. in our model, did exhibit a trend toward activation of the PPARα isoform. Modest but significant activation of PPARα by mangiferin has previously been reported using the HEK293 cell model at 25−100 μM mangiferin (*11*), and our lack of significant increase in PPARα may be explained by the different cell models used in the two studies.

Our initial studies suggested that quercetin and norathyriol, but not mangiferin, at 100 μM, could significantly inhibit the ability of isoform-specific ligands to activate their respective PPAR targets. To further characterize this effect, we studied the ability of quercetin, norathyriol, and mangiferin, including concentrations ranging from 100 nM to 1 mM, to antagonize PPAR transactivation. These studies showed that quercetin was capable of inhibiting the transactivation of all PPAR isoforms with IC_{50} values ranging from 56.3 to 76.9 μM. Consistent with this result, another group showed that quercetin was able to inhibit PPAR isoforms in primary keratinocytes, although no characterization of respective potency was determined (*37*). Mangiferin, in contrast, showed no ability to inhibit PPARβ transactivation and showed only a weak effect on the transactivation of PPARγ and PPARα (IC_{50} values > 1 mM). Norathyriol, the putative metabolite of mangiferin, inhibited the transactivation of all three isoforms (IC_{50} values ranging from 92.8 to 153.5 μM). Norathyriol was a weaker inhibitor of PPAR transactivation for each isoform than quercetin. Quercetin was the most potent at inhibiting PPARγ transactivation, whereas norathyriol showed the least potency against PPARγ.

Mangiferin was less active than its putative metabolite norathyriol at inhibiting PPAR transactivation, highlighting the importance of conversion to the aglycone metabolite for activity. Studies with bacteria isolated from human feces show the ability for mangiferin to be cleaved to norathyriol in vitro (*25*). Results of metabolic studies with rabbits given oral mangiferin describe the presence of metabolites in the urine that suggest that norathyriol is produced in vivo (*38*). Hence, the relative importance of the apparent activation of PPARα by mangiferin versus the inhibition of PPAR isoforms by norathyriol is likely to dictate the overall physiological response to ingested mangiferin. Further complexity could arise from individual variations in intestinal flora (*39*), which would also influence the physiological outcome, as would the concomitant presence of specific endogenous and exogenous activators of PPAR isoforms.

The ability of quercetin and norathyriol to inhibit, to some degree, all three PPAR isoforms suggests that the target for these agents may not necessarily be the PPAR protein but may involve some component of the transcriptional machinery, such as the heterodimerization partner RXR or shared cofactors. Antagonist-mediated actions on steroid hormone receptors may simply involve competition for agonist binding or an active situation in which corepressors are recruited to the receptor (*41*). Additionally, RXR antagonists can abolish the effects of PPARγ agonists (*40*). Hence, a possible mechanism for quercetin or norathyriol to antagonize all PPARs could be mediated through shared corepressors or antagonism of the essential heterodimerization partner, RXR.

The concentration of compounds such as flavonoids required to modulate gene expression in vitro is quite often significantly higher than the steady state concentrations reached in vivo (*42*). However, such compounds may accumulate in cells with long-term ingestion allowing them to modulate their target transcription factors (*42*). Moreover, there may be other transcription factors that may be more sensitive to the effects of mango components such as quercetin and mangiferin and the putative metabolite norathyriol. Future studies will require the in vivo assessment of norathyriol levels after mango ingestion and the consequences of these agents on other transcription factors. Collectively, the constituents of mango and their metabolites may alter gene transcription and contribute to positive health benefits.

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LITERATURE CITED

(1) Michels, K. B.; Edward, G.; Joshipura, K. J.; Rosner, B. A.; Stampfer, M. J.; Fuchs, C. S.; Colditz, G. A.; Speizer, F. E.; Willett, W. C. Prospective study of fruit and vegetable consumption and incidence of colon and rectal cancers. *J. Natl. Cancer Inst.* **2000**, *92*, 1740–1752.

(2) Kris-Etherton, P. M.; Hecker, K. D.; Bonanome, A.; Coval, S. M.; Binkoski, A. E.; Hilpert, K. F.; Griel, A. E.; Etherton, T. D. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *Am. J. Med.* **2002**, *113*, 71–88.

