



The Effect of an Iron Supplement on Lycopene Metabolism and Absorption During Digestion in Healthy Humans

Rachel Kopec, Catherine Caris-Veyrat, Marion Nowicki, Jean-Paul Bernard, Sophie Morange, Chureeporn Chitchumroonchokchai, Béatrice Gleize, Patrick Borel, Jean-paul Bernard

► To cite this version:

Rachel Kopec, Catherine Caris-Veyrat, Marion Nowicki, Jean-Paul Bernard, Sophie Morange, et al.. The Effect of an Iron Supplement on Lycopene Metabolism and Absorption During Digestion in Healthy Humans. *Molecular Nutrition and Food Research*, 2019, 63 (22), pp.1900644. 10.1002/mnfr.201900644 . hal-02487069

HAL Id: hal-02487069

<https://amu.hal.science/hal-02487069>

Submitted on 14 Sep 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

The effect of an iron supplement on lycopene metabolism and absorption during digestion in healthy humans

Rachel E. Kopec^{1,2*}, Catherine Caris-Veyrat¹, Marion Nowicki³, Jean-Paul Bernard⁴, Sophie Morange⁴, Chureeporn Chitchumroonchokchai², Beatrice Gleize¹, Patrick Borel³

¹INRA UMR408, University of Avignon, Avignon, France

²Division of Human Nutrition, The Ohio State University, Columbus, Ohio, U.S.A.

³INRA, INSERM, Aix Marseille Univ, C2VN, Marseille, France

⁴Hôpital de la Conception, Marseille, France

***To whom correspondence and request for reprints should be addressed:**

Dr. Rachel Kopec, The Ohio State University, 1787 Neil Ave., Columbus, OH, 43210

email:kopec.4@osu.edu, phone: +(1).614.688.0954

Keywords: carotenoid, apo-lycopenals/apo-lycopenoids, gastrointestinal lumen, ferrous sulfate, bioavailability

Abbreviations: AUC = Area under the time-versus-concentration curve, BCO2 = β -carotene oxygenase 2, DCM = dichloromethane, EDTA = ethylenediaminetetraacetic acid, LC-MS/MS = high pressure liquid chromatography-tandem mass spectrometry, methanol = MeOH, methyl *tert*-butyl ether = MTBE, TRL = triglyceride-rich lipoprotein fraction

ABSTRACT

Scope: To investigate the formation and absorption of lycopene (LYC) metabolites in the human upper gastrointestinal lumen, in the absence and presence of iron.

Methods: Healthy males (n=7) consumed test meals delivering ~22 mg LYC + ~0.3 mg apo-lycopenals from oleoresin without (-FeSO₄) and with ferrous sulfate (160 mg, +FeSO₄).

Subjects were intubated with a naso-gastric/naso-duodenal tube. Digesta, blood plasma, and the triglyceride-rich lipoprotein (TRL) fractions of plasma were analyzed using LC-MS/MS, to measure lycopene and apo-lycopenoids.

Results: Digesta LYC concentrations increased with time ($P = 1.2 \times 10^{-7}$), decreased with time*iron ($P = 1.1 \times 10^{-5}$), and remained ~200x higher than apo-lycopenals/lycopenone. Digesta apo-8'-, -10'-, -12'-, -14'-, -15-lycopenal and apo-13-lycopenone concentrations increased with time ($P < 0.01$), apo-12'-, -14'-, -15-lycopenal, apo-13-lycopenone increased with iron ($P < 0.05$), and time*iron decreased apo-8'-, -10'-, -12'-, -14'-, -15-lycopenal, apo-13-lycopenone concentrations ($P < 0.01$). A 1.9-fold decrease in LYC TRL area-under-the-time-concentration-curve was observed after the test meal +FeSO₄ vs. the test meal -FeSO₄ ($P = 0.02$). Apo-lycopenals were detected in later TRL fractions. No apo-lycopenols or apo-lycopenoic acids were observed in any samples.

Conclusions: FeSO₄ reduces lycopene absorption. Apo-lycopenals appear to be absorbed from foods, and not made in significant quantities during digestion.

INTRODUCTION

Numerous studies, previously reviewed^[1], have associated increased lycopene (LYC) consumption with reduced chronic disease risk. However, only a small portion of LYC, the pigment conferring red hues to tomatoes, watermelon, and pink grapefruit, is absorbed by humans. Under even the most ideal meal conditions, not more than 23%-33.9% is taken up.^[2, 3] Low solubility resulting from its physical-chemical properties reduces absorption,^[4] and a portion may also be lost to enzymatic or chemical degradation during digestion. Trace metals in water or meal co-factors like iron, which remain active in the acidic environment of the stomach, have been shown to induce lipid and LYC oxidation and co-oxidation in some,^[5-9] but not all,^[10] *in vitro* models of digestive conditions. Likewise, body temperature, peristaltic gastric contractions, the presence of oxygen and lipid or peroxy radicals, and the shift from immiscible liquid phases to an emulsion system together favor LYC oxidation.^[11, 12] In studies showing oxidation under digestive conditions, the LYC is ultimately consumed by oxidative reactions,^[8, 13, 14] and metabolites are presumably formed in the process.

Limited LYC absorption also suggests that the portion remaining in the digestive tract could protect the gastrointestinal system from oxidative damage. Higher intakes of lycopene have been associated with a reduced fecal blood, mucus and pus in subjects with ulcerative colitis in remission.^[15] Likewise, animal studies of colitis have demonstrated a reduction in myeloperoxidase, a marker of colon inflammation, after lycopene administration both with^[16] or without^[17] iron. One plausible mechanism of action is that LYC acts directly to protect the gastrointestinal lumen. However, it is also possible that LYC is converted to bioactive metabolites^[18, 19] in the intestinal tract which confer protection.^[20, 21] Recombinant studies have demonstrated that recombinant β -carotene oxygenase 1 (BCO1) obtained from humans cleaves

LYC *in vitro*,^[22] and the enzyme β -carotene oxygenase 2 (BCO2) obtained from mouse and ferret has the ability to cleave LYC,^[23] with a preference for *cis*-LYC isomers.^[24] Its expression by human intestinal cells further supports the hypothesis that it may act during digestion.^[25] Indeed, LYC metabolites have been found circulating in human plasma,^[26–28] although the origin of these metabolites has not been established.

In vitro studies reveal ~20% loss of LYC over the digestive period,^[10, 29] regardless of presence or absence of iron. However, *in vivo* studies of the loss of LYC during digestion in the presence of dietary iron have not been performed. Likewise, the formation and potential absorption of LYC metabolites made during digestion in healthy humans has not been observed. Thus, the primary objective of this study was to follow LYC metabolism in the upper digestive tract of healthy humans, in the absence and presence of dietary iron. Postprandial concentrations of LYC and apo-lycopenoid metabolites (i.e. apo-lycopenals, apo-lycopenols, or apo-lycopenoic acids) in the gastrointestinal lumen and in the chylomicron-rich triglyceride-lipoprotein (TRL) fraction of blood (representing newly absorbed lipophilic analytes^[30]) were measured.

MATERIALS & METHODS

Chemicals

Food-grade oleoresin (4.5% lycopene by weight, product number 1EAA9272) was kindly provided by Dr. Simona Birtic of Naturex (Montfavet, France). The oleoresin was extracted from tomato and consisted of tomato phospholipids, in addition to minor amounts of tocopherols, phytoene and phytofluene. The isomer composition was 78% all-*trans*, 11% 5-*cis*, and 11% as other *cis*-isomers. MS grade methanol and LC grade methyl tert-butyl ether (MTBE) were purchased from Fisher Scientific (Illkirch, France). Dichloromethane (DCM) was purchased from Fisher Scientific (Pittsburgh, PA, USA). Double deionized water was produced by a Millipore Q-Plus. MS grade formic acid (>98% pure) was purchased from Sigma-Aldrich (Saint-Quentin-Fallavier, France). Apo-6'-, -8'-, and -12'-lycopenal standards were purchased from CaroteNature (Münsingen, Switzerland). Apo-10'-lycopenal, apo-10'-lycopenol, apo-10'-lycopenoic acid, apo-14'-lycopenal, apo-14'-lycopenol, apo-14'-lycopenoic acid, apo-11'-lycopenal standards were synthesized previously.^[31] The glycerol phosphate oxidase assay was from Abliance (Compiègne, France).

