Dietary Lycopene Downregulates Carotenoid 15,15′-Monooxygenase and PPAR-γ in Selected Rat Tissues[1–3]

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ABSTRACT In vitro studies have suggested that lycopene is an efficient substrate for carotenoid 9′10′-monooxygenase II (CMO2) but an inhibitor of carotenoid 15,15′-monooxygenase I (CMO1). The objectives of this study were to clone the rat CMO2 gene, determine whether feeding lycopene for different lengths of time (3–37 d) altered the expression of genes related to carotenoid cleavage [CMO1, CMO2 and peroxisomal proliferator-activated receptor γ (PPAR-γ)] or increased the activity of selected phase I and phase II detoxification enzymes in rat tissues. The cloned rat CMO2 gene was 92 and 82% homologous to the mouse and human CMO2 nucleotide sequence, respectively. The relative abundance of CMO1, CMO2, and PPAR-γ were differentially expressed among rat tissues. CMO1 and PPAR-γ expression were decreased in the kidney and adrenal with lycopene intake ($P < 0.05$), whereas CMO2 expression was reduced only in the kidney. Lycopene did not alter hepatic phase I activity, but hepatic quinone reductase activity increased after 3 and 7 d of lycopene feeding ($P < 0.05$). Lycopene intake decreased a PPAR-γ target gene, fatty acid binding protein 3 (FABP3), in the kidney and adrenal ($P < 0.05$). Thus, these data show that although the intake of 0.25 g lycopene/kg diet does not induce hepatic P450 detoxification enzymes, lycopene feeding alters CMO1, PPAR-γ, and FABP3 mRNA expression in selected rat tissues with a moderate effect on kidney CMO2 expression. These data suggest that lycopene may play an important role in the modulation of β-carotene, retinoid, and/or lipid metabolism. J. Nutr. 136: 932–938, 2006.

KEY WORDS: • *lycopene* • *β-carotene 15,15′- monooxygenase* • *β-carotene 9′,10′-monooxygenase* • *rats* • *prostate*

Carotenoids are C_{40} isoprenoid compounds responsible for many of the pigments found throughout the plant and animal kingdoms. Although the full nutritional relevance of carotenoids has yet to be elucidated, as early as 1930, it was reported that the carotenoid β-carotene could be cleaved into vitamin A in the small intestine of mammals (1). In 1965, carotenoid 15,15′-monooxygenase (CMO1[5]; formerly termed β-carotene 15,15′-dioxygenase) was found in the small intestinal homogenates of rats and was reported to be the primary enzyme responsible for the central cleavage of β-carotene into 2 molecules of the vitamin A precursor, retinal (2,3). In 2001, an alternative carotenoid cleavage enzyme, carotenoid 9′,10′-monooxygenase (CMO2), was cloned from mice and expressed in β-carotene or lycopene synthesizing and accumulating *Escherichia coli* (4). CMO2 was reported to significantly cleave lycopene (4). Unlike the conversion of β-carotene to vitamin A, which is catalyzed primarily by CMO1, in vitro studies showed that lycopene, conversely, is an inefficient substrate for CMO1 (5). Although there is a great deal of research concerning the transcriptional regulation of CMO1 (5–13), other carotenoid cleavage enzymes have yet to be fully evaluated.

CMO1 was identified as a peroxisome proliferator-activated receptor-γ (PPAR-γ) target gene in mice (14). This was determined by identifying a peroxisomal proliferator response element (PPRE) in the promoter region of the CMO1 gene, which is a recognition site for PPAR-γ. PPRE is a key regulator of the CMO1 gene, and its deletion or mutation causes CMO1 promoter activity to return to basal levels (14). Moreover, either the retinoid X receptor (RXR)/RXR homodimerization or the PPAR/RXR heterodimerization is necessary for transcription of CMO1 (14), although CMO1 is most efficiently transcribed when heterodimerization occurs. Vitamin A and

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[5] Abbreviations used: CMO1, carotenoid 15,15′-monooxygenase I; CMO2, carotenoid 9′10′-monooxygenase II; Ct, critical threshold; EST library, expressed sequence tags (single pass sequence reads from cDNA libraries); FABP3, fatty acid binding protein 3; lycopene-3 d, rats fed a control diet for 30 d and then switched to a lycopene diet for 3 d; lycopene-7 d, rats fed a control diet for 30 d and then switched to a lycopene diet for 7 d; lycopene 30 d, rats fed a lycopene diet for 30 d; lycopene 37 d, rats fed a lycopene diet for 37 d; lycopene-control-7 d, rats fed a lycopene diet for 30 d and then switched to a control diet for 7 d; PPAR-γ peroxisome proliferator-activated receptor-γ; PPRE, peroxisomal proliferator response element; QR, quinone reductase; RXR, retinoid X receptor.

