RESEARCH ARTICLE

Vitamin E tocotrienols improve insulin sensitivity through activating peroxisome proliferator-activated receptors

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Vitamin E is comprised of two classes of compounds: tocopherols and tocotrienols. Tocotrienol-enriched palm oil has been shown to help reduce blood glucose levels in patients and preclinical animal models. However, the mechanistic basis for tocotrienol action is not well established. Peroxisome proliferator-activated receptors α, γ, and δ (PPARα, PPARγ, and PPARδ) are ligand-regulated transcription factors that play essential roles in energy metabolism. Importantly, synthetic PPARα and PPARγ ligands are currently used for treating hyperlipidemia and diabetes. In this study, we present data that tocotrienols within palm oil functioned as PPAR modulators. Specifically, both α- and γ-tocotrienol activated PPARα, while δ-tocotrienol activated PPARα, PPARγ, and PPARδ in reporter-based assays. Tocotrienols enhanced the interaction between the purified ligand-binding domain of PPARα with the receptor-interacting motif of coactivator PPARγ coactivator-1α. In addition, the tocotrienol-rich fraction of palm oil improved whole body glucose utilization and insulin sensitivity of diabetic *Db/Db* mice by selectively regulating PPAR target genes. These lines of evidence collectively suggested that PPARs represent a set of molecular targets of tocotrienols.

Keywords:
Diabetes / Peroxisome proliferator-activated receptor / Tocotrienols

1 Introduction

Vitamin E is comprised of two classes of compounds, tocopherols and tocotrienols. Known for its anti-oxidant action, α-tocopherol is the most studied vitamin E, while the actions of tocotrienols are less well understood. However, recent studies on tocotrienols suggested that tocotrienols are anti-inflammatory *in vitro* [1] and neuro-protective in preclinical animal models [2]. Notable is that tocotrienols improve metabolic parameters in patients [3].

Peroxisome proliferator-activated receptors α, γ, and δ (PPARα PPARγ, and PPARδ) are ligand-regulated transcription factors belonging to the nuclear hormone receptor superfamily [4]. Like other members in this superfamily, PPARγ, for example, contains a ligand-independent transcriptional domain at its *N* terminus, a centrally located DNA-binding domain (DBD), a variable hinge region, and a C-terminally located ligand-binding domain (LBD). The LBD is responsible for recognizing and binding to a variety of structurally distinctive endogenous and exogenous ligands. PPARs recruit cofactors such as PPARγ coactivator-1α (PGC-1α) through its receptor-interacting motifs (LXXLL motifs) in a ligand-dependent manner to modulate the transcriptional levels of target genes [5].

PPARs play essential roles during the developments of diabetes through regulating energy metabolism. First,

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Abbreviations: CPT2, carnitine palmitoyl transferase 2; **ITT,** insulin tolerance test; **LBD,** ligand-binding domain; **OGTT,** oral glucose tolerance test; **PPARαγδ,** peroxisome proliferator-activated receptors αγδ; **PGC-1α,** PPARγ coactivator-1α; **TRF,** tocotrienol-rich fraction; **UCP3,** uncoupling protein 3

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endogenous ligands for PPARs such as unsaturated fatty acid, prostaglandins, and lysophosphatidic acid, are key physiological regulators of metabolic pathways [6–11]. Secondly, genetic mutations of PPARγ are associated with insulin resistance and diabetes in human [12]. Thirdly, manipulating PPAR expression levels in rodents suggested that PPAR activities participate in improving insulin sensitivity, conferring resistance to high-fat-diet-induced obesity [13–15]. Importantly, synthetic ligands of PPARα and PPARγ are prescription drugs for dyslipidemia and diabetic treatments [16], while synthetic ligands for PPARδ are currently under development for metabolic diseases [17].

Since some of the known beneficial effects of tocotrienols overlap with those of PPAR ligands and the chemical structures of tocotrienols partially resemble that of a known PPARγ agonist troglitazone, we tested the hypothesis that tocotrienols function as PPAR modulators to exert their health beneficial effects. Using both *in vitro* receptor–coactivator interaction assays and cell-based functional assays, we established that tocotrienols are PPAR ligands. In addition, using a tocotrienol-rich fraction (TRF) derived from palm oil, we confirmed that TRF behaved as tissue- and target gene-selective PPAR modulators in diabetic *Db/Db* mice *in vivo*.

2 Materials and methods

2.1 Chemical reagents

Rosiglitazone, WY14643, GW0742, and oleic acid were purchased from Sigma Chemicals; DMEM and penicillin-streptomycin from GIBCO-BRL Life Technologies; FBS Hyclone; Lipofectamine 2000, Trizol and SuperScriptTM III Reverse Transcriptase kits from Invitrogen Life Science Technologies; random primers and SYRB green kit Takara; Dual-Luciferase Reporter Assay System from Promega; protease inhibitor cocktail III from Merck; Cremophor EL from BASF; and human insulin from Eli Lilly. The TRF composed of 23.54% α-tocotrienol, 43.16% γ-tocotrienol, 9.83% δ-tocotrienol, and 23.5% α-tocopherol was kindly provided by Dr. Kah Hay Yuen of University Sains Malaysia and used as described [18]. Purified tocotrienols were kindly provided by Dr. Abdul Gapor of Malaysian Palm Oil Board and used as described [19].

