Variations in Plasma Lycopene and Specific Isomers over Time in a Cohort of U.S. Men[1]

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ABSTRACT Epidemiologic and laboratory studies suggest a possible role for tomato products, a rich source of the carotenoid lycopene, in the prevention of certain cancers and cardiovascular disease. Lycopene is consumed primarily as the all-*trans*-isomer, but the majority of lycopene in blood and tissue exists as a variety of *cis*-isomers. Specific isomers may be involved in different biological reactions, and patterns of isomers may provide insight into the risk or pathogenesis of disease processes. Total lycopene concentration and the concentrations of the *cis*- and *trans*-lycopene isomers were measured by HPLC in plasma samples taken 3–4 y apart from 144 mostly nonsmoking male participants in the Health Professionals Follow-up Study. Correlations between plasma concentrations determined 3–4 y apart ranged from 0.55 (all-*trans*-isomer) to 0.70 (*cis*-isomer 5 -*cis*) ($P < 0.001$). For total lycopene, the correlation was 0.63 ($P < 0.001$). Total *cis*-lycopene contributed ~67% of total lycopene (range 50–79%). At each time point, the various lycopene isomer concentrations were highly correlated with one another with Spearman correlation coefficients ranging from 0.90 to 0.99 ($P < 0.001$). Plasma concentrations of total lycopene and its most abundant isomers in samples taken 3–4 y apart were strongly correlated, indicating that dietary patterns and metabolic processes defining lycopene concentrations are stable over time. Because the patterns of lycopene isomers showed limited between-person variability, our results suggest that measuring specific lycopene isomers in epidemiologic studies may not provide additional information beyond that provided by total lycopene concentration. Single plasma samples quantitating plasma lycopene are a valid predictor of long-term exposure for epidemiologic studies. J. Nutr. 133: 1930–1936, 2003.

KEY WORDS: • *lycopene* • *isomers* • *reproducibility*

Epidemiologic studies have suggested that higher intake of tomatoes and tomato products may protect against cardiovascular disease (1–5) and reduce the risk of several types of cancer, particularly those of the prostate, lung and digestive tract (2,3,6,7). The most abundant carotenoid in tomatoes is lycopene (8), which demonstrates a potent ability to quench reactive oxygen in vitro (9–12). Thus, lycopene has been hypothesized to mediate in part the potential health benefits of tomato products (3). However, knowledge gained from in vivo studies in experimental models or from clinical studies is insufficient to define the mechanisms through which lycopene may influence carcinogenesis or cardiovascular disease. Although much of the emphasis is directed toward antioxidant pathways (8,13–15), some in vitro data also suggest that lycopene may influence other cellular processes related to cancer or vascular damage (16–18).

In foods, lycopene occurs mainly in the all-*trans* form, which is the most thermodynamically stable form (19,20). However, in human and rodent plasma, the *cis*-form predominates and accounts for ~50–70% of total lycopene (19,21). Although retaining their hydrophobic characteristics, the *cis*- and *trans*-isomers may distribute differently within lipoproteins and lipid bilayers of cell organelles and membranes, leading to unique biological effects (3,22). All-*trans* lycopene and a number of different *cis*-isomers have been measured in human plasma, but no information on the variability of plasma measures within individuals over time is available in the literature (19,23–26). Typically, plasma concentrations of lycopene are measured at one time point in nested case-control epidemiologic studies, which may or may not represent long-term

