# Lycopene bioavailability and metabolism in humans: an accelerator mass spectrometry study[1–3]

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## ABSTRACT

**Background:** To our knowledge, there is no direct information on lycopene metabolism in humans.

**Objective:** The objective of this study was to quantify the long-term human bioavailability of lycopene in plasma and skin after a single dose of $^{14}$C-lycopene and to profile the metabolites formed.

**Design:** We preselected 2 male subjects as lycopene absorbers and gave them an oral dose of 10 mg synthetic lycopene combined with ≈6 μg [6,6′,7,7′-$^{14}$C]lycopene (≈30,000 Bq; 92% *trans* lycopene). The appearance of $^{14}$C in plasma, plasma triacylglycerol–rich lipoprotein (TRL) fraction, urine, expired breath carbon dioxide, and skin biopsies was measured over 42 d. The $^{14}$C in lycopene-isomer fractions from plasma and TRL fraction was measured to assess the isomerization of lycopene in vivo.

**Results:** We quantified $^{14}$C from $^{14}$C-lycopene in plasma, the plasma TRL fraction, expired carbon dioxide, urine, and skin. The time to maximum concentration ($t_{max}$) of total $^{14}$C-lycopene in plasma was 6 h, and the elimination half-life ($t_{1/2}$) was 5 d, which were different from the $t_{max}$ and $t_{1/2}$ of unlabeled lycopene (0.5 and 48 d, respectively). $^{14}$C-Lycopene was extensively isomerized after dosing as a 92% all-*trans* isomer at dosing but changed to 50% *trans*, 38% 5 *cis*, 1% 9 *cis*, and 11% other *cis* isomers after 24 h. A similar pattern of isomerization was seen in plasma TRL fractions.

**Conclusions:** Lycopene was extensively isomerized after dosing and rapidly metabolized into polar metabolites excreted into urine with the rapid peak of $^{14}CO_2$ after dosing, which implies that β-oxidation was involved in the lycopene metabolism. Lycopene or its metabolites were detected in skin for up to 42 d. *Am J Clin Nutr* 2011;93:1263–73.

## INTRODUCTION

Lycopene is the most abundant carotenoid present in tomatoes and one of the main carotenoids present in the human diet (1). Lycopene has been of nutritional interest since it was first suggested as having a role in the prevention of prostate cancer (2) and has subsequently been suggested to play a role in the prevention of cardiovascular disease (3–5) and skin cancer (6) and the reduction of oxidative stress (7, 8). These effects have been suggested to be mediated by the antioxidant capacity of the lycopene molecule via signaling effects of lycopene metabolites (1, 9).

The study of lycopene's role in human nutrition is complicated because the all-*trans* isomer predominates in the main dietary source of lycopene (ie, tomatoes), but blood, plasma, and tissues contain relatively greater concentrations of *cis* isomers (10). The processing of tomatoes by heating converts the all-*trans* lycopene to various *cis* isomers. *cis*-Lycopene isomers are regarded as being more bioavailable because they are more soluble and better absorbed from the intestinal lumen than is the all-*trans* isomer (11), although the isomerization may also take place in vivo. The gastrointestinal lumen (12, 13), liver (14), and enterocytes (15) were identified as potential sites of lycopene isomerization in vivo.

The use of accelerator mass spectrometry (AMS) to detect an ultralow dose of the $^{14}$C-labeled lycopene tracer could help answer some of the questions surrounding lycopene bioavailability. AMS instruments measure the ratio or abundance of rare isotopes and have only been used to a limited extent in nutritional science (16–24). AMS can accurately measure ratios at 1% of the natural $^{14}$C abundance ("modern carbon": the ratio of $^{14}$C:$^{12}$C before 1950 or $9.8 \times 10^{-16}$ mol $^{14}$C/g carbon) (25). This sensitivity [the low attomole ($10^{-18}$) range of $^{14}$C] makes AMS useful in studies on micronutrients in which a few micrograms of tracer can be used rather than the larger amounts (milligrams) often required for $^{13}$C-labeled tracers. Sample sizes required for measurements are very low [eg, a few microliters of blood or a few milligrams of tissue (24)]. The whole-body radiation exposure from nutritional $^{14}$C-microdosing experiments that used 3700 Bq $^{14}$C ranged from 1.3 to 5.2 μSv compared with 20 μSv exposure from a 4-h airline flight (26), which confirmed that the risk from radiation $^{14}$C-microdosing studies was negligible.

Labeled lycopene, in addition to the study of plasma bioavailability, makes it possible to follow the kinetics of excretion in urine and study the changes in the isomer pattern postabsorption

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as well as to gain information about metabolism. It also enables the determination of pharmacokinetic variables for lycopene without the possible confounding of endogenous stores of the carotenoid. In this article, we described a study that used $^{14}$C-labeled lycopene and AMS to gain a new understanding of the lycopene bioavailability and metabolism in humans and evaluated the use of different AMS instruments for nutrition research.

## SUBJECTS AND METHODS

### Chemicals and reagents

[6,6′,7,7′-$^{14}$C]Lycopene ($^{14}$C-lycopene) was provided by DSM (Kaiseraugst, Switzerland) and was purified before use with a 97.2% radiopurity (by radio-HPLC) and >96% chemical purity by nuclear magnetic resonance spectroscopy (Selcia, Essex, United Kingdom). The specific activity was 6.26 MBq/mg. $^{14}$C-lycopene was stored in amber bottles under nitrogen gas at −20°C. The isomeric composition of the $^{14}$C-lycopene was 92% all-*trans* and 8% *cis* (predominantly 5-*cis*) determined by HPLC and liquid scintillation counting (LSC) (*see* below). Synthetic unlabeled lycopene was provided by DSM (27) and had a similar isomeric composition of 90% all-*trans* and 10% 5-*cis* isomers. The $^{14}$C-lycopene was formulated for human consumption through the mixture of it with 10 mg unlabeled lycopene, dissolution in dichloromethane before homogenization in a gelatin and sucrose matrix, and removal of dichloromethane under a reduced pressure at 40°C. The exact amount of $^{14}$C present in the dose was determined by LSC. The doses were flushed with argon, kept away from light, and stored at −20°C. All solvents used were of HPLC grade (Merck, Darmstadt, Germany). Standard lycopene (95% all-*trans*) was from Sigma (Buchs, Switzerland). Mixed *cis*-lycopene-isomer tomato oleoresin (28) was used to identify the retention time of *cis*-lycopene isomers.

