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Adherence to cancer prevention recommendations and antioxidant and inflammatory status in premenopausal women

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Abstract

For cancer prevention, the World Cancer Research Fund and American Institute for Cancer Research (WCRF/AICR) emphasise recommendations to improve individual behaviour, including avoidance of tobacco products, maintaining a lean body mass, participating in physical activity, consuming a plant-based diet, and minimising the consumption of energy-dense foods, such as sodas, red and processed meats and alcohol. In the present study of 275 healthy premenopausal women, we explored the association of adherence scores with levels of three biomarkers of antioxidant and inflammation status: serum C-reactive protein (CRP), serum γ-tocopherol and urinary F2-isoprostane. The statistical analysis applied linear regression across categories of adherence to WCRF/AICR recommendations. Overall, seventy-two women were classified as low (≤4), 150 as moderate (5–6), and fifty-three as high adherers (≥7). The unadjusted means for CRP were 2·7, 2·0 and 1·7 mg/l for low, moderate and high adherers ($P_{trend} = 0{\cdot}03$); this association was strengthened after adjustment for confounders ($P_{trend} = 0{\cdot}006$). The respective values for serum γ-tocopherol were 1·97, 1·63 and 1·45 μg/ml ($P_{trend} = 0{\cdot}02$ before and $P_{trend} = 0{\cdot}03$ after adjustment). Only for urinary F2-isoprostane, the lower values in high adherers (16·0, 14·5, and 13·3 ng/ml) did not reach statistical significance ($P_{trend} = 0{\cdot}18$). In an analysis by BMI, overweight and obese women had higher biomarker levels than normal weight women; the trend was significant for CRP ($P_{trend} < 0{\cdot}001$) and γ-tocopherol ($P_{trend} = 0{\cdot}003$) but not for F2-isoprostane ($P_{trend} = 0{\cdot}14$). These findings suggest that both adherence to the WCRF/AICR guidelines and normal BMI status are associated with lower levels of biomarkers that indicate oxidative stress and inflammation.

Key words: Chronic inflammation: Cancer prevention: Nutrition: Lifestyle: Recommendations

Nutritional and lifestyle factors are thought to be associated with a higher risk for cancer and other chronic conditions, but little is known whether guidelines from different agencies are related to indicators of lower disease risk. The World Cancer Research Fund and American Institute for Cancer Research (WCRF/AICR) emphasise recommendations to improve individual behaviour, including avoidance of tobacco products, maintaining a lean body mass, participating in moderate physical activity, consuming a primarily plant-based diet and minimising the consumption of energy-dense foods and drinks, red and processed meats and alcohol[1]. In two large cohort studies, participants experienced a 9 to 10% lower mortality for each WCRF/AICR recommendation that was met[2,3]. Based on evidence that chronic inflammation plays a major role in cancer development[4,5], we evaluated the diet of 275 healthy premenopausal women who completed a FFQ and explored the association of adherence scores with levels of three biomarkers of antioxidant and inflammation status: serum C-reactive protein (CRP), urinary F2-isoprostane and serum γ-tocopherol. CRP represents a non-specific indicator of inflammation[6] that has been associated with cancer incidence[7] and survival[8]. Among markers of oxidative stress, F2-isoprostanes are considered the 'gold standard' because they are stable and specific and are only formed directly by chemical oxidation from NO_x generated *in vivo*[9–11]. Owing to their antioxidant activity, tocopherol isomers may shield against oxidative damage[12,13]. In particular, γ-tocopherol selectively protects cells from the DNA-damaging effects of NO_x[14–16], possesses anti-inflammatory activity[17], and rises in response to inflammation[18,19]. High circulating levels of γ-tocopherol, that is, >2·5 μg/ml, do not appear to reflect dietary intake, rather they represent a response to

Abbreviations: BEAN Study, Breast, Estrogen, and Nutrition Study; CRP, C-reactive protein; WCRF/AICR, World Cancer Research Fund and American Institute for Cancer Research.

