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## Original Research

# Newly developed dietary assessment tools for lutein and zeaxanthin are correlated with 24-hour diet recalls, but are not a valid measure of intake in Australian and United Kingdom adults

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## ABSTRACT

Habitual dietary intake measurement of carotenoids lutein and zeaxanthin (L/Z) has often been omitted or attempted with tools of unknown validity in past research. It was hypothesized that the dietary assessment tool, the L/Z screener, developed as part of this study, would be valid with agreement within 0.25 mg/day when compared against multiple 24-hour diet recalls in healthy Australian and United Kingdom adults. Two screeners with 91 food items were developed, 1 with a recall timeframe of a month and the other a week. Over 4 weeks, 56 Australian and 47 United Kingdom participants completed 4 weekly screeners, 2 monthly screeners, and eight 24-hour diet recalls. Validity was assessed through Bland-Altman plot analysis. L/Z intake measured by all tools was significantly correlated, with correlation coefficients from 0.58 to 0.83. Despite these correlations, the screeners were not valid, with poor Bland-Altman plot agreement when compared with the diet recalls. The Australian weekly screener performed best, demonstrating a mean difference of 0.51 mg/day and 95% limits of agreement between -1.46 mg/day and 2.49 mg/day of L/Z intake. Baby spinach, broccoli, and pumpkin provided the greatest proportion of L/Z intake. The low validity may be explained by high rates of misestimation or missed capture of moderate to high L/Z containing foods such as baby spinach. Prior research reliant on correlational

Abbreviations: 24DR, 24-hour diet recall; CWS, combined weekly screeners; FCT, food composition tables; FFQ, food frequency questionnaire; LOA, limits of agreement; L/Z, lutein and zeaxanthin; MS, monthly screener; MS1, baseline monthly screener; MS2, week 4 monthly screener; USDA, US Department of Agriculture; WS, weekly screener.

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statistics for L/Z tool validity should be interpreted with caution, and future screener development should prioritize accurate capture of high contribution foods.

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## 1. Introduction

The 2 carotenoids, lutein and zeaxanthin (L/Z), belong to a subgroup of non-Vitamin A-forming carotenoids known as xanthophylls [1]. L/Z are not found ubiquitously across all foods. Foods rich in L/Z include leafy vegetables, broccoli, corn, eggs, and goji berries [2,3]. The ratio of L to Z is variable between foods. For example, green leafy vegetables may have 17 times more L than Z [4]. Comparatively, orange capsicums may be dominant in Z, with 5 times more Z than L [5]. In humans, L/Z have shown direct and indirect antioxidant functions, such as quenching singlet oxygen species and blue light absorption [1]. As such, dietary and supplemental intake of L/Z have been investigated for their role in ocular function, cognitive function, reducing risk of Alzheimer disease, and reducing risk and severity of age-related macular degeneration [6–8].

Populations in the highest percentile of dietary intake (upwards of 3 mg/day) or consuming an L/Z supplement (10 mg L/2 mg Z) were shown to have reduced risk or severity of age-related macular degeneration [9–11]. However, habitual dietary L/Z intake in recent observational, epidemiological, and clinical studies was often not monitored or was captured with tools not specifically validated for L/Z [11–15]. Previous attempts to validate the measurement of dietary L/Z intake have been either unsuccessful or not specific to L/Z, for example, capturing total intake of many different carotenoids rather than L/Z exclusively [16–20]. The current lack of specific and valid tools to quantitatively monitor habitual dietary L/Z intake is an identified barrier to advancing understanding of the diet–disease and dose–response relationships between L/Z and macular health [15].

Methods to capture dietary intake most commonly rely on self-report and include tools such as the 24-hour diet recall (24DR), screeners, and food frequency questionnaires (FFQ) [21]. These tools, although cost-effective and low burden for respondents, have well-established validity and reliability limitations [22,23]. One limitation is their reliance on accurate recall of intake by the respondent. Accurate reporting is limited by difficulties in estimating volumes or weights of food, high inter-day intake variability, and social desirability bias for certain foods [21,24]. Developing new tools and improving existing ones is an active area of research to assist the understanding of diet–disease relationships, especially when the focus is on specific food constituents such as L/Z.

A screener is a type of diet assessment tool designed to capture a specific or small number of nutrients and is thus appropriate for capturing episodically consumed dietary constituents [21,25]. The nonubiquitous presence and varied concentration of L/Z across foods increases the likelihood of episodic consumption [3]. This report describes the de-

velopment and validation of a dietary screener designed to quantitatively capture habitual L/Z dietary intake for use in epidemiological and intervention studies. Two formats of an L/Z screener were developed: 1 with a recall timeframe of a month (monthly screener [MS]) and the other a week (weekly screener [WS]). The aim of this study was to develop the L/Z screeners and investigate whether daily dietary L/Z intake measured by the screeners was valid with agreement within 0.25 mg/day compared with intake measured from multiple 24DRs in adults residing in Australia and the United Kingdom. Validity was tested by Bland-Altman plot analysis [26,27]. These screeners are the first tools designed specifically for L/Z and address an identified gap of questionnaire tools needed to advance the understanding of the diet–disease relationship between L/Z and macular health [15].

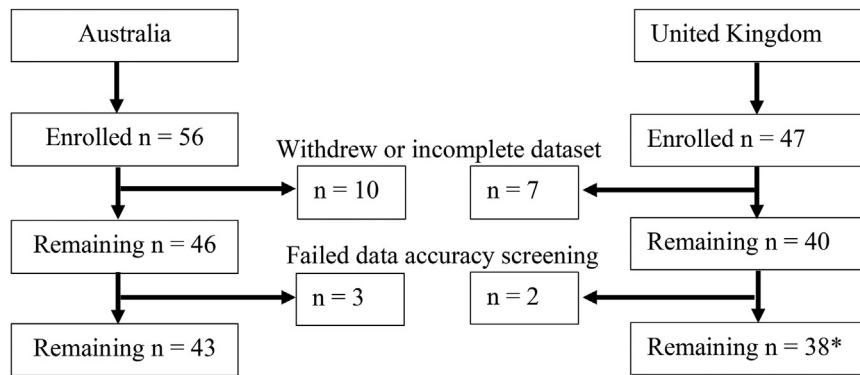
## 2. Methods and materials

Procedures for this study were in accordance with the Declaration of Helsinki and were approved by the University of Queensland Low and Negligible Risk ethics committee and the Sport and Health Sciences ethics committee at the University of Exeter (#2020001764). All participants provided written informed consent.

