Public Health Nutrition: 8(3), 298–308 DOI: 10.1079/PHN2004688

Evaluation of brief dietary questions to estimate vegetable and fruit consumption – using serum carotenoids and red-cell folate

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Submitted 26 May 2004: Accepted 13 October 2004

Abstract

Objective: To evaluate responses to self-administered brief questions regarding consumption of vegetables and fruit by comparison with blood levels of serum carotenoids and red-cell folate.

Design: A cross-sectional study in which participants reported their usual intake of fruit and vegetables in servings per day, and serum levels of five carotenoids (α-carotene, β-carotene, β-cryptoxanthin, lutein/zeaxanthin and lycopene) and red-cell folate were measured. Serum carotenoid levels were determined by high-performance liquid chromatography, and red-cell folate by an automated immunoassay system.

Settings and subjects: Between October and December 2000, a sample of 1598 adults aged 25 years and over, from six randomly selected urban centres in Queensland, Australia, were examined as part of a national study conducted to determine the prevalence of diabetes and associated cardiovascular risk factors.

Results: Statistically significant ($P < 0.01$) associations with vegetable and fruit intake (categorised into groups: ≤1 serving, 2–3 servings and ≥4 servings per day) were observed for α-carotene, β-carotene, β-cryptoxanthin, lutein/zeaxanthin and red-cell folate. The mean level of these carotenoids and of red-cell folate increased with increasing frequency of reported servings of vegetables and fruit, both before and after adjusting for potential confounding factors. A significant association with lycopene was observed only for vegetable intake before adjusting for confounders.

Conclusions: These data indicate that brief questions may be a simple and valuable tool for monitoring vegetable and fruit intake in this population.

Keywords
Vegetables
Fruit
Dietary intake methods
Serum carotenoids
Red-cell folate
Antioxidants
Biological markers
Brief questions
Short questions
Surveys

There is convincing evidence that high intakes of vegetables and fruit are associated with lower risk of chronic diseases[1–5]. International[1] and national health organisations[6,7] have recommended increasing the consumption of vegetables and fruit as an important health and nutrition priority. Numerous government agencies around the world have instituted health promotion programmes aimed at increasing consumption of vegetables and fruit at the population level[8–10].

Several methods of monitoring vegetable and fruit consumption are currently being used in various settings. Dietary assessment methods range from detailed weighed food diaries, to 24-hour recalls conducted by trained interviewers, to self-administered food-frequency questionnaires (FFQs) and brief food behaviour checklists. In Australia, the National CATI Technical Reference Group has recommended several specific dietary questions related to vegetable and fruit consumption for inclusion in CATI (computer-assisted telephone interview) surveys[11]. CATI surveys offer the benefit of using brief questionnaires on a relatively large sample of people. The short dietary questions used in the present study were first used in the 1995 National Nutrition Survey conducted by the Australian Bureau of Statistics[12] and again in the 2001 National Health Survey[13] to monitor vegetable and fruit intake of the population. Several studies have evaluated these brief vegetable and fruit intake questions using other dietary assessment methods, such as 3-day weighed food records[14], 24-hour recalls[15] or FFQs[16], with promising results. However, only a few 'brief' questionnaires

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concerning vegetable and fruit intake have been evaluated using biological measures[17–19]. Biochemical measures have been used to validate increased intake of folate, vitamin B_6 and ascorbic acid in an indigenous community[20]. Concentrations of several nutrients in the blood (serum carotenoids, and red-cell folate) are considered reliable markers of vegetable and fruit dietary intake[21–28].

The purpose of the present study was to evaluate responses to self-administered brief questions regarding consumption of vegetables and fruit in comparison with circulating levels of serum carotenoids and red-cell folate.

Materials and methods

The study was conducted in Queensland, Australia between October and December 2000, as part of a national study – The Australian Diabetes, Obesity and Lifestyle Study (AusDiab) – to determine the prevalence of diabetes and associated cardiovascular risk factors among adults aged 25 years and over[29]. The International Diabetes Institute and The University of Queensland ethics committees approved the study. Six urban sites were randomly selected from census collector districts in Queensland. Trained interviewers conducted house-to-house interviews and eligible participants were invited to attend a biomedical and physical examination, which included anthropometric measurements, collection of a blood sample and standardised questionnaires related to sociodemographic and lifestyle- or health-related characteristics. All respondents gave informed consent to participate in the survey upon arrival at the testing site. Details of the sampling framework and overall study design have been published elsewhere[30]. A total of 1634 persons (approximately 30% of those estimated to be eligible) completed the examination. Complete data for the serum carotenoids, red-cell folate and brief dietary questions were available for 1598 adults.

Brief dietary questions were administered at the testing site. Participants were asked 'How many servings of vegetables do you *usually* eat each day? including fresh, frozen or tinned vegetables. *A serving* = 1/2 cup of cooked vegetables or 1 cup of salad vegetables'. Usual daily consumption of fruit was assessed by the question, 'How many servings of fruit do you *usually* eat each day? including fresh, frozen or tinned fruit. *A serving* = 1 medium piece or 2 small pieces of fruit or 1 cup of diced pieces of fruit'. Possible responses were: *don't eat vegetables/fruit*, *eat 1 serving or less*, *eat 2 to 3 servings*, *eat 4 to 5 servings*, *eat 6 servings or more*.