2.2 Animals and administration

Male C57BLKS/J-*Lepr Db/Db* mice (18-wk-old) were purchased from National Resource Center for Mutant Mice and randomized into two groups of eight housed at $22 \pm 2^{\circ}C$ on a 12-h light–dark cycle. Mice were treated daily with an administration of vehicle (1:10 dilution of 1:1 ethanol and Cremophor EL in phosphate-buffered saline) or 50 mg/kg TRF by gavage for 2 wk. Animal studies were conducted in accordance with the Animal Research Committee of the Guangzhou Institute of Biomedicine and Health (SYXK, Guangdong, China).

2.3 Oral glucose and insulin tolerance tests

For oral glucose tolerance tests (OGTT), mice were fasted for 6 h and glucose levels from tail vein blood were determined immediately using an Accu-chek advantage glucometer (Roche) at 0, 15, 30, and 60 min after an oral administration of 1 g/kg glucose. For insulin tolerance tests (ITT), 6-h-fasted mice were intraperitoneally injected with 1.5 U/kg insulin. Glucose levels were determined as above at 0, 15, 30, and 60 min after injection.

2.4 Quantitative real-time PCR analysis

Muscle samples of *Db/D*b mice were collected and stored at –80°C. Total RNA was extracted by Trizol reagent and cDNA was generated with random primers by SuperScriptTM III Reverse Transcriptase kits according to the manufacturer's protocol. Real-time PCR reactions were performed with a SYRB green kit in an MJ research PTC 200. An 18S rRNA was used as an internal control.

2.5 Transient transfection and luciferase reporter assays

Human kidney 293-FT cells (American Type Culture Collection) were cultured in DMEM with 10% FBS and 100 U/mL penicillin–streptomycin under humidified air containing 5% CO_2 at 37°C. A 293-FT cells (2×10^4 *per* well) were plated in 96-well plates in culture medium. After an overnight cultivation, cells were transfected with 2 ng *per* well pCMV-GAL4-DBD-hPPARα, hPPARγ, or hPPARδ-LBD expression vectors, 25 ng *per* well pFR-Luc reporter plasmid, and 2 ng *per* well *Renilla*-Luc plasmid using 0.25 μL *per* well Lipofectamine 2000. Ligands at indicted concentrations were added 6 h later and incubated for an additional 24 h before luciferase activities were measured using the Dual-Luciferase Reporter Assay System according to the manufacturer's instructions.

2.6 Protein expression and purification

The LBD of hPPARα (amino acids 176–468) was cloned between *Bam*HI and *Hin*dIII sites of pET-30a vector (Stratagene). N-terminal His_6-tagged hPPARα-LBD was expressed from freshly transformed *E. coli* BL21(DE3) in LB media at 37°C to an OD of 0.6. The culture was then induced with 0.05 mM IPTG and grown at 16°C for 16 h. Cells were harvested and re-suspended in buffer A (20 mM

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Tris, pH 8.0, 150 mM NaCl, 10% glycerol, 5 mM 2-mercaptoethanol). Cells were disrupted by sonication in the presence of protease inhibitors (cocktail III and PMSF), and the soluble fraction was isolated by centrifugation (10 000 × *g*, 30 min). The pH of the supernatant was checked and the supernatant was loaded onto Ni–NTA (1/100 mL culture) and eluted with a gradient of 0–300 mM imidazole over 20 column volumes of buffer A. The fractions containing His_6-tagged hPPARα-LBD were dialyzed over buffer A to remove imidazole.

2.7 Receptor and coactivator interaction analysis

Interactions between His_6-tagged hPPARα-LBD and PGC-1α coactivator peptides in the presence of different ligands were analyzed using a BIAcore 3000. Biotinylated PGC-1α peptides (HD Biosciences, China): EEPSLAKKAALAPAN (NR2 negative control); and EEPSLLKKLLLAPAN (NR2) were captured onto the streptomycin-immobilized surface of an SA chip (PerkinElmer) in flow cells 1 and 2, respectively. After stabilization of the surface by injection of 0.05% SDS, 300 nM His6-tagged hPPARα-LBD incubated with various ligands in running buffer (50 mM Tris, pH 8.0, 150 mM NaCl, 1 mM $MgCl_2$, 0.002% NP-40, 0.2 mM DTT) were injected into flow cells for 3 min at a flow rate of 20 μL/min at 25°C. Surface plasmon resonance changes in resonance units were monitored simultaneously in all four-flow cells. The surface was regenerated at the end of each cycle by a quick injection of 0.05% SDS. Sensorgrams were generated by BIAcontrol software 4.1 using double referencing to eliminate responses from the reference surface and buffer-only control. Specific changes in resonance unit were generated with nonspecific interaction between LBD and NR2 negative control mutant peptide deducted.

2.8 Statistical analysis

Data were analyzed with an unpaired Student's *t*-test, and figures were produced by Origin 8.0. Asterisks indicate significant differences (* $p<0.05$, ** $p<0.01$, *** $p<0.001$).

3 Results

3.1 TRF ameliorates whole body glucose utilization and improves insulin sensitivity in diabetic *Db/Db* mice

Palm oil is a natural source of tocotrienol and has been widely used in Asian countries as a health supplement. The TRF of palm oil improved metabolic parameters in patients [20]. To examine if TRF mediated some of its beneficial effects through altering energy metabolism, we first treated diabetic *Db/Db* mice with TRF and then measured glucose disposal and insulin sensitivity. *Db/Db* mice ($n=8$ *per* group) were daily dosed by gavage with vehicle or 50 mg/kg *per* day TRF for 2 wk. We found no significant changes in body weight and liver weight upon TRF treatment (data not shown). OGTT and ITT were used to assess the glucose and insulin sensitivities of these animals. We found that TRF at this dose was able to lower the fasting blood glucose level and reduce the areas under the curve in both OGTT (Fig. 1A) and ITT (Fig. 1B), indicating improvements in both glucose and insulin sensitivities.