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9-*cis* retinoic acid status (a potential metabolite of β-carotene) in rats and chickens was shown to be involved in the transcriptional regulation of CMO1; this is expected, given that metabolites often play an important role in feedback regulation (6). Without this close control over metabolite concentration, a cell's viability could be compromised.

The regulation of the CMO2 gene has yet to be identified. Much like the transcriptional regulation of CMO1 by β-carotene metabolites, it is conceivable that lycopene and/or its metabolites may play a critical role in the transcriptional regulation of CMO2. To learn more about lycopene's potential health benefits, therefore, it is necessary to know more about how lycopene intake can affect the regulation of potential lycopene cleavage enzymes, such as CMO1 and CMO2.

The specific aims of the current study were to partially clone the rat CMO2 gene, and to determine whether lycopene feeding for varying lengths of time resulted in the regulation of CMO1, CMO2, or PPAR-γ in various tissues or resulted in the activation of selective phase I or phase II detoxification enzymes in selected tissues in F344 rats.

MATERIALS AND METHODS

Experimental design. The University of Illinois Laboratory Animal Care and Use Committee approved the animal protocol and all principles of laboratory care were followed. At 40 d of age, male F344 rats ($n = 56$) were randomly assigned to 2 groups based on body weight and were fed either an AIN-93G control or AIN-93G lycopene-enriched diet (0.25 g lycopene/kg diet; Dyets) for 30 d (15). Subsequently, 14 rats from each group were killed at 70 d of age. The remaining 14 rats that had consumed a control diet were switched to a lycopene diet for 3 or 7 d ($n = 7$). The remaining rats that had consumed a lycopene diet were switched to a control diet for 7 d or were continued to consume a lycopene diet for an additional 7 d ($n = 7$). Blood and tissue collection procedures were followed as previously described (16). Stomach contents, small and large intestinal mucosa (flushed with 1.15% potassium chloride), heart, liver (perfused with ice cold 1.15% potassium chloride), lung, adrenal, seminal vesicles, kidney, prostate (sectioned into dorsolateral, ventral and anterior lobes), and testes were dissected, snap-frozen, and stored at −80°C.

Total RNA extraction and cDNA synthesis. All materials related to cloning and mRNA expression were obtained from InVitrogen unless otherwise specified. Total RNA from 0.025–0.03 g of tissue was extracted using the RNeasy Kit (Qiagen). RNA concentrations were determined spectrophotometrically at 260 nm. The RNA samples were reverse transcribed into complementary DNA by Superscript II reverse transcriptase using random hexamers (Applied Biosystems). The reaction was carried out at 25°C for 10 min, 42°C for 50 min, 70°C for 15 min, and 15°C until removed from the thermal cycler. cDNA from a control liver was used to clone CMO2.

PCR cloning of rat CMO2. Forward and reverse PCR primers (MWG) for rat CMO2 were designed based on the mouse (GenBank™ accession number NM_133217) and human (GenBank accession number NM_031938) CMO2 gene conserved regions. The following forward and reverse primers were selected (see Supplemental Figure 1): forward primer 1–5′ GAGCAAGTTTCTACAGAGTGA, reverse primer 1–5′ TTGTCATTCCAAAGCTATGGTAGT, forward primer 2–5′ ATTGCTGTGAATGGAGC, reverse primer 2–5′ AGGCATTGATTTGATGA, forward primer 3–5′ TCATCAAATCAATGCCTT and reverse primer 3–5′ TCTGGTTGGGAGTGATCACCACAGA.

Each probe sequence was amplified using PCR. The PCR mixtures consisted of autoclaved water, 10X LA buffer, dNTP, rat cDNA, LA Taq Polymerase, and the selected primer pairs. PCR products were checked for purity using 2% agarose gel electrophoresis.

The entire amount of each amplified PCR product was isolated and gel purified using the QIAEXII kit and then ligated into a modified T-vector. The pBlueScript (Stratagene) vector was modified by the addition of a single deoxythymidine ("T") residue at the 3′ end. The ligated recombinant plasmid was transformed into *E-coli* DH10B chemical component cells, which were inoculated onto ampicillin-containing agar plates and then incubated at 37°C overnight.

Ampicillin-resistant, white colonies were amplified in LB media by incubating at 37°C for 11 h. After centrifugation (8000 × *g*; 10 min), cells were used for plasmid preparation with QIAprep Miniprep plasmid DNA purification kit (Qiagen). Once the plasmids were purified, restriction enzyme digestion was performed to confirm the size of the inserts. Clones were sequenced by ABI 3730XL capillary sequencer (Applied Biosystems).