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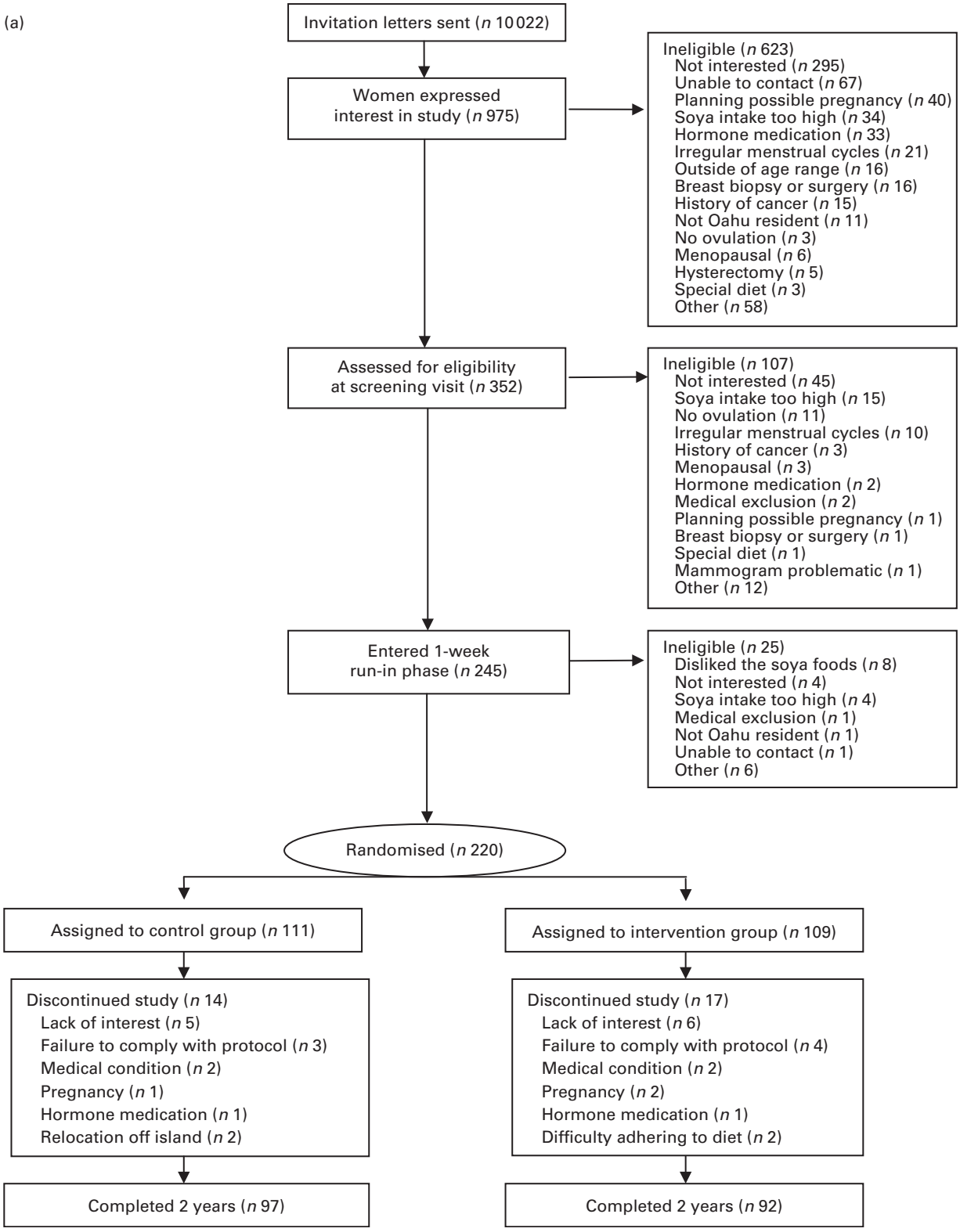
the presence of an inflammatory stimulus. Individuals with circulating levels of γ-tocopherol >2·5 μg/ml are considered hyper γ-tocopherolaemic[20]; such elevated levels are associated with low vitamin D status[20,21], obesity[22], age[23], smoking[20] and CRP[22]. Thus, γ-tocopherol may be an excellent overall marker of health risk. We hypothesised that women with high adherence to the WCRF/AICR recommendations have a more favourable inflammatory biomarker profile. In addition, we explored the association between BMI and the same biomarkers of antioxidant and inflammatory status.

(a)