Participants were categorised into three groups according to their responses. Although there were five possible response categories, the two extreme categories contained only a very small number of responses while the middle category contained most of the responses. Therefore those reporting *don't eat* or *eat 1 serving or less* formed one group, those reporting *2 to 3 servings* formed a second group, and those reporting *4 to 5 servings* or *6 servings or more* formed a third group.

Participants arrived for the study examination having fasted for at least 12 h and, if not taking hypoglycaemic medication, completed a 2-h oral glucose tolerance test (OGTT) after consuming a drink containing 75 g glucose. Blood was drawn for the serum carotenoid and red-cell folate determinations at the time of the 2-h OGTT or 2 h after the fasting sample for those who did not complete the OGTT. Serum samples for the carotenoid determinations were handled meticulously and protected from light at each stage of processing to prevent deterioration and degradation[31]. The serum was pipetted, frozen, packed in dry ice and shipped to a laboratory in Brisbane for analysis. Serum samples were analysed within 3 weeks of collection. Five serum carotenoids (α-carotene, β-carotene, β-cryptoxanthin, lutein/zeaxanthin and lycopene) were assayed simultaneously using the high-performance liquid chromatography procedure described by Talwar *et al.*[32]. Red-cell folate was measured using the Bayer Advia Centuar automated immunoassay system (Bayer, Melbourne, Australia). Lipids (total cholesterol, high-density lipoprotein (HDL)-cholesterol and triglycerides) were measured enzymatically on an Olympus AU 600. Low-density lipoprotein (LDL)-cholesterol was calculated from the Friedwald formula[33]:

$$\text{LDL-cholesterol} = \text{total cholesterol} - [\text{HDL-cholesterol} + (\text{triglycerides}/5)].$$

Demographic and other lifestyle variables were collected using standardised questionnaires and were categorised as follows. Age was divided into 10-year age groupings. Educational status was categorised as *post-graduate qualification*, *trade certificate or bachelor's degree*, and *secondary school or less*. Body mass index (BMI) was categorised as *underweight* (BMI $< 20\,\text{kg m}^{-2}$), *normal* (BMI ≥ 20 to $< 25\,\text{kg m}^{-2}$), *overweight* (BMI ≥ 25 to $< 30\,\text{kg m}^{-2}$) and *obese* (BMI $\geq 30\,\text{kg m}^{-2}$). Smoking status was categorised as *never* (smoked less than 100 cigarettes during lifetime), *former smoker* (smoke less than daily for at least the last 3 months, but used to smoke daily) and *current smoker* (smoke at least daily)[29]. Physical activity beneficial to health was categorised as *sufficiently active* (greater than 150 min of 'physical activity time' in the previous week), *insufficiently active but not sedentary* (less than 150 min of 'physical activity time' in the previous week) and *sedentary* (no participation in physical activity in the previous week). 'Physical activity time' was calculated as the sum of the time spent walking or performing moderate activity, plus double the time spent in vigorous activity to reflect its greater intensity[34]. Vitamin supplement use during the previous 24 h was categorised as *yes* for respondents who indicated that they took any vitamin or mineral supplements on the previous day, and *no* for respondents who indicated they did not. Alcohol

consumption was categorised as *none*, *60 or fewer standard drinks per month* or *greater than 60 standard drinks per month*.

Plasma lipids were categorised using the criteria for abnormal lipid levels based on recommendations by the National Heart Foundation[35] and the Australian Diabetes Society[36]. Dietary intake of total and saturated fat was estimated using the validated Cancer Council of Victoria FFQ, which is a self-administered, semi-quantitative questionnaire containing 80 food and beverage items with 10 frequency options ranging from *never* to *three or more times a day*[37].

Statistical analyses

To account for unequal probability of selection and for non-response, data were weighted to the Queensland population for the survey year. The weighted data were analysed using the survey (SVY) commands (for survey data) available in STATA Statistical Software version 8[38]. These commands take into account the cluster survey design in the calculation of point estimates, variance and standard errors.

Distributions of serum carotenoids and red-cell folate were skewed and therefore were logarithmically transformed to better approximate normal distribution for regression analyses. Results are expressed as geometric means and proportions. The *t*-test and analysis of variance were used to estimate differences in means between groups. Pearson's chi-square statistic was used to test for significant differences in proportions.

The association between serum carotenoids and red-cell folate versus reported number of servings of vegetables and fruits was estimated by performing multiple regression, in which the log-transformed serum carotenoids and red-cell folate were the outcome variables and reported number of servings of vegetables and fruit were the explanatory variables. Analysis was performed for each serum carotenoid and red-cell folate separately, adjusting for potential confounders. Potential confounders or covariates were those variables identified that were significantly associated with serum carotenoids and red-cell folate, as well as vegetable and fruit intake (age, sex and vitamin use), and also variables that were significantly associated with serum carotenoids and red-cell folate only (sex, age, BMI, smoking, vitamin use, alcohol intake, total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides). The covariates were included simultaneously into the regression model.

All tests were two-tailed with significance criterion of $P < 0.01$. Due to missing values, the sample size was not the same for all variables.

Results

Table 1 provides the unadjusted geometric mean levels of serum carotenoids and red-cell folate by sociodemographic, anthropometric and health-related behaviour variables, and categories of plasma lipids. Differences between groups were considered significant at $P < 0.01$ level.

Mean serum carotenoids (with the exception of lycopene) and red-cell folate levels were significantly lower for males, younger adults, the obese (except red-cell folate), current smokers, those who reported no vitamin or supplement use (except β-cryptoxanthin, lutein/zeaxanthin) and those who reported consuming more than 60 standard drinks per month (except red-cell folate). Lycopene levels were significantly lower for the elderly, those without tertiary education, obese individuals and those not usually consuming alcohol.

