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Dietary predictors of serum total carotene in low-income women living in São Paulo, south-east Brazil

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Abstract

Objective: Dietary intake and nutritional status of antioxidant vitamins have been reported to protect against some cancers. The objective of the present study was to assess the correlations between serum levels of carotenoids (including β-, α- and γ-carotene), lycopene, retinol, α- and γ-tocopherols, and dietary intakes estimated by an FFQ, among low-income women in the Brazilian Investigation into Nutrition and Cervical Cancer Prevention (BRINCA) study.

Design: Cross-sectional study of data for 918 women aged 21–65 years participating in the BRINCA study in São Paulo city. Multiple linear regression models were used with serum nutrient levels as the dependent variable and dietary intake levels as the independent variable, adjusted for confounding factors.

Results: In energy-adjusted analyses, the intakes of dark green and deep yellow vegetables and fruits (partial $R^2 = 4{\cdot}8\%$), total fruits and juices (partial $R^2 = 1{\cdot}8\%$), vegetables and fruits (partial $R^2 = 1{\cdot}8\%$), carrots (partial $R^2 = 1{\cdot}4\%$) and citrus fruits and juices only (partial $R^2 = 0{\cdot}8\%$) were positively correlated only with serum total carotene levels, after adjusting for serum total cholesterol concentration, age, hospital attended, smoking status, BMI and presence of cervical lesions. Multiple-adjusted serum levels of carotenoids were positively correlated with intake quartiles of dark green and deep yellow vegetables and fruits and total fruits and juices independent of smoking status.

Conclusions: The intake of specific fruits and vegetables was an independent predictor of serum total carotene levels in low-income women living in São Paulo.

Keywords
Fruits
Vegetables
Dietary intake
β-Carotene

Several lines of evidence suggest that fruits and vegetables (F&V) convincingly decrease the risk of CVD and probably protect against some cancers (mouth, pharynx, larynx, oesophagus and stomach)[1]. In addition to other strategies to prevent diet-related chronic diseases (i.e. obesity, diabetes mellitus, CVD, hypertension, stroke and some cancers), the WHO recommends increasing the consumption of F&V to a minimum of 400 g/d. The protective effects of high F&V intake against the above-mentioned chronic diseases can possibly be explained by the reduction of total energy intake, the presence of blood pressure-lowering substances (K and phytochemicals), and the protection against reactive oxidants[2]. Carotenoids and tocopherols are natural antioxidants present mainly in F&V among other dietary sources. Overall, 600 carotenoids have been isolated from natural sources, including pro-vitamin A carotenoids (α-carotene, β-carotene and β-cryptoxanthin)[3].

It has been shown that populations may have different plasma carotenoid profiles according to the type and amount of F&V consumed and other demographic factors[4], such as income and educational level[5–7]. Many epidemiological studies show that diet quality follows a socio-economic gradient, since socio-economic status is likely to affect all aspects of energy balance from access to healthy foods to opportunities for physical activity[8]. Many studies carried out in developed countries have found correlations between serum vitamin concentrations (carotenoids, lycopene, tocopherols) and dietary factors[5,6,9–13]. Dietary intake of F&V has been described as a good predictor for some biomarkers[4,14–16]. However, only a few studies have been conducted in low-income individuals in both developed[7,17,18] and developing nations[19–21]. The results of these studies should be carefully interpreted due to small sample size[19,20] or

† See Appendix for list of members of the Brazilian Investigation into Nutrition and Cervical Cancer Prevention (BRINCA) Study Team.

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methodological limitations related to the use of a non-validated FFQ[21].

In Brazil, data from family budget surveys carried out in all metropolitan areas in 1989 and 1996 suggested that the contributions derived from total fruits, fruit juices and vegetables to total energy intake were declining[22]. A more recent family budget survey conducted in 2002–3 estimated the individual acquisition of fruits and vegetables to be about 67 g/d and 79 g/d, respectively[23]. These estimates are much lower than what has been recommended to reach nutritional needs for good health and the prevention of diet-related chronic diseases. It has been suggested that circulating antioxidant vitamins may be important in the natural history of cervical carcinogenesis in populations with lower intake levels of β-carotene[24]. In the present study, to report dietary food sources of serum carotenoids, retinol, α- and γ-tocopherols of low-income women, we investigated the determinants of these vitamins in the Brazilian Investigation into Nutrition and Cervical Cancer Prevention (BRINCA) study.

