A pilot study to investigate if New Zealand men with prostate cancer benefit from a Mediterranean-style diet

Sharon Erdrich, Karen S Bishop, Nishi Karunasinghe, Dug Yeo Han, Lynnette R Ferguson

Carcinoma of the prostate is the most commonly diagnosed malignancy and the third leading cause of mortality in New Zealand men, making it a significant health issue in this country. Global distribution patterns suggest that diet and lifestyle factors may be linked to the development and progression of this cancer. Twenty men with diagnosed prostate cancer adhered to a Mediterranean diet, with specific adaptations, for three months. Prostate-specific antigen, C-reactive protein and DNA damage were evaluated at baseline and after three months of following the diet. Dietary data were collated from diet diaries and an adaptation of a validated Mediterranean diet questionnaire. A significant reduction in DNA damage compared to baseline was apparent, with particular benefit noted for overall adherence to the diet ($p = 0.013$), increased intake of folate ($p = 0.023$), vitamin C ($p = 0.007$), legumes ($p = 0.004$) and green tea ($p = 0.002$). Higher intakes of red meat and dairy products were inversely associated with DNA damage ($p = 0.003$ and $p = 0.008$ respectively). The results from this small feasibility study suggest that a high-antioxidant diet, modelled on Mediterranean traditions, may be of benefit for men with prostate cancer. Protection against DNA damage appears to be associated with the diet implemented, ostensibly due to reduction in reactive oxidant species. These findings warrant further exploration in a longer trial, with a larger cohort.

2 # A Pilot Study to Investigate if New Zealand Men with
3 # Prostate Cancer Benefit from a Mediterranean-Style Diet

4 **Sharon Erdrich[1,*], Karen S. Bishop[2], Nishi Karunasinghe [3], Dug Yeo Han[3]and**
5 **Lynnette R.Ferguson[1,3]**

6 [1] Discipline of Nutrition, FM&HS, University of Auckland, Private Bag 92019, Auckland