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The bioavailability of tetra-*cis*-lycopene in humans and tetra-*cis* lycopene concentrations in selections of heritage tomatoes

McGhie T, Bentley-Hewitt K, Herath T, Smith H, Martell S, Middlemiss-Kraak S.

June 2014

Confidential report for:
Central Tree Crops Research Trust

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Executive summary

The bioavailability of tetra-*cis*-lycopene in humans and tetra-*cis*-lycopene concentrations in selections of heritage tomatoes

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Plant & Food Research: Palmerston North

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The common red tomato contains all-*trans*-lycopene, whereas some golden/orange coloured heritage tomatoes contain a different isomer of lycopene: tetra-*cis*-lycopene. There is some published scientific evidence to suggest that tetra-*cis*-lycopene is absorbed into the bloodstream more efficiently than all-*trans*-lycopene when processed tomato products are consumed.

The primary objective of this study was to determine the relative bioavailability of all-*trans*-lycopene and tetra-*cis*-lycopene when tomatoes are consumed in a fresh, unprocessed state.

This study found that, in humans, more tetra-*cis*-lycopene is absorbed from fresh tomatoes into the bloodstream than all-*trans*-lycopene, thus the bioavailability of tetra-*cis*-lycopene is greater than all-*trans*-lycopene.

The concentration of tetra-*cis*-lycopene in golden/orange tomato cultivars varies, however some cultivars contain high concentrations of tetra-*cis*-lycopene. The maximum concentration of tetra-*cis*-lycopene detected was 7.2 mg/100 g fresh weight.

The high concentrations of tetra-*cis*-lycopene in some golden/orange tomatoes and the greater bioavailability of tetra-*cis*-lycopene suggest that the health benefits associated with lycopene may be greater when golden/orange tomatoes, such as the cultivar ‘Moonglow’, are consumed compared with consumption of the common red tomato.

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1 Introduction

A diet rich in fruits and vegetables is well known to be associated with a reduced risk of several diseases, such as cardiovascular disease and some cancers. Plant foods, including fruits such as tomato, contain chemical compounds (metabolites or phytochemicals) that are considered to be beneficial for the health of human consumers. The metabolite composition within each plant type varies by cultivar, climate and production system. There is also a view that modern fruit cultivars contain lower concentrations of health-promoting metabolites than older 'heritage' cultivars.

In previous years Plant & Food Research (PFR) has measured carotenoids and polyphenolic compounds in tomato cultivars and found that all of the red tomatoes tested contain the isomer of lycopene known as 'all-*trans*-lycopene' whereas a smaller number of cultivars contain a different isomer of lycopene known as 'tetra-*cis*-lycopene' (C^4-lycopene). Tomatoes containing tetra-*cis*-lycopene tend to be golden/orange (also described as tangerine) coloured, however not all golden/orange tomatoes contain tetra-*cis*-lycopene.

The health benefits provided by phytochemicals to human consumers are critically dependent on the absorption of the phytochemical in to the human body usually by means of digestion and transport into the blood circulation.

The aims of this study were twofold.

1. Measure metabolites in heritage cultivars of tomato with a focus on carotenoids and specifically the two isomers of lycopene (all-*trans*-lycopene and tetra-*cis*-lycopene).
2. Measure the bioavailability of both all-*trans*-lycopene and tetra-*cis*-lycopene in humans when consumed as a fresh fruit.

2 Materials and Methods

Carotenoid Analysis:

Samples of tomato and grapefruit samples were collected by Mark Christensen, Central Tree Crops Research Trust, and sent by courier to Plant & Food Research, Palmerston North. All samples were frozen (-18°C) on arrival and stored frozen until analysis.

Tomato samples were homogenised using a Hobart food chopper, while being kept frozen using dry ice.

Portions of the homogenised samples were extracted with tetrahydrofuran:methanol, with the addition of anhydrous Na_2SO_4 and Na_2CO_3 and the carotenoid composition was measured by High Performance Liquid Chromatography (HPLC).