### Subject selection

This was a proof-of-concept study that used AMS to determine the bioavailability and metabolism of lycopene in humans. Because it is known that some people absorb lycopene poorly, subjects were prescreened to ensure that they had a good postprandial absorption of lycopene before starting the study. Healthy males subjects were recruited who were normolipidemic (fasting plasma triglyceride concentrations <200 mg/dL) and had an age range of 40–50 y, a body mass index (in kg/m$^2$) of 20–27, and skin type II or III (white, fair to brown hair, and skin that gets sunburned easily). These skin types were selected because they were the most commonly used in previous studies on lycopene concentrations in skin (29, 30). Exclusion criteria were smoking, the regular consumption of alcohol, use of medication, regular use of vitamin, mineral, or antioxidant supplements, gastrointestinal surgery (including appendectomy) or disturbances, exposure to artificial ultraviolet or greater than regular exposure to sunlight, participation in a previous $^{14}$C-tracer study, an allergy to fish or peanut products, and an aversion to tomatoes and tomato-based products. Subjects were asked to avoid all lycopene-rich foods, including tomatoes and tomato-based products, during the prescreening and study periods to increase the proportional lycopene bioavailability.

Four male subjects were recruited for prescreening to ensure that they were lycopene absorbers. After donating a fasting blood sample they were given a standardized meal that contained 25 g fat in 2.93 MJ (700 kcal) and 33 g tomato paste (25 mg lycopene). Three hours later, another blood sample was taken. The blood was separated into plasma, and the triacylglycerol-rich lipoprotein (TRL) fraction was isolated by using the method of van Vliet et al (31) and analyzed for lycopene (*see* section entitled "Separation of lycopene isomers"). Two subjects were selected on the basis of a >100% increase in lycopene in both plasma and the TRL fraction 3 h after consumption of the tomato paste. Written informed consent was obtained from volunteers, and the study was approved by the Aspire Independent Review Board LLC (San Diego, CA). The clinical study was carried out at the Covance Clinical Research Unit (Honolulu, HI) in 2006.

### Dosing, specimen collection, and sample preparation

The 2 selected male subjects (40 and 60 y of age with a body mass index of 26.2 and 26.7, respectively) were asked to abstain from lycopene-containing foods for 1 wk before the start of the study and then for the duration of the study (6 wk) to obtain conditions similar to a previous study (32). On the test day, the subjects came to the metabolic unit after an overnight fast. Baseline samples of urine (overnight), fasting blood, expired air, and a 4-mm diameter skin-punch biopsy (lower back below the beltline) were taken. The subjects were given an oral dose of 37 kBq $^{14}$C-lycopene in 10 mg unlabeled lycopene with a meal (a milkshake that comprised 300 g banana, 100 mL skim milk, 25 g olive oil, and 12.6 g sucrose; 573 kcal, 7 g protein, 86 g carbohydrates, and 8 g fiber). Blood samples were taken at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 9, and 12 h postdose. Urine and expired air were collected at 4 and 12 h postdose. Fasting blood, 24-h urine, and expired air were collected daily on days 2–7 postdose and subsequently on days 13, 21, 28, 35, and 42 postdose. Skin biopsies were taken on days 0, 4, 7, and 14 for subject 1 and on days 0, 14, 28, and 42 for subject 2 (*see* supplemental Figure 1 under "Supplemental data" in the online issue for an overview of the analyses).

#### *Blood plasma*

Blood was collected in lithium-heparin coated tubes and centrifuged for 10 min at 3000 × *g*. Samples were protected from light as far as possible and stored at −40°C. Plasma samples were either directly analyzed for $^{14}$C by AMS (both types of AMS instrument) or extracted and individual lycopene isomers separated and collected for AMS analysis by using HPLC (*see* below).

#### *Urine*

Subjects were given bottles with which to store their urine and requested to keep samples at 4°C. All urine from subjects was collected during the first week and then spot urine samples were taken at all time points afterward. Urine samples were analyzed directly by AMS (Newton Scientific Inc [NSI (Cambridge, MA)] and Massachusetts Institute of Technology [MIT (Cambridge, MA)] (NSI-MIT) system; *see* below for details).

#### *Breath carbon dioxide*

Two methods for breath carbon dioxide collection were used. One method was adapted from the method of Gunnarsson et al

(33) by using 2 perspex tubes; the first tube was filled with Drierite (Sigma), which is a material that traps water, and the second tube was filled with Ascarite II (Sigma), which is a material that traps carbon dioxide. Silica tubing attached the 2 perspex tubes together, and subjects were instructed to blow into the tubes through a plastic drinking straw. The second method for the collection of expired air for the AMS analysis has been described by Schulze-König et al (34). In this method, the carbon dioxide of the exhaled breath was captured on a trap with type 13× zeolite followed by analyses in which the carbon dioxide was directly released into the hybrid ion source of the MIni radioCArbon DAting System (MICADAS) AMS system [Swiss Federal Institute of Technology, Zürich (ETHZ), Switzerland] via the controlled heating of the trap while flushing with helium gas.

*Skin biopsies*

Four-millimeter-diameter full-thickness punch biopsies were taken from the lower back, just below the beltline, weighed, washed with 0.9% saline solution to remove any contaminating blood and adipose tissue, and stored at −70°C before being shipped for analysis by AMS (bio-MICADAS system; ETHZ).

*TRL fraction*

The TRL fraction of plasma mainly contained chylomicrons and a small amount of VLDL. This fraction was isolated from plasma by ultracentrifugation by using the method of van Vliet et al (31): Four milliliters of plasma was overlaid with 4 mL sodium bromide solution with a density of 1.006 g/mL, and TRL was separated by ultracentrifugation for 30 min at 100,000 × *g* at 15°C. Samples were extracted by using the same method as plasma, and TRL lycopene isomers were separated by HPLC before gas-fed AMS analysis. Care was taken to protect the samples from light during the procedure, and samples were stored at −40°C before analysis.

**Separation of lycopene isomers**

The $^{14}C$ in lycopene isomers and TRL was measured at the following time points: 0, 3, 6, 9, 12, 24, 48, 72, 96, 144, and 336 h. Lycopene isomers in the test dose, plasma, and TRL were isolated by HPLC by using the method of Schierle et al (35). Briefly, 200 μL plasma or 400 μL TRL solution was made up to 1120 μL with distilled water in an amber tube, 1200 μL ethanol was added to denature the proteins, and the sample was mixed by vortex. The sample was extracted 3 times by using 0.05% butylated hydroxytoluene in hexane, and the extracts were evaporated and dissolved in 10 μL dichloromethane and 90 μL hexane. Lycopene isomers were separated by normal-phase HPLC as follows: 50 μL of extracted sample was injected onto a 90-cm Nucleosil HPLC column (3, 300 × 4.6-mm, 5-μm particle-size columns in a series; Machery-Nagel, Düren, Germany) with a Nucleosil precolumn (Machery-Nagel), and lycopene was eluted with an isocratic flow of *n*-hexane with 0.1% *n*-ethyldiisopropylamine at 1 mL/min. A Hewlett-Packard 1050 HPLC (Hewlett-Packard, Santa Clara, CA) with a variable wavelength detector (quantification) and diode-array detector (spectral confirmation) was used. Fractions were collected with a FRAC-100 fraction collector (Pharmacia, Uppsala, Sweden). Lycopene isomers were quantified at 470 nm on the basis of a standard curve determined by using all-*trans* lycopene, with the assumption of a similar absorption coefficient for all lycopene isomers (35). Standard curves were repeated twice a week to ensure repeatability. Lycopene isomers were determined on the basis of previously published chromatograms (35), and a tomato-oleoresin sample was enriched with *cis* isomers of lycopene (28), with isomers confirmed by mass spectrometry/mass spectrometry (MS/MS) analysis (15).