Methods

Study population and design

Participants were from the BRINCA study, a case–control hospital-based study designed to investigate the relationship among dietary factors, serum vitamin concentrations and cervical cancer in São Paulo city, São Paulo state, Brazil. The exclusion criteria were pregnancy, breast-feeding, hysterectomy, positive test for HIV, bleeding, mental disturbance, and radiotherapy or chemotherapy treatments. From March 2003 to May 2005, 1676 women completed the study protocol by filling out a questionnaire in three major public hospitals: the Brazilian Institute for Cancer Research, Hospital Leonor Mendes de Barros and Hospital Perola Byington. In the main study, we aimed to recruit prospectively newly diagnosed cases of cervical intraepithelial neoplasia (CIN) and invasive cancer. Eligible women were residents of São Paulo aged 18–65 years who had no prior hysterectomy, previous treatment for cervical neoplasia or cancer history. During the same period, control women were selected from among those attending screening in the same clinics where cases were diagnosed. Cases and controls were invited to participate in the study and interviewed before the colposcopy examination to minimize differential recall bias. The Institutional Review Board of the School of Public Health of the University of São Paulo and the Medical Ethical Committees of all participating hospitals approved the study protocol, and written informed consent was obtained from each participant.

For the present analysis we excluded the following participants because of concern that cancer treatment could have affected the associated factors under investigation: 148 with any diagnosis of invasive cancer, fifty-two who reported haemorrhage in the last 6 months, four HIV-positive, one with lupus, four younger (<21 years), one older (>65 years), and thirty-one without any information in medical records. We also excluded eight participants considered outliers based on the reported dietary intake distribution (<2929 kJ/d (700 kcal/d) or >25 105 kJ/d (6000 kcal/d), corresponding to the <2·5 or >97·5 percentile, respectively). Of the 1427 eligible participants for the present study, 918 (64·3 %) provided blood samples.

General and anthropometric information

A general questionnaire was used to obtain data on medical and reproductive history, socio-economic characteristics, lifestyle, smoking and drinking habits. Self-reported ethnicity/race was defined in three categories used by the Instituto Brasileiro de Geografia e Estatística: white, black or mulatto (person with one black parent and other white parent). Physical activities were assessed by a structured physical activity questionnaire used in a previous study with good reproducibility (Spearman coefficient ranging from 0·51 to 0·82)[25]. The questionnaire was developed using 24 h activity recalls to select the most frequent physical activities reported from people attending in a public health centre. Women were asked about frequency and time spent in practices of gym, physical fitness, cycling, sports, leisure-time physical activities, usual work, walking and household activities and care activities (child <5 years old or elderly). Time spent in each activity in hours per week was multiplied by energy expenditure, expressed in metabolic equivalents of task (MET), and then summed over all activities, to yield total MET × h/week.

Anthropometry was performed with subjects wearing light clothes and no shoes using calibrated electronic scales (model MEA-07400; Measurement Specialities, Hampton, VA, USA). Height was measured with a tape measure fixed to a flat wall. Both measures were repeated, and the mean values were used to calculate BMI (kg/m^2), which was then classified as normal (BMI < 25 kg/m^2), overweight (BMI = 25·00–29·99 kg/m^2) or obese (BMI ≥ 30·00 kg/m^2) according to the WHO guidelines[26].

Dietary data

Food consumption was assessed using a previously validated FFQ adapted to the present study[25,27]. In summary, we conducted a validation study in a random sub-sample of ninety-six cases and controls from the BRINCA study (FFQ1), using three 24 h dietary recalls (DR) obtained during a year reported in a second FFQ (FFQ2). The energy-adjusted intra-class correlations between FFQ1 and FFQ2 (one-year interval) ranged from 0·4–0·6 (B vitamins, Fe, Zn, Mg, P and Ca) to 0·7 (vitamin A and folate). The energy-adjusted and de-attenuated Pearson correlations (*r*) between FFQ and DR ranged from 0·3–0·4 (macronutrients and B vitamins) to 0·5–0·8 (fibre, vitamin A, Ca, folate and P; MA Cardoso, EC Laguna, LY Tomita and V D'Almeida, unpublished results).

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Subjects were asked by trained nutritionists about the usual frequency of food consumption (seventy-six items) and portion sizes (small, medium, large and extra large) during the previous year. The nutrient composition of the diets was determined using the Dietsys software version 4·01[28]. The nutrient database was based primarily on the US Department of Agriculture publications supplied by Dietsys and supplemented by the Brazilian Standard Food Composition Table only for the typical Brazilian recipe of *feijoada* (black beans cooked with pork and beef)[29]. The subjects were also questioned about the use of vitamin supplements (commercial name or brand, frequency and duration) within the past year. For the present study, we investigated macronutrients, vitamins, minerals and five food groups: (i) dark green and deep yellow vegetables and fruits (green salad, kale, broccoli, spinach, pumpkin, carrot, sweet potatoes, papaya and mango); (ii) total fruits and juices; (iii) citrus fruits and juices only; (iv) total vegetables; and (v) total F&V.