Authentic carotenoid standards (lutein, β-carotene, and all-trans-lycopene) were used to identify and quantify each compound by retention time and spectral properties. Tetra-*cis*-lycopene was identified by reference to previously published research on tomatoes and was quantified as all-*trans*-lycopene equivalents. It is important to note that the method used to calculate the concentration of tetra-*cis*-lycopene in this current work differs from that used in previous years. The spectral properties of all-*trans*-lycopene and tetra-*cis*-lycopene differ with both the wavelength for absorption and the intensities (extinction coefficient) of absorption being different (Table 1).

Table 1. Spectral properties of lycopene isomers

Lycopene isomer	Wavelength (nm)	Extinction Coefficient
all-*trans*-lycopene	470	184,900
tetra-*cis*-lycopene	436	102,900

Lycopene Bioavailability:

A human clinical study was undertaken where participants were fed a single meal of either red ‘Rosalita’ or orange ‘Moonglow’ tomatoes. The trial involved 13 volunteers and was of a crossover design with the details as shown in Figure 1.

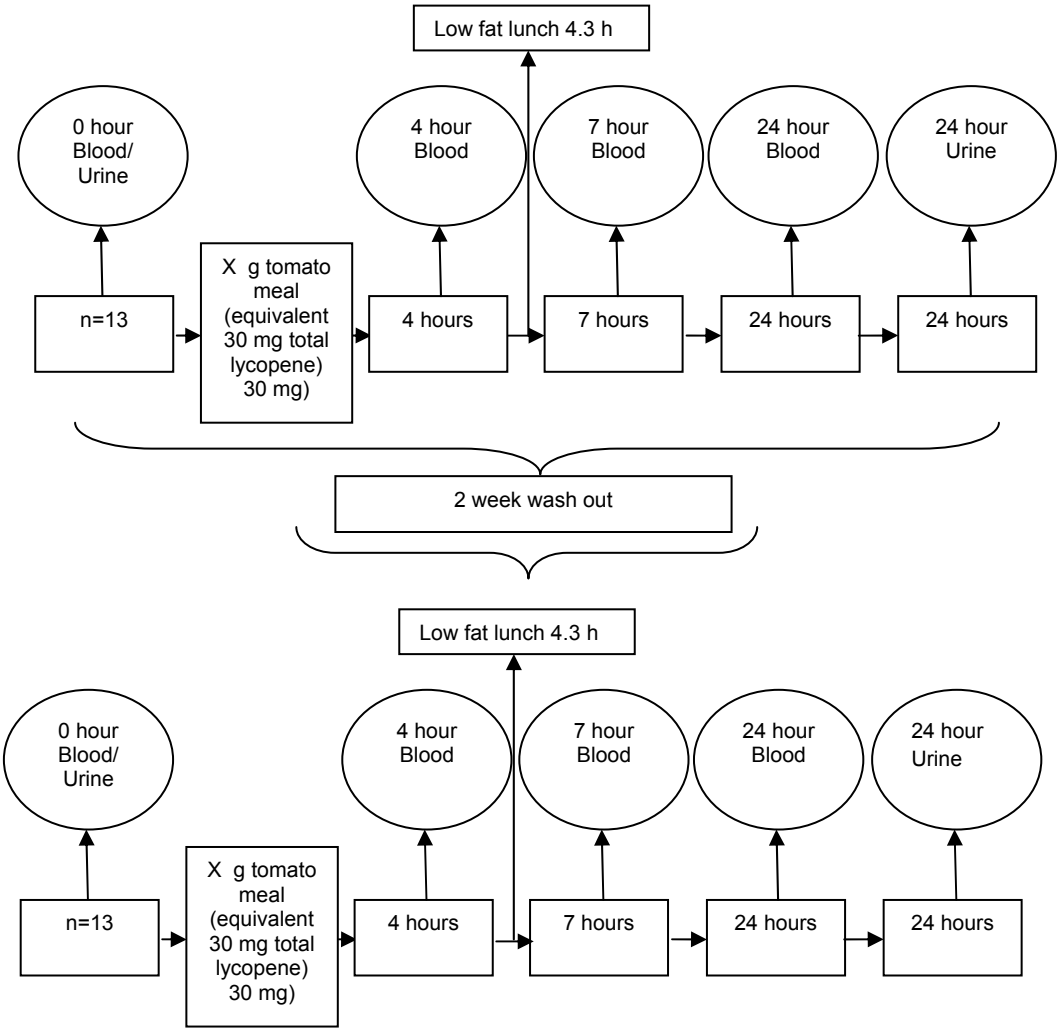

Figure1. Schematic of clinical trial design.

Collected plasma samples from the two stages of the clinical trial were stored at -80◦C until analysis by HPLC/UHPLC-UV/vis. All-*trans*-lycopene was measured in plasma samples for the 'Rosalita' arm of the study by HPLC using a Waters C30 (4.6 i.d. x 250 mm) column. Tetra-*cis*-lycopene was measured in plasma samples from the 'Moonglow' arm of the study by UHPLC using a Thermo Accurcore C30 (2.1 i.d. x 150 mm) column. Concentrations of all-*trans*-lycopene were calculated using an authentic all-*trans*-lycopene; concentrations of tetra-*cis*-lycopene were calculated by reference to the all-*trans*-lycopene with corrections for the difference in spectral absorption characteristics as outlined above.

