JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Induction of Peroxisome Proliferator-Activated Receptor γ (PPARγ)-Mediated Gene Expression by Tomato (*Solanum lycopersicum* L.) Extracts

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Supporting Information

ABSTRACT: Since beneficial effects related to tomato consumption partially overlap with those related to peroxisome proliferator-activated receptor γ (PPARγ) activation, our aim was to test extracts of tomato fruits and tomato components, including polyphenols and isoprenoids, for their capacity to activate PPARγ using the PPARγ2 CALUX reporter cell line. Thirty tomato compounds were tested; seven carotenoids and three polyphenols induced PPARγ2-mediated luciferase expression. Two extracts of tomato, one containing deglycosylated phenolic compounds and one containing isoprenoids, also induced PPARγ2-mediated expression at physiologically relevant concentrations. Furthermore, enzymatically hydrolyzed extracts of seven tomato varieties all induced PPARγ-mediated expression, with a 1.6-fold difference between the least potent and the most potent variety. The two most potent varieties had high flavonoid content, while the two least potent varieties had low flavonoid content. These data indicate that extracts of tomato are able to induce PPARγ-mediated gene expression *in vitro* and that some tomato varieties are more potent than others.

KEYWORDS: *peroxisome proliferator-activated receptor gamma (PPARγ), tomato extracts, polyphenols, carotenoids, fatty acids, luciferase reporter gene assay, mixture effects*

1. INTRODUCTION

Peroxisome proliferator-activated receptor γ (PPARγ, NR1C3) belongs to the nuclear receptor superfamily. Upon activation by agonists, PPARγ forms a heterodimer with retinoid X receptor (RXR) and binds to a peroxisome proliferator-responsive element (PPRE) in the regulatory domain of target genes, thereby affecting their expression.[1,2] PPARγ is known to be an important stimulator of adipogenesis.[2,3] In addition, thiazolidinediones (TZDs) are a group of PPARγ agonists shown to improve insulin sensitivity.[4,5] Other beneficial health effects that have been related to PPARγ activation include, for example, anti-inflammatory activity,[6,7] effects on cholesterol levels,[8,9] and reduced risk of atherosclerosis.[10,11] Furthermore, PPARγ agonists have been related to inhibition of the development of prostate, breast, and colon cancer,[12,13] although it is not yet clearly established to what extent the anticancer effects are PPARγ-mediated.[14−16]

Tomato is one of the most consumed vegetables in the Western world and is a major component of the healthy Mediterranean diet.[17,18] The consumption of tomato has been related to reduced risk of prostate cancer and several other cancer types.[17,19] High intake of tomato is believed to modify lipid profiles toward a healthier pattern[20,21] and to lower the risk of atherosclerosis and cardiovascular diseases.[22,23] In addition, some phytochemicals present in tomato have been related to improved glucose and insulin levels.[24,25] Tomato fruits contain many bioactive phytochemicals, for example, flavonoids, including quercetin, kaempferol, and naringenin chalcone, and carotenoids, including β-carotene and lycopene.[26−30]

Given the overlap between the beneficial health effects associated with PPARγ activation and those associated with tomato consumption, we hypothesized that bioactive components of tomato fruit may be able to activate PPARγ. Therefore, the aim of the present study was to investigate whether tomato components, including polyphenols, isoprenoids, and fatty acids, as well as tomato extracts are able to activate PPARγ-mediated gene expression. To that end, the recently developed PPARγ2 CALUX reporter cell line[31] was used to investigate whether flavonoid-rich and isoprenoid-rich tomato extracts as well as individual compounds known to be present in tomato fruits are able to induce PPARγ-mediated gene expression.

Received: July 27, 2012
Revised: February 15, 2013
Accepted: February 19, 2013
Published: February 19, 2013