Mean serum carotenoid and red-cell folate levels were significantly lower for those with total cholesterol $< 5.5\,\text{mmol}\,l^{-1}$ (β-cryptoxanthin and lutein/zeaxanthin only), HDL-cholesterol $< 1.0\,\text{mmol}\,l^{-1}$ (except lycopene and red-cell folate), LDL-cholesterol $< 3.5\,\text{mmol}\,l^{-1}$ (except α-carotene and red-cell folate) and those with triglycerides $\geq 2.0\,\text{mmol}\,l^{-1}$ (except β-cryptoxanthin, lutein/zeaxanthin and red-cell folate). Red-cell folate levels were significantly lower for those with triglyceride levels $< 2.0\,\text{mmol}\,l^{-1}$. There was no relationship between any serum carotenoid and total dietary fat and saturated fat at the $P < 0.01$ level (data not shown in Table 1).

Table 2 provides the distribution of selected categorical variables by vegetable intake. The result indicates that females were more likely than males to report usually consuming 4 or more servings of vegetables, as were those who reported vitamin/supplement use. The proportion of respondents who reported consuming 4 or more servings of vegetables also increased significantly with age.

Table 3 provides the distribution of selected categorical variables by fruit intake. Females were more likely than males to report consuming 4 or more servings of fruit, as were those who did not smoke, those who reported consuming none or fewer than 60 alcoholic drinks per month, those who reported sufficient physical activity and those who reported vitamin/supplement use in the previous 24 h. The proportion of respondents who reported consuming 4 or more servings of fruit also increased significantly with age.

Table 4 provides the unadjusted and adjusted geometric mean levels of serum carotenoids and red-cell folate by vegetable and fruit intake adjusting for potential confounders. The unadjusted geometric means of α-carotene, β-carotene, β-cryptoxanthin, lutein/zeaxanthin and red-cell folate increased significantly with increasing frequency of vegetable and fruit intake. This association remained after adjusting for the variables associated with the blood nutrient levels as well as vegetable and fruit intakes (adjustment 1: sex, age and vitamin use). The association also remained after adjusting for all the variables associated with serum carotenoids and red-cell folate only (adjustment 2: sex, age, BMI, smoking, vitamin

Table 1 Unadjusted geometric mean concentrations of serum carotenoids ($\mu mol\,l^{-1}$) and red-cell folate ($nmol\,l^{-1}$) by selected variables for adults, aged 25 years and over, in the Queensland AusDiab study, 2000 ($n = 1598$)