(3) Griffiths, G.; Trueman, L.; Crowther, T.; Thomas, B.; Smith, B. Onions—a global benefit to health. *Phytother. Res.* **2002**, *16*, 603–615.

(4) Kassie, F.; Uhl, M.; Rabot, S.; Grasl-Kraupp, B.; Verkerk, R.; Kundi, M.; Chabicovsky, M.; Schulte-Hermann, R.; Knasmuller, S. Chemoprevention of 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ)-induced colonic and hepatic preneoplastic lesions in the F344 rat by cruciferous vegetables administered simultaneously with the carcinogen. *Carcinogenesis* **2003**, *24*, 255–261.

(5) Gorinstein, S.; Zemser, M.; Haruenkit, R.; Chuthakorn, R.; Grauer, F.; Martin-Belloso, O.; Trakhtenberg, S. Comparative content of total polyphenols and dietary fiber in tropical fruits and persimmon. *J. Nutr. Biochem.* **1999**, *10*, 367–371.

(6) Barua, A. B.; Olson, J. A. Reversed-phase gradient high-performance liquid chromatographic procedure for simultaneous analysis of very polar to nonpolar retinoids, carotenoids and tocopherols in animal and plant samples. *J. Chromatogr., B: Biomed. Sci. Appl.* **1998**, *707*, 69–79.

(7) Kermanshai, R.; McCarry, B. E.; Rosenfeld, J.; Summers, P. S.; Weretilnyk, E. A.; Sorger, G. J. Benzyl isothiocyanate is the chief or sole anthelmintic in papaya seed extracts. *Phytochemistry* **2001**, *57*, 427–435.

(8) Pott, I.; Breithaupt, D. E.; Carle, R. Detection of unusual carotenoid esters in fresh mango (*Mangifera indica* L. cv. "Kent"). *Phytochemistry* **2003**, *64*, 825–829.

(9) Formica, J. V.; Regelson, W. Review of the biology of quercetin and related bioflavonoids. *Food Chem. Toxicol.* **1995**, *33*, 1061–1080.

(10) Duarte, J.; Perez-Palencia, R.; Vargas, F.; Angeles Ocete, M.; Perez-Vizcaino, F.; Zarzuelo, A.; Tamargo, J. Antihypertensive effects of the flavonoid quercetin in spontaneously hypertensive rats. *Br. J. Pharmacol.* **2001**, *133*, 117–124.

(11) Huang, T. H.; Peng, G.; Li, G. Q.; Yamahara, J.; Roufogalis, B. D.; Li, Y. Salacia oblonga root improves postprandial hyperlipidemia and hepatic steatosis in Zucker diabetic fatty rats: activation of PPAR-α. *Toxicol. Appl. Pharmacol.* **2006**, *210*, 225–235.

