British Journal of Nutrition (2013), **109**, 1544–1556
doi:10.1017/S0007114513000263

British Journal of Nutrition

Systematic Review with Meta-Analysis

Effect of plant foods and beverages on plasma non-enzymatic antioxidant capacity in human subjects: a meta-analysis

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(Submitted 25 May 2012 – Final revision received 8 January 2013 – Accepted 8 January 2013 – First published online 14 March 2013)

Abstract

Non-enzymatic antioxidant capacity (NEAC) represents a sensitive biomarker measuring the *in vivo* antioxidant potential of vegetable foods. To evaluate the effectiveness of plant-derived foods and beverages on the plasma non-enzymatic antioxidant system, we analysed all literature published upto May 2010. Data were extracted by two authors independently, and the effect size was summarised using standardised mean differences by a random-effects model. For the analysis, eighty-eight studies were included, reporting a total number of 122 interventions and involving 2890 subjects. There was overall evidence of the effectiveness of fruit, vegetables, dietary patterns based on plant foods, red wine and tea in increasing plasma NEAC. No changes were found for chocolate and fruit juices. We observed an overall effect size three times higher in subjects with risk factors when compared with healthy subjects. Total radical-trapping antioxidant parameter, oxygen radical absorbance capacity and ferric-reducing antioxidant power methods showed a similar increase in plasma NEAC following dietary supplementation, whereas Trolox equivalent antioxidant capacity did not respond to dietary supplementation. Data from the present meta-analysis show that plant-derived foods represent an effective strategy to enhance an endogenous antioxidant network in humans. This is particularly evident in the presence of oxidative stress-related risk factors.

Key words: **Plant foods: Antioxidant capacity: Oxidative stress: Human nutrition: Flavonoids**

A large body of epidemiological evidence strongly suggests a primary role for plant-based dietary patterns in reducing the risk of diseases[1]. However, the identification of the molecules involved in the protective effect of vegetable food and their mechanism of action is far from being understood[2]. Phytochemicals contained in the plant kingdom are hypothesised to reduce free radical-related cellular damage, potentiating redox defence of the body and contributing to reduction of the risk of developing oxidative stress-related diseases[3]. On the basis of the so-called 'antioxidant hypothesis', long-term clinical trials have been developed to investigate the effect of supplementation with natural antioxidants to reduce the development of oxidative stress-related diseases. However, clinical trials have produced extremely contrasting results, highlighting the difficulties in mimicking the healthy effect of plant-derived foods through the usage of natural supplements[4,5]. Moreover, a negative effect in increasing mortality rates of overall mortality and cancer has been observed in specific meta-analyses, raising strong concerns about the use of galenic antioxidants for disease prevention[5,6].

Antioxidant defences of the body are composed of molecular and enzymatic players; however, the composition of this network markedly differs, in terms of concentration and components, between body compartments[7]. The multifunctional

Abbreviations: EXP, experimental studies; FRAP, ferric-reducing antioxidant power; NEAC, non-enzymatic antioxidant capacity; ORAC, oxygen radical absorbance capacity; OSRRF, oxidative stress-related risk factor; Q-EXP, quasi-experimental studies; SMD, standardised mean difference; TEAC, Trolox equivalent antioxidant capacity; TRAP, total radical-trapping antioxidant parameter.

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properties of the antioxidant network highlight the crucial importance of dynamic interactions among the components of the network in protecting body fluids from oxidative stress. Non-enzymatic antioxidant capacity (NEAC), defined as the moles of oxidants neutralised by 1 litre of plasma, represents a biomarker measuring the antioxidant potential, including synergistic interactions, of body fluids and tissues[7]. In terms of participation of individual components to the network, the main contributor to NEAC is uric acid (40–55 %), followed by thiol groups (10–24 %), ascorbic acid (8–15 %) and vitamin E (less than 10 %)[8]. The contribution of single antioxidants to overall NEAC leaves unexplained a percentage ranging from 20 to 40 % that might be accounted for by dietary components and/or synergistic interactions. NEAC methodology has been widely used to investigate the role of diet in modulating antioxidant function in humans, though criticisms have been raised on the real ability of NEAC to be a proper biomarker of antioxidant function *in vivo*[8,9]. Moreover, the large number of assays available for the measurement of NEAC in biological matrices has become a major obstacle in understanding the pros and cons of NEAC as a biomarker for assessing antioxidant capacity *in vivo*.

The main point of concern relies on the lack of a clear association between the ingestion of plant foods rich in antioxidants and changes in plasma NEAC. Plasma NEAC was shown to respond to the ingestion of dietary food items rich in antioxidants such as tea, wine, chocolate, fruit and vegetables in the majority of acute ingestion studies[8]. However, if we analyse chronic intervention studies, the picture is much more complex and results are not homogeneous. Recently, it has been suggested that the efficiency of antioxidants in modulating NEAC in chronic trials depends on the 'healthy status' of the subjects, with larger effects occurring in subjects characterised by risk factors (i.e. smoking), pollutant exposure[10] or pathologies (HIV^+ and CVD)[8]. Healthy subjects might have a lower responsiveness to antioxidant supplementation because they do not have any oxidative stress conditions in which a higher intake of redox molecules might be required.

In order to clarify these concerns, we performed a meta-analysis of human intervention studies testing the effect of vegetable food and beverage consumption on plasma NEAC. Moreover, the effect of oxidative stress-related risk factors (OSRRF) and of the applied methodology was also investigated.

Experimental methods

Search strategy

An extensive search for 'antioxidant capacity', 'antioxidant potential', 'antioxidant status' and diet or specific food/beverages (fruit, fruit juice, vegetable, tea, chocolate and wine) was performed, collecting dietary intervention studies up to May 2010. Papers suitable for inclusion were identified by systematic research on the MEDLINE and EMBASE electronic databases. Searches were limited to human subjects and results reported in the English language. In addition, a manual search of references from reports of clinical trials or review articles was performed to identify relevant studies.

Study selection

In the present meta-analysis, we included dietary intervention studies with an experimental design (EXP) as well as with a quasi-experimental design (Q-EXP). EXP studies were randomised, with a control group and a parallel or cross-over design. Observational studies (pre- and post-intervention or pre- and post-data), non-randomised or uncontrolled study design were included in the Q-EXP study category[11]. Q-EXP studies were pooled together with EXP studies only after assessing whether they were in agreement with EXP studies[12,13].

