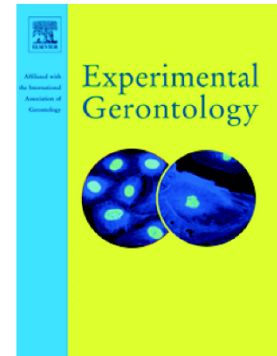


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Plasma lutein and zeaxanthin concentrations associated with musculoskeletal health and incident frailty in The Irish Longitudinal Study on Ageing (TILDA)

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None.

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ABSTRACT

Introduction: Lutein and zeaxanthin are diet-derived carotenoids that are proposed to help mitigate frailty risk and age-related declines in musculoskeletal health via their anti-oxidant and anti-inflammatory properties. Therefore, this study aimed to investigate the association between lutein and zeaxanthin status and indices of musculoskeletal health and incident frailty among community-dwelling adults aged ≥ 50 years in the Irish Longitudinal Study on Ageing (TILDA).

Methods: Cross-sectional analyses ($n = 4,513$) of plasma lutein and zeaxanthin concentrations and grip strength, usual gait speed, timed up-and-go (TUG), probable sarcopenia (defined as grip strength < 27 kg in men, < 16 kg in women), and bone mass (assessed using calcaneal broadband ultrasound stiffness index) were performed at Wave 1 (2009 – 2011; baseline). In the longitudinal analyses ($n = 1,425 - 3,100$), changes in usual gait speed (at Wave 3, 2014 - 2015), grip strength (Wave 4, 2016) and timed up-and-go (TUG; Wave 5, 2018), incident probable sarcopenia (at Wave 4) and incident frailty (Fried's phenotype, Frailty Index, FRAIL Scale, Clinical Frailty Scale-classification tree, at Wave 5) were determined. Data were analysed using linear and ordinal logistic regression, adjusted for confounders.

Results: Cross-sectionally, plasma lutein and zeaxanthin concentrations were positively associated with usual gait speed (B [95% CI] per 100-nmol/L higher concentration: Lutein 0.59 [0.18, 1.00], Zeaxanthin 1.46 [0.37, 2.55] cm/s) and inversely associated with TUG time (Lutein -0.08 [-0.11, -0.03], Zeaxanthin -0.14 [-0.25, -0.04] s; all $p < 0.01$), but not with grip strength or probable sarcopenia ($p > 0.05$). Plasma lutein concentration was positively associated with bone stiffness index (0.54 [0.15, 0.93], $p < 0.01$). Longitudinally, among participants who were non-frail at Wave 1, higher plasma lutein and zeaxanthin concentrations were associated lower odds of progressing to a higher frailty category (e.g. prefrailty or frailty) by Wave 5 (ORs 0.89 – 0.57,

$p < 0.05$) based on the Fried's phenotype, FRAIL Scale and the Clinical Frailty Scale, and in the case of zeaxanthin, Frailty Index. Neither plasma lutein nor zeaxanthin concentrations were associated with changes in musculoskeletal indices or incident probable sarcopenia ($p > 0.05$).

Conclusion: Higher plasma lutein and zeaxanthin concentrations at baseline were associated with a reduced likelihood of incident frailty after ~8 years of follow up. Baseline plasma lutein and zeaxanthin concentrations were also positively associated with several indices of musculoskeletal health cross-sectionally but were not predictive of longitudinal changes in these outcomes over 4-8 years.

Keywords: carotenoids, ageing, frailty, musculoskeletal health, nutrition, sarcopenia

INTRODUCTION

Ageing is associated with declines in musculoskeletal health including reductions in skeletal muscle mass and function, declines in bone mineral density, and the microstructural deterioration of bone tissue. These adverse age-related changes in skeletal muscle and bone can lead to the diseases of sarcopenia and osteoporosis, respectively, resulting in the increased risk of poor physical performance, physical disability, falls, fractures, and reduced quality of life [1,2]. Furthermore, sarcopenia is a major contributor to frailty, a multidimensional geriatric syndrome that is characterised by declines in multiple physiological systems and increased susceptibility to stressors [3,4].

It is well established that adequate nutrition, in addition to other healthy lifestyle factors (e.g. physical activity), plays an important role in mitigating musculoskeletal declines [5,6] and frailty risk [7] in ageing. Healthier dietary patterns that are rich in fruit and vegetables are associated with better preservation of musculoskeletal health [8-10] and lower frailty incidence [11-13] in older adults in observational studies. Carotenoids represent a class of phytochemicals present in fruit and vegetables that may contribute to these health-promoting effects. Carotenoids are colourful pigments that possess anti-oxidant and anti-inflammatory properties [14]. As carotenoids cannot be synthesised endogenously, the carotenoid concentrations of blood and other tissues provide a biomarker of carotenoid dietary intake, which occurs predominantly via fruit and vegetables consumption [14]. Plasma carotenoid concentrations are concentration biomarkers, meaning that while they cannot be used to estimate absolute dietary intakes of carotenoids, they correlate with dietary intake and can be used to rank individuals [15]. The major carotenoids found in human blood can be broadly classified into carotenes (β -carotene, α -carotene, lycopene) and xanthophylls (lutein, zeaxanthin, and β -Cryptoxanthin) [14]. Lutein and

zeaxanthin have long been implicated in improving visual function and attenuating disease progression in patients with age-related macular degeneration [16,17]. Emerging evidence now suggests that these compounds may also play a protective role in other conditions with an inflammatory and oxidative stress aetiology, including frailty, sarcopenia, and osteoporosis.

In a cross-sectional study, Semba et al. observed that lower plasma lutein and zeaxanthin concentrations were associated with impaired grip, hip, and knee strength in community-dwelling older women [18]. Furthermore, plasma lutein and zeaxanthin concentrations were recently shown to be inversely associated with prefrailty and frailty, across several frailty instruments (Fried's Phenotype [19], the Rockwood Frailty Index [20], FRAIL scale [21]), in a cross-sectional analysis of The Irish Longitudinal Study of Ageing (TILDA) [22]. These findings are consistent with two further cross-sectional studies showing that circulating lutein and zeaxanthin concentrations were associated with Fried-defined frailty in European and North American older populations [23,24]. However, data on the longitudinal association between circulating lutein and zeaxanthin concentrations and changes in musculoskeletal health and incident frailty are limited. Moreover, the relationship between carotenoid status and sarcopenia has never been investigated, to our knowledge. Therefore, the aims of this study were to investigate: 1) the cross-sectional and longitudinal associations between baseline plasma lutein and zeaxanthin concentrations and indices of musculoskeletal health and probable sarcopenia, and 2) the longitudinal association between baseline plasma lutein and zeaxanthin concentrations and incident frailty, among community-dwelling older adults in TILDA.