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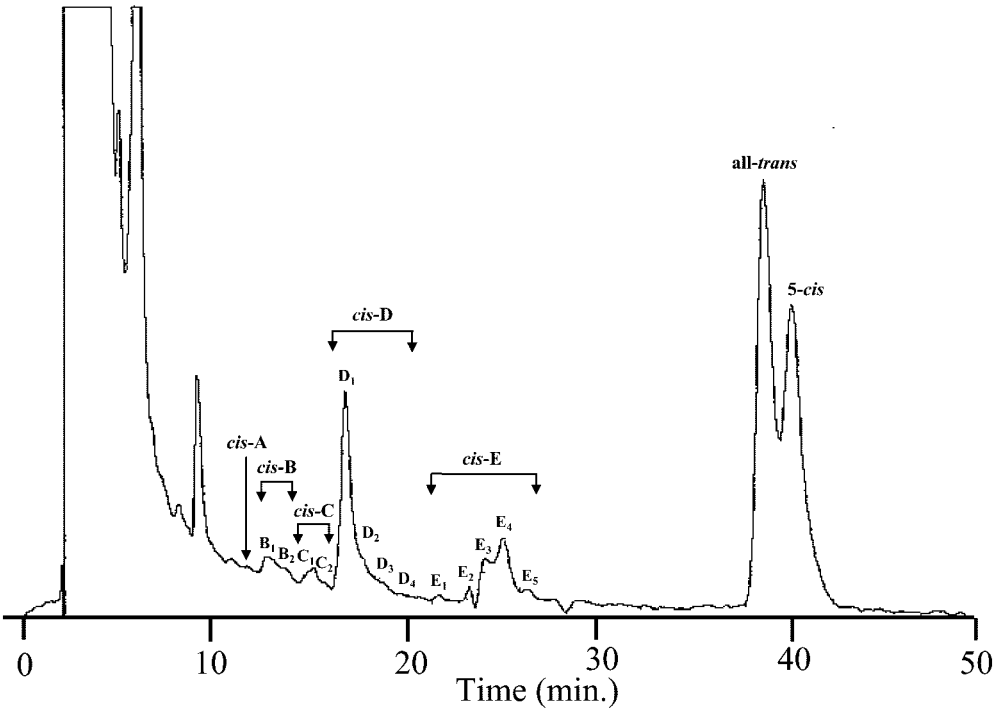

FIGURE 1 HPLC chromatogram on designation of lycopene isomers. Representative HPLC electrochemical detection chromatogram of 50 μL human plasma. Peak identifications: all-*trans* lycopene; 5-*cis* lycopene. Designations *cis*-A, B, C, D and E are for referencing groups of isomers by elution order and similarity in spectral characteristics.

dietary exposures. Thus, long-term within-person variation may be a source of measurement error and may weaken measures of association with risk or pathogenesis of disease. Information concerning lycopene isomer concentrations and their variability over time are therefore important for future epidemiologic studies investigating associations between specific lycopene isomers and human diseases (3). Furthermore, nested case-control studies also must consider possible associations of lycopene concentrations with certain lifestyle and demographic factors, such as age, body mass index (BMI) or alcohol intake, because these factors may be potential confounders (6,27). For cancers that vary by race, such as prostate cancer (27,28), information on possible variation in lycopene concentrations by race might also help to explain such variation in cancer risk.

Using blood samples taken 3 to 4 y apart in an ongoing large male cohort, the Health Professionals Follow-up Study, we investigated the variability of concentrations of lycopene and its major isomers in human plasma over time. We also examined whether certain lifestyle and demographic characteristics are associated with concentrations of total lycopene or specific isomers.

MATERIALS AND METHODS

Study population. The Health Professionals Follow-Up Study (HPFS) was initiated in 1986 when 51,529 male U.S. health professionals returned a mailed questionnaire inquiring about lifestyle factors (including information on smoking status, ethnicity, weight and height) and medical history. A 131-item food-frequency questionnaire (FFQ) was also added to the questionnaire. Every 2 y, information on lifestyle factors and medical history was updated by follow-up questionnaires and every 4 y, another FFQ was added to the follow-up questionnaires (29). Between 1993 and 1995, we requested that surviving cohort members provide a blood specimen. Participants were mailed a blood collection kit and asked to return a blood sample, chilled in ice, by a prepaid overnight courier. Approximately 18,159 men returned EDTA-preserved blood samples. On receipt by our laboratory, the blood specimens were separated into buffy coats, plasma and RBC and frozen in liquid nitrogen. In 1997, 75 Caucasian, 75 Asian and 63 African-American men, who had donated blood earlier were asked to donate a second blood sample. Of those, 150 participants returned a second blood specimen resulting in 150 paired samples, which were analyzed for lycopene and specific isomers. Before blood donation, all participants were required to give written consent and this study was approved by the Human Subject Committee of the Harvard School of Public Health. For this analysis, we used information from the 1994 follow-up questionnaire and the 1994 FFQ.