Real-time quantitative PCR. mRNA expressions of the selected genes were measured using a real-time quantitative PCR method with SYBR green fluorescence dye. Primer pairs for each gene of interest were designed by Primer Express software (Applied Biosystems). The selected primer pairs were designed to measure CMO1 (NM_053648): Forward-5′-CCTAGAGCTCCCTCGGATAAATTA and Reverse-5′-GTTGGGACTGGACTCCATTGTACT (amplicon length 89 bp); CMO2 (DQ083174): Forward 5′-ACCATCTCCCAGTTTTGAAGAAC and Reverse-5′- GCAATGCACGGCAGACTCT (amplicon length 85 bp); PPAR-γ (NM013124): Forward-5′- AGGATTCATGACCAGGGAGTT and Reverse-5′-AGCAAACTCAAACTTAGGCTCCAT (amplicon length 78 bp); fatty acid binding protein 3 (FABP3; AF144090.2): Forward-5′-AAGCCCGGCTCACATTGA and Reverse-5′-CCCACTGAACTTTTCCATTGGT (amplicon length 138 bp) and 18S (×00686): Forward-5′-GATCCATTGGAGGGCAAGTCT and Reverse-5′ AACTGCAGCAACTTTAATATACGCTATT (amplicon length 79 bp). A validation experiment was performed for each set of primers (MWG), to confirm efficiency, amplification of a single gene (no primer dimers), and to optimize primer concentrations. The rRNA 18S was selected as the housekeeping gene. cDNA from each tissue sample was used in the SYBR Green assay. A standard curve for CMO1, CMO2, and PPAR-γ ranging from 625–40,000 ng/L was prepared using a cDNA sample obtained from the small intestinal mucosa.

The PCR reaction mixture contained 10 ng of cDNA, 500 nmol/L forward and reverse primers of selected genes (or 300 nmol/L of 18S primers), and 1X SYBR Green PCR Master Mix (Applied Biosystems). The mRNA expression of CMO1, CMO2, FABP3, and PPAR-γ was measured in select tissues using the Applied Biosystem's 7900HT Fast Real-Time PCR detection system (method followed as described in the manufacturer's instructions). The relative standard curve method was used to quantify mRNA concentrations of each gene of interest in relation to 18S rRNA (method followed as described in the manufacturer's instructions). In brief, a standard curve for each gene of interest and 18S was included in each run. The standard curves were individually calculated based on the critical threshold (Ct) values, which allowed correction of differences in amplification efficiencies between the target gene and 18S. The sample mRNA for each gene was determined by inserting the Ct values into the standard curve and then dividing the RNA amount of the gene of interest by that of 18S for each sample (relative abundance). The calculated relative abundance was further converted to a fold of the control group in **Table 1**.

Phase I detoxification enzyme analysis. Ethoxyresorufin-, methoxyresorufin-, and benzyloxyresorufin-*O*- deethylase (Sigma-Aldrich) activity in hepatic tissue was measured using a fluorometric method as described by Pohl and Fouts (17) and later modified by Breinholt et al. (18). The end-point product, resorufin, was measured using an excitation wavelength of 550 nm and an emission wavelength of 585 nm; it was then quantified using a freshly made resorufin standard curve.

Phase II detoxification activity assays. Quinone reductase (QR) activity was measured in the kidney, lung, and hepatic tissue by using the substrate, 2,6-dichlorophenolindophenol and following the method of Ernster (19) as modified by Benson et al. (20). All samples were analyzed in triplicate for both quinone reductase activity and for nonspecific activity. The Bio-Rad assay was used to measure the protein concentrations based on the Bradford method (21) using a Quant microplate reader (Bio-Tek Instruments). The Bio-Rad assay was performed in both the phase I and phase II detoxification assays.

TABLE 1

Relative CMO1, CMO2. and PPAR-γ mRNA expression in tissues of rats fed lycopene for varying lengths of time[1]