		α-Carotene		β-Carotene		β-Cryptoxanthin		Lutein/zeaxanthin		Lycopene		Red-cell folate	
	n†	Mean‡	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
Sociodemographic variables													
Gender													
Male	674	0.10*	0.09–0.11	0.40*	0.36–0.43	0.17*	0.15–0.18	0.37*	0.35–0.39	0.45	0.42–0.48	588.1*	568.8–608.1
Female	924	0.14	0.13–0.15	0.59	0.56–0.63	0.21	0.20–0.22	0.40	0.39–0.42	0.43	0.41–0.45	626.0	607.7–644.9
Age group (years)													
25–34	190	0.10*	0.08–0.11	0.38*	0.33–0.45	0.14*	0.12–0.16	0.33*	0.30–0.36	0.53	0.47–0.61	557.4*	519.1–598.6
35–44	334	0.11	0.10–0.13	0.44	0.40–0.49	0.17	0.15–0.18	0.36	0.34–0.38	0.51	0.46–0.55	583.9	561.7–606.9
45–54	395	0.12	0.11–0.13	0.51	0.47–0.56	0.19	0.17–0.20	0.39	0.37–0.41	0.44	0.41–0.48	639.0	614.9–664.0
55–64	329	0.13	0.11–0.14	0.58	0.52–0.65	0.25	0.22–0.29	0.45	0.42–0.48	0.40	0.36–0.44	636.7	609.7–665.0
65–74	240	0.12	0.11–0.14	0.58	0.51–0.66	0.25	0.21–0.28	0.48	0.45–0.52	0.33	0.29–0.37	649.5	618.3–682.3
75+	111	0.15	0.13–0.18	0.73	0.60–0.88	0.32	0.26–0.40	0.47	0.41–0.54	0.24*	0.20–0.29	654.7	596.2–719.0
Educational status													
Postgraduate	118	0.11	0.10–0.12	0.57	0.50–0.65	0.24	0.21–0.28	0.38	0.35–0.42	0.51	0.44–0.59	620.5	581.1–662.5
Trade certificate, bachelor's degree	850	0.12	0.11–0.13	0.48	0.45–0.52	0.18	0.17–0.20	0.38	0.36–0.40	0.49	0.44–0.50	599.2	577.7–621.6
Secondary school or less	628	0.14	0.12–0.16	0.47	0.42–0.53	0.19	0.17–0.20	0.40	0.38–0.42	0.38*	0.35–0.42	617.4	597.4–638.1
Anthropometry													
Body mass index													
Underweight	92	0.13	0.10–0.16	0.56	0.47–0.68	0.19	0.15–0.23	0.39	0.34–0.45	0.41	0.34–0.50	598.2	543.3–658.7
Normal	550	0.14	0.12–0.15	0.59	0.53–0.65	0.22	0.20–0.24	0.40	0.38–0.42	0.45	0.41–0.49	606.5	585.0–628.7
Overweight	575	0.12	0.11–0.13	0.48	0.44–0.52	0.19	0.17–0.21	0.40	0.38–0.42	0.47	0.44–0.51	591.2	567.4–616.0
Obese	381	0.08*	0.07–0.09	0.36*	0.32–0.39	0.14*	0.13–0.16	0.35*	0.33–0.37	0.38*	0.35–0.41	638.2	610.9–666.6
Health-related behaviours													
Smoking status													
Never	827	0.13	0.13–0.14	0.56	0.53–0.60	0.21	0.20–0.23	0.41	0.39–0.43	0.45	0.42–0.48	619.7	597.9–642.2
Former	535	0.13	0.11–0.14	0.52	0.47–0.57	0.21	0.19–0.23	0.40	0.38–0.42	0.46	0.43–0.49	630.1	609.1–651.9
Current	231	0.06*	0.05–0.07	0.29*	0.24–0.35	0.11*	0.09–0.12	0.31*	0.29–0.34	0.39	0.34–0.44	530.5*	500.8–561.9
Physical activity													
Sufficiently active	788	0.12	0.11–0.13	0.53	0.49–0.57	0.20	0.19–0.22	0.39	0.37–0.41	0.46	0.43–0.49	602.7	584.7–621.2
Insufficiently active	493	0.11	0.10–0.13	0.46	0.42–0.51	0.18	0.17–0.20	0.39	0.37–0.41	0.43	0.40–0.47	605.7	582.5–629.9
Sedentary	317	0.10	0.09–0.12	0.42	0.36–0.49	0.16	0.14–0.18	0.37	0.34–0.39	0.39	0.35–0.45	620.3	582.7–660.4
Vitamin use during previous 24 h													
Yes	481	0.14	0.12–0.15	0.63	0.58–0.70	0.22	0.20–0.24	0.41	0.38–0.43	0.43	0.39–0.47	703.6	679.1–729.0
No	1008	0.11*	0.10–0.12	0.45*	0.42–0.48	0.18	0.17–0.20	0.38	0.37–0.40	0.45	0.43–0.48	574.8*	557.3–592.8
Alcohol intake													
None	361	0.13	0.11–0.14	0.53	0.48–0.59	0.21	0.19–0.23	0.40	0.37–0.43	0.36*	0.32–0.40	591.7	564.0–621.0
$\leq$ 60 drinks $month^{-1}$	1060	0.12	0.12–0.13	0.54	0.51–0.57	0.20	0.19–0.21	0.40	0.38–0.41	0.48	0.45–0.50	615.4	597.6–633.8
$>$ 60 drinks $month^{-1}$	177	0.06*	0.05–0.08	0.24*	0.19–0.30	0.11*	0.09–0.13	0.32*	0.29–0.35	0.38	0.31–0.46	586.9	545.0–626.2
Plasma lipids ($mmol\,l^{-1}$)													
Total cholesterol $<$5.5	728	0.11	0.10–0.12	0.45	0.41–0.49	0.16*	0.15–0.18	0.34*	0.33–0.36	0.42	0.39–0.45	612.3	592.5–632.7
Total cholesterol $\geq$5.5	871	0.12	0.11–0.13	0.52	0.49–0.57	0.21	0.20–0.23	0.44	0.42–0.46	0.46	0.43–0.49	601.5	580.1–623.6
HDL-cholesterol $<$1.0	181	0.08*	0.06–0.09	0.33*	0.29–0.38	0.15*	0.12–0.17	0.33*	0.30–0.37	0.39	0.34–0.44	627.1	586.1–671.1
HDL-cholesterol $\geq$1.0	1418	0.12	0.11–0.13	0.51	0.48–0.55	0.19	0.18–0.21	0.40	0.38–0.41	0.45	0.42–0.47	604.2	589.2–619.5
LDL-cholesterol $<$3.5	760	0.11	0.10–0.12	0.45*	0.41–0.49	0.17*	0.16–0.18	0.35*	0.34–0.37	0.41*	0.38–0.44	625.0	607.4–643.1
LDL-cholesterol $\geq$ 3.5	781	0.13	0.12–0.14	0.56	0.52–0.61	0.22	0.20–0.24	0.43	0.41–0.45	0.48	0.45–0.51	584.1	560.3–608.9
Triglycerides $<$2.0	1224	0.12	0.11–0.13	0.53	0.49–0.56	0.19	0.18–0.21	0.39	0.37–0.40	0.45	0.43–0.48	593.4*	574.6–610.5
Triglycerides $\geq$2.0	376	0.09*	0.08–0.10	0.36*	0.32–0.40	0.17	0.15–0.19	0.39	0.37–0.42	0.39*	0.36–0.43	660.2	633.1–688.5

CI – confidence interval; HDL – high-density lipoprotein; LDL – low-density lipoprotein.
* Significant at $P < 0.01$ level; variance adjusted for cluster design.
† Because of missing values, total *n* is not the same for all variables.
‡ Weighted by age and sex to represent the Queensland population for the survey year.