Blood collection and laboratory analyses

Fasting venous blood samples, protected from light, were collected into anticoagulant-free tubes, centrifuged at 1300 rpm for 13 min within the first hour of collection and stored at −70°C until analysis. Unfortunately, we were not able to separate β-, α- and γ-carotene when the analysis was run. As β-carotene is considered the most prevalent carotenoid in plasma, in the present study serum samples were analysed for total carotene including β-, α- and γ-carotene, lycopene, α- and γ-tocopherols and retinol by reversed-phase HPLC (HP-1100 system; Hewlett Packard, Palo Alto, CA, USA)[30]. As previously described[31], lipid extracts from serum samples were prepared with methanol/hexane. The pellet obtained after final solvent evaporation was dissolved in 0·4 ml methanol–ethanol (1:1, v/v) for injection into the chromatograph. Aliquots of 20 μl were injected on to a 3·9 mm × 150 mm C8 Nova-Pak column, under isocratic mobile phase delivery (20 mM-$LiClO_4$ in methanol–water (98:2, v/v), 0·7 ml/min). An electrochemical detector (Bioanalytical Systems, Inc., West Lafayette, IN, USA) was used, with an oxidation potential of 0·6 V. The observed wavelengths and retention times were, respectively, 325 nm and 2 min 0 s (±0·2 s) for retinol, 280 nm and 3 min 33 s (±0·2 s) for γ-tocopherol, 280 nm and 3 min 56 s (±0·2 s) for α-tocopherol, 450 nm and 5 min 54 s (±0·2 s) for lycopene, 450 nm and 7 min 34 s (±0·2 s) for β-carotene, in a single run (total run of 10 min).

The peaks for carotenoids that were under the quantification limits were set to zero (five samples for total carotene, one for lycopene and two for γ- tocopherol; detectable levels of total carotene, lycopene and γ- tocopherol were respectively 0·5, 0·1 and 0·2 μmol/l). Serum total cholesterol was measured enzymatically using an automatic device (ADVIA 1650; Bayer, East Walpole, MA, USA). All samples were analysed within 6 months of collection. The laboratory assayed internal and external blinded quality control specimens in every run. From the control specimens, the accuracy and inter-assay CV for all of these analytes were within 8 %.

Statistical methods

Dietary intakes and biomarker concentrations were log-transformed before analysis. The dietary nutrient intakes were adjusted for total energy intake using the residual method[32]. Serum vitamin concentrations did not differ according to supplement use owing to the small number of current users (*n* 13, 1·4 %). Monthly per capita income was converted from Reals (Brazilian currency) to US dollars using the mean monthly exchange conversion rate. Cervical cytology was classified in accordance with WHO criteria.

Pearson correlation coefficients were used to assess potential correlations between total carotene, lycopene, α- and γ-tocopherols and retinol concentrations and the continuous variables: age (years), education (years of schooling), BMI, fasting time (h), total serum cholesterol concentration and dietary intake. Tests for linear trend were calculated by assigning a median value for each serum micronutrient and modelling this as a continuous variable across categories of race/ethnicity, income, smoking status, oral contraceptive use, alcohol intake, season of blood collection and cervical cytological classification. Pearson correlation coefficients were also used in assessing correlations between the dietary factors and serum micronutrients. These correlations were further investigated using multiple linear regression models, with dietary intake as the independent variable and serum carotene, tocopherols and retinol as the dependent variable. The regression coefficients (β_1) and the coefficients of determination (adjusted R^2) were estimated using multiple separate models for each serum vitamin, adjusting for potential confounding variables selected in a stepwise forward procedure based on $P < 0{\cdot}05$ for estimated β_1 and change in adjusted R^2. Estimated food groups and nutrient intakes were included in linear multiple regression models in the last step to determine their independent effect on serum micronutrient levels. Calculation of partial R^2 was used to assess the degree of variability each dietary variable contributed to serum micronutrients, expressed as a percentage. Stratified analyses by smoking status (non-smoker *v.* current smoker) were conducted to test whether a significant interaction existed between smoking, food group intakes and serum total carotene levels. Adjusted mean intake of food groups and serum total carotene were compared in smokers *v.* non-smokers using ANOVA. The independent effect of smoking on serum total carotene concentrations was estimated and assessed for linear trend across quartiles of food intake after adjusting for confounding variables. None of the variables were collinear. Statistical significance was estimated using $P < 0{\cdot}05$. All analyses were performed using the STATA statistical software package version 9·0 (Statacorp, College Station, TX, USA).

