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Cinnamon Polyphenol Extract Regulates Tristetraprolin and Related Gene Expression in Mouse Adipocytes

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ABSTRACT: Cinnamon (*Cinnamomum verum*) has been widely used in spices, flavoring agents, and preservatives. Cinnamon polyphenol extract (CPE) may be important in the alleviation of chronic diseases, but the molecular evidence is not substantial. Tristetraprolin (TTP) family proteins have anti-inflammatory effects through the destabilization of pro-inflammatory mRNAs. TTP expression is reduced in fats of obese people with metabolic syndrome and brains of suicide victims. This study used quantitative real-time PCR to explore the effects of CPE on the regulation of TTP, VEGF, and related gene expression in mouse 3T3-L1 adipocytes. CPE (100 μg/mL) increased TTP mRNA levels by up to 10-fold, and this stimulation was sustained over 16 h. The levels of VEGF mRNA, a putative target of TTP, were decreased 40−50% by CPE. It also affected the expression of other genes coding for ZFP36L1 and ZFP36L3 (TTP homologues), GM-CSF, COX2, IL6, APP, G-CSF, and PAI1. This study demonstrated that CPE rapidly induces TTP mRNA and reduces VEGF mRNA and affects the expression of a number of other genes in the cultured adipocytes.

KEYWORDS: adipocytes, cinnamon polyphenol extract, gene expression, inflammation, tristetraprolin, vascular endothelial growth factor

INTRODUCTION

Cinnamon (*Cinnamomum verum*) is a small evergreen tree 10−15 m tall with greenish flowers. It was mentioned in Chinese texts as long as 4000 years ago and imported to Egypt as early as 2000 B.C.[1] Common cinnamon (*C. verum*, *Cinnamomum zeylanicum*) and cassia (*Cinnamomum aromaticum*) have a long history of uses as spices, flavoring agents, preservatives, and pharmacological agents. Recent studies have also demonstrated that cinnamon polyphenol extract (CPE) may be important in the alleviation of chronic diseases[2] and inflammation by attenuating TNF-α-induced intestinal lipoprotein ApoB48 overproduction through regulating inflammatory, insulin, and lipoprotein pathways in enterocytes.[3] However, the molecular evidence is not substantial.

Tristetraprolin/zinc finger protein 36 (TTP/ZFP36) family proteins regulate gene expression at the post-transcriptional level. TTP family proteins consist of three well-known members in mammals (ZFP36 or TTP, ZFP36L1 or TIS11B, and ZFP36L2 or TIS11D) and a fourth member in mouse and rat but not in humans (ZFP36L3).[4,5] TTP binds to AU-rich elements (AREs) found in some mRNAs and destabilizes those transcripts. The mRNAs encoding TNFα and GM-CSF are stabilized in TTP knockout mice and in cells derived from them.[6,7] Excessive levels of these cytokines in TTP knockout mice result in a severe systemic inflammatory syndrome including arthritis, autoimmunity, and myeloid hyperplasia.[8,9] Up-regulation of TTP reduces inflammatory responses in macrophages.[10] These lines of evidence support the conclusion that TTP is an anti-inflammatory protein and arthritis suppressor.

Agents that induce TTP gene expression may have potential therapeutic value for the prevention and/or treatment of inflammation-related diseases. TTP mRNA and protein levels are induced by a number of agents, including growth factors (insulin, insulin-like growth factor I, epidermal growth factor, fibroblast growth factor, platelet-derived growth factor, and fetal calf serum),[11,12] cytokines (TNFα, GM-CSF, and interferon-γ (INFγ)),[6,10,12,13] and zinc.[14] However, TTP gene expression is also induced by tumor promoters (phorbol 12-myristate 13-acetate and tetradecanoyl phorbol acetate)[11,13] and bacterial endotoxin lipopolysaccharide (LPS).[6,15−17] Finally, TTP gene expression is induced by viral infection.[18] The fact that most of these agents increase the expression levels of anti-inflammatory TTP and pro-inflammatory cytokines such as TNFα in the same cells and tissues[6,19] may limit the therapeutic potential of these agents. Therefore, it is important to search for other agents with the potential to favor anti-inflammatory but disfavor pro-inflammatory gene expression.