2. EXPERIMENTAL SECTION

2.1. Chemicals. Rosiglitazone (≥98%, CAS no. 122320-73-4) and palmitic acid (≥98%, CAS no. 57-10-3) were obtained from Cayman Chemical (Ann Arbor, MI, USA). α-Linolenic acid (ALA, ≥99%, CAS no. 463-40-1), oleic acid (OA, ≥99%, CAS no. 112-80-1), linoleic acid (LA, ≥99%, CAS no. 60-33-3), stearic acid (SA, ≥95%, CAS no. 57-11-4), myristic acid (MA, ≥95%, CAS no. 544-63-8), quercetin dihydrate (≥98%, CAS no. 6151-25-3), ferulic acid (≥99%, CAS no. 537-98-4), chlorogenic acid (≥95%, CAS no. 327-97-9), β-carotene (≥97%, CAS no. 7235-40-7), RRR-α-tocopherol (CAS no. 59-02-9), RRR-γ-tocopherol (≥96%, CAS no. 54-28-4), and RRR-δ-tocopherol (≥90%, CAS no. 119-13-1) were purchased from Sigma Aldrich (St. Louis, MO, USA). Kaempferol (≥99%, CAS no. 520-18-3), kaempferol-3-*O*-rutinoside (≥98%, CAS no. 17650-84-9), cyanidin chloride (≥96%, CAS no. 528-58-5), delphinidin chloride (≥97%, CAS no. 528-53-0), lycopene (≥98%, CAS no. 502-65-8), and lutein (≥95%, CAS no. 127-40-2) were obtained from Extrasynthase (Genay, France). Caffeic acid (≥99%, CAS no. 331-39-5) and rutin (≥97%, CAS no. 153-18-4) were obtained from Acros Organics (Geel, Belgium). Naringenin (95%, CAS no. 480-41-1) was obtained from ICN Biomedicals (OH, USA). Naringenin chalcone as a mixture with naringenin was purchased from Apin Chemicals (Oxon, UK), since the pure compound was not commercially available. Phytoene (98%, CAS no. 540-04-5), phytofluene (95%, CAS no. 540-05-6), neoxanthin (97%, CAS no. 14660-91-4), violaxanthin (95%, CAS no. 126-29-4), neurosporene (95%, CAS no. 502-64-7), γ-carotene (96%, CAS no. 472-93-5), and δ-carotene (95%, CAS no. 472-92-4) were obtained from Carotenature (Lupsingen, Switzerland).

Rosiglitazone and all phenolic compounds and tocopherols were dissolved in dimethylsulfoxide (DMSO, 99.9%, CAS no. 67-68-5, Acros Organics, Geel, Belgium). The fatty acids were dissolved in ethanol (absolute, CAS no. 64-17-5, Merck KGaA, Darmstadt, Germany), and the carotenoids were dissolved in tetrahydrofuran (THF; ≥99%, CAS no. 109-99-9, Acros Organics, Geel, Belgium).

2.2. Extraction of Tomato Samples. *2.2.1. Tomato Extracts Containing Semipolar Compounds.* The model tomato sample "tomato mix" was previously described.[32] In short, ripe beef, cherry, and round tomatoes were pooled and snap frozen in liquid nitrogen. Then, the frozen tomatoes were ground to a powder using an analytical mill (type A11 basic, IKA, Staufen, Germany). The powder was stored at −80 °C until further use. A glycosidase-treated tomato extract that contains enzymatically hydrolyzed phenolic compounds was prepared from the tomato mix. To this end 300 μL of 0.1 M sodium acetate (pH 4.8) and 100 μL of Viscozyme L (Sigma Aldrich, St. Louis, MO, USA) were added to 0.6 g of tomato mix and incubated at 37 °C for 1 h. Then, 3 mL of methanol was added, and the Viscozyme-treated tomato mix was put in an ultrasonic bath for 10 min and centrifuged at 1000*g* for 10−15 min. The supernatant was filtered using 0.2 μm polytetrafluoroethylene filters (M-filter, Tiel, The Netherlands) and dried under a nitrogen stream and stored at −80 °C. The Viscozyme-treated extract, referred to as enzymatically hydrolyzed tomato extract (containing hydrolyzed phenolic compounds), was dissolved in DMSO/assay medium (1:4 v/v) just before analysis in the PPARγ2 CALUX cells.

In addition to the tomato mix, nine different tomato varieties, which were kindly provided by Syngenta (Enkhuizen), were investigated. These nine tomato varieties vary in their polyphenolic content and included two high-pigment tomato varieties (varieties G and H). Enzymatically hydrolyzed extracts of the nine tomato varieties were prepared using the method described above. These extracts were tested in the PPARγ2 CALUX cell line for their potency to induce PPARγ-mediated gene expression. In addition, chemical analyses of the seven extracts that appeared active in the PPARγ2 CALUX assay without showing cytotoxicity (varieties A−H) were performed using LC-MS.

2.2.2. Tomato Extract Containing Isoprenoids. Roma tomatoes were purchased at a local supermarket and were pooled, snap frozen in liquid nitrogen, and subsequently ground in an analytical mill. The powder, which will be further referred to as Roma tomato mix, was stored at −80 °C until further use. A chloroform extract containing isoprenoids was prepared from the Roma tomato mix as described before.[26] In short, 0.5 g of Roma tomato mix was dissolved in 4.5 mL of methanol/chloroform (2.5:2.0 v/v) and put on ice for 10 min. Then, 2.5 mL of precooled Tris-HCl (50 mM, pH 7.4) was added, and the sample was mixed. After centrifugation for 10 min at 1000*g*, the chloroform phase was transferred to a new tube. Extraction was repeated twice by adding fresh chloroform to the remaining methanol/Tris phase, mixing, and centrifugation of the tubes. The three chloroform fractions were combined and dried under a stream of nitrogen and stored at −80 °C. Prior to analysis in the PPARγ2 CALUX cells, the isoprenoid-containing tomato extract was dissolved in THF/assay medium (1:1 v/v).