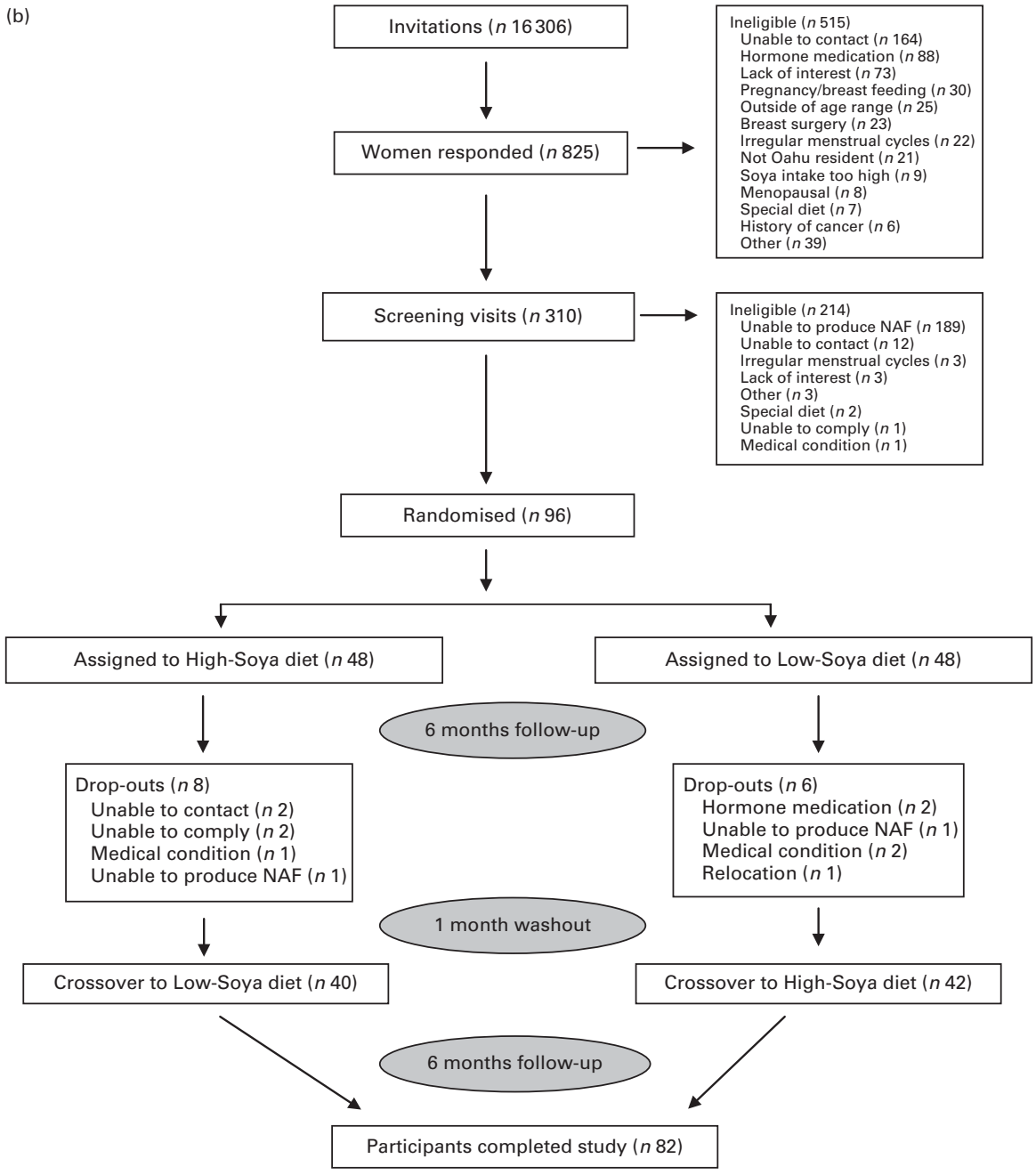


Fig. 1. Flow chart for recruitment and study population of the Breast, Estrogen, and Nutrition 1 (BEAN1) Study (a) and the Breast, Estrogen, and Nutrition 2 (BEAN2) Study (b). NAF, nipple aspirate fluid.

Methods

Study design and population

The current analysis used baseline data from two dietary intervention studies (Fig. 1): the Breast, Estrogen, and Nutrition (BEAN1), which randomised 220 women to a 2-year clinical trial to examine the effects of two daily soya servings on sex steroids and mammographic densities[24], and BEAN2, which was conducted in a cross-over design with eighty-two women[25]. Only data collected at baseline before randomisation to an intervention were analysed. The protocols for both studies were approved by the University of Hawaii Committee on Human Studies and by the Institutional Review Boards of the participating hospitals. All participants signed an informed consent form before entry into the trials. As described in detail previously[24,25], eligibility criteria for both studies included a normal mammogram, no breast implants, no current oral contraceptive use or pregnancy, no previous cancer diagnosis, intact uterus and ovaries, regular menstrual cycles, and low soya intake. Additional criteria for BEAN2 included the ability to produce at least 10 μl nipple aspirate fluid, one of the study outcomes[25]. After exclusion

Table 1. Scoring for World Cancer Research Fund and American Institute for Cancer Research (WCRF/AICR) recommendations based on data from baseline FFQ (Number of women and percentages)

