Screening of carrot callus and suspension culture cell lines for tracer production

Table 1S. Summary of lutein yields in carrot callus culture and suspension culture. Data for each tissue type are means and SEM from 1-10 biological samples and 2 analytical (HPLC measurement) sub-samples. [a]Tissues shaded in gray were not initiated into suspension culture. [b]Only one callus line was generated from Amarillo true leaf, preventing calculation of standard deviation of yield. [c]Two of four replicates had levels of lutein below limits of quantification, while the remaining two replicates accumulated greater than 5.0 µg/g lutein. Therefore, while only the two lutein-accumulating callus lines were subsequently initiated into solution culture, the callus tissue mean is listed here. n.q., not quantifiable.

		Callus culture		**Solution culture[a]**			
		µg/g cells		**µg/g cells**		**mg/L solution**	
Varietal	**Tissue**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**
Atomic Red	Hypocotyl	0.07	0.07				
	Root	n.q.	-				
Amarillo	Hypocotyl	3.05	1.11				
	Root	0.96	0.41	4.14	0.10	1.59	0.04
	True Leaf [b]	3.24	-				
Jaune Obtuse du Doubs	Hypocotyl	6.83	3.13	5.17	0.69	0.76	0.17
	Root	1.91	0.77				
Yellow Solaris	Hypocotyl	4.10	0.30				
	Root [c]	2.55 [c]	1.47	2.85 [c]	0.55	0.69	0.09
Yellowstone	Hypocotyl	3.63	1.46	2.50	0.57	0.82	0.18
	Root	3.59	1.23	0.93	0.35	0.43	0.16
Purple Dragon	Hypocotyl	2.39	0.60				
	Root	1.64	0.66				
Purple Sun	Cotyledon	2.20	0.18				
	Hypocotyl	1.84	0.38				
	Root	1.55	0.42				
	True Leaf	2.31	0.79				

UHPLC-TQ-MS investigation of lutein-containing fractions of monkey liver and carrot extracts

Table 2S. Unlabeled lutein is detected in two LC fractions of the extracts from the liver of the ^{13}C-lutein fed monkey. We confirm the detection of lutein by multiple reaction monitoring (MRM) using seven transitions and comparing the endogenous compound with a lutein standard (xanthophyll). The results corroborate with HPLC-PDA measurements of the fractions where lutein signal was lower in the fraction #1. Correlation coefficients are calculated by comparing peak areas from the samples versus standards. The ions shown in the table have been reported by MASSBANK ((http://www.massbank.jp/jsp/Dispatcher.jsp?type=disp&id=CA000067&site=3) for fragmentation of lutein $[M]^{+\cdot}$ by FAB-EBEB). The ion at m/z 145 is one of most intense ions reported there.

Analyte	**Monitored ion**	**Targeted MRM transition**	**Peak area (a.u.)**		
			Lutein standard	**Liver, LC fraction 1**	**Liver, LC fraction 2**
Lutein	$[M]^{+\cdot}$	568.4->145	125600	8430	50800
Lutein	$[M+H-H_2O]^+$	551.5->345.3	80200	3640	39050
Lutein	$[M+H-H_2O]^+$	551.5->533	61100	3350	26070
Lutein	$[M+H-H_2O]^+$	551.5->429.5	49200	1670	17700
Lutein	$[M]^{+\cdot}$	568.4->338	23000	970	11300
Lutein	$[M+H]^+$	569.4->146	17600	1000	6050
Lutein	$[M+H]^+$	569.4->339	7320	0	5260
		Correlation coefficient		0.97	0.99

Table 3S. ^{13}C-labeled lutein is detected in an LC fraction of extract from liver of ^{13}C-lutein fed monkey. We confirm the detection of labeled lutein using MRM transitions determined by characterizing ^{13}C-lutein in carrot extract fractions (confirmed by LDI-MS and HPLC-PDA analysis) and an endogenous compound with the same m/z from the liver. The results corroborate with HPLC-PDA measurements of the fractions where lutein signal was lower in the fraction #1. Correlation coefficients are calculated by comparing peak areas from the samples versus standards. Signals of some of targeted MRM transitions were not detected, therefore, not shown in the table.

