

### RESEARCH ARTICLE

## The Dietary Inflammatory Index<sup>®</sup> and Alternative Healthy Eating Index 2010 in relation to leucocyte telomere length in postmenopausal women: a cross-sectional study

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### Abstract

Telomeres are nucleoprotein complexes that form the ends of eukaryotic chromosomes where they protect DNA from genomic instability, prevent end-to-end fusion and limit cellular replicative capabilities. Increased telomere attrition rates, and relatively shorter telomere length, is associated with genomic instability and has been linked with several chronic diseases, malignancies and reduced longevity. Telomeric DNA is highly susceptible to oxidative damage and dietary habits may make an impact on telomere attrition rates through the mediation of oxidative stress and chronic inflammation. The aim of this study was to examine the association between leucocyte telomere length (LTL) with both the Dietary Inflammatory Index<sup>®</sup> 2014 (DII<sup>®</sup>) and the Alternative Healthy Eating Index 2010 (AHEI-2010). This is a cross-sectional analysis using baseline data from 263 postmenopausal women from the Alberta Physical Activity and Breast Cancer Prevention (ALPHA) Trial, in Calgary and Edmonton, Alberta, Canada. No statistically significant association was detected between LTL z-score and the AHEI-2010 ( $P = 0.20$ ) or DII<sup>®</sup> ( $P = 0.91$ ) in multivariable adjusted models. An exploratory analysis of AHEI-2010 and DII<sup>®</sup> parameters and LTL revealed anthocyanidin intake was associated with LTL ( $P < 0.01$ ); however, this association was non-significant after a Bonferroni correction was applied ( $P = 0.27$ ). No effect modification by age, smoking history, or recreational physical activity was detected for either relationship. Increased dietary antioxidant and decreased oxidant intake were not associated with LTL in this analysis.

**Key words:** Telomeres: Alternative Healthy Eating Index: Dietary Inflammatory Index<sup>®</sup>: Chronic inflammation: Oxidative stress

Telomeres are nucleoprotein structures that form the ends of eukaryotic chromosomes where they maintain genomic stability<sup>(1)</sup> and limit the replicative capabilities of the cell<sup>(2–4)</sup>.

Telomere length at birth is an inherited trait; however, telomeres shorten approximately 50–100 bp in every round of DNA replication because of the inability of DNA polymerase

**Abbreviations:** AHEI-2010, Alternative Healthy Eating Index 2010; ALPHA, Alberta Physical Activity and Breast Cancer Prevention; C-DHQ-I, Canadian Dietary History Questionnaire-I; DII<sup>®</sup>, Dietary Inflammatory Index<sup>®</sup>; DXA, dual-energy X-ray absorptiometry; LTL, leucocyte telomere length.

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to synthesise chromosomal ends completely<sup>(5,6)</sup>. Telomeres therefore shorten naturally with each cell division or replication, eventually leading to cellular senescence<sup>(7,8)</sup>. Leucocyte telomere length (LTL) may experience accelerated attrition directly through oxidative damage, and indirectly through increased cellular replication during chronic inflammation, both of which are hypothesised to mediate the association between lifestyle and LTL<sup>(7)</sup>. Demographic and lifestyle variables, including dietary habits, physical activity levels and substance abuse, mediate levels of chronic inflammation and oxidative stress, and thus may be associated with LTL. LTL may serve as a marker of biological, rather than chronological, age, and may also provide insight into the risk of chronic diseases associated with oxidative stress and chronic inflammation, including CVD and several cancers<sup>(9–15)</sup>.

Telomeric DNA is rich in guanine nucleotides, which are prone to be selectively oxidised to 8-oxo-2'-deoxyguanosine and 2,6-diamino-4-oxo-5-formamidopyrimidine by reactive oxygen species<sup>(16,17)</sup>. Reactive oxygen species may also produce single-stranded DNA breaks directly, or single-stranded breaks may occur during the repair of oxidative base modifications<sup>(17)</sup>. In addition to these mechanisms, single-strand breaks in telomeric DNA are less efficiently repaired by DNA repair machinery than in non-telomeric genomic DNA. It has been hypothesised that the susceptibility of telomeres to damage by reactive oxygen species is, in fact, an important mechanism whereby telomeres act as 'sentinels' of excessive oxidative damage, disallowing the continued proliferation of damaged and potentially oncogenic cells<sup>(7,18)</sup>. Lymphocyte LTL is further affected by oxidative stress because of the increased proliferation (and coinciding telomere loss) of these cells during the inflammatory response triggered by oxidative stress<sup>(19)</sup>.

Current research is aimed at understanding the relationship between advanced telomere attrition and anthropometric measures including increased BMI and intra-abdominal fat<sup>(20)</sup>, as well as lifestyle factors including smoking<sup>(21)</sup>, alcohol intake<sup>(22)</sup> and suboptimal psychosocial health<sup>(23)</sup>, contributing through the impact of oxidative stress and chronic inflammation. Diet is an important source of oxidants and antioxidants and may affect levels of oxidative stress and inflammation in the blood and intestinal environment<sup>(24–27)</sup>. Diets rich in fruit and vegetables high in antioxidants are associated with lower intracellular concentrations of inflammatory biomarkers and reduced risk of chronic diseases, cancer and CVD<sup>(18,25,28,29)</sup>. Thus, telomere length may be a biomarker of dietary oxidant/antioxidant status<sup>(17)</sup>. Traditionally, nutritional epidemiology has focused on the association of specific nutrients or food groups with telomere length and other health outcomes; however, the study of dietary patterns has recently emerged as a more holistic approach at assessing these relationships<sup>(30,31)</sup>.

The Alternative Healthy Eating Index 2010 (AHEI-2010) is a score that provides an assessment of the diet's capacity to prevent chronic disease<sup>(32)</sup>. Designed as an alternative to the Healthy Eating Index (HEI) which quantified adherence to the US 1995 Dietary Guidelines, the AHEI-2010 was based on foods and nutrients predictive of chronic disease risk<sup>(32)</sup>. Higher AHEI-2010 scores have been associated with less weight gain over time<sup>(33)</sup>, lower risk of acute myocardial

infarction<sup>(34)</sup>, lower risk of chronic obstructive pulmonary disease<sup>(35)</sup>, lower long-term chronic inflammation<sup>(36)</sup>, lower cancer risk<sup>(37)</sup>, lower all-cause mortality<sup>(38)</sup> and longer LTL<sup>(39)</sup>.