### 2.1. Screener development

Two formats of an L/Z screener were developed with differing timeframes based on L/Z plasma half-lives, applicability to typical intervention trial lengths, and reduction of memory recall bias [21,28–30]. Plasma half-life of L/Z has been reported to be between 5 and 76 [29–31]. Therefore, recall timeframes of 1 and 4 weeks were considered to increase the potential that the screener would closely reflect circulating plasma L/Z levels. Five factors were considered when developing the screeners: timeframe of participant recall [21,29,30,32], reference food composition tables (FCT) [3], foods to include, serving sizes [33], and frequency of intake. After initial development, an internal test of face validity was conducted with volunteers [34]. The MS and WS both contained 91 food items with defined serving sizes. Reference serving sizes were listed in both a volumetric and gram weight, for example “1 apple (165 g).” Participants could report frequency of food servings per week or per month for the MS, and solely per week for the WS. The FCTs from the United States [3] and Australia [35] were used to identify foods rich in L/Z. Foods with more than 100 µg/100 g of L/Z were prioritized for inclusion in addition to 20 foods with little or no L/Z. The inclusion of low-L/Z foods aimed to reduce social desirability bias by increasing the range of foods reported [36]. The 91 food items were a mixture of cooked and raw foods, and included 25 fruits, 39 vegetables, 6 grains, 12





**Fig. 2 – Flow chart of study participant selection and completion in the Australian and UK cohorts. In the Australian cohort, 56 participants enrolled, 10 had incomplete data, and 3 failed data accuracy screening; 43 completed. In the UK cohort, 47 participants enrolled, 7 had incomplete data, and 2 failed data accuracy screening; 38 completed. \*Indicates missing monthly screener 2 data for all UK participants.**

assess the accuracy of participant recall and identify over or underreporting as per methods described elsewhere [26,27]. As shown in Fig. 2, participant datasets were removed for identified over- or underreporting using the Goldberg cutoffs in combination with review of any participant reported reasons for unusual eating days and weight-related goals such as weight gain or loss. For the Australian and combined cohort Bland-Altman plot analysis, participants with fewer than eight 24DR or 4 CWSs were removed. For the combined cohort analysis of 4 CWSs and eight 24DR, the calculated intake difference between the tools was not normally distributed even after logarithmic base 10 transformation, except when an outlier participant reporting a difference between tools of 11.96 mg/day was removed. Results of the Bland-Altman plot analysis are presented with this outlier participant removed. For the UK cohort, participants were only removed if fewer than six 24DR or 3 CWSs were available. This increased the data available for analysis substantially because only 8 participants completed all 4 WSs and eight 24DRs. The comparison between six 24DRs and 3 CWSs was deemed appropriate because the Australian cohort showed no significant difference in intake between 6 or eight 24DRs and 3 or 4 CWSs.

## 2.8. Statistical analyses

Statistical analysis was conducted using SPSS (28.0) [41]. Results are presented both combined and individually for the Australian and UK cohorts. Data normality was tested with the Shapiro-Wilk test. Differences between cohort participant characteristics and L/Z intake were tested with a  $\chi^2$  test, 2-tailed independent samples *t*-test, or Mann-Whitney *U*-test. Because intake of L/Z from each individual food was calculated, the percentage contribution to total L/Z intake of each food group and individual food was calculated. An independent samples *t*-test for difference of means of the dietary L/Z intake reported between each of the tools was conducted. The outcomes were not significant; thus, no assumptions were violated for a Bland-Altman plot analysis. To determine validity, a Bland-Altman plot analysis of the mean daily L/Z intake was performed to compare between the 24DR, MS2, and CWSs

[42,43]. The MS2 was used such that the timeframe in which L/Z intake was recalled was aligned with intake reported from the diaries. Predetermined limits of agreement (LOA) did not exist on which to benchmark validity of the screeners. Informed by prior research, validity was therefore determined by whether the agreement with 24DR intakes was such that the screeners would have utility to detect changes in habitual intake at values that have been reported to impact macular L/Z concentrations in intervention studies. Dietary or supplemental intervention trials have reported providing as little as 0.5 mg/day L/Z and observed change to macular concentrations [12,15]. Therefore, the 95% LOA needed to be equal to or less than  $\pm 0.25$  mg/day to adequately capture any impactful fluctuations in habitual dietary intake. Cronbach alpha and 2-way mixed-effects model absolute intraclass correlation coefficient was performed for test-retest reliability between the MS1 and MS2. Normally distributed data are presented as mean  $\pm$  standard deviation and nonnormally distributed data as median and 25th to 75th percentile. Results were considered statistically significant at  $P < .05$ .

## 3. Results

### 3.1. Participant characteristics

Fifty-six Australian and 47 UK adults enrolled in the study. Ten Australian participants and 7 UK participants withdrew or failed to complete the required screeners and 24DRs (Fig. 2). The median age of Australian participants was 25 (25–29) years, 73% were female, and 64% had a tertiary education (Table 1). The median age of UK participants was 46 (40–50) years, 98% were female, and 77% had a tertiary education. The age and tertiary education status of the UK participants was significantly higher than the Australian cohort,  $P < .001$ . The analysis of UK screeners and 24DRs was a female-only cohort because the only male participant in the UK cohort did not meet the Goldberg cutoffs and was removed.