METHODS

Design and setting

This study analysed data from TILDA, a nationally representative prospective cohort of community-dwelling adults age ≥ 50 years in the Republic of Ireland. An overview of the study is available at: <https://tilda.tcd.ie/about/where-are-we-now/>. The full study sampling procedures and cohort profile have been described previously [25,26]. Briefly, Wave 1 (baseline) of data collection was conducted between October 2009 and July 2011, and subsequent data was collected approximately biannually over four longitudinal Waves (Wave 2: February 2012 to March 2013; Wave 3: March 2014 to October 2015; Wave 4: January to December 2016; Wave 5: January to December 2018). Waves 1 and 3 included a detailed health assessment conducted at a health centre. Waves 2, 4 and 5 were non-health centre Waves, during which a briefer battery of assessments were completed in participants' homes. At Wave 1, 8,173 adults aged ≥ 50 years completed a computer-aided personal interview, representing a response rate of 62%. Approximately 72% ($n = 5894$) of participants who took part in the interviews also participated in a health assessment, of which 26% ($n = 5655$) provided blood samples for biobank archiving. Study participants provided written informed consent in accordance with the Declaration of Helsinki guidelines, and ethical approval was granted for each Wave by the Faculty of Health Sciences Research Ethics Committee at Trinity College Dublin, Ireland.

Analytic sample

The cross-sectional analysis (Wave 1) included participants with a valid lutein and zeaxanthin plasma concentration measurement, complete data on the cross-sectional outcome measures (grip strength, usual gait speed, TUG performance, and bone stiffness index), and complete data on

relevant covariates at Wave 1 ($n = 4,513$; Figure 1). In the longitudinal analyses of the musculoskeletal outcomes, we included participants from the cross-sectional analytic sample who had data on the respective outcome at follow up, which was Wave 3 for usual gait speed and bone stiffness index, Wave 4 for grip strength, and Wave 5 for TUG (Figure 1). For longitudinal analyses of incident probable sarcopenia, only participants who did not have probable sarcopenia at Wave 1 were included (Figure 1). Finally, for the outcome of frailty, a cross-sectional analysis of its relationship with lutein and zeaxanthin plasma concentrations was not performed, as this has been reported previously [22]. However, novel longitudinal analyses of the association between lutein and zeaxanthin plasma concentrations and incident frailty at Wave 5 were conducted. These analyses included participants with complete data on frailty status at Wave 1 and Wave 5 using all four frailty instruments (Fried's phenotype, the Rockwood Frailty Index, Clinical Frailty Scale-classification tree, and FRAIL Scale), who had a valid lutein and zeaxanthin plasma concentration measurement at Wave 1, who had complete data on relevant covariates, and who were non-frail at Wave 1 based on all four instruments ($n = 1,425$, Figure 2). For the 9-point CFS, non-frail was defined as a score of < 4 . Further details on the four frailty measures as operationalised in TLDA are described below and elsewhere [22,27].

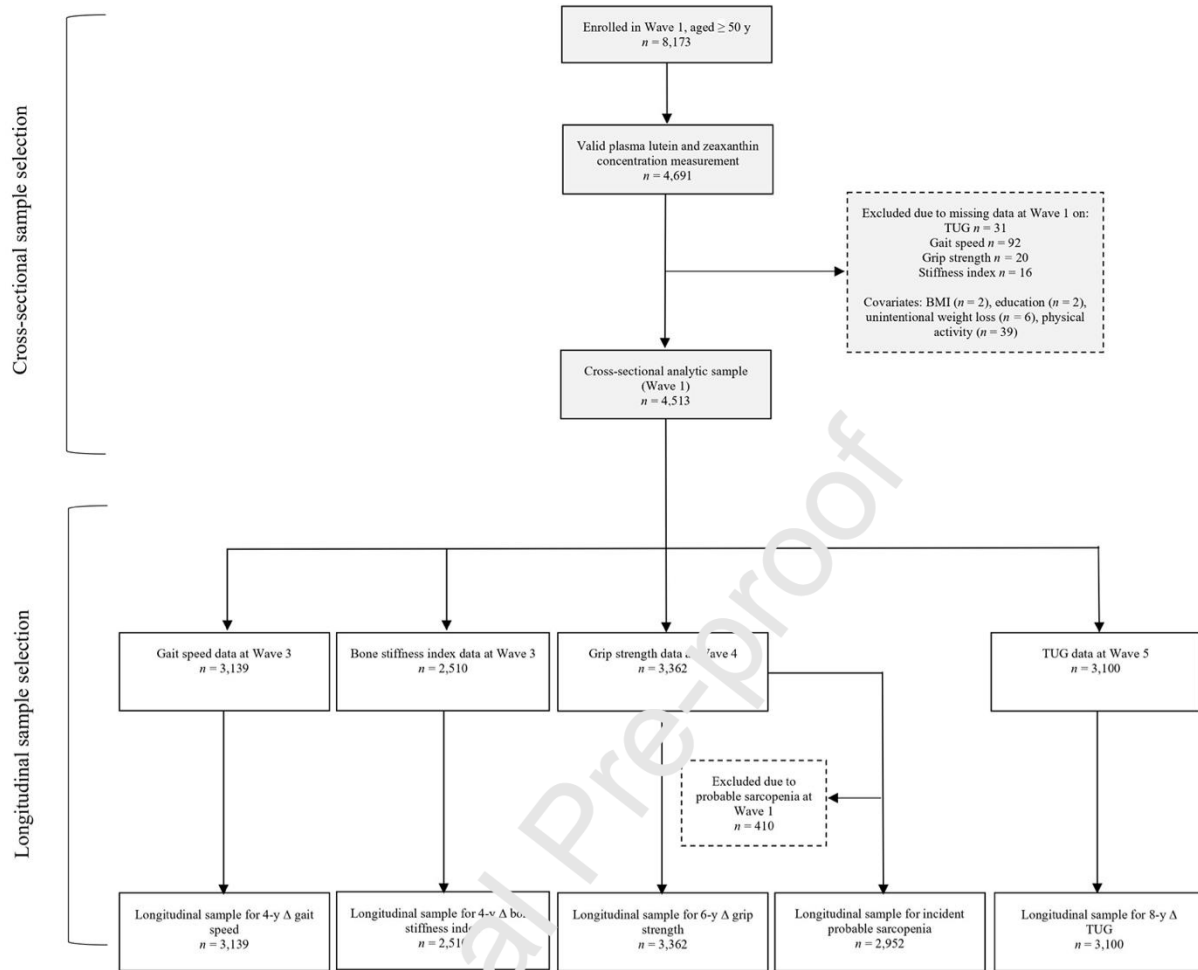


Figure 1. Participant flow chart for the cross-sectional and longitudinal musculoskeletal outcome analyses. TUG, timed up-and-go.