The Dietary Inflammatory Index<sup>®</sup> (DII<sup>®</sup>) is a tool for assessing diet quality based on inflammatory potential<sup>(40)</sup>. The inflammatory capacity of the diet is assessed by estimating the intake of forty-five micro- and macronutrients, multiplying each by an overall inflammatory effect score and then centring each value on global mean intake values<sup>(40)</sup>. In this study, each inflammatory effect score was estimated based on the results of a rigorous literature review (about 2000 articles read and scored)<sup>(40)</sup>. Higher DII<sup>®</sup> scores have been associated with higher incidence of cancer, CVD and all-cause mortality<sup>(41,42)</sup> and lower LTL<sup>(43,44)</sup>.

The aim of the present study was to assess the association between the intake of foods and nutrients predictive of chronic disease (AHEI-2010), and of the overall inflammatory potential of the diet (DII<sup>®</sup>) and LTL in postmenopausal, healthy Canadian women. While use of the DII<sup>®</sup> aligns more closely with the objectives of this study, but because relationships between AHEI-2010 scores and LTL have been extensively explored in the literature, we therefore chose to implement both. We hypothesised that AHEI-2010 scores would be positively associated with LTL and DII<sup>®</sup> scores inversely associated with LTL. The secondary objective was to assess intake of the individual components of both the AHEI-2010 and DII<sup>®</sup> for association with LTL, with the hypothesis that certain food groups or nutrients were driving any associations detected in the primary objective.

## Methods

### Subjects

We conducted a cross-sectional analysis using the baseline data from the Alberta Physical Activity and Breast Cancer Prevention (ALPHA) Trial<sup>(45)</sup>. Women were recruited for the ALPHA Trial through mailings to participants in the Alberta Breast Screening Programme, media campaigns and brochures and posters distributed through the Alberta Family Physicians Research Network. The main inclusion criteria included: aged 50–74 years, English speaking, residents in the Calgary or Edmonton areas, postmenopausal, no previous cancer diagnosis except non-melanoma skin cancer, no major co-morbidities, acceptable heart and lung function (as determined in baseline fitness test), BMI 22.0 to 40.0 kg/m<sup>2</sup>, breast tissue density  $\geq 0\%$ , able to undertake unrestricted physical activity as determined through completion of the Physical Activity Readiness-Medical Exam (PAR-MedX) Questionnaire, inactive lifestyle, not currently planning to undertake a weight control/loss programme, not currently, previously (<12 months) or planning to take drugs related to oestrogen metabolism or breast tissue growth, not currently or planning on taking weight loss medications, not a current smoker or excessive drinker. Baseline assessments were performed from 2003 through 2007<sup>(45)</sup>.

### Ethics

Research ethics boards at the Alberta Cancer Board and Universities of Calgary and Alberta approved the protocol



for this ancillary study of the ALPHA Trial. The Conjoint Health Research Ethics Board at the University of Calgary and the Health Research Ethics Board of Alberta Health Services approved the LTL analysis protocol. The ALPHA Trial was conducted according to the Declaration of Helsinki, and all participants provided written informed consent. The trial was registered at ClinicalTrials.gov (identifier: NCT00522262).

### Health and lifestyle assessments

Participants completed four separate questionnaires focused on their baseline health, quality of life and psychosocial factors, physical activity in the past year and dietary intake. The Baseline Health Questionnaire measured health and sociodemographic variables including menstrual and reproductive history; medical and health history; past hormone replacement therapy; medication and vitamin/supplement use; previous benign breast disease; alcohol intake and smoking history. The General Health Questionnaire captured psychosocial measures using the self-administered RAND 36-item Health Survey<sup>(46)</sup>, which assessed overall quality of life, as well as mental and physical health. The Past Year Total Physical Activity Questionnaire<sup>(47)</sup> assessed all types of physical activity done in the past 12 months. Finally, the Canadian Dietary History Questionnaire-I (C-DHQ-I) assessed dietary intake over the previous 12 months<sup>(48)</sup>. This questionnaire was based on the United States National Cancer Institute's Diet History Questionnaire and adapted for use in Canadian populations to reflect differences in food availability and fortification practices<sup>(48)</sup>. It includes 124 items and generates estimates of food and supplemental macro- and micronutrient intake.

A sub-maximal modified Balke test<sup>(49)</sup> was used to estimate maximum oxygen consumption from sub-maximal exercise intensities to measure cardiopulmonary fitness. Anthropometric measurements were taken by Certified Exercise Physiologists using standardised methods and equipment. These include height and weight (used to estimate BMI) and waist and hip circumferences (used to estimate waist:hip ratio). A dual-energy X-ray absorptiometry (DXA) scan was used to assess overall percentage body fat and fat distribution with a Hologic 4500 DXA Scanner (Calgary) or GE Lunar Prodigy High Speed Digital Fan Beam X-ray-based Bone Densitometer (Edmonton). A single-slice computed tomography (CT) scan was used to measure central adiposity (total abdominal fat, intra-abdominal fat and subcutaneous fat) using a Marconi PQ5000 Vision Master/Picker (Calgary) or Phillips/Marconi MX8000 multi-slice scanner (Edmonton).

### Measurement of Alternative Healthy Eating Index 2010 and Dietary Inflammatory Index\*

DII<sup>®</sup> and AHEI-2010 scores were estimated for each participant using data derived from the 124-item C-DHQ-I using Diet\*Calc software (version 1.4.3) downloaded from the United States National Cancer Institute's Website, Division of Cancer Control and Population Sciences. The DII<sup>®</sup> was scored for all participants using methods previously described by

Shivappa *et al.*<sup>(40)</sup>. Briefly, mean intake was standardised using the global mean and standard deviation, converted to represent a range between  $-1$  (maximally anti-inflammatory) and  $+1$  (maximally pro-inflammatory), multiplied by the overall inflammatory effect score, and summed to generate an overall dietary inflammatory effect score for each participant. A total of thirty-eight of the forty-five dietary parameters were available for the DII<sup>®</sup> estimation (Table 1) and included both dietary and supplemental intake. AHEI-2010 score was estimated using methods described by Chiuve *et al.*<sup>(32)</sup>. Intake of eleven food groups/macronutrients was used to calculate the corresponding score out of 10 and summed to obtain a score between 0 (minimally 'healthy') and 110 (maximally 'healthy')<sup>(32)</sup>.