General inclusion criteria were as follows: (1) human intervention studies assessing the effectiveness of foods, beverages or dietary patterns on plasma NEAC; (2) studies with both acute and chronic supplementation periods; (3) chronic studies with a treatment period longer than 7 d; (4) healthy people and subjects characterised by OSRRF (smoking, dyslipidaemia, heart transplant, obesity and ageing) or pathologies (HIV^+ and CVD); (5) numerical or graphical information about results, study duration (chronic studies) or time point (acute studies) and dosage of the plant-derived foods and beverages used. Studies were excluded when only abstracts were available; experimental meal did not fall into the categories that we considered; data presentation was incomplete; treatment effects on NEAC were reported as AUC; subjects were <18 years; information about the dose of food and beverages was incomplete; the washout time was not considered or was less than 7 d (only for the cross-over design).

When necessary, efforts were made to contact investigators for clarification or additional data.

Quality assessment and data extraction

For data extraction, two investigators (D. L.-B. and G. M.) independently screened the titles and abstracts resulting from the search strategies. Articles with titles or abstracts clearly irrelevant were rejected at first screening. The full text of potentially relevant articles was reviewed to assess eligibility for inclusion in the meta-analysis with any disagreement resolved by consensus. Considering that the present meta-analysis included different types of studies, it was performed following both PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) and MOOSE (Meta-Analysis of Observational Studies in Epidemiology) checklists[14].

Quality assessment of the included studies was performed using study design as the main quality variable (parallel, cross-over studies or pre–post-intervention studies, placebo, studies with or without a control group). Moreover, other features related to human dietary intervention studies, such as the number of participants enrolled, the length of studies for chronic ones and the description of experimental meal, were considered. Score quality was not used as a criterion for the selection of trials, but it was only used for allocation purpose in high-, fair or low-bias risk. Trials with an adequate study design were classified as low-bias risk (good quality: >7 points), whereas those with an adequate design but with one or more unclear quality components were classified as fair quality (4–7 points). Trials with an inadequate study

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Table 1. Criteria utilised to assess the quality of the studies included in the meta-analysis

Trials	Study design			No. of participants enrolled	Length of the study (for chronic studies)	Description of experimental meal		Quality assessment		
	Controlled (placebo or control with food, diet and beverages without antioxidant)	Randomised (with parallel or cross-over design)	Pre–post-data	>10 subjects	>12 d	Main characteristics	Funding	Good	Fair	Poor
Tea	11/12	8/12	1/12	9/12	8/8	5/12	2/12	8	3	1
Fruit juices	7/19	7/19	12/19	14/19	13/14	12/19	4/19	8	0	11
Red wine	11/16	6/16	/16	9/19	3/4	2/16	6/16	5	5	6
Chocolate	9/11	7/11	2/11	11/11	5/5	10/11	4/11	7	1	3
Fruit	8/11	6/11	3/11	8/11	4/4	4/11	4/11	6	1	4
Vegetables	4/12	2/12	8/12	9/12	8/9	4/12	3/12	1	2	9
Dietary patterns	5/7	5/7	2/7	7/7	7/7	5/7	0/7	2	2	3

design were considered as high-bias risk or poor quality (<4 points; Tables 1 and 2). Extracted data include: name of the first author; year of publication; experimental meal; number of participants; health status of the enrolled subjects; type and design of the study and time point considered for data analysis; daily dosage; assay used; study quality.

Data analysis

Relevant data of the studies available for formal meta-analytic evaluation were entered into Comprehensive Meta-Analysis software (Biostat) for evaluation of the meta-analysis.

In particular, for parallel or cross-over studies, when results were reported as post-data only, we used mean, standard deviation and sample size in each group, or difference in means, sample size and *P* value between groups. When results were reported as pre- and post-data, we used mean, standard deviation, sample size in each group and correlation between baseline and end-point intervention period, or mean change, standard deviation difference, sample size in each group, correlation between baseline and end-point intervention period. For observational studies considering only one group (pre–post-intervention data), we used mean difference, standard deviation of difference and sample size. For all data, standard deviations were calculated, when necessary, from standard error or CI, and data not provided in numerical form were estimated from figures. In all types of dietary intervention studies, we assumed the correlation between baseline and end-point study period to be 0·5 to produce the most conservative estimate[13,15].

When in the same study the effect on plasma NEAC of two different doses of the same experimental meal was evaluated, our approach was to create a synthetic variable for each study to compute a combined effect based on these variables (defined as the mean of the effect size), and we used it as the effect size in the analysis. The same procedure was used in chronic studies that reported the effect on NEAC of the same experimental meal, in two or more periods, as well as in acute studies that showed the effect on NEAC of the same experimental meal, in two or more time points.

For acute studies, the significant change (positive or negative) on plasma or serum NEAC was considered as the time point to statistical analysis, while if there were no reported significant changes, we have considered the time point at the end of the study. For chronic studies, we considered the end point of the intervention.

All studies addressed the same outcome (plasma antioxidant capacity or NEAC), used different methods of analysis (total radical-trapping antioxidant parameter (TRAP), ferric-reducing antioxidant power (FRAP), oxygen radical absorbance capacity (ORAC) and Trolox equivalent antioxidant capacity (TEAC)) and presented the results in different scales. To enable a joint comparison, the same index of treatment effects was used, and in each individual study, the standardised mean difference (SMD) with 95 % CI was calculated[16]. In the present analysis, a positive SMD indicates an increase in plasma NEAC after consumption of plant-derived foods and beverages (experimental meal). When an author published the plasma NEAC value detected with two or more methods, we evaluated separately the effect size in relation to the assay used.

Meta-analysis was performed using DerSimonian & Laird's 'random-effects models'. Under the random-effects model, effect sizes are assumed not to be common to all studies; they vary under a normal distribution model[17]. We used a random-effects model because there was evidence of heterogeneity between studies based on the χ^2 test for heterogeneity at the significant level of $P=0{\cdot}10$ and the I^2 statistic, percentage of total variation across studies due to heterogeneity, rather than chance, of more than 50 %[18].