				Women (*n* 275)	
WCRF/AICR recommendation	Associated recommendations used in this study	Met/did not meet recommendation if:	Score	*n*	%
Body fatness: be as lean as possible without becoming underweight	Maintain body weight in the normal BMI range	Met: BMI between 18·5 and 25 kg/m^2	1	138	50·2
		Did not meet: BMI $<$18·5 or $\geq$25 kg/m^2	0	137	49·8
Physical activity: be physically active for at least 30 min every day	Be moderately or strenuously physically active at least 3·5 h/week	Met: $\geq$3·5 h/week	1	178	64·7
		Did not meet: $<$3·5 h/week	0	97	35·3
Energy density: limit consumption of energy dense foods: avoid sugary drinks	Limit the consumption of sugary drinks to $<$250 g/d	Met: $<$250 g/d	1	196	71·3
		Did not meet: $\geq$250 g/d	0	79	28·7
Plant foods: eat more of a variety of vegetables, fruits, whole grains and legumes such as beans	Eat 5 servings of fruits and vegetables and 1 serving or more of whole grains	Met: $\geq$5 servings/d $\geq$1 serving/d	1	87	31·6
		Did not meet: $\geq$5 servings/d $<$1 serving/d	0	18	6·5
		Did not meet: $<$5 servings/d	0	170	61·9
Red meat: limit consumption of red meats (such as beef, pork and lamb) and avoid processed meats	Limit red/processed meat consumption to $<$2·5 servings/d (2·5 servings based on 500 g/week)	Met: $<$2·5 servings/d	1	243	88·4
		Did not meet: $\geq$2·5 servings/d	0	32	11·6
Alcohol: limit alcoholic drinks	If alcoholic drinks are consumed, limit consumption to no more than 1 drink/d	Met: $\leq$1 drink/d	1	248	90·2
		Did not meet: $<$1 drink/d	0	27	9·8
Salty foods: limit consumption of salt; avoid moldy grains or legumes	Limit consumption of salty and processed foods to $<$2400 mg/d	Met: $<$2400 mg/d	1	132	48·0
		Did not meet: $\geq$2400 mg/d	0	143	52·0
Smoking: do not smoke and avoid tobacco smoke	Do not smoke or quit smoking if you are a present smoker	Met: Never	1	179	65·1
		Met: Past	1	17	6·2
		Did not meet: current	0	79	28·7
Supplements: aim to meet nutritional needs through diet alone	Dietary supplements are not recommended for cancer prevention	Not operationalised	N/A		

N/A, not available.

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of dropouts and women with incomplete data, the current analysis included 275 women who provided biological samples and complete nutritional information from the baseline FFQ that could be used to score adherence to the WICR/AICR recommendations (Table 1).

Data collection

All participants completed a previously validated FFQ at baseline that included questions on habitual dietary intake during the last year, physical activity, smoking status, medical history and reproductive characteristics[26]. The FFQ had eight frequency categories for foods and nine for beverages. Respondents could choose from three typical serving sizes and photographs were included to help visualise proportions for selected foods. BMI was calculated from baseline weight and height. The FFQ were analysed utilising the Food Composition Table maintained by the Nutrition Support Shared Resource at the Cancer Center[27]; the databases represent an extensive list of local foods consumed by the various ethnic populations of Hawaii and the Pacific. Food servings were defined according to the Food Guide Pyramid[28].

Sample collection and assays

Serum and urine samples were collected during the midluteal phase using ovulation kits in BEAN1 and confirmation by serum progesterone levels[24] and self-reported menstruation information in BEAN2[25]. For this analysis, CRP and serum γ-tocopherol were available for BEAN1 participants only and urinary F2-isoprostane levels for BEAN2 women only. All specimens were stored at −80°C after aliquoting. The CRP assay was based on a latex particle enhanced immunoturbidimetric method using a Cobas MiraPlus clinical autoanalyser and a kit from Pointe Scientific, Inc., with a detection limit of 0·1 mg/l[29]. Serum samples were analysed for γ-tocopherol by reverse phase HPLC with photodiode array detection between 220 and 600 nm[30,31]. The assay was regularly validated during the analysis by inclusion of external standards within each sample batch and through successful participation in the quality assurance program organised by US National Institute of Standards and Technology. Levels of urinary 15-F_{2t}-isoprostane were measured using an enzyme-linked immunosorbent assay kit (Oxford Biomedical Research) in six batches[32].

Statistical analysis

All statistical analyses were performed using SAS, release 9.4 (SAS Institute, Inc.). A scoring system for eight of the ten recommendations by the WCRF/AICR for cancer prevention[1] was modeled similar to a previous publication[3]. Participants were given a score of 1 or 0 depending on if they met or did not meet a recommendation (Table 1), and the adherence scores were classified as low (<5), moderate (5–6), and high (≥7). Analyses for BMI status were based on pre-determined BMI categories: normal (<25 kg/m^2), overweight (25– < 30 kg/m^2) and obese (≥30 kg/m^2). Because only

Table 2. Baseline characteristics of 275 premenopausal women from two intervention studies*

(Number of premenopausal women and percentages; mean values and standard deviations)