2.3. Cell Culture. The construction and validation of the PPARγ2 CALUX cell line (BioDetection Systems, Amsterdam, The Netherlands) was described before.[31] In short, human U2OS osteosarcoma cells were stably transfected with an expression vector for PPARγ2 and a reporter construct containing a luciferase gene under transcriptional control of 3xPPRE-tata. PPARγ2 CALUX cells were grown in culture medium (DMEM/F12 glutamax medium, Invitrogen, Breda, The Netherlands) supplemented with 7.5% fetal calf serum (Invitrogen), nonessential amino acids (Invitrogen), and penicillin/streptomycin (Invitrogen) (final concentrations 10 U/mL and 10 μg/mL, respectively). Once per week 200 μg/mL G418 (Duchefa Biochemie, Haarlem, The Netherlands) was added to the culture medium in order to maintain selection pressure. Cells were cultured at 37 °C and 5% CO_2 in a humid atmosphere.

2.4. Reporter Gene Assays. The ability of tomato extracts or individual compounds to induce PPARγ2-mediated luciferase expression was tested by measuring luciferase activity in the PPARγ2 CALUX reporter cells. To this end, PPARγ2 CALUX cells were seeded in 96-well plates (Corning Incorporated, Cambridge, MA, USA) at a density of 10 000 cells per well in 100 μL of assay medium (DMEM/F12 without phenol red, Invitrogen) supplemented with 5% fetal calf serum treated with dextran-coated charcoal (Thermo Scientific, Waltham, MA, USA), nonessential amino acids (Invitrogen), and penicillin/streptomycin (Invitrogen) (final concentrations 10 U/mL and 10 μg/mL, respectively). Before exposure to the fatty acids, the plated cells were treated with vitamin E by adding 20 μL of a 50 mM RRR-α-tocopherol solution to 20 mL of assay medium (final concentration 50 μM vitamin E). Vitamin E serves as an antioxidant to prevent oxidation of the unsaturated fatty acids. After 24 h, when the cells formed a monolayer, 100 μL of fresh assay medium supplemented with the test compounds was added to the wells. When testing the individual compounds, the percentage of solvent in the exposure medium was kept at or below 0.5%. Only for testing the highest concentrations of 100 μM, 1% of solvent had to be used. On each plate, 1 μM rosiglitazone was included as a positive control. All individual compounds were tested in concentrations up to 100 μM. Only the fatty acids were tested in concentrations up to 200 μM. During the exposure to fatty acid, the cells were coexposed to 50 μM freshly added vitamin E and 0.1% BSA (Sigma Aldrich, St. Louis, MO, USA). BSA facilitates the solubility and cellular availability of the fatty acids. After 24 h of exposure the medium was removed and low-salt buffer (30 μL per well) was added. The plates were subsequently frozen overnight at −80 °C in order to lyse the cells. Luciferase activity was measured using a luminometer (Luminoscan Ascent, Thermo Scientific, Waltham, MA, USA) by adding 100 μL of flash mix (20 mM Tricine, 1.07 mM $(MgCO_3)_4Mg(OH)_2$, 2.67 mM $MgSO_4$, 0.1 mM EDTA, 2.0 mM dithiothreitol, 470 μM luciferine, 5.0 mM ATP) per well and measuring the light production as relative light units (RLU).

All individual tomato compounds were tested for cytotoxicity using the Cytotox CALUX cell line as described by Van der Linden et al.[33] These Cytotox CALUX cells are U2OS cells with an invariant luciferase expression and respond to cytotoxicity with a decreased luciferase activity compared to the solvent control. Only noncytotoxic concentrations of the individual tomato compounds were used for testing in the PPARγ2 CALUX cell line. The Cytotox CALUX assay was also used to confirm that, under the conditions tested, there was

no effect of the tomato extracts or tomato compounds on luciferase activity itself.

2.5. Chemical Analysis Using LC-PDA-QTOF-MS. Using LC-PDA-QTOF-MS, semipolar compounds in the sample can be separated using liquid chromatography (LC) and detected using photodiode array (PDA). In the next step, the compounds are ionized and masses are detected using quadrupole time-of-flight (QTOF) in combination with mass spectrometry (MS). This LC-PDA-QTOF-MS method provides insight into the semipolar compounds present in the (tomato) sample since individual compounds can be identified on the basis of their retention time and mass. LC-PDA-QTOF-MS analysis was performed as described before by De Vos and colleagues [34] in order to detect semipolar compounds present in the enzymatically hydrolyzed extracts of the seven noncytotoxic tomato varieties. In short, 5 μL of the redissolved and filtered deglycosylated tomato extracts was injected onto a C_{18} column. Chromatographic separation was performed using ultrapure water (eluent A) and acetonitrile (eluent B), both acidified with 0.1% formic acid, using a linear gradient starting at 5% B up to 35% B in 45 min with a flow rate of 0.190 mL min^{-1}. Thereafter, the column was washed and equilibrated for 15 min at the starting conditions, before the next injection. After separation and detection of the semipolar compounds by LC-PDA, ionization was performed using an electrospray ionization source and masses were detected in positive mode (ESI+) using quadrupole time-of-flight high-resolution mass spectrometry (QTOF-MS). Ion chromatograms obtained from LC-PDA-QTOF-MS were analyzed using MassLynx 4.1 (Waters) software.