Table 2 Distribution of number of servings of vegetables usually consumed by selected sociodemographic and health variables for adults, aged 25 years and over, in the Queensland AusDiab study, 2000 ($n = 1598$)

	Reported number of servings of vegetables *usually* consumed						
	≤1 serving day^{-1}		2–3 servings day^{-1}		≥ 4 servings day^{-1}		
	n†	%‡	*n*	%	*n*	%	*P*-value*
Sociodemographic variables							
Gender							0.0001
Male	145	21.2	386	59.0	137	19.8	
Female	110	12.7	477	52.4	343	34.9	
Age group (years)							0.0001
25–34	43	20.8	120	63.8	27	15.4	
35–44	59	17.9	178	54.8	90	27.3	
45–54	64	16.1	208	53.1	121	30.8	
55–64	37	12.6	168	51.1	127	36.3	
65–74	28	11.8	135	55.0	82	33.2	
75+	24	19.3	54	47.1	33	33.6	
Educational status							0.1621
Postgraduate	13	13.7	73	63.0	31	23.3	
Trade certificate, bachelor's degree	140	17.8	463	56.3	241	25.9	
Secondary school or less	99	15.7	324	53.1	206	31.2	
Anthropometry							
Body mass index							0.1123
Underweight	13	14.8	63	68.2	23	17.0	
Normal	86	17.6	286	51.6	177	30.8	
Overweight	98	17.4	307	56.4	167	26.2	
Obese	58	15.4	207	56.5	113	28.1	
Health-related behaviours							
Smoking status							0.1353
Never	116	14.7	448	57.6	256	27.7	
Former	87	17.1	284	54.2	167	28.7	
Current	51	22.6	127	53.3	51	24.1	
Physical activity							0.0501
Sufficient	105	13.8	425	56.7	254	29.5	
Insufficiently active	88	19.7	267	55.4	136	24.9	
Sedentary	62	20.3	171	53.0	90	26.6	
Vitamin use during previous 24 h							0.0114
Yes	61	15.1	251	51.9	174	33.0	
No	182	17.8	573	57.6	271	24.6	
Alcohol intake							0.4343
None	57	18.3	183	52.8	115	28.9	
≤ 60 drinks month^{-1}	160	15.6	586	57.1	313	27.4	
> 60 drinks month^{-1}	37	20.9	92	52.3	51	26.8	
Plasma lipids (mmol l^{-1})							
Total cholesterol < 5.5	117	16.6	393	56.5	217	26.9	0.8068
Total cholesterol ≥ 5.5	138	17.1	470	54.7	263	28.2	
HDL-cholesterol < 1.0	41	23.2	95	55.7	40	21.0	0.0560
HDL-cholesterol ≥ 1.0	214	16.0	768	55.6	440	28.4	
LDL-cholesterol < 3.5	119	16.2	405	56.0	240	27.8	0.9499
LDL-cholesterol ≥ 3.5	119	16.7	427	55.1	231	28.2	
Triglycerides < 2.0	187	15.9	654	55.5	386	28.6	0.0979
Triglycerides ≥ 2.0	68	20.3	209	56.0	94	23.6	

HDL – high-density lipoprotein; LDL – low-density lipoprotein.
* Variance adjusted for cluster design.
† Because of missing values, total *n* is not the same for all variables.
‡ Weighted by age and sex to represent the Queensland population for the survey year.

use, alcohol intake, serum total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides). Unadjusted geometric means of lycopene increased significantly, but in a non-linear manner, with vegetable intake, but not with fruit intake. After adjustment, the significant association between lycopene and increased frequency of vegetable intake disappeared. Serum lycopene did not show a significant association with frequency of fruit intake either before or after adjustment for potential confounders.

Discussion

Our data indicate that brief questions related to usual intake of vegetables and fruit may be useful tools for monitoring key dietary behaviours. We found that the

Table 3 Distribution of number of servings of fruit usually consumed by selected sociodemographic and health variables for adults, aged 25 years and over, in the Queensland AusDiab study, 2000 ($n = 1598$)

	Reported number of servings of fruit *usually* consumed						
	≤1 serving day^{-1}		2–3 servings day^{-1}		≥ 4 servings day^{-1}		
	n†	%‡	*n*	%	*n*	%	*P*-value*
Sociodemographic variables							
Gender							0.0003
Male	312	47.5	282	41.6	71	10.9	
Female	320	36.6	485	51.0	122	12.4	
Age group (years)							0.0001
25–34	98	52.3	76	40.2	15	7.5	
35–44	165	49.0	133	40.4	31	10.6	
45–54	159	40.1	192	48.9	42	11.0	
55–64	110	33.2	170	52.0	52	14.8	
65–74	70	28.0	136	55.7	36	16.3	
75 +	30	26.6	60	54.8	17	18.6	
Educational status							0.0385
Postgraduate	35	28.5	61	54.2	21	17.3	
Trade certificate, bachelor's degree	338	43.5	410	46.1	93	10.4	
Secondary school or less	252	41.8	295	45.5	79	12.7	
Anthropometry							
Body mass index							0.1260
Underweight	52	55.5	36	36.1	10	8.4	
Normal	207	40.7	269	46.8	70	12.5	
Overweight	227	41.6	269	45.5	73	12.8	
Obese	146	39.9	193	50.6	40	9.5	
Health-related behaviours							
Smoking status							0.0001
Never	284	36.4	421	50.0	111	13.6	
Former	201	40.1	269	48.7	65	11.2	
Current	140	60.4	77	33.6	13	5.9	
Physical activity							0.0013
Sufficient	271	37.4	390	48.1	119	14.5	
Insufficiently active	208	44.4	236	46.4	46	9.2	
Sedentary	153	49.9	141	41.9	28	8.2	
Vitamin use during previous 24 h							0.0001
Yes	170	36.2	234	46.4	80	17.4	
No	429	44.0	494	47.1	101	8.9	
Alcohol intake							0.0041
None	126	37.6	178	48.8	47	13.6	
≤ 60 drinks month^{-1}	407	40.6	522	48.1	128	11.3	
> 60 drinks month^{-1}	95	55.8	67	33.5	18	10.7	
Plasma lipids (mmol l^{-1})							
Total cholesterol <5.5	288	41.0	356	48.5	79	10.5	0.2229
Total cholesterol ≥5.5	344	42.9	411	44.2	114	12.9	
HDL-cholesterol <1.0	85	49.6	78	41.6	14	8.8	0.1432
HDL-cholesterol ≥1.0	547	40.9	689	47.0	179	12.1	
LDL-cholesterol <3.5	299	41.3	374	47.6	88	11.1	0.4331
LDL-cholesterol ≥3.5	303	41.9	366	44.9	105	13.3	
Triglycerides <2.0	474	41.7	591	46.4	157	11.9	0.8362
Triglycerides ≥2.0	158	43.0	176	46.3	36	10.7	