	BEAN1 and BEAN2	
Characteristic	*n*	%
Ethnicity		
White	112	40·7
Asian	99	36·0
Native Hawaiian	38	13·8
Other	26	9·5
Age (years)	41·9	4·5
BMI (kg/m^2)	26·1	5·7
Parity		
No	204	74·2
Yes	71	25·8
Biospecimen collection in luteal phase		
No	37	13·5
Yes	238	86·5
Smoking status		
Never	179	65·1
Past	17	6·2
Current	79	28·7
Serum C-reactive protein (mg/l)†	2·1	3·2
Serum γ-tocopherol (μg/ml)†	1·69	0·96
Urinary F2-isoprostane (ng/ml)†	14·6	5·3
Dietary intake from baseline FFQ		
Total energy (kcal/d)		
Mean	1911	
SD	893	
Red/processed meat (servings/d)		
Mean	1·3	
SD	1·1	
Whole grain (servings/d)		
Mean	1·9	
SD	1·5	
Dietary fibre (g/d)		
Mean	20·1	
SD	11·6	
Total energy from fat (%)		
Mean	32·6	
SD	6·0	
Fruit (servings/d)		
Mean	1·5	
SD	1·6	
Vegetables (servings/d)		
Mean	3·3	
SD	2·4	
Fruit and vegetables (servings/d)		
Mean	4·8	
SD	3·4	
Alcohol (drinks/d)		
Mean	0·3	
SD	0·6	
Regular soda (g/d)		
Mean	50	
SD	140	
Na (mg/d)		
Mean	2781	
SD	1469	
Physical activity (h/week)		
Mean	7·7	
SD	8·0	

BEAN, Breast, Estrogen, and Nutrition.

* Percentages may not add to 100 due to rounding.

† Serum γ-tocopherol and C-reactive protein (CRP) were available for BEAN1 only and urinary F2-isoprostane levels for BEAN2 only. Data were missing for serum γ-tocopherol (*n* 23) and serum CRP (*n* 12).

three women had a BMI < 18·5 kg/m^2, these participants were excluded from the analyses. An indicator variable with a cut-off of 2·5 μg/ml for hyper γ-tocopherolaemic status was created. Means and standard deviations for dietary and lifestyle habits as well as biomarkers were computed by level of adherence and by BMI status. Non-normally distributed variables were log-transformed to obtain *P*-values for trend tests across categories using analysis of variance. In adjusted models, we included age, parity, ethnicity and timing of biospecimen collection within the luteal phase (yes or no) as covariates and expressed the differences as least square means with 95% CI. Finally, we examined the distribution of hyper γ-tocopherolaemia by adherence category and computed OR and 95% CI in a logistic regression model with low adherers as the reference group.

Results

The study population (Table 2) consisted of 41% whites, 36% Asians, 14% Native Hawaiians and 9% Others with a mean age of 41·9 (SD 4·5) years. The mean BMI was 26·1 (SD 5·7) kg/m^2 with 27% classified as overweight and 22% as obese. Of all biospecimens, 87% were collected during the luteal phase. The majority of participants (Table 1) were never smokers (65%), met the physical activity guideline (65%) and reported drinking one or fewer alcoholic beverages per day (90%). As to nutritional recommendations, 88% adhered to low red/processed meat intake, 71% to low soda intake, but only 48% limited salt intake to < 2400 mg/d, and 32% consumed adequate amounts of vegetables, fruits and whole grains. The respective means for CRP, γ-tocopherol and F2-isoprostances were 2·1 mg/l, 1·7 μg/ml and 14·6 ng/ml. Levels of serum γ-tocopherol and CRP were significantly correlated 0·24 (P=0·001).

Overall, seventy-two women were classified as low-adherers, 150 as moderate adherers, and fifty-three as high-adherers (Table 3). The low adherers had a mean BMI of 30·3 (SD 6·0) kg/m^2, while the mean BMI of the high adhering women was 22·2 (SD 2·0) kg/m^2. High adherers were also more physically active (P_{trend} < 0·0001), consumed less red/processed meat (P_{trend} < 0·0001), lower percentage total energy from fat (P_{trend} = 0·002), less salt (P_{trend} < 0·0001) and more dietary fibre (P_{trend} = 0·01) and more fruits and vegetables (P_{trend} = 0·002). The unadjusted means for CRP (BEAN1 only) were 2·7, 2·0 and 1·7 mg/l for low, moderate and high adherers (P_{trend} = 0·03); this association was strengthened after adjustment for potential confounders with least square means for log-transformed CRP values of 1·12, 0·87 and 0·71 mg/l (P_{trend} = 0·006). The respective values for serum γ-tocopherol (BEAN1 only) were 1·97, 1·63 and 1·45 μg/ml (P_{trend} = 0·02) before and 1·10, 0·96, and 0·91 μg/ml (P_{trend} = 0·03) after adjustment. However, for urinary F2-isoprostane (BEAN2 only), the higher values in low adherers (16·0, 14·5, and 13·3 ng/ml) did not reach statistical significance

Table 3. Characteristics of women by adherence to World Cancer Research Fund and American Institute for Cancer Research recommendations*