The objective of this study was to explore the molecular effects of CPE on the regulation of TTP, VEGF, and related gene expression in cultured adipocytes. qRT-PCR was used to investigate the effects of CPE on the expression of the selected genes in mouse 3T3-L1 adipocytes. This study demonstrated that CPE rapidly induces TTP mRNA and reduces VEGF mRNA and affects the expression of a number of other genes in the cultured adipocytes.

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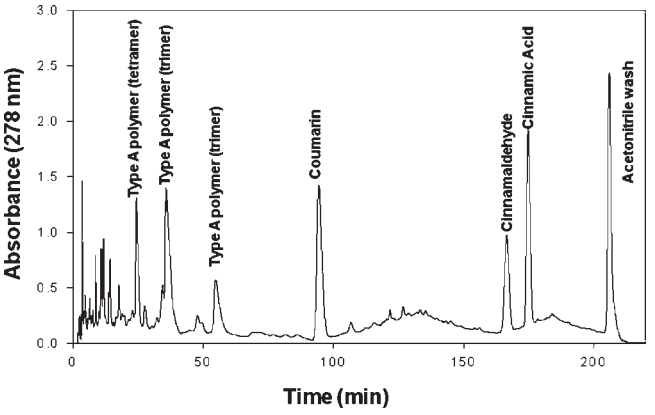

Figure 1. HPLC separation of cinnamon polyphenol extract. CPE (1 mL, 29 mg/mL in 0.1 N acetic acid) was separated by a Symmetry Prep C18 column (7.8 × 300 mm Waters WAT 066235) using a Waters HPLC chromatography system with Millennium 2100 software and a Waters 996 ultraviolet absorbance detector. The sample was eluted with a program with the following steps in 0.1 N acetic acid: 8% acetonitrile for 90 min, 8−15% acetonitrile for 40 min, 15% acetonitrile for 30 min, 15−20% acetonitrile for 20 min, 20% acetonitrile for 20 min, and 100% acetonitrile for 8 min. Type A polymers were determined by mass spectrometry as described.[21] The CPE contained 2.01% of type A tetramer and 3.83 and 1.64%, respectively, of the two type A trimers.

MATERIALS AND METHODS

Cell Line and Reagents. Mouse 3T3-L1 preadipocytes were purchased from American Type Culture Collection and maintained at 37 °C in a humidified incubator. Dulbecco's modified Eagle's medium (DMEM) was from Gibco BRL. Recombinant human insulin, dexamethasone, and 1-isobutyl-3-methylxanthine were from Sigma. TRI_{ZOL} reagent and SuperScript II reverse transcriptase were from Invitrogen. RNA 6000 Ladder was from Ambion. PCR primers and TaqMan probes were designed using Primer Express software (Applied Biosystems) and synthesized by Biosearch Technologies. Absolute QPCR Mix was from ABgene House.

Cinnamon Polyphenol Extract. CPE was prepared from ground cinnamon (*Cinnamomum burmannii*).[20,21] CPE powder was reconstituted at 100 mg/mL in 100% dimethyl sulfoxide (DMSO). The compositions of CPE mixture were analyzed by HPLC using a Symmetry Prep C_{18} column (Figure 1). The structure of a polyphenol trimer (M_r 864 Da) was shown previously.[21]

Cell Culture. Mouse 3T3-L1 preadipocytes were differentiated into adipocytes.[20] More than 90% of the cells accumulated lipid drops (Figure 2), indicating differentiation from preadipocytes to adipocytes.[20] The cells were serum-starved in DMEM without any supplementation for 3−4 h before CPE (10 and 100 μg/mL) and DMSO (the vehicle control, 0.01 and 0.1%) were added to the medium for various times. The CPE doses were selected on the basis of our previous studies showing significant effects of CPE in adipocytes[20] and macrophages.[22] Lower concentrations of pure component may be effective if active compound is identified in CPE and used in the experiments instead of the polyphenolic mixture.