HPLC eluent was collected every minute, and peaks were matched to each appropriate fraction. Because some of the lycopene-isomer peaks were spread out over more than one fraction, $^{14}C$ counts for the relevant fractions were summed for each isomer. 13- and 15-*cis* Lycopene isomers that were not fully separated by the method were reported together as the sum of the 2 peaks.

**Separation of lycopene metabolites**

A total of 10 mL urine collected between 12 and 24 h (the greatest enrichment of $^{14}C$) from both subjects was treated with β-glucuronidase and sulphatase (25 kU and 250 U, respectively) (Sigma) in 5 mL 50 mmol/L phosphate-buffered solution (pH 7), and incubated overnight at 37°C to deconjugate any possible lycopene metabolites. Urine was lyophilized to near dryness and 1 mL 5% methanol was added. The deconjugated urine sample was passed through a 0.2-μm filter, and 50 μL of the sample was injected into a Hewlett-Packard 1050 HPLC under the following conditions: 0–5 min, 100% solvent A (100% water), followed by a gradient to 100% solvent B (100% methanol) over 25 min, and then 100% solvent B for 10 min. The system was returned to 100% solvent A over 10 min, and the column re-equilibrated with 100% solvent A for a further 10 min. The flow rate was 1 mL/min, and the column used was a 250 × 4.6-mm, 5-μm particle size Zorbax SB-Aq $C_{18}$ column (Agilent). The absorbance was monitored with a photodiode-array detector that measured between 210 and 500 nm, and fractions were collected every 1 min for analysis by liquid scintillation.

Fractions with a greater than background radioactivity were further analyzed with a nanospray full scan and tandem mass experiments (MS/MS in product-ion mode by selecting relevant deprotonated and protonated ions). Analyses were carried out on a TSQ Quantum 7000 triple-quadrupole instrument (ThermoFisher, San Jose, CA) equipped with a static-nanospray interface. Dried fractions of interest were resuspended in 50 μL $H_2O$/methanol (1:1, vol:vol) that contained 0.1% formic acid. A total of 2 μL was loaded into the nanospray needle and manually centrifuged to allow the droplets to reach the tip of the needle. From the full-scan mass measurements (*m/z* 50–800 in a scan time of 1 s while applying a spray voltage between 700 and 1200 V) in both positive- and negative-ionization modes, ions that corresponded to known lycopene metabolites (36–41) and potential products that resulted from β-oxidation of lycopene were submitted to subsequent MS/MS analyses.

**AMS**

Bio-AMS is fundamentally a technique for the determination of the radiocarbon content of a biological sample. This is typically accomplished by measuring the ratio of $^{14}C$ to either of the

2 stable carbon isotopes ($^{12}C$ or $^{13}C$). Less typically, it can be accomplished by counting $^{14}C$ atoms with quantification by comparison with standards run under the same conditions. Each approach for determining the $^{14}C$ content in a sample has advantages and disadvantages compared with the other; the isotope ratio (IR) approach is far more sensitive and accurate but requires rigorous contamination control, whereas the $^{14}C$ counting methodology is less sensitive and prone to greater variability but has better throughput, and contamination is less problematic. Because these 2 approaches to the quantification of minute quantities of $^{14}C$ have never, to our knowledge, been compared for a biological dataset, an ancillary objective was to compare pharmacokinetic results of the 2 bio-AMS methods in plasma.

The following 2 types of AMS instruments were used in this study: the MIT AMS system and the MICADAS AMS system (ETHZ). At MIT, an instrument designed and constructed by the NSI was used. Samples analyzed at MIT included whole blood, plasma, urine, expired air carbon dioxide, TRL, and lycopene isomers in plasma and TRL. At ETHZ, IR AMS analyses were conducted with a MICADAS instrument, and comprised plasma, skin samples, and samples of exhaled air carbon dioxide.

### *Gas-fed $^{14}C$-counting AMS measurements*

The NSI-MIT system incorporates a laser-induced combustion interface that produces carbon dioxide directly from samples for online introduction into the gas-fed ion source of the AMS instrument. This system and its operation have been described in detail in previous publications (42, 43). Expired carbon dioxide that was trapped on Ascarite (Sigma) was analyzed after its release by treatment with concentrated sulfuric acid within a septum-sealed vial and released carbon dioxide was introduced directly into the AMS source without laser combustion. The quantification of combusted liquid-phase samples was based on the radioisotope abundance rather than the IR, whereas the quantification of breath samples was made by the IR that was calibrated by using identically treated $Na_2[^{14}C]CO_2$ reference samples.

### *IR AMS measurements*

IR AMS measurements were carried out on a MICADAS instrument (ETHZ) that has been described elsewhere (44, 45). Liquid and solid samples were combusted to carbon dioxide before reduction to a fullerene solid and analysis by IR AMS. Samples of expired breath carbon dioxide were directly introduced in the gas-accepting ion source of the MICADAS instrument (ETH Zurich). Details of the procedure have been previously published (34). Because of the lower limit of quantification of the AMS instrument (ETH Zurich) (≈10% of background $^{14}C$; 0.1 modern) and high precision (46), this was used for skin biopsy samples when a low enrichment of $^{14}C$ was anticipated.

### *LSC*

LSC was performed with a Packard Tri-Carb 2100 TR Liquid Scintillation Analyzer (Packard, Waltham, MA). Samples were mixed with 10 mL liquid scintillation cocktail (Ultima Gold; Perkin Elmer, Shelton, CT), and counts were measured for 10 min.

## Pharmacokinetics and data analysis

The incremental area under the curve (AUC) was determined by a mixed log-linear trapezoidal model. The mixed log-linear trapezoidal model performed the AUC calculation in the following way: the linear rule (trapezoidal) was applied when the concentration was ascending and the log linear rule was applied when the concentration was descending. Noncompartmental analyses were performed per Gustin et al (32). The AUC, maximum concentration ($C_{max}$), time to the $C_{max}$ ($t_{max}$), and elimination half-life ($t_{1/2}$) were calculated with Kinetica (version 5.0; ThermoFisher Scientific, Billerica, MA).

Samples for lycopene analysis were extracted in duplicate, and AMS analysis of fractions from each replicate, along with total $^{14}C$ analyses, were analyzed in triplicate. When appropriate, analytic data were reported as means (±SDs). No statistical comparisons were performed because only 2 subjects were studied.