2.6. Data Analysis. Each test compound or extract was tested in at least two independent experiments, and one representative curve is presented (unless stated otherwise). In each of the independent experiments, all data points were performed in triplicate. The RLU data were converted into percentages of the positive control (1 μM rosiglitazone) and presented as mean values ± standard error (SE). Fold inductions were calculated by dividing the luciferase activity of the sample by the luciferase activity of the solvent control sample. Individual compounds giving less than 2-fold induction at the maximum concentration (100 μM or the highest concentration that could be tested without cytotoxicity) were considered unable to induce PPARγ2-mediated gene expression. Statistical significance was assessed using the one-sided Student's t test and a cutoff value of $p \leq 0.05$. Given p-values are per-comparison error rates.

3. RESULTS AND DISCUSSION

3.1. PPARγ Activation by Individual Phytochemicals. In order to investigate whether compounds that are known to be present in tomato have the capacity to function as PPARγ agonists, PPARγ2 CALUX reporter cells were exposed for 24 h to increasing concentrations of 30 individual compounds. These 30 compounds were selected on the basis of their presence in tomato fruit (Table 1) and include 11 polyphenols, 10 carotenoids, three tocopherols, and six fatty acids. Of all 30 compounds tested, the phenolic compounds kaempferol, naringenin, and naringenin chalcone and the carotenoids violaxanthin, phytofluene, neurosporene, lycopene, β-carotene, γ-carotene, and δ-carotene were able to induce PPARγ2-mediated expression of luciferase (Table 1 and Figures S1−S3 in the Supporting Information). The other phenolic compounds and carotenoids as well as the three tocopherols were not able to activate PPARγ2-mediated luciferase expression (Table 1). Induction of PPARγ by β-carotene, lycopene, kaempferol, naringenin, and naringenin chalcone is in line with findings in the literature reporting that these compounds can bind to PPARγ when tested in receptor binding assays.[35−37] These earlier findings, however, indicate binding to the PPARγ receptor, which does not automatically lead to activation of PPARγ and to subsequent changes in PPARγ-mediated gene expression patterns. For example, although quercetin was reported to bind to PPARγ,[35,37] it was not able to stimulate PPARγ-mediated gene expression in PPARγ2 CALUX cells. Of the polyphenols and isoprenoids tested in the current research, kaempferol, naringenin, and naringenin chalcone have been shown to influence PPARγ-mediated gene expression before.[38−40] In addition, cyanidin was reported to induce binding of mouse PPARγ to the PPRE,[41] while in our studies cyanidin was not able to induce PPARγ-mediated gene expression. This may be due to a species difference between human and mouse,

Table 1. Overview of Tomato Compounds Selected to Be Tested and Summary of the Results Obtained in the PPARγ2 CALUX Reporter Cell Line

compound	reference(s) for presence in tomato	fold induction at 1 μM	fold induction at 10 μM	maximal fold induction (concentration)
Polyphenolic Compounds				
kaempferol	28, 30	1.5	3.7	
kaempferol-3-*O*-rutinoside	28	inactive	inactive	
quercetin	26, 30	inactive	inactive	
rutin	26, 28	inactive	inactive	
naringenin	28, 30, 49	1.5	5.5	
naringenin chalcone	26, 28	1.5	2.6	
delphinidin[a]	61	inactive	inactive	
cyanidin[a]	62	inactive	inactive	
chlorogenic acid	28, 49	inactive	inactive	
ferulic acid	49, 63	inactive	inactive	
caffeic acid	49, 63	inactive	inactive	
Carotenoids				
lutein	26, 27	inactive	inactive	
neoxanthin	26, 27	inactive	inactive	
violaxanthin	26, 27	1.2	1.2	2.6 (100 μM)
phytoene	27, 29	inactive	inactive	
phytofluene	27, 29	1.0	1.6	
neurosporene	27, 29	1.2	1.7	2.4 (30 μM)
lycopene	27, 29	1.2	1.5	2.6 (100 μM)
β-carotene	26, 27	1.3	2.3	
γ-carotene	27	1.6	3.9	4.6 (100 μM)
δ-carotene[a]	44, 64	1.1	2.5	3.6 (100 μM)
Tocopherols				
α-tocopherol	26, 65	inactive	inactive	
β-tocopherol	65, 66	inactive	inactive	
δ-tocopherol	26, 65	inactive	inactive	
Fatty Acids				
α-linolenic acid (ALA; C18:3n3)	67, 68	1.2[b]	2.6[b]	
oleic acid (OA; C18:1n9)	67, 68	1.3[b]	3.8[b]	
linoleic acid (LA; C18:2n6)	67, 68	1.2[b]	2.8[b]	
palmitic acid (PA; C16:0)	67, 68	inactive	inactive	
stearic acid (SA; C18:0)	67, 68	inactive	inactive	
myristic acid (MA; C14:0)	67	inactive	inactive	