3.2 TRF regulates the expression of PPAR target genes in muscle tissue

Since muscle is responsible for metabolizing the majority of glucose, we then subjected half of the animals to 12 h of fasting and collected skeletal muscle samples to examine the mRNA expression patterns of key metabolic regulatory genes by quantitative RT-PCR. Glucose transporter 4 (Glut4) is primarily responsible for insulin-stimulated glucose uptake in skeletal muscle [21]. We found that TRF treatment enhanced the expression levels of Glut4 under both fasting and fed states (Fig. 2A); however, the inductions did not reach statistical significance. Muscle also metabolizes fatty acid through β-oxidation as a source of energy. Similarly, we found that the expression level of carnitine palmitoyl transferase 2 (CPT2), which is responsible for initiating long-chain fatty acids β-oxidation in the mitochondria [22], was induced by TRF in the fed state but did

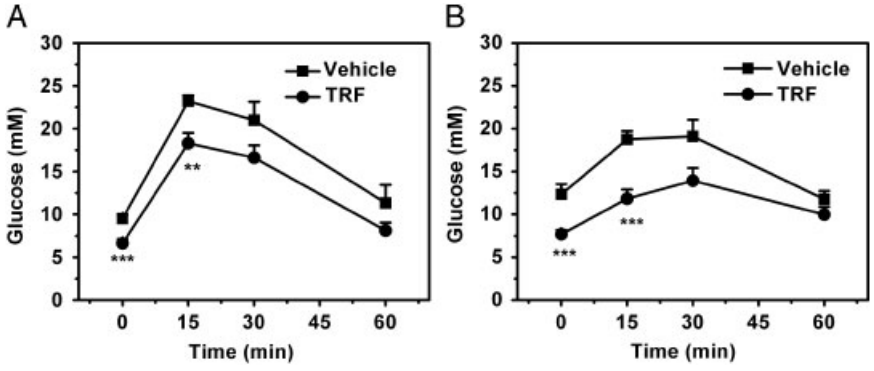

Figure 1. TRF improves insulin sensitivity in *Db/Db* mice. *Db/Db* mice ($n=8$) were orally dosed with 50 mg/kg body weight TRF or vehicle for 2 wk. Mice were subjected to an OGTT (A) and an ITT (B) as described in Section 2. Values are shown as mean ± SEM. Asterisks indicate significant differences. (** $p<0.01$, *** $p<0.001$).

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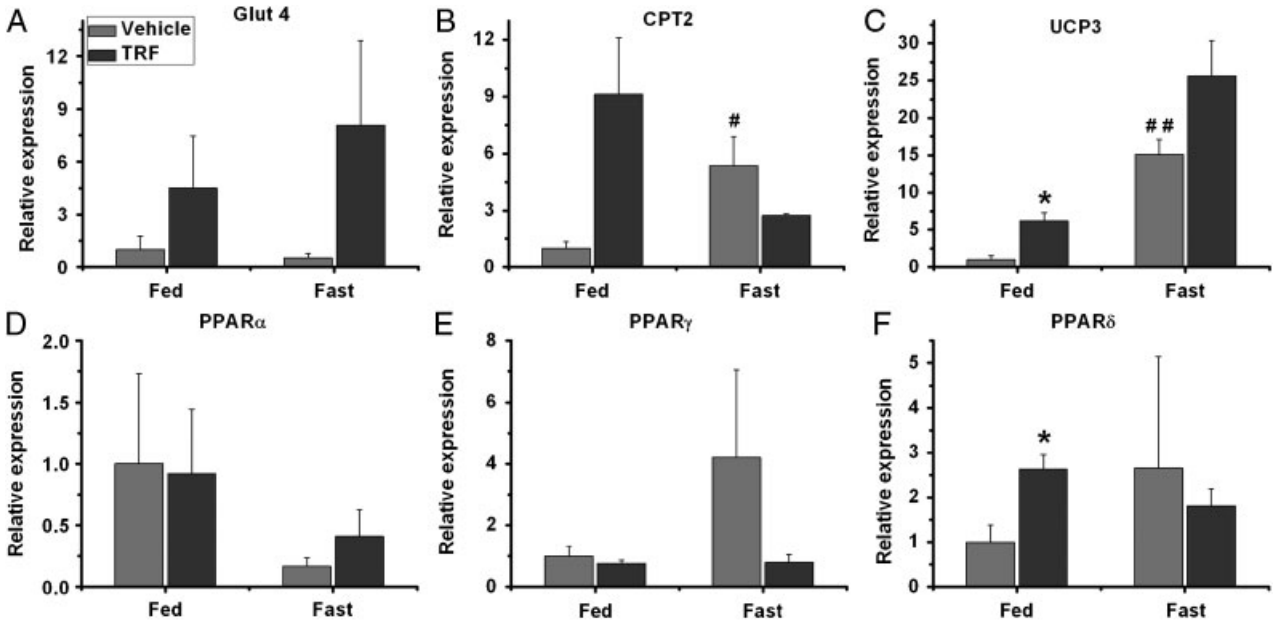