	Fold of the control[2]							
	Small intestinal wall	Liver	Testes	Adrenal	Ventral prostate	Dorsolateral plus anterior prostate	Lung	Kidney
CMO1								
Control	1.0 ± 0.003	1.0 ± 0.1^{b}	1.0 ± 0.3	1.0 ± 0.2^{a}	1.0 ± 0.1	1.0 ± 0.5^{a}	1.0 ± 0.6^{a}	1.0 ± 0.4^{a}
Lycopene-3 d	1.0 ± 0.3	1.4 ± 0.2^{ab}	1.4 ± 0.3	0.2 ± 0.1^{b}	0.6 ± 0.1	0.6 ± 0.1^{ab}	0.8 ± 0.3^{a}	0.1 ± 0.03^{b}
Lycopene-7 d	1.1 ± 0.9	1.7 ± 0.1^{ab}	1.1 ± 0.2	0.4 ± 0.1^{b}	0.6 ± 0.2	0.36 ± 0.07^{ab}	0.2 ± 0.1^{b}	0.3 ± 0.1^{b}
Lycopene-30 d	1.3 ± 0.5	1.1 ± 0.1^{ab}	1.1 ± 0.1	0.6 ± 0.1^{ab}	0.9 ± 0.3	0.5 ± 0.2^{ab}	0.3 ± 0.1^{ab}	ND[3]
Lycopene-37 d	0.9 ± 0.3	1.5 ± 0.2^{ab}	1.2 ± 0.1	0.4 ± 0.1^{b}	0.8 ± 0.4	0.44 ± 0.17^{ab}	0.8 ± 0.2^{a}	ND[3]
Lycopene-Control-7 d[4]	2.0 ± 0.7	2.3 ± 0.2^{a}	1.1 ± 0.2	0.4 ± 0.1^{b}	0.4 ± 0.1	0.25 + 0.14^{b}	ND[3]	0.1 ± 0.01^{b}
CMO2								
Control	1.0 ± 0.5^{ab}	1.0 ± 0.2	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.2	1.0 ± 0.5^{a}	1.0 ± 0.5^{a}	1.0 ± 0.3^{a}
Lycopene-3 d	1.5 ± 0.1^{a}	1.6 ± 0.2	0.8 ± 0.1	1.0 ± 0.1	2.0 ± 0.6	0.5 ± 0.1^{ab}	1.5 ± 0.9^{a}	0.4 ± 0.1^{b}
Lycopene-7 d	1.2 ± 0.3^{ab}	1.5 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	1.1 ± 0.2	0.3 ± 0.1^{ab}	1.4 ± 0.7^{a}	0.5 ± 0.1^{b}
Lycopene-30 d	0.54 ± 0.14^{ab}	1.3 ± 0.4	0.7 ± 0.1	1.1 ± 0.1	1.7 ± 0.6	0.5 ± 0.2^{ab}	0.4 ± 0.1^{a}	ND[3]
Lycopene-37 d	0.47 ± 0.1^{b}	1.3 ± 0.1	1.0 ± 0.1	1.3 ± 0.2	0.9 ± 0.1	0.5 ± 0.2^{ab}	0.2 ± 0.1^{b}	ND[3]
Lycopene-Control-7 d[4]	0.8 ± 0.2^{ab}	1.1 ± 0.1	1.1 ± 0.2	1.3 ± 0.2	2.0 ± 0.7	0.2 ± 0.1^{b}	ND[3]	0.4 ± 0.2^{b}
PPARγ								
Control	1.0 ± 0.5^{ab}	1.0 ± 0.2	1.0 ± 0.1	1.0 ± 0.3^{a}	1.0 ± 0.1	1.0 ± 0.5^{a}	1.0 ± 0.4^{a}	1.0 ± 0.4^{a}
Lycopene-3 d	1.6 ± 0.1^{a}	0.8 ± 0.01	1.2 ± 0.3	0.2 ± 0.1^{b}	0.6 ± 0.1	0.6 ± 0.1^{ab}	0.6 ± 0.3^{a}	0.2 ± 0.05^{b}
Lycopene-7 d	1.2 ± 0.2^{ab}	1.0 ± 0.01	1.3 ± 0.3	0.36 ± 0.07^{b}	1.0 ± 0.3	0.3 ± 0.1^{ab}	0.3 ± 0.2^{ab}	0.5 ± 0.2^{ab}
Lycopene-30 d	0.6 ± 0.1^{ab}	2.1 ± 0.8	1.2 ± 0.1	0.6 ± 0.1^{ab}	0.9 ± 0.3	0.5 ± 0.2^{ab}	0.6 ± 0.1^{a}	ND[3]
Lycopene-37 d	0.5 ± 0.1^{b}	1.1 ± 0.001	1.0 ± 0.04	0.42 ± 0.12^{ab}	0.5 ± 0.2	0.5 ± 0.2^{ab}	0.2 ± 0.1^{b}	ND[3]
Lycopene-Control-7 d[4]	0.8 ± 0.2^{ab}	2.0 ± 0.2	0.4 ± 0.2	0.38 ± 0.13^{b}	0.5 ± 0.1	0.2 ± 0.2^{b}	ND[3]	0.2 ± 0.04^{b}
FABP3[5]								
Control	ND[3]	ND	ND	1.0 ± 0.2^{a}	ND	ND	ND	1 ± 0.4^{a}
Lycopene-3 d	ND	ND	ND	0.2 ± 0.1^{b}	ND	ND	ND	0.1 ± 0.03^{b}
Lycopene-7 d	ND	ND	ND	0.4 ± 0.1^{b}	ND	ND	ND	0.5 ± 0.2^{ab}
Lycopene-30 d	ND	ND	ND	0.3 ± 0.1^{b}	ND	ND	ND	ND
Lycopene-37 d	ND	ND	ND	0.4 ± 0.1^{b}	ND	ND	ND	ND
Lycopene-Control-7 d[5]	ND	ND	ND	0.1 ± 0.03^{b}	ND	ND	ND	0.2 ± 0.04^{b}

[1] Values are means ± SEM, $n = 5$ or 6. Means in a column for a gene with superscripts without a common letter differ, $P < 0.05$.
[2] 18S was used as the endogenous control.
[3] ND, not determined.
[4] The lycopene-control-7 d group was fed lycopene for 30 d and then switched to a control diet for 7 d.
[5] Measured in selected tissues.