(Number of women and percentages; mean values and standard deviations)

	Low adherence (≤4)		Moderate adherence (5–6)		High adherence (≥7)			
	n	%	*n*	%	*n*	%	$P_{unadjusted}$†	$P_{adjusted}$†
n	72		150		53			
Age (years)							0·15	
Mean	42·8		41·6		41·8			
SD	3·3		4·7		5·0			
Ethnicity							0·16	
White	23	31·9	62	41·3	27	50·9		
Asian	29	40·3	51	34·0	19	35·9		
Native Hawaiian	12	16·7	24	16·0	2	3·8		
Other	8	11·1	13	8·7	5	9·4		
Serum CRP (mg/l)‡							0·03	0·006
Mean	2·7		2·0		1·7			
SD	3·5		3·2		2·7			
Serum γ-tocopherol (μg/ml)‡							0·02	0·03
Mean	1·97		1·63		1·45			
SD	1·01		0·94		0·83			
Hyper γ-tocopherolaemia (≥2·5 μg/ml)	12	25	15	17	4	12	0·29	
Urinary F2-isoprostane (ng/ml)‡							0·18	0·21
Mean	16·0		14·5		13·3			
SD	7·2		4·8		3·8			

CRP, C-reactive protein.

* Percentages may not add to 100 due to rounding.

† P_{trend} values were computed for continuous variables using log-transformed data except for age and % total energy from fat. *P* values for categorical variables were computed using χ^2 or Fisher's exact test. Adjusted model included age, parity, ethnicity, and timing of biospecimen collection within the luteal phase (yes or no) as covariates.

‡ Serum γ-tocopherol and CRP were available for Breast, Estrogen, and Nutrition 1 (BEAN1) only and urinary F2-isoprostane levels for Breast, Estrogen, and Nutrition 2 (BEAN2) only. Data were missing for serum γ-tocopherol (*n* 23) and serum CRP (*n* 12).

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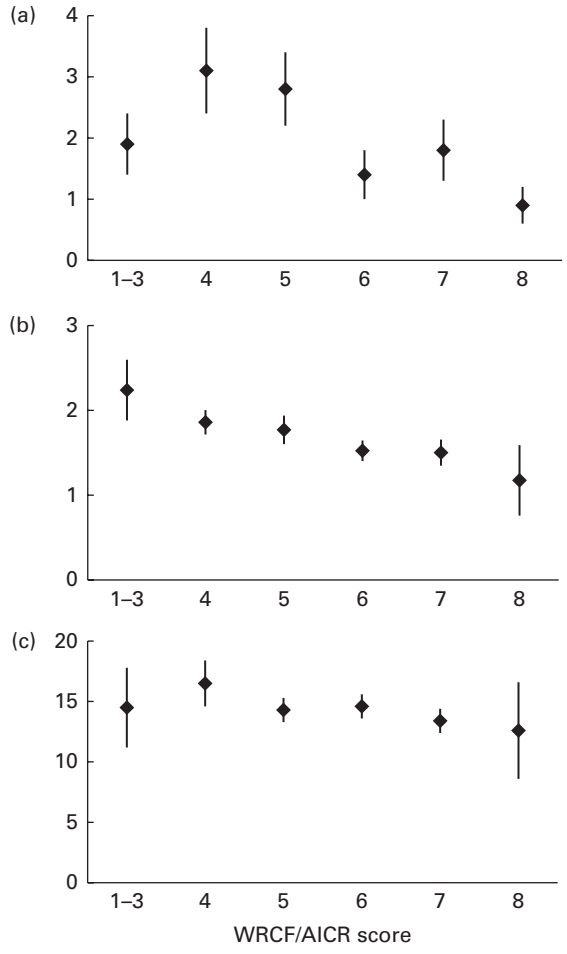

Fig. 2. Mean biomarker levels and standard errors by World Cancer Research Fund and American Institute for Cancer Research (WCRF/AICR) score. (a) Serum C-reactive protein (mg/l, $P_{trend} < 0{\cdot}01$), (b) serum α-tocopherol (μg/ml, $P_{trend} < 0{\cdot}01$) and (c) urinary F2-isoprostane (ng/l, $P_{trend} = 0{\cdot}27$). Missing data were recorded for (a) (*n* 23) and (b) (*n* 12). P_{trend} values were computed using log-transformed biomarker levels.

($P_{trend} = 0{\cdot}18$). In the adjusted models, the respective F2-isoprostane values were 2·74, 2·66 and 2·60 ng/ml ($P_{trend} = 0{\cdot}21$).