Results

The distribution of participants across hospitals was 511 (55·7 %) from Brazilian Institute for Cancer Research, 331 (36·0 %) from Perola Byington and seventy-six (8·3 %) from Leonor Mendes de Barros. Most of the participants were housewives or unemployed, 420 (45·8 %), with a median monthly income of $US 63·5. A high proportion of overweight and obesity for a developing country was observed: 278 (30·3 %) and 147 (16·0 %), respectively. Other general characteristics, dietary intake and the biomarker concentrations are presented in Table 1.

Serum concentrations of carotenoids, retinol and tocopherols were positively correlated with serum total cholesterol and age ($P < 0·005$). The strongest correlation coefficients were observed between serum total carotene and BMI ($r = 0·60$, $P = 0·02$). Serum total carotene levels were strongly correlated with serum lycopene ($r = 0·42$, $P < 0·001$), serum α-tocopherol ($r = 0·45$, $P < 0·001$) and serum γ-tocopherol ($r = 0·34$, $P < 0·001$). The medians and interquartile ranges of serum vitamins according to the main characteristics of the participants are shown in Table 2. Alcohol consumption was not significantly different across quartiles of serum vitamin levels. The proportion of current and former smokers was high (49·6 %), with statistically significant differences in serum total carotene concentration according to smoking status ($P = 0·005$).

Serum total carotene concentrations were positively correlated with intakes of dark green and deep yellow vegetables and fruits ($r = 0·27$), total fruits and juices ($r = 0·18$), carrots ($r = 0·18$), total F&V ($r = 0·17$) and citrus fruits and juices only ($r = 0·12$). They were negatively correlated with sweets and snacks ($r = -0·12$; $P < 0·001$ for all). Among energy-adjusted nutrients, the highest Pearson correlation coefficients were observed between serum total carotene concentrations and dietary intakes of pro-vitamin A carotenoids ($r = 0·24$), vitamin A (IU; $r = 0·23$), β-carotene ($r = 0·23$) and α-carotene ($r = 0·21$; $P < 0·001$ for all).

The results of multiple linear regression analyses between dietary factors and serum total carotene levels are summarized in Table 3. The intake of vegetables and fruits, mainly dark green and deep yellow ones, was the strongest dietary predictor of serum total carotene concentration in our study population, after adjusting for confounding variables. Ham and sausage consumption was a negative determinant of serum total carotene levels after adjusting for confounding variables. For serum concentrations of tocopherols, only dietary pro-vitamin A carotenoids and vitamin C were positively associated with α-tocopherol levels (partial $R^2 =$ 0·5 % and 0·6 %, respectively, adjusted for age, serum total cholesterol, season of blood collection and BMI); while the intake of ham and sausages (partial $R^2 = 0·5$ %) and sweets (partial

Table 1 Characteristics of the study population: low-income women (*n* 918) aged 21–65 years participating in the Brazilian Investigation into Nutrition and Cervical Cancer Prevention (BRINCA) study

Variable	Median	IQR
Age (years)	38·0	29·8, 47·0
Schooling (years)	7·0	4·0, 11·0
Monthly per capita income ($US)	63·5	35·0, 113·0
BMI (kg/m^2)	24·7	22·0, 28·2
Total physical activity (MET × h/week)	137·7	83·5, 205·0
Biomarkers		
Total carotene (μmol/l)	0·66	0·37, 1·17
Lycopene (μmol/l)	0·90	0·50, 1·46
Retinol (μmol/l)	1·73	1·30, 2·38
α-Tocopherol (μmol/l)	14·80	10·90, 19·72
γ-Tocopherol (μmol/l)	1·80	1·30, 2·30
Total cholesterol (mg/dl)	191·0	167·0, 222·0
Daily nutrient, food group and alcohol intakes		
Total energy (kJ)	8657·9	7070·7, 10 925·0
Protein (% of energy)	14·7	12·6, 16·8
Carbohydrate (% of energy)	52·5	47·8, 57·8
Fat (% of energy)	31·9	27·7, 35·4
Total fibre (g)	14·1	10·4, 18·4
Folate (μg)	185·7	137·9, 244·9
Vitamin C (mg)	118·7	74·3, 190·8
Vitamin E (mg)	7·2	5·9, 9·0
Pro-vitamin A carotenoids (mg)	2139·0	1132·5, 3501·5
α-Carotene (mg)	140·8	59·0, 280·7
β-Carotene (mg)	1748·2	933·1, 2940·8
Lycopene (mg)	976·5	552·2, 1621·3
Vitamin A (IU)	8763	8313, 9214
Total fruits and juices (g/d)	148·1	63·3, 286·8
Dark green & deep yellow vegetables & fruits (g/d)	25·0	9·6, 51·5
Total vegetables (g/d)	98·9	61·6, 149·6
Alcohol (g/d)	0·2	0, 24·7

IQR, interquartile range.