3 Results

Carotenoid Analysis

Table 2 shows the concentrations of carotenoids present in the 26 samples provided. Overall, the carotenoid concentrations show large variations and the concentration ranges are similar to those reported previously by PFR.

Table 2. Concentrations of carotenoids in tomato cultivars provided by the Central Tree Crops Research Trust.

	Carotenoid concentrations (mg/100 g FW)				
Cultivar Name	lutein	β-carotene	lycopene	C^4-lycopene	Total
'Barnes Mountain Yellow'*	n.d.	n.d.	n.d.	6.93	6.93
'Burstyn'	n.d.	n.d.	n.d.	2.27	2.27
'Ceylon'	0.09	0.67	0.76	n.d.	1.52
'Cherries'	0.15	1.24	3.37	0.02	4.78
'Chester'	0.34	0.17	n.d.	0.04	0.55
'Columbia Orange'	0.15	1.48	4.58	0.04	6.25
'Dark Rose'	0.15	0.88	5.87	0.03	6.93
'Galapagos Cherries'	0.21	0.67	0.11	0.03	1.01
'Golden Currant'	0.24	0.26	n.d.	0.04	0.54
'Golden Egg'	0.12	0.27	0.04	0.10	0.53
'Hirshels' Cherry'	0.19	1.59	4.16	0.04	5.98
'Ilses' Yellow Latvian'*	0.04	n.d.	0.01	7.22	7.28
'Jubilee'	n.d.	n.d.	n.d.	0.76	0.76
'Mountain Gold'	0.01	n.d.	n.d.	5.53	5.54
'Oaxacen Jewel'	0.17	0.44	1.10	0.03	1.73
'Peruvian Wild'	0.21	1.41	0.58	0.05	2.25
'Poma Amoris Minora Lutea'	0.15	0.15	0.01	0.03	0.35
'Red Ruffled'	0.17	0.67	4.75	0.03	5.62
'Regmi Orange'	0.18	1.48	0.81	0.03	2.50
'Sorin Cherry'	0.13	0.38	1.12	0.05	1.68
'T.C. Jones'	0.07	0.04	n.d.	n.d.	0.11
'Teardrop'	0.26	1.88	0.77	0.06	2.96
'Tess' Landrace Currant'	0.19	1.94	7.32	0.07	9.53
'Virginia Sweets'	0.01	0.07	0.02	n.d.	0.10
'Yellow Pear'	0.15	0.07	n.d.	n.d.	0.22
'Orange Russian'	0.10	0.98	1.22	0.15	2.45
n.d. = not detected					
lycopene = all-*trans*-lycopene					
C^4-lycopene = tetra-*cis*-lycopene					