The median daily L/Z intake reported from each of the tools ranged from 2.4 to 3.3 mg for the Australian cohort and

**Table 1 – Participant characteristics of Australian and UK healthy adults.**

|                               | Australian (n = 56) | UK (n = 47)      | Combined (n = 104) |
|-------------------------------|---------------------|------------------|--------------------|
| Age, y                        | 27 (25–29)          | 46 (40–50)*      | 33 (26–48)         |
| Sex, female                   | 73%                 | 98%              | 85%                |
| BMI, kg/m <sup>2</sup>        | 24 (22.6–26.5)      | 24 (22.5–30.7)   | 24 (22–28)         |
| Physical activity, hours/week | 7 (4.9–9.0)         | 6 ± 3.9          | 7 (4–9)            |
| Education, tertiary educated  | 65%                 | 77% <sup>a</sup> | 84%                |

Abbreviation: BMI, body mass index.

Data are presented as median (25th–75th percentile), mean ± standard deviation, or a percentage.

\* Parameter significantly different between cohorts,  $P < .001$ .

**Table 2 – Daily lutein and zeaxanthin intake from the monthly diet screener, 4 combined weekly diet screeners, and 8 combined 24-hour diet recalls in Australian and UK healthy adult cohorts individually and combined.**

| Tool             | Australia |               | UK |               | Combined |               |
|------------------|-----------|---------------|----|---------------|----------|---------------|
|                  | n         | Intake        | n  | Intake        | n        | Intake        |
| MS1              | 49        | 3.2 (2.2–5.3) | 38 | 3.7 (2.1–5.4) | 87       | 3.4 (2.1–5.3) |
| MS2              | 42        | 2.7 (1.7–3.5) | -  | -             | -        | -             |
| 4 CWS            | 35        | 2.8 (2.1–4.3) | 15 | 2.8 (1.6–3.9) | 50       | 2.8 (1.9–4.3) |
| 8 combined 24DRs | 32        | 2.4 (1.6–3.1) | 9  | 2.6 ± 0.76    | 41       | 2.4 (1.6–3.1) |

Abbreviations: 24DR, 24-hour diet recalls; CWS, combined weekly screener; MS1, monthly screener 1; MS2, monthly screener 2.

Intake data presented as median (25th–75th percentile) or mean ± standard deviation mg/day of lutein and zeaxanthin.

2.6 to 3.7 mg for the UK cohort (Table 2). Within a cohort, daily dietary L/Z intake captured by each tool was significantly correlated (Table 3). The strongest correlation was in the Australian cohort between the MS2 and CWSs:  $R = 0.83$ ,  $R^2 = 0.75$  ( $P < .001$ ). There was also strong correlation between the Australian MS1 and MS2:  $R = 0.81$ ,  $R^2 = 0.75$  ( $P < .001$ ). The weakest correlation was between the CWSs and 24DRs in the UK cohort:  $R = 0.62$ ,  $R^2 = 0.11$  ( $P = .002$ ).

### 3.2. Comparison of screeners with 24DRs

The Bland-Altman plot analyses indicated poor agreement of daily L/Z intake between the screeners and 24DRs, with modest mean differences but large 95% LOA (Table 3). In the Australian cohort, between the CWSs and MS, the CWSs had better agreement with the 24DRs. Participants were more likely to report higher L/Z intake with the CWSs compared with the 24DRs, with a mean difference of 0.51 mg/day and 95% LOA of -1.46 to 2.49 mg/day. The Bland-Altman plot analysis between the MS2 and 8 combined 24DRs indicated a mean difference in daily L/Z intake of 0.33 mg/day and 95% LOA of -2.91 to 3.58 mg/day (Table 3). Seven participants reported a mean L/Z intake above 4 mg/day (Fig. 3A). Three of these 7 participants reported differences between the 2 tools greater than the 95% LOA. A small number of outlier differences were also present in the UK cohort. Three UK participants reported much higher intakes in the CWSs compared with the 24DRs with differences of 5.59 mg/day, 6.16 mg/day, and 11.96 mg/day.

The MS in the Australian cohort indicated a high test-retest reliability with a Cronbach  $\alpha = 0.86$  and 2-way mixed-effects model absolute intraclass correlation coefficient of 0.85. Despite being highly correlated, when divided into tertiles, there

was differences in classification of at least 30% in either direction between all tools (see Supplemental materials).

The contribution to L/Z intake from all food groups was consistent between the 2 screeners and cohorts (Table 4). The vegetable food group contributed the most to total L/Z dietary intake, with the contribution ranging from 87% to 91%. Fruits and the meat and alternatives groups were the next highest contributing sources, contributing between 3% and 6% to total L/Z intake.

The foods that contributed the most to total L/Z intake were similar between the Australian and UK cohorts (Table 5). In the Australian cohort, baby spinach contributed the most with between 13% and 22% to total L/Z intake across the screeners. Additionally, baby spinach, cooked pumpkin, and cooked broccoli combined made up approximately one quarter (23%–31%) of total L/Z intake across the screeners. Other contributing foods included cooked zucchini, carrot, lettuce, and cooked egg. In the UK cohort, the major contribution was more evenly distributed among 6 foods, with cooked broccoli, cooked green peas, baby spinach, and lettuce combined contributing 19% to 22% of total L/Z intake across the screeners. Other high-contribution foods included cooked egg and cooked and raw orange carrot.