The inverse associations for the biomarkers with higher adherence (Fig. 2) were more pronounced for CRP ($P_{trend} < 0{\cdot}01$) and γ-tocopherol ($P_{trend} < 0{\cdot}01$) than for F2-isoprostane ($P_{trend} = 0{\cdot}27$). Whereas the mean levels by adherence score were more or less flat for F2-isoprostane, serum γ-tocopherol showed a continuous decline from >2·0 to <1·5 μg/ml across categories. We observed smaller proportions of women with hyper γ-tocopherolaemia among moderate and high adherers than in low adherers (17 and 12 *v.* 25 %, respectively). However, the respective OR of 0·60 (95 % CI 0·26, 1·41) and 0·41 (95 % CI 0·12, 1·42) for moderate and high adherers *v.* low adherers were not statistically significant. For CRP, the levels were up to twice as high for the three lower categories than for women scoring 6 to 8.

When participants were grouped by BMI category (Table 4), red/processed meat, percentage of total energy from fat and Na intake were significantly higher and physical activity significantly lower in overweight and obese than normal weight women. AICR scores not including BMI were lowest in the obese group, followed by overweight and normal weight women with respective values of 4·3 (SD 1·0), 4·8 (SD 1·2) and 5·1 (SD 1·0). The levels of CRP, γ-tocopherol and F2-isoprostane were substantially higher in overweight and obese than normal weight women; these trends remained unchanged after adjustment for age, ethnicity, parity and luteal phase. However, the linear trend was only statistically significant for CRP and γ-tocopherol with log-transformed values of 0·56, 0·96 and 1·38 ($P_{trend} < 0{\cdot}0001$) and 7·20, 7·40 and 7·55 ($P_{trend} = 0{\cdot}008$), respectively. The respective least square means for F2-isoprostane were 2·64, 2·57 and 2·83 ($P_{trend} = 0{\cdot}21$) across BMI categories.

Discussion

In the present study of multi-ethnic premenopausal women, many women met a high proportion of the eight recommendations operationalised according to the WCRF/AICR cancer prevention guidelines, in particular, limiting the consumption of energy-dense food such as sodas, red/processed meat and alcohol. Women with lower adherence scores had a higher BMI and reported higher intakes of unfavourable foods and behaviours. The results of this study suggest a weak inverse relation between adherence to nutritional and lifestyle recommendations and two of the three markers of antioxidant and inflammatory status examined. Specifically, γ-tocopherol and CRP, but not F2-isoprostane, were lower in women with higher adherence scores. In a similar pattern, overweight and obese women had substantially higher levels of all three biomarkers than normal weight women; in particular, CRP was nearly fivefold higher in obese women. In response to the inflammatory stress marked by low adherence scores and excess body weight, γ-tocopherol appears to rise as a physiological mechanism to protect the body against inflammation-related damage. Although a well-established marker of inflammation, urinary F2-isoprostane showed little association with adherence scores and BMI status.

To our knowledge, this is the first analysis to examine the association between WCRF/AICR recommendations and biomarkers of inflammation and antioxidant status. Previous studies investigated adherence to WCRF/AICR recommendations with morbidity and mortality in women. For example, a 22 to 24 % lower cancer-specific mortality for meeting one to two recommendations and 61 % lower for meeting five to six recommendations were reported[3]. A larger cohort observed similar results with a 34 % lower mortality for individuals who met six to seven recommendations[2]. The association between excess body weight and elevated CRP levels is well known and has been described for many populations[33,34].

The association of overweight with the biomarkers may be due to cytokines produced in adipose tissue or due to direct effects of dietary components associated with a high BMI. For example, obese women consumed more red/processed meat, fat and salt, food components that were related to higher γ-tocopherol in a previous report[35]. While dietary intake of γ-tocopherol and blood levels are poorly correlated[36–39], red/processed meat consumption is thought to be related to

Table 4. Characteristics of women by BMI category*

(Mean values and standard deviations; number of women and percentages)