* Although these cultivars have 'yellow' in their name, it would be more accurate to describe them as 'orange' in colour

Lycopene Bioavailability

Five men and seven women aged 23–59 y (Table 1-sex age BMI) recruited in house at Plant & Food Research, Palmerston North, New Zealand completed the trial with no ill effects from the treatments. All participants completed the trial. Ethical approval for the trial was obtained from the Health and Disabilities Ethics Committee, New Zealand (reference 13/CEN/164).
All participants gave written informed consent and were deemed healthy by health screen questionnaires and assessment of normal blood lipids (cholesterol and triglycerides). Participants were excluded if they were pregnant, smokers, had a diagnosis of a long-term medical condition, were taking regular prescribed medication or dietary supplements containing carotenoids, regularly (more than once a week) took antacids, laxatives, proton pump inhibitors or medication which affected lipid absorption, or had fasting cholesterol greater than 5.3 mmol/L or fasting triglycerides greater than 2.3 mmol/L. A wash out period of 4 days prior to the start of the study excluded foods potentially high in lycopene from the diet (e.g. tomatoes, guava and watermelon). These dietary restrictions were continued until the end of the study.
The participants were allocated into a non randomised, single group study, in which 150 g of 'Rosalita' tomatoes (containing approximately 16 mg of all-*trans*-lycopene) was consumed for breakfast with 2 slices of wholemeal toast (Ploughman™ Mixed grains-toast). and 10 mL olive oil (SumOlives, extra virgin olive oil, Sum Oil Ltd, Wanganui) followed by a 2 week wash out and then 150 g of 'Moonglow' tomatoes (containing approximately 16 mg of tetra-*cis*-lycopene) served with toast and olive oil as previously described. Subsamples of each tomato type were analysed 2 days before feeding to quantify approximate lycopene levels in each variety, before feeding. The trial was conducted from 5 March to 21 March 2014. Each treatment period consisted of participants donating baseline peripheral blood samples (1 x 6 ml) in EDTA mineral free tubes, after they had fasted for at least 12 hours. Immediately after providing the baseline blood samples, participants were fed a tomato meal and then donated further blood samples at 4, 7 and 24 h post-meal. Two morning urine samples were provided by participants on the day, and the following day of the treatment. These were stored at -80 once delivered. Plasma was immediately prepared from blood by centrifugation of the blood samples at 2000 x g for 20 min at 4 °C and stored at -80 °C for lycopene analysis.

Table 3. Participant characteristics at the onset of the study (mean values with their standard errors)

	Women (n=7)		Men (n=5)	
	Mean	SEM	Mean	SEM
Age (years)	40.5	4.9	41.8	5.0
BMI (kg/m^3)	23.1	0.8	26.3	0.5
Cholesterol (mmol/L)	4.5	0.3	4.8	0.3
Triglycerides (mmol/L)	0.74	0.1	1.38	0.4

Methods to measure the two lycopene compounds (all-*trans* and tetra-*cis*) were adapted from published methods to optimise detection limits and recovery of both lycopene isomers through the sample preparation process. Most previous studies concerning the bioabsorption of carotenoids from tomato products have considered all-*trans*-lycopene only. Initially it proved difficult to obtain compound separation between β-carotene and tetra-*cis*-lycopene. Detection by liquid chromatography – mass spectrometry (LC-MS) was unsatisfactory as all three compounds (all-*trans*-lycopene, tetra-*cis*-lycopene, and β -carotene) have the same elemental composition and therefore same accurate molecular weight. This meant that these compounds cannot be distinguished from each other by mass spectrometry and physical separation by