Laboratory analysis. Specimens were divided into aliquots under dim light and arranged in pairs. Paired quality control samples (n = 10), obtained from pooled plasma samples were interspersed randomly among the 150 paired samples. Lycopene analysis was conducted in the laboratory of Dr. Steven Schwartz at the Department of Food Science and Technology at The Ohio State University, Columbus, OH. Aliquots (100 μL) of blood plasma were deproteinated with incorporation of 100 μL ethanol containing a mass fraction of 0.1% BHT. Carotenoids were then extracted with two 500-μL portions of acetone/hexane (volume fraction 1:1) containing a mass fraction of 0.02% BHT. Each portion was mixed using a vortex for 30 s, after which the hexane layers were removed and combined. Individual extracts were dried under a stream of nitrogen at ambient temperature and analyzed immediately by reversed-phase HPLC using a C30 column (prepared at the National Institute of Standards and Technology, Gaithersburg, MD; commercially available from Waters, Milford, MA) with an electrochemical detector (ESA Coularray, Chelmsford, MA) following the methodology of Ferruzzi et al. (30). Because lycopene is transported in the plasma via lipoproteins (31), we also measured plasma cholesterol concentrations at the laboratory of Dr. Steven Clinton at The Ohio State University using an Infinity Total Cholesterol enzymatic assay kit (Sigma Diagnostics, St. Louis, MO) according to the manufacturer's recommendations. The mean intrapair CV for plasma cholesterol measurements based on 10 quality control samples was 7.5%.

Statistical analysis. Lycopene concentrations were right skewed; therefore nonparametric tests were employed. Differences between time 1 and time 2 concentrations were assessed using the Wilcoxon sign rank test, and correlations between paired lycopene and isomer concentrations were examined by calculating Spearman partial correlation coefficients. We adjusted for factors that might influence lycopene concentrations at time 1 and time 2 such as age, race, month of blood sampling, BMI and cholesterol concentrations. We

assessed differences in lycopene concentrations by lifestyle and demographic characteristics using the Wilcoxon rank-sum test (2 categories) or the Kruskal-Wallis test (3 or more categories). Because age, BMI, hours since last meal and alcohol intake (information on these variables was obtained from the 1994 questionnaire) are continuous variables, we also performed a trend test by regressing these variables on lycopene concentrations. Six paired samples were excluded from our final analysis because of technical problems when analyzing the samples. Mean intrapair CV were calculated on the basis of the 10 paired quality control samples. *P*-values are reported for two-sided tests. A *P*-value of <0.05 was considered significant.

RESULTS

Of the 144 participants included in our analysis, 44% donated their first blood sample in 1993, 51% in 1994 and 5% in 1995 (time 1). Except for one participant who donated his second blood sample in 1996, all second blood samples were donated in 1997 (time 2). The vast majority of men in this study were nonsmokers; only 5 of the 144 participants were current smokers at both times 1 and 2. A representative HPLC chromatogram is shown in **Figure 1** and labeled to illustrate our designation of lycopene isomers. Unequivocal identifications of the geometrical isomer configurations were not made for all lycopene isomers separated using the HPLC method in this manuscript. Detailed nuclear magnetic resonance experiments are required for this analysis. Thus, the legend in Figure 1 lists the two isomers that have been identified (all-*trans* and 5-*cis*) and clarifies the designation for groups of isomers that elute with similar spectral characteristics.