### Measurement of telomere length

Blood samples were collected at study baseline using standardised blood collection, processing, storage and shipment protocols<sup>(45)</sup>. Nucleic acid extraction was automated on the Hamilton STARlet (Hamilton Robotics Inc.) using the Macherey-Nagel NucleoMag Blood 200  $\mu$ l kit. DNA was eluted in nuclease-free water and quantified using a Qubit dsDNA HS Assay Kit (ThermoFisher Scientific Inc.).

LTL (T) relative to a standard single-copy  $\beta$ -globin gene (S; *36B4*) reference was quantified and expressed as a ratio (T:S). Three reference samples were run on each plate: wild-type BJ (human foreskin fibroblast primary cells), BJ + hTERT (human foreskin fibroblast primary cells transformed to express hTERT), and a pooled standard DNA to estimate inter-plate variation. Pooled standard DNA was prepared by pooling thirty buffy coats selected at random into one sample, and DNA extracted using a QIAmp DNA Mini Kit. All samples were measured in triplicate, and laboratory staff was blinded to the participant ID and time point. Final telomere length measurements were normalised to correct for between-run variation using methods described previously<sup>(50)</sup>.

### Statistical analysis

Multivariable linear regression analysis was used to model the association between DII<sup>®</sup> and AHEI-2010 scores with relative LTL. Relative LTL was square root transformed to achieve a more Gaussian distribution and converted to  $z$ -score to simplify interpretation and comparison between studies (Fig. 1 (a)), since the calculation of the T:S ratio results in the right skewed distribution inherent of relative data. The inter-plate CV from our quantitative PCR analysis were relatively large, with values for our standard pooled DNA and wild-type human foreskin fibroblast primary cells reference DNA of 39 and 27 %, respectively. After applying a previously described normalisation factor<sup>(51)</sup>, inter-plate variation was reduced to 10 % for standard pooled DNA and 12 % for the wild-type BJ reference DNA. Finally, square root-transformed relative telomere lengths were normalised using  $z$ -scores for ease of interpretation and for comparison with existing literature. Relative, square root-transformed, normalised LTL is referred to simply as LTL from this point forth.



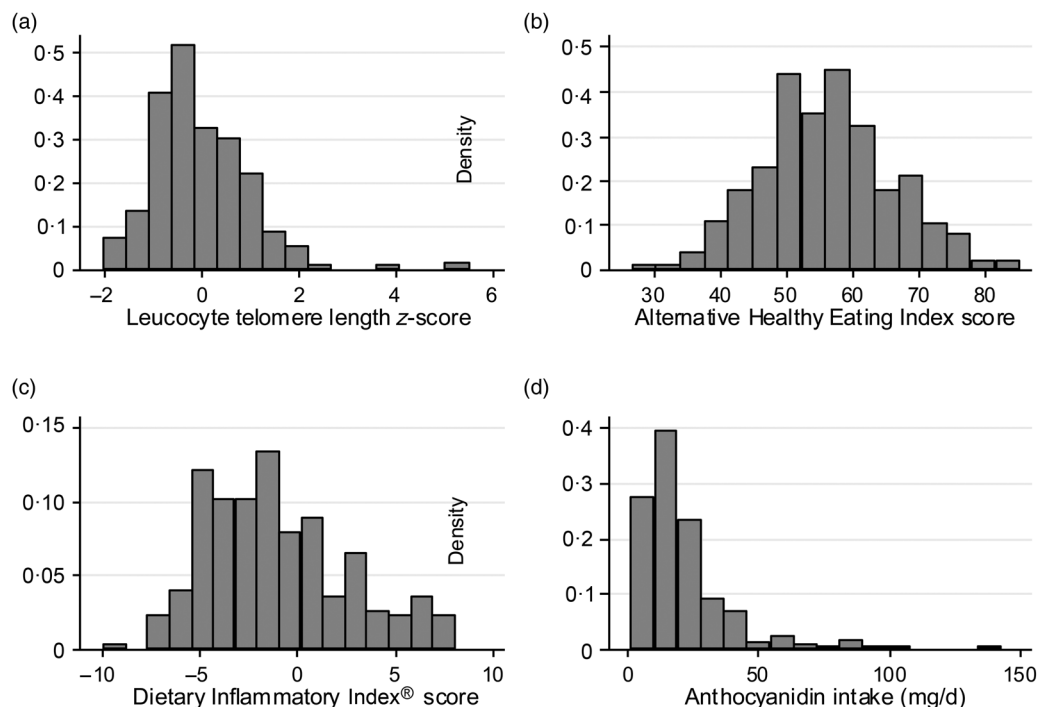
**Table 1.** Association between Alternative Healthy Eating Index 2010 score and Dietary Inflammatory Index<sup>®</sup> score with leucocyte telomere length (LTL) z-score for continuous and categorised dietary indices in in Alberta Physical Activity and Breast Cancer Prevention (ALPHA) Trial participants, Alberta, Canada (2003–2006)

Dietary index	Age adjusted (n 275)			Multivariable adjusted* (n 261)					
	$\beta$ ( $\times 10^{-1}$ )	95 % CI	P	$\beta$ ( $\times 10^{-1}$ )	95 % CI	P			
Alternative Healthy Eating Index 2010	-0.07	-0.19, 0.06	0.29	-0.09	-0.22, 0.04	0.20			
Dietary Inflammatory Index <sup>®</sup>	-0.06	-0.39, 0.27	0.71	0.02	-0.34, 0.38	0.91			
Pairwise comparisons of multivariable adjusted* LTL z-score by quarters									
Dietary index	Quartile of index score	Before adjustment (n)	After adjustment (n)	Index score median	LTL z-score median	$\beta$ ( $\times 10^{-1}$ )	95 % CI	P	P-trend
Alternative Healthy Eating Index	Q1	68	64	45.01	0.06	Reference		Reference	0.07
	Q2	69	65	52.48	-0.30	-0.32	-0.65, 0.15	0.06	
	Q3	69	66	58.89	-0.09	-0.30	-0.64, 0.35	0.08	
	Q4	69	68	68.06	-0.18	-0.30	-0.64, 0.03	0.08	
Dietary Inflammatory Index <sup>®</sup>	Q1	68	66	-4.98	0.04	Reference		Reference	0.57
	Q2	69	67	-2.69	-0.28	-0.08	-0.41, 0.25	0.63	
	Q3	69	65	-0.35	-0.01	0.27	-0.07, 0.60	0.12	
	Q4	69	65	3.55	-0.17	-0.05	-0.39, 0.28	0.75	

\* Model adjusted for age, years since menopause, smoking history, physical activity from recreational activities, cardiopulmonary fitness ( $VO_{2max}$ ), BMI, intra-abdominal fat area, self-perceived psychosocial health, current employment status and marital status.