chromatography is a critical requirement for accurate quantification. Furthermore, the HPLC method used for analysis of tetra-*cis*-lycopene in tomato fruit was not suitable for plasma samples because β-carotene and tetra-*cis*-lycopene are only just separated and the comparatively larger amounts of β-carotene than tetra-*cis*-lycopene in plasma meant that β -carotene interfered with the measurement of tetra-*cis*-lycopene. The issues associated with compound separation were overcome by the adoption of two separate analytical methods, one for all-*trans*-lycopene/'Rosalita' plasma samples and another for tetra-*cis*-lycopene/'Moonglow' plasma samples. Examples of chromatograms obtained for the time course for a typical single subject are shown in Figure 2.

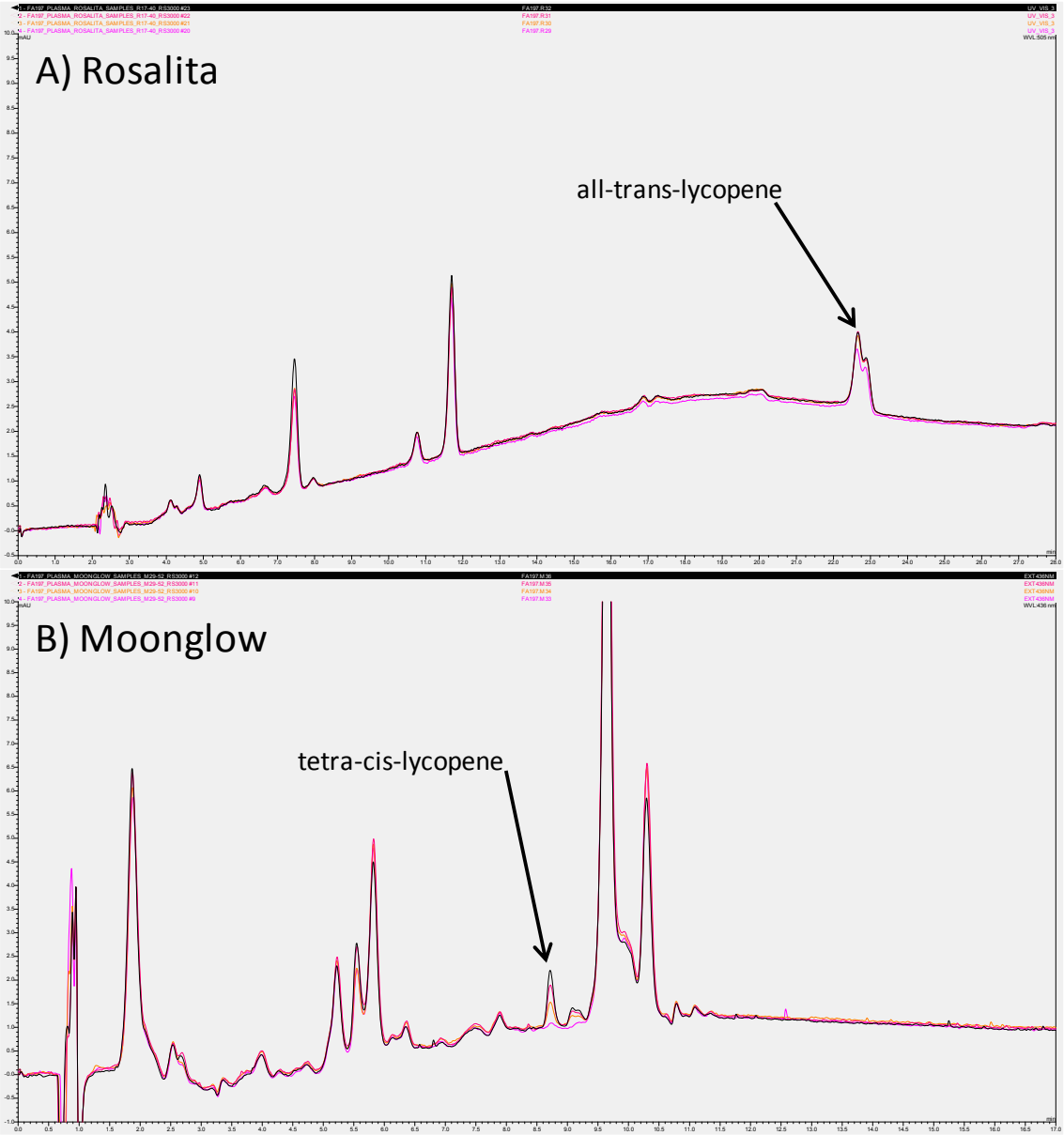

Figure 2. Typical examples of HPLC/UHPLC chromatograms of plasma carotenoid showing A) changes in all-*trans*-lycopene concentrations following a meal of 'Rosalita' tomatoes; and B) changes in tetra-*cis*-lycopene concentrations following a meal of 'Moonglow' tomatoes.

The concentrations of all-*trans*-lycopene and tetra-*cis*-lycopene present in plasma following the consumption of a single meal of either 'Rosalita' or 'Moonglow' are presented in Table 4 and summarised in Figure 3. These data show that at time zero, the plasma concentrations of all-

trans-lycopene had declined to a mean of 0.17 μmol L-1. This is similar to other reports and shows that in general the study participants had followed instructions and avoided the consumption of tomato products. In contrast, and as expected, tetra-*cis*-lycopene was not detected in baseline plasma, confirming the view that this carotenoid is currently not a component of the typical human diet because of the current scarcity of golden/orange heritage tomato varieties.

Following consumption of a single meal of 'Rosalita' tomatoes, the plasma concentration of all-*trans*-lycopene increased, however the response was variable among the participants. In contrast, following the consumption of 'Moonglow' tomatoes the plasma concentration of tetra-*cis*-lycopene invariably increased. Furthermore the magnitude of the mean increase in plasma lycopene concentration was greater for tetra-*cis*-lycopene from 'Moonglow' than for all-*trans*-lycopene from 'Rosalita' (Figure 3).

Table 4. Concentrations of lycopene in human plasma following a single dose of either red 'Rosalita' tomatoes or orange 'Moonglow' tomatoes.