Potential publication bias (i.e. the association of publication probability with the statistical significance of study results) was

Table 2. Quality score used in the meta-analysis

	Score
Randomised (with a parallel or cross-over design)	2
Group controlled without an antioxidant meal (with a parallel or cross-over design)	2
Placebo controlled (with a parallel or cross-over design)	2
Blinding	1
Funding	0
Length of the study (for chronic studies)	1
No. of participants enrolled (>10 subjects)	1
Description of experimental meal	1

investigated using visual assessment of the funnel plot calculated by Comprehensive Meta-Analysis software[19] (Biostat).

Results

We identified 716 potential literature citations through the database searches (Fig. 1). At first, 156 studies met our inclusion criteria and five additional studies potentially meeting our inclusion criteria were identified through scanning reference lists. Of the 161 references, seventy-three papers were excluded for the following reasons: outcome measures not suitable for meta-analysis, continuous variables of pre- and post-intervention data or mean difference for only post-data not included in the publication; NEAC values expressed as AUC; chronic intervention trials were less than 7 d or intervention studies performed with dietary supplements. The remaining eighty-eight studies, reporting a total number of 122 interventions met our selection criteria and were included in the meta-analysis (Table S1, available online).

The following three main categories of intervention, on the basis of type of food, were identified: beverages (*n* 47); plant foods (*n* 34); plant food-based dietary patterns (*n* 7). Considering the criteria used to assess the quality of studies included in the present meta-analysis, thirty-six studies were of good quality, fifteen studies were fair and thirty-seven studies were of poor quality. Moreover, forty of the included studies had an experimental design (EXP), whereas forty-eight studies had a quasi-experimental design (Q-EXP). Plasma NEAC was assessed in 2890 subjects. Moreover, all subjects were stratified into two main subgroups: healthy subjects (*n* 1880) and subjects with risk factors or diseases (*n* 1010). After evaluating the agreement between Q-EXP and EXP studies through the view of effect size direction (data not shown), both Q-EXP and EXP studies were processed together[13].

Beverages

Intervention studies involving red wine (*n* 16), fruit juices (*n* 20) and tea (*n* 12) are described in Fig. 2. There was clear evidence on the efficacy of red wine consumption in increasing plasma NEAC, both in acute feeding studies (SMD 1·118, 95 % CI 0·976, 1·325; $P<0·000$) and in chronic studies (SMD 1·130, 95 % CI 0·818, 1·617; $P<0·001$). Fruit juice ingestion was able to increase plasma NEAC in acute feeding studies (SMD 1·028, 95 % CI 0·446, 1·702; $P=0·001$), but was ineffective in chronic intervention studies (SMD 0·157, 95 % CI −0·275, 0·422; $P=0·382$). Tea consumption induced a similar increase in plasma NEAC both after acute (SMD 0·703, 95 % CI 0·154, 1·248; $P=0·012$) and chronic ingestion (SMD 0·664, 95 % CI 0·249, 1·078; $P=0·002$). The results showed that green tea had a stronger antioxidant effect than black tea (SMD 0·957, 95 % CI 0·447, 1·466; $P<0·0001$ for green tea and SMD 0·487, 95 % CI 0·072, 0·901; $P=0·021$ for black tea) (data not shown).

Plant foods

The effect of chocolate (*n* 11), fruits (*n* 10) and vegetables (*n* 12) on plasma NEAC is described in Fig. 3. Chocolate

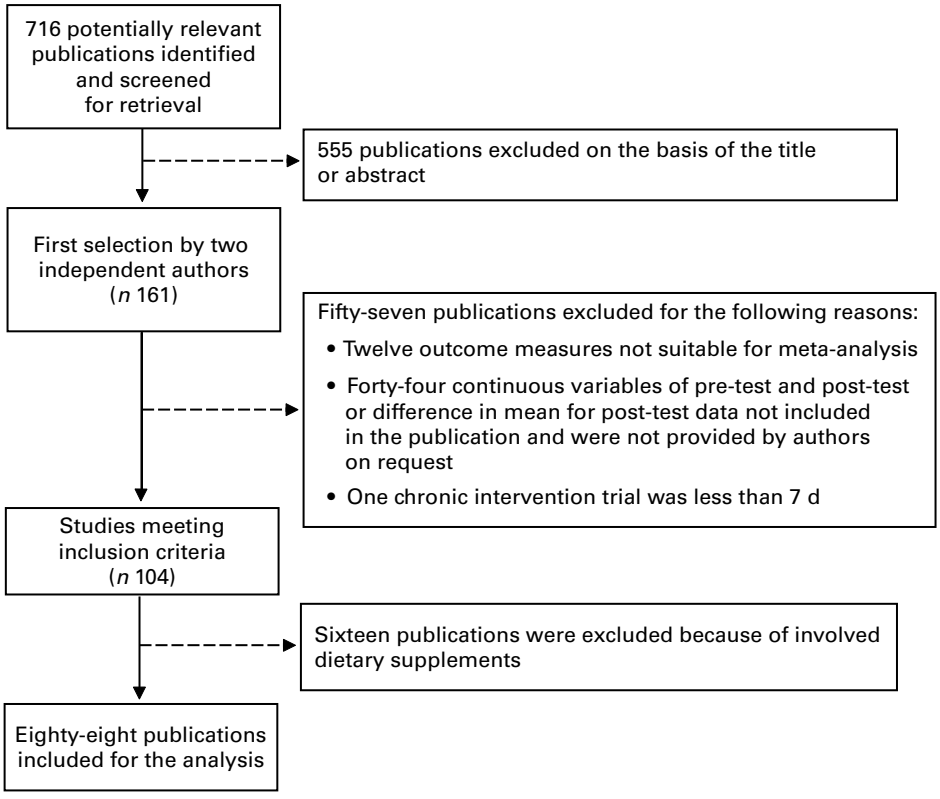

Fig. 1. Flow chart of the study identification and selection.