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Table 2 Levels of serum antioxidant vitamins (μmol/l) according to characteristics of the participants at the time of blood collection: low-income women (*n* 918) aged 21–65 years participating in the Brazilian Investigation into Nutrition and Cervical Cancer Prevention (BRINCA) study

		Total carotene		Lycopene		Retinol		α-Tocopherol		γ-Tocopherol	
Variable	*n*	Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median	IQR
Race/ethnicity											
White	299	0·70	0·40, 1·18	1·03	0·60, 1·60	1·80	1·40, 2·40	16·50	12·40, 21·67	1·90	1·50, 2·50
Mulatto	537	0·65	0·33, 1·12	0·82	0·47, 1·35	1·70	1·26, 2·30	14·00	10·42, 18·61	1·70	1·30, 2·30
Black	42	0·74	0·33, 1·33	1·18	0·60, 2·07	1·62	1·17, 2·16	15·97	10·86, 20·03	1·70	1·23, 2·24
P for trend	–		0·68		0·31		0·49		<0·001		0·002
Monthly per capita income ($US)											
0–35	228	0·62	0·31, 1·10	0·78	0·45, 1·32	1·65	1·20, 2·20	14·54	10·31, 18·54	1·70	1·30, 2·27
36–63	225	0·59	0·31, 1·10	0·80	0·43, 1·44	1·75	1·22, 2·30	13·92	10·34, 19·04	1·60	1·26, 2·20
64–113	229	0·70	0·40, 1·27	0·99	0·61, 1·50	1·70	1·40, 2·40	14·63	11·01, 20·30	1·90	1·40, 2·40
114–850	224	0·72	0·40, 1·14	1·10	0·60, 1·60	1·90	1·40, 2·50	15·90	12·50, 20·41	1·86	1·40, 2·50
P for trend	–		0·11		<0·001		0·002		0·005		0·01
Smoking											
Never	463	0·78	0·41, 1·21	0·92	0·50, 1·50	1·80	1·35, 2·34	15·01	11·10, 20·30	1·80	1·40, 2·30
Former	186	0·60	0·33, 1·14	0·90	0·50, 1·44	1·70	1·30, 2·30	15·73	11·29, 19·91	1·90	1·38, 2·50
Smoker	269	0·60	0·30, 1·00	0·89	0·52, 1·43	1·70	1·23, 2·42	14·15	10·69, 18·99	1·60	1·30, 2·23
P for trend	–		0·005		0·31		0·80		0·16		0·12
Oral contraceptive use											
Never	215	0·70	0·36, 1·20	0·85	0·48, 1·42	1·63	1·20, 2·20	15·34	11·20, 20·10	1·80	1·30, 2·30
Former	512	0·70	0·42, 1·24	0·90	0·50, 1·44	1·70	1·26, 2·26	15·40	11·20, 20·30	1·82	1·40, 2·40
Current user	190	0·50	0·26, 0·93	0·90	0·53, 1·47	2·07	1·60, 2·68	13·39	10·20, 17·63	1·50	1·20, 2·00
P for trend	–		0·001		0·87		<0·001		0·002		0·002
Season of blood collection											
Spring	289	0·70	0·40, 1·20	1·00	0·60, 1·50	1·70	1·21, 2·20	13·20	10·16, 17·85	1·60	1·20, 2·10
Summer	177	0·80	0·50, 1·30	0·85	0·50, 1·20	1·70	1·28, 2·15	17·00	13·20, 21·65	1·90	1·50, 2·50
Autumn	155	0·53	0·29, 1·10	0·90	0·46, 1·70	1·85	1·48, 2·51	17·20	12·80, 21·20	2·00	1·50, 2·50
Winter	249	0·60	0·31, 1·01	0·81	0·46, 1·40	1·78	1·20, 2·59	12·90	10·38, 18·03	1·60	1·20, 2·26
P for trend	–		0·38		0·14		0·002		0·09		0·09
Cervical cytological classification											
Normal	408	0·80	0·13, 1·40	1·02	0·60, 1·60	1·80	1·40, 2·40	16·60	12·57, 22·05	1·90	1·50, 2·40
Equivocal atypia	39	0·63	0·50, 1·00	0·90	0·50, 1·30	1·69	1·38, 2·09	14·20	10·43, 19·62	1·90	1·40, 2·63
CIN1*	131	0·60	0·35, 1·07	0·73	0·44, 1·30	1·71	1·30, 2·40	14·94	10·95, 18·50	1·60	1·30, 2·30
CIN2*	120	0·57	0·31, 0·91	0·79	0·60, 1·55	1·70	1·11, 2·30	11·91	9·65, 16·76	1·50	1·20, 2·24
CIN3*	220	0·52	0·29, 0·99	0·76	0·42, 1·29	1·72	1·24, 2·42	12·91	9·92, 17·62	1·60	1·20, 2·10
P for trend	–		<0·001		<0·001		0·86		<0·001		<0·001