Sunflower oil, white granular sugar, and demineralized water were purchased at local supermarkets (Avignon and Marseille, France). Lecico, Inc. kindly donated the Ovolife IF 50 phospholipid (Hamburg, Germany), consisting of 50% w/w phospholipid derived from egg yolk. The ferrous sulfate (FeSO₄) tablet (Tardyferon®, 80 mg of iron per capsule) was purchased from a local pharmacy and powdered before blending with the test meal, described below.

Preparation of LYC test meals

The LYC-containing oleoresin was mixed with sunflower oil, aliquoted into amber glass ampoules (targeting 10 mg LYC + 0.25 mg apo-lycopenals/apo-lycopenone in 2.5 g total oil),

closed under argon, and stored under refrigeration (4° C) before test meal preparation. For iron-containing meals, Tardyferon® tablets were finely powdered and the equivalent of 2 tablets sealed in separate amber ampoules under argon.

Test and control meal preparations and macronutrient composition were previously published,^[32] with LYC replacing ¹³C β-carotene for this study. For test meals containing iron (i.e. +FeSO₄), just before serving, an ampoule of powdered Tardyferon® (delivering 160 mg Fe⁺²) was added, and the meal blended for an additional 30 s.

Because LYC crystals in the oleoresin complicated the aliquoting of LYC-rich oil into each ampoule, samples of each individual test meal (0.5 mL) were taken to measure the final concentration of LYC and apo-lycopenoids dosed (see **Table 1**).

Clinical Subjects and Experimental Design

The study was performed at the Clinical Investigation Center of the Hôpital de la Conception in Marseille, France. The regional Institutional Review Board Comité de Protection des Personnes Sud Méditerranée I, France (Protocol #2013-A01398-37) approved the study, which was in accordance with the Declaration of Helsinki of 1975 as revised in 1983. Further details are provided at clinicaltrials.gov, study #NCT03492593. Baseline characteristics of the subjects are provided in **Table 2**. Criteria of inclusion and exclusion match those published previously,^[32] with an additional exclusion criteria of LYC supplement consumption in the last 3 mo. Written, informed consent was obtained from all subjects before participation. Twelve healthy male subjects were screened for the study, with 9 subjects meeting the inclusion/exclusion criteria and randomly assigned to consume the test or control meals by the study coordinator (n = 7 for LYC-containing test meals, n=2 for control meals, i.e. the test meal with sunflower oil in place of the LYC-rich oleoresin). Sample size was estimated based on

previously published work of the study of carotenoids and vitamins A and E in the human digestive tract.^[33, 34]

Overnight fasted subjects arrived at the clinic, and a nasoduodenal tube was inserted and positioned in the antrum of the stomach or the inferior descending duodenum, as confirmed by X-ray. Afterward, a blood sample (0 h) was taken. Test meal subjects then consumed either the liquid test meal alone (i.e. -FeSO₄) or liquid test meal +FeSO₄. Likewise, control meal subjects consumed a liquid control meal -FeSO₄, or a liquid control meal +FeSO₄. Meals were drunk in 5 min. and chased with demineralized water (50 mL) used to rinse the meal preparation glassware. Samples of digesta (either gastric or duodenal) were taken as previously described^[32] at 30 min, 1, 2, 3, and 4 hours postprandially. Exact digesta sample volume was measured, to which an aqueous “inhibitor” solution (containing 0.5% pyrogallol and 0.7% EDTA) was added at half the volume. Samples were immediately vortexed, aliquoted, and stored at -80 °C. Plasma samples were taken at 0, 1, 2, 4, 5, 6, and 7 h, and TRL fractions were prepared as previously described.^[32] Samples were transported on dry ice between facilities, and held at -80 °C until extraction. Subjects consumed the same meal -FeSO₄ twice, and the same meal +FeSO₄ twice (once to obtain gastric digesta and a second time to obtain duodenal digesta, randomized for order). Because subjects were required to wait ≥ 2 weeks between the consumption of each meal, the total study period for each subject was ≥ 6 weeks.

Digesta, Plasma, and TRL extraction

Samples were thawed for 10 min in cold tap water, and extracted as previously described.^[32] Extracts were immediately dried under argon and stored for ≤ 2 days at -80 °C before LC-MS/MS analysis.

LC-MS/MS analyses

Extracts of digesta, plasma, TRL and test meal were redissolved in 1:1 MTBE/MeOH, and analyzed using the same LC-MS parameters described previously,[32] except for those noted below. The gradient method began with 0% B, holding 0% B for 1 min, increasing to 30% B over 4 min, increasing to 55% B over 3 min., increasing to 90% B over 7 min, increasing to 100% B over 2 min., holding at 100% B for 4 min, and returning and holding 0% B for 3 min. Quadrupole 1 resolution = 0.7 units, quadrupole 3 resolution = 2.0 units, for all precursor>product combinations monitored (provided in **Supplemental Table 1**).

Identities of apo-6'-, -8'-, -10'-, -12'-, -14'- and -11-lycopenal, apo-10'-, -14'- and apo-10'-, -14'-lycopenoic acid were confirmed with retention time and precursor > product ions coincident with authentic standards, and quantitated with external standard curves. The retention times, precursor ions, and product ions of apo-15-lycopenal and apo-13-lycopenone were determined using a LYC extract rich in apo-lycopenals, and quantitated using apo-14'-lycopenal equivalents. Putative retention times, precursor and product ions of the remaining apo-lycopenols and apo-lycopenoic acids *not* available as authentic standards were determined by studying ionization, fragmentation, and retention time behavior of the available acids and alcohols (see **Online Supplemental Material** and **Supplemental Table 1** for additional details of quantitation).

Caco-2 cell experiments

LYC was oxidized as previously described.^[35] Approximately 0.4 mg of the resulting apo-lycopenals/lycopenone mixture was solubilized in chloroform (0.5 mL) before incorporation into artificial mixed micelles composed of oleic acids, bile salts, α -tocopherol, and phosphatidylcholines, as previously described.^[36] The mixed micelles were diluted 1:4 with DMEM containing 500 μ mol/L phenol red, and the solution immediately incubated with Caco-2 cells (2 mL/well), as previously described.^[36]

Caco-2 human intestinal cells (HTB 37, passages 27-31) were grown in 6-well plates on membrane inserts for 21 days after reaching confluence. The freshly prepared DMEM containing the apo-lycopenals/lycopenone micelle solution was applied to the apical surface of the cells and incubated at 37 °C in an atmosphere of 5% CO₂ and 95% air for 7 h. Monolayer integrity was monitored by phenol red transfer from the apical to basolateral compartments, as detailed previously.^[37] After incubation, the apical and basolateral media, as well as the washed cell monolayer, were collected and frozen at -80 °C.