RNA Extraction and Real-Time PCR Analysis. RNA was isolated from mouse adipocytes treated with DMSO and CPE using TRI_{ZOL} reagent. RNA concentrations and integrity were determined using RNA 6000 Nano Assay Kit and the Bioanalyzer 2100 (Agilent Technologies) with RNA 6000 Ladder as the standards. The cDNAs were synthesized from total RNA using SuperScript II reverse transcriptase.[20] The gene names, GenBank accession numbers, amplicon sizes, and the sequences (from 5′ to 3′) of the forward primers, TaqMan probes (TET-BHQ1), and reverse primers have been described.[23] TaqMan reaction mixtures and the thermal cycle conditions were identical to those described.[20] qRT-PCR reactions were performed in 96-well plates in an ABI Prism 7700 real time PCR instrument (Applied Biosystems). The $\Delta\Delta C_T$ method of relative quantification was used to determine the fold change in expression.[20]

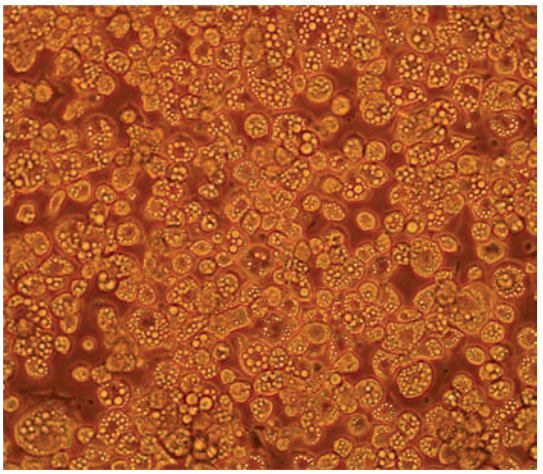

Figure 2. Differentiated mouse adipocytes. Mouse 3T3-L1 fibroblasts were differentiated in the induction medium containing insulin, dexamethasone, and 1-isobutyl-3-methylxanthine. Microscopic observation indicated that approximately 90% of the cells accumulated lipid drops, an indication of differentiation from preadipocytes to adipocytes.[20].

Statistical Analyses. The gene expression data were analyzed by SigmaStat 3.1 software (Systat Software) using ANOVA or ANOVA on ranks. Multiple comparisons were performed with the Student−Newman−Keuls method.[22]

RESULTS

Expression Profiles of Selected Genes in Mouse Adipocytes. On the basis of microscopic observation, it is estimated that >90% of the 3T3-L1 cells differentiated from preadipocytes to adipocytes in which lipid drops accumulated (Figure 2). qRT-PCR assays on mRNA levels of adipocyte-specific cytokines, adiponectin, leptin, and leptin receptor provided molecular evidence for the differentiation of preadipocytes into lipid-filling adipocytes (data not shown).

To provide a basis for the comparison of the effects of CPE on the expression of various genes, we analyzed the relative expression levels of the selected genes in untreated adipocytes (Table 1). In the TTP family, TTP mRNA levels were approximately 10−15% those of ZFP36L1 and ZFP36L2 and were 100-fold that of ZFP36L3. Hu antigen R/embryonic lethal, abnormal vision-like 1 (HuR/ELAVL1), and vascular endothelial growth factor (VEGF) mRNA was 4.5−12-fold that of TTP. TTP mRNA levels were approximately 30-fold that of COX2, 125-fold that of GM-CSF, and 100000-fold that of TNF in the adipocytes.

CPE Effects on TTP Family mRNA Levels. TTP mRNA levels were increased up to 10-fold that of the control by CPE (100 μg/mL) treatments for 0.5−1.5 h and were still 2-fold those of the control after 16 h of treatment (Figure 3). The effects of CPE on the expression of TTP homologues (ZFP36L1, ZFP36L2, and ZFP36L3) in the same cells were generally small. CPE increased ZFP36L1 mRNA levels by approximately 60% in cells treated for 60 min but decreased those treated for 16 h