[a]Although not found in conventional tomato, these compounds are found in special varieties such as purple tomato (delphinidin and cyanidin) and Delta tomato (δ-carotene).[44,61,64] [b]For fatty acids, the fold induction at 10 and 100 μM is presented (instead of 1 and 10 μM).

dx.doi.org/10.1021/jf304790a | J. Agric. Food Chem. 2013, 61, 3419−3427

or it may indicate that the binding of PPARγ to the PPRE as induced by cyanidin is not adequate to produce a change in gene expression.

It is of interest to note that none of the flavonoid glycosides were able to induce PPARγ2-mediated expression, whereas some of the corresponding aglycones are active agonists (Table 1). For example, the aglycone kaempferol was able to induce PPARγ2-mediated expression, while its glycoside kaempferol-3-*O*-rutinoside was not. As metabolism of polyphenols and isoprenoids may affect their bioactivity, future research could focus on metabolites of these compounds. Although our findings show that flavonoid glycosides do not show activity, this does not necessarily imply that glucuronidated flavonoid metabolites would be without effect as well. Previous studies in an EpRE reporter gene cell line have demonstrated that, for example, glucuronides of quercetin can induce EpRE-mediated gene expression up to 5-fold and that this EpRE-mediated gene induction in the reporter cell line by these glucuronides includes their efficient deglucuronidation.[42] Whether similar results would be obtained for the PPARγ2 CALUX reporter cell line remains to be established.

Of the six fatty acids tested for their ability to activate PPARγ2, α-linolenic acid, oleic acid, and linoleic acid were found to act as PPARγ2 agonists, while palmitic acid, stearic acid, and myristic acid were not functioning as PPARγ2 agonists (Table 1). These findings are in line with data reported by Chou et al. showing that ALA, OA, and LA function as PPARγ agonists and that palmitic acid and stearic acid do not activate PPARγ.[43] Chou and colleagues, however, report that myristic acid also functions as a PPARγ agonist, while our data did not show PPARγ agonism for this compound. Of all tested compounds, naringenin, kaempferol, and γ-carotene were the most active inducers of PPARγ2-mediated luciferase gene expression: these compounds induce PPARγ2-mediated luciferase expression at concentrations of 1 μM or higher and showed the highest fold induction at a concentration of 10 μM (Table 1).

3.2. PPARγ Activation by Extracts of Tomato Fruit. To investigate the potential of tomato fruit to induce PPARγ2-mediated luciferase gene expression, two different types of tomato extracts were tested: one extract containing isoprenoids obtained by chloroform extraction (further referred to as isoprenoid-containing extract) and one extract containing semipolar compounds, including flavonoids and other phenolic compounds. As the results with the individual compounds indicated that only the aglycones (and not the glycosides) were able to activate PPARγ, the tomato powder sample was treated with Viscozyme L before extraction with methanol in order to remove the glycosyl residues from the bioactive phenolic components and will be further referred to as enzymatically hydrolyzed tomato extract. After 24 h of exposure, the isoprenoid-containing tomato extract was able to induce PPARγ2-mediated luciferase in a dose-dependent manner (Figure 1). The PPARγ2-activating effect of the isoprenoid-containing tomato extract may be due to the presence of the carotenoids violaxanthin, phytofluene, neurosporene, lycopene, β-carotene, γ-carotene, and δ-carotene, which were all found to function as PPARγ2-agonists (Table 1) and are known to be present in tomato fruits.[26,27,29,44] In addition, also the enzymatically hydrolyzed tomato extract was able to induce PPARγ2-mediated luciferase in a dose-dependent manner (Figure 1). In the enzymatically hydrolyzed tomato extract kaempferol, naringenin, and naringenin chalcone may contribute to the activity since these compounds were able to activate PPARγ2 when tested as pure compounds. Previous studies have reported the presence of these compounds in tomato fruit.[28,30] In humans, enzymatic hydrolysis of flavonoid glycosides also occurs in the intestine before uptake.[45,46]

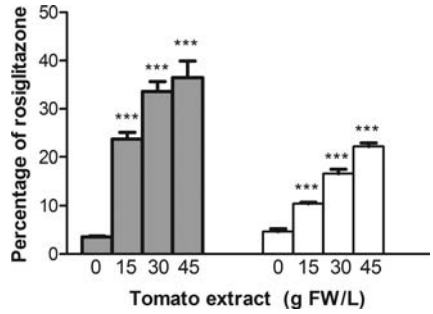

Figure 1. Enzymatically hydrolyzed tomato extract (gray bars) and isoprenoid-containing tomato extract (white bars) induce dose-dependent significant PPARγ2-mediated luciferase expression in the PPARγ2 CALUX cells. The amount of tomato extract is expressed as gram fresh weight per liter (g FW/L). Luciferase activity is expressed as percentage of the positive control (1 μM rosiglitazone). Data are corrected for background luciferase activity and are expressed as mean ± SEM ($n = 3$). Asterisks indicate the level of significance compared to the solvent control: *$p < 0.05$; **$p < 0.01$; and ***$p < 0.001$.