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Red wine

	Study	Dose	Quality	Participants (*n*)	SMD	*P*
Acute	Vukovic, 2009[39]	3 ml/kg	Good	10	1·377	0·006
Acute	Maxwell, 1994[40]	5-7 ml/kg	Fair	10	1·599	0·002
Acute	Modun, 2008[41]	3 ml/kg	Good	9	1·263	0·014
Acute	Arendt, 2005[42]	200 ml	Good	19	0·000	1·000
Acute	Serafini, 1998[43]	113 ml	Good	19	1·708	0·001
Acute	Blackhurst, 2006[45]	About 200 ml	Good	15	–0·168	0·646
Acute	Cao, 1998_a[66]	300 ml	Poor	16	2·304	0·000
Acute	Cao, 1998_b[66]	300 ml	Poor	16	0·632	0·218
Acute	Day, 1995[47]	250 ml	Poor	6	1·388	0·000
Acute	Fernández-Pachón, 2005_a[48]	300 ml	Poor	8	0·883	0·000
Acute	Fernández-Pachón, 2005_b[48]	300 ml	Poor	8	1·065	0·000
Acute	Pinzani, 2010[49]	300 ml	Poor	5	0·451	0·079
Acute	Simonetti, 2001[50]	About 200 ml	Poor	5	1·552	0·000
Acute	Kiviniemi, 2007[54]	0·75 g/kg	Poor	18	2·018	0·000
Acute					1·118	0·000
Chronic	Avellone, 2006[44]	250 ml	Fair	48	1·875	0·000
Chronic	Otaolaurruchi, 2007_a[51]	300 ml	Fair	16	0·365	0·470
Chronic	Otaolaurruchi, 2007_b[51]	300 ml	Fair	16	1·150	0·033
Chronic	Tsang, 2005[52]	375ml	Fair	20	0·496	0·284
Chronic	Guarda, 2005[53]	250 ml	Fair	20	1·610	0·002
Chronic					1·130	0·001
Overall					1·121	0·000

Fruit juice

	Study	Dose	Quality	Participants (*n*)	SMD	*P*
Acute	Chaves, 2009_a[103]	2·5 cups	Fair	10	0·557	0·388
Acute	Netzel, 2002[95]	400 ml	Poor	6	0·850	0·001
Acute	Netzel, 2005_a[96]	300 ml	Poor	16	1·133	0·035
Acute	Netzel, 2005_b[96]	300 ml	Poor	16	4·665	0·000
Acute	Pedersen, 2000_a[97]	500 ml	Good	18	1·248	0·000
Acute	Pedersen, 2000_b[97]	500 ml	Good	18	0·164	0·371
Acute	Prior, 2007[98]	315 ml	Good	6	0·637	0·282
Acute	Vinson, 2008[100]	240 ml	Good	10	0·846	0·070
Acute					1·028	0·001
Chronic	Arendt, 2001_a[83]	1000 ml	Poor	13	1·973	0·000
Chronic	Arendt, 2001_b[83]	1000 ml	Poor	12	0·397	0·168
Chronic	Borochov-Negri, 2006[93]	200 ml	Poor	10	0·577	0·091
Chronic	Bub, 2003 _a[85]	330 ml	Poor	27	–0·088	0·648
Chronic	Bub, 2003 _b[85]	330 ml	Poor	27	0·211	0·278
Chronic	Castilla, 2006_a[84]	100 ml	Poor	15	0·934	0·003
Chronic	Castilla, 2006_b[84]	100 ml	Good	38	0·841	0·020
Chronic	Chaves, 2009_b[103]	2·5 cups	Fair	10	3·804	0·000
Chronic	Cilla, 2009_a[86]	500 ml	Poor	32	–4·068	0·000
Chronic	Cilia, 2009_b[86]	500 ml	Poor	32	–5·4608	0·000
Chronic	Duthie, 2006[87]	750 ml	Good	20	–0·272	0·547
Chronic	Gorinstein, 2004[94]	150 ml	Good	72	0·148	0·609
Chronic	Guo, 2008_a[88]	250 ml	Poor	13	0·571	0·056
Chronic	Guo, 2008_b[88]	250 ml	Poor	13	–0·074	0·790
Chronic	O'Byrne, 2002[89]	10 ml/kg	Poor	31	0·866	0·000
Chronic	Park, 2009[90]	5·5 ml/kg	Good	40	0·600	0·064
Chronic	Riso, 2005[91]	600 ml	Good	16	0·170	0·499
Chronic	Ruel, 2005[99]	7 ml/kg	Poor	21	0·846	0·001
Chronic	Wilms, 2007[101]	1000 ml	Poor	168	0·371	0·000
Chronic	Young, 1999_a[92]	1083 ml	Poor	5	–0·006	0·989
Chronic	Young, 1999_b[92]	1083 ml	Poor	5	–0·096	0·830
Chronic					0·157	0·382
Overall					0·556	0·220

Tea

	Study	Dose	Quality	Participants (*n*)	SMD	*P*
Acute	Maxwell, 1996[35]	500 ml	Poor	10	0·680	0·053
Acute	Hodgson, 2000_b[32]	4 cups	Good	40	0·176	0·580
Acute	Kyle, 2007[37]	400 ml	Fair	5	0·953	0·055
Acute	Panza, 2008[29]	600 ml	Fair	28	0·385	0·313
Acute	Hodgson, 2000_a[32]	4 cups	Good	40	0·306	0·336
Acute	Serafini, 1996_b[33]	300 ml	Good	10	1·619	0·026
Acute	Serafini, 1996_a[33]	300 ml	Good	10	1·923	0·012
Acute					0·703	0·012
Chronic	Davies, 2003[34]	5 cups	Good	15	0·000	1·000
Chronic	Duffy, 2001_a[36]	900 ml	Good	42	0·350	0·261
Chronic	Duffy, 2001_b[36]	900 ml	Good	42	0·532	0·090
Chronic	Van het hof, 1997_b[31]	900 ml	Fair	32	0·500	0·164
Chronic	Widlansky, 2005_a[38]	900 ml	Good	44	0·306	0·313
Chronic	Widlansky, 2005_b[38]	900 ml	Good	44	0·440	0·150
Chronic	Bertipaglia de santana, 2008[27]	500 ml	Good	50	3·232	0·000
Chronic	Erba, 2005[28]	2 cups	Good	24	0·362	0·379
Chronic	Coimbra, 2006[30]	1000 ml	Fair	68	0·231	0·343
Chronic	van het Hof, 1997_a[31]	900 ml	Fair	32	1·125	0·003
Chronic					0·666	0·002
Overall					0·680	0·000