The apical and basolateral fractions were extracted as described in *Digesta, Plasma, and TRL extraction*. Cells were extracted by combining with an equal volume of MeOH and probe sonication (Microson XL-2000, Misonix, Farmingdale, NY) for 30 sec, placed on ice for 1 min, repeated twice more. Then DCM (4 parts) was added, followed by 30 sec of probe sonication and centrifugation (Allegra 6R, Beckman Coulter, Indianapolis IL) at 4 °C for 5 min at 162 x g. The lower DCM layer was transferred to a clean glass vial and the extraction repeated, with DCM layers combined. All extracts were dried under argon gas and analyzed immediately using the same LC-MS/MS method described on a Vanquish UHPLC interfaced with a Quantiva triple quadrupole (Thermo Fisher Scientific, Waltham, MA). Species were identified by comparison with an oxidized apo-lycopenal/lycopenone mixture.^[26]

Statistics

Analyses were performed using R, version 3.1.0.^[38] Visual inspection of residual plots confirmed assumptions of homoscedasticity and normality. LYC bioavailability in the TRL fraction was baseline-corrected before calculation of the area under the time-concentration curve (AUC) using trapezoidal approximation. To determine the effect of iron on LYC bioavailability within the same subject, AUC values of the test meal -FeSO₄ vs. the test meal +FeSO₄ were

compared using a paired t-test, with $P < 0.05$ considered statistically significant. Repeated measures ANOVA assessed the effect of time, iron, and time* iron on the percentage of all-*trans*-, 5-*cis*-, and other-*cis*-LYC isomers in the TRL fraction.^[10] Because of the absence of duodenal digesta samples in different subjects (due to inability to aspirate sample at that time point), a mixed model was chosen to determine the influence of digesta type, time, iron, and time*iron on metabolite formation. The lme4 package with mixed effects regression^[39] was used to determine the relationship between digesta type, time, iron, and time*iron on the percentage of all-*trans*-, 5-*cis*-, and other-*cis*-LYC to total LYC in digesta. The relationship between digesta type, time, iron, and time*iron on the absolute values of total LYC, LYC isomers, and apo-lycopenals/apo- lycopene in digesta was also determined using this approach. Fixed effects included sampling time (0, 0.5, 1, 2, 3, 4 h), digesta type (gastric or duodenal), and iron (+FeSO₄ or -FeSO₄) were considered, and the mixed effect time*iron was modeled as a new variable. Random effects included subject, for which individual intercepts were determined. The likelihood ratio test (LRT) of the full model was compared to the model minus the effect in question to understand the influence of the fixed effects on the goodness of fit of the model, with $P < 0.05$ statistically significant. To calculate percentage loss between the two meals, LYC digesta levels of the test meal -FeSO₄ vs. the test meal +FeSO₄ within the gastric and duodenal compartments were made using AUC comparisons ($P < 0.05$).

RESULTS

Subject Recruitment and Sample Collection

Supplemental Figure 1 provides an overview of subject randomization, sampling, and attrition.

LC-MS/MS method development

Details are provided in **Online Supplemental Material** and **Supplemental Figures 2, 3, and 4**. The method limit of detection (LOD), defined as signal/noise = 3, was ~0.4 fmol on column for apo-6', -10'-lycopenal, and ~2 fmol on column for apo-8', -12', -14'-lycopenal.

Digesta concentrations of LYC and apo-lycopenals/apo-lycopenone

Digesta concentrations of total LYC increased 4.5-fold in the test meal -FeSO₄ and remained the same in the test meal + FeSO₄, between initial concentration (t = 0) and after 4 h of digestion (**Figure 1A**). Over the course of 4 hours of digestion, the test meal +FeSO₄ resulted in 44% less LYC in the gastric compartment and 66% less LYC in the duodenal compartment, as compared to the test meal -FeSO₄ (as determined by comparing digesta AUC).

With regards to isomer composition, the test meal -FS delivered 84.4% of the LYC dose as all-*trans*-, 8.7% as 5-*cis*-, and 6.9% as other-*cis*-LYC. The test meal +FeSO₄ delivered 83.8% of the LYC dose as all-*trans*-, 7.9% as 5-*cis*-, and 8.3% as other-*cis*-LYC. There was no statistical significance between the LYC isomer composition between the two test meals, although the absolute level of LYC delivered was higher in the test meal -FeSO₄ as compared to the test meal +FeSO₄ (**Table 1**). There was no significant change in LYC isomer profile within a subject over time, regardless of the presence or absence of iron, or the digesta type sampled.

The test meal also delivered apo-lycopenals/apo-lycopenone, noted in **Table 1**. The test meal +FS delivered significantly more apo-10', -12', -14', -15-lycopenal and apo-13-

lycopenone as compared to the test meal $-\text{FeSO}_4$ ($P < 0.05$). **Figure 1A** shows the concentrations of total LYC and **Figure 1B-H** each of the apo-lycopenals/apo-lycopenone in the gastric and duodenal digesta samples after the test meal fed $-\text{FS}$ and $+\text{FS}$. A breakdown of digesta LYC by isomer type is shown in **Supplemental Figure 5**. The mixed modeling results are provided in **Table 3**. Across all subjects, time was positively associated with the digesta concentrations of total LYC, as well as the concentrations of each isomer individually. There was a non-significant trend of total LYC concentration being higher in gastric digesta relative to duodenal digesta. In contrast, the interaction factor of time*iron was significantly negatively associated with digesta total LYC concentrations and the concentrations of each individual isomer.

Digesta concentrations of apo-lycopenals/apo-lycopenone increased up to 9-fold more than initial test meal concentrations at their peak (**Figure 1B-H**), however they remained ~200 fold lower than LYC concentration in the same samples. A non-significant trend was seen for gastric digesta type positively associated with the concentrations of apo-8', -12'-lycopenal and apo-13-lycopenone (**Table 3**). The concentrations of digesta apo-8', -10', -12', -14', -15-lycopenal and apo-13-lycopenone were significantly increased with time. Iron was significantly associated with increased digesta concentrations of the shorter apo products, i.e. apo-12', -14', -15-lycopenal and apo-13-lycopenone. In contrast, the time*iron effect was significantly associated with decreased concentrations of apo-8', -10', -12', -14', -15-lycopenal and apo-13-lycopenone. Interestingly, no factors were significantly associated with digesta concentrations of apo-6'-lycopenal.

Plasma and TRL concentrations of LYC and apo-lycopenals/lycopenone

LYC was observed circulating in the fasted plasma of all subjects ($0.43 \pm 0.31 \mu\text{mol/L}$). Similarly, apo-lycopenals/apo-lycopenone, but no apo-lycopenols nor apo-lycopenoic acids, were detected in the plasma of control meal and test meal subjects, and concentrations remained stable over the 7 h after test-meal consumption. Mean concentrations at 0 h, i.e. after an overnight fast, for apo-6'-lycopenal = $0.53 \pm 0.57 \text{ nmol/L}$, apo-8'-lycopenal = $0.17 \pm 0.10 \text{ nmol/L}$, apo-10'-lycopenal = $0.34 \pm 0.38 \text{ nmol/L}$, apo-12'-lycopenal = $0.40 \pm 0.54 \text{ nmol/L}$, apo-14'-lycopenal = $0.74 \pm 0.76 \text{ nmol/L}$, and apo-13-lycopenone = $0.47 \pm 0.59 \text{ nmol/L}$. Apo-15-lycopenal was only consistently detectible in the plasma of 2 subjects. These levels remained constant throughout the study.

The AUC of total LYC observed over 7 hours in the TRL fraction after test meal -FeSO₄ consumption was on average $118 \pm 58.7 \text{ nmol}\cdot\text{h/L}$ plasma, and after test meal +FeSO₄ consumption was $62.9 \pm 20.2 \text{ nmol}\cdot\text{h/L}$ plasma (**Figure 2**). This ~1.9 fold lower AUC after test meal +FeSO₄ consumption was determined to be statistically significant using a paired t-test ($P = 0.0218$). It should also be noted that LYC was more bioavailable from the meal -FeSO₄ vs. the meal + FeSO₄ in 6 of 7 subjects tested. The LYC isomer profile remained fairly stable over 0-7 hours, with a non-significant trend towards an increase in all-*trans*-LYC and a decrease in other-*cis*-LYC over time ($P = 0.06$) in the TRL fraction of plasma (**Supplemental Figure 6**).