# RESULTS

## Prestudy

Three of the 4 subjects in the prestudy had an increase (>100%) in lycopene in the plasma TRL fraction. The baseline plasma sample for the fourth subject was visibly fatty, and had high baseline TRL lycopene indicating that the subject had not fasted as requested and therefore, was not considered for the study. Two subjects were selected for participation in the study. Baseline characteristics of the subjects are presented in **Table 1**.

## AMS analysis of $^{14}C$ in plasma, whole blood, skin, expired air carbon dioxide, and urine

$^{14}C$ in plasma appeared rapidly and was present even at 30 min, with a $C_{max}$ of 153–177 Bq/L, $t_{max}$ of 3–6 h, and $t_{1/2}$ of 380–899 h (**Table 2**; **Figure 1**). Generally, pharmacokinetic variables measured for total $^{14}C$ in plasma were similar for both AMS instruments used. $^{14}C$ from the oral lycopene dose was still measurable in plasma 42 d after intake with both AMS instruments, although below the limit of quantification for the NSI-MIT AMS instrument. $^{14}C$ measured in lycopene accounted

**TABLE 1**
Baseline characteristics of the 2 subjects at the start of the study[1]

| | Age | BMI | Fasting plasma lycopene | Fasting plasma TRL lycopene | Total cholesterol | HDL cholesterol | LDL cholesterol | Triglycerides |
|---|---|---|---|---|---|---|---|---|
| | *y* | *kg/m²* | *μmol/L* | *μmol/mmol TG* | *mmol/L* | *mmol/L* | *mmol/L* | *mmol/L* |
| Subject 1 | 40 | 26.2 | 0.147 | 0.15 | 4.59 | 1.50 | 2.95 | 0.84 |
| Subject 2 | 60 | 26.7 | 0.237 | 0.16 | 5.10 | 1.12 | 3.57 | 1.49 |

[1] TRL, triacylglycerol-rich lipoprotein; TG, triglycerides.

for 6 and 10% of the total $^{14}C$ AUC and between 10% and 30% of the total $^{14}C$ measured in plasma for subjects 1 and 2, respectively.

$^{14}C$ could be quantitatively measured in urine by AMS for ≤14 d and detected ≤21 d after dosing, with the peak excretion of $^{14}C$ from lycopene occurring around 24 h (**Figure 2**). The average $C_{max}$, $t_{max}$, $t_{1/2}$, and $AUC_{0–1008\ h}$ were 2138 Bq, 24 h, 16.25 h, and 5,588,965 Bq · h, respectively. The recovery of $^{14}C$ in urine was 19% for subject 1 and 17% for subject 2.

$^{14}C$ in breath carbon dioxide could be detected 4 h after dosing, with values that fell below detection limits at 144 h (**Figure 3**). The average $C_{max}$, $t_{max}$, $t_{1/2}$, and $AUC_{0–144\ h}$ were 60 Bq/h, 4 h, 27.8 h, and 932 Bq · h, respectively. The recovery of $^{14}C$ in breath carbon dioxide was 3.6% and 2.4% for subjects 1 and 2, respectively.

An increase in $^{14}C$ could be measured in skin biopsies after 4 d and remained elevated over the baseline sample after 42 d (**Figure 4**), with the $C_{max}$ observed at 4–7 d. This amount of $^{14}C$ corresponded to an increase of ≈2.1 fmol $^{14}C$-lycopene/g skin at 144 h, with the assumption that all $^{14}C$ measured in skin was lycopene. The use of the same ratio of intake to response in skin for a dose of 10 mg lycopene would lead to an increase of 4.3 pmol lycopene/g skin.

### Analysis of lycopene isomers in plasma and TRLs

The analysis of isolated lycopene isomers in plasma and TRL fractions indicated that all-*trans* $^{14}C$-lycopene was converted to *cis* isomers because $^{14}C$ was present in 9-, 13-, and 15-*cis* fractions, although these isomers were not present in the lycopene fed to subjects. 5-*cis* Lycopene was also a major $^{14}C$-lycopene isomer in plasma (on average, 34% of total $^{14}C$-lycopene) when only 8% of the fed $^{14}C$-lycopene was present as this isomer. Between 3 and 96 h, ≈50% of the $^{14}C$ in lycopene was present as all-*trans* isomer in both subjects. At 144 and 336 h in one subject, this amount fell to 40% of the total $^{14}C$-lycopene, whereas for the second subject, the $^{14}C$ in all-lycopene isomers was below the limit of quantification after 96 h. In both subjects, the amount of $^{14}C$ in 13- + 15-*cis* isomers increased over time, with the average percentage of 4.3% between 3 and 24 h and 12.7% between 48 and 336 h.

The $t_{max}$ for all unlabeled lycopene isomers was higher (12 h) than those measured as $^{14}C$-lycopene, whereas the $t_{max}$ and $t_{1/2}$ of total $^{14}C$ were shorter than both unlabeled and $^{14}C$-lycopene (Table 2). The $^{14}C$ specifically detected as lycopene by HPLC separation and offline AMS detection accounted for 10–30% of the total $^{14}C$ detected at the same time points in plasma.

The pattern of lycopene isomers in the TRL fraction differed between the 2 subjects, with one subject always having >50% *cis* isomers in the TRL between 3 and 96 h, whereas between 3 and 12 h, the other subject had <40% *cis* isomers in their TRL fraction, which increased to >50% between 24 and 48 h (**Table 3**). Both subjects had similar total $^{14}C$-lycopene and total lycopene in their TRL fractions. The plasma unlabeled lycopene-isomer distribution did not change during the study for either subject.

### Urinary lycopene metabolites

The HPLC analysis of deconjugated urinary metabolites after the $^{14}C$-lycopene dose showed 2 peaks with $^{14}C$ above background amounts (**Figure 5**). The first peak occurred between 2 and 5 min with minimal retention on a $C_{18}$ column with 100%

**TABLE 2**
Noncompartmental pharmacokinetic variables for total $^{14}C$, $^{14}C$-lycopene, and unlabeled lycopene in plasma in 2 subjects (S1 and S2) after a single dose of $^{14}C$-labeled lycopene[1]