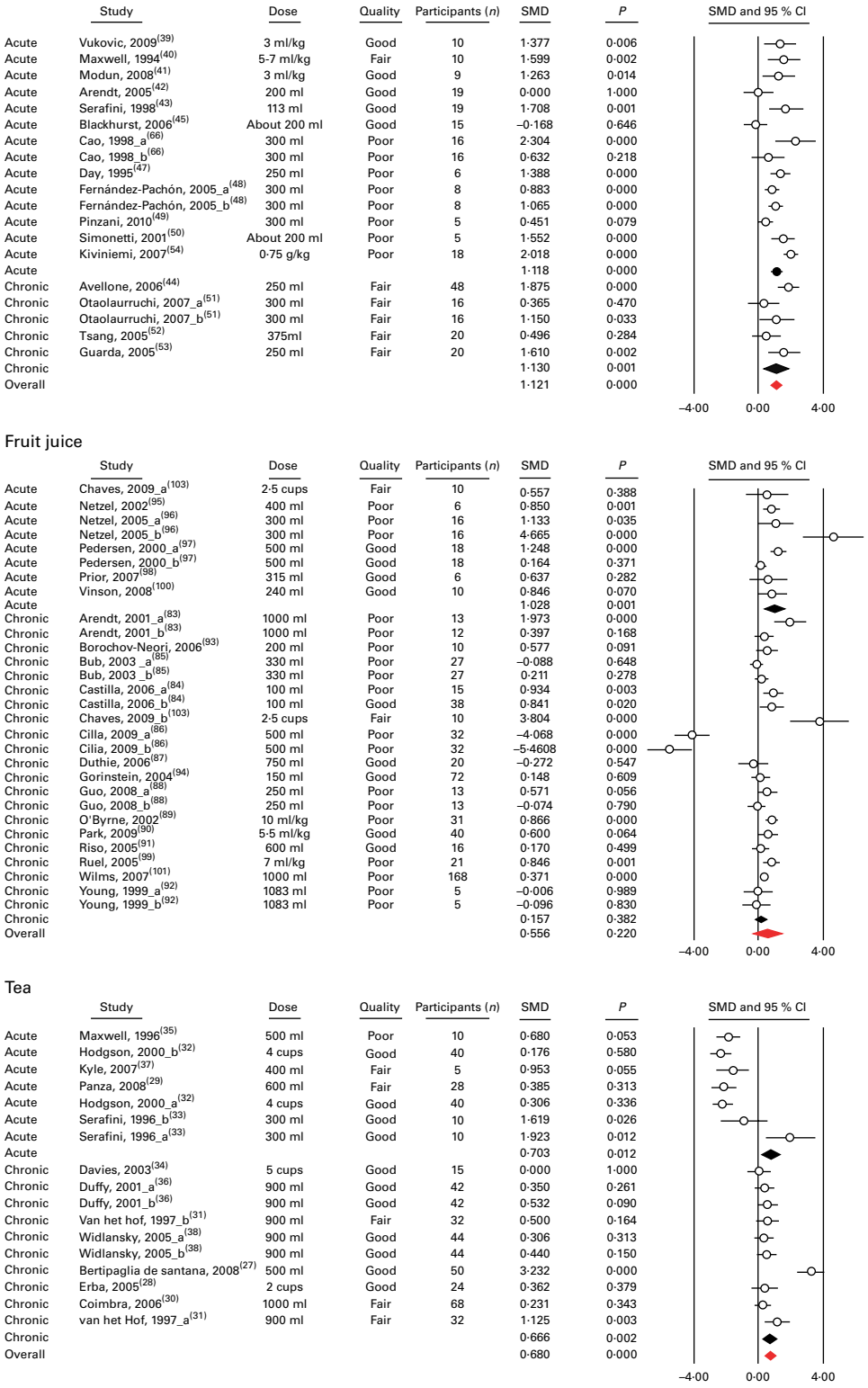

Fig. 2. Effect of beverage ingestion on plasma non-enzymatic antioxidant capacity (NEAC). Studies were stratified according to the design of the study (acute or chronic). A positive standardised mean difference (SMD) indicates an increase in plasma NEAC, whereas a negative SMD indicates a decrease in plasma NEAC. The terms a, b or c was used when the same author measured plasma NEAC with different methods. (A colour version of this figure can be found online at http://www.journals.cambridge.org/bjn).