Apo-lycopenals/lycopenone were also detected in some of the TRL fractions of plasma in test meal subjects (but none observed in any TRL fractions of control meal subjects). **Figure 3** depicts an LC-MS/MS chromatogram of a separated TRL fraction extract at 6 h from one subject after consuming the test meal -FeSO₄. Apo-8'-, -10'-, -12'-, -and -14'-lycopenal were detectible in the TRL of all subjects at hours 5, 6, and 7, however only ~35% of the samples were above the quantitative signal to noise cutoff (i.e. signal to noise ≥ 10 , or $\geq \sim 67 \text{ pmol/L}$). Apo-6'-

lycopenal and apo-13-lycopenone were only detected in the TRL fraction a few subjects, and apo-15-lycopenal was only detected at a few time points in two subjects.

Apo-lycopenols and apo-lycopenoic acids were not observed in any of the digesta, plasma, or TRL extracts. The possibility that apo-lycopenals may be reduced and esterified to produce apo-lycopenyl esters circulating in plasma was also investigated using a mild saponification procedure to test plasma from 2 subjects. However, no apo-lycopenols were observed in these saponified plasma extracts.

Caco-2 cell experiments

The oxidized LYC mixture contained apo-8', -10', -12', -14', -15-lycopenal and apo-13-lycopenone, but no LYC or apo-6'-lycopenal remained after the reaction. The same metabolites were visible in the media applied to the apical membrane at 0 h and after 7 h of incubation (**Supplemental Figure 7**). In the cell and basolateral extracts, the same metabolites minus apo-10'-lycopenal were observed.

DISCUSSION

The metabolism of LYC in the upper digestive tract of healthy humans was observed, exploring the effects of digestion alone and for the first time digestion in the presence of the common dietary oxidant iron. Ferrous sulfate was chosen because it is prescribed as a supplemental source for those who are iron deficient,^[40] and a dose of 160 mg per day is in line with the 150-200 mg/day dose prescribed by medical professionals for these individuals.^[41] Likewise, consumption of inorganic iron from sources also rich in ascorbic acid (like fruits and vegetables) results in ferric iron being reduced to ferrous iron during digestion.^[42]

An initial decrease in LYC digesta concentrations from 0 h (i.e. test meal) to the first aspirated sample at 0.5 h was observed, as previously noted in a similar study with β -carotene,^[32] and is hypothesized to be a “dilution effect” as endogenous fluid is secreted into the digestive lumen. After this initial drop, increasing concentrations of LYC in the gastric compartment over time may be due to LYC floating with lipid droplets in the early stages of digestion,^[43] and better access to this lipid phase observed as the compartment is emptied. This result contrasts with a previous study where tomato puree LYC (10 mg) slowly decreased over 3 h of gastric digestion.^[33] Differences could be attributed to differences of LYC localization in the food matrix, with tomato puree LYC remaining in the food matrix and thus emptied regularly from the stomach into the duodenum^[33] as compared to LYC already dissolved in oil. Duodenal digesta LYC concentration also increased with time, similar to a previous study with ^{13}C β -carotene,^[32] and may have occurred as bigger lipid droplets are transferred to the duodenum at the end of the gastric emptying phase.

Over time, the test meal +FeSO₄ led to a significant decrease in digesta total LYC (and each isomer individually) relative to the test meal –FeSO₄. This result is in contrast to *in vitro*

studies where no change in the loss of LYC (-20%) was observed over 2-3 h, regardless of presence of Fe^{2+} .^[10, 29] At 2 h, the study herein observed a 15% loss in stomach and a 40% loss in the duodenum of LYC, after the $+\text{FeSO}_4$ as compared to the $-\text{FeSO}_4$ meal (**Figure 1A**), suggesting oxidation of LYC directly via Fe^{2+} , or co-oxidation with meal lipid, did occur in the human system. Besides oxidation, the Fe^{2+} physical-chemical properties as a divalent metal may also reduce LYC micellarization and uptake, as supported by an *in vitro* study where a dose-wise reduction in micellarization of LYC was observed with increasing concentrations up to 12.5 mmol Fe^{2+} /L digesta.^[44] Significant reductions were also observed in a human study when LYC was fed with Ca^{2+} , another divalent metal, plausibly due to change in zeta-potential absolute charge.^[45] Similarly, complexation of iron with free fatty acids and/or bile salts could also reduce micelle formation, and ultimately LYC uptake.^[44, 46] LYC bioavailability (as measured by TRL AUC) was significantly lower with the test meal $+\text{FeSO}_4$ as compared to the test meal $-\text{FeSO}_4$, further supporting the loss of LYC during digestion in the presence of iron. The isomer profile in the TRL had a non-significant trend towards an increase in all-*trans* LYC and decrease in other-*cis* LYC over time, confirming previous work demonstrating that the isomer profile is dependent on the profile of the test meal fed.^[2, 3, 47, 48]

With regard to digesta concentrations of apo-lycopenals/lycopenone, a presumed “dilution effect” was also observed between the test meal concentrations of apo-lycopenals and the 0.5 h digesta sampling. Time was positively associated with increasing apo-lycopenal/lycopenone concentrations for all except apo-6'-lycopenal in digesta, which matches the trend observed for LYC. Iron was expected to result in greater concentrations of all apo-lycopenal/lycopenone in the digesta due to increased oxidation of lycopene.^[11, 12] While iron increased concentrations in the initial test meal preparation (**Table 1**), iron was positively

correlated with only shorter chain apo-products in the digesta (i.e. apo-12'-, -14'-, -15-lycopenals and apo-13-lycopenone, which could be derived either from LYC or from apo-6'-, -8'-, or -10'-lycopenal). Conversely, time*iron was associated with decreased concentrations of all apo-lycopenals/lycopenone except apo-6'-lycopenal, as demonstrated both in **Figure 1** and **Table 1**. This loss in the digesta was most markedly observed in the concentration drop from 2 h to 4 h in the presence of iron, likely due to continued oxidation. Indeed, pre-formed apo-lycopenals inhibit lipid peroxidation induced by another form of dietary iron, metmyoglobin, and are likely destroyed in the process.^[6] Thus, apo-lycopenals may be transient metabolites of LYC when iron is present, as noted by their increase in the test meal prepared +FeSO₄ vs. -FeSO₄ (**Table 1**), but further as yet un-identified metabolites produced from their oxidation during digestion.

The concentrations of apo-lycopenals/lycopenone noted in blood plasma (both fasting and post-prandial) are on par with those reported previously.^[26] It was unanticipated that the source of LYC used for the test meal would be rich in apo-lycopenals, providing 10-100x more apo-lycopenals than that delivered from a raw tomato or tomato-product,^[26] and providing an unexpected opportunity to study whether apo-lycopenals are absorbed directly. Apo-lycopenals/lycopenone were observed in later TRL fractions, regardless of test meal consumption -FeSO₄ or +FeSO₄. These results were surprising considering the lack of ¹³C apo-lycopenals reported in blood plasma after the feeding of a single 12 mg dose of ¹³C LYC in oil (no ¹³C apo-lycopenals were reported in the dose).^[2] A study by Cooperstone et al. detected apo-6'-apo-lycopenal in 29 subjects, of which it was quantifiable in 14 (averaging 0.82 nmol/L) after 4 weeks of daily tomato juice consumption, but no other apo-lycopenals/lycopenone were observed.^[49] The study reported herein fed 3.5 times more apo-6'-lycopenal, an equivalent dose of apo-8'-lycopenal, and 3-40 times more of the remaining apo-lycopenals/lycopenone, all

delivered with a large quantity of lipid. Furthermore, the LOD is estimated at ~2-20 pmol apo-lycopenals/lycopenone per L of plasma, as compared to an LOD of 100 pmol/L plasma estimated by Cooperstone et al. Thus, the difference in dose and instrument sensitivity are the most plausible reasons for the detection of apo-lycopenals in the TRL of these study subjects.