HDL – high-density lipoprotein; LDL – low-density lipoprotein.
* Variance adjusted for cluster design.
† Because of missing values, total *n* is not the same for all variables.
‡ Weighted by age and sex to represent the Queensland population for the survey year.

responses to these two relatively simple questions had positive associations with objective markers of vegetable and fruit intake.

Several biological measures, particularly serum carotenoids, have been shown to have good agreement with vegetable and fruit intakes as assessed by more intensive methods of dietary assessment. In a small study using weighed food records, Polsinelli *et al.*[39] found moderate correlations between total vegetable and fruit intake in servings per day and β-carotene, lutein and α-carotene ($r = 0.48$, 0.60 and 0.73, respectively). Positive and modest agreement has also been found between individual serum carotenoid levels (except lycopene) and vegetable and fruit intake as assessed by 24-hour recall methods[18]. Researchers have reported moderate correlations between carotenoids and usual intake of vegetables and/or fruit from food frequencies in several countries: Mexico[40], Japan[41], the USA[18,19,25,42,43] and the UK[44].

Table 4 Unadjusted and adjusted geometric mean concentrations of serum carotenoids and red-cell folate by reported number of servings of vegetables and fruit for adults, aged 25 years and over, in the Queensland AusDiab study, 2000

	Reported number of servings of vegetables *usually* consumed							Reported number of servings of fruit *usually* consumed						
	≤1 serving day^{-1}		2–3 servings day^{-1}		≥4 servings day^{-1}			≤1 serving day^{-1}		2–3 servings day^{-1}		≥4 servings day^{-1}		
	Mean†	95% CI	Mean	95% CI	Mean	95% CI	*P*-value*	Mean†	95% CI	Mean	95% CI	Mean	95% CI	*P*-value*
Serum carotenoids (μmol l^{-1})														
α-Carotene														
Unadjusted	0.08	0.07–0.08	0.12	0.11–0.13	0.14	0.13–0.16	0.0001	0.09	0.08–0.09	0.14	0.13–0.15	0.18	0.16–0.21	0.0001
Adjust (1)	0.08	0.07–0.09	0.13	0.12–0.14	0.14	0.13–0.16	0.0001	0.09	0.08–0.10	0.14	0.13–0.15	0.19	0.16–0.21	0.0001
Adjust (2)	0.09	0.08–0.10	0.13	0.12–0.14	0.15	0.13–0.16	0.0001	0.10	0.09–0.11	0.14	0.13–0.15	0.18	0.15–0.20	0.0001
β-Carotene														
Unadjusted	0.38	0.34–0.42	0.49	0.46–0.53	0.59	0.53–0.67	0.0001	0.37	0.34–0.41	0.57	0.54–0.61	0.77	0.68–0.88	0.0001
Adjust (1)	0.44	0.39–0.48	0.55	0.51–0.58	0.59	0.52–0.66	0.0003	0.42	0.39–0.45	0.59	0.56–0.63	0.80	0.70–0.90	0.0001
Adjust (2)	0.46	0.41–0.51	0.55	0.52–0.59	0.60	0.55–0.67	0.0023	0.45	0.42–0.48	0.60	0.57–0.64	0.75	0.67–0.85	0.0001
β-Cryptoxanthin														
Unadjusted	0.15	0.14–0.17	0.19	0.17–0.20	0.22	0.20–0.25	0.0001	0.12	0.11–0.13	0.24	0.23–0.26	0.34	0.29–0.39	0.0001
Adjust (1)	0.18	0.16–0.20	0.21	0.20–0.22	0.23	0.20–0.25	0.0064	0.14	0.13–0.15	0.25	0.24–0.27	0.37	0.32–0.41	0.0001
Adjust (2)	0.18	0.16–0.20	0.21	0.20–0.23	0.23	0.21–0.25	0.0086	0.14	0.13–0.15	0.25	0.24–0.27	0.35	0.31–0.40	0.0001
Lutein/zeaxanthin														
Unadjusted	0.33	0.30–0.36	0.37	0.36–0.39	0.45	0.43–0.48	0.0001	0.34	0.32–0.36	0.41	0.39–0.43	0.44	0.39–0.49	0.0001
Adjust (1)	0.36	0.33–0.39	0.40	0.38–0.41	0.46	0.44–0.49	0.0001	0.37	0.35–0.39	0.43	0.41–0.45	0.46	0.42–0.50	0.0001
Adjust (2)	0.35	0.33–0.38	0.40	0.38–0.41	0.46	0.44–0.49	0.0001	0.37	0.35–0.39	0.43	0.41–0.45	0.45	0.41–0.49	0.0001
Lycopene														
Unadjusted	0.43	0.39–0.48	0.47	0.44–0.50	0.40	0.36–0.43	0.0043	0.44	0.41–0.48	0.44	0.42–0.47	0.44	0.39–0.49	0.9852
Adjust (1)	0.40	0.36–0.44	0.44	0.42–0.47	0.39	0.35–0.43	0.0470	0.40	0.37–0.53	0.43	0.40–0.46	0.46	0.40–0.52	0.0852
Adjust (2)	0.41	0.37–0.45	0.44	0.42–0.47	0.39	0.36–0.43	0.0832	0.40	0.37–0.43	0.43	0.41–0.46	0.44	0.39–0.50	0.2117
Red-cell folate (nmol l^{-1})														
Unadjusted	557.8	530.0–587.1	605.1	584.9–626.0	648.3	622.7–674.9	0.0001	555.2	532.2–579.3	644.5	626.8–662.8	674.3	633.7–717.6	0.0001
Adjust (1)	572.7	544.4–602.4	625.5	607.2–644.3	647.8	623.7–672.8	0.0009	575.8	554.7–597.6	652.4	634.6–670.6	671.3	632.9–711.9	0.0001
Adjust (2)	570.1	542.5–599.1	622.9	605.0–641.2	645.8	622.9–669.5	0.0005	575.3	554.2–597.2	647.3	630.0–665.1	671.7	634.0–711.6	0.0001