		'Rosalita'			'Moonglow'		
Participant #	Time (hr)	ATLyc (μg/mL)	ATLyc Δ (ng/mL)	ATLyc (μmol L^{-1})	TCLyc (μg/mL)	TCLyc Δ (ng/mL)	TCLyc (μmol L^{-1})
L01	0	0.044	0.0	0.082	0.000	0.0	0.000
L01	4	0.052	8.0	0.097	0.025	24.8	0.046
L01	7	0.112	68.0	0.209	0.043	42.7	0.080
L01	24	0.064	20.0	0.119	0.048	47.6	0.089
L02	0	0.092	0.0	0.171	0.000	0.0	0.000
L02	4	0.104	12.0	0.194	0.019	19.4	0.036
L02	7	0.112	20.0	0.209	0.035	35.1	0.065
L02	24	0.088	-4.0	0.164	0.045	44.7	0.083
L03	0	0.088	0.0	0.164	0.000	0.0	0.000
L03	4	0.088	0.0	0.164	0.005	5.2	0.010
L03	7	0.076	-12.0	0.142	0.010	10.3	0.019
L03	24	0.088	0.0	0.164	0.030	29.9	0.056
L04	0	0.076	0.0	0.142	0.000	0.0	0.000
L04	4	0.092	16.0	0.171	0.017	16.9	0.032
L04	7	0.072	-4.0	0.134	0.025	25.3	0.047
L04	24	0.088	12.0	0.164	0.021	21.3	0.040
L05	0	0.104	0.0	0.194	0.000	0.0	0.000
L05	4	0.104	0.0	0.194	0.017	17.4	0.032
L05	7	0.104	0.0	0.194	0.020	19.6	0.037
L05	24	0.088	-16.0	0.164	0.040	39.5	0.074
L06	0	0.156	0.0	0.291	0.000	0.0	0.000
L06	4	0.152	-4.0	0.283	0.023	22.8	0.043
L06	7	0.168	12.0	0.313	0.027	26.5	0.049
L06	24	0.168	12.0	0.313	0.059	58.9	0.110
L07	0	0.116	0.0	0.216	0.000	0.0	0.000
L07	4	0.128	12.0	0.238	0.036	35.6	0.066
L07	7	0.144	28.0	0.268	0.069	69.0	0.128
L07	24	0.136	20.0	0.253	0.051	50.6	0.094
L08	0	0.06	0.0	0.112	0.000	0.0	0.000
L08	4	0.072	12.0	0.134	0.027	26.7	0.050
L08	7	0.076	16.0	0.142	0.044	44.3	0.083
L08	24	0.072	12.0	0.134	0.046	46.4	0.086
L09	0	0.12	0.0	0.224	0.000	0.0	0.000
L09	4	0.116	-4.0	0.216	0.015	15.3	0.028
L09	7	0.116	-4.0	0.216	0.029	29.0	0.054
L09	24	0.112	-8.0	0.209	0.040	40.4	0.075
L10	0	0.024	0.0	0.045	0.001	1.3	0.002
L10	4	0.044	20.0	0.082	0.004	4.4	0.008
L10	7	0.1	76.0	0.186	0.008	8.3	0.015
L10	24	0.044	20.0	0.082	0.022	21.8	0.041
L11	0	0.144	0.0	0.268	0.000	0.0	0.000
L11	4	0.14	-4.0	0.261	0.006	6.2	0.012
L11	7	0.056	-88.0	0.104	0.013	13.0	0.024
L11	24	0.156	12.0	0.291	0.032	31.9	0.059
L12	0	0.052	0.0	0.097	0.000	0.0	0.000
L12	4	0.068	16.0	0.127	0.013	13.5	0.025
L12	7	0.08	28.0	0.149	0.038	37.6	0.070
L12	24	0.08	28.0	0.149	0.045	45.3	0.084
L13	0	0.116	0.0	0.216	0.000	0.0	0.000
L13	4	0.132	16.0	0.246	0.013	13.0	0.024
L13	7	0.128	12.0	0.238	0.022	21.5	0.040
L13	24	0.132	16.0	0.246	0.032	32.1	0.060
Means	**0**	**0.092**	**0.0**	**0.171**	**0.000**	**0.1**	**0.000**
	4	**0.099**	**7.7**	**0.185**	**0.017**	**17.0**	**0.032**
	7	**0.103**	**11.7**	**0.193**	**0.029**	**29.4**	**0.055**
	24	**0.101**	**9.5**	**0.189**	**0.039**	**39.3**	**0.073**