3.3. PPARγ Activation by Seven Different Tomato Varieties. In addition to the mixture of beef, cherry, and round tomatoes, enzymatically hydrolyzed extracts of nine tomato varieties, designated A–I, were tested for their potency to induce PPARγ-mediated gene expression. These nine tomato varieties included two high-pigment tomato varieties (G and H). Two tomato varieties, H and I, showed cytotoxicity at 45 g FW/L and were therefore not included in further analysis. The other seven varieties were all able to significantly induce PPARγ-mediated gene expression at 45 g FW/L (Figure 2). There was a 1.6-fold difference in the induction found with the least potent variety (variety A) and the most potent variety (variety G) (Figure 2).

The extracts of the seven tomato varieties were chemically analyzed using LC-MS, and chromatograms representing the total ion signal of each variety are presented in the Supporting

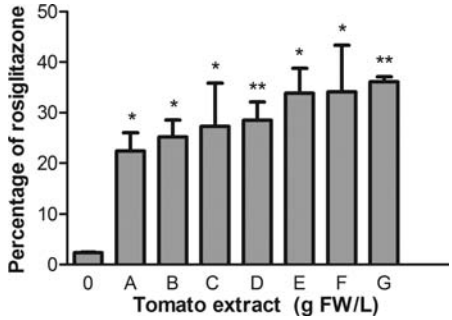

Figure 2. Enzymatically hydrolyzed extracts of seven tomato varieties induce PPARγ2-mediated luciferase expression in the PPARγ2 CALUX cells. The extracts were tested at 45 g FW/L. The solvent control (0) is included. Luciferase activity is expressed as a percentage of the positive control (1 μM rosiglitazone). Data are corrected for background luciferase activity and are expressed as mean ± SEM of three independent experiments. Asterisks indicate the level of significance compared to the solvent control: *$p < 0.05$; **$p < 0.01$; and ***$p < 0.001$.