## 4. Discussion

Intakes reported between the screeners and 24DRs indicated poor agreement via Bland-Altman plot analysis but significant moderate correlations (Table 3). The 95% LOA of the MS and CWSs compared with the 24DRs were at minimum greater than 0.25 mg/day, indicating that the screeners were

**Table 3 – Agreement of mean daily lutein and zeaxanthin intake between the monthly diet screener, combined weekly screeners, and multiple combined 24-hour diet recalls determined by Bland-Altman plot analysis in Australian and UK healthy adults.**

| Tool comparison       |   | Mean difference <sup>h</sup> | Lower 95% LOA <sup>h</sup> | Higher 95% LOA <sup>h</sup> | R      | R <sup>2</sup> |
|-----------------------|---|------------------------------|----------------------------|-----------------------------|--------|----------------|
| AU                    | MS2 vs 24DR <sup>(6)</sup>                | 0.33                         | -2.91                      | 3.58                        | 0.58*  | 0.35           |
|                       | (n = 31) <sup>a</sup>                     | (0.00–0.67)                  | (-3.24 to -2.58)           | (3.24–3.91)                 |        |                |
|                       | CWS <sup>(4)</sup> vs 24DR <sup>(6)</sup> | 0.51                         | -1.46                      | 2.49                        | 0.70*  | 0.67           |
|                       | (n = 28) <sup>b</sup>                     | (0.00–1.03)                  | (-1.97 to -0.95)           | (1.97–3.00)                 |        |                |
|                       | MS2 vs CWS <sup>(4)</sup>                 | -0.48                        | -2.4                       | 1.45                        | 0.83*  | 0.75           |
| (n = 34) <sup>c</sup> | (-0.95 to 0.00)                           | (-2.88 to -1.93)             | (0.98–1.93)                |                             |        |                |
| MS1 vs MS2            | 0.65                                      | -3.21                        | 4.51                       | 0.81*                       | 0.59   |                |
|                       | (n = 42) <sup>d</sup>                     | (0.00–1.3)                   | (-3.86 to -2.56)           | (3.86–5.17)                 |        |                |
| UK                    | CWS <sup>(3)</sup> vs 24DR <sup>(6)</sup> | 1.32                         | 0.37                       | 4.64                        | 0.62** | 0.12           |
|                       | (n = 23) <sup>e, f</sup>                  | (1.00–1.74)                  | (0.28–0.49)                | (3.52–6.11)                 |        |                |
| CC                    | CWS <sup>(4)</sup> vs 24DR <sup>(6)</sup> | 1.23                         | 0.57                       | 2.66                        | 0.75*  | 0.57           |
|                       | (n = 35) <sup>g</sup>                     | (1.00–1.51)                  | (0.46–0.69)                | (2.17–3.27)                 |        |                |

Abbreviations: 24DR, 24-hour diet recall; AU, Australia; CC, combined cohorts; CI, confidence interval; CWS, combined weekly screeners; MS1, monthly screener 1; df, degrees of freedom; LOA, limits of agreement; MS2, monthly screener 2; SEM, standard error of the mean; <sup>(4)</sup>mean intake per day from the 4 weekly screeners, <sup>(6)</sup> mean intake per day from the eight 24-hour diet recalls, <sup>(3)</sup> mean intake per day from 3 or more weekly screeners, <sup>(6)</sup> mean intake per day from 6 or more 24-hour diet recalls.

<sup>a</sup> AU MS2 vs 24DR<sup>(6)</sup>: SEM = 0.30, t value (30 df) = 1.12.

<sup>b</sup> AU CWS<sup>(4)</sup> vs 24DR<sup>(6)</sup>: SEM = 0.19, t value (27 df) = 2.70.

<sup>c</sup> AU MS2 vs CWS<sup>(4)</sup>: SEM = 4.7, t value (33 df) = -2.8.

<sup>d</sup> AU MS1 vs MS2: SEM = 8.5, t value (41 df) = 2.1.

<sup>f</sup> UK CWS<sup>(3)</sup> vs 24DR<sup>(6)</sup>: SEM = 0.06, t value (22 df) = 2.06.

<sup>g</sup> CC CWS<sup>(4)</sup> and 24DR<sup>(6)</sup>: SEM = 0.03, t value (38 df) = 3.07.

<sup>h</sup> Data presented as mg/day (95% CI).

<sup>^</sup> Bland-Altman plot analysis values back transformed after Log10 transformation.

\* P < .001.

\*\* P = .002.

**Table 4 – Percentage contribution to total lutein and zeaxanthin intake by the 6 food groups from the monthly diet screeners and combined weekly diet screeners in Australian and UK healthy adults.**

| Food group                             | Australia                     |                               |                               | United Kingdom                 |                               | Combined cohorts              |                               |
|--|-------------------------------|-------------------------------|-------------------------------|--------------------------------|-------------------------------|-------------------------------|-------------------------------|
|  | MS1                           | MS2                           | CWS <sup>(4)</sup>            | MS1                            | CWS <sup>(3)</sup>            | MS1                           | CWS <sup>(4)</sup>            |
| Vegetables                             | 89.7 (80.7–93.0) <sup>a</sup> | 91.2 (85.5–92.4) <sup>a</sup> | 89.2 (80.9–92.3) <sup>a</sup> | 88.0 (80.7–91.3) <sup>a</sup>  | 87.1 (82.1–92.6) <sup>a</sup> | 88.3 (81.0–92.3) <sup>a</sup> | 87.1 (82.2–91.9) <sup>a</sup> |
| Fruits                                 | 3.1 (1.1–7.0) <sup>b</sup>    | 3.5 (1.7–5.6) <sup>b</sup>    | 3.4 (2.1–5.2) <sup>b</sup>    | 5.7 (2.0–10.1) <sup>b</sup>    | 5.0 (2.5–8.5) <sup>b</sup>    | 4.1 (1.5–8.8) <sup>b</sup>    | 3.8 (2.3–7.1) <sup>b</sup>    |
| Grains                                 | 1.6 (0.8–3.2) <sup>b</sup>    | 1.5 (0.9–2.8) <sup>b</sup>    | 2.0 (1.1–3.3) <sup>b</sup>    | 1.8 (1.1–2.9) <sup>c</sup>     | 2.4 ± 1.5 <sup>c</sup>        | 1.7 (0.9–2.9) <sup>c</sup>    | 2.0 (1.2–3.1) <sup>c</sup>    |
| Meat and alternatives                  | 3.2 (1.8–6.1) <sup>b</sup>    | 3.9 (1.9–6.0) <sup>b</sup>    | 4.5 (2.7–7.9) <sup>b</sup>    | 3.3 (1.6–6.0) <sup>b</sup>     | 3.5 (1.8–7.1) <sup>b</sup>    | 3.3 (1.8–6.0) <sup>b</sup>    | 4.6 (2.7–7.8) <sup>b</sup>    |
| Milk, yogurt, cheese, and alternatives | 0.3 (0.1–0.6) <sup>c</sup>    | 0.3 (0.1–0.5) <sup>c</sup>    | 0.3 (0.2–0.7) <sup>c</sup>    | 0.3 (0.0–0.5) <sup>d</sup>     | 0.3 (0.1–0.8) <sup>d</sup>    | 0.3 (0.1–0.6) <sup>d</sup>    | 0.3 (0.2–0.7) <sup>d</sup>    |
| Discretionary foods                    | 0.3 (0.2–0.6) <sup>c</sup>    | 0.3 (0.2–0.6) <sup>c</sup>    | 0.4 (0.3–0.7) <sup>c,1</sup>  | 0.2 (0.1–0.4) <sup>d,1,2</sup> | 0.4 (0.1–0.7) <sup>d</sup>    | 0.3 (0.1–0.4) <sup>d</sup>    | 0.4 (0.3–0.7) <sup>d,2</sup>  |