HPLC analysis of lycopene and tissue lycopene extraction. Total tissue lycopene extraction and analysis was performed as indicated earlier (16). In brief, the analysis of tissue and serum lycopene concentrations was accomplished by combining the sample with KOH/ethanol solution containing 0.01% BHT. Tissue samples were then saponified at 60°C for 30 min (serum was not saponified). The samples were then immediately placed on ice and 3 mL of double deionized water was added. Lycopene was extracted with 7 mL of hexane 4 times. After extraction, the samples were dried in a Speedvac concentrator (model AS160; Savant), flushed with argon, and stored at −80°C for <2 d before HPLC analysis. A Waters 991 detector (Millipore) with monitoring from 250–550 nm, a Rainin Dynamics gradient pump system model SD-200, a Varian Prostar model 210, and a C30, 4.6 × 150 nm column (YMC) were used for the detection of lycopene.

Statistical analysis. A complete randomized design was used to assign rats to treatment groups. Differences among treatment groups were analyzed by 1-way ANOVA and post hoc Tukey's test. Results were expressed as mean fold of the control ± SEM. All statistical analyses were conducted using SAS (version 8.1; SAS Institute) with an α level ≤0.05. For HPLC analysis, 2 prostate and seminal vesicle tissues were pooled within groups, resulting in $n = 3$.

RESULTS

Partial cloning of the rat CMO2 gene. A search of the expressed sequence tag (EST) library for a putative rat CMO2 gene was unsuccessful. The approach used for partial cloning of the rat CMO2 gene relied on the identification of conserved regions within the mouse and human CMO2 genes. Three sets of primers with overlapping regions were selected to allow alignment of the 3 separate fragments. The partially cloned CMO2 gene was 1269 bp in length and was located on chromosome 8, which was different from that reported in mice (chromosome 9) and humans (chromosome 11). The comparison of the rat, human, mouse, and ferret CMO2 genes showed that the rat CMO2 gene was 82% homologous to that of humans and ferrets and 92% homologous to the mouse nucleotide sequence. The rat CMO2 gene was 35% homologous to the mouse CMO1 nucleotide sequence and 34% homologous to the rat and human CMO1 nucleotide sequence, respectively. The start codon was identified by using an EST (GenBank accession number CK3371.1), allowing for the documentation of a putative amino acid sequence (GenBank accession number AAY85350).

Relative Abundance. The mRNA relative abundance of CMO1, CMO2 and PPAR-γ compared with 18S rRNA was evaluated in the liver, testes, lung, kidney, adrenal, ventral prostate lobe, and dorsolateral plus anterior prostate lobes in rats fed a control diet for 30 d (**Fig. 1**). The relative abundance of all the measured genes showed tissue-specific expression. The relative abundance of CMO1 was greatest in the adrenal, followed by the prostate, kidney, lung, small intestinal mucosa, liver, and testes. The relative abundance of CMO2 was greatest in the adrenal, liver, and testes, followed by low abundance in the kidney, lung, prostate,