The parent Caco-2 cell line does not express BCO1,^[50] and has not been reported to express BCO2. Likewise, mammals do not synthesize lycopene (it can only be obtained from the diet).^[51] Thus, the uptake and basolateral secretion of apo-lycopenals observed in the Caco-2 experiments is due to absorption, and not from further BCO1 or BCO2 cleavage during incubation. These results further support the hypothesis that these products are being absorbed from the test meal. Taken together with the human results, evidence collectively suggests that the bulk of apo-lycopenals observed in circulation originate from foods.

One limitation of this study was the lack of labeled apo-lycopenals, which would more conclusively prove their absorption from the digestive tract. Future studies feeding a labeled dose ≥ 1 mg of each of the apo-lycopenal/lycopenone species would better capture the kinetics of uptake and transport, and could provide TRL concentrations above the limit of quantitation. Also, this study was conducted in healthy male subjects, and differences could be observed in other demographic groups. Finally, only apo-lycopenoids (i.e. < 40 carbon metabolites) were studied in this work, to the exclusion of other reported LYC epoxides and diols, which have been previously examined.^[27, 28]

In summary, LYC from a lipid-rich test meal appeared to be robust to loss and to isomerization during digestion, and apo-lycopenal/lycopenone concentrations were largely constant relative to LYC concentrations over 4 h. The feeding of inorganic iron resulted in a loss of LYC both in the digesta and in the newly-absorbed TRL fraction, and reduced digesta apo-

404 lycopenal/lycopenone concentrations over time. The presence of apo-lycopenals/lycopenone in
405 the later chylomicron-rich TRL fractions supports absorption from the test meal, and a lack of
406 apo-lycopenols and apo-lycopenoic acids in all biological samples tested suggests a limit of
407 detection that is too high, or that apo-lycopenals may be metabolized quite differently from
408 retinal. In short, if maximal absorption of LYC and/or apo-lycopenals/apo-lycopenone is
409 desired, then LYC-rich meals should be consumed in the absence of inorganic iron.

410

ACKNOWLEDGEMENTS

We thank Simona Birtic of Naturex, Inc. for providing the lycopene-enriched oleoresin and Lecico, Inc. for providing the egg phospholipids. This work was supported by grants from the PACA (Provence-Alpes-Côte d’Azur) Region and the OSU Ohio Agricultural Research and Development Center. Rachel Kopec received support from the European Union, in the framework of the Marie-Curie FP7 COFUND People Programme, through an AgreenSkills fellowship (under grant agreement n° 267196). R.E.K., P.B., C.C.V. designed research, R.E.K., M.N., C.C., J.P.B., S.M., B.G. conducted research, R.E.K. analyzed data and performed statistical analyses, R.E.K. wrote paper, R.E.K. and P.B. have primary responsibility for final manuscript content. All authors have read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

- [1] Story, E.N., Kopec, R.E., Schwartz, S.J., Harris, G.K., *Annu. Rev. Food Sci. T.* **2010**, *1*, 189.
- [2] Moran, N.E., Cichon, M.J., Riedl, K.M., Grainger, E.M., Schwartz, S.J., Novotny, J.A., Erdman, J.W., Jr., Clinton, S.K., *Am. J. Clin. Nutr.* **2015**, *102*, 1436.
- [3] Diwadkar-Navsariwala, V., Novotny, J.A., Gustin, D.M., Sosman, J.A., Rodvold, K.A., Crowell, J.A., Stacewicz-Sapuntzakis, M., Bowen, P.E., *J. Lipid Res.* **2003**, *44*, 1927.
- [4] McClements, D.J., *Adv. Colloid Interface Sci.* **2012**, *174*, 1.
- [5] Müller, L., Goupy, P., Fröhlich, K., Dangles, O., Caris-Veyrat, C., Böhm, V., *J. Agric. Food Chem.* **2011**, *59*, 4504.
- [6] Goupy, P., Reynaud, E., Dangles, O., Caris-Veyrat, C., *N. Journal Chem.* **2012**, *36*, 575.
- [7] Goupy, P., Vulcain, E., Caris-Veyrat, C., Dangles, O., *Free Radic. Biol. Med.* **2007**, *43*, 933.
- [8] Sy, C., Caris-Veyrat, C., Dufour, C., Boutaleb, M., Borel, P., Dangles, O., *Food Funct.* **2013**, *4*, 698.
- [9] Boon, C.S., McClements, D.J., Weiss, J., Decker, E.A., *J. Agric. Food Chem.* **2009**, *57*, 2993.
- [10] Kopec, R.E., Gleize, B., Borel, P., Desmarchelier, C., Caris-Veyrat, C. *Food Funct.* **2017**, *8*, 1494.
- [11] Lee, M.T., Chen, B.H., *Food Chem.* **2002**, *78*, 425.
- [12] Boon, C.S., Xu, Z., Yue, X., McClements, D.J., Weiss, J., Decker, E.A., *J. Agric. Food Chem.* **2008**, *56*, 1408.
- [13] Sy, C., Dangles, O., Borel, P., Caris-Veyrat, C., *Arch. Biochem. Biophys.* **2015**, *572*, 89.
- [14] Sy, C., Dangles, O., Borel, P., Caris-Veyrat, C., *Mar. Drugs.* **2015**, *13*, 7020.
- [15] Głąbska, D., Guzek, D., Zakrzewska, P., Włodarek, D., Lech, G., *Nutrients.* **2016**, *8*, 613.
- [16] Reifen, R., Nissenkorn, A., Matas, Z., Bujanover, Y., *J. Gastroenterol.* **2004**, *39*, 514.
- [17] Reifen, R., Nur, T., Matas, Z., Halpern, Z., *Int. J. Vitam. Nutr. Res.* **2001**, *71*, 347.
- [18] Carail, M., Caris-Veyrat, C., *Pure Appl. Chem.* **2006**, *78*, 1493.
- [19] Erdman, J.W., Jr., Ford, N.A., Lindshield, B.L., *Arch. Biochem. Biophys.* **2009**, *483*, 229.
- [20] Cheng, J., Miao, B., Hu, K.-Q., Fu, X., Wang, X.D., *J. Nutr. Biochem.* **2018**, *56*, 26.
- [21] Harrison, E.H., Quadro, L., *Ann. Rev. Nutr.* **2018**, *38*, 153.
- [22] dela Peña, C., Narayanasamy, S., Riedl, K.M., Curley, R.W., Jr, Schwartz, S.J., Harrison, E.H., *J. Biol. Chem.* **2013**, *288*, 37094.
- [23] Keifer, C., Hessel, S., Lampert, J.M., Vogt, K., Lederer, M.O., Breithaupt, D.E., von Lintig, J., *J. Biol. Chem.* **2001**, *276*, 14110.
- [24] Hu, K.Q., Liu, C., Ernst, H., Krinsky, N.I., Russell, R.M., Wang, X.D., *J. Biol. Chem.* **2006**, *281*, 19327.
- [25] Lindqvist, A., He, Y.-G., Andersson, S., *J. Histochem. Cytochem.* **2005**, *53*, 1403.
- [26] Kopec, R.E., Riedl, K.M., Harrison, E.H., Curley, R.W., Jr, Hruszkewycz, D.P., Clinton, S.K., Schwartz, S.J., *J. Agric. Food Chem.* **2010**, *58*, 3290.
- [27] Khachik, F., Beecher, G.R., Goli, M.B., Lusby, W.R., Smith, J.C., Jr., *Anal. Chem.* **1992**, *64*, 2111.
- [28] Cichon, M.J., Moran, N.E., Riedl, K.M., Schwartz, S.J., Clinton, S.K., *Metabolites.* **2018**, *8*, E24.
- [29] Blanquet-Diot, S., Soufi, M., Rambeau, M., Rock, E., Alric, M. *J. Nutr.* **2009**, *139*, 876.
- [30] Borel, P., Desmarchelier, C., Nowicki, M., Bott, R., *Free Radic. Biol. Med.* **2015**, *83*, 238.
- [31] Reynaud, E., Aydemir, G., Rühl, R., Dangles, O., Caris-Veyrat, C., *J. Agric. Food Chem.* **2011**, *59*, 1457.
- [32] Kopec, R.E., Caris-Veyrat, C., Nowicki, M., Gleize, B., Carail, M., Borel, P., *Am. J. Clin. Nutr.* **2018**, *108*, 803.
- [33] Tyssandier, V., Reboul, E., Dumas, J.-F., Bouteloup-Demange, C., Armand, M., Marcand, J., Sallas, M., Borel P., *Am. J. Physiol. Gastrointest. Liver Physiol.* **2003**, *284*, G913.
- [34] Borel, P., Pasquier, B., Armand, M., Tyssandier, V., Grolier, P., Alexandre-Gouabau, M.C., Andre, M., Senft, M., Peyrot, J., Jaussan, V., Lairon, D., Azais-Braesco, V., *Am. J. Physiol. Gastrointest. Liver Physiol.* **2001**, *280*, G95.
- [35] Caris-Veyrat, C., Schmid, A., Carail, M., Böhm, V., *J. Agric. Food Chem.* **2003**, *51*, 7318.
- [36] Chitchumroonchokchai, C., Schwartz, S.J., Failla, M.L., *J. Nutr.* **2004**, *134*, 2280.