Abbreviations: CWS, combined weekly screeners; L/Z, lutein and zeaxanthin; MS1, monthly screener 1; MS2, monthly screener 2; <sup>(4)</sup> 4 combined weekly screeners; <sup>(3)</sup> 3 or more combined weekly screeners.

Data presented as median (25th–75th percentile) or mean ± standard deviation percentage (%) contribution to total L/Z intake.

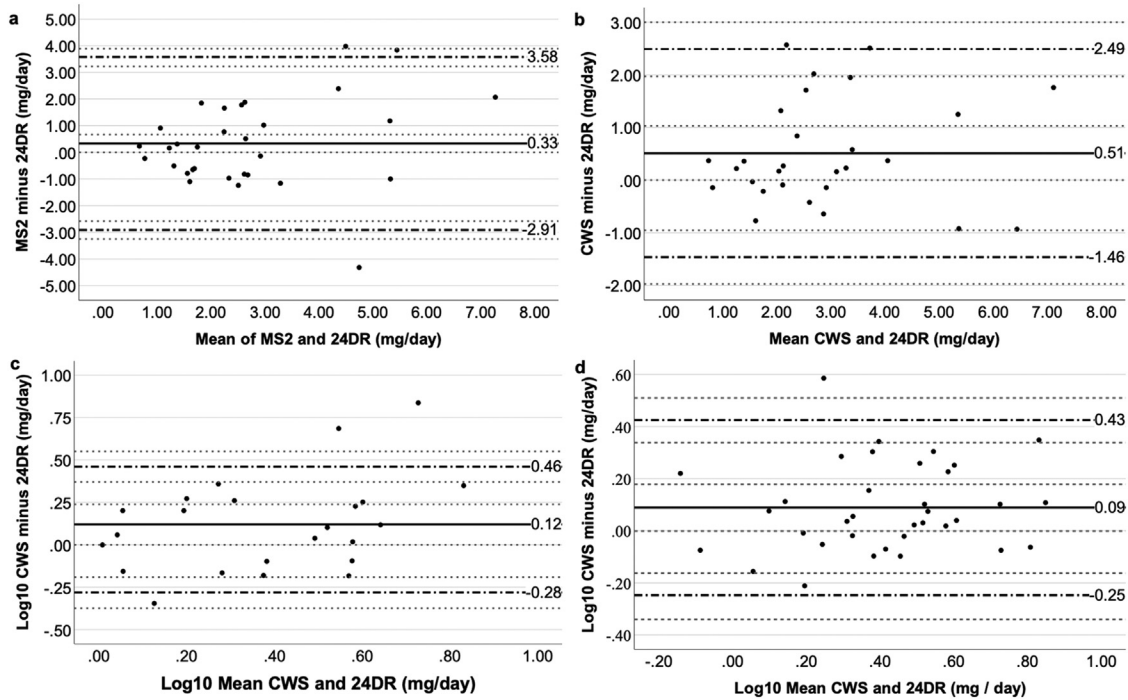
<sup>a, b, c, d</sup> Within a column, cells with the same superscript letter were not significantly different to each other.

<sup>1,2</sup> Indicate within a row a significant difference between tools with the same number.

not valid in the population observed [12,15]. The WS agreed best with the 24DRs, reporting a mean difference of 0.51 mg/day and 95% LOA between -1.46 and 2.49 mg/day in the Australian cohort. There was no clear trend in the direction of differences reported between any of the tools. The mean differences between the tools were trending toward the screeners reporting higher L/Z intakes compared with the 24DRs. This aligns with similar studies comparing an FFQ

or screener intake against 24DRs or diet records outlined later [17,18]. The median dietary L/Z intake of the combined cohorts was between 2.4 and 3.4 mg/day (Table 2). This intake aligns with mean intakes of 0.5 to 4.5 mg/day measured by FFQ in previous Western country populations [11,44–46].