Age-only and multivariable-adjusted estimates were reported. Final models were adjusted for: age, years since menopause, smoking history (<100 lifetime cigarettes; former casual smoker; former smoker), recreational physical activity

(metabolic equivalent (MET)-h/week per year), cardiopulmonary fitness ( $VO_{2max}$ ), BMI, intra-abdominal fat area ( $cm^2$ ), self-perceived psychosocial health, current employment (dichotomous) and marital status. For the secondary analysis



**Fig. 1.** Histogram of baseline data from participants in the Alberta Physical Activity and Breast Cancer Prevention (ALPHA) Trial (n 275) in Alberta, Canada (2003–2007) (a) Square root transformed relative leucocyte telomere length z-score, (b) Alternative Healthy Eating 2010 score, (c) Dietary Inflammatory Index<sup>®</sup> score and (d) anthocyanidin intake (mg/d).



of the eleven individual components of the AHEI-2010 and thirty-eight available components of the DII<sup>®</sup>, a Bonferroni correction<sup>(51)</sup> was applied to adjust for multiple comparisons. Based on an extensive review of the literature, age, smoking history and past-year recreational physical activity (metabolic equivalent (MET)-h/week per year) were assessed for potential effect modification by including an interaction term in the multivariable linear regression model for each potential modifier. A *P* value <0.05 for the interaction term was considered statistically significant evidence for effect modification that would have resulted in stratum-specific estimates being reported. Non-linear relationships for each index and LTL were assessed using restricted cubic spline analysis with five knots at the 5th, 27.5th, 50th, 72.5th and 95th percentiles of diet score.

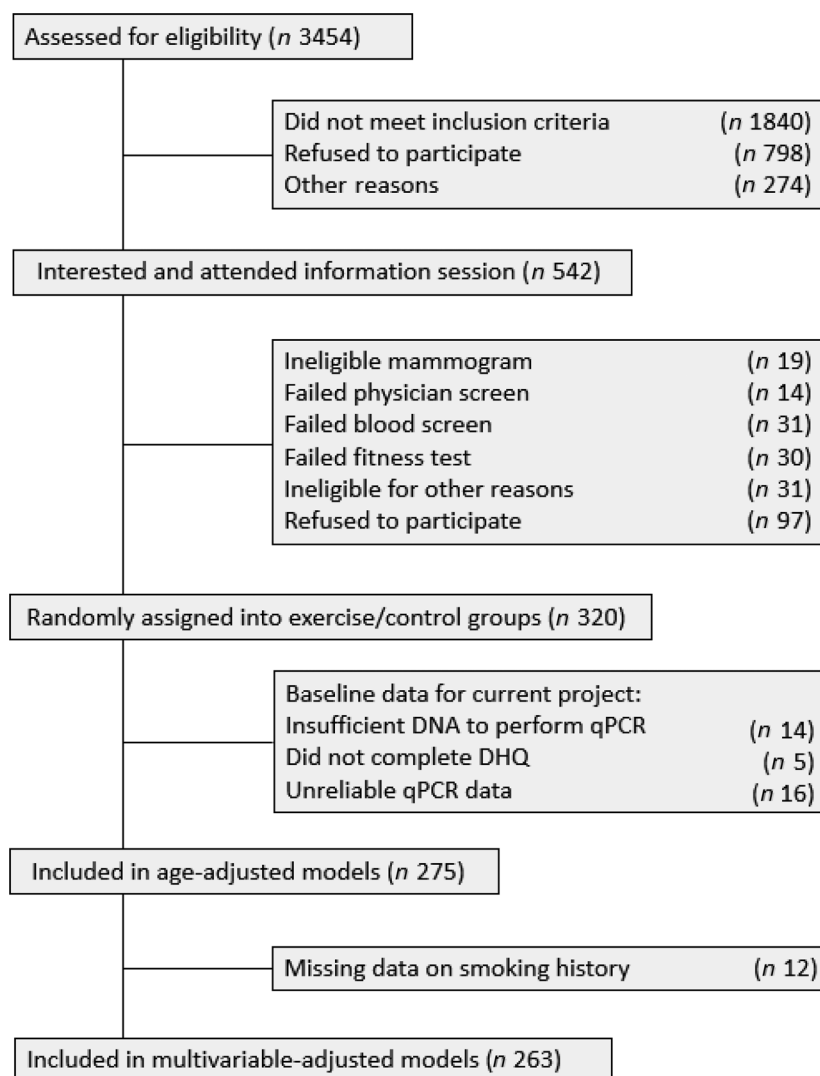
Sensitivity analyses were conducted to explore the effect of the DII<sup>®</sup> estimated with food only (no supplements), age-adjusted models for those for whom smoking data were available only to match the multivariable-adjusted population, and AHEI-2010 models additionally adjusted for total energy

intake. Statistical analyses were performed using STATA (Intercooled (IC) version 14.0 for Mac), *P* values were two-sided, with  $\alpha$ -level = 0.05.

## Results

Dietary, LTL and demographic data were available for 263 women. A full project flow chart can be visualised in Fig. 2. The mean age was 60.9 (SD 5.5) years (range 50–74 years) and BMI was 29.3 (SD 4.4) kg/m<sup>2</sup>. Women were primarily Caucasian (91.6%), non-smokers (69.2% had smoked less than 100 lifetime cigarettes) and relatively well educated (67.6% with college/trade diploma or university degree). Detailed population characteristics are summarised in Table 2.