Analyte	**Monitored ion**	**Targeted MRM transition**	**Peak area (a.u.)**		
			Carrot extract, LC fraction	**Liver, LC fraction 1**	**Liver, LC fraction 2**
^{13}C lutein	$[M+H-H_2O]^+$	591.6->143.6	4724000	17890	151800
^{13}C lutein	$[M]^{+\cdot}$	608.6->123.3	3111000	0	17200
^{13}C lutein	$[M]^{+\cdot}$	608.6->389.1	111100	0	6130
^{13}C lutein	$[M+H]^+$	609.6->150.9	160990	0	3960
^{13}C lutein	$[M+H]^+$	609.6->243.5	85040	0	4320
^{13}C lutein	$[M+H]^+$	609.6->430	15330	0	3370
		Correlation coefficient		0.81	0.85

Table 4S. Unlabeled lutein is detected in two LC fractions from livers of two female rhesus macaques fed ^{13}C-lutein-free diets. We confirm the detection of lutein by MRM using seven transitions and comparing the endogenous compound with the lutein standard. Correlation coefficients are calculated for the peak areas of the targeted signals acquired in the carrot extract, the liver fractions, and the lutein standard. Signals of several targeted MRM transitions were not detected, and therefore, not shown in the table ions. Animal #2 had 10 times more unlabeled lutein in its diet than did Animal #1, which is the most likely cause for the difference in areas of lutein signal acquired from the samples.

Analyte	**Monitored ion**	**Targeted MRM transition**	**Peak area (a.u.)**			
			Lutein standard	**Carrot extract, LC fraction**	**Liver 1, LC fraction**	**Liver 2, LC fraction**
Lutein	$[M]^{+\cdot}$	568.4->145.0	320500	0	1070	5490
Lutein	$[M+H-H_2O]^+$	551.5->345.3	267400	0	451	5070
Lutein	$[M+H-H_2O]^+$	551.5->429.5	169100	0	280	2140
Lutein	$[M+H-H_2O]^+$	551.5->533	152300	41	691	2650
Lutein	$[M+H]^+$	569.4->146.0	48900	15	103	707
Lutein	$[M]^{+\cdot}$	568.4->338	48730	185	181	1260
Lutein	$[M+H]^+$	569.4->339	20720	0	69	423
Lutein	$[M]^{+\cdot}$	568.4->476.4	3980	0	0	0
Lutein	$[M+H]^+$	569.4->468	153	0	0	0

Correlation coefficients

	Lutein standard	**Carrot extract, LC fraction**	**Liver 1, LC fraction**	**Liver 2, LC fraction**
Lutein standard	1.00			
Carrot extract, LC fraction	-0.20	1.00		
Liver 1, LC fraction	0.89	-0.07	1.00	
Liver 2, LC fraction	0.99	-0.12	0.88	1.00

Table 5S. ^{13}C-labeled lutein is not detected in two LC fractions of extract from livers of two monkeys fed control diet. Conclusion is made due to low level of correlation between MRM signals of ^{13}C-lutein in carrot extract fraction (confirmed by LDI-MS and HPLC-PD) and endogenous compound with the same m/z as well as strong mismatch in most prominent ions detected for the compounds. The results corroborate with HPLC-PDA measurements. Information on all detected signals as well as background levels is shown in the table. Correlation coefficients are calculated for all peak area columns.

Analyte	**Monitored ion**	**Targeted MRM transition**	**Peak area (a.u.)**			
			Lutein standard	**Carrot extract, LC fraction**	**Liver 1, LC fraction**	**Liver 2, LC fraction**
^{13}C lutein	$[M+H-H_2O]^+$	591.6->143.6	6130	82660	105	227
^{13}C lutein	$[M]^{+\cdot}$	608.6->389.1	3250	1520	0	0
^{13}C lutein	$[M+H-H_2O]^+$	591.6->487	352	1420	0	0
^{13}C lutein	$[M+H]^+$	609.6->243.5	2440	1320	45	157
^{13}C lutein	$[M]^{+\cdot}$	608.6->123.3	10780	1030	3550	5640
^{13}C lutein	$[M+H-H_2O]^+$	591.6->503.2	298	876	68	0
^{13}C lutein	$[M+H]^+$	609.6->430	3940	871	516	438
^{13}C lutein	$[M]^{+\cdot}$	608.6->176.7	9820	637	0	79
^{13}C lutein	$[M+H]^+$	609.6->150.9	7410	385	218	414

Correlation coefficients

	Xanthophyll standard	**Carrot extract, LC fraction**	**Liver 1, LC fraction**	**Liver 2, LC fraction**
Xanthophyll standard	1.00			
Carrot extract, LC fraction	0.11	1.00		
Liver 1, LC fraction	0.58	-0.13	1.00	
Liver 2, LC fraction	0.60	-0.11	1.00	1.00