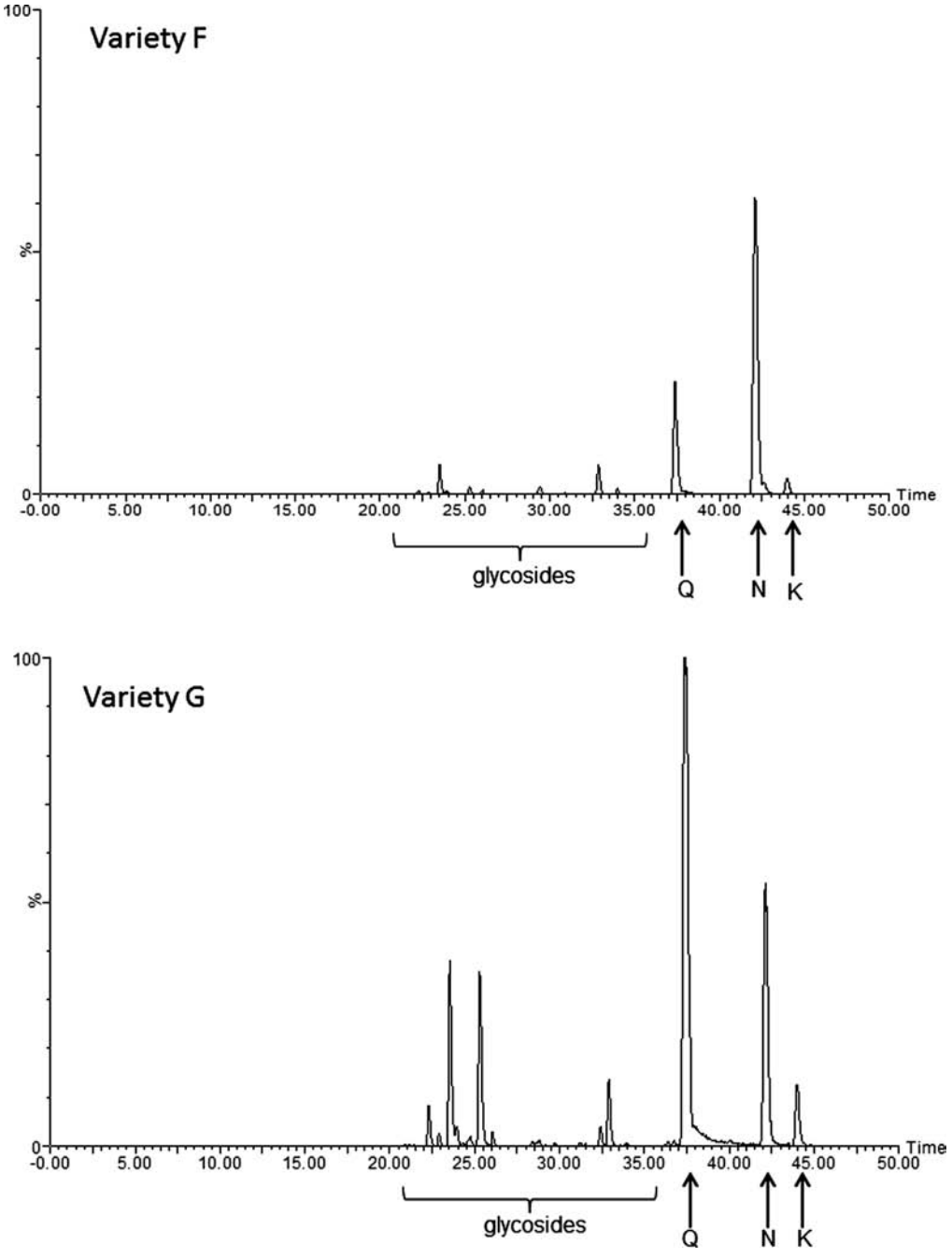

Figure 3. Chromatograms of enzymatically hydrolyzed extracts of the most potent tomato varieties (F and G), representing the total ion signal of selected masses: 273.075 (naringenin); 287.055 (kaempferol); 303.050 (quercetin). Maximal intensity (100%) of 15 000. Indicated with arrows are N = naringenin; K = kaempferol; Q = quercetin; glycosides = flavonoid glycosides. For complete chromatograms, see the Supporting Information.

Information. Chromatograms showing selected masses of flavonoid aglycones indicated that considerable amounts of kaempferol, naringenin, and quercetin were present in the most potent tomato varieties, F and G (Figure 3). In addition, levels of kaempferol and naringenin, both found to function as PPARγ2 agonists when tested individually, were quantified in the enzymatically hydrolyzed extracts of the seven tomato varieties (Table 2). It is interesting to note that the least potent varieties, A and B, had the lowest content of kaempferol and naringenin, while the two most potent varieties, F and G, had the highest content of kaempferol and naringenin. These results suggest that the differences in potency to induce PPARγ-mediated gene expression may be partly explained by the differences in levels of kaempferol and naringenin and/or other flavonoids.

3.4. PPARγ Activation by Phytochemical Mixtures. Most of the individual compounds induced PPARγ2-mediated luciferase expression at concentrations of 1 μM and higher. These concentrations seem relatively high, since the concentrations of these compounds in human plasma normally do not exceed 1 μM.[47,48] After tomato consumption, plasma concentrations of several phytochemicals, including β-carotene, lycopene, and naringenin, increase, but still stay below 1 μM.[21,47,49] The total concentration of phytochemicals, however, is higher than 1 μM[50,51] and may be high enough to activate PPARγ and lead to PPARγ-mediated changes in gene

dx.doi.org/10.1021/jf304790a | J. Agric. Food Chem. 2013, 61, 3419−3427

Table 2. Concentrations of Kaempferol and Naringenin Found in Enzymatically Hydrolyzed Extracts of Seven Tomato Varieties

tomato variety	kaempferol (μM)[a]	naringenin (μM)[a]
A	0.07	0.59
B	0.04	0.04
C	1.13	7.76
D	0.03	1.30
E	0.02	1.59
F	0.31	13.19
G[b]	1.33	10.56

[a]Kaempferol and naringenin are presented as μM present in extract dissolved and diluted to 45 g FW/L, which is the concentration at which the extracts were tested in the PPARγ2 CALUX cell line. [b]High-pigment variety.

expression. To investigate whether an additive effect of different tomato compounds on PPARγ2 activation can be expected, induction of PPARγ2-mediated gene expression by combinations of kaempferol, naringenin, and β-carotene was investigated. This reveals that upon combining different compounds at concentrations of 1 μM that individually do not induce PPARγ2-mediated gene expression, induction of PPARγ2-mediated expression can be obtained (Figure 4). The effect