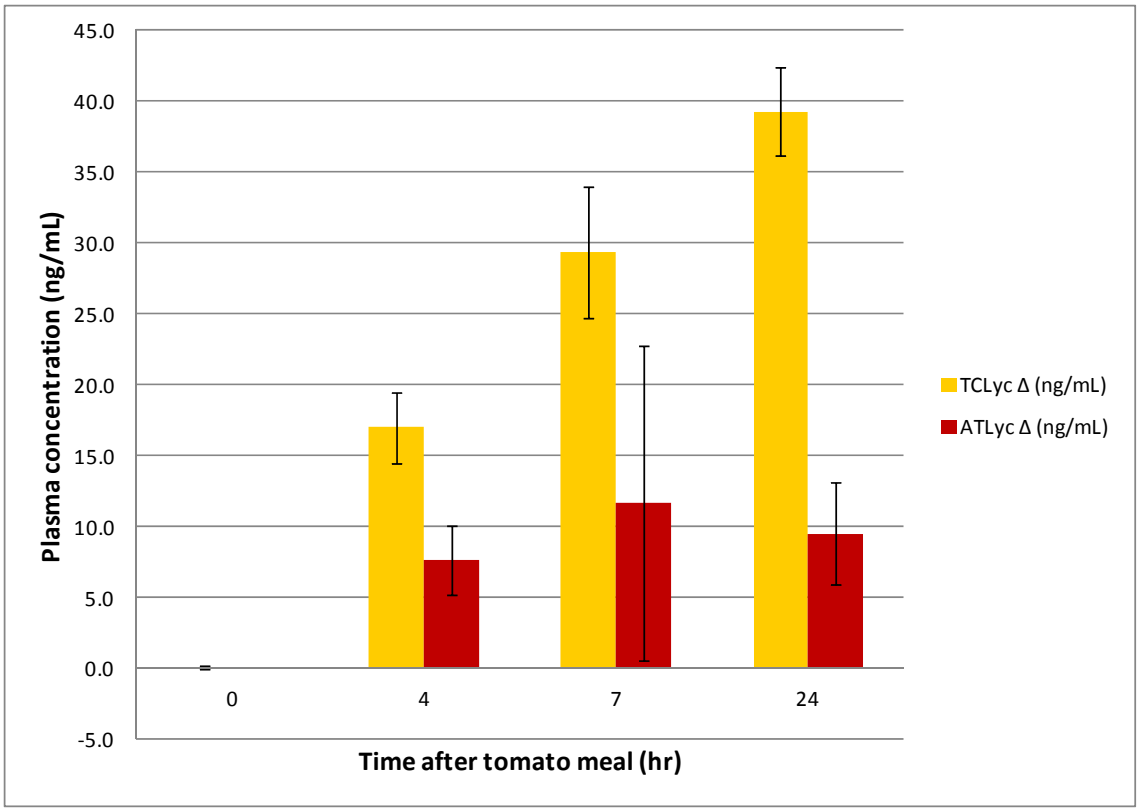

Figure 3. Change in lycopene concentrations in human plasma following a single meal of either red 'Rosalita' tomatoes (all-*trans*-lycopene) or orange 'Moonglow' tomatoes (tetra-*cis*-lycopene). * indicates statistical significance ($p<0.05$) between TCLyc Δ and ATLyc Δ means.

4 Discussion

The main objective of this study was to determine the relative bioavailability of all-*trans*-lycopene and tetra-*cis*-lycopene when tomatoes are consumed in a fresh, unprocessed state. The results show that following a single meal containing ca. 16 mg of carotene, more tetra-*cis*-lycopene is absorbed into the bloodstream than is all-*trans*-lycopene. Consequently, if these lycopene isomers have similar health effects, then the benefits associated with the consumption of lycopene may be greater when tetra-*cis*-lycopene is consumed.

In this study the source of tetra-*cis*-lycopene was the tomato cultivar 'Moonglow', which has orange, or golden/orange fruit distinct from the traditional 'red' tomato. Although most commercial tomatoes accumulate the all-*trans*-lycopene isomer, some genotypes and cultivars have a variation in the carotenoid biosynthetic genes that leads to the lycopene isomer with four 'cis' double bonds. Tomato genotypes either produce all-*trans*-lycopene or tetra-*cis*-lycopene, but not both. Analysis of 26 tomato cultivars (Table 2), and previous studies, show that tetra-*cis*-lycopene-containing cultivars are less common than all-*trans*-lycopene-containing cultivars. However, some tetra-*cis*-lycopene-containing tomato cultivars contain substantial amounts of tetra-*cis*-lycopene. Interestingly, there are yellow tomatoes, in addition to red and golden/orange tomatoes, that contain very little lycopene and their colour appears to be mainly a result of β-carotene and another carotenoid, lutein. Therefore, chemical analysis is the best method to confirm the presence of tetra-*cis*-lycopene in tomato cultivars.

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