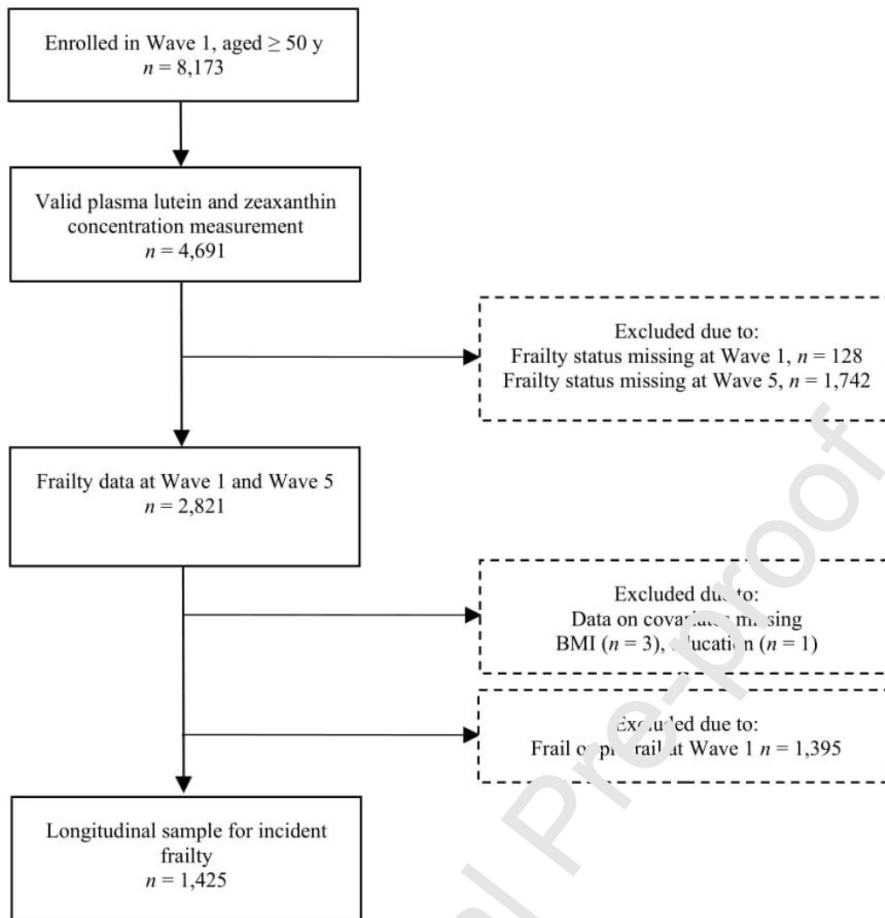


Figure 2. Participant flow chart for the incident frailty analyses.

Plasma lutein and zeaxanthin concentrations

Non fasting venous blood samples were collected between 09:30 and 16:30 into 10 mL K₂EDTA tubes (BD, Becton, Dickinson Limited, Oxford, UK) during the health assessments at Wave 1. Samples were covered in tin foil to protect from direct light and were transported at 2°C to 8°C. Plasma and Buffy coats were separated from the blood samples within 48 hours of collection and stored at -80°C until analysed. The carotenoids lutein and zeaxanthin were measured by the reversed phase high performance liquid chromatography method. Details of extraction procedures and high performance liquid chromatography analysis were previously described [28]. Average inter-assay coefficients of variation (CV) were 4.0% and 6.8% for lutein and zeaxanthin, respectively.

Strength and physical performance

Handgrip strength was measured using a hydraulic hand dynamometer (Baseline, Fabrication Enterprises, Inc., White Plains, NY, USA) while the participant was standing with the forearm positioned at a right angle to the upper arm. Respondents with swelling, inflammation, severe pain, or recent injury to their hand/wrist, and those with surgery to their hand/wrist in the last 6 months were excluded from handgrip strength measurements. At Wave 1, during the health assessment, maximum grip strength was measured from four tests (two on each hand). At Wave 4, during the home assessment, only one measurement was taken using the respondent's dominant hand. Probable sarcopenia was defined using the 2019 European Working Group on Sarcopenia in Older People (EWGSOP2) criteria [1] as grip strength < 16 kg for women and < 27 kg for men.

TUG was measured according to standard protocols [29]. Briefly, participants were asked to rise from the chair, walk 3 metres at normal pace to a line clearly marked on the floor, turn around, walk back to the chair, and sit down again. Walking aids were allowed if required, and no instructions were given about the use of participants' arms. At the Wave 1 health assessments that took place in the health centre, the TUG was measured using a chair with armrests and a seat of height of 46 cm. At Wave 5, the TUG was performed in the participants' own homes using an available chair that matched these dimensions as closely as possible (seat height 40–50 cm). Usual gait speed was measured using a 4.88-m electronic walkway with embedded pressure sensors (GAITRite, CIR Systems, Inc., Havertown, PA, USA) during health assessments at Wave 1 and Wave 3. Participants started 2.5 m before and finished 2 m after the walkway to allow for acceleration and deceleration. Average gait speed was calculated from two walks performed at normal pace.

Bone health

Bone mass was measured at Waves 1 and 3 during the health assessments using quantitative ultrasound of the heel bone (Achilles Heel Ultrasound, Lunar, Madison, WI, USA).

Measurements were taken barefoot using the non-dominant foot. The Achilles Heel Ultrasound measures the broadband ultrasound attenuation and the speed of sound through bone which are combined to provide an index of bone stiffness. This has been shown to be a significantly better predictor of fractures than broadband ultrasound attenuation or speed of sound alone [30].