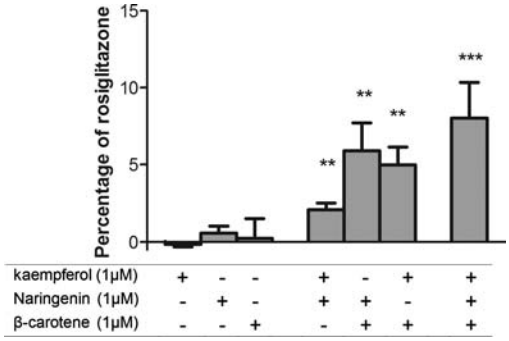

Figure 4. PPARγ2-mediated luciferase expression in the PPARγ2 CALUX cells induced by individual phytochemicals and combinations of phytochemicals at 1 μM each. Luciferase activity is expressed as percentage of the positive control (1 μM rosiglitazone). Data are corrected for background luciferase activity and for luciferase activity observed with the solvent control. Data are expressed as mean ± SEM of five independent experiments. Asterisks indicate the level of significance compared to the solvent control: *$p < 0.05$ and **$p < 0.01$.

was most striking for the combination of β-carotene with either kaempferol or naringenin: the individual compounds were not able to induce PPARγ2-mediated expression at 1 μM, but the combination of 1 μM β-carotene with either 1 μM kaempferol or 1 μM naringenin resulted in a significant induction. Our results suggest an additive effect when combining individual phytochemicals. This additive effect might be achieved by the fact that these phytochemicals all act by binding to the same receptor, and therefore their concentrations can be added up. Additive effects between phytochemicals have been reported before.[52] Another explanation could be that the mixtures of phytochemicals are also able to activate RXR, which is the dimerization partner for PPARγ, leading to an additive effect in activation of the PPARγ–RXR complex.

Furthermore, phytochemicals are present not only in plasma but also in tissues. In tissues the total carotenoid level has been reported to reach concentrations of 5.1, 9.4, and 7.6 nmol/g wet tissue (equal to 5.1, 9.4, and 7.6 μM, assuming that 1 kg of wet tissue corresponds to 1 L) in liver, adrenal glands, and testes, respectively.[53] These concentrations correspond to the concentrations of carotenoids that were found to induce PPARγ2-mediated gene expression. However, the functional effects of the food compounds and extracts still need to be substantiated *in vivo*. This is true for carotenoids, but especially for polyphenols that do not occur as aglycones in plasma.

The beneficial effects of tomato consumption have often been linked to lycopene.[54] Our data indicate that many other phytochemicals present in tomato may be involved in possible PPARγ-mediated beneficial health effects of tomato fruits.

3.5. Tomato, PPARγ, and Health. Induction of PPARγ-mediated gene expression has frequently been suggested to play a role in insulin sensitization,[55] protection against prostate, breast, and colon cancer,[12,13] and protection against atherosclerosis.[10,11] Although our data indicate that tomato extracts are able to induce PPARγ-mediated changes in gene expression, additional data are needed to confirm that tomato consumption *in vivo* leads to PPARγ-mediated gene expression and to PPARγ-related beneficial health effects. Several *in vivo* studies provide a link between tomato consumption and beneficial effects on lipid peroxidation rate,[56] lipid profile,[19] and blood pressure.[57,58] A role for PPARγ-mediated gene expression in end points such as serum levels of free fatty acids and HDL cholesterol, blood pressure, and glucose tolerance has been reported,[4,8,59] and activation of PPARγ may thus provide a potential mode of action of several of the beneficial health effects of tomato consumption.

Using reporter gene assays, it was previously demonstrated that PPARγ1 and PPARγ2 are activated by PPARγ agonists in a similar way.[31,60] This suggests that tomato compounds and tomato extracts that are able to activate PPARγ2 may be able to also activate PPARγ1.

In conclusion, our data show that isolated tomato compounds as well as the enzymatically hydrolyzed tomato extract containing phenolic compounds and the isoprenoid-containing tomato extract were able to induce PPARγ2-mediated gene expression. Taking into account concentrations at which PPARγ activation was detected and reported physiological levels of PPARγ-activating compounds in plasma and various tissues, our results indicate that beneficial health effects associated with tomato consumption may be (partly) mediated by PPARγ2-mediated induction of gene transcription.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Concentration–response curves of some polyphenolic compounds (Figure S1), carotenoids (Figure S2), and fatty acids (Figure S3) as well as chromatograms of enzymatically hydrolyzed extracts of tomato varieties A–G, representing the total ion signal (Figure S4). This information is available free of charge via the Internet at http://pubs.acs.org.

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Funding

This work was supported by the Food and Nutrition Delta (grant number FND07007).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Syngenta for providing fruits of the nine tomato varieties.

■ ABBREVIATIONS USED

ALA, α-linolenic acid; DMSO, dimethyl sulfoxide; FW, fresh weight; LA, linoleic acid; LC-MS, liquid chromatography−mass spectrometry; LC-PDA-QTOF-MS, liquid chromatography−photodiode array−quadrupole time-of-flight−mass spectrometry; MA, myristic acid; OA, oleic acid; PA, palmitic acid; PPARγ, peroxisome proliferator-activated receptor γ; PPRE, peroxisome proliferator-responsive element; RLU, relative light units; RXR, retinoid X receptor; SA, stearic acid; THF, tetrahydrofuran; TZDs, thiazolidinediones

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