| | $C_{max}$ | | $t_{max}$ | | $t_{1/2}$ | | $AUC_{0–last\ sample}$ | | $C_{max}$ | | $AUC_{0–336\ h}$ | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | S1 | S2 | S1 | S2 | S1 | S2 | S1 | S2 | S1 | S2 | S1 | S2 |
| | *Bq/L* | | *h* | | *h* | | *Bq/L · h* | | *μmol/L* | | *μmol/h* | |
| Total $^{14}C$ | | | | | | | | | | | | |
| Total plasma $^{14}C$[2] | 153 | 172 | 6 | 4 | 380 | 514 | 45,311 | 39,999 | — | — | — | — |
| Total plasma $^{14}C$[3] | 177 | 174 | 5 | 3 | —[4] | 899 | 53,628 | 45,763 | — | — | — | — |
| $^{14}C$-Lycopene | | | | | | | | | | | | |
| Total plasma lycopene | 43.8 | 47 | 6 | 6 | 131 | 110 | 4494 | 2260 | — | — | — | — |
| All-*trans* lycopene | 17.3 | 19.9 | 6 | 6 | 115 | 99.6 | 2018 | 1178 | — | — | — | — |
| 5-*cis* Lycopene | 21.6 | 19.9 | 6 | 6 | 159 | 69.8 | 1546 | 622 | — | — | — | — |
| 9-*cis* Lycopene | 0.59 | 0.60 | 6 | 6 | 249 | —[4] | 49.9 | 25.6 | — | — | — | — |
| 13- and 15-*cis* Lycopene | 2.24 | 3.81 | 144 | 72 | —[4] | —[4] | 507 | 244 | — | — | — | — |
| Other lycopene isomers | 2.31 | 3.62 | 6 | 6 | 147 | —[4] | 362 | 176 | — | — | — | — |
| Unlabeled lycopene | | | | | | | | | | | | |
| Total plasma lycopene | — | — | 12 | 12 | —[4] | 956 | — | — | 1.01 | 1.26 | 31,124 | 36,279 |
| All-*trans* lycopene | — | — | 12 | 12 | —[4] | 1264 | — | — | 0.582 | 0.757 | 17,815 | 21,420 |
| 5-*cis* Lycopene | — | — | 12 | 12 | —[4] | 981 | — | — | 0.153 | 0.166 | 5131 | 5232 |
| 9-*cis* Lycopene | — | — | 12 | 12 | —[4] | 776 | — | — | 0.061 | 0.068 | 1820 | 1943 |
| 13- and 15-*cis* Lycopene | — | — | 12 | 12 | —[4] | 391 | — | — | 0.079 | 0.112 | 2390 | 2751 |
| Other lycopene isomers | — | — | 12 | 12 | —[4] | 825 | — | — | 0.132 | 0.159 | 3975 | 4924 |

[1] $C_{max}$, maximum concentration; $t_{max}$, time to the maximum concentration; $t_{1/2}$, elimination half-life; AUC, area under the curve; —, no value was calculated for this parameter.
[2] Obtained by using the MICADAS accelerator mass spectrometry system (Swiss Federal Institute of Technology Zürich, Zurich, Switzerland).
[3] Obtained by using the $^{14}C$ counting accelerator mass spectrometry system (Massachusetts Institute of Technology, Cambridge, MA)
[4] Not possible to calculate because of the shape of the curve.

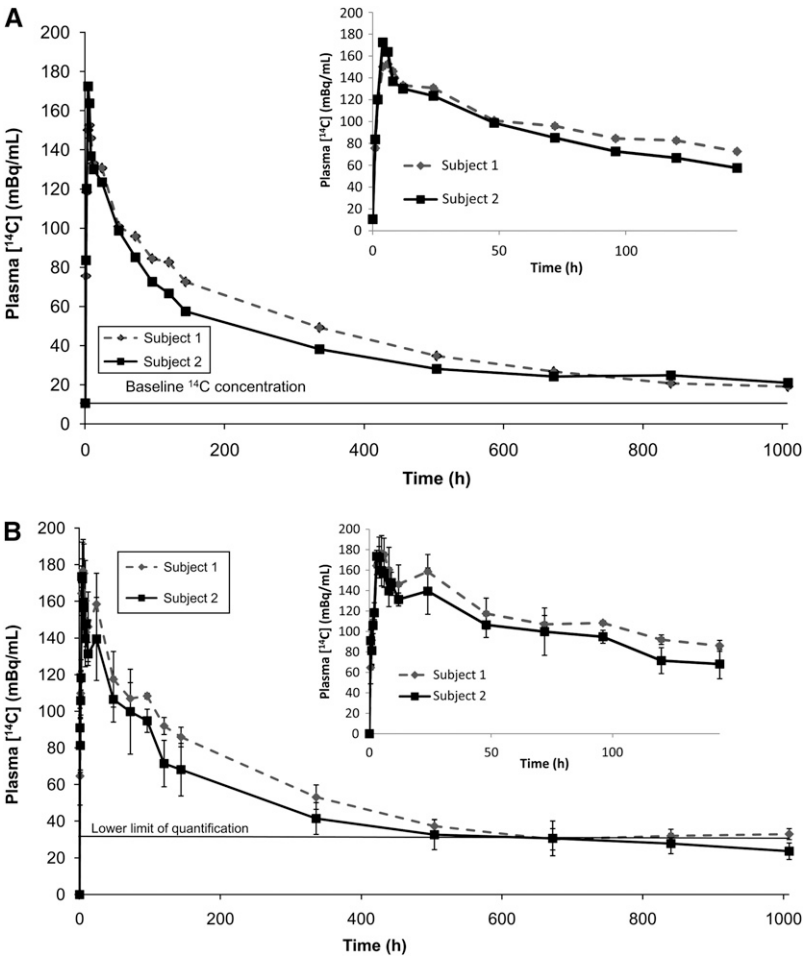



**FIGURE 1.** Detection of $^{14}C$ in plasma by using isotope-ratio (A) and $^{14}C$-counting (B) accelerator mass spectrometry analysis after a single dose of 30 kBq $^{14}C$-lycopene. The inset graphs show the curves between 0 and 144 h. Error bars represent SD from triplicate measurements. Isotope-ratio measurements (MICADAS instrument; ETH Zurich, Zurich, Switzerland) typically have a precision of ≈1%, and thus corresponding error bars are not visible.

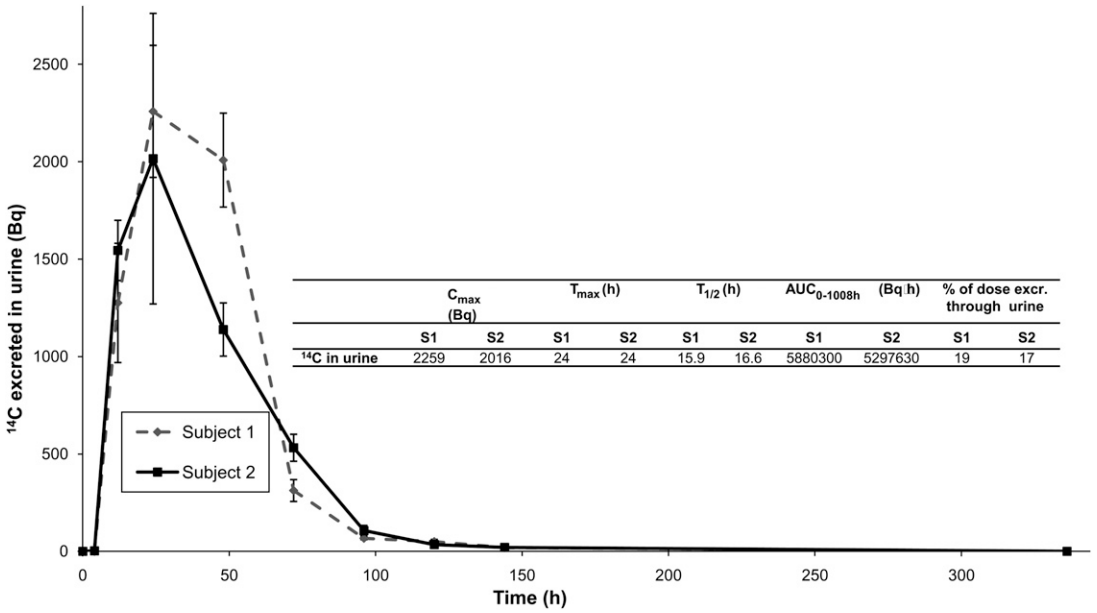
water, which indicated that these metabolites were highly polar and probably had a low molecular weight. A second peak was observed at 22–23 min, which indicated more hydrophobic metabolites. The analysis of these fractions by full-scan nanospray mass spectrometry and MS/MS to confirm the presence of known or potential $\beta$-oxidation metabolite masses did not provide or confirm that previously published or potential metabolites were present.