Table 1. Relative Levels of TTP Family and Other Selected mRNA in Untreated Mouse 3T3-L1 Adipocytes[a]

class	mRNA	cycle of threshold C_T	expression ratio fold of TTP or APP
housekeeping gene	RPL32	17.16 ± 0.11	1.00
TTP/ZFP36 family	TTP (ZFP36/TIS11)	24.63 ± 0.45	1.00
	ZFP36L1 (TIS11B)	21.86 ± 0.25	6.82
	ZFP36L2 (TIS11D)	21.36 ± 0.21	9.65
	ZFP36L3	31.59 ± 0.06	0.01
inflammation	COX2	29.80 ± 0.55	0.03
	GM-CSF	31.61 ± 0.17	0.008
	HuR	21.04 ± 0.11	12.04
	TNF	41.35 ± 0.81	1×10^{-5}
VEGF family	VEGFA	22.45 ± 0.04	4.53
	VEGFB	21.10 ± 0.61	11.55
other mRNA	APP	21.02 ± 0.11	1.00
	CRP	36.29 ± 0.40	3×10^{-5}
	G-CSF	32.76 ± 0.40	3×10^{-4}
	IFNγ	37.59 ± 2.47	1×10^{-5}
	IL1A	undetectable	undetectable
	IL6	28.94 ± 0.16	4×10^{-3}
	IL12B	32.73 ± 0.16	3×10^{-4}
	PAI1	23.70 ± 0.06	0.16
	TAU	34.65 ± 0.45	8×10^{-5}

[a] Values are the mean ± SD, $n = 2-4$.

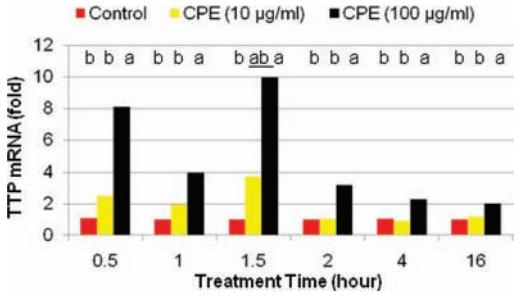

Figure 3. Cinnamon polyphenol extract increased TTP mRNA levels in adipocytes. Total RNAs were isolated from mouse 3T3-L1 adipocytes. RNA-derived cDNA (25 ng) was used for the quantitative detection of mRNA levels using q-RT- PCR. The relative ratios of mRNA levels were calculated using the $\Delta\Delta C_T$ method normalized with the RPL32 C_T value as the internal control and the control as the calibrator. Values are the mean ± SD, $n = 2-4$. Values with different lower case letters displayed above the columns of the figure are significantly different at $p < 0.05$. (For example, "b b a" on top of the 0.5 h column indicates significant difference between the 100 μg/mL treatment and the 10 μg/mL treatment or the control but not between the 10 μg/mL treatment and the control.)

(Table 2). ZFP36L2 mRNA levels were not affected by CPE treatment, but ZFP36L3 mRNA levels were decreased after longer treatment (Table 2).

CPE Effects on TTP-Related VEGF and HuR mRNA Levels. The mRNA of VEGF (a proangiogenic cytokine) is a target of TTP family proteins,[24,25] and HuR/ELAVL1 is a mRNA-stabilizing protein with distinct but overlapping binding specificity with TTP.[26] CPE significantly decreased VEGFA mRNA levels by >50% in cells treated for various times from 0.5 to 4 h (Figure 4) and decreased VEGFB mRNA levels by approximately 40% in cells treated for 1−1.5 h (data not shown). The effects of CPE on the levels of mRNAs encoding HuR in adipocytes were minimal (data not shown).

CPE Effects on TTP-Targeted Pro-inflammatory Cytokine mRNA Levels. TTP is a mRNA destabilizing factor for a number of immediate-early response genes, such as TNF and GM-CSF.[4] Higher concentrations of CPE (100 μg/mL) increased GM-CSF mRNA levels by approximately 2-fold that of the control after 2 h of treatment (Table 2). Its effects on COX2 and IL6 mRNA levels in the same cells were up to 26- and 4-fold that of the control after 2 h of treatment, respectively (Table 2). IFNγ and TNF mRNA levels in the cells were too low to be reliably compared (data not shown).