- [37] Bumrungpert, A., Kalpravidh, R.W., Suksamrarn, S., Chaivisuthangkura, A., Chitchumroonchokchai, C., Failla, M.L. *Mol. Nutr. Food Res.* **2009**, 53 Suppl 1, S54.
- [38] R Core Team, R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. **2013**.
- [39] Bates, D., Maechler, M., Bolker, B., lme4: Linear mixed-effects models using S4 classes. *R Package Version 0.999999-0*. **2012**.
- [40] Clark, S.F., *Curr. Opin. Gastroenterol.* **2009**, 25, 122.
- [41] Alleyne, M., Horne, M.K., Miller, J.L., *Am. J. Med.* **2008**, 121, 943.
- [42] Hurrell, R., Egli, I., *Am. J. Clin. Nutr.* **2010**, 91, 1461S.
- [43] Armand, M., Borel, P., Dubois, C., Senft, M., Peyrot, J., Salducci, J., Lafont, H., Lairon, D., *Am. J. Physiol.* **1994**, 266, G372.
- [44] Biehler, E., Hoffmann, L., Krause, E., Bohn, T., *J. Nutr.* **2011**, 141, 1769.
- [45] Borel, P., Desmarchelier, C., Dumont, U., Halimi, C., Lairon, D., Page, D., Sébédio, J.L., Buisson, C., Buffière, C., Rémond, D. *Br. J. Nutr.* **2016**, 116, 2091.
- [46] Bengtsson, A., Scheers, N., Andlid, T., Alminger, M.L., Sandberg, A.S., Svanberg, U., *Int. J. Food Sci. Nutr.* **2009**, 60 Suppl 5, 125.
- [47] Richelle, M., Lambelet, P., Rytz, A., Tavazzi, I., Mermoud, A.F., Juhel, C., Borel, P., Bortlik, K., *Br. J. Nutr.* **2012**, 107, 1482.
- [48] Cooperstone, J.L., Ralston, R.A., Riedl, K.M., Haufe, T.C., Schweiggert, R.M., King, S.A., Timmers, C.D., Francis, D.M., Lesinski, G.B., Clinton, S.K., Schwartz, S.J., *Mol. Nutr. Food Res.* **2015**, 59, 658.
- [49] Cooperstone, J.L., Novotny, J.A., Riedl, K.M., Cichon, M.J., Francis, D.M., Curley, R.W., Jr., Schwartz, S.J., Harrison, E.H., *Am. J. Clin. Nutr.* **2018**, 108, 784.
- [50] During, A., Nagao, A., Terao, J., *J. Nutr.* **1998**, 128, 1614.
- [51] Britton, G., Liaaen-Jensen, S., Pfander, H., Carotenoids: Biosynthesis and Metabolism, Birkhauser Verlag, Basel, Boston, Berlin **1998**.

Table 1. Lycopene and apo-lycopenals/apo-lycopenone measured in the test meals¹

Carotenoid	-FeSO ₄ (mg/meal)	+FeSO ₄ (mg/meal)
Total Lycopene	23.1 ± 1.4	21.3 ± 1.5
all- <i>trans</i> -lycopene	19.5 ± 1.3	17.9 ± 1.4
5- <i>cis</i> -lycopene	2.0 ± 0.30	1.7 ± 0.25
other- <i>cis</i> -lycopene	1.6 ± 0.16	1.8 ± 0.22
Apo-6'-lycopenal	0.16 ± 0.029	0.15 ± 0.030
Apo-8'-lycopenal	0.043 ± 0.009	0.048 ± 0.007
Apo-10'-lycopenal ²	0.012 ± 0.002	0.023 ± 0.002
Apo-12'-lycopenal ²	0.017 ± 0.002	0.027 ± 0.002
Apo-14'-lycopenal ²	0.029 ± 0.006	0.078 ± 0.007
Apo-15-lycopenal ³	0.009 ± 0.004	0.019 ± 0.005
Apo-13-lycopenone ^{2,3}	0.032 ± 0.010	0.080 ± 0.013

¹Values are means ± SEM, $n = 11$ for both test meals, with sampling taken just after meal preparation (see *Methods* for details). Note that neither apo-lycopenols nor apo-lycopenoic acids were observed in the test meals.

²The amount of this compound between meal types was statistically different (two-tailed unpaired Student's t-test ($P < 0.05$)).

³Provided as apo-14'-lycopenal equivalents.

Table 2. Characteristics of the subjects¹

	Age (years)	Weight (kg)	BMI (kg/m ²)	Plasma total cholesterol concentration (mg/dL)	Plasma triacylglycerol concentration (mg/dL)	Plasma glucose (mg/dL)	Hemoglobin (g/dL)
Test Meals	43.0 ± 6.8	80 ± 7.9	24.1 ± 2.3	162 ± 38.9	55.7 ± 23.0 ²	90 ± 6.1	15.4 ± 0.87
Control Meals	27.5 ± 4.9	77 ± 3.7	23.9 ± 2.3	170 ± 14.1	80.0 ± 0 ²	83 ± 4.2	15.8 ± 0.21

¹Values are means ± standard deviation at the screening visit of subjects who completed the study, $n = 7$ for the two test meals, i.e. one with lycopene oleoresin -FeSO₄, the other with lycopene oleoresin +FeSO₄, $n = 2$ for the control meals, i.e. one -FeSO₄ and one +FeSO₄.

²Characteristics between meal types are statistically different from one another using a two-tailed Welch's (i.e. unpaired) t-test ($P < 0.05$)

BMI = Body mass index.