The MS and WS had poor validity for ranking participants by intake. High misclassification rates of 38% to adjacent tertiles were observed with the CWSs when ranked by the MS2



**Fig. 3 – Bland-Altman plot analyses demonstrating poor agreement of daily dietary lutein and zeaxanthin intake between the monthly screener, combined weekly screeners, and multiple combined 24-hour diet recalls. (A) Australian second monthly screener versus 8 combined 24-diet recalls. (B) Australian 4 combined weekly screeners versus 8 combined 24-hour diet recalls. (C) United Kingdom log base 10 transformed 3 or more combined weekly screeners versus 6 or more combined 24-hour diet recalls. (D) Combined cohort log base 10 transformed 4 combined weekly screeners versus eight 24-hour diet recalls. For each figure, black solid line indicates the mean difference, the black dashed lines indicate the 95% limits of agreement, and the gray dashed and dotted lines indicate the 95% confidence intervals for mean difference and 95% limits of agreement. Abbreviations: 24DR, 24-hour diet recalls; CWS, combined weekly screeners; Log10, logarithmic base 10; MS2, second monthly screener.**

**Table 5 – Top 6 ranked foods in percentage contribution to total lutein and zeaxanthin intake from the monthly diet screeners and combined weekly screeners in Australian and UK healthy adults.**

| Tool |     | 1st             | 2nd                     | 3rd            | 4th                     | 5th                    | 6th                    |                        |
|------|-----|-----------------|-------------------------|----------------|-------------------------|------------------------|------------------------|------------------------|
| AU   | MS1 | Food            | B. spinach <sup>b</sup> | Broccoli       | Pumpkin                 | Zucchini               | O. carrot <sup>b</sup> |                        |
|      |     | %               | 17.6 (2.8–26.8)         | 5.4 (2.1–8.8)  | 4.2 (0.0–10.9)          | 4.0 (0.0–8.1)          | 2.6 (0.0–7.0)          | Lettuce <sup>b c</sup> |
|      | MS2 | Food            | B. spinach <sup>b</sup> | Pumpkin        | Broccoli                | O. carrot <sup>b</sup> | Zucchini               | Lettuce <sup>b c</sup> |
|      | %   | 21.9 (0.0–30.1) | 5.0 (0.0–10.2)          | 4.5 (1.7–9.1)  | 2.9 (0.0–9.3)           | 2.7 (0.0–5.4)          | 2.6 (0.0–6.1)          |                        |
|      | CWS | Food            | B. spinach <sup>b</sup> | Pumpkin        | Broccoli                | Egg                    | Lettuce <sup>b c</sup> | Zucchini               |
|      | %   | 13.6 (5.6–35.3) | 5.8 (0.0–12.1)          | 4.2 (0.7–8.9)  | 3.1 (1.2–4.8)           | 2.6 (0.3–6.1)          | 2.5 (0.0–7.0)          |                        |
| UK   | MS1 | Food            | Broccoli                | Green peas     | B. spinach <sup>b</sup> | Lettuce <sup>b c</sup> | O. carrot              | Egg                    |
|      |     | %               | 6.8 (3.2–12.4)          | 5.0 (2.5–10.5) | 3.7 (0.0–5.8)           | 3.4 (0.0–9.3)          | 2.6 (1.2–4.9)          | 2.6 (0.9–4.6)          |
|      | CWS | Food            | Broccoli                | Green peas     | Lettuce <sup>b c</sup>  | O. carrot <sup>b</sup> | B. spinach             | O. carrot              |
|      | %   | 7.7 (4.1–9.9)   | 5.7 (1.4–14.7)          | 5.3 (2.4–10.2) | 5.0 (0.8–7.7)           | 3.6 (0.0–10.1)         | 2.7 (1.6–5.9)          |                        |
| CC   | MS1 | Food            | B. spinach <sup>b</sup> | Broccoli       | Green peas              | O. carrot <sup>b</sup> | Egg                    | Egg                    |
|      |     | %               | 14.0 (0.0–22.2)         | 7.8 (2.8–1.4)  | 3.8 (0.0–6.7)           | 2.6 (0.0–5.9)          | 2.2 (0.4–3.6)          | 2.1 (1.0–4.5)          |
|      | CWS | Food            | B. spinach <sup>b</sup> | Broccoli       | Lettuce <sup>b c</sup>  | Egg                    | O. carrot <sup>b</sup> | B. spinach             |
|      | %   | 8.1 (0.0–22.6)  | 5.8 (2.4–9.4)           | 3.2 (1.5–7.1)  | 3.0 (1.3–4.9)           | 2.4 (0.0–7.0)          | 2.3 (0.0–9.9)          |                        |

Abbreviations: B, baby; CC, combined; CWS, combined weekly screeners; MS1, monthly screener 1, MS2, monthly screener 2; O, orange. Data presented as median (25th–75th percentile).

<sup>b</sup> Indicates a raw food, all other foods in cooked form.

<sup>c</sup> Type of lettuce Cos or Romaine.

(see Supplemental materials). The inability to rank participants into tertiles between MS2 and CWSs indicates that these 2 screeners cannot be used interchangeably. Logarithmic base 10 transformation and reliability testing of the MS1 and MS2 data resulted in a normal data distribution, a Cronbach alpha of 0.88, and absolute intraclass correlation coefficient of 0.78. Despite a high absolute intraclass correlation coefficient, the 31% misclassification observed between the MS1 and MS2 was higher than previous similar validation research [18]. In the validation study by Satia et al. [18], an FFQ with a recall timeframe of a month ranked participants intakes into quartiles. Of all antioxidant nutrients investigated, the range of classification into the same or adjacent quartile was between 65% and 89%, and only 0% to 12% misclassification into the opposite quartile [18]. Exact rates of misclassification for L/Z were not reported. The multidirectional high misclassification of the MS and WS observed in the present study indicates the screeners were not able to rank participants consistently by intake and are thus not valid for ranking participants in intervention or observational study designs.