Figure 2. TRF selectively enhances the expression of PPAR target genes. Muscle samples from fasted and nonfasted (Fed) mice were collected and mRNA expression levels of PPARs and their target genes were examined by quantitative RT-PCR. Relative expression levels of Glut4 (A), CPT2 (B), UCP3 (C), PPARα (D), PPARγ (E), and PPARδ (F) were normalized to 18S rRNA and presented with the fed state vehicle control set as 1. Relative expression levels of target genes are shown as mean ± SEM of three individual mice. # and ## indicate significant differences ($p<0.05$ and $p<0.01$) induced by fasting. Asterisk indicates significant difference ($p<0.05$) induced by TRF treatment.

not reach statistical significance (Fig. 2B). On the other hand, we found that the expression level of uncoupling protein 3 (UCP3), which is responsible for fine tuning mitochondrial membrane potential and promoting β-oxidation [23], was significantly enhanced in the fed state, while the fasting-induced expression was not significantly altered (Fig. 2C).

Since the transcriptional regulations of Glut4, CPT2, and UCP3 in the muscle has been demonstrated to be regulated in part by PPARs [24–26], we next measured if their mRNA expression levels were altered by TRF. We found that TRF did not significantly alter PPARα expression (Fig. 2D); however, the fasting-induced PPARγ expression appeared to be suppressed by TRF treatment, although without reaching statistical significance (Fig. 2E). Importantly, TRF produced a statistically significant enhancement of PPARδ expression in the fed but not fast state (Fig. 2F). Although some of the TRF-mediated inductions of metabolic genes did not reach statistical significance, the data still collectively suggest that glucose uptake and β-oxidation may be enhanced by TRF, associated with altered expression levels of PPARs to promote energy utilization.

3.3 Tocotrienols enhance PPARs activities

In addition to alternation in mRNA expression levels, the overall activity levels of PPARs are governed by ligands that function as agonists or antagonists. We examined if TRF and its primary constituent tocotrienols could function as modulators of PPARs. We transiently transfected 293-FT human kidney cells with a luciferase reporter plasmid under the control of a Gal4 response element, expression vectors for Gal4-DBD fusions of human PPARα, PPARγ or PPARδ LBDs, an internal control *Renilla* luciferase plasmid, and then treated these cells with either a PPARα ligand WY14643, a PPARγ ligand rosiglitazone, a PPARδ ligand GW0742, or dilutions of TRF. We found that TRF dose dependently and potently activated the activity of PPARα (Fig. 3A), and modestly activated the activities of PPARγ (Fig. 3B) and PPARδ (Fig. 3C). We next examined if the primary constituent of TRF, *i.e.* tocotrienols, could stimulate the activities of PPARs. We tested the abilities of purified α-, γ-, and δ-tocotrienols to function as PPAR agonists in the Gal4-DBD-PPAR-LBD system. We found that α-,γ-, and δ-tocotrienols dose dependently and potently enhanced the activity of PPARα (Fig. 3D), whereas δ-tocotrienol modestly enhanced the activities of PPARγ (Fig. 3E) and PPARδ (Fig. 3F), recapitulating the pan-agonistic activities of TRF. Since TRF contains α-tocopherol in addition to tocotrienols, it is conceivable that α-tocopherol is actually responsible for binding to and activating PPARs. We tested for this and found that α-tocopherol was not able to modulate the activities of PPARs in the Gal4-DBD-PPAR-LBD system (data not shown).

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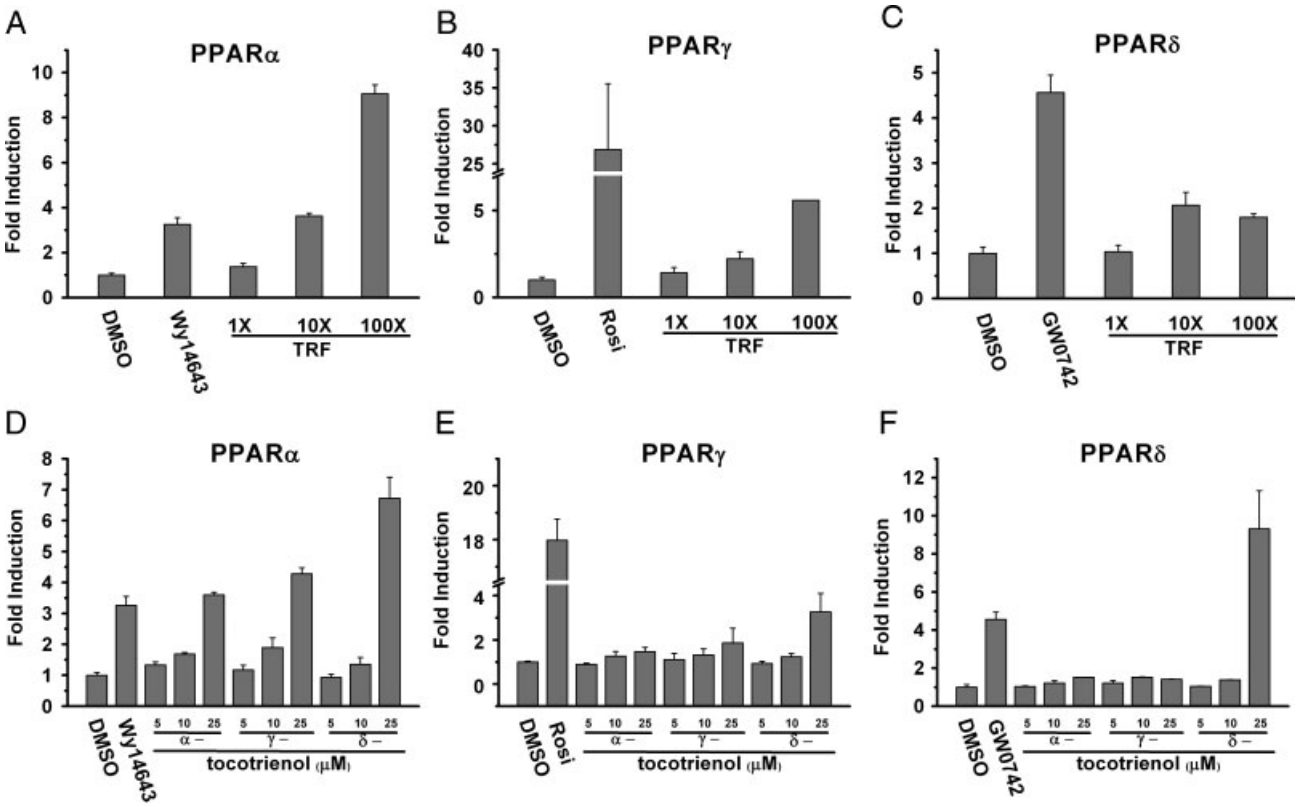