Frailty

Frailty was assessed using four widely used instruments: Fried's frailty phenotype, Frailty Index, FRAIL Scale and Clinical Frailty Scale-classification tree. Fried's frailty phenotype is defined by exhaustion, unexplained weight loss, weakness, slowness, and low physical activity [19]. Three or more components define frailty, and one or two define prefrailty. The operationalization of Fried's frailty phenotype was conducted using TILDA population-specific cut-points, as described previously [22,31]. The Frailty Index operationalises frailty as an accumulation of age-related deficits including symptoms, health behaviours, clinical signs, diagnoses, and functional limitations which plausibly contribute to poor health [20]. A 32-item Frailty Index was constructed using self-reported health measures available in TILDA, as described previously [22]. Scores of <0.10 , 0.10 to <0.25 and ≥ 0.25 were classified as non-frail, prefrail, and frail, respectively. The FRAIL scale is based on self-reported responses on five items: fatigue, resistance, ambulation, illnesses, and weight loss. Three or more components define frailty, and one or two define prefrailty. The criteria published in the original model [21] were adapted based on self-reported measures collected during TILDA interviews, as described previously [22]. The CFS summarises the overall level of frailty based on specific geriatric dimensions including multimorbidity, physical function, physical activity, level of dependency, and cognition to generate a frailty score ranging from 1 (very fit) to 9 (terminally ill). The CFS was operationalised in TILDA according to the published CFS classification tree, as described previously [27].

Covariates

Directed acyclic graphs (DAGs) were constructed using online Dagitty software (<http://www.dagitty.net>) [32] to identify confounding factors in the association between the

exposure (Wave 1 plasma lutein and zeaxanthin concentrations) and the outcomes (Supplementary figures 1 and 2) [33]. DAGs provide a structured, visual representation of the causal research question and of the related causal assumptions that can be used identify variables that, if controlled for, are sufficient to resolve confounding [34]. Compared to traditional methods for identifying confounding, DAGs improve transparency by making underlying assumptions explicit [34]. The DAG-derived covariates included in the models of the cross-sectional and longitudinal association between plasma lutein and zeaxanthin concentrations and grip strength, physical performance and bone health were: age, sex, BMI, education, physical activity, number of chronic diseases, alcohol consumption, smoking, and unintentional weight loss, all assessed at Wave 1. As weight loss represents one of the components of frailty defined using Fried's phenotype and FRAIL Scale, weight loss was not included as a covariate in models exploring the relationship between plasma lutein and zeaxanthin concentrations and frailty, however the other covariates were the same as listed for the outcomes above (Supplementary Figure 2). Self-reported age (years, continuous), sex (male/female), highest level of educational attainment (primary/secondary/tertiary level), and information on smoking habits (current smoker yes/no) and weight loss (self-reported unintentional weight loss of 4.5 kg or more in past year, yes/no) were derived from a computer-aided personal interview. Participants were asked about their alcohol intake using a self-completion questionnaire. Based on their responses, participants categorised into one of four groups according to the Irish Department of Health low risk alcohol guidelines: alcohol non-consumers (0 standard units/week), low risk alcohol consumer (<11 standard units/week in women, < 17 standard units/week in men), high risk alcohol consumer (\geq 11 standard units/week in women, \geq 17 standard units/week in men), or alcohol nonresponse (no response provided by the participant to alcohol questions). One standard unit was calculated as

half a pint of beer, 100 mL of wine, or a standard pub measure (35.5 mL) of spirits; equivalent to 10 g of pure alcohol. Height was measured using a wall-mounted measuring rod (SECA 240, SECA, Birmingham, UK) and body mass was measured using electronic floor scales (SECA, Birmingham, UK) during the health assessment, and were used to derive body mass index (kg/m^2). Chronic conditions were collected by self-reported doctor's diagnosis. The number of chronic conditions, based on lung disease, asthma, arthritis, osteoporosis, cancer, Parkinson's disease, peptic ulcer, hip fracture, heart disease (heart attack or heart failure or angina), cataracts, hypertension, high cholesterol, stroke, and diabetes, was included in the analyses as a continuous variable. Physical activity was measured in MET minutes (continuous) using the short form International Physical Activity Questionnaire [35].

Plasma 25-hydroxyvitamin D [25(OH)D] concentration and plasma c-reactive protein (CRP) concentration, measured in non-fasting samples collected during the Wave 1 health assessment, were included as covariates in the sensitivity analyses. Concentrations of 25(OH)D (D_2 and D_3) were quantified by LC-MS/MS (API 4000; AB SCIEX). CRP concentrations were measured on a Roche Cobas c 701 analyser with a proprietary immunoturbidimetric assay (Roche Diagnostics Ireland, Tina-quant® C-Reactive Protein 3rd Gen). Information on nutritional supplement use was collected in the computer-aided personal interview and was used as a descriptive variable but not included as a covariate in any of the models.

Statistical analyses

Multivariate linear regression models were used to examine the cross-sectional associations between plasma lutein and zeaxanthin concentrations and maximal grip strength, usual gait speed, TUG, calcaneal stiffness index at Wave 1 (2010), as well the longitudinal associations

between Wave 1 plasma lutein and zeaxanthin concentrations and the change over time in usual gait speed (at Wave 3, 2014), grip strength (Wave 4, 2016) and timed up-and-go (TUG; Wave 5, 2018). For each linear regression model, homoscedasticity of residuals and the presence of a linear relationship between the outcome variable and the independent variables were checked using scatterplots. P-P plots were used to verify that residuals were approximately normally distributed. Binary logistic regression models were used to examine the association between plasma lutein and zeaxanthin concentrations and probable sarcopenia (yes/no) at Wave 1, as well as incident probable sarcopenia at Wave 4. In each binary logistic regression model, linearity of continuous variables with respect to the logit of the dependent variable were assessed via the Box-Tidwell procedure. In all linear and logistic regression analyses, multicollinearity was determined by inspecting the variance inflation factor (VIF). All VIFs were < 2.5 [36].