	Normal (18·5–<25·0 kg/m^2)		Overweight (25·0–29·9 kg/m^2)		Obese (>30 kg/m^2)			
	Mean	SD	Mean	SD	Mean	SD	$P_{unadjusted}$†	$P_{adjusted}$†
n	138		74		60			
Age (years)	41·6	4·7	42·6	3·9	41·7	4·6	0·62	
Smoking status							0·13	
Never								
n	96		48		35			
%	68·1		64·8		58·3			
Past								
n	9		7		1			
%	6·4		95		1·7			
Current								
n	36		19		24			
%	25·5		25·7		40·0			
Serum CRP (mg/l)‡	1·0	1·1	2·3	2·8	4·7	4·9	<0·001	<0·0001
Serum γ-tocopherol (μg/ml)‡	1·49	0·91	1·79	1·04	2·00	0·88	0·003	0·008
Urinary F2-isoprostane (ng/ml)‡	14·2	4·6	13·6	5·0	17·4	6·9	0·14	0·21
Dietary intakes from baseline FFQ								
Total energy (kcal/d)	1810	887	1944	798	2069	1001	0·10	
Red/processed meat (servings/d)	1·1	1·0	1·4	1·1	1·7	1·0	<0·0001	
Whole grains (servings/d)	1·6	1·8	1·8	1·6	1·8	1·6	0·44	
Dietary fibre (g/d)	20·7	12·8	19·4	9·3	19·4	11·5	0·49	
Total energy from fat (%)	31·2	6·0	34·1	5·1	34·1	6·3	0·0003	
Fruit (servings/d)	1·6	1·8	1·6	1·4	1·3	1·2	0·18	
Vegetables (servings/d)	3·4	2·7	3·4	2·2	2·9	1·7	0·31	
Fruit and vegetables (servings/d)	5·0	4·0	4·9	3·0	4·2	2·6	0·18	
Alcohol (drinks/d)	0·4	0·6	0·4	0·8	0·2	0·4	0·17	
Regular soda (g/d)	58	179	30	68	61	99	0·18	
Na (mg/d)	2679	1557	2672	1294	3162	1457	0·01	
Physical activity (h/week)	8·9	8·7	7·1	6·8	5·7	7·1	0·001	
WCRF/AICR score without BMI§	5·1	1·0	4·8	1·2	4·3	1·0	<0·0001	

CRP, C-reactive protein; WCRF/AICR, World Cancer Research Fund and American Institute for Cancer Research.

* Percentages may not add to 100 due to rounding. Three women with a BMI <18·5 kg/m^2 were not included in the analysis.

† P_{trend} values were computed for continuous variables using log-transformed data except for age and % total energy from fat. *P* values for categorical variables were computed using χ^2 or Fisher's exact test. Adjusted model included age, parity, ethnicity, and timing of biospecimen collection within the luteal phase (yes or no) as covariates.

‡ Serum γ-tocopherol and CRP levels are available for Breast, Estrogen, and Nutrition 1 (BEAN1) only and urinary F2-isoprostane levels are available for Breast, Estrogen, and Nutrition 2 (BEAN2) only. Missing data were recorded for serum γ-tocopherol (*n* 23) and serum CRP (*n* 12).

§ BMI individual score was removed from the AICR score.

inflammatory responses during digestion, which may lead to an increase in circulating γ-tocopherols[40]. The stimulation of inflammatory cytokines by excess adiposity may also influence γ-tocopherol levels[20,41]. Among antioxidants, γ-tocopherol appears to be unique, in that higher circulating levels are directly associated with oxidative stress, whereas most antioxidants are reduced under conditions of oxidative stress[18,19]. The wide range of adverse conditions associated with elevated γ-tocopherol, and the general correlation with risk factors in the present study indicate it may be a useful integrative marker for assessing future disease risk. The occurrence of hyper γ-tocopherolaemia was weakly related to lower overall adherence scores without reaching statistical significance, suggesting a higher cutoff for defining this state might be indicated. Since very few supplements contain γ-tocopherol, it is unlikely that high circulating γ-tocopherol levels are due to supplement intake. While γ-tocopherol accounts for 80 % of tocopherols in the diet, most is excreted or metabolised in healthy people[36,39].

Strengths of the present study include the ethnic diversity of the women, as well as the use of a previously validated FFQ[26]. We analysed baseline nutritional data prior to implementation of the study interventions, thereby capturing usual dietary and lifestyle habits from the year before dietary changes were initiated. Given the generally good health status of the participants, it is unlikely that unknown, underlying conditions affected the biomarker levels. By contrast, given the healthy status of the women, their high adherence to lifestyle recommendations, and the strict eligibility criteria, the participants probably represented a relatively narrow range in biomarker levels. Therefore, the results may not be generalisable to a population with a higher prevalence of chronic conditions. The study was also limited by the small sample size and the low statistical power, the lack of a general energy-density variable, and the absence of information on dietary supplement intake.

Diet–disease associations are difficult to determine due to the length of time required for quantifiable symptoms to be expressed and chronic diseases to develop. Elevated levels of biomarkers in healthy people may help to identify persons at a higher risk for chronic disease development later in life. The fact that WCRF/AICR adherence scores and BMI status

showed similar associations with the three biomarkers suggests that the type of foods consumed may be less important in determining future disease risk than the excess body weight resulting from poor dietary patterns. The current findings suggest that adherence to the WCRF/AICR guidelines is associated with lower levels of biomarkers that indicate oxidative stress and inflammatory status, in particular, serum CRP and γ-tocopherol, and may lower future disease risk.

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The authors' contributions are as follows: G. M. conceived the original studies, obtained funding, supervised the data analysis and finalised the manuscript. R. V. C. and A. A. F. provided laboratory results and interpreted the results. Y. M. and F. B. collated the statistical information and drafted the manuscript. All authors read and approved the findings of the study.

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