CPE Effects on Other Selected mRNA Levels. Several other gene targets were selected to evaluate the effects of CPE on 3T3-L1 adipocytes. APP mRNA levels were decreased 49% that of the control in the adipocytes by CPE treatment (100 μg/mL) for 1.5 h (Table 2). The mRNA levels of G-CSF, IL6, and PAI1 were increased by 3-, 4-, and 7-fold those of the controls, respectively, by CPE treatment (100 μg/mL) for 2 h (Table 2). CPE did not affect the expression of CRP, IL12B, or TAU in the adipocytes, and IL1A mRNA levels were too low to be reliably quantified (data not shown).

DISCUSSION

Cinnamon species have a long history of uses as spices, flavoring agents, preservatives, and pharmacological agents.

Table 2. Effects of CPE on TTP Homologues, TTP-Targeted Pro-Inflammatory Cytokines, and Other Selected mRNA Levels in Mouse 3T3-L1 Adipocytes[a]

mRNA	time (h)	control	10 μg/mL fold of control	100 μg/mL
ZFP36L1	0.5	1.03 ± 0.28	0.95 ± 0.47	1.14 ± 0.46
	1.0	1.00 ± 0.10 c	1.32 ± 0.16 b	1.60 ± 0.12 a
	1.5	1.04 ± 0.31	1.06 ± 0.58	1.35 ± 0.64
	2.0	1.00 ± 0.10	1.04 ± 0.18	1.56 ± 0.19
	4.0	1.02 ± 0.21 b	1.36 ± 0.18 a	1.34 ± 0.26 a
	16.0	1.00 ± 0.06 a	0.95 ± 0.19 a	0.52 ± 0.00 b
ZFP36L3	0.5	1.02 ± 0.24	0.85 ± 0.42	0.91 ± 0.54
	1.0	1.04 ± 0.33	1.12 ± 0.66	1.05 ± 1.09
	1.5	1.06 ± 0.40	0.92 ± 0.60	0.92 ± 0.74
	2.0	1.00 ± 0.32	0.97 ± 0.51	1.08 ± 0.65
	4.0	1.01 ± 0.15 a	1.00 ± 0.13 a	0.60 ± 0.04 b
	16.0	1.02 ± 0.21 a	1.02 ± 0.15 a	0.12 ± 0.05 b
GM-CSF	0.5	1.01 ± 0.10	0.80 ± 0.16	1.01 ± 0.17
	1.0	1.00 ± 0.12	1.06 ± 0.11	0.81 ± 0.23
	1.5	1.03 ± 0.32	1.12 ± 0.18	0.96 ± 0.61
	2.0	1.02 ± 0.28 b	0.93 ± 0.31 b	2.18 ± 0.26 a
	4.0	1.02 ± 0.23 b	1.66 ± 0.29 a	1.54 ± 0.22 a
COX2	0.5	1.05 ± 0.37	1.43 ± 0.46	1.35 ± 0.29
	1.0	1.02 ± 0.22 b	1.83 ± 0.39 b	4.76 ± 2.00 a
	1.5	1.01 ± 0.13 c	1.29 ± 0.14 b	6.12 ± 0.09 a
	2.0	1.00 ± 0.05 b	3.53 ± 1.56 b	25.59 ± 6.43 a
	3.5	1.00 ± 0.14 b	0.58 ± 0.25 b	4.32 ± 1.76 a
IL6	0.5	1.06 ± 0.09	1.63 ± 0.14	1.63 ± 0.20
	1.0	1.00 ± 0.10	3.16 ± 1.21	2.50 ± 0.73
	1.5	1.01 ± 0.12 b	2.97 ± 0.79 a	4.22 ± 0.00 a
	2.0	1.00 ± 0.00	2.48 ± 1.10	3.79 ± 0.87
	3.5	1.01 ± 0.20 b	0.72 ± 0.33 b	2.08 ± 0.76 a
APP	0.5	1.01 ± 0.19 a	0.99 ± 0.07 a	0.75 ± 0.32 a
	1.0	1.00 ± 0.05 a	1.05 ± 0.13 a	0.61 ± 0.07 b
	1.5	1.00 ± 0.06 a	0.35 ± 0.12 b	0.51 ± 0.00 b
	2.0	1.00 ± 0.02 a	0.98 ± 0.00 a	0.81 ± 0.00 b
	3.5	1.01 ± 0.21 b	0.73 ± 0.06 b	0.75 ± 0.05 b
G-CSF	0.5	1.02 ± 0.30	0.88 ± 0.34	0.82 ± 0.07
	1.0	1.01 ± 0.21	0.81 ± 0.10	1.11 ± 0.60
	1.5	1.05 ± 0.45	0.91 ± 0.50	0.52 ± 0.00
	2.0	1.00 ± 0.00 b	1.72 ± 0.34 ab	2.67 ± 0.57 a
	3.5	1.02 ± 0.28 b	0.68 ± 0.21 b	3.13 ± 0.99 a
PAI1	0.5	1.00 ± 0.02 b	1.44 ± 0.01 ab	2.62 ± 0.67 a
	1.0	1.00 ± 0.07 b	2.04 ± 0.40 b	5.45 ± 2.09 a
	1.5	1.01 ± 0.20 c	1.62 ± 0.25 b	2.90 ± 0.00 a
	2.0	1.00 ± 0.00 b	3.22 ± 1.23 b	7.39 ± 0.48 a
	3.5	1.00 ± 0.06 b	0.64 ± 0.12 b	1.86 ± 0.36 a