Ordinal logistic regression was used to interrogate the associations between Wave 1 lutein and zeaxanthin concentrations and incident prefrailty/frailty, defined using Fried's phenotype, the Frailty Index, and the CES-classification tree, at Wave 5. Separate models were used for each frailty instrument. Where the assumption of proportional odds was violated, as assessed using a full likelihood ratio test comparing the fit of the proportional odds model to a model with varying location parameters, multinomial regression was used as a sensitivity analysis. Due to the low incidence of frailty at Wave 5 according to the FRAIL Scale instrument, the prefrail and frail groups were pooled, and binary logistic regression was performed instead of ordinal logistic regression. In all cases, separate models were conducted for lutein and zeaxanthin. All models were fully adjusted for covariates, as outlined under the "covariates" section.

Four sensitivity analyses were performed. The first sensitivity analysis determined the effect of removing outliers. The second sensitivity analysis determined the effect of including plasma CRP concentration at Wave 1 as a covariate, to assess whether changes in systemic inflammation mediated the relationship between plasma lutein and zeaxanthin concentrations and musculoskeletal and frailty outcomes. In the case of bone stiffness index, a third sensitivity analysis was performed to determine the effect of including plasma 25-hydroxyvitamin D [25(OH)D] concentration at Wave 1 as a covariate. Finally, in a fourth sensitivity analysis, repeated measures analyses were performed whereby the longitudinal associations between Wave 1 plasma lutein and zeaxanthin concentrations and the musculoskeletal and frailty outcomes over the follow up period were analysed via generalized estimating equations (GEE). Data from the intermediate Waves were included in the GEE analyses (e.g. in the case of the frailty outcomes data from Waves 1 – 5 were included). Grip strength was excluded from the repeated measures analyses due to variability regarding the calibration of the dynamometers across TILDA waves, making direct comparison of the raw values across waves problematic. Data analyses were performed using SPSS (version 27.0, Chicago, IL, USA). Results were considered statistically significant at $p < 0.05$.

RESULTS

Analytical compared to excluded sample

The characteristics of the participants included in the cross-sectional analyses (at Wave 1) are shown in Table 1. Participants had a mean age of 62 ± 8 years and were 53% female. On average, participants were well functioning, based on the comparison of their mean grip strength, TUG, and gait speed measurements to the EWGSOP cut-off points for sarcopenia (grip strength < 16 kg for women and < 27 kg for men, TUG ≥ 20 s, gait speed ≤ 0.8 m/s [37]). The prevalence of probable sarcopenia was 14%. The prevalence of prefrailty was 30%, 31%, and 18%, and prevalence of frailty was 2%, 10%, and 1% based on the Fried's phenotype, Frailty Index and FRAIL Scale tools, respectively. In addition, 23% of participants had scores of 4 (very mild frailty) to 7 (severe frailty) on the 9-point CFS-classification tree.

Compared to the excluded sample, the cross-sectional analytical sample was younger, had a lower proportion of females, were less likely to be current smokers, had higher levels of physical activity and educational attainment, and a greater proportion reported taking nutritional supplements. The cross-sectional analytical sample also had lower levels of frailty, prefrailty, probable sarcopenia and chronic conditions, better grip strength and TUG performance, but lower usual gait speed, compared to the excluded sample. In addition, a greater proportion of participants were categorised as having low risk and high risk alcohol consumption in the analytical sample, whereas more participants in the excluded sample reported being alcohol non-consumers or did not respond to the alcohol consumption questions. There were no differences in BMI.

TABLE 1

Characteristics of participants included in the cross-sectional analysis at Wave 1

	Total	Men	Women
<i>n</i>	4,513	2,102	2,411
Age (%)			
50 - 64 y	64.5	62.1	66.5
65 - 74 y	26.8	29.0	24.9
≥ 75 y	8.7	8.8	8.6
BMI (kg/m ²)	28.5 ± 4.9	29.1 ± 4.4	28.0 ± 5.1
Education (%)			
Primary / none	21.7	24.2	19.6
Secondary	42.0	41.3	42.6
Tertiary	36.3	34.5	37.8
Physical activity (%)			
Low	27.1	22.2	30.4
Moderate	36.2	32.2	39.6
High	36.7	44.5	29.9
Alcohol			
Non consumer (%)	20.4	17.1	23.2
Low risk consumer (%)	56.0	56.6	55.5
High risk consumer (%)	11.1	13.5	9.1
Did not respond (%)	12.5	12.8	12.2
Current smokers (%)	14.7	14.7	14.8
Number of chronic conditions (%)			
0	23.8	26.2	21.7
1	29.7	29.7	28.3
≥2	47.2	44.1	50.0
Lutein (nmol/L)	209 ± 131	190 ± 109	225 ± 146
Zeaxanthin (nmol/L)	57 ± 49	52 ± 42	61 ± 54
Handgrip strength (kg)	27.5 ± 9.8	35.3 ± 8.0	20.8 ± 5.1
TUG (s)	8.6 ± 2.0	8.6 ± 2.0	8.6 ± 2.0
Usual gait speed (cm/s)	136 ± 21	138 ± 20	134 ± 21
Stiffness index	90 ± 19	98 ± 19	83 ± 17
Unintentional weight loss (%)	6.0	5.2	6.7
Probable sarcopenia (%)	14.4	13.3	15.3
Prefrail			
Fried (%) [†]	29.7	28.5	30.8
Frailty index (%)	30.5	28.6	32.1
FRAIL scale (%) ^{††}	18.3	14.9	21.3
Frail			
Fried (%) [†]	1.8	1.7	1.9
Frailty index (%)	9.5	8.4	10.5
FRAIL scale (%) ^{††}	1.2	1.3	1.2
CFS classification tree [‡]			
CFS1 – Very fit (%)	11.7	12.1	11.4
CFS2 – Fit (%)	43.0	45.8	40.5
CFS3 – Managing well (%)	22.5	17.8	26.6
CFS4 – Very mild frailty (%)	14.3	15.0	13.6

CFS5 – Mild frailty (%)	2.2	1.7	2.7
CFS6 – Moderate frailty (%)	5.7	6.9	4.6
CFS7 – Severe frailty (%)	0.6	0.7	0.5
Nutritional supplements (%)			
≥1	18.8	11.1	25.6

Values are mean \pm SD or (%). [†] $n = 4482$ ($n = 2092$ men, $n = 2390$ women), ^{††} $n = 4510$ ($n = 2101$ men, $n = 2409$ women), [‡] $n = 4511$ ($n = 2101$ men, $n = 2410$ women). CFS, Clinical Frailty Scale; TUG, timed up-and-go.