Several demographic and anthropometric characteristics were statistically significantly associated with LTL including recreational physical activity ( $\beta = 0.01$ ; *P* = 0.01) and intra-abdominal fat area ( $\beta < -0.01$ ; *P* = 0.04) but no association was found with age ( $\beta = -0.02$ ; *P* = 0.06; Table 2). The inflammatory biomarker IL-6 was inversely associated with LTL ( $\beta = -0.15$ ; pg/ml;



**Fig. 2.** Participant flow chart showing recruitment and inclusion of participants into the Alberta Physical Activity and Breast Cancer Prevention (ALPHA) Trial, including baseline participants included in the present study. Alberta, Canada (2003–2007). qPCR, quantitative PCR; DHQ, Dietary History Questionnaire.



**Table 2.** Baseline characteristics for participants (*n* 275) and univariate associations between baseline characteristics with Alternative Healthy Eating Index 2010 (AHEI-2010) score, Dietary Inflammatory Index<sup>®</sup> (DII<sup>®</sup>) score and leucocyte telomere length (LTL) in the Alberta Physical Activity and Breast Cancer Prevention (ALPHA) Trial, Alberta, Canada (2003–2006)

Characteristic	<i>n</i>	Mean			Median	Q1, Q3		% With characteristic	Association with AHEI-2010 score		Association with DII <sup>®</sup> score <sup>®</sup>		Association with LTL z-score	
		Mean	sd	sd		β	<i>P</i>		β	<i>P</i>	β	<i>P</i>		
Characteristic (continuous)														
Age at baseline (years)	275	60.9	5.5	60.4	56.6, 64.4				0.06	0.58	-0.05	0.17	-0.02	0.06
Time since menopause (years)	274	12.7	8.3	10.6	6.0, 17.7				0.05	0.51	0.04	0.12	-0.02	0.03
Self-perceived general health (/100)	272	82.4	13.3	82.0	77.0, 92.0				0.04	0.36	0.01	0.59	<-0.01	0.91
Self-perceived psychosocial health (/100)	275	83.6	11.8	88.0	80.0, 92.0				0.04	0.44	-0.01	0.59	<-0.01	0.67
Fasting glucose														
mmol/l	275	5.6	1.1	4.9	4.9, 6.0				<0.01	0.75	<0.01	0.37	<0.01	0.21
mg/dl	275	100.3	20.7	97	89.0, 108.0				0.01	0.75	<0.01	0.37	<0.01	0.21
Insulin (μIU/ml)	275	7.3	5.8	6.0	3.4, 8.9				-0.09	0.41	0.01	0.81	<-0.01	0.61
IL-6 (pg/ml)	275	1.8	1.2	1.4	1.1, 2.0				-0.35	0.49	0.04	0.81	-0.15	<0.01
TNF-α (pg/ml)	275	1.6	0.6	1.4	1.2, 1.8				-0.88	0.39	0.65	0.08	-0.04	0.72
CRP (mg/l)	275	3.0	4.0	1.0	0.7, 3.0				-9.40	0.48	2.90	0.56	-1.80	0.20
Total energy intake														
kJ/d	275	6527.5	2282.8	6266.8	4945.5, 7729.1				<0.01	0.22	<-0.01	<0.01	<-0.01	0.83
kcal/d	275	1560.1	545.6	1497.8	1182.0, 1847.3				<0.01	0.22	<-0.01	<0.01	<-0.01	0.83
Alcohol intake (g/d)	275	4.7	6.7	1.9	0.6, 6.0				0.14	0.11	-0.09	<0.01	0.01	0.13
Total physical activity (MET-h/week)	375	123.2	71.2	108.3	76.0, 150.0				0.14	0.62	<-0.01	0.41	<-0.01	0.17
Employment physical activity (MET-h/week)	275	52.8	55.3	45.0	2.4, 86.4				-0.01	0.28	<-0.01	<0.04	<-0.01	0.90
Household physical activity (MET-h/week)	275	58.8	44.9	50.3	35.0, 69.4				0.0004	0.98	-0.02	<0.01	<-0.01	0.10
Recreational physical activity (MET-h/week)	275	10.9	12.7	6.6	2.0, 15.3				0.10	0.04	-0.03	0.03	0.01	0.01
Cardiopulmonary fitness (VO <sub>2max</sub> )	274	26.9	6.0	27.5	23.8, 30.7				0.26	<0.01	-0.04	0.30	0.01	0.38
BMI (kg/m <sup>2</sup> )	275	29.3	4.4	28.7	25.9, 32.0				-0.30	0.03	0.11	0.02	-0.02	0.23
Total body fat (%)	275	42.3	5.4	42.5	38.5, 45.7				-0.22	<0.05	0.08	0.06	-0.01	0.38
Intra-abdominal fat area (cm <sup>2</sup> )	275	101.9	56.9	94.9	55.0, 14.05				-0.02	0.04	<-0.01	0.16	<-0.01	0.04
Characteristic (categorical)														
Ever pregnant (% yes)	274	-	-	-	-			92.7	-0.30	0.90	-0.44	0.60	0.04	0.87
Family history of breast cancer (% yes)	274	-	-	-	-			21.2	1.67	0.25	0.14	0.80	0.23	0.12
Smoking history (% former smoker)	263	-	-	-	-			20.9	-1.93	0.20	0.39	0.48	0.12	0.44
Ethnicity (% Caucasian)	274	-	-	-	-			91.6	2.02	0.34	-0.11	0.98	0.02	0.91
Education (% with college or university degree)	273	-	-	-	-			67.8	3.16	0.01	-0.91	0.05	0.17	0.18
Employment status (% employed)	263	-	-	-	-			52.9	-0.35	0.77	0.96	0.03	0.02	0.88
Marital status (% married/common law)	274	-	-	-	-			74.1	-0.084	0.95	0.28	0.57	-0.06	0.65
Past hormone medication use (% ever)	275	-	-	-	-			45.0	-0.32	0.79	-0.07	0.88	0.03	0.79
Past hormone replacement therapy use (% ever)	274	-	-	-	-			44.2	-0.57	0.63	-0.11	0.80	0.02	0.88
High cholesterol or TAG (% ever)	274	-	-	-	-			32.5	0.58	0.64	0.08	0.86	-0.17	0.18

CRP, C-reactive protein; MET, metabolic equivalent.