Total *cis*-lycopene isomer concentrations were calculated by summing the concentrations of all measured *cis*-isomers. For the most part, median concentrations of lycopene isomers did not differ considerably between times 1 and 2 (**Table 1**). The magnitude of the CV appeared to be related to the concentration of the isomer; higher CV were observed for the lycopene isomers with lower concentrations, where more measurement error is expected. CV $< 15\%$ were found only for the more abundant lycopene isomers: all-*trans* isomer (8.9%), *cis*-isomer D1 (14.1%) and *cis*-isomer 5-*cis* (14.5%). Therefore, we focused our subsequent analysis on these more abundant lycopene isomers.

We also examined lycopene isomer concentrations by lifestyle and demographic characteristics and by season and time of day of blood sampling (**Table 2**). Total lycopene, all-*trans* lycopene and total *cis*-lycopene concentrations did not differ significantly by age, race, BMI, season of blood sampling, time of day of blood sampling or alcohol intake. However, the sample size in some categories was small, limiting our power to detect subtle effects. Our data suggest an inverse trend for hours since the last meal with lycopene concentrations, typically reflecting the sampling of blood early in the morning after an overnight fast. There was also a significant positive trend for cholesterol concentrations with lycopene concentrations. In all categories, results were similar when lycopene concentrations were examined for times 1 and 2 separately, rather than as their mean (data not shown).

Plasma cholesterol concentration was modestly correlated with total lycopene concentration (time 1: $r = 0.27$, $P = 0.001$; time 2: $r = 0.24$, $P = 0.004$). No correlations were observed between total lycopene concentration and age, BMI or alcohol intake at time 1 (age: $r = -0.0008$; BMI: $r = 0.07$; alcohol $r = -0.10$) or time 2 (age; $r = -0.06$; BMI: $r = 0.07$; alcohol: $r = 0.05$); all *P*-values were >0.1. Correlations between cholesterol, age, BMI or alcohol intake and the more abundant lycopene isomers in times 1 and 2 were similar to those observed between these factors and total lycopene (data not shown).

TABLE 1

Plasma concentrations and intrapair CV for lycopene and its isomers in participants in the Health Professionals Follow-up Study[1,2]

	Time 1[3]	Time 2	Mean Intrapair CV[4]
	nmol/L		*%*
Total lycopene	221.4	213.2	8.9
All-*trans*	74.9	67.6	10.8
Total-*cis*	146.5	146.1	13.1
Isomer A	2.2	2.0	34.5
Isomer B_1	2.4	2.4	47.6
Isomer B_2	0.6	0.8	53.0
Isomer C_1	2.1	1.7	38.5
Isomer C_2	0	0	35.1
Isomer D_1	31.9	30.1	14.1
Isomer D_2	6.1	5.6	21.1
Isomer D_3	1.9	1.9	60.8
Isomer D_4	0	0	141.4
Isomer E_1	0	0	94.7
Isomer E_2	1.5	1.4	30.2
Isomer E_3	5.5	5.5	22.8
Isomer E_4	11.3	11.4	27.2
Isomer E_5	1.1	1.1	59.0
Isomer 5-*cis*	71.0	75.3	14.5

[1] Values are medians.
[2] Median concentrations for all isomers except for isomer 5-*cis* were calculated on all 144 paired samples; mean concentrations for isomer 5-*cis* were calculated on 142 paired samples.
[3] Times 1 and 2 refer to the time points 3–4 y apart at which blood samples were obtained.
[4] CV were calculated on 10 paired quality control (reference) samples.

Correlations between total lycopene and the major isomers for the two blood sampling times ranged from 0.55 to 0.70 ($P < 0.01$) (**Table 3**). Total lycopene concentrations and the major isomers were also strongly correlated with one another ($r = 0.90$ to 0.99) ($P < 0.01$) (**Table 4**).