Figure 3. Tocotrienols act as pan-PPAR agonists and 293-FT cells were transiently transfected with a pFR-luciferase reporter plasmid, expression vectors for Gal4-DBD-PPARα (A, D), -PPARγ (B, E), or -PPARδ (C, F) LBDs, and internal control *Renilla* luciferase plasmid. (A–C) TRF dilutions (10^{-5} dilution of TRF as 1X), 50 μM WY 14643, 1 μM Rosiglitazone, 10 nM GW0742, or DMSO as a control were added for 24 h before luciferase assays. (D–F) Purified α-, γ-, and δ-tocotrienols at concentrations indicated were tested as in (A–C). Relative luciferase activity indicating the ratio of pFR-luciferase *versus Renilla* luciferase activities was calculated and the induction by ligands were presented as fold induction *versus* DMSO. All transfection experiments were performed in triplicate wells and each experiment has been repeated at least three times with essentially identical results. Data shown are from representative experiments. Results represent mean ± SD.

3.4 Tocotrienols directly bind to PPARα LBD

Additionally, we employed a cell-free *in vitro* system to confirm if α-, γ-, and δ-tocotrienols could bind to the LBD of PPARα. The LBD of PPARα was expressed in *E. coli* as a His_6-tag fusion protein and purified to more than 95% pure using a Ni–NTA column. The purified PPARα-LBD was then incubated with either vehicle, unsaturated fatty acid oleic acid as a positive control, or different forms of tocotrienols. These ligand-bound LBDs were then allowed to pass through a sensor chip on which the NR2 LXXLL motif or control mutant peptides of coactivator PGC-1α were immobilized. The specific interactions between the coactivator LXXLL peptides with that of LBDs were monitored by measuring the changes in surface resonance, with the nonspecific interaction between the LBDs and mutant peptides deducted. Similar to positive control oleic acid (Fig. 4A), we found that α-, γ-, and δ-tocotrienols were able to enhance the interaction between PPARα-LBD and PGC-1α LXXLL peptide (Figs. 4B–D) in dose-dependent manners, whereas α-tocopherol did not (Fig. 4A). It is therefore likely that α-, γ-, and δ-tocotrienols are direct agonists for PPARs with a preference for PPARα.

4 Discussion

Vitamin E has been used as a dietary supplement primarily due to its anti-oxidant property. Although vitamin E is composed of two classes of chemicals, tocopherols and tocotrienols, most of the vitamin E supplement currently available on the market is in the form of α-tocopherol. However, there are doubts about the effectiveness of α-tocopherol to reduce cardiovascular risk and prevent cancer in human and preclinical animal models [27, 28]. To complicate this further, there is increasing concern over the use of α-tocopherol in particular regarding its ability to stimulate drug-metabolizing enzymes through activating xenobiotic receptors [29]. On the other hand, tocotrienols have been shown to alleviate the symptoms of metabolic disorder through lowering lipid and cholesterol levels in type II diabetic patients [3]. Using preclinical animal

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models, tocotrienols have also been shown to have anti-cancer and neuro-protective properties [30, 31].

The mechanistic action behind the ability of tocotrienol to lower serum cholesterol level had been attribute to its suppressive action on HMG-CoA reductase [32], a key enzyme in the cholesterol synthesis pathway. However, this mechanism leaves unexplained the abilities of tocotreinols to improve insulin sensitivity. In our present study, we established that tocotrienols possess yet another mechanism of action; they are selective PPAR modulators (SPPARMs). Using purified receptor–coactivator interaction assays, we confirmed that tocotrienols, which differ from tocopherols by possessing three double bonds in the phytyl side chain, bound to and enhanced the ability of PPARα to interact with PGC-1α coactivator LXXLL peptides (Fig. 4). We also demonstrated the abilities of tocotrienols to alleviate insulin resistance *in vivo* (Fig. 1) by dually modulating the expression levels of PPARs and a panel of PPAR target genes (Fig. 2). Specifically, UCP3 has been demonstrated to be induced by PPARα and PPARδ ligands [26, 33]. Intriguingly, δ-tocotrienol activates both PPARα and PPARδ in our cell-based reporter assay (Fig. 3). It is therefore likely that the tocotrienols within TRF are responsible for mediating the changes in gene expression through PPARs *in vivo*.