## DISCUSSION

Although many studies have examined at the bioavailability of lycopene, particularly by focusing on the differences between various sources of lycopene (eg, natural compared with synthetic) and its metabolism, it has remained difficult to absolutely prove the fate of oral lycopene in humans beyond the appearance in plasma. In this study, we used AMS to show the first in-human proof that oral all-*trans* lycopene was converted into *cis* isomers after ingestion, lycopene and/or postabsorption lycopene metabolites were transported to skin, lycopene was probably, at least in part, metabolized by $\beta$-oxidation into carbon dioxide, and polar metabolites were excreted in urine.

Both $^{14}C$-lycopene and unlabeled lycopene in plasma followed first-order kinetics overall. Absolute pharmacokinetic results generally differed from previous studies, although the $C_{max}$ was similar to that in a study with a similar study design and dose of lycopene (32) with the diverse range of study designs, doses, and matrices used being the likely reason for differences in lycopene kinetics (47–49). The finding of $^{14}C$ from lycopene in plasma 1004 h after the dose was unexpected, although there was evidence for a slow-turnover tissue compartment that may have explained this finding (50). Pharmacokinetic variables from the TRL-rich fraction of plasma also differed between $^{14}C$- and unlabeled-lycopene, although this result was less conclusive because the baseline for unlabeled lycopene was not reached in one subject.

The presence of mainly *cis* rather than all-*trans* lycopene isomers in plasma and tissue was suggested to be due to the preferential absorption of *cis*-lycopene isomers and/or isomerization of all-*trans* lycopene in the intestinal lumen (12, 13), enterocytes (15), or liver (14). The results from this study supported the idea that all-*trans* lycopene is extensively isomerized in vivo because labeled 5-, 9-, 13-, and 15-*cis* lycopene isomers were detected in plasma, even though the starting $^{14}C$-labeled lycopene was 92% all-*trans* and 8% 5-*cis* isomers. Although these results did not rule out the possibility that *cis* lycopene was preferentially absorbed, the results provided further evidence



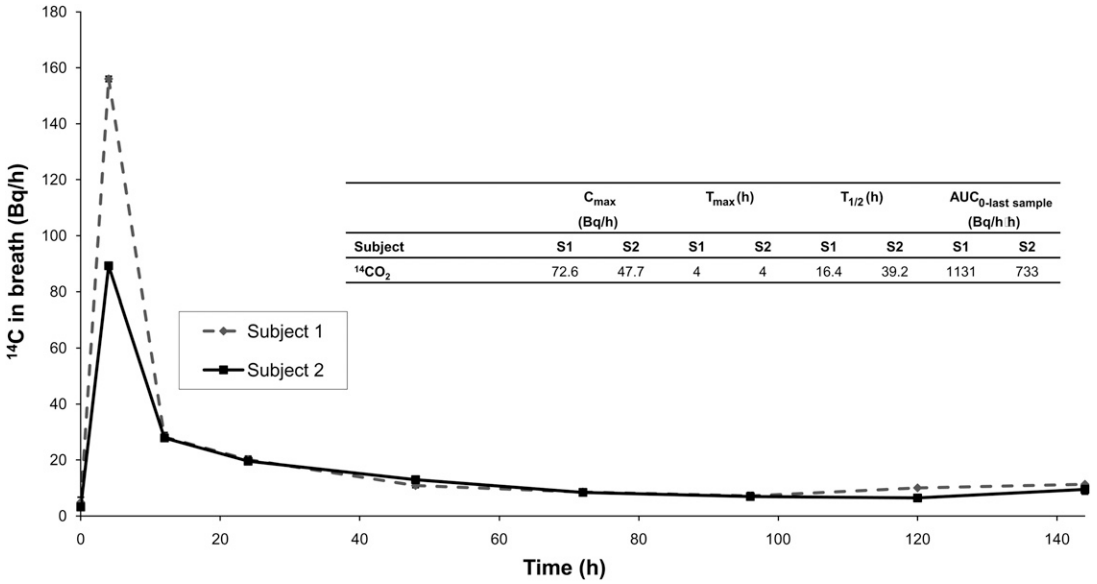

| | $C_{max}$ (Bq) | | $T_{max}$ (h) | | $T_{1/2}$ (h) | | $AUC_{0\text{-}1008h}$ (Bq·h) | | % of dose excr. through urine | |
|---|---|---|---|---|---|---|---|---|---|---|
| | S1 | S2 | S1 | S2 | S1 | S2 | S1 | S2 | S1 | S2 |
| $^{14}$C in urine | 2259 | 2016 | 24 | 24 | 15.9 | 16.6 | 5880300 | 5297630 | 19 | 17 |

**FIGURE 2.** Detection of $^{14}$C in urine in 2 subjects (S1 and S2) after a single dose of $^{14}$C-labeled lycopene, measured by using $^{14}$C-counting accelerator mass spectrometry. All $^{14}$C in urine was assumed to be in the form of lycopene metabolites. Error bars represent SD from triplicate measurements. $C_{max}$, maximum concentration; $T_{max}$, time to the $C_{max}$; $T_{1/2}$, elimination half-life; AUC, area under the curve; excr., excreted.

that the in vivo isomerization of all-*trans* lycopene occurs in humans, most notably to 13- and 15-*cis* isomers.

In rats, ≈55% of an absorbed $^{14}$C-lycopene dose was present in tissues in the form of undefined polar metabolites, and ≤92% of an absorbed $^{14}$C-lycopene dose was present in some tissues after 3 h, which suggested that lycopene was extensively metabolized (36) and possibly explained a large proportion of the 70–90% difference between total $^{14}$C and $^{14}$C-lycopene observed in this study. The shorter kinetics for total $^{14}$C compared with $^{14}$C-lycopene suggested that this metabolism occurred rapidly. Some losses may have occurred during the extraction and analysis, although the recovery with pure $^{14}$C-lycopene on the HPLC was determined to be 90%, and thus, with the assumption of a similar recovery for other isomers (35), the analytic error unlikely explained the observed difference.