$P < 0.01$ ), yet not with the AHEI-2010 ( $\beta = -0.35$ ;  $P = 0.49$ ) or the DII<sup>®</sup> ( $\beta = 0.04$ ;  $P = 0.81$ ). C-reactive protein was not associated with LTL ( $\beta = -1.80$ ;  $P = 0.20$ ), the AHEI-2010 ( $\beta = -0.94$ ;  $P = 0.48$ ) or DII<sup>®</sup> ( $\beta = 0.29$ ;  $P = 0.56$ ). A complete summary of univariate associations between AHEI-2010, DII<sup>®</sup> score and LTL with baseline demographic and anthropometric characteristics are shown in Table 2.

AHEI-2010 score was approximately normally distributed, with a mean score of 56.3 (SD 9.75) (Fig. 1(b)). Results from the age-adjusted model estimated that for a one-unit increase in AHEI-2010 score, LTL decreased by approximately 0.07 (95 % CI  $-0.019$ , 0.06) standard deviations ( $P = 0.29$ ). Similarly, no significant association was detected between LTL and AHEI-2010 in the fully adjusted model, where a one-unit increase in AHEI-2010 score corresponded to an estimated decrease of 0.09 (95 % CI  $-0.22$ , 0.04) standard deviations of LTL ( $P = 0.20$ ). There was no statistical evidence of effect modification for age, smoking history or recreational physical activity. Primary results are summarised in Table 1. Restricted cubic spline regression analysis provided no evidence of a non-linear relationship between AHEI-2010 and LTL  $\chi$ -score ( $P = 0.37$ ). Results of the multivariable adjusted pairwise comparisons of AHEI-2010 quartiles with LTL (Table 1) indicated no statistically significant relationship between the first and second quartiles ( $\beta = -0.32$ ;  $P = 0.06$ ), third quartile ( $\beta = -0.30$ ;  $P = 0.08$ ) or fourth quartile ( $\beta = -0.30$ ;  $P = 0.08$ ). The test for linear trend across quartiles was also non-statistically significant ( $P$ -trend = 0.07). An exploratory analysis which assessed the eleven individual parameters that comprise the index as individual exposures also found no significant association between dietary intake from any parameter and LTL in age-adjusted or multivariable adjusted models (Table 3). AHEI-2010 scores correlated inversely ( $r = -0.44$ ;  $P < 0.01$ ) with DII<sup>®</sup> scores (Fig. 3). DII<sup>®</sup> score was approximately normally distributed as illustrated in Fig. 1(c), with a mean score of  $-1.01$  (SD 3.58). As indicated in Table 1, the age-adjusted model estimated that for a one-unit increase in DII<sup>®</sup> score, the LTL decreased by approximately 0.06 (95 % CI  $-0.39$ , 0.27) standard deviations ( $P = 0.71$ ). Similarly, no statistically significant association was detected between LTL and the DII<sup>®</sup> in the multivariable adjusted model where a one-unit increase in DII<sup>®</sup> score corresponded to an estimated increase of 0.02 (95 % CI  $-0.34$ , 0.38) standard deviations of the LTL ( $P = 0.91$ ). There was no statistical evidence of effect modification by age, smoking history or recreational physical activity. Restricted cubic spline regression analysis provided no evidence of a non-linear relationship between the DII<sup>®</sup> and LTL ( $P = 0.56$ ). Multivariable adjusted pairwise comparisons of DII<sup>®</sup> quartiles with LTL (Table 3) indicated no statistically significant relationships for any of the quartiles of DII<sup>®</sup> and there was no statistically significant linear trend.

An exploratory analysis that assessed each of the thirty-eight available parameters of the DII<sup>®</sup> (Table 3) as individual potential predictors of LTL found that anthocyanidin intake was significantly associated with LTL  $\chi$ -score in both age-adjusted ( $\beta = 0.01$ ;  $P = 0.01$ ) and multivariable adjusted ( $\beta = 0.09$ ;  $P < 0.01$ ) models, but not in Bonferroni corrected ( $P = 0.23$ ) models. All sensitivity analyses conducted had a trivial effect

on the interpretation of the results and conclusions of the study (data not shown).

## Discussion

This study found neither DII<sup>®</sup> nor AHEI-2010 scores to be associated with LTL in this population of healthy, postmenopausal women. Additionally, none of the eleven components of the AHEI-2010 were associated with LTL. LTL was not statistically significantly correlated with age, and neither DII<sup>®</sup> nor AHEI scores were associated with the inflammatory biomarkers C-reactive protein or IL-6 as expected.

Only one study has examined the relationship between the AHEI-2010 and LTL in 4676 disease-free women from the Nurses' Health Study (NHS), in which the authors found higher AHEI-2010 scores to be associated with longer LTL<sup>(39)</sup>. Results from two other studies that had previously examined the association between DII<sup>®</sup> score and LTL indicated a statistically significant inverse association between baseline DII<sup>®</sup> and LTL<sup>(43,44)</sup>. Shivappa *et al.*<sup>(41)</sup> found that higher DII<sup>®</sup> scores were associated both with shorter LTL, and with C-reactive protein (serving as a construct validation of the DII<sup>®</sup>) in a cross-sectional analysis of 7215 National Health and Nutrition Examination Survey (NHANES) participants. Garcia-Calzon *et al.*<sup>(43)</sup> found that an increase in DII<sup>®</sup> score was associated with a two-fold increased LTL attrition over a 5-year follow-up in 520 Spanish men and women from the PREvención con DIeta MEDiterránea (Prevention with Mediterranean Diet Study; PREDIMED-NAVARRA) study. Conversely, in 2018, De Meyer *et al.*<sup>(52)</sup> published cross-sectional results indicating no association between DII<sup>®</sup> scores and LTL in 2509 healthy men and women, concluding an at most limited association between dietary patterns and LTL in the general population. Similarly, Meinilä *et al.*<sup>(53)</sup> recently reported no association between DII<sup>®</sup> scores in either cross-sectional analyses or longitudinal assessments over 10 years in their cohort of 1046 elderly men and women who participated in the Helsinki Birth Cohort Study. Our study differs in sample size ( $n$  263 *v.* 4676 in the NHS) and design (cross-sectional *v.* prospective with change in LTL measured) to studies that reported relationships between the DII<sup>®</sup> and LTL, we could conclude that our analyses limited our ability to detect differences in LTL. Our study findings do, however, align with the findings of Meinilä *et al.*<sup>(53)</sup> and De Meyer *et al.*<sup>(52)</sup> and therefore we must consider that the inflammatory potential of the diet does not directly or indirectly influence LTL as hypothesised.