As SPPARMs, α-, and γ-tocotrienols function as PPARα-selective agonists; however, δ-tocotrienol functions as a pan-agonist with partial agonistic activities on PPARγ and PPARδ compared with synthetic full agonists (Fig. 3). The abilities of tocotrienols to enhance PPAR transcriptional activities are likely to be subjected to cell type modification. Comparing the abilities of tocotrienols to enhance receptor and coactivator interaction *in vitro* (Fig. 4) to that of activating receptor activities in tissue culture cells (Fig. 3) suggested that cell type-specific factors may enhance the abilities of tocotrienols to stimulate PPAR activities. Fatty acid-binding proteins (FABPs), which bind to a number of unsaturated fatty acids, have been demonstrated to promote the abilities of these endogenous ligands to activate PPARs [34]. It is therefore reasonable to suggest that these cellular factors may function to modify the abilities of tocotrienols to function as PPAR ligands. In addition, α-tocopherol transfer protein (α-TTP), tocopherol-binding protein (TBP), tocopherol-associated proteins 1, 2, 3 (TAP1/2/3) can bind to and modulate the uptake and distribution of vitamin E [35–38]. Thus, the absorption, distribution, metabolism, and excretion of tocotrienols would likely contribute to another level of regulation *in vivo*.

Besides changing the expression of target genes involved in carbohydrate and lipid metabolism, PPAR ligands are also anti-inflammatory in part through down-regulating the NF-κB pathway [39]. Particularly, the lipopolysaccharide-(LPS-) mediated enhancement of inducible NO synthase is suppressed by PPARγ [40]. Intriguingly, tocotrienols are also known to possess anti-inflammatory activities like suppressing the expression of inducible NO synthase induced by LPS [1]. Our results imply that the anti-inflammatory effects of tocotrienols may be mediated in part through modulating the activities of PPARs. Importantly, chronic inflammation is associated with metabolic diseases like diabetes. The anti-diabetic action of TRF *in vivo* may

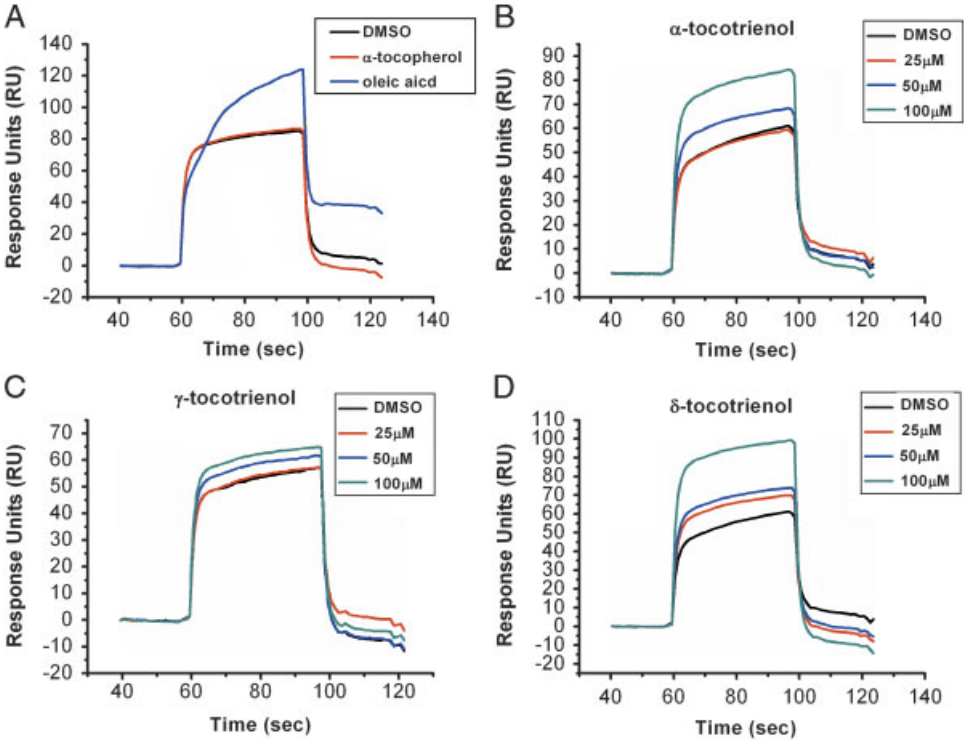

Figure 4. Tocotrienols enhance the interaction between PPARα and PGC-1α. (A–D) Interactions between purified His_6-tagged hPPARα-LBD and NR2 motif of PGC-1α coactivator in the presence of different tocotrienols were analyzed by surface plasmon resonance as described in Section 2. Interactions were tested in the presence of DMSO, 100 μM oleic acid as a positive control and 50 μM α-tocopherol (A). Dose-dependent interactions of α-tocotrienol (B), γ-tocotrienol (C), and δ-tocotrienol (D) were tested compared with basal interaction in the presence of DMSO.

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also be associated with suppressing the extent of inflammation through PPARs.

PPARγ ligands like rosiglitazone and pioglitazone are currently used for management of diabetes, while PPARα ligands like gemfibrozil and benzafibrate are prescribed for dyslipidemia. However, side effects ranging from edema, weight gain, to compromised heart function contributes to the debate regarding the risks and benefits of using PPARγ synthetic ligands for diabetes [41]. Importantly, a dual PPARα and PPARγ agonist actually increased cardiovascular risk and marketing was terminated even after getting regulatory approval [42]. Since tocotrienol supplements have not been associated with adverse cardiovascular events but in fact reduce cardiovascular risk factors in small clinical trials, our results also raise an intriguing question whether these natural PPAR ligands may be better medicines for management of metabolic syndrome compared with synthetic PPAR ligands. Additionally, our results call into question whether the vitamin E supplement taken by millions of people daily in the form of α-tocopherol is the right form of vitamin E for conferring the greatest beneficial effect in metabolic diseases like diabetes.