We also calculated the proportions of total *cis*-lycopene and all-*trans* lycopene relative to total lycopene concentrations using the mean of concentrations at times 1 and 2. Regardless of actual concentration of total lycopene, total *cis*-lycopene demonstrated remarkable consistency and contributed ~67% (range 50–79%) to total lycopene (**Fig. 2**). These proportions did not vary by race or other demographic variables (data not shown). In addition, we also investigated whether the contribution of total *cis*-and all-*trans*-isomers to total lycopene changed over time by calculating differences in proportions of total *cis*-lycopene and all-*trans* lycopene to total lycopene between times 2 and 1. The differences in the all *trans*/total lycopene ratio between times 2 and 1 did not exceed 10% (**Fig. 3**) for 84% of participants. All reported results were similar after we excluded the 5 current smokers from the analysis.

DISCUSSION

To our knowledge, this is the first study investigating the concentrations of plasma lycopene isomers in a cohort of men and documenting their changes over time. Several interesting findings emerged from this study. First, modern HPLC technology with very sensitive electrochemical detection allows investigators to assess a vast array of mono- and poly-*cis*-

TABLE 2

Plasma total lycopene and lycopene isomer concentrations in male participants in the Health Professionals Follow-up Study stratified by lifestyle and demographic characteristics[1]

Characteristic	*n*	Total lycopene	Total *trans*-lycopene	Total *cis*-lycopene
		nmol/L		
Age in 1994, *y*				
<50	21	240.9	64.7	158.1
51–55	23	208.2	68.0	138.5
56–60	26	248.6	83.0	160.7
61–65	27	250.5	73.2	165.6
66–70	19	194.9	61.0	129.2
>70	28	206.0	68.1	139.7
Race				
Caucasian	54	204.3	72.1	133.5
African-American	41	190.6	66.6	125.9
Asian	49	240.9	73.3	163.7
Body mass index in 1994, *kg/m*^{2}				
16.4–22.7	24	184.0	62.0	117.3
22.8–24.1	25	208.8	65.7	139.7
24.3–25.5	25	236.1	74.2	152.0
25.7–27.8	24	231.5	72.6	151.2
27.9–34.5	25	230.6	72.9	155.1
Month of blood sampling,[2]				
November–January	23	200.6	61.4	143.0
February–April	27	227.4	82.8	148.6
May–July	52	233.2	72.7	158.3
August–October	42	219.6	75.9	144.5
Time since last meal,[2] *h*				
1–2	16	278.9	99.9	176.1
3–4	17	239.1	94.6	146.4
5–6	12	173.6	67.5	108.0
>7	92	221.43	73.9	147.63
Time of blood sampling,[2]				
0700–0900 h	68	236.1	78.2	155.8
1000–1300 h	43	206.3	78.4	144.8
1400–0600 h	17	210.0	59.9	143.0
Plasma cholesterol,[3] *mg/dL*				
122.2–186.9	28	178.5	55.8	114.7
187.3–209.1	29	198.6	71.4	126.5
209.3–221.6	29	236.1	74.2	157.7
222.3–240.1	29	248.9	79.6	158.1
240.3–307.8	29	267.7*†	86.0*†	177.5†
Alcohol intake in 1994, *g/d*				
None	37	237.9	73.2	159.4
0.88–1.96	16	226.9	76.9	157.0
2.00–6.73	28	260.5	87.8	166.3
6.87–14.54	26	175.4	63.7	112.0
15.48–71.38	25	200.7	63.6	134.9

[1] Values are medians. Medians were calculated using each participant's mean lycopene and lycopene isomer concentrations at times 1 and 2. * $P < 0.05$ for differences in lycopene concentrations by lifestyle and demographic characteristics using the Wilcoxon rank-sum test (2 categories) or the Kruskal-Wallis test (3 or more categories); † P for trend < 0.05 [trend tests were calculated by regressing lycopene concentrations as a continuous variable on age, body mass index, hours since last meal, plasma cholesterol and alcohol intake (also as a continuous variable)]. Not all numbers add up to 144 because of missing information (1994 questionnaire).