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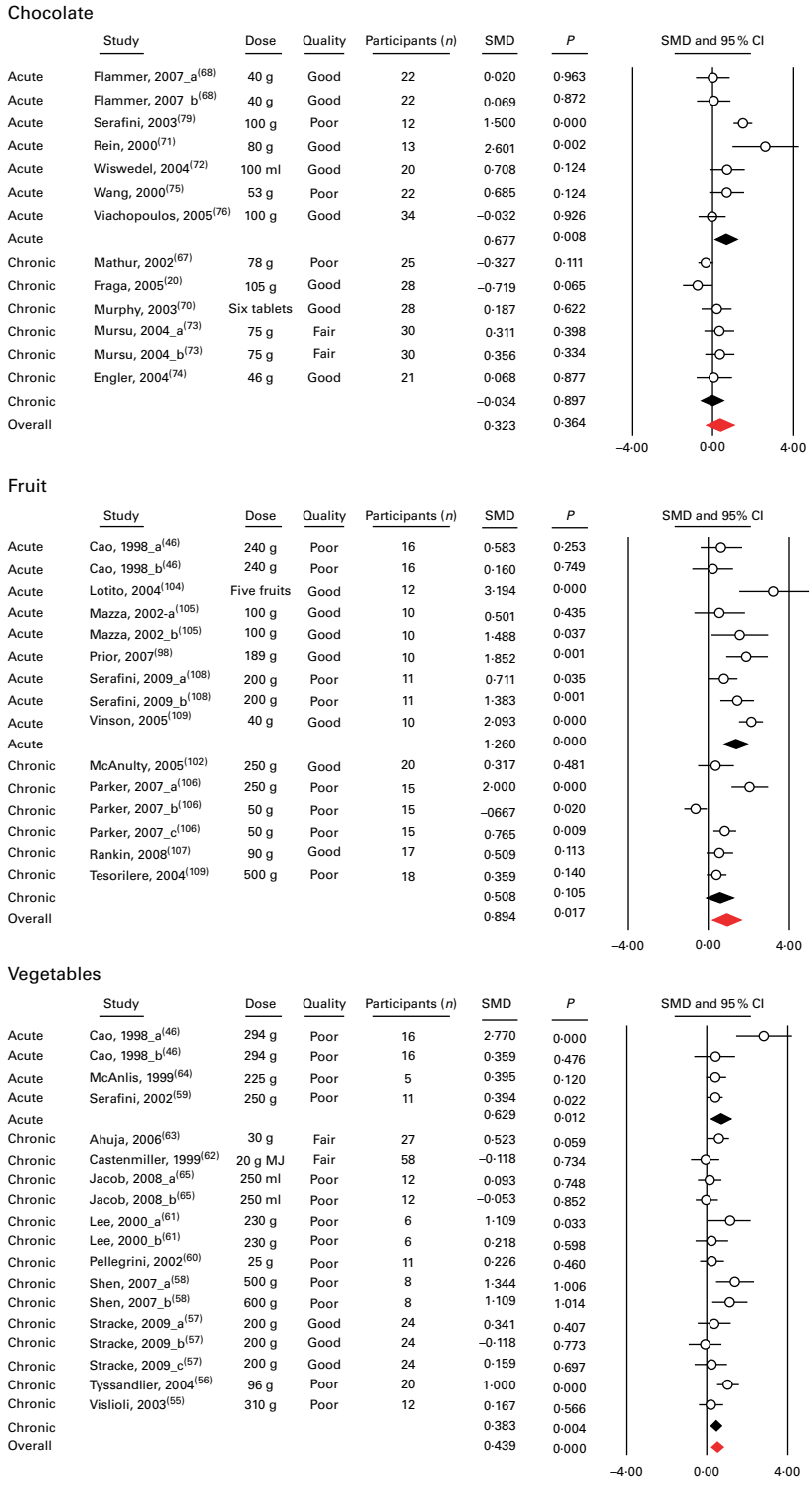

Fig. 3. Effect of plant food ingestion on plasma non-enzymatic antioxidant capacity (NEAC). Studies were stratified according to the design of the study (acute or chronic). A positive standardised mean difference (SMD) indicates an increase in plasma NEAC, whereas a negative SMD indicates a decrease in plasma NEAC. The terms a, b or c was used when the same author measured plasma NEAC with different methods. (A colour version of this figure can be found online at http://www.journals.cambridge.org/bjn).

increased plasma NEAC in acute studies (SMD 0·677, 95 % CI 0·509, 1·066; $P<0·008$), but failed to enhance plasma NEAC after chronic ingestion (SMD −0·034, 95 % CI −0·540, 0·473; $P=0·897$). Similarly, antioxidants from fruits massively increase endogenous antioxidant defences in acute studies (SMD 1·260, 95 % CI 0·946, 1·535; $P<0·000$), while no effect was revealed in chronic feeding studies (SMD 0·508, 95 % CI 0·133, 0·938; $P=0·105$). On the other hand, vegetables

induced a significant increase in NEAC both after acute (SMD 0·629, 95 % CI 0·141, 1·117; $P<0·012$) and chronic consumption (SMD 0·383, 95 % CI 0·120, 0·647; $P=0·004$).

Plant food-based dietary patterns

In agreement with the results observed for fruit and vegetables, dietary patterns based on fruit and vegetables had a strong impact in modulating positively plasma NEAC (SMD 0·965, 95 % CI 0·809, 1·122; $P<0·000$; Fig. 4).

Effect of oxidative stress-related risk factors on plasma non-enzymatic antioxidant capacity

As described in Table 3, participants enrolled in chronic studies were divided into healthy subjects (no detectable exposure to oxidative stress-related risk factors) and subjects exposed to oxidative stress-related risk factors. For the beverage category, an effect on plasma NEAC was clearly detected in the risk factor category (SMD 0·765, 95 % CI 0·310, 1·220; $P<0·001$), whereas no changes in plasma NEAC were observed in healthy subjects (SMD 0·177, 95 % CI −0·154, 0·508; $P<0·296$). On the contrary, in the food group (chocolate, fruit, vegetables and plant food-based dietary patterns), a significant increase in plasma NEAC in both categories was observed ($P<0·001$ for healthy and OSRRF subjects). However, the efficiency of dietary supplementation was much higher in subjects characterised by risk factors (SMD 1·253, 95 % CI 0·685, 1·820) than in healthy subjects (SMD 0·502, 95 % CI 0·235, 0·769; $P<0·001$). Finally, when all the available data (beverages and foods) were grouped, we observed that the increase in plasma NEAC was three times higher in subjects with risk factors (SMD 0·937, 95 % CI 0·592, 1·281; $P<0·001$) when compared with healthy subjects (SMD 0·367, 95 % CI 0·162, 0·572; $P<0·001$).

Effect of methodology on plasma non-enzymatic antioxidant capacity

The influence of methodology on the response of plasma NEAC after dietary supplementation with antioxidant-rich plant foods and beverages is described in Table 4. When methodologies based on the hydrogen atom transfer reaction were analysed, a positive response was detected in TRAP and ORAC, both in acute (SMD 1·113, 95 % CI 0·486, 1·741; $P=0·001$; SMD 1·308, 95 % CI 0·704, 1·911; $P<0·001$) and chronic studies (SMD 0·574, 95 % CI 0·078, 1·071; $P=0·023$; SMD 0·653, 95 % CI 0·304, 1·001; $P=0·001$). When single-electron transfer-based methods were tested, FRAP was increased both in acute (SMD 1·059, 95 % CI 0·612, 1·505; $P<0·001$) and chronic periods (SMD 0·366, 95 % CI 0·076, 0·656; $P=0·013$). On the contrary, the TEAC assay was unable to detect changes in plasma NEAC after dietary supplementation with vegetable foods (acute studies: SMD 0·08, 95 % CI −0·660, 0·819; $P=0·819$ and chronic studies: SMD 0·318, 95 % CI −0·075, 0·711; $P=0·113$).