Approximately 18% of the total $^{14}$C dose was excreted via the urine in this study. In studies in rats, this figure has been much lower (ie, ≈2% of the total dose over 168 h) (36). To our knowledge, no previous studies have reported the presence of lycopene metabolites in human urine, and the results obtained confirmed that lycopene was metabolized to polar metabolites in mammals (36, 37). Given the difference between total $^{14}$C and




| | $C_{max}$ (Bq/h) | | $T_{max}$ (h) | | $T_{1/2}$ (h) | | $AUC_{0\text{-last sample}}$ (Bq/h·h) | |
|---|---|---|---|---|---|---|---|---|
| Subject | S1 | S2 | S1 | S2 | S1 | S2 | S1 | S2 |
| $^{14}CO_2$ | 72.6 | 47.7 | 4 | 4 | 16.4 | 39.2 | 1131 | 733 |

**FIGURE 3.** Detection of $^{14}$C in breath samples as $^{14}CO_2$ in 2 subjects (S1 and S2) after a single dose of $^{14}$C-labeled lycopene measured by using $^{14}$C-counting accelerator mass spectrometry. Error bars that represented SDs from triplicate measurements were not visible due to the low relative error. $C_{max}$, maximum concentration; $T_{max}$, time to the $C_{max}$; $T_{1/2}$, elimination half-life; AUC, area under the curve.

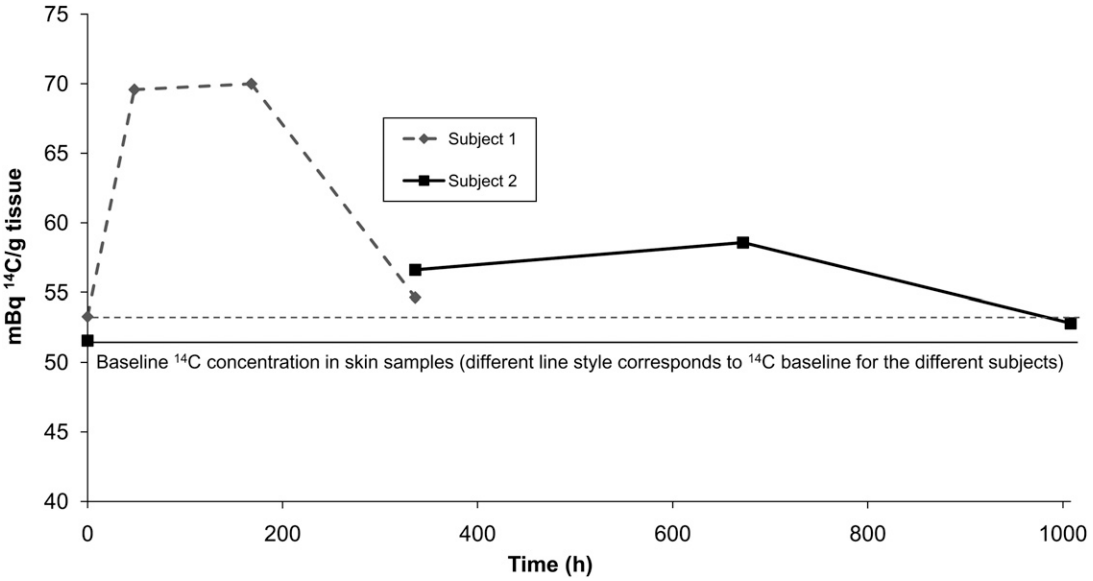



**FIGURE 4.** Detection of $^{14}C$ in skin biopsy samples in 2 subjects after a single dose of 30 kBq $^{14}C$-labeled lycopene measured by using isotope-ratio accelerator mass spectrometry. The limited number of biopsies that could be taken from each subject precluded the sampling of each subject at all time points. Error bars that represented SDs from triplicate measurements were not visible due to the low relative error.

$^{14}C$-lycopene in plasma and the rapid peak of $^{14}CO_2$ in expired breath (4 h), it appeared that at least part of the absorbed lycopene was rapidly metabolized. Several metabolite products of lycopene have been proposed and detected in plasma, with some because of the oxidation of lycopene [2,6-cyclolycopene-1,5-diol A (51)], as well as products from the eccentric cleavage of acetate from lycopene (apo-lycopenals) (52). The overall dose of lycopene fed in this study (10 mg) was relatively low, and we did not expect to see large peaks in urine derived from lycopene metabolites. The separation by one HPLC step was not sufficient to obtain a semipure fraction because of the number of highly polar metabolites in urine. Because the $^{14}C$ enrichment of the urine samples was low, the detection of purified metabolites after repeated purification steps with LSC would not have been possible. In rat studies that used $^{14}C$-lycopene to study the lycopene distribution and metabolism, metabolites present in tissues were apparently mostly highly polar metabolites (36, 37), which fit with finding the major peak of $^{14}C$ extracted from urine eluting in water. The acetate cleavage of lycopene via $\beta$-oxidation was the most likely metabolic route because of the finding of lycopenals in plasma (52) and the detection of a rapid peak of breath $^{14}CO_2$ in this study. The future discovery of human lycopene metabolites is of importance because several studies attributed biological activities to proposed metabolites (53).

The finding of elevated $^{14}C$ concentrations in skin indicated that lycopene or lycopene metabolites reached the skin after a single oral dose. Other studies that used unlabeled lycopene have shown that chronic supplementation with lycopene did elevate skin lycopene concentrations (54), and lycopene contributed to skin color and protection against skin photodamage (29, 55). Because skin biopsies were taken from the lower

**TABLE 3**
Noncompartmental pharmacokinetic variables for $^{14}C$-lycopene and unlabeled lycopene in the plasma triacylglycerol-rich lipoprotein (TRL) fraction in 2 subjects (S1 and S2) after a single dose of $^{14}C$-labeled lycopene[1]