While the statistically significant association between anthocyanidin intake and LTL was no longer statistically significant after correction for multiple testing, some discussion of this finding is warranted. Anthocyanidins are common plant pigments that reflect light in the red to blue range and are responsible for the pigmentation in foods such as blueberries, raspberries, red onions and purple yams<sup>(54,55)</sup> (list not exhaustive). Anthocyanidins are considered the most important subcategory of flavonoids because of their strong *in vitro* and *in vivo* antioxidant activity<sup>(28,54,55)</sup>. While this finding may be a result of type I error from multiple testing, further investigation into this highly biologically plausible association is justified.



**Table 3.** Associations between Alternative Healthy Eating Index 2010 component dietary intake and Dietary Inflammatory Index<sup>®</sup> component with leucocyte telomere length z-score in Alberta Physical Activity and Breast Cancer Prevention (ALPHA) Trial participants, Alberta, Canada (2003–2006)\*

	Overall inflammation effect score	Daily intake		Age adjusted (n 275)		Multivariable adjusted† (n 261)	
		Mean	SD	$\beta$ ( $\times 10^{-1}$ )	P	$\beta$ ( $\times 10^{-1}$ )	P
<b>Alternative Healthy Eating Index 2010 component</b>							
Vegetables‡ (servings/d)	–	4.28	2.67	–0.10	0.65	–0.09	0.70
Fruit§ (servings/d)	–	2.68	2.04	0.21	0.45	0.17	0.53
Whole grains (servings/d)	–	1.01	0.73	0.24	0.77	0.41	0.66
Sugar-sweetened beverages and fruit juices (servings/d)	–	0.69	0.96	–0.32	0.61	<–0.10	0.91
Nuts and legumes (servings/d)	–	1.10	1.35	–2.21	0.09	–2.31	0.08
Red/processed meats (servings/d)	–	0.50	0.44	–0.12	0.93	1.59	0.35
Trans-fats (% of energy)	–	3.22	1.89	–0.07	0.93	0.13	0.89
Long-chain (n-3) fats   (mg/d)	–	93.67	84.85	<0.01	0.48	<0.01	0.38
PUFA (% of energy)	–	13.30	7.22	–0.33	0.19	–0.33	0.23
Na (mg/d)	–	2421.75	1035.07	<0.01	0.97	<0.01	0.93
Alcohol (drinks/d)	–	4.66	6.70	0.14	0.11	0.07	0.50
<b>Dietary Inflammatory Index<sup>®</sup> Component</b>							
<b>Energy intake</b>							
kJ/d	0.180	6527.54	2286.97	<0.01	0.95	<0.01	0.67
kcal/d	0.180	1560.12	546.60	<0.01	0.95	<0.01	0.67
Total fat (g/d)	0.298	57.07	27.55	<0.01	0.76	<0.01	0.85
Total carbohydrate (g/d)	0.097	202.06	74.51	<0.01	0.89	<0.01	0.62
Total protein (g/d)	0.021	63.17	24.94	<0.01	0.97	<0.01	0.96
Alcohol (g/d)	–0.278	4.66	6.70	0.14	0.10	0.07	0.50
Cholesterol (mg/d)	0.110	194.27	116.93	<0.01	0.52	<0.01	0.44
Saturated fat (g/d)	0.373	17.53	8.89	0.03	0.63	0.07	0.31
Monounsaturated fat (g/d)	–0.009	21.74	10.96	–0.03	0.61	<–0.01	0.92
Polyunsaturated fat (g/d)	–0.337	13.30	7.22	–0.08	0.35	–0.04	0.67
Trans-fatty acids (g/d)	0.229	3.21	1.89	0.06	0.84	0.21	0.55
n-3 Fatty acids (g/d)	–0.436	1.33	0.69	–0.15	0.86	0.34	0.73
n-6 Fatty acids (g/d)	–0.159	11.87	6.56	–0.09	0.33	–0.05	0.62
Total dietary fibre (g/d)	–0.663	19.40	9.58	–0.02	0.72	–0.01	0.80
Vitamin A (retinol equivalents/d)	–0.401	1990.62	1197.34	<0.01	0.20	<0.01	0.07
$\beta$ -Carotene ( $\mu$ g/d)	–0.584	4755.50	4215.64	<0.01	0.23	<0.01	0.23
Vitamin D ( $\mu$ g/d)	–0.446	10.34	6.08	0.05	0.61	–0.04	0.74
Vitamin E (mg/d)	–0.419	12.57	7.75	<0.01	0.48	<0.01	0.99
Vitamin C (mg/d)	–0.424	316.92	303.11	<0.01	0.15	<0.01	0.43
Thiamine (mg/d)	–0.098	2.89	2.45	0.13	0.61	0.09	0.74
Riboflavin (mg/d)	–0.068	2.95	1.83	0.18	0.57	0.06	0.87
Niacin (mg/d)	–0.246	29.97	15.40	–0.01	0.84	–0.02	0.62
Vitamin B <sub>6</sub> (mg/d)	–0.365	7.43	12.27	0.06	0.20	0.06	0.30
Total folate ( $\mu$ g/d)	–0.190	616.00	270.40	<0.01	0.40	<0.01	0.19
Vitamin B <sub>12</sub> ( $\mu$ g/d)	0.106	6.61	3.52	–0.02	0.91	–0.13	0.49
Mg (mg/d)	–0.484	364.04	126.41	<0.01	0.55	<0.01	0.39
Fe (mg/d)	0.032	19.57	9.84	–0.08	0.21	–0.09	0.16
Zn (mg/d)	–0.313	16.31	9.40	<–0.01	0.97	–0.03	0.67
Se (mg/d)	–0.191	82.78	34.11	<0.01	0.98	<0.01	0.75
Caffeine (g/d)	–0.110	3.90	3.46	0.21	0.22	<0.01	0.16
Flavonols (mg/d)	–0.467	27.94	20.46	0.02	0.44	0.01	0.63
Flavones (mg/d)	–0.616	2.11	1.72	0.13	0.71	–0.03	0.93
Isoflavones (mg/d)	–0.593	6.95	30.61	–0.02	0.37	–0.03	0.22
Anthocyanidins (mg/d)	–0.131	21.40	17.98	0.09	0.01	0.09	<0.01
Flavanones (mg/d)	–0.250	31.98	27.82	<0.01	0.80	–0.02	0.50
Flavan-3-ol (mg/d)	–0.415	345.38	488.57	<0.01	0.41	<0.01	0.52
Onion (g/d)	–0.301	11.05	16.38	–0.02	0.51	–0.03	0.50
Pepper (g/d)	–0.131	7.32	12.17	–0.05	0.28	–0.07	0.21
Green/black tea (g/d)	–0.536	0.92	1.11	0.49	0.37	0.35	0.54