Previous validation studies have returned poor tool validity when attempting to capture total dietary or antioxidant intake, sometimes inclusive of L/Z [16–20]. Comparison with prior studies is difficult because of the frequent use of correlation statistics rather than assessing agreement through a Bland-Altman plot analysis. Similar to the present study, prior research has often relied on the USDA FCT to calculate L/Z dietary intake [17,18]. A study in 28 Australian adults compared an FFQ with a 6-month recall timeframe with 12 days of diet records completed over 1 year. Mean  $\pm$  standard deviation daily L/Z intake reported from the diet records and FFQ were  $0.52 \pm 0.26$  mg and  $1.63 \pm 1.17$  mg, respectively. The reported intakes were significantly correlated, with a correlation coefficient of 0.40 ( $P < .05$ ). Plasma L/Z was also measured and used to report a validity coefficient calculated by the method of triads. The low validity coefficient (95% confidence interval) for L/Z of 0.19 (0.05–0.71) indicated that the FFQ did not provide a valid measure of L/Z intake [17]. The small sample size and misaligned timeframes of dietary data collection were proposed as explanations for the poor validity. The diet records were completed after the FFQ and plasma measurement. In the present study, the timeframes of dietary data collection were more closely aligned with the WS and MS. Participants were asked to recall intake over the same timeframe, during which the 24DRs were collected. This closer alignment is reflected in the higher correlation coefficients of 0.58 ( $P < .001$ ) between the MS and 24DRs and 0.70 ( $P < .001$ ) between the CWSs and 24DRs in the Australian cohort. Another study that used closely aligned recall timeframes was conducted in 81 White and 83 African American adults. It compared the data from an FFQ with a recall timeframe of 1 month against 4 telephone-administered 24DRs. Two of the 24DRs were completed on a weekday and 2 on weekend days in the month preceding the FFQ. Median (25th–75th percentile) daily L/Z intake reported by the FFQ was 3.03 (1.61–4.84) mg for White participants and 1.94 (1.06–3.98) mg for African American participants. Median (25th–75th percentile) daily L/Z intake reported by the 24DRs was 2.41 (1.20–3.69) for White participants and 1.63 (0.93–2.91) for African American participants. The significant adjusted correlation coefficient between the 2 tools was

0.49 for White participants and 0.51 for African American participants,  $P \leq .0001$  [18]. Intake representative of 1 month may have been difficult to capture with just four 24DRs because of inter-day intake variability [22]. In the present study, the large number of 24DR days captured may explain the stronger correlations observed between tools. The Australian and combined cohorts CWSs and 24DRs comparison indicated correlation coefficients of 0.70 ( $R^2 = 0.67$ ) and 0.75 ( $R^2 = 0.57$ ), respectively. The moderate correlation but poor Bland-Altman agreement observed raises concerns regarding the utility of results obtained in prior L/Z validation studies reliant on correlational statistics. The linear relationship between 2 dietary intake tools measuring the same component as demonstrated by correlation statistics is arguably not enough to demonstrate validity [42]. Unlike a Bland-Altman plot, correlation statistics do not provide an indication of the bias between tool differences or an indication as to what degree of difference is appropriate [43]. As demonstrated in this study, the MS and CWSs were both moderately correlated with the 24DRs; however the Bland-Altman plot demonstrated poor agreement, reasons for that poor agreement, and therefore the tools' invalidity. Without the use of a Bland-Altman plot, correlation statistics would have overestimated the validity of the MS and WS. Prior L/Z or antioxidant questionnaire validation studies, solely reliant on correlational statistics to determine validity, should be interpreted with caution. The absence of a validated tool to capture habitual dietary L/Z intake remains a barrier to understanding the diet–disease and dose–response relationships between dietary L/Z intake and conditions such as age-related macular degeneration. It also precludes identifying a daily dietary intake recommendation for L/Z [15].

The poor Bland-Altman agreement and the screeners' inability to rank participants by intake compared with the 24DRs may be explained by misestimation or missed capture of a small subset of foods such as those listed in Table 5. Misestimation refers to the incorrect recall of the amount or frequency of intake of a food. Missed capture refers to true intake of a food not being captured because of the timeframe being observed through a particular tool. The misestimation or missed capture of foods may partially explain the emerging trends of higher L/Z intakes being reported through FFQ or screener tools compared with 24DR or diet record tools. Some of these foods, including baby spinach, are high-L/Z concentration foods that are sporadically consumed in amounts difficult to estimate by volume or weight. The misestimation or missed capture of such foods was particularly obvious in participants reporting high consumption of L/Z. Seven Australian participants reported a combined MS2 and 24DR mean daily L/Z intake greater than 4 mg/day and were more likely to report larger differences in intake between the MS2 and 24DR. Three of these 7 participants reported differences between the 2 tools greater than the 95% LOA (Fig. 3A). These larger differences occurred through poor agreement in reported vegetable consumption, particularly green leafy vegetables. For example, the participant with a difference of  $-4.32$  mg/day between the MS and 24DRs reported that 90% of L/Z intake was from vegetables in the MS2. The top 3 foods had 34.6% from cooked frozen baby spinach, 16.5% from cooked kale, and 14.9% from raw baby spinach. Similarly, 3 UK participants reported



high L/Z intake and large differences between the CWSs and 24DRs. The differences in L/Z intake between the CWSs and 24DRs for these 3 participants were 5.59 mg/day, 6.16 mg/day, and 11.96 mg/day. These differences related to green leafy vegetables (kale, baby spinach, rocket, silver beet), broccoli, green pea, and carrot intake. More representative capture of these high-contribution foods is needed in future validation attempts.

Understanding how errors have occurred is necessary to improving how intake is captured more accurately from these vegetables. Differences may have occurred through repeat errors with moderate concentration foods such as carrot (0.3 mg cooked and 0.7 mg raw of L/Z per 100 g food) or infrequent errors with high-concentration foods such as baby spinach (>0.6 mg/100 g L/Z) [3]. The impact of misestimating intake of a high-concentration food such as baby spinach can be observed in 1 participant's reported WS and MS intake. Across the 4 WSs completed, this participant reported 5 servings of baby spinach, equaling a total of 13.3 mg of L/Z for the month. Comparatively, in the MS2 this participant only reported 4 servings of baby spinach; a total of 10.6 mg L/Z and difference of 2.7 mg (or 0.1 mg/day) to the CWSs. The difference in baby spinach intakes reported between the CWSs and MS2 demonstrates the impact of memory recall bias and difficulty in estimating food volumes [21,24]. In particular, green leafy vegetables appear to pose an issue. Their inclusion in mixed dishes, their light but voluminous nature in a raw state, and stark volume shrinkage when cooked, make it difficult for participants to estimate intake weight or volume in metric cups. To improve the validity of the MS and WS, the inclusion of a photographic atlas with real-size food portions, including portion in multi-ingredient dishes, to visually assist participants when estimating food intake is justified [44,47].