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Cross-sectional associations between plasma lutein and zeaxanthin concentrations and musculoskeletal health

Plasma lutein and zeaxanthin concentrations were positively associated with usual gait speed and TUG performance (all $p < 0.01$), but not with maximal grip strength ($p > 0.05$; Table 2) at Wave 1. In addition, neither plasma concentration of lutein (OR 0.95, 95% CI 0.89, 1.02, $p = 0.17$, per 100 nmol/L increase) nor zeaxanthin (OR 0.95, 95% CI 0.78, 1.14, $p = 0.56$, per 100 nmol/L increase) were cross-sectionally associated with probable sarcopenia. Lutein, but not zeaxanthin, concentration was positively associated with calcaneal stiffness index at Wave 1 ($p < 0.01$; Table 2), indicative of a positive relationship with bone mass. In the sensitivity analyses, removing outliers or including plasma CRP concentration as a covariate in the model did not influence the results for any of the cross-sectional outcomes (Supplementary Table 1 & 2). In the case of bone stiffness index, including plasma 25(OH)E concentration at Wave 1 as a potential confounder in the model did not impact the results (Supplementary Table 3).

TABLE 2

Multivariate regression analysis of the cross-sectional associations between plasma lutein and zeaxanthin concentrations and musculoskeletal health outcomes at Wave 1 in older community-dwelling participants

	Lutein			Zeaxanthin		
	B	95% CI	p-value	B	95% CI	p-value
Gait speed (cm/s)	0.59	0.18, 1.00	<0.01	1.46	0.37, 2.55	<0.01
Stiffness index	0.54	0.15, 0.93	<0.01	0.86	-0.17, 1.90	0.10
Grip strength (kg)	0.10	-0.04, 0.25	0.16	0.04	-0.33, 0.42	0.82
TUG (s)	-0.07	-0.11, -0.03	<0.01	-0.14	-0.25, -0.04	<0.01

Models were adjusted for age, sex, smoking status, BMI, alcohol intake, physical activity, unintentional weight loss, number of chronic diseases and education. CI, confidence interval. Data were analysed using multiple linear regression. Separate regression models were used for each outcome (*i.e.* grip strength, gait speed, TUG, and stiffness index). B coefficients were calculated per 100 nmol/L higher plasma concentration. TUG, timed up-and-go.

Longitudinal associations between plasma lutein and zeaxanthin concentrations and changes in musculoskeletal health

The 4-year change in usual gait speed (at Wave 3) was -2.46 ± 14.65 cm/s, the 4-year change in stiffness index (at Wave 3) was -1.41 ± 9.54 , the 6-year change in grip strength (at Wave 4) was -0.36 ± 5.82 kg, and the 8-year change in TUG (at Wave 5) was 2.23 ± 3.06 s, respectively.

There were no associations between Wave 1 plasma lutein and zeaxanthin concentrations and the longitudinal changes in usual gait speed, grip strength, TUG or stiffness index over follow up ($p > 0.05$, Table 3). Among participants who did not have probable sarcopenia at Wave 1, after 6 years of follow up the incidence of probable sarcopenia was 9.1 %. There were no associations between plasma concentrations of lutein (OR 1.01, 95% CI 0.91, 1.11, $p = 0.90$, per 100 nmol/L increase) or zeaxanthin (OR 1.06, 95% CI 0.83, 1.27, $p = 0.66$, per 100 nmol/L increase) and incident probable sarcopenia. In the sensitivity analyses, removing outliers or including plasma CRP concentration as a covariate in the model did not influence the results regarding the association between plasma lutein and zeaxanthin and the change in musculoskeletal outcomes (Supplementary Table 4) or incident sarcopenia (Supplementary Table 5). Furthermore, including plasma 25(OH)D concentration at Wave 1 as a covariate in the model of the association between plasma lutein and zeaxanthin and the 4-year change in stiffness index did not impact the results (Supplementary Table 6). In the fourth sensitivity analysis, the relationship between plasma lutein and zeaxanthin concentrations and the repeated measures of musculoskeletal outcomes over the follow-up period were analysed using generalised estimating equations (GEE). Similar to the primary analyses, Wave 1 plasma lutein and zeaxanthin concentrations were not associated with gait speed, stiffness index (Supplementary Table 7).or probable sarcopenia (Supplementary Table 8) over the follow up. However, in contrast to the

primary analyses, the GEE analyses showed that higher Wave 1 plasma lutein and zeaxanthin concentrations were associated with lesser decline in TUG performance over the 8 years of follow-up, although the treatment effect was small (Supplementary Table 8).

TABLE 3

Multivariate regression analysis of the longitudinal associations between Wave 1 plasma lutein and zeaxanthin concentrations and changes in musculoskeletal health outcomes over 4 – 8 years in older community-dwelling participants

	Lutein			Zeaxanthin		
	B	95% CI	p-value	B	95% CI	p-value
Gait speed (4-y Δ)	-0.06	-0.48, 0.36	0.78	0.01	-1.04, 1.05	0.99
Stiffness index (4-y Δ)	0.03	-0.29, 0.35	0.84	0.04	-0.74, 0.83	0.92
Grip strength (6-y Δ)	-0.03	-0.19, 0.13	0.74	-0.11	-0.51, 0.29	0.59
TUG (8-y Δ)	-0.05	-0.13, 0.04	0.28	-0.13	-0.33, 0.08	0.22

Models were adjusted for age, sex, smoking status, BMI, alcohol intake, physical activity, unintentional weight loss, number of chronic diseases and education. Data were analysed using multiple linear regression. Separate regression models were used for each outcome (*i.e.* grip strength, gait speed, TUG, and stiffness index). B coefficients were calculated per 100 nmol/L higher plasma concentration. CI, confidence interval. Δ , change.