IQR, interquartile range.
*Cervical intraepithelial neoplasia grades of evolution 1, 2 and 3 (cancer precursor lesions).

Table 3 Dietary predictors of serum concentrations of total carotene: low-income women (*n* 918) aged 21–65 years participating in the Brazilian Investigation into Nutrition and Cervical Cancer Prevention (BRINCA) study

	Serum total carotene*		
Dietary intake	β_1	95 % CI	Partial R^2 (%)
Food groups			
Dark green & deep yellow vegetables & fruit (g/d)	0·059‡	0·041, 0·078	4·8
Total fruits and juices (g/d)	0·034‡	0·017, 0·052	1·8
Total fruits and vegetables (g/d)	0·054‡	0·027, 0·082	1·8
Carrots (g/d)	0·029‡	0·012, 0·045	1·4
Total vegetables (g/d)	0·050‡	0·020, 0·080	1·3
Fish and seafood (g/d)	0·027‡	0·011, 0·044	1·3
Ham and sausage (g/d)	−0·027§	−0·046, −0·008	0·9
Tomato and watermelon (g/d)	0·027‖	0·007, 0·046	0·9
Citrus fruits and juices only (g/d)	0·018‖	0·004, 0·032	0·8
Energy-adjusted nutrients†			
Vitamin A (IU)	0·104‡	0·071, 0·136	4·5
Pro-vitamin A carotenoids (mg)	0·077‡	0·051, 0·103	3·9
β-Carotene (mg)	0·074‡	0·048, 0·099	3·6
α-Carotene (mg)	0·047‡	0·029, 0·065	3·2
Vitamin B_{12} (mg)	0·066‡	0·037, 0·095	2·4
Niacin (mg)	0·153‡	0·065, 0·240	1·4
β-Cryptoxanthin (mg)	0·031‡	0·013, 0·050	1·3
Vitamin C (mg)	0·052‡	0·020, 0·084	1·2
Vitamin B_6 (mg)	0·212‡	0·083, 0·341	1·2
Folate (μg)	0·102§	0·037, 0·167	1·1
Fat (g)	−0·155§	−0·268, −0·042	0·9
K (mg)	0·109‖	0·023, 0·194	0·7
Fe (mg)	0·150‖	0·031, 0·272	0·7

*Adjusted for age (years), serum total cholesterol (continuous), hospital, cervical cytological classification, BMI (continuous) and smoking status (never, former, current).
†Energy adjustment by the residual method.
‡$P \leq 0{\cdot}001$.
§$P \leq 0{\cdot}005$.
‖$P < 0{\cdot}05$.

$R^2 = 0{\cdot}5\,\%$) was inversely correlated with γ-tocopherol levels (adjusted for the same covariates as for α-tocopherol plus cervical cytological classification and race/ethnicity). No dietary predictors were observed for serum lycopene and retinol in our study population. The strongest positive predictors of serum concentrations of lycopene were income, hospital, cervical lesion, alcohol consumption and serum total cholesterol (adjusted $R^2 = 0{\cdot}078$); while those of serum retinol levels were income, alcohol consumption, serum total cholesterol and season of blood collection (adjusted $R^2 = 0{\cdot}052$).

As expected, the highest median dietary intakes of total fruits and juices and dark green and deep yellow vegetables and fruits were observed in non-smokers compared with smokers: 161·40 and 123·0 g/d for total fruits and juices, and 26·4 and 21·1 g/d for dark green and deep yellow vegetables and fruits, respectively. Figure 1 illustrates the association between adjusted mean serum total carotene concentrations and intake quartiles of dark green and deep yellow vegetables and fruits and total fruits and juices according to smoking status, adjusted for confounding variables. A significant trend was found, showing increasing serum total carotene values with increasing quartiles of dietary intake of both food groups in non-smokers (P for trend $<0{\cdot}001$) and smokers (P for trend $<0{\cdot}001$). Median (interquartile range) serum total carotene concentration (μmol/l) adjusted for confounding variables and intake of total fruits and juices was 0·45 (0·38, 0·52) and 0·51 (0·44, 0·59) for smokers and non-smokers, respectively ($P < 0{\cdot}001$); and adjusted for dark green and deep yellow vegetables and fruits was 0·48 (0·39, 0·55) and 0·53 (0·45, 0·62) for smokers and non-smokers, respectively ($P < 0{\cdot}001$).