[a] Values are the mean ± SD, $n = 2-4$. Means in a row without a common letter differ, $p < 0.05$. IFNγ and IL1A mRNA levels were too low to be reliable (data not shown). ZFP36L2, CRP, IL12B, and TAU mRNA levels were not significantly different among the treatments at any of the time points (data not shown).

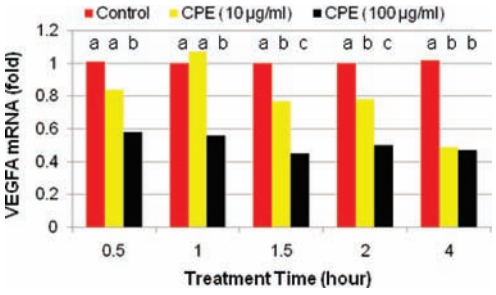

Figure 4. Cinnamon polyphenol extract decreased VEGF mRNA levels in adipocytes. Total RNA isolation, RNA-derived cDNA synthesis, and q-RT-PCR procedures were described in the caption of Figure 3. The relative ratios of mRNA levels were calculated using the $\Delta\Delta C_T$ method normalized with RPL32 C_T value as the internal control and the control as the calibrator. Values are the mean ± SD, $n = 2-4$. Values with different lower case letters displayed above the columns of the figure are significantly different at $p < 0.05$. (For example, "a a b" on top of the 0.5 h column indicates significant difference between the 100 μg/mL treatment and the 10 μg/mL treatment or the control but not between the 10 μg/mL treatment and the control.)

CPE has been shown to have potential effects on the alleviation of chronic diseases.[2] It was shown recently that CPE reverses TNF-α-induced overproduction of intestinal apoB48 by regulating gene expression involving inflammatory, insulin, and lipoprotein signaling pathways.[3] However, detailed analyses at the molecular levels are insufficient. In this paper, we investigated the effects of CPE on the expression of anti-inflammatory TTP and a number of related genes in 3T3-L1 adipocytes, a widely used cell model for mechanistic studies of lipid metabolism. Although variations on the levels of gene expression exist, the main results based on either significant differences or the trends of CPE effects on gene expression indicate that CPE increased TTP, decreased VEGF, and affected other related gene expression in the cultured adipocytes.

One of the major results is that CPE increased TTP gene expression in mouse 3T3-L1 adipocytes, and its stimulation on TTP expression was sustained over longer time. CPE increased TTP expression in the cultured adipocytes based on a short-time course of study (within 2 h of treatment).[20] In this paper, we extended the analysis to include much longer exposure of CPE. CPE increased TTP mRNA levels up to 10-fold during a 30−90 min treatment. TTP mRNA levels were still 2-fold that of the control after 16 h of treatment by CPE at 100 μg/mL. It is known that TTP gene expression is rapidly induced by insulin, but the induction pattern is different from that of CPE. TTP mRNA levels are increased 7-fold that of the control with 30 min of treatment with insulin, but the levels return to that of the control within 2 h of treatment.[23] The effects of CPE on the expression of three TTP homologous genes were minimal, with <50% those of the controls within 2 h of treatment, and the expression of TTP homologues was decreased after longer treatments with CPE. TTP has anti-inflammatory properties with therapeutic potential for the prevention and treatment of inflammation-related diseases. Our results suggest that CPE may be useful in the alleviation of diseases associated with inflammation.