Longitudinal association between plasma lutein and zeaxanthin concentrations and incident frailty

The frailty analyses included participants who were non-frail at Wave 1. The incidence of prefrailty was 34.6%, 25.3%, and 11.2%, and incidence of frailty was 2.5%, 2.3% and 0.1% based on the Fried's phenotype, Frailty Index and FRAIL Scale tools, respectively, after 8 years of follow up at Wave 5. In addition, 6.6% of participants developed scores of 4 (very mild frailty) to 6 (living with moderate frailty) on the 9-point CFS-classification tree at Wave 5. No participants in the analytical sample developed CFS-classification tree scores > 6. Increases in plasma lutein and zeaxanthin concentrations at Wave 1 (per 100 nmol/L increase) were associated with a decrease in the odds of progressing to a higher frailty category at Wave 5, based on the Fried's Phenotype, FRAIL Scale and CFS-classification tree instruments (all $p < 0.05$, Table 4). Increased zeaxanthin concentrations were also associated with a decrease in the odds of progressing to a higher Frailty Index-defined frailty category at Wave 5 ($p = 0.01$), however the association between lutein concentration and Frailty Index-defined frailty category did not reach statistical significance ($p = 0.06$, Table 4). Progressing to a higher frailty category can be interpreted as developing incident prefrailty or frailty defined by the Fried's phenotype, FRAIL Scale and Frailty Index instruments, or an increased score on the 9-point CFS.

In the ordinal logistic analyses, the assumption of proportional odds was met, except for in the plasma lutein and Fried-defined frailty model. Therefore, as a sensitivity analysis, a multinomial logistic regression analysis was performed. This showed that lutein concentration was associated incident Fried-defined frailty (OR 0.41, 95% CI 0.24, 0.68, $p < 0.01$, per 100 nmol/L increase), but not prefrailty (OR 0.93, 95% CI 0.84, 1.04, $p = 0.21$, per 100 nmol/L increase). Removing outliers or including plasma CRP concentrations as a covariate did not alter

the frailty outcome results (Supplementary Table 9). When the data were analysed via GEE, Wave 1 plasma lutein and zeaxanthin concentrations were associated with lower odds of progressing to a higher frailty category according to all four frailty instruments across the 8 years of follow up (Supplementary Table 10).

TABLE 4

Multivariate regression analysis of the longitudinal associations between Wave 1 plasma lutein and zeaxanthin concentrations and frailty status after 8 y of follow up based on the Fried's Phenotype, CFS-classification tree, Frailty Index, and FRAIL Scale instruments

	Lutein			Zeaxanthin		
	OR	95% CI	p-value	OR	95% CI	p-value
Fried	0.89	0.80, 0.99	0.03	0.72	0.56, 0.94	0.01
CFS	0.87	0.79, 0.95	0.01	0.73	0.60, 0.89	0.01
Frailty index	0.89	0.79, 1.01	0.06	0.68	0.50, 0.92	0.01
FRAIL scale	0.80	0.67, 0.96	0.01	0.57	0.35, 0.93	0.02

Models were adjusted for age, sex, smoking status, BMI, alcohol intake, physical activity, number of chronic diseases and education. Data were analysed via ordinal logistic regression except for the FRAIL Scale data which were analysed via binary logistic regression. Only $n = 2$ participants were classified as having incident frailty using the FRAIL Scale, so the prefrail and frail groups were pooled for analysis. Separate regression models were used for each frailty instrument. ORs were calculated per 100 nmol/L higher plasma concentration. CFS, Clinical Frail Scale.

DISCUSSION

This study from a large longitudinal cohort of community-dwelling older adults demonstrated that lower plasma concentrations of lutein and zeaxanthin were predictive of incident frailty after ~8 years of follow up. In addition, at baseline, plasma lutein and zeaxanthin concentrations were positively associated with several indices of musculoskeletal health including usual gait speed, timed up-and-go performance and, in the case of lutein, calcaneal stiffness index, but were not predictive of changes in these outcomes over 4-8 years of follow-up. There were no associations between plasma concentrations of lutein and zeaxanthin concentrations and grip strength or EWGSOP-defined sarcopenia, either cross-sectionally or longitudinally.

The current study extends previous work based on the nationally representative TILDA cohort which demonstrated that, at baseline, lower plasma lutein and zeaxanthin concentrations were cross-sectionally associated with frailty measured using three different instruments (Fried's phenotype, FRAIL Scale, and Frailty Index) [22]. Building on these findings in the current study, we show that among participants who were non-frail at baseline, lower plasma lutein and zeaxanthin concentrations were longitudinally associated with increased odds of progressing to a higher frailty category (e.g. prefrailty or frailty) after 8 y of follow up, after adjusting for sociodemographic, lifestyle, and health factors. This finding was consistent across all four frailty instruments used in the current study (Fried's Phenotype, FRAIL Scale, Frailty Index, CFS-classification tree), with the exception that the association between lutein concentration and incident Frailty Index-defined prefrailty/frailty did not reach statistical significance.

Nevertheless, the directionality of this association was similar to the other instruments.

Specifically, each 100 nmol/L increase in baseline plasma lutein and zeaxanthin concentration was associated with an 11-20% and 27-43% lower odds of progressing to a higher frailty

category, respectively, across the four frailty instruments. Relative to mean concentrations at baseline, a 100 nmol/L increase is equivalent to approximately a 48% increase in lutein and a ~1.75-fold increase in zeaxanthin concentration. To put this in context, a 100 nmol/L increase in plasma lutein concentrations is roughly equivalent to the increase observed in a previous study following the consumption of an additional ~2 portions/d of lutein-rich vegetables (green beans, pumpkin, and sweetcorn) for 4 weeks [38].

Few longitudinal studies have explored the association between carotenoid status and frailty onset. Consistent with the current findings, low serum concentrations of total carotenoids (where lutein and zeaxanthin represented two of the six measured carotenoids) were associated with higher risk of incident Fried-defined frailty over 3 years of follow up among older, moderately to severely disabled women in the Women's Health and Ageing Study I [39]. Prior to the current study, only one longitudinal study has explored the association between circulating concentrations of lutein and zeaxanthin and incident frailty [23], to our knowledge. In that study, which included older adults from a combination of four European cohort studies, lower circulating concentrations of lutein and zeaxanthin were associated with higher odds of Fried-defined frailty at baseline but were not associated with frailty onset over 2-5 years of follow up [23]. The reason for the discordance between the findings of that study and the present study is unclear, however it may relate to differences in the sample size (915 versus 1,425) or the follow up time (2-5 versus 8 years) between the previous [23] and the current study, respectively.