Missed capture of impactful foods such as baby spinach, must also be addressed to improve the validity of the screeners. The previously mentioned participant who reported 4 or 5 baby spinach servings over the month also demonstrated a likely example of missed capture. The total number of baby spinach serves reported from all eight 24DRs combined was only 1.75 servings. The 24DRs may have underestimated mean L/Z intake over the month. In this case, poor agreement between the 24DRs and screeners occurred because of the presence of an irregularly consumed food and utilization of a dietary intake method that did not adequately capture habitual intake [21,48]. It appears 8 nonconsecutive days of 24DRs over 4 weeks was insufficient to capture interday variation in dietary L/Z intake. Missed capture of L/Z intake by the 24DRs may mean the validity of screeners has been underestimated and that the screeners may actually be better at capturing habitual dietary L/Z intake than the Bland-Altman plot analysis suggested. Future studies would be strengthened with the addition of a biological marker such as blood L/Z concentration to correlate with the L/Z intake captured by the screeners [17]. Additionally, the dietary intake method selected to perform relative validity against the screeners should consider the impact of missed capture observed in this study. Future studies should consider balancing participant burden, and the benefit from capturing greater number of days of dietary intake through 24DRs or alternative methods such as nonconsecutive repeated 3-day diet records [49].

The limitations of this study include the origin of the FCT used for intake analysis, the substantial missing data and attrition rates, low demographic diversity of the cohort, and the lack of a biological marker. The use of the USDA FCT is a limitation to determining accurate intake because the data on food composition likely differs from the local food supply of the study cohorts. It may differ for many reasons, including variation in plant cultivar, growing and food storage conditions, and extraction and analysis methods to determine concentrations [50–52]. The use of the USDA FCT was unavoidable in this study because of the paucity of data about L/Z concentrations in the Australian and UK food composition databases [3,35,53]. Using local data is critical to accurately represent intake. For example, cooked green peas have an L/Z value of 2590 µg/100 g in the USDA FCT (identification 170420), 1134 µg/100 g in the UK McCance and Widdowson's dataset (food code 13-527), and 620 µg/100 g in the Australian FSANZ table (identification F006538). The missing data and high attrition rates limited the strength of validity testing across all tools and cohorts. The goal of 30 or more participants per the Australian and UK cohort was only achieved for the comparison of the MS2 against the 24DR and MS1 against the MS2 in the Australian cohort. The high study burden was reported as a reason for attrition. Additionally, the predominantly female and tertiary-educated characteristics of participants who did complete the study are not generalizable to the overall Australian or UK population. Finally, measuring concomitantly blood L/Z concentration as a biological marker was not considered because of COVID-19 pandemic restrictions at data collection. Future research to validate the screeners should aim to capture a more diverse population and include a biological marker of L/Z intake to allow for the triad method of validation. To reduce participant burden, the use of less intensive dietary intake collection tools spaced out over a longer timeframe such as 6, 12, or 24 months could be considered. The longer timeframe would also allow for greater likelihood of capturing habitual intake because L/Z-containing foods were observed to be episodically consumed in this study, and consumption may change seasonally across the year.

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## 5. Conclusion

A valid tool to capture habitual dietary L/Z intake is important to progressing the understanding of the diet–disease and dose–response relationships between dietary L/Z intake and conditions such as age-related macular degeneration [15]. These L/Z specific screeners were not valid, demonstrating poor agreement and ability to rank participants according to intake compared with L/Z intake derived from multiple 24DRs. Dietary L/Z intake between the screeners and 24DRs for the Australian and UK cohorts both Individually and combined were moderately to strongly correlated. Despite significant correlations, the Bland-Altman plots indicated that participants were unable to accurately recall intake of L/Z containing foods, particularly green leafy vegetables. The phenomenon of strong correlation but poor Bland-Altman plot agreement observed in this study suggests that results from prior research reliant only on correlation statistics must be interpreted with caution. Only a small number of foods,

such as baby spinach and broccoli, contributed markedly to dietary L/Z intake in this study. Accurate representation of these high contribution foods in local FCT and capture of intake through screeners should be the focus of future validation attempts. In addition, to improve the validity of the screeners, future studies would benefit from a larger, more diverse study sample, a lower participant burden study design to reduce attrition rates, the addition of a photographic atlas to assist with accurate food volume estimation, the use of a local FCT data, and the use of a concomitant biological marker.

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### Author Declarations

None.

### Data availability statement

The dataset supporting the conclusions of this article is available in the UQeSpace repository and is permitted for reuse with a share alike requirement, <https://doi.org/10.48610/f9416b1>

### CRedit authorship contribution statement

**Naomi Kathleen Fitzpatrick:** Conceptualization, Resources, Methodology, Investigation, Formal analysis, Data curation, Writing – original draft, Writing – review & editing, Visualization, Project administration. **Sandra Capra:** Conceptualization, Investigation, Resources, Supervision, Validation, Writing – review & editing. **Angela Shore:** Conceptualization, Resources, Supervision, Validation, Writing – review & editing. **David Briskey:** Conceptualization, Resources, Supervision, Validation, Writing – review & editing. **Sarah Jackman:** Conceptualization, Resources, Supervision, Validation, Writing – review & editing. **Joanna Bowtell:** Conceptualization, Resources, Supervision, Validation, Writing – review & editing. **Veronique Chachay:** Conceptualization, Investigation, Project administration, Resources, Supervision, Validation, Writing – review & editing.

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### Supplementary materials

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