Discussion

In the present study, we found that dark green and deep yellow vegetables and fruits, total fruits and juices, citrus fruits and juices only and carrots were independent predictors of serum total carotene levels in low-income Brazilian women. The median F&V intake (about 250 g/d) was lower than the WHO recommendation of 400 g/d for this food group[2] and the intake of wholegrain cereals was unusual. In the present population, serum retinol levels were in the normal range as established by the Food and Nutrition Board criteria[33], and serum concentrations of total carotene and lycopene were higher than the values observed in the Third National Health and Nutrition Examination Survey (NHANES III) among non-smoking women[34]. However, lower intakes of F&V similar to the levels in our study population were found in a study conducted among adolescents in Costa Rica, where daily consumption of one portion of fruit (112 g)

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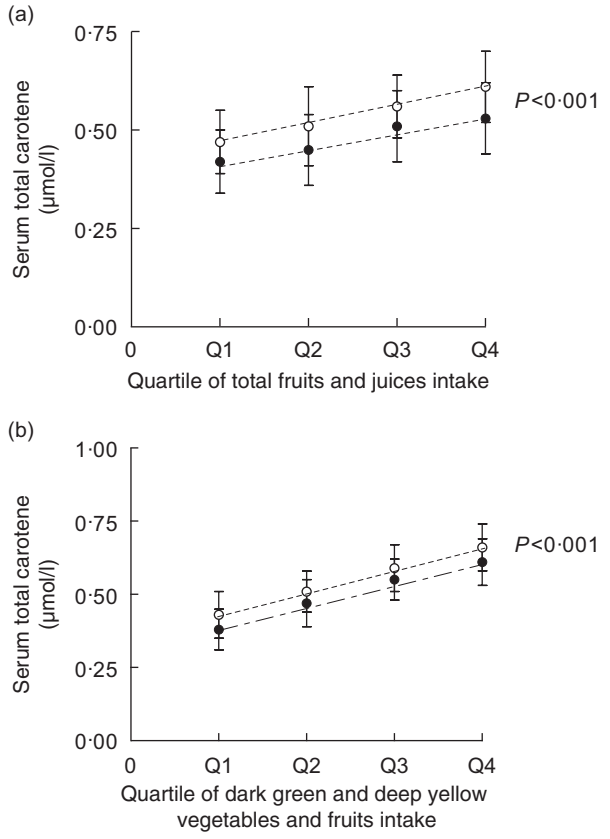

Fig. 1 Adjusted mean (95 % CI) serum total carotene level by intake quartile of total fruits and juices (a) and dark green and deep yellow vegetables and fruits (b) according to smoking status (○, non-smokers; ●, smokers) among low-income women (*n* 918) aged 21–65 years participating in the Brazilian Investigation into Nutrition and Cervical Cancer Prevention (BRINCA) study. Mean serum concentrations of total carotene were adjusted for age, serum cholesterol, BMI, hospital and cervical cytological classification. Range of dietary intakes (g/d), Q1 to Q4: 0–63·2, 63·3–147·8, 147·9–286·8 and 286·9–490·8 for total fruits and juices; 0–9·5, 9·6–24·9, 25·0–51·5 and 51·6–362·3 for dark green and deep yellow vegetables and fruit

was reported by ~84 % and that of green vegetables (23 g) by ~34 % of the participants[20].

In our study, differences in serum total cholesterol, age, hospital attended, cervical cytological classification, BMI, smoking status and intake of dark green and deep yellow vegetables and fruits explained 14·4 % of the variation of serum total carotene concentrations. Previous studies explained 10·7 % to 39·4 % in multivariate models that included age, gender, smoking, alcohol intake, serum cholesterol and/or TAG, total energy intake, and vegetable and fruit or β-carotene intake[4,7,13,14,16,35]. Smoking has been negatively correlated with circulating β-carotene levels[4,7,12–13,17,36,37], with reported lower dietary intake of β-carotene in smokers compared with non-smokers[12,34,35,38–40]. In our study, there was no evidence of the above interaction, and a similar trend of increasing serum total carotene concentrations with increasing intakes of dietary food sources of carotenes was noted in both smoking and non-smoking participants after adjusting for confounding variables. However, a slight decrease in serum total carotene concentrations was observed among smokers compared with non-smokers at the same dietary intake levels probably due to destruction of carotenes by highly oxidative tobacco smoke[41].