The putative protective effect of lutein and zeaxanthin, and indeed other carotenoids, on frailty risk is believed to be due to their antioxidant and anti-inflammatory properties. Frailty is associated with increased concentrations of biomarkers of oxidative stress and inflammation, such as urinary 8-epi-PGF₂a isoprostanes and plasma IL-6 [40]. Oxidative stress is proposed to

contribute to frailty onset through oxidative damage to cellular macromolecules (carbohydrates, lipids, proteins, and DNA), cellular senescence and the upregulation of proinflammatory cytokine expression, resulting the functional decline of multiple tissues and organs [41]. As ageing is associated with a reduction in the endogenous antioxidant defence system [41], increased intake of exogenous antioxidants, such as carotenoids, via the diet may help redress redox balance and reduce oxidative damage. Indeed, the carotenoid content of fruit and vegetables represents one of the components that may mediate the protective effects of higher fruit and vegetable intakes on frailty risk reported in the literature [11].

Our observation that plasma lutein and zeaxanthin concentrations were modestly associated with several indices of physical performance (usual gait speed, TUG) cross-sectionally, but were not associated with changes in these outcomes over time, suggests that plasma lutein and zeaxanthin concentrations may be markers but not predictors of physical performance in older adults. Counter to this notion, in the InCHIANTI prospective cohort of older adults in Italy, lower plasma concentrations of total carotenoids (which included lutein and zeaxanthin) were associated with slower walking speed cross-sectionally [42], as well as a steeper decline in walking speed and increased odds of developing severe walking disability [42] and low hip, knee, and grip strength [43] after 6 years of follow up.

Prior to the current study, no longitudinal studies had examined the association between circulating lutein and zeaxanthin concentrations *per se* and physical performance outcomes. However, a recent longitudinal analysis of participants aged 33-88 years in the Framingham Heart Study Offspring cohort reported that higher intakes of combined lutein + zeaxanthin were associated with a small, but significantly greater annualised increase in grip strength and walking speed over ~12 years of follow up [44]. Thus, it is possible that a longer follow-up period than

was used in the current study (4-8 years depending on the outcome) may be required to detect an effect of lutein and zeaxanthin status on changes in physical performance over time. As such, it will be interesting to explore these relationships further in future Waves of TILDA. The mechanisms by which carotenoids may affect muscle function are unclear, but are mainly hypothesised to relate to attenuated age-related skeletal muscle mitochondrial DNA damage resulting from oxidative stress, leading to the atrophy, loss and impaired function of muscle fibres [45,46]. There is also some recent evidence that lutein may influence synaptic function independent of its antioxidant activity, in conditions of mitochondrial dysfunction [47].

There is evidence suggesting that lutein stimulates bone formation and inhibits bone resorption *in vitro* [48,49] and that lutein supplementation enhances bone mineral density in mice *in vivo* [49]. Nonetheless, the results of human epidemiologic studies on the associations of intakes and circulating concentrations of individual carotenoids and bone mineral density are inconsistent [50-54]. In the present study, at baseline, lutein concentrations were positively associated with calcaneal ultrasound-derived stiffness index, a measure of bone mass that is predictive of fracture risk [55]. In line with these data, combined lutein + zeaxanthin intakes were positively associated with greater femoral neck, total hip and lumbar spine bone mineral density measured via dual energy x-ray absorptiometry (DXA) among adults aged ≥ 50 y in the NHANES population-based cohort in the US [53]. Conversely, another study reported no association between circulating lutein + zeaxanthin concentrations and DXA-derived bone mineral density in older adults in China [54]. As such, further work is required to elucidate the impact of lutein and zeaxanthin on bone health in ageing.

Strengths of this study include the large representative sample of adults aged ≥ 50 years, the use of four widely accepted frailty instruments, the use of DAGs to select the appropriate

covariates to include in the models, and the use of an objective measurement of lutein and zeaxanthin status using gold standard laboratory methodologies. Plasma carotenoid concentrations are considered to be a good biomarker of intake, particularly when compared to the limitations associated with self-reported dietary intake data [56]. Plasma concentrations of lutein and zeaxanthin are influenced, not only by dietary intakes of lutein and zeaxanthin, but also by their bioavailability (e.g. due to the food matrix, lipid co-ingestion, interindividual variability in digestion and absorption) and factors such as smoking, adiposity, health status, alcohol intake, age, and sex [14]). However, we adjusted for many of the factors that can confound the association between dietary intake and plasma concentrations in our analytical models.

There were several limitations of the current study. The shorter (4-6 years) time interval between repeated measures of usual gait speed, bone stiffness index and grip strength, and differences in the testing environment / procedures between repeated measures of grip strength and TUG, may have influenced the precision of the estimate of change in these outcomes. In addition, plasma concentrations of lutein and zeaxanthin were measured at one time point only (baseline) and therefore do not capture chronic exposure. As carotenoids enter the blood stream within 3 - 4 h of food consumption, the collection of non-fasting blood samples in the current study could, speculatively, have attenuated the associations between plasma concentrations and the outcome measures. Nonetheless, previous studies have used both fasting [42,43] and non-fasting [18,39] blood samples to demonstrate associations between circulating carotenoid concentrations and frailty / musculoskeletal health in older adults. Finally, as lutein and zeaxanthin are predominantly consumed through fruit and vegetables, it is possible that the associations presented in the current study are caused by other unmeasured factors associated

with higher fruit and vegetable intake (e.g. higher intake of other carotenoids and bioactives, higher overall dietary quality, other healthy lifestyle behaviours etc.), rather than the lutein and zeaxanthin *per se*.

In conclusion, we report that lower plasma concentrations of lutein and zeaxanthin were predictive of incident frailty after ~8 years of follow up in a large cohort of community dwelling older adults in Ireland. In addition, plasma lutein and zeaxanthin concentrations were positively associated with several indices of musculoskeletal health including usual gait speed, timed up-and-go performance and bone stiffness index at baseline, but were not predictive of changes in these outcomes over 4-8 years of follow-up. This study is the among the first to evaluate the longitudinal associations between plasma lutein and zeaxanthin concentrations and incident frailty and changes in musculoskeletal outcomes in older adults.

CRedit authorship contribution statement

Murphy: Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review and editing, Project Administration, Funding acquisition. **Duggan:** Methodology, Writing – review and editing. **Davis:** Methodology, Writing – review and editing. **O’Halloran:** Methodology, Writing – review and editing. **Knight:** Methodology, Writing – review and editing. **Kenny:** Resources, Writing – review and editing. **McCarthy:** Conceptualization, Writing – review and editing, Supervision. **Romero-Ortuno:** Conceptualization, Methodology, Resources, Writing – review and editing, Supervision.

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