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The authors have declared no conflict of interest.

5 References

[1] Shu-Jing Wu, P.-L. L., Lean-Teik, N.g., Tocotrienol-rich fraction of palm oil exhibits anti-inflammatory property by suppressing the expression of inflammatory mediators in human monocytic cells. *Mol. Nutr. Food Res.* 2008, *52*, 921–929.

[2] Sen, C. K., Khanna, S., Roy, S., Tocotrienols in health and disease: the other half of the natural vitamin E family. *Mol. Aspects Med.* 2007, *28*, 692–728.

[3] Baliarsingh, S., Beg, Z. H., Ahmad, J., The therapeutic impacts of tocotrienols in type 2 diabetic patients with hyperlipidemia. *Atherosclerosis* 2005, *182*, 367–374.

[4] Desvergne, B., Wahli, W., Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocr. Rev.* 1999, *20*, 649–688.

[5] Puigserver, P., Wu, Z., Park, C. W., Graves, R. *et al.*, A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell* 1998, *92*, 829–839.

[6] Gupta, R. A., Tan, J., Krause, W. F., Geraci, M. W. *et al.*, Prostacyclin-mediated activation of peroxisome proliferator-activated receptor delta in colorectal cancer. *Proc. Natl. Acad. Sci. USA* 2000, *97*, 13275–13280.

[7] Yu, K., Bayona, W., Kallen, C. B., Harding, H. P. *et al.*, Differential activation of peroxisome proliferator-activated receptors by eicosanoids. *J. Biol. Chem.* 1995, *270*, 23975–23983.

[8] Keller, H., Dreyer, C., Medin, J., Mahfoudi, A. *et al.*, Fatty acids and retinoids control lipid metabolism through activation of peroxisome proliferator-activated receptor-retinoid X receptor heterodimers. *Proc. Natl. Acad. Sci. USA* 1993, *90*, 2160–2164.

[9] Forman, B. M., Chen, J., Evans, R. M., Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors a and g. *Proc. Natl. Acad. Sci. USA* 1997, *94*, 4312–4317.

[10] Forman, B. M., Tontonoz, P., Chen, J., Brun, R. P. *et al.*, 15-Deoxy-[Delta]12,14-Prostaglandin J2 is a ligand for the adipocyte determination factor PPAR[gamma]. *Cell* 1995, *83*, 803–812.

[11] Kliewer, S. A., Lenhard, J. M., Willson, T. M., Patel, I. *et al.*, A prostaglandin J2 metabolite binds peroxisome proliferator-activated receptor [gamma] and promotes adipocyte differentiation. *Cell* 1995, *83*, 813–819.

[12] Stumvoll, M., Haring, H., The peroxisome proliferator-activated receptor-gamma2 Pro12Ala polymorphism. *Diabetes* 2002, *51*, 2341–2347.

[13] Wang, Y. X., Lee, C. H., Tiep, S., Yu, R. T. *et al.*, Peroxisome-proliferator-activated receptor delta activates fat metabolism to prevent obesity. *Cell* 2003, *113*, 159–170.

[14] Lee, C.-H., Olson, P., Hevener, A., Mehl, I. *et al.*, PPARdelta regulates glucose metabolism and insulin sensitivity. *Proc. Natl. Acad. Sci. USA* 2006, *103*, 3444–3449.

[15] Jones, J. R., Barrick, C., Kim, K.-A., Lindner, J. *et al.*, Deletion of PPARgamma in adipose tissues of mice protects against high fat diet-induced obesity and insulin resistance. *Proc. Natl. Acad. Sci. USA* 2005, *102*, 6207–6212.

[16] Staels, B., Fruchart, J.-C., Therapeutic roles of peroxisome proliferator-activated receptor agonists. *Diabetes* 2005, *54*, 2460–2470.

[17] Oliver, W. R., Jr., Shenk, J. L., Snaith, M. R., Russell, C. S. *et al.*, A selective peroxisome proliferator-activated receptor delta agonist promotes reverse cholesterol transport. *Proc. Natl. Acad. Sci. USA* 2001, *98*, 5306–5311.

[18] Rasool, A., Rahman, A., Yuen, K., Wong, A., Arterial compliance and vitamin E blood levels with a self emulsifying preparation of tocotrienol rich vitamin E. *Arch. Pharmacol. Res.* 2008, *31*, 1212–1217.

[19] McIntyre, B. S., Briski, K. P., Gapor, A., Sylvester, P. W., Antiproliferative and apoptotic effects of tocopherols and tocotrienols on preneoplastic and neoplastic mouse mammary epithelial cells. *Proc. Soc. Exp. Biol. Med.* 2000, *224*, 292–301.

[20] Sen, C. K., Khanna, S., Roy, S., Tocotrienols: vitamin E beyond tocopherols. *Life Sci.* 2006, *78*, 2088–2098.

[21] Ishiki, M., Klip, A., Mini review: recent developments in the regulation of glucose transporter-4 traffic: new

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signals, locations, and partners. *Endocrinology* 2005, *146*, 5071–5078.