(12) Guha, S.; Ghosal, S.; Chattopadhyay, U. Antitumor, immunomodulatory and anti-HIV effect of mangiferin, a naturally occurring glucosylxanthone. *Chemotherapy* **1996**, *42*, 443–451.
(13) Sanchez, G. M.; Re, L.; Giuliani, A.; Nunez-Selles, A. J.; Davison, G. P.; Leon-Fernandez, O. S. Protective effects of *Mangifera indica* L. extract, mangiferin and selected antioxidants against TPA-induced biomolecules oxidation and peritoneal macrophage activation in mice. *Pharmacol. Res.* **2000**, *42*, 565–573.
(14) Yoshikawa, M.; Ninomiya, K.; Shimoda, H.; Nishida, N.; Matsuda, H. Hepatoprotective and antioxidative properties of *Salacia reticulata*: preventive effects of phenolic constituents on CCl4-induced liver injury in mice. *Biol. Pharm. Bull.* **2002**, *25*, 72–76.
(15) Rau, O.; Wurglics, M.; Paulke, A.; Zitzkowski, J.; Meindl, N.; Bock, A.; Dingermann, T.; Abdel-Tawab, M.; Schubert-Zsilavecz, M. Carnosic acid and carnosol, phenolic diterpene compounds of the labiate herbs rosemary and sage, are activators of the human peroxisome proliferator-activated receptor gamma. *Planta Med.* **2006**, *72*, 881–887.
(16) Ricketts, M. L.; Moore, D. D.; Banz, W. J.; Mezei, O.; Shay, N. F. Molecular mechanisms of action of the soy isoflavones includes activation of promiscuous nuclear receptors. A review. *J. Nutr. Biochem.* **2005**, *16*, 321–330.
(17) Michalik, L.; Auwerx, J.; Berger, J. P.; Chatterjee, V. K.; Glass, C. K.; Gonzalez, F. J.; Grimaldi, P. A.; Kadowaki, T.; Lazar, M. A.; O'Rahilly, S.; Palmer, C. N. A.; Plutzky, J.; Reddy, J. K.; Spiegelman, B. M.; Staels, B.; Wahli, W. International Union of Pharmacology. LXI. Peroxisome proliferator-activated receptors. *Pharmacol. Rev.* **2006**, *58*, 726–741.
(18) Berger, J. P.; Akiyama, T. E.; Meinke, P. T. PPARs: therapeutic targets for metabolic disease. *Trends Pharmacol. Sci.* **2005**, *26*, 244–251.
(19) Issemann, I.; Prince, R. A.; Tugwood, J. D.; Green, S. The retinoid X receptor enhances the function of the peroxisome proliferator activated receptor. *Biochimie* **1993**, *75*, 251–256.
(20) Kliewer, S. A.; Umesono, K.; Noonan, D. J.; Heyman, R. A.; Evans, R. M. Convergence of 9-cis retinoic acid and peroxisome proliferator signalling pathways through heterodimer formation of their receptors. *Nature* **1992**, *358*, 771–774.
(21) Perissi, V.; Rosenfeld, M. G. Controlling nuclear receptors: the circular logic of cofactor cycles. *Nat. Rev.* **2005**, *6*, 542–554.
(22) Zandbergen, F.; Plutzky, J. PPARα in atherosclerosis and inflammation. *Biochim. Biophys. Acta* **2007**, *1771*, 972–982.
(23) Kim, H.; Haluzik, M.; Asghar, Z.; Yau, D.; Joseph, J. W.; Fernandez, A. M.; Reitman, M. L.; Yakar, S.; Stannard, B.; Heron-Milhavet, L.; Wheeler, M. B.; LeRoith, D. Peroxisome proliferator-activated receptor-α agonist treatment in a transgenic model of type 2 diabetes reverses the lipotoxic state and improves glucose homeostasis. *Diabetes* **2003**, *52*, 1770–1778.
(24) Kim, D. J.; Bility, M. T.; Billin, A. N.; Willson, T. M.; Gonzalez, F. J.; Peters, J. M. PPARβ/δ selectively induces differentiation and inhibits cell proliferation. *Cell. Death Differ.* **2005**, *13*, 53–60.
(25) Sanugul, K.; Akao, T.; Li, Y.; Kakiuchi, N.; Nakamura, N.; Hattori, M. Isolation of a human intestinal bacterium that transforms mangiferin to norathyriol and inducibility of the enzyme that cleaves a C-glucosyl bond. *Biol. Pharm. Bull.* **2005**, *28*, 1672–1678.
(26) Pinto, M. M.; Sousa, M. E.; Nascimento, M. S. Xanthone derivatives: new insights in biological activities. *Curr. Med. Chem.* **2005**, *12*, 2517–2538.
(27) Chun-Nan, L.; Cheng-Hsiung, C.; Arisawa, M.; Shimizu, M.; Morita, N. Two new xanthone glycosides from *Tripterospermum lanceolatum*. *Phytochemistry* **1982**, *21*, 205–208.