\* Data unavailable for garlic, ginger, rosemary, saffron, thyme/oregano, turmeric and eugenol intake while calculating Dietary Inflammatory Index<sup>®</sup> score.

† Model adjusted for age, years since menopause, smoking history, physical activity from recreational activities, cardiopulmonary fitness ( $VO_{2max}$ ), BMI, intra-abdominal fat area, self-perceived psychosocial health, current employment status and marital status.

‡ Not including potatoes/French fries.

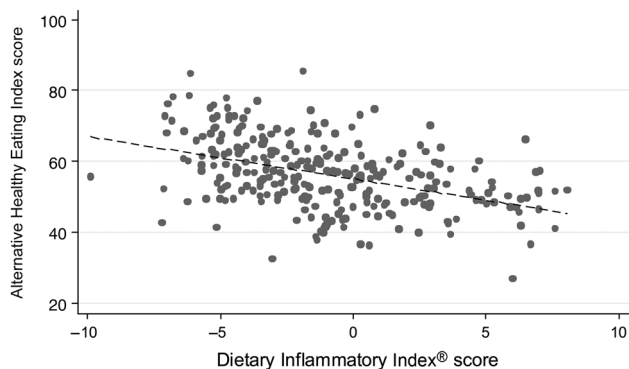
§ Not including fruit juices.

|| EPA and DHA.

This study had several strengths of note. First, we were able to evaluate several well-measured potential confounders. For example, direct measurements of body size and composition

were taken including a DXA scan for overall percentage body fat and fat distribution and a computed tomography (CT) scan for central adiposity (total abdominal fat, intra-abdominal





**Fig. 3.** Scatterplot demonstrating correlation ( $r = -0.44$ ;  $P < 0.001$ ) between Alternative Healthy Eating Index 2010 (y-axis) scores and Dietary Inflammatory Index<sup>®</sup> scores (x-axis) from participants in the Alberta Physical Activity and Breast Cancer Prevention (ALPHA) Trial ( $n = 275$ ) in Alberta, Canada (2003–2007). ---, Fitted values.

fat and subcutaneous fat). Self-reported physical activity was assessed using the reliable and validated Past Year Total Physical Activity Questionnaire<sup>(47)</sup>. Moreover, the stringent exclusion criteria of the underlying ALPHA Trial reduced potential confounding by those restricted variables (e.g. alcohol consumption, current smoking, etc.). In addition, we had thirty-eight of the forty-five DII<sup>®</sup> parameters available to calculate the DII<sup>®</sup> score, where examples from the literature often include as few as twenty parameters<sup>(56)</sup>. Extensive dietary data were also available for potentially important vitamins, minerals and flavonoids.

These results are limited by using self-reported dietary measurements which may be biased to over-reporting of healthy foods and under-reporting of unhealthy foods (social desirability bias)<sup>(57)</sup>. There was some underreporting of total energy intake, which is common for FFQ such as the C-DHQ-I. In addition, neither dietary index was associated with the key inflammatory biomarkers C-reactive protein or IL-6, thus the DII<sup>®</sup> and AHEI-2010 are not validated by the present study. Measurement error is a common limitation when measuring telomere length, regardless of the assay chosen<sup>(58)</sup> and LTL did not correlate significantly with age, compromising the results of the present study. The cross-sectional study design prohibits temporality from being established, and generalisability is limited since this study was conducted on a relatively small sample of primarily Caucasian, healthy, postmenopausal women of relatively high education and socio-economic status. The inclusion of postmenopausal women only may have also limited our ability to detect associations, since the association between LTL and inflammation may be less pronounced in postmenopausal than pre-menopausal women<sup>(59)</sup>. Hypothesised mechanisms underlying this finding include differing insulin resistance and leptin levels in pre- *v.* postmenopausal women and decreased circulating oestrogen in postmenopausal women<sup>(59)</sup>. Oestrogen may be linked to LTL through its anti-inflammatory and oxidant properties, and its ability to stimulate telomerase, a telomere-elongating enzyme<sup>(59)</sup>. The use of a homogeneous population in terms of age (50 to 74 years), ethnicity, anthropometric characteristics, physical activity and sociodemographic factors may have limited the range of telomere length variability such that

only studies with large sample sizes, or exposures that have an important impact on LTL, would be able to detect any significant changes. For the same reasons mentioned above, the results of this study have limited generalisability. Finally, given the initial design of the ALPHA Trial and its primary purpose, these analyses were exploratory in nature which we acknowledge as a limitation of this study.

This study did not provide evidence of an association between the AHEI-2010 or DII<sup>®</sup> and LTL in this cohort. However, this research alone does not discount the association between dietary patterns and reduced oxidative damage and longer telomeres. Larger studies, or longer-term cohort or randomised controlled trials, would be an ideal alternative to understand this putative relationship more fully, as detectable changes in telomere attrition rates due to environmental stressors may take several years to occur<sup>(60)</sup>. Variations or SNP in genes involved in the inflammatory response should be considered for future studies. Finally, our findings corroborate recent research which suggest that the diet's inflammatory potential may have limited impact on telomere length and telomere attrition rates<sup>(52,53)</sup>.

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The authors of this paper declare no conflicts of interest.



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