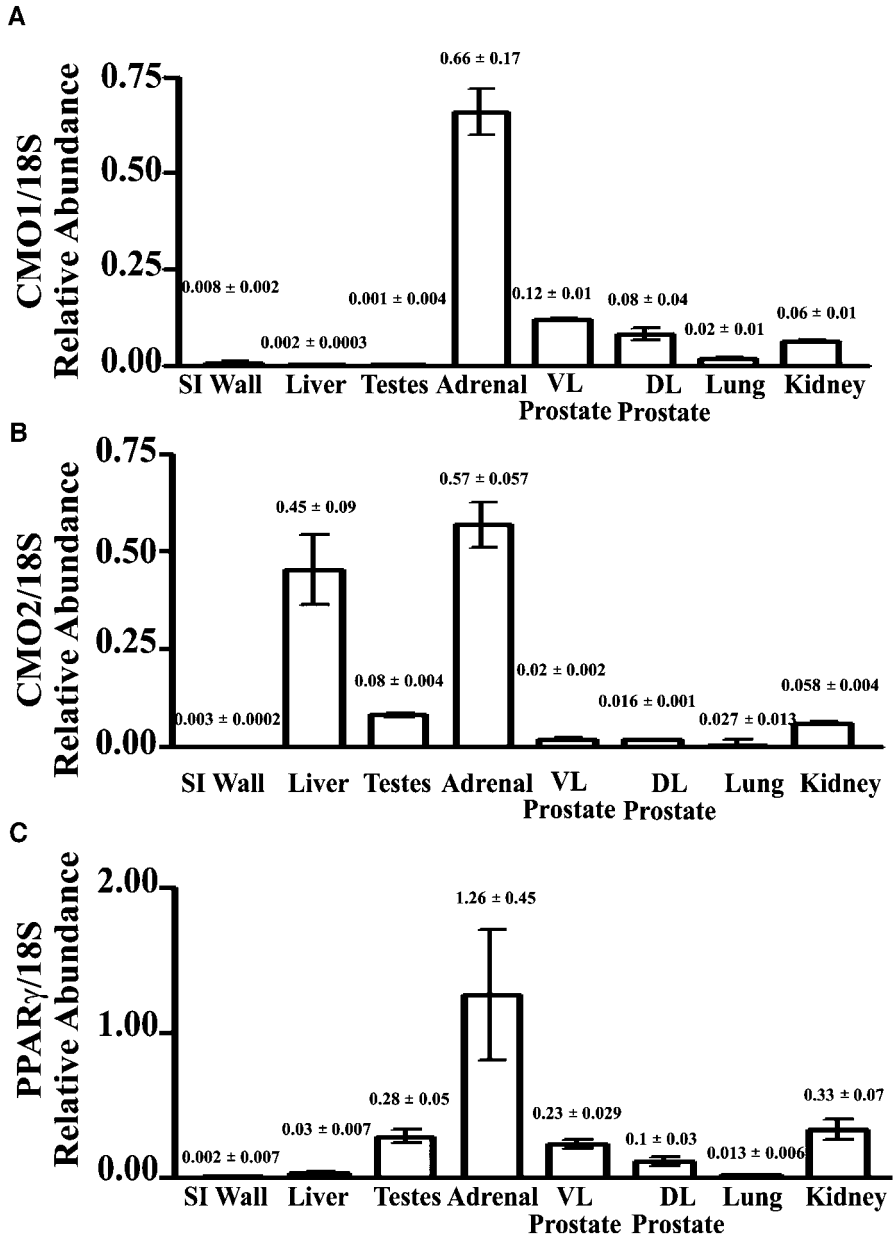

FIGURE 1 Relative abundance of CMO1 (*A*), CMO2 (*B*), and PPAR-γ (*C*) mRNA expression in different tissues from F344 rats fed a control AIN-93G diet for 30 d. Values are means ± SEM, $n = 6$.

and small intestinal mucosa. The PPAR-γ relative abundance was greatest in the adrenal, kidney, testes, ventral prostate lobe, and dorsolateral plus anterior prostate lobes, followed by low abundance in the liver, lung, and small intestinal mucosa.

Effect of lycopene on gene expression. CMO1 expression was downregulated by lycopene intake in the adrenal gland and kidney (**Table 1**). There were decreases in PPAR-γ ($P < 0.05$) and FABP3 ($P < 0.05$) expression (a PPAR-γ target gene) in the adrenal gland and kidney parallel to that of CMO1. PPAR-γ expression in the mucosal cells decreased when lycopene was fed for 37 d compared with 3 d ($P < 0.05$).

Lycopene intake did not affect CMO2 gene expression in the adrenal glands (Table 1) or in most other tissues. In the kidney, CMO2 expression was lower in rats fed lycopene than in controls ($P < 0.05$). CMO2 expression was lower in the mucosal cells when lycopene was fed for 37 d compared with 3 d ($P < 0.05$). Moreover, CMO2 expression was lower in the dorsolateral prostate lobe of rats that were switched from a lycopene diet for 30 d to a control diet for 7 d ($P < 0.05$) compared with controls (Table 1). CMO2 expression also was lower in lung after 37 d of lycopene intake than in control.

Detoxification enzymes. The activities of hepatic phase I detoxification enzymes were not altered with lycopene feeding despite a range of tissue lycopene concentrations (46–150 nmol/g). Hepatic QR activity (data not shown) was increased in rats fed a lycopene diet for 3 or 7 d compared with rats fed lycopene for 0, 30, or 37 d ($P < 0.05$). Hepatic QR activity was significantly decreased after lycopene was removed from the diet for 7 d compared with the rats fed lycopene for 30 d ($P < 0.05$). Lycopene feeding did not affect QR activity in kidney or lung tissues (data not shown).

Tissue lycopene concentrations. Hepatic lycopene concentration increased with increased length of lycopene feeding **(Table 2).** Prostate and adrenal lycopene concentrations were not affected by increased length of feeding. The mean liver and serum lycopene concentrations (Table 2) at 37 d of lycopene intake in the current study were similar or slightly higher than the rat liver [16–138 nmol/g; (15,16,22–25)] and serum

TABLE 2

Serum and tissue lycopene concentrations from rats fed lycopene for varying lengths of time[1]