(28) Forman, B. M.; Tontonoz, P.; Chen, J.; Brun, R. P.; Spiegelman, B. M.; Evans, R. M. 15-Deoxy-Δ12,14-prostaglandin J2 is a ligand for the adipocyte determination factor PPARγ. *Cell* **1995**, *83*, 803–812.
(29) Gopisetty Venkata, N.; Robinson, J. A.; Cabot, P. J.; Davis, B.; Monteith, G. R.; Roberts-Thomson, S. J. Mono(2-ethylhexyl)phthalate and mono-*n*-butyl phthalate activation of peroxisome proliferator activated-receptors α and γ in breast. *Toxicol. Lett.* **2006**, *163*, 224–234.
(30) Duez, H.; Lefebvre, B.; Poulain, P.; Torra, I. P.; Percevault, F.; Luc, G.; Peters, J. M.; Gonzalez, F. J.; Gineste, R.; Helleboid, S.; Dzavik, V.; Fruchart, J.-C.; Fievet, C.; Lefebvre, P.; Staels, B. Regulation of human ApoA-I by gemfibrozil and fenofibrate through selective peroxisome proliferator-activated receptor α modulation. *Arterioscler. Thromb. Vasc. Biol.* **2005**, *25*, 585–591.
(31) Faddy, H. M.; Robinson, J. A.; Lee, W. J.; Holman, N. A.; Monteith, G. R.; Roberts-Thomson, S. J. Peroxisome proliferator-activated receptor α expression is regulated by estrogen receptor α and modulates the response of MCF-7 cells to sodium butyrate. *Int. J. Biochem. Cell Biol.* **2006**, *38*, 255–266.
(32) Lambe, K. G.; Tugwood, J. D. A human peroxisome-proliferator-activated receptor-gamma is activated by inducers of adipogenesis, including thiazolidinedione drugs. *Eur. J. Biochem.* **1996**, *239*, 1–7.
(33) Brown, P. J.; Stuart, L. W.; Hurley, K. P.; Lewis, M. C.; Winegar, D. A.; Wilson, J. G.; Wilkison, W. O.; Ittoop, O. R.; Willson, T. M. Identification of a subtype selective human PPARα agonist through parallel-array synthesis. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1225–1227.
(34) Sznaidman, M. L.; Haffner, C. D.; Maloney, P. R.; Fivush, A.; Chao, E.; Goreham, D.; Sierra, M. L.; LeGrumelec, C.; Xu, H. E.; Montana, V. G.; Lambert, M. H.; Willson, T. M.; Oliver, W. R.; Sternbach, D. D. Novel selective small molecule agonists for peroxisome proliferator-activated receptor delta (PPARδ)—synthesis and biological activity. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1517–1521.
(35) Sarkar, F. H.; Li, Y. Mechanisms of cancer chemoprevention by soy isoflavone genistein. *Cancer Metastasis Rev.* **2002**, *21*, 265–280.
(36) Manna, S. K.; Mukhopadhyay, A.; Aggarwal, B. B. Resveratrol suppresses TNF-induced activation of nuclear transcription factors NF-κB, activator protein-1, and apoptosis: potential role of reactive oxygen intermediates and lipid peroxidation. *J. Immunol.* **2000**, *164*, 6509–6519.
(37) Thuillier, P.; Brash, A. R.; Kehrer, J. P.; Stimmel, J. B.; Leesnitzer, L. M.; Yang, P.; Newman, R. A.; Fischer, S. M. Inhibition of peroxisome proliferator-activated receptor (PPAR)-mediated keratinocyte differentiation by lipoxygenase inhibitors. *Biochem. J.* **2002**, *366*, 901–910.
(38) Krishnaswamy, N. R.; Seshadri, T. R.; Tahir, P. J. Metabolism of some polyphenolic compounds related to calophyllolide in rabbits. *Indian J. Exp. Biol.* **1971**, *9*, 458–461.
(39) Gibson, G.; Roberfroid, M. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J. Nutr.* **1995**, *125*, 1401–1412.
(40) Tarrade, A.; Schoonjans, K.; Pavan, L.; Auwerx, J.; Rochette-Egly, C.; Evain-Brion, D.; Fournier, T. PPARγ/RXRα heterodimers control human trophoblast invasion. *J. Clin. Endocrinol. Metab.* **2001**, *86*, 5017–5024.
(41) Jackson, T. A.; Richer, J. K.; Bain, D. L.; Takimoto, G. S.; Tung, L.; Horwitz, K. B. The partial agonist activity of antagonist-occupied steroid receptors is controlled by a novel hinge domain-binding coactivator L7/SPA and the corepressors N-CoR or SMRT. *Mol. Endocrinol.* **1997**, *11*, 693–705.
(42) Kuo, S. M. Flavonoids and gene expression in mammalian cells. *Adv. Exp. Med. Biol.* **2002**, *505*, 191–200.

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