Table 3. Mixed model results for digesta concentrations of lycopene and apo-lycopenals/apo-lycopenone¹

Analyte	Coefficient (± SE)	<u>Time</u>		Coefficient (± SE)	<u>Digesta</u>		Coefficient (± SEM)	<u>Iron</u>		Coefficient (± SEM)	<u>Time*Iron</u>	
		χ^2	P-value ²		χ^2	P-value ²		χ^2	P-value ²		χ^2	P-value ²
total lycopene ²	1.0E+5(±1.8E+4)	28.1	1.2E-7 ³	6.4E+4(±3.7E+4)	3.08	0.08	6.6E+4(±5.5E+4)	1.47	0.23	-8.1E+4(±2.5E+4)	10.7	1.1E-5 ³
all- <i>trans</i> - lycopene ²	1.0E+5(±1.8E+4)	21.1	4.3E-6 ³	6.3E+4(±3.6E+4)	2.78	0.10	6.6E+4(±5.4E+4)	1.19	0.27	-8.1E+4(±2.4E+4)	7.22	7.2E-3 ³
5- <i>cis</i> - lycopene ²	2.3E+4(±4.6E+3)	22.9	1.7E-6 ³	1.4E+4(±9.1E+3)	2.36	0.12	1.5E+4(±1.4E+4)	1.15	0.28	-1.9E+4(±6.3E+3)	9.24	2.4E-3 ³
other- <i>cis</i> - lycopene ²	2.9E+4(±5.0E+3)	31.2	2.3E-8 ³	1.0E+4(±1.4E+4)	1.1	0.30	1.9E+4(±1.5E+4)	1.47	0.23	-2.3E+4(±6.9E+3)	11.53	6.8E-4 ³
apo-6'- lycopenal	1.2E+5(±1.1E+5)	1.11	0.29	2.8E+5(±2.2E+5)	1.50	0.22	3.0E+5(±3.4E+5)	0.74	0.39	-4.4E+4(±1.5E+5)	0.08	0.77
apo-8'- lycopenal	4.4E+4(±1.5E+4)	9.21	2.4E-3 ³	4.8E+4(±2.9E+4)	2.86	0.09	4.4E+4(±4.4E+4)	1.01	0.32	-4.2E+4(±2.0E+4)	4.48	3.4E-2 ³
apo-10'- lycopenal	1.8E+4(±5.4E+3)	11.6	6.6E-4 ³	1.7E+4(±1.1E+4)	2.43	0.12	2.8E+4(±1.6E+4)	2.87	0.09	-1.9E+4(±7.3E+3)	6.65	9.9E-3 ³
apo-12'- lycopenal	3.9E+4(±9.8E+3)	15.3	9.0E-5 ³	3.5E+4(±2.0E+4)	3.13	0.08	6.2E+4(±3.0E+4)	6.25	0.012 ³	-4.0E+4(±1.3E+4)	9.10	2.5E-3 ³
apo-14'- lycopenal	1.5E+5(±3.2E+4)	20.0	7.8E-6 ³	1.6E+4(±6.4E+4)	0.06	0.81	2.5E+5(±9.7E+4)	6.38	0.012 ³	-1.7E+5(±4.3E+4)	14.3	1.5E-4 ³
apo-15- lycopenal ⁴	8.0E+4(±1.8E+4)	18.5	1.7E-5 ³	1.3E+4(±3.7E+4)	0.12	0.73	1.1E+5(±5.6E+4)	4.08	4.3E-2 ³	-7.3E+4(±2.5E+4)	8.73	3.1E-3 ³
apo-13- lycopenone ⁴	1.5E+5(±3.7E+4)	16.7	4.3E-5 ³	1.3E+5(±7.5E+4)	3.08	0.08	3.3E+5(±1.1E+5)	8.35	3.8E-3 ³	-1.9E+5(±5.0E+4)	14.1	1.7E-4 ³

¹All test subject-samples at were included in determining the mixed model coefficients provided above ($n=7$ gastric -FeSO₄, $n=7$ gastric +FeSO₄, $n=4$ duodenal -FeSO₄, and $n=6$ duodenal +FeSO₄ subject samples at $t = 0, 0.5, 1, 2, 3, 4$)

²As determined by the likelihood test ratio, degrees of freedom = 1 for all factor evaluations

³ $P < 0.05$ was considered statistically significant.

⁴Provided as apo-14'-lycopenal equivalents

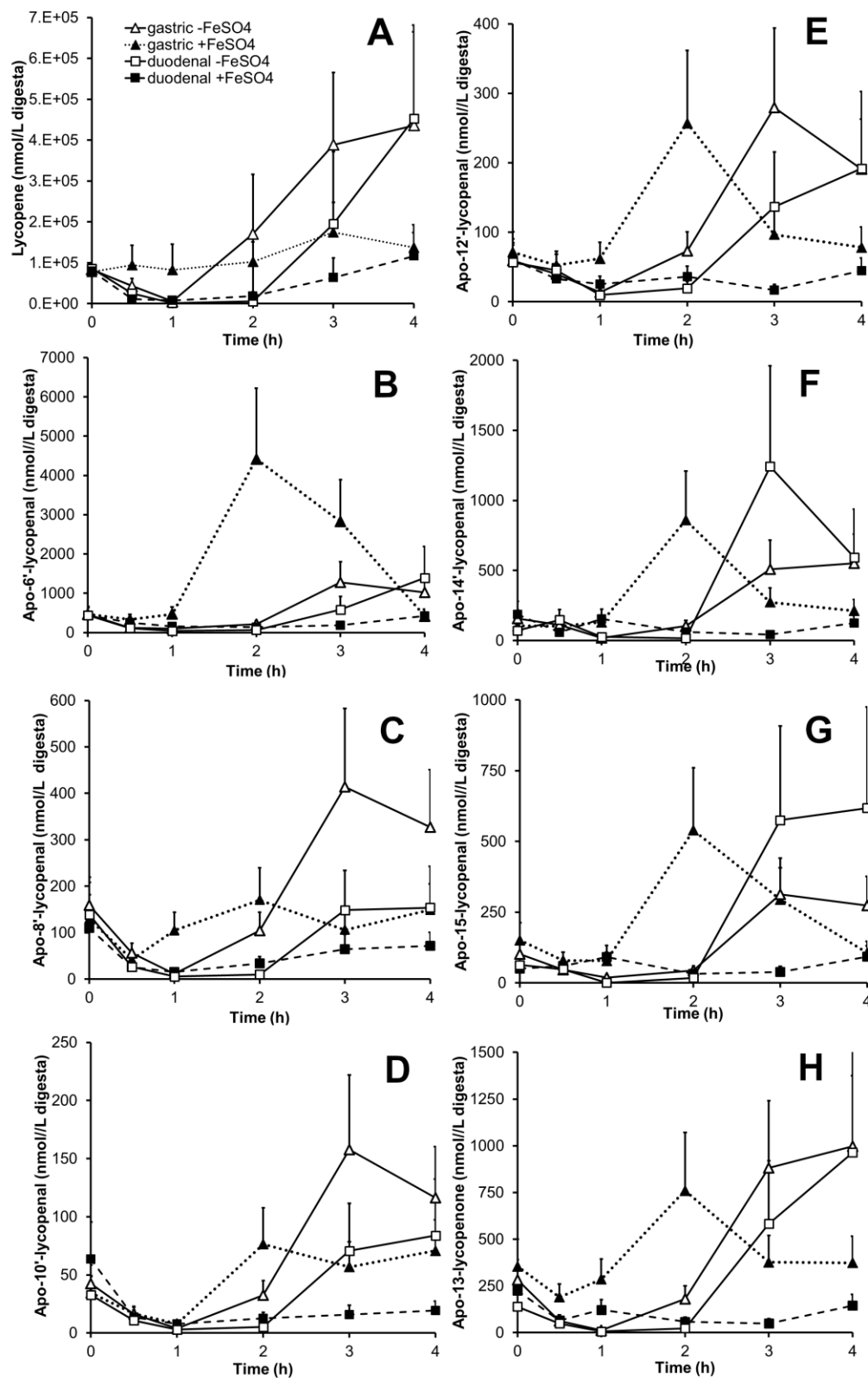


Figure 1. The concentrations (nmol/L) of lycopene and apo-lycopenals/lycopenone in digesta of subjects over 4 h. Sampling from gastric $-FeSO_4$ (Δ), gastric $+FeSO_4$ (\blacktriangle), duodenal $-FeSO_4$ (\square) and duodenal $+FeSO_4$ (\blacksquare) compartments are shown. Concentrations of lycopene and apo-lycopenals/lycopenone are represented as means \pm SEM, $n = 7$ for gastric $-FeSO_4$, $n = 7$ for gastric $+FeSO_4$, $n = 4$ for duodenal $-FeSO_4$, $n = 6$ for duodenal $+FeSO_4$. **A)** total lycopene, **B)** apo-6'-lycopenal, **C)** apo-8'-lycopenal, **D)** apo-10'-lycopenal, **E)** apo-12'-lycopenal, **F)** apo-14'-lycopenal, **G)** apo-15-lycopenal i.e. acyclo-retinal, **H)** apo-13-lycopenone. The relative impact of digesta type, time, iron, and time*iron on the mixed model describing the concentration of each isomer are provided in Table 3.