Other variables identified in previous studies, such as oral contraceptive (OC) use, were not significant in multiple linear models in our study. It has been reported that OC users have lower levels of serum β-carotene[42,43]. In a representative sample of US women, OC users had lower dietary intake of carotenoids, were more likely to smoke and drink, were married and highly educated compared with non-users of OC, after adjusting for confounding variables[40]. One explanation suggested for the lower levels of β-carotene among OC users is related to the decrease in LDL levels used to carry β-carotene in plasma. The negative correlation between alcohol consumption and blood β-carotene concentrations has been explained by the oxidative stress mechanisms among regular drinkers[4,12–14,44,45].

Similar seasonal variation in serum concentrations of total carotene and tocopherols found in the present study was reported previously[4,46]. The positive correlation between temperature and plasma carotene was explained by the variation in dietary sources according to season, with light and heat contributing to increase the carotenoid contents of specific F&V[46]. In the south-eastern part of Brazil, important differences in food availability, environmental temperature and humidity are expected at least between summer and winter. Higher consumption of energy-dense foods in winter could be responsible for the increased concentrations of α- and γ-tocopherols, which in turn was strongly correlated with serum total cholesterol.

Few earlier studies have assessed the correlation between serum levels of micronutrients and dietary intakes in multiple models. In Brazil, one previous study with 100 women in the sub-cohort sample living in São Paulo city mentioned strong crude correlations ($r > 0{\cdot}40$; data not shown) between the consumption of carrots and serum α-carotene and β-carotene levels, and between citrus fruit intake and serum lycopene levels[47]. However, few studies have correlated dietary intakes and blood carotene levels after adjusting for confounding variables. In the New York Women's Health Study involving 228 women[16] and in the European Prospective Investigation into Cancer and Nutrition with 2974 participants[4], total vegetable intake and tomato and its products accounted for 7 % and 14 % of blood lycopene levels, respectively, after adjusting for confounding variables. In a sub-sample of NHANES III (*n* 3413), the frequency of consumption of pizza, pasta and tomato was a significant determinant of serum lycopene levels[6]; and in Massachusetts Hispanic elders, total carotenoid intake was a predictor of blood lycopene levels[7]. Other

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studies did not find any correlation, possibly due to small sample size ($n < 400$), low intakes or poor accuracy in estimates of the main food sources of lycopene[10,11,14,17].

Similarly to our study, determinants of serum retinol such as income, alcohol consumption, serum total cholesterol concentration and season of blood collection were found in other studies[13]. Determinants of blood levels of α-tocopherol reported previously were age, education, body weight or BMI, blood cholesterol and/or TAG, cigarette use, total energy, alcohol and fat intake[9,12,36]. Only two studies in adults and children found a significant correlation between dietary intake of vitamin E and plasma α-tocopherol levels[34,48]. The lack of significant dietary predictors for circulating tocopherols after adjusting for confounders may be related to the inaccuracy of the FFQ to capture total intakes of vegetable oil, its major dietary source. In our study, we included additional items about type and frequency of vegetable oil use, but it may not have been sufficient to better estimate vitamin E dietary sources.

It is possible that the estimated β_1 in our cross-sectional study was lower than the true slope since we collected only one blood sample and blood micronutrient levels vary on a daily basis. This daily variability can attenuate the relationship between the dietary exposure and the blood levels. In a previous study, Block *et al.*[49] estimated that two or three independent blood samples for β-carotene and two to five samples for lycopene are necessary to minimize the attenuation effect of measures, which is difficult to perform in large population studies. These authors also recommended the use of blood samples collected in 2- to 4-week intervals for non-smokers or passive smokers with dietary intakes of F&V less than 4 servings/d. A single measurement of plasma carotenoids could introduce non-differential misclassification, which will bias the association towards the null[50]. This may also explain the relatively low correlation between dietary carotenoids and plasma carotenoids in the present study.

To our knowledge, the present study is the first one to look for determinants of serum total carotene levels in a Brazilian population. Our findings were similar to those of previous studies conducted in participants with higher educational and income levels in developed countries. Since carotenoids lack a regulatory homeostatic mechanism, their serum level has been considered the best biomarker for F&V consumption[33].Thus, measures of serum carotene concentrations should be included to complement FFQ validation studies in populations with low F&V intake.

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