Discussion

The present meta-analysis shows that the ingestion of antioxidants from most vegetable foods and beverages differently modulates plasma NEAC in human subjects. The antioxidant effect is more prominent in subjects exposed to cardiovascular risk factors or affected by oxidative stress-related pathologies.

Analysing the plant-derived beverage category, we noted that red wine, black tea and green tea displayed an excellent antioxidant effect, in both acute and chronic studies. In agreement with epidemiological evidence, studies analysing the antioxidant potential of red wine, included in the present meta-analysis, used a moderate amount of red wine (about 250 ml), suggesting that the protective role of moderate red wine intake could be linked to its ability to enhance the body's antioxidant system. On the other hand, fruit juice

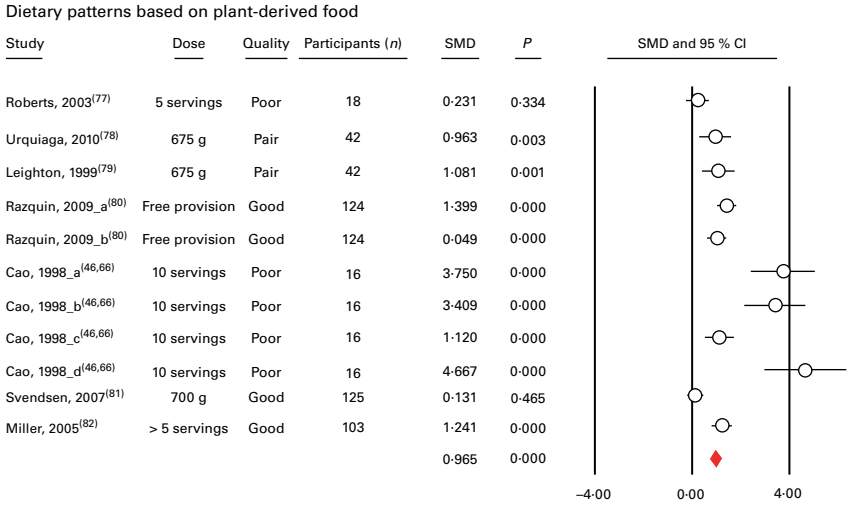

Fig. 4. Effect of chronic ingestion of plant food-based dietary patterns on plasma non-enzymatic antioxidant capacity (NEAC). A positive standardised mean difference (SMD) indicates an increase of plasma NEAC, whereas a negative SMD indicates a decrease in plasma NEAC. Terms a, b or c was used when the same author measured plasma NEAC with different methods. (A colour version of this figure can be found online at http://www.journals.cambridge.org/bjn).

Table 3. Chronic dietary supplementation studies with beverages and solid foods containing antioxidants and plasma non-enzymatic antioxidant capacity in human subjects: effect of oxidative stress-related risk factors (OSRRF)
(Number of interventions and 95 % confidence intervals)

Treatments	Status	No. of interventions	Results	Effect size		
				SMD	95 % CI	*P**
Beverages (tea, fruit juices and red wine)	Healthy	23	↔	0·177	−0·154, 0·508	0·296
	OSRRF	12	↑	0·765	0·310, 1·220	0·001
Food (chocolate, fruit, vegetables and dietary patterns based on plant-derived food)	Healthy	30	↑	0·502	0·235, 0·769	<0·001
	OSRRF	7	↑	1·253	0·685, 1·820	<0·001
Overall	Healthy	53	↑	0·367	0·162, 0·572	<0·001
	OSRRF	19	↑	0·937	0·592, 1·281	<0·001

SMD, standardised mean difference; ↔, unchanged; ↑, increased.
* Test for the overall effect.

failed to display any antioxidant action in chronic trials, differently from single-time ingestion where an antioxidant effect is detected. The heterogeneity of fruit juice composition could be one of the reasons for the variability in plasma NEAC following chronic consumption. Moreover, fruit juices rich in fructose or enriched with high-fructose maize syrup, inducing free radical production, could decrease the antioxidant properties of fruit juice[20].

The plant-derived food category, such as chocolate, fruits and vegetables, showed a clear antioxidant response after acute ingestion, whereas only vegetables were able to increase plasma antioxidant capacity after chronic intervention trials.

Despite more evidence being needed, variability of fruit composition and presence of potential oxidative stress inducers, such as fructose, might be the basis of the lack of effect displayed by fruit and fruit juices. Chocolate, despite the high content in flavonols and antioxidants, failed to display any effect in chronic studies. However, if we analyse the chronic studies, we observe that in Fraga's[21] study, milk chocolate, losing antioxidant properties *in vivo*, was tested. Moreover, subjects enrolled in five chronic intervention trials were characterised by a lack of risk factors for oxidative stress, which is a factor able to affect the *in vivo* antioxidant response of foods[7]. In order to determine whether cocoa could represent a valid source of antioxidant ingredients, more studies on people exposed to oxidative stress risk factors are needed.

Epidemiological data linked the protective role of plant foods on several diseases with their ability to ameliorate the body's redox network. In line with evidence suggested in these studies, we reported a strong increase in plasma NEAC in dietary patterns rich in the plant food category.

Considering the overall effects, we observed a difference in terms of NEAC responses on the basis of the study design. In particular, when compared with chronic designs, plasma NEAC is promptly increased after vegetable food ingestion in acute studies. Acute ingestion trials rely on the importance of having a short experimental window free from variables such as physical activity, diet and homeostasis that might affect NEAC in chronic studies. However, as for all the kinetics, once a peak of effect is reached, between 30 and 60 min for beverages and after 2 h for solid foods, plasma levels turn back to baseline levels in few hours. Acute ingestion models allow us to draw relatively fast conclusions about the 'potentiality' of the food to display an antioxidant action in human subjects. It might be considered a useful pilot study before a chronic trial, still representing the golden standard for confirming the effect observed in an acute trial. On the other hand, the increase in plasma NEAC detected in chronic studies suggests a positive association between endogenous and dietary antioxidants. In chronic

Table 4. Chronic supplementation studies with beverages and solid foods containing antioxidants and plasma non-enzymatic antioxidant capacity in human subjects: effect of methodology
(Number of interventions, participants and 95 % confidence intervals)

Assays	Type of studies	No. of interventions	No. of participants	Results	Effect size		
					SMD	95 % CI	*P**
TRAP	Acute	12	148	↑	1·113	0·486, 1·741	0·001
	Chronic	15	269	↑	0·574	0·078, 1·071	0·023
FRAP	Acute	14	226	↑	1·059	0·612, 1·505	<0·001
	Chronic	23	674	↑	0·366	0·076, 0·656	0·013
ORAC	Acute	11	115	↑	1·308	0·704, 1·911	<0·001
	Chronic	19	686	↑	0·653	0·304, 1·001	0·001
TEAC	Acute	6	96	↔	0·08	−0·660, 0·819	0·819
	Chronic	21	707	↔	0·318	−0·075, 0·711	0·113

SMD, standardised mean difference; TRAP, total radical-trapping antioxidant parameter; ↑, increased; FRAP, ferric-reducing antioxidant potential; ORAC, oxygen radical absorbance capacity; TEAC, Trolox equivalent antioxidant capacity; ↔, unchanged.
* Test for the overall effect.