	Serum	Liver	Adrenals	Dorsolateral plus anterior prostate	Ventral prostate
	nmol/L		*nmol/g*		
Control[2]	0	0	0	0	0
Lycopene-3 d	523 ± 87^{ab}	43 ± 5.1^{b}	4.2 ± 2.3	0.9 ± 0.3	0.3 ± 0.2
Lycopene-7 d	228 ± 64^{bc}	47 ± 5.4^{b}	5.4 ± 2.0	0.3 ± 0.1	0.4 ± 0.2
Lycopene-30 d	725 ± 63^{a}	102 ± 15.0^{a}	4.6 ± 3.0	0.3 ± 0.2	1.1 ± 0.1
Lycopene-37 d	530 ± 29^{ab}	113 ± 12.6^{a}	8.0 ± 1.5	0.2 ± 0.1	0.6 ± 0.02
Lycopene-control-7 d[3]	94 ± 8.7^{c}	84 ± 10.1^{ab}	3.4 ± 1.5	0.3 ± 0.1	0.2 ± 0.2

[1] Values are means ± SEM; serum (n = 6), hepatic tissue (n = 12), dorsolateral plus anterior prostate, and ventral prostate (n = 3). Means in a column with superscript without a common letter differ, $P < 0.05$.
[2] Lycopene was not detectable; data were not included in the statistical analysis.
[3] Fed lycopene for 30 d and then switched to a control diet for an additional 7 d.

(74–600 nmol/L) lycopene concentrations previously reported by our laboratory and by others (15,16,22–25) in lycopene-fed rats. Total prostate lycopene concentration was 0.4 ± 0.06 nmol/g in the current study, which was consistent with the 0.38 nmol lycopene/g in rat prostate tumors (26) and 0.46 nmol lycopene/g in normal rat prostate glands (27) as reported by others after lycopene feeding.

DISCUSSION

The selective tissue accumulation of ^{14}C-lycopene metabolites in androgen-related tissues noted in our previous studies (15,16), led to the current study objectives which were as follows: *1*) to clone the rat CMO2 gene; *2*) to determine whether lycopene feeding for varying lengths of time would result in the altered expression of CMO1, CMO2, FABP3, and/or PPARγ; and *3*) to determine whether lycopene feeding would result in the activation of selective phase I or phase II detoxification enzymes in selected F344 rat tissues. The level of dietary lycopene fed in the current study (0.25 g lycopene/kg diet) is the same as that used for previous experimental trials with rats (15,16,22–24).

The relative abundance of the measured genes was differentially distributed among rat tissues. The relative abundance of CMO1 and CMO2 from the current study correlated well with a previous study involving male and female BALB/c mice (4); however, in the previous study, these researchers did not evaluate expression in the adrenal or prostate glands. The relative abundance of CMO1 was lower in the rat liver than in the rat extrahepatic tissues as was reported for mice (4), which may be explained by species differences or by cell-type differences of the measured samples. All sections of the small intestinal mucosa were pooled and measured for CMO1 mRNA concentration; because CMO1 was reported previously to be greatest in the duodenum and jejunum of mice and rats, respectively (13,28), the lower relative abundance reported in this paper is likely a result of diluted CMO1 mRNA concentration.

The adrenal gland contained the greatest relative abundance of all of the measured genes. Recently, CMO1 and CMO2 were reported to be highly concentrated in the human mineralocorticoid-producing zona glomerulosa and in the glucocorticoid-producing zona fasciculata of the adrenal cortex, suggesting that CMO1 and/or CMO2 may be responsible for local bioconversion of carotenoids and that they may be involved in steroidogenesis (29). PPAR-γ expression was reported previously to be high in adipose tissue, adrenal glands, and spleen in mice (30–32); thus we expected to find a high relative abundance of PPAR-γ in these tissues.

Both CMO1 (human) and PPAR-γ (mouse) relative abundances were also high in the prostate, and previous reports showed that these 2 genes were localized in the epithelium of the prostate gland (29,32). Differentiation and development of the prostate is dependent on androgen-driven processes within the epithelial and stroma cells. Although a functional androgen receptor is present in both cells, the high transcriptional activity in the epithelium is associated with a higher tumor grade compared with the lower androgen receptor expression in the stroma (33,34). The presence of CMO1 and PPARγ within the prostate epithelium suggests their possible role in androgen function, which could be important in prostate cancer. In fact, lycopene was shown to downregulate 5-α-reductase (an enzyme responsible for converting testosterone to its active form, dihydrotestosterone) in the prostate (26), and this observation may be correlated with the high CMO1 and/or PPARγ mRNA concentration in this gland. The high relative abundance of CMO2 and the lower relative abundance of CMO1 in the liver suggests that CMO2 acts as a back-up system for CMO1 to cleave provitamin A carotenoids into vitamin A and/or retinoids under conditions of low vitamin A status in the liver.