[2] For the categories hours since last meal, and time and month of blood sampling, only time 1 concentrations are shown. Times 1 and 2 refer to the time points 3–4 y apart at which blood samples were obtained.

[3] Mean of plasma cholesterol concentrations at times 1 and 2. To convert mg/dL to mmol/L, multiply by 0.02586.

TABLE 3

Plasma total lycopene and isomer concentrations and correlations of participants in the Health Professionals Follow-up Study between times 1 and 2

				Spearman rank correlations	
Lycopene	Time 1	Time 2	*P*-value[2]	Crude[3]	Partial[3,4]
	nmol/L				
Total Lycopene	221.4	213.2	0.12	0.58	0.63
Total *cis*-isomer	146.5	146.1	0.27	0.62	0.66
Cis-isomer D1	31.9	30.1	0.01	0.66	0.70
Cis-isomer 5-*cis*	71.0	75.3	0.36	0.67	0.70
All-*trans*-isomer	74.9	67.6	0.02	0.47	0.55

[1] Values are medians; $n = 144$.

[2] *P*-values on differences between time 1 and time 2 concentrations were calculated using the Wilcoxon sign-rank test. Times 1 and 2 refer to the time points 3–4 y apart at which blood samples were obtained.

[3] All $P < 0.001$.

[4] Spearman rank correlations adjusted for age, race, season of blood sampling at both time points, as well as differences in body mass index between times 1 and 2 and differences in cholesterol concentrations between times 1 and 2.

lycopene isomers in plasma, and we have presented data for the 12 *cis*-isomers most commonly found in men. The mean intrapair CV were <15% only for the more abundant lycopene isomers. This is expected because at very low concentrations of isomers, small variations in sample processing, carotenoid extraction and HPLC analysis will accentuate the imprecision inherent in trying to quantitate concentrations of molecules found at such low concentration in biological samples. Thus, our work indicates that several isomers with CV of 15% can be quantified with appropriate precision to be applied to epidemiologic studies.

Although the sample size was small, our study did not suggest strong relationships between lycopene isomer concentrations and certain lifestyle and demographic characteristics, such as age, race, BMI or alcohol intake. We did observe a significant inverse trend between hours since last meal and lycopene concentrations. Lipoprotein clearance after overnight fasting is expected and because no other known plasma transport mechanisms exist for lycopene transport, this finding illustrates that standardizing sampling to a specific time point postfasting is one way to reduce variability in lycopene blood concentrations, thereby improving the precision of epidemiologic studies.

Because prostate cancer risk is greater in African American men and reduced in Asian American men compared with Caucasians (27,28), some of the differences in risk might be related to differences in tomato product intake or perhaps differences in carotenoid/lycopene metabolism. Ethnic variation in dietary intake of carotenoid containing fruits and vegetables and differences in plasma carotenoids have been reported (32–35). Our study showed no major differences between plasma lycopene concentrations or patterns and concentrations of major lycopene isomers. This study is limited to health professionals with similar education and socioeconomic status, and strongly suggests that there are no major ethnic determinants of lycopene metabolism. However, our study does not eliminate the possibility that the larger spectrum of socioeconomic status may contribute to ethnic differences in

TABLE 4

Correlation matrix for total lycopene and specific isomers in plasma samples taken 3–4 y apart from 144 male participants in the Health Professionals Follow-up Study

	Spearman correlation coefficient[1]				
	Total lycopene	All-*trans*-lycopene	Total *cis*-lycopene	D1-*cis*-lycopene	5-*cis*-Lycopene
			Time 1, Time 2		
Total lycopene	—	0.96, 0.95	0.99, 0.99	0.96, 0.96	0.97, 0.96
All-*trans*-lycopene		—	0.92, 0.91	0.90, 0.90	0.91, 0.89
Total *cis*-lycopene			—	0.96, 0.96	0.98, 0.98
D1-*cis*-lycopene				—	0.96, 0.93
5-*cis*-Lycopene					—

[1] All $P < 0.001$.

dietary patterns involving tomato products and lycopene, which in turn may mediate overall prostate cancer risk.