[22] Brady, P. S., Ramsay, R. R., Brady, L. J., Regulation of the long-chain carnitine acyltransferases. *FASEB J.* 1993, *7*, 1039–1044.

[23] Bezaire, V., Seifert, E. L., Harper, M. E., Uncoupling protein-3: clues in an ongoing mitochondrial mystery. *FASEB J.* 2007, *21*, 312–324.

[24] Ye, J.-M., Doyle, P. J., Iglesias, M. A., Watson, D. G. *et al.*, Peroxisome proliferator-activated receptor (PPAR)-alpha activation lowers muscle lipids and improves insulin sensitivity in high fat-fed rats: comparison with PPAR-gamma activation. *Diabetes* 2001, *50*, 411–417.

[25] Muoio, D. M., Way, J. M., Tanner, C. J., Winegar, D. A. *et al.*, Peroxisome proliferator-activated receptor-alpha regulates fatty acid utilization in primary human skeletal muscle cells. *Diabetes* 2002, *51*, 901–909.

[26] Dressel, U., Allen, T. L., Pippal, J. B., Rohde, P. R. *et al.*, The peroxisome proliferator-activated receptor beta/delta agonist, GW501516, regulates the expression of genes involved in lipid catabolism and energy uncoupling in skeletal muscle cells. *Mol. Endocrinol.* 2003, *17*, 2477–2493.

[27] The HOPE and HOPE-TOO Trial Investigators, Effects of long-term vitamin E supplementation on cardiovascular events and cancer: a randomized controlled trial. *J. Am. Med. Assoc.* 2005, *293*, 1338–1347.

[28] Shaish, A., George, J., Gilburd, B., Keren, P. *et al.*, Dietary? Carotene and alpha-tocopherol combination does not inhibit atherogenesis in an ApoE deficient mouse model. *Arterioscler. Thromb. Vasc. Biol.* 1999, *19*, 1470–1475.

[29] Traber, M. G., Vitamin E, nuclear receptors and xenobiotic metabolism. *Arch. Biochem. Biophys.* 2004, *423*, 6–11.

[30] Mishima, K., Tanaka, T., Pu, F., Egashira, N. *et al.*, Vitamin E isoforms [alpha]-tocotrienol and [gamma]-tocopherol prevent cerebral infarction in mice. *Neurosci. Lett.* 2003, *337*, 56–60.

[31] Wada, S., Satomi, Y., Murakoshi, M., Noguchi, N. *et al.*, Tumor suppressive effects of tocotrienol *in vivo* and *in vitro*. *Cancer Lett.* 2005, *229*, 181–191.

[32] Parker, R., Pearce, B., Clark, R., Gordon, D., Wright, J., Tocotrienols regulate cholesterol production in mammalian cells by post-transcriptional suppression of 3-hydroxy-3-methylglutaryl-coenzyme A reductase. *J. Biol. Chem.* 1993, *268*, 11230–11238.

[33] Muoio, D. M., MacLean, P. S., Lang, D. B., Li, S. *et al.*, Fatty acid homeostasis and induction of lipid regulatory genes in skeletal muscles of peroxisome proliferator-activated receptor (PPAR) alpha knock-out mice. Evidence for compensatory regulation by PPARdelta. *J. Biol. Chem.* 2002, *277*, 26089–26097.

[34] Wolfrum, C., Borrmann, C. M., Borchers, T., Spener, F., Fatty acids and hypolipidemic drugs regulate peroxisome proliferator-activated receptors a- and g-mediated gene expression *via* liver fatty acid binding protein: A signaling path to the nucleus. *Proc. Natl. Acad. Sci. USA* 2001, *98*, 2323–2328.

[35] Yoshida, H., Yusin, M., Ren, I., Kuhlenkamp, J. *et al.*, Identification, purification and immunochemical characterization of a tocopherol-binding protein in rat liver cytosol. *J. Lipid Res.* 1992, *33*, 343.

[36] Arita, M., Sato, Y., Miyata, A., Tanabe, T. *et al.*, Human alpha-tocopherol transfer protein: cDNA cloning, expression and chromosomal localization. *Biochem. J.* 1995, *306*, 437.

[37] Kempn, P., Zingg, J.-M., Ricciarelli, R., Hierl, M. *et al.*, Cloning of novel human SEC14p-like proteins: ligand binding and functional properties. *Free Rad. Biol. Med.* 2003, *34*, 1458–1472.

[38] Zimmer, S., Stocker, A., Sarbolouki, M. N., Spycher, S. E. *et al.*, A novel human tocopherol-associated protein. Cloning, *in vitro* expression, and characterization. *J. Biol. Chem.* 2000, *275*, 25672–25680.

[39] Hong, C., Tontonoz, P., Coordination of inflammation and metabolism by PPAR and LXR nuclear receptors. *Curr. Opin. Genet. Dev.* 2008, *18*, 461–467.

[40] Ricote, M., Li, A. C., Willson, T. M., Kelly, C. J., Glass, C. K., The peroxisome proliferator-activated receptor-[gamma] is a negative regulator of macrophage activation. *Nature* 1998, *391*, 79–82.

[41] Nissen, S. E., Wolski, K., Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. *N. Engl. J. Med.* 2007, *356*, 2457–2471.

[42] Nissen, S. E., Wolski, K., Topol, E. J., Effect of muraglitazar on death and major adverse cardiovascular events in patients with type 2 diabetes mellitus. *J. Am. Med. Assoc.* 2005, *294*, 2581–2586.

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