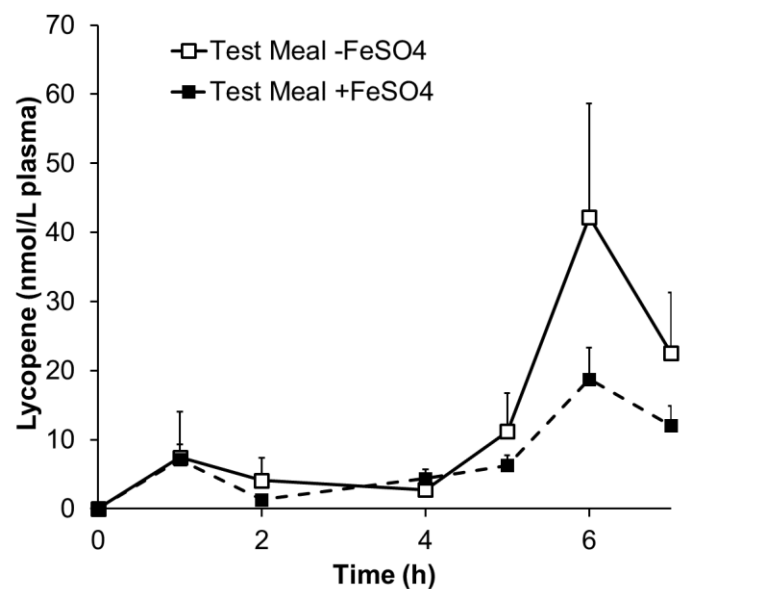


Figure 2. Average baseline-corrected plasma triglyceride-rich lipoprotein (TRL) concentrations of total lycopene over the course of 7 h after test meal consumption $-FeSO_4$ (\square) and test meal $+FeSO_4$ (\blacksquare). Values represent the average of 7 subjects at each time point \pm SEM. The test meal $+FeSO_4$ resulted in a statistically significant decrease in AUC relative to the test meal $-FeSO_4$ (paired t-test, $P = 0.0218$).

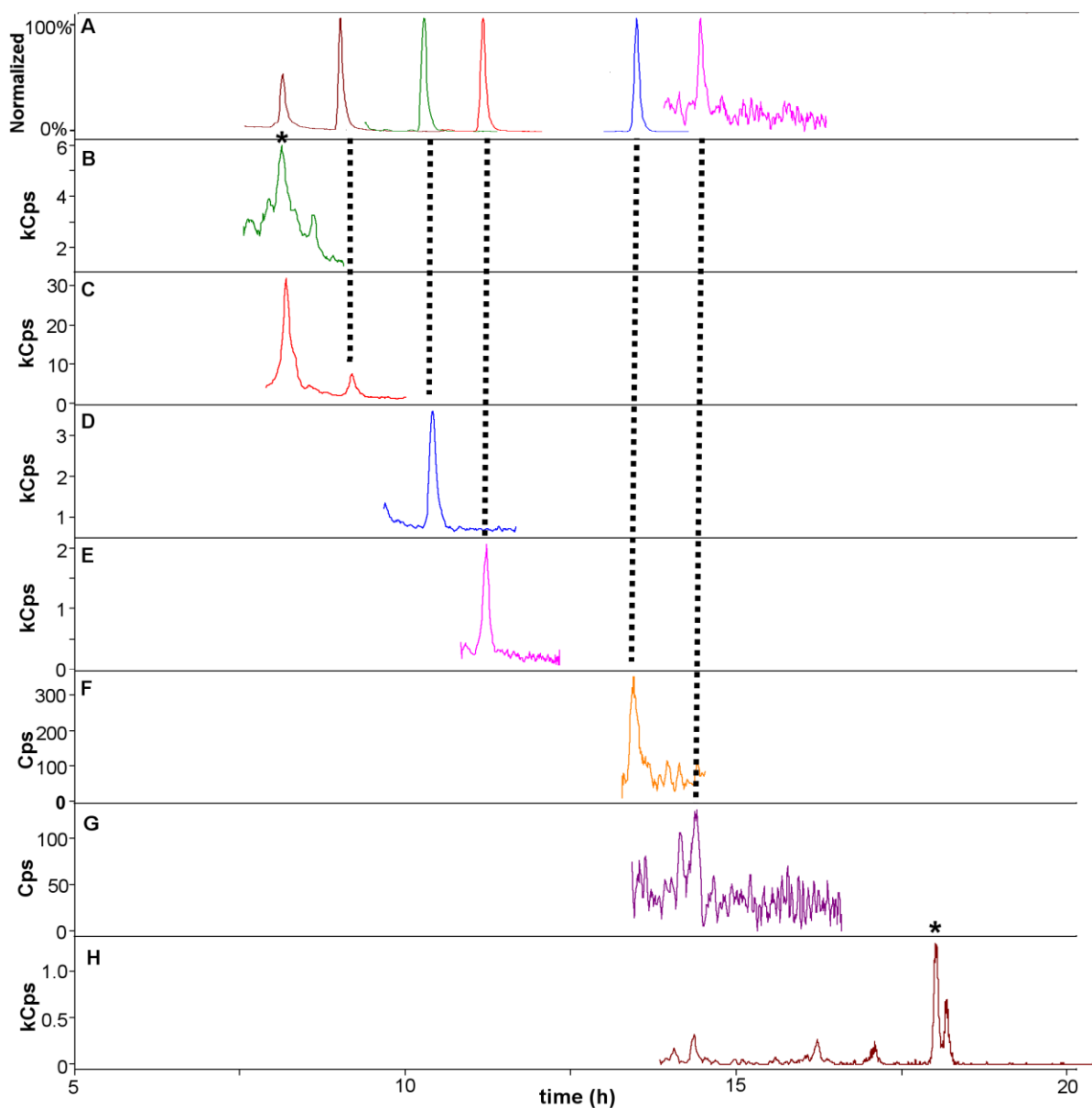


Figure 3. A representative LC-MS/MS chromatogram depicting **A)** overlaid MRMs of apo-lycopenals from the standards mixture normalized to the peak of highest intensity in each SRM and **B-H)** metabolites observed in the TRL fraction of a subject 6 h after meal consumption, shown in ion counts per second, Cps or kilo-ion counts per second, kCps (**B** = apo-13-

lycopenone denoted with asterisk, **C** = apo-14'-lycopenal, **D** = apo-12'-lycopenal, **E** = apo-10'-lycopenal, **F** = apo-8'-lycopenal, **G** = apo-6'-lycopenal, **H** = lycopene, all-*trans*-lycopene denoted with an asterisk). The precursor > product ion transition used for quantitation was selected to display (see **Supplemental Table 1** for further details).