The majority of published studies that have examined the relationships between demographic factors and plasma lycopene concentrations found significant inverse associations between total lycopene concentration and age (36–41). These observations may be due to age-related changes in dietary patterns or a decrease in total food intake in addition to changes in metabolism associated with aging, age-related diseases and medications (36–41). However, in this cohort of relatively healthy professional men, we did not observe any age-related trends. Although alcohol is hypothesized to modulate lycopene absorption or metabolism (37), we did not observe any associations with lycopene concentrations or isomer patterns in our study.

This study is the largest one to examine the variation in concentrations of plasma lycopene and its major isomers over time. We observed significant moderate correlations of 0.55–0.70 for total lycopene and major isomers in blood samples taken 3–4 y apart. Several previous studies examined total lycopene concentrations at different time points but within a period ≤ 1 y (38,42,43). In a study by Peng et al. (38), an intraclass correlation of 0.85 was found in 96 healthy Caucasians based on 3 lycopene measurements over a 1-mo period. A correlation of 0.57 between lycopene concentrations among 29 healthy volunteers over an interval of 1 y was observed in another study (43). Interestingly, in a group of 42 British women, mean total lycopene concentrations differed significantly by the season in which the sample was obtained. In that study, higher total lycopene concentrations were found in summer and autumn than in winter and spring (44). On the other hand, another study (42) reported slightly higher lycopene levels in the winter, but differences were not significant. Neither total lycopene concentrations nor all-*trans* or total *cis*-lycopene concentrations differed significantly by month of blood sampling in our group of U.S. health professionals.

Finally, we found that lycopene isomer concentrations at each time of blood sampling were highly correlated with one another and that the proportion of total *cis*-lycopene and all-*trans* lycopene to total lycopene did not vary substantially among most subjects. In our study, total *cis*-lycopene contributed ~60–80% to total lycopene concentrations. These proportions are consistent with some recent studies published using the advanced HPLC technology that allows the separation and quantitation of the lycopene isomers (19,23,25,45).

