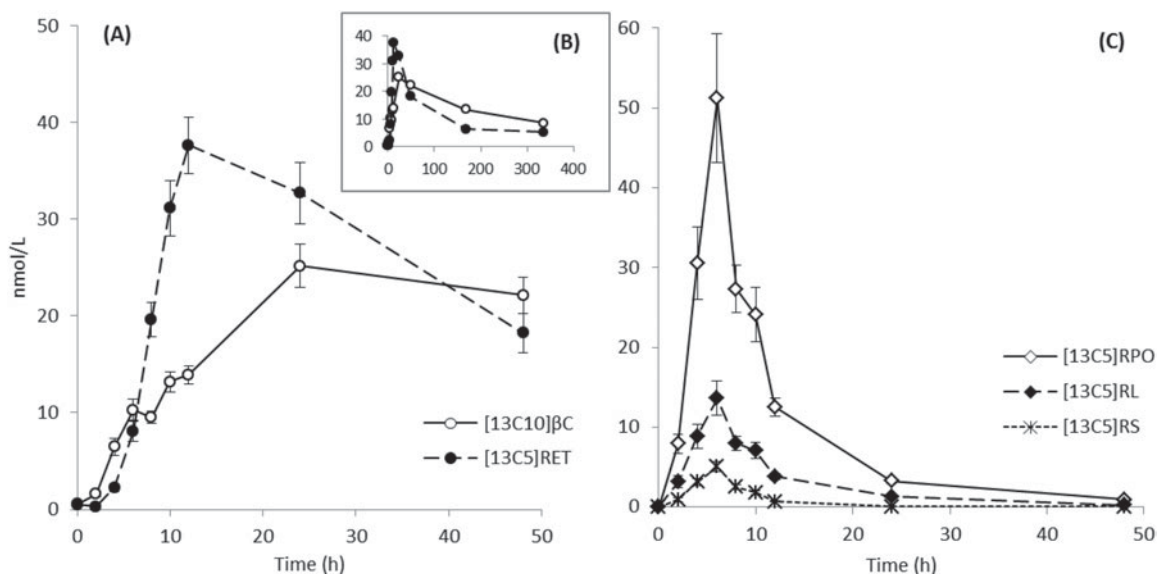


## An LC-MS/MS method for stable isotope dilution studies of $\gamma$ -carotene bioefficacy and vitamin A status in humans

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Isotope dilution is currently the most accurate technique in humans to determine vitamin A status and bioavailability/bioconversion of provitamin A carotenoids<sup>(1)</sup>. However, limits of MS detection, coupled with extensive isolation procedures, have hindered investigations of physiologically-relevant doses of stable isotopes<sup>(2)</sup>. We developed a sensitive liquid chromatography-tandem-mass spectrometry (LC-MS/MS) analytical method to study the plasma response from co-administered oral doses of 2 mg [<sup>13</sup>C<sub>10</sub>]- $\beta$ -carotene and 1 mg [<sup>13</sup>C<sub>10</sub>]-retinyl acetate in human subjects. A single one-phase solvent extraction, with no saponification or purification steps, left retinyl esters intact for determination of intestinally-derived retinol in chylomicrons versus retinol from the liver bound to retinol-binding protein (RBP).



**Fig. 1.** Quantitative LC-MS/MS analysis of mean plasma responses from 45 human subjects (+/- SEM) over the first 48 h (A, C) and whole 14 day study period (B). Administered [<sup>13</sup>C<sub>10</sub>]- $\beta$ -carotene ( $\beta$ C) and resulting [<sup>13</sup>C<sub>5</sub>] cleavage products (RET, retinol; RL, retinyl linoleate; RPO, retinyl palmitate + oleate; RS, retinyl stearate) are shown in A, B, and C.

Co-administration of [<sup>13</sup>C<sub>10</sub>]-retinyl acetate with [<sup>13</sup>C<sub>10</sub>]- $\beta$ -carotene not only acts as a reference dose for inter-individual variations in absorption and chylomicron clearance rates, but also allows for simultaneous determination of an individual's vitamin A status. In summary, this new analytical method enables the detection of physiological concentrations of provitamin A carotenoids, their cleavage products as well as preformed vitamin A for a period of at least 2 weeks post dose administration and allows high throughput analysis due to its simplicity and short run times.

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