CI – confidence interval.
Adjust (1) – adjusted for sex, age, and vitamin use simultaneously.
Adjust (2) – adjusted for sex, age, body mass index, smoking, vitamin use, alcohol intake, serum total cholesterol, high-density lipoprotein-cholesterol, low-density lipoprotein-cholesterol and triglycerides simultaneously.
Serum carotenoids and red-cell folate were log-transformed for regression analyses.
* Variance adjusted for cluster design.
† Weighted by age and sex to represent the Queensland population for the survey year.

A study by Carroll *et al.*[44] found poor correlations (except among younger males) between intake of individual dietary carotenoids estimated from an FFQ and plasma carotenoids. The low correlations in this study may have been due to factors such as incomplete food composition data for individual carotenoids and not controlling for confounding factors, particularly smoking and alcohol intake.

Correlations between fruit intake and serum carotenoids, particularly β-cryptoxanthin, have been shown to be higher than correlations with serum ascorbic acid[27]. Whether serum carotenoids are better markers of fruit intake is, however, controversial, as Block *et al.* reported that serum vitamin C is more strongly correlated with total vegetable and fruit consumption[22].

While significant associations were shown between most of the carotenoids and vegetable and fruit intake in the current study, these were not found for serum lycopene. This supports previous studies, which have found poor or negative associations between serum lycopene and intake of vegetables and fruit[25,39,45]. A possible reason for this is the differing distribution of food sources for lycopene and differing bioavailability compared with other carotenoids. Lycopene is found predominantly in tomatoes and tomato-based products such as tomato paste, tomato sauce and in mixed dishes containing these ingredients, such as pasta sauces and pizzas[46]. In addition, bioavailability from these mixed dishes may be higher as the heating and homogenisation of tomatoes has been shown to increase the bioavailability of lycopene. Absorption of lycopene is also higher from tomato juice than from raw tomatoes[47,48]. Lycopene is also better absorbed when the tomatoes are consumed together with oil[49].

Serum carotenoids have been shown in intervention studies to respond to increases in vegetable and fruit intakes. In studies where subjects have been given a test diet or counselled to increase vegetable and fruit intake over the short (3–4 week) and medium term (over 6 months) or longer (12 months), serum carotenoids (other than lycopene) have shown significant increases when compared with control groups[21,50,51]. Serum carotenoids have also been shown to decrease in response to reduced vegetable and fruit intake when subjects are placed on diets low in vegetables and fruit over similar time periods[21,52].

Several studies have also found good agreement between brief questionnaires of vegetable and fruit intake and nutrient biomarkers. Resnicow *et al.*[18] assessed the performance of three different vegetable/fruit FFQs and one or more 24-hour recalls using serum carotenoids among African American adults. Although the two-item vegetable and fruit questionnaire performed less well than more detailed questionnaires or 24-hour recalls, significant positive correlations were found, ranging from $r = 0.27$ for cryptoxanthin to $r = 0.31$ for β-carotene. There was no correlation between lycopene and any of the questionnaires or the 24-hour recall methods.

Cappuccio *et al.*[19] assessed a two-item vegetable and fruit questionnaire using plasma ascorbic acid, β-carotene, α-tocopherol and 24 h urine potassium excretion on 271 general practice patients in London. They found a significant positive correlation between total vegetable and fruit intake and urinary potassium excretion ($r = 0.23$, $P < 0.001$), but not with β-carotene. However, when intakes of vegetable and fruit were analysed separately, fruit intake but not vegetable intake correlated with plasma levels of β-carotene ($P = 0.04$). Although the simple questionnaire had low sensitivity – only 36% of those in the upper tertile of reported vegetable and fruit intake (5 or more servings per day) also had higher levels of biomarkers, it did have high specificity: low concentrations of biomarkers were observed in 76–83% of those in the lower two tertiles of reported intake.