CPE also decreased VEGF mRNA levels in mouse 3T3-L1 adipocytes. VEGF is an important mitogenic and angiogenic factor associated with tumor progression, collateral vessel formation in ischemic tissues, inflammation, and development of diabetic retinopathy.[27] VEGF is also a key regulator of adipogenesis in obesity.[28] VEGF mRNA is destabilized by TTP family proteins in intact cells.[24,29] It was reported recently that TTP might represent a novel antiangiogenic and antitumor agent acting through its destabilizing activity on VEGF mRNA because TTP decreases RasVal12-dependent VEGF expression and development of vascularized tumors in nude mice.[25] A recent study has demonstrated that CPE is a potent inhibitor of VEGFR2 kinase activity, inhibits VEGF-induced endothelial cell proliferation, migration, and tube formation in vitro, sprout formation from aortic ring ex vivo, and

tumor-induced blood vessel formation in vivo.[30] It is not clear if CPE-induced TTP in 3T3-L1 adipocytes directly mediated the VEGF mRNA reduction. Nevertheless, that CPE-treated adipocytes showed increased TTP mRNA and decreased VEGF gene expression suggests a potential role for CPE in restricting blood supply to adipose tissue.[31]

The relative ratios of anti- and pro-inflammatory proteins are proposed to be important in the modulation of inflammatory responses.[32] Previous studies have shown that TTP gene expression is induced in mammalian cells by a wide range of agents.[4] However, most of the same agents also increase the expression levels of pro-inflammatory cytokines in the same cells and tissues.[6,19] In this study, we determined that CPE increased anti-inflammatory TTP expression up to 10-fold as discussed previously, but also modestly increased the levels of mRNAs coding for some pro-inflammatory cytokines in adipocytes. The mRNA levels of pro-inflammatory cytokine GM-CSF, COX2, and IL6 were increased up to 2-, 26-, and 4-fold that of the control, respectively, by CPE treatment at 100 μg/mL (Table 2). Because TTP mRNA levels in untreated adipocytes were 20−125-fold of COX2, IL6, and GM-CSF mRNA levels (Table 1), the net increases of TTP mRNA molecules were still larger than the pro-inflammatory cytokine mRNAs in the same cells. Therefore, CPE may have benefits in improving inflammation-related diseases, assuming there is a positive correlation between mRNA and protein levels in adipocytes. However, it is a complex relationship between TTP mRNA and protein levels. The interactions between TTP protein and its pro-inflammatory cytokine mRNA target molecules are also being explored. Further studies are required to support this conclusion.

In summary, the results presented here demonstrate that CPE increases the expression of TTP mRNA and decreases the expression of VEGF mRNA. Our results further demonstrate that CPE regulates the expression of multiple other TTP-related genes in adipocytes. Therefore, it would be interesting to investigate how its multiple effects on the regulation of important genes including the mRNA binding and destabilization factor TTP and proangiogenic factor VEGF in mouse adipocytes relate to the claims of health benefits of cinnamon.

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ABBREVIATIONS USED

APP, amyloid beta precursor protein; ARE, AU-rich element; CPE, cinnamon polyphenol extract; COX2, cyclooxgenase-2; CRP, C-reactive protein; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethyl sulfoxide; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; HuR, Hu antigen R; IFNγ, interferon-gamma; IL, interleukin; INS, insulin; INSR, insulin receptor; LPS, lipopolysaccharide; PAI1, plasminogen activator inhibitor I; qRT-PCR, quantitative real-time PCR; RPL32, ribosomal protein L32; TAU, microtubule-associated protein tau; TNF, tumor necrosis factor; TTP, tristetraprolin; VEGF, vascular endothelial growth factor; ZFP36, zinc finger protein 36; ZFP36L, ZFP36-like.

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