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studies, we face a physiological mechanism of homeostatic control, aimed to tune an endogenous antioxidant network. More specifically, it might be possible that in a 'healthy condition' such as the absence of specific risk factors for oxidative stress, the body does not require an upload of dietary antioxidants. In order to test this hypothesis, we divided all chronic intervention studies on the basis of the 'healthy status' of the subjects.

Interestingly, and in agreement with previous evidence[7], we observed a strong antioxidant response in the 'OSRRF' category that is three times higher than the 'healthy' category. In particular, plant-derived beverages (tea, fruit juices and red wine) are unable to increase plasma NEAC in healthy subjects, whereas plant-derived foods (chocolate, fruit, vegetables and dietary patterns based on plant-derived food) enhance plasma NEAC both in the healthy and OSRRF groups, with a double effect size in the last category. In this view, when chronic intervention studies were conducted on healthy subjects characterised by the lack of specific OSRRF, the body's antioxidant defences might better cope with free radical formation, preventing the need for dietary antioxidants. On the contrary, an increased intake of dietary antioxidant compounds could be identified as a helpful strategy to potentiate endogenous redox defences if pro-oxidant conditions are present.

In order to evaluate NEAC, several methodological approaches were performed so as to give birth to a major obstacle in understanding the pros and cons of NEAC as a biomarker for assessing antioxidant capacity *in vivo*. Based on hydrogen atom transfer (TRAP and ORAC) or single-electron transfer (FRAP and TEAC), these methods could provide information related to the redox network in the plasma compartment after antioxidant ingestion[7,9]. Our data revealed that all methods, except TEAC, showed a similar trend following plant-derived food and beverage consumption. In particular, TRAP and ORAC displayed a double response in plasma NEAC in acute studies when compared with chronic studies, whereas the FRAP method showed a three times higher antioxidant response in acute studies than in chronic studies. On the contrary, the TEAC assay did not reveal any change in plasma NEAC neither in acute nor in chronic trials. The ORAC and TRAP assays measure the antioxidant inhibition of peroxyl radical-induced oxidation and therefore reflect classical radical chain-breaking antioxidant activity by hydrogen atom transfer. On the other hand, the TEAC assay is based on the ability of antioxidants in scavenging the radical cation 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid). Biases of this method are related to plasma dilution and to non-physiological conditions of analysis that strongly reduce its sensitivity to detect plasma NEAC modulation in biological fluids. In fact, the peroxidase process does not mimic radical formation *in vivo* and the assay uses a non-physiological radical. Although the methods used revealed a good reproducibility in measuring plasma NEAC, among all the methods to detect the hydrogen atom transfer reaction, we suggest to use the TRAP and/or ORAC assays, whereas FRAP could be more sensitive than the TEAC assay (single-electron transfer-based methods) to assess plasma NEAC. Finally, the present results highlight the importance of assessing plasma non-enzymatic antioxidant defences through a battery of methodologies furnishing complementary information on different modalities of action of dietary antioxidants[22,23].

Although several meta-analyses have shown a decreased risk of oxidative stress-related disease after vegetable food consumption[24–26], there are no meta-analyses measuring the impact of vegetable foods on plasma antioxidant markers in human subjects. In the present meta-analysis, we evaluated 122 intervention studies with 2890 analysed subjects, so that the precision and power of the analysis is increased. Moreover, about 86 % of the included trials were performed with more than ten participants; this highlights the validity of the present results[6].

However, the present meta-analysis has some limitations. First, the reviewed studies in the present meta-analysis are extremely heterogeneous, as they have different study designs. Indeed, in the meta-analysis, about 62·5 % of the total intervention studies were not randomised and controlled, whereas about 46·6 % of the included studies had a randomised and controlled design. Finally, about 37·5 % of all the intervention trials were randomised and controlled with a longitudinal study design.

Second, due to the difficulties in planning an appropriate placebo, clear evidence about the antioxidant potential of plant-derived foods and beverages is still far to come. However, on the basis of the above-described considerations, in order to evaluate the risk of overestimating intervention effects, all trials were stratified according to the study design (experimental and quasi-experimental design), and both EXP and Q-EXP studies have been processed together only after evaluating that their direction effects were similar[13].

In conclusion, our data suggest that ingestion of plant foods and beverages increases plasma antioxidant potential in human subjects. We also showed that the antioxidant effect of plant foods is most conspicuous in subjects exposed to OSRRF, compared with those who are not exposed. These findings suggest the importance of considering exposure to oxidative stress as an important variable in deriving the benefits from the antioxidant action of plant foods. Accordingly, the present findings suggest the importance of introducing the concept of the 'redox necessity for a redox effectiveness' of antioxidants, beneficial when the body is under stress and not in all phases of life, if a plant food-based diet is routinely followed. Further chronic intervention studies involving subjects sharing a common need for dietary antioxidants are warranted to unravel the relative contributions of dietary and endogenous antioxidants.

Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S0007114513000263

Acknowledgements

D. L.-B. designed the study and directed its implementation, including quality assurance and control. F. T. contributed to

the analysis of the data and revised the article. A. S. contributed to the interpretation of the data and to the writing and revising of the manuscript. G. M. conducted the literature review and contributed to the writing and revising of the manuscript. M. S. helped in supervising the project, designing the study's analytic strategy and writing and revising of the manuscript. No conflict of interest exists for each author. No funding was received.

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