While our study showed that short question responses can accurately rank people according to higher or lower intakes of fruit and vegetables when compared with concentrations of red-cell folate, this has not been consistently reported in the literature. In Finland, Silaste *et al.*[53] reported that mean concentrations of serum and red-cell folate were low on a diet of 1 serving of vegetables and/or fruit per day, and higher on a diet of at least 7 servings of vegetables, berries and citrus fruit per day. Some other studies have also suggested that a dietary pattern associated with higher intakes of vegetables and fruit, as measured by an FFQ, is associated with higher levels of red-cell folate[54]. Another study from the UK[55] investigated the effectiveness of three routes of folate intake (folate from food, from foods fortified with folic acid or given as a supplement) to optimise red-cell folate status. A relationship with red-cell folate was found only between folate from supplements and from folate-fortified foods.

Plasma lipids are important determinants of plasma carotenoids. The present study showed a consistent association between plasma lipid levels and concentrations of serum carotenoids. Plasma β-carotene has been associated with plasma cholesterol in a number of studies[56]. This association is explained by an understanding of the absorption of β-carotene, which has been summarised by Nierenberg *et al.*[57]. When β-carotene is absorbed intact (rather than cleaved and metabolised to retinol and retinoic acid), it is transported via chylomicrons to the liver. It is then secreted with very low-density lipoprotein (VLDL) into the bloodstream but most β-carotene in the blood is actually found in LDL, as VLDL is converted to LDL in the blood.

Study strengths and limitations

Our study was conducted on a large sample of adults, both females and males, across a range of ages. Because the study was part of a large diabetes and cardiovascular risk factor study, extensive demographic and other biomedical variables were also collected and could be assessed as potential confounding factors. The brief

questions related to vegetable and fruit consumption were compared with objective biological markers of diet which are independent of memory, subject compliance and respondent bias[58]. Because the errors associated with the use of biological markers of diet are independent of those associated with questionnaire or recall methods of dietary assessment, the association between diet and these markers is unlikely to be due to correlated errors[59].

The biological indicators measured in this study were based on a single blood sample. Some studies using biological markers have used multiple blood samples collected at different time points to better characterise an individual's nutrient status[59]. However, Van Kappel *et al.*[24], who conducted a longer-term study to investigate the reproducibility of serum levels of carotenoids, found that a single sample could accurately rank individuals for α-carotene, β-carotene and lutein.

The blood samples in the current study were collected after an overnight fast, and are thus unlikely to be affected by carotenoid or red-cell folate intake at the previous meal. Studies have shown that while plasma levels of α- and β-carotene are altered within a few hours following a carotene-rich meal[60], a single dose of β-carotene does not result in continuously raised β-carotene concentrations 5 or 10 days later. Thus, apart from an initial rise following a meal, carotenoid levels reflect the long-term intake[61]. Red-cell folate also is considered to represent folate status over a longer term, perhaps months[62].

While our study, along with others, have found moderate correlations between plasma carotenoids and reported usual intake of total vegetables and total fruit, other studies have found stronger associations between specific vegetables and specific fruit intakes and individual carotenoids. For example, Michaud *et al.*[43] showed that intake of carrots measured by FFQ was a strong predictor of plasma α- and β-carotene levels in plasma, while oranges and orange juice intakes predicted plasma β-cryptoxanthin, and plasma lutein was predicted by lettuce and spinach intakes. Other studies have shown higher correlations between calculated intakes of individual carotenoids and plasma concentration, compared with correlations with total vegetable and fruit intakes[42,43]. While circulating levels of carotenoids may be more strongly correlated with intakes of specific vegetables and fruit, and intakes of individual carotenoids, they are still appropriate as independent estimates of vegetable and fruit intakes for evaluating the brief questions.

Conclusion

Questions similar to those evaluated in the present study are currently used widely in Australia and other countries in a variety of surveys and settings as a cost-effective way to monitor vegetable and fruit consumption at a population level. While biomarkers do not provide accurate point estimates of vegetable and fruit consumption, they provide an independent means of ranking intakes, against which short questions on fruit and vegetable intake can be compared. By comparison with biomarkers, the short questions evaluated here appear to rank individuals reasonably well. These results provide credibility for the continued widespread use of these brief questions in a variety of population-based surveys and settings to monitor trends in vegetable and fruit consumption.

Acknowledgements

The national AusDiab study was conducted by the International Diabetes Institute with partial funding from the Commonwealth Department of Health and Aged Care, State and Territory governments and pharmaceutical companies (Eli Lilly (Aust) Pty Ltd, Janssen-Cilag (Aust) Pty Ltd, Knoll Australia Pty Ltd, Merck Lipha SA, Alphapharm Pty Ltd, Merck Sharp & Dohme (Aust), Pharmacia and Upjohn Pty Ltd, Roche Diagnostics, Servier Laboratories (Aust) Pty Ltd, SmithKline Beecham International, BioRad Laboratories Pty Ltd, HITECH Pathology Pty Ltd), Qantas Airways Ltd and the Australian Kidney Foundation. The Queensland phase of the study was partially funded by units within Queensland Health (Public Health Services, the Health Information Centre, the Health Service Districts of Cairns, Gold Coast, Prince Charles Hospital, Sunshine Coast, Royal Brisbane Hospital and Toowoomba Health Districts) and the Chemical Pathology Laboratory at Princess Alexandra Hospital.

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