The effect of lycopene intake for varying lengths of time on the modulation of gene expression and detoxification enzyme activity in rat tissues. CMO1 gene expression generally decreased in the kidney and adrenal with lycopene intake. Lycopene metabolites were previously reported to be high on a percentage basis in the adrenal and prostate glands compared with the small intestinal mucosa of rats fed a lycopene diet identical to that fed in the current study (15). We demonstrated that prefeeding F344 rats lycopene resulted in decreased absorption of a single oral dose of ^{14}C-lycopene compared with prefeeding a control diet, showing that lycopene absorption was influenced by prior dietary treatment (15). The previous study also demonstrated that the cleavage of ^{14}C-lycopene was not affected by prior lycopene intake. Consistent with this observation, CMO2 expression was affected only moderately by lycopene feeding in the current study compared with the control (Table 1). The presence of ^{14}C-lycopene metabolites in tissues as early as 3–5 h after feeding suggested local cleavage of lycopene in the prostate, adrenal, and seminal vesicles (15,16). In vitro,

CMO2 was reported to cleave lycopene, whereas CMO1 was shown to cleave β-carotene (4). Moreover, it was documented that the concentration of β-carotene metabolites (e.g., vitamin A and retinoic acid) could regulate the expression of its major cleavage enzyme, CMO1, in rats. The results of the current in vivo trial did not provide evidence that CMO2 expression is substantially affected by lycopene feeding. We reported a 50% depression in expression of CMO2 in the kidney with lycopene feeding but no effect in the adrenal gland. The decrease in CMO1 expression in the kidney and adrenal gland of lycopene-fed rats suggests feedback inhibition of CMO1 by lycopene/lycopene metabolites.

FABP3 expression, a PPAR-γ target gene, was also measured in select tissues to determine whether lycopene would downregulate a PPAR-γ target gene that is not known to be involved in carotenoid metabolism. Lycopene intake significantly decreased the expression of PPAR-γ and its target gene, FABP3, in the adrenal glands and kidney, suggesting that lycopene acts through downregulation of PPAR-γ expression. The biochemical evaluation of the CMO2 promoter region has not yet been completed in any species, and it is currently unknown whether a PPRE is present within its promoter region or if CMO2 is a PPAR-γ target gene. We evaluated the mouse CMO2 regulatory region using the transcription element search software and the transcription factor database (35)and a PPRE was not found within the CMO2 promoter region, which suggests that CMO2 is not a PPAR-γ target gene. This may further explain why CMO2 was not significantly affected by lycopene intake in vivo, but future mechanistic studies are clearly needed.

In the current study, both PPAR-γ1 and PPAR-γ2 isoforms were measured as total PPAR-γ; thus the changes in gene expression could result from either isoform. No reports have been published regarding lycopene's ability to downregulate PPARγ in specific tissues; however, a β-carotene metabolite (*all-trans*-retinoic acid) was reported to inhibit the expression of PPARγ−2 and CCAAT/enhancer binding protein-α in adipose tissue. Moreover, retinoic acid was shown to induce DEC1/Stra13, which was also suggested to mediate the repression of PPAR-γ2 (37). Although lycopene is comprised of an open carbon chain and β-carotene contains 2 β-ionone rings, both compounds are hydrophobic and contain 40 carbons and thus may result in the same biological outcome.

The downregulation of PPAR-γ in the adrenal and kidney in the current study may also occur through mechanism(s) similar to those reported in adipose tissue.

The lack of induction in hepatic phase I activity in the current study, in contrast to a previous study (18), suggests that the dietary lycopene concentration in the current study was physiological and not excessive. These results also suggest that the changes in the expression of the genes measured were not secondary to phase I metabolism of lycopene. The hepatic QR activity results suggest that short-term lycopene feeding may have transient, health beneficial effects.

The novel findings presented here have several implications: first, the relative abundance of CMO1 and CMO2 is dependent on tissue type and suggests quite different functions of these 2 enzymes. Second, the tissue-specific expression patterns of CMO1, CMO2, and PPAR-γ in the current study suggest that not only could carotenoid cleavage and regulation occur in the liver and small intestinal mucosa as previously noted (1–4) but they may also occur locally within extrahepatic tissues. Third, the downregulation of CMO1 expression in the adrenal gland and kidney with lycopene intake suggests that lycopene affects β-carotene and/or retinoid metabolism in these tissues. Fourth, the decrease in PPAR-γ target genes in select tissues implies an effect of lycopene on lipid metabolism. Fifth, the minimal effects of lycopene on CMO2 gene expression suggest that lycopene and/or its metabolites may not be primary substrates for CMO2 in rats. This finding was unexpected but it is possible that lycopene might be a preferred substrate for a currently unidentified cleavage enzyme. It is also possible that lycopene could modulate the protein expression, activity, and/or cellular location of CMO1 and/or CMO2. We cannot conclude that lycopene or its metabolites are not primary substrates from CMO2 gene expression data alone. Further research evaluating protein concentration and substrate binding would help to expand upon the present mRNA results.

Overall, lycopene was fed at concentrations that did not induce hepatic P450 detoxification enzymes but did alter CMO1, PPAR-γ, and FABP3 expression in selective tissues with a moderate effect on kidney CMO2 expression. These data suggest that lycopene may play an important role in the modulation of β-carotene, retinoid, and/or lipid metabolism.

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