Our findings do not provide insight into the mechanisms

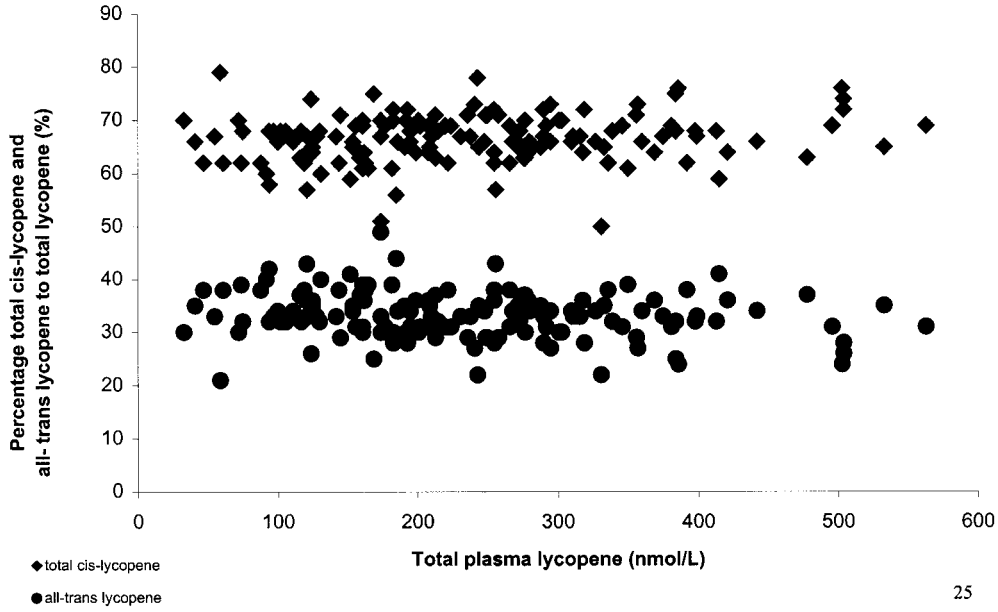

FIGURE 2 Proportion of total *cis*-lycopene and all-*trans*-lycopene to total lycopene using mean plasma concentrations in two samples taken 3–4 y apart from 144 mostly nonsmoking male participants in the Health Professionals Follow-up Study.

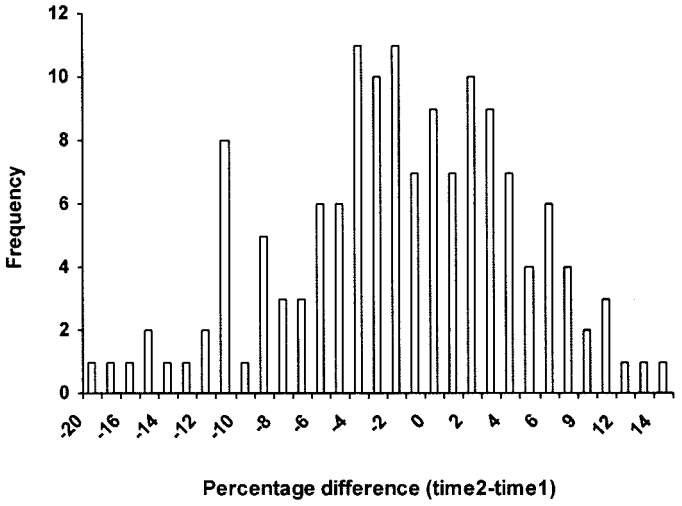

FIGURE 3 Distribution of differences in the proportion of all-*trans*-lycopene to total lycopene in plasma samples taken 3–4 y apart from 144 mostly nonsmoking male participants in the Health Professionals Follow-up Study.

underlying the formation, interconversion or biological roles of various lycopene isomers. Tomato products contain lycopene primarily in the all-*trans* form (19,20), a thermodynamically stable isomer, and we have again documented that the *cis*-form predominates in plasma. Although harsh cooking methods may contribute to *cis*-isomer formation, it appears that the majority of isomerization occurs in vivo (19–21). For *cis*-isomers ingested or formed during digestion, it appears that the structural change enhances the bioavailability, perhaps through improving solubility into micelles (21,46). In rodents, androgen status and energy intake modulate lycopene isomer metabolism (47,48). The limited within-person and between-person variability in lycopene isomer patterns suggests the activity of undefined biologic processes that maintain stability in this equilibrium (21). Future efforts will be directed toward elucidating details of lycopene isomer formation and biological function, issues that remain enigmatic (3,22).

In summary, reasonably high correlations between plasma concentrations of total lycopene and its most common isomers in plasma samples drawn 3–4 y apart were observed in mostly nonsmoking health professionals. Much additional laboratory and clinical research is required to define the mechanisms leading to *cis*-isomer formation and the biological functions of the various isomers. However, the very strong correlations among these lycopene isomers and the limited between-person variability in the proportions of total *cis*-lycopene and all-*trans* lycopene to total lycopene, suggest that it will be difficult to separate the independent effects of each of the isomers in the course of epidemiologic studies. At the present time, the additional effort and costs of measuring different lycopene isomers in epidemiologic studies are not likely to provide predictive information beyond that provided by total lycopene.

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