| | $C_{max}$ | | $t_{max}$ | | $t_{1/2}$ | | $AUC_{0–96\ h}$ | | $C_{max}$ | | $AUC_{0–96\ h}$ | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | S1 | S2 | S1 | S2 | S1 | S2 | S1 | S2 | S1 | S2 | S1 | S2 |
| | *mBq/mmol TG* | | *h* | | *h* | | *mBq/mmol TG · h* | | *μmol/mmol TG* | | *μmol/mmol TG · h* | |
| $^{14}C$-Lycopene | | | | | | | | | | | | |
| Total TRL lycopene | 17.5 | 10.0 | 3 | 3 | 299 | 614 | 525 | 601 | — | — | — | — |
| All-*trans* lycopene | 5.96 | 7.11 | 3 | 3 | 111 | —[2] | 164 | 303 | — | — | — | — |
| 5-*cis* Lycopene | 8.15 | 2.84 | 3 | 24 | 190 | 124 | 218 | 154 | — | — | — | — |
| Other lycopene isomers | —[2] | 1.99 | —[2] | 9 | —[2] | 78.5 | 140 | 135 | — | — | — | — |
| Unlabeled lycopene | | | | | | | | | | | | |
| Total TRL lycopene | —[2] | 1.85 | —[2] | 9 | —[2] | —[2] | 128 | 113 | —[2] | 1.85 | 128 | 113 |
| All-*trans* lycopene | —[2] | 1.19 | —[2] | 9 | —[2] | 166 | 65.5 | 62.6 | —[2] | 1.19 | 65.5 | 62.6 |
| 5-*cis* Lycopene | —[2] | 0.73 | —[2] | 0 | —[2] | —[2] | 63.5 | 50.6 | —[2] | 0.73 | 63.5 | 50.6 |
| Other lycopene isomers | —[2] | —[2] | —[2] | —[2] | —[2] | —[2] | —[2] | —[2] | —[2] | —[2] | —[2] | —[2] |

[1] $C_{max}$, maximum concentration; $t_{max}$, time to the maximum concentration; $t_{1/2}$, elimination half-life; AUC, area under the curve; TG, triglycerides.
[2] Not possible to calculate because of the shape of the curve.

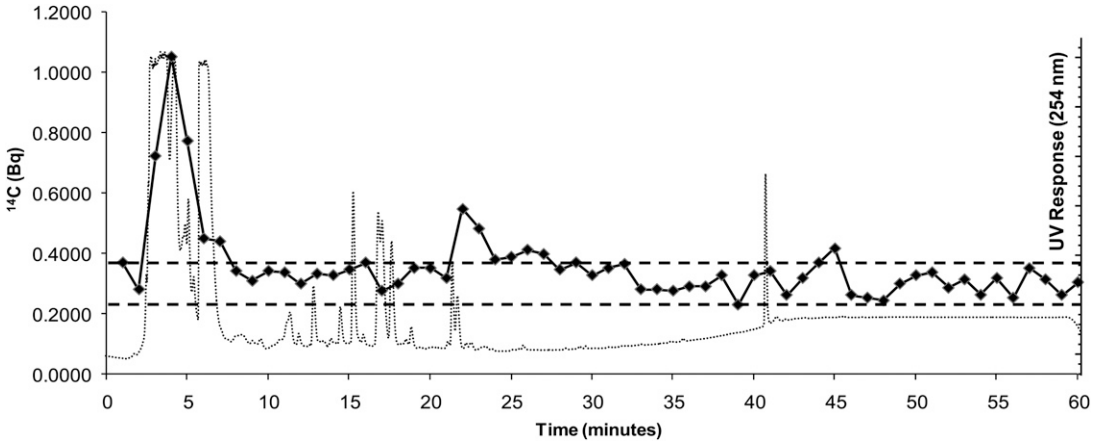



**FIGURE 5.** Representative chromatogram of the detection of $^{14}$C in an extract of deconjugated urine metabolites 12 h after a single dose of $^{14}$C-lycopene. The dotted line is the ultraviolet detector response at 254 nm, whereas the solid line is the amount of $^{14}$C detected in fractions collected every 30 s. The 2 dashed lines are the range for background $^{14}$C. The HPLC gradient was from 100% water through to 100% methanol. Two major peaks of $^{14}$C were observed at 5 and 22.5 min. The $^{14}$C measurements were made by using liquid scintillation counting. UV, ultraviolet.

lumbar region, which is an area that is generally not exposed to sunlight, it is probable that concentrations would have been higher had biopsies been taken in other regions such as the forehead or hand (54), although this was not practicable for ethical and cosmetic purposes. Skin concentrations of lycopene have been reported to be between 0.13 and 0.22 pmol/mg wet weight (30, 56–58), and supplementation with lycopene for up to 7 weeks has been shown to increase tissue skin lycopene concentrations in humans by 150% (59), and thus, it is probable that the $^{14}$C detected in skin biopsy samples was at least partly present as lycopene.

To our knowledge, this study is the first in which $^{14}$C-counting and IR AMS instruments were used to analyze a common sample set. Results from measurements of plasma with both instruments were comparable, although the greater sensitivity of the IR AMS instrument meant that measurements at 42 d were still well above the lower limit of quantification (LLOQ), whereas these measurements were below the LLOQ (but above the lower limit of detection) for the $^{14}$C-counting AMS instrument. Results for the calculated pharmacokinetic variables were similar; between-instrument differences were no greater than the difference between the 2 subjects. These results highlight the comparability of the 2 types of instruments for measurements over the same range of $^{14}$C. For most samples in this study, the sensitivity of the $^{14}$C-counting AMS instrument was sufficient, which meant that it was possible to take advantage of the higher throughput and less sample preparation required for this type of AMS. For tissue samples, time points distant to the time of dosing, and other biological samples in which the dilution of the radioisotope by the abundance of stable isotope in the sample pushed the IR closer to the background $^{14}$C, the greater IR precision of IR-AMS was an advantage. These results suggested that the 2 types of AMS instruments can be considered complimentary in the context of this type of research study.

With the use of an appropriate AMS instrumentation, it was possible to successfully follow the fate of a microdose of $^{14}$C-lycopene in humans, even in tissue samples (skin). This underlined the potential of this instrumentation in further micronutrient research (24). Furthermore, as the size (60), sensitivity (61) and hyphenation (62) of AMS instruments are improved, the possibilities for application of AMS in nutrition research will expand.

In conclusion, this work presents the first use of AMS applied to measuring lycopene kinetics in humans and demonstrates the power of this technique to understand what happens in the body to compounds that are present at low concentrations in food. From this study, it is evident that lycopene was, to some extent, isomerized after ingestion and is rapidly metabolized. Lycopene or its metabolites are transported to skin and may remain there for several days before being turned over. AMS has an enormous potential for furthering the knowledge of the bioavailability of nutrient compounds, especially with the availability of smaller and dedicated bio-AMS instruments.

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The authors' responsibilities were as follows—GW, SK, and LBF: conceived the study, MR, GW, and ABR: designed the study, KW and RR: provided and prepared essential material; LTV and JR: performed the clinical trial; ABR, AB, HAS, TS-K, RGL, PLS, SRT, and PAG: performed analyses; ME and ILFN: performed data analyses; and ABR and LTV: wrote the manuscript. All authors: read and approved the final manuscript. ABR, AB, PAG, ME, ILFN, SK, MR, LBF, and GW are employees of Nestlé, which is a food company that sells products enriched in lycopene. KW and RR are employees of DSM, which is a supplier of nutrient ingredients, including lycopene. Nestlé Research Center employees were involved in the instigation, design, execution, analysis, and interpretation of the results. None of the other authors declared a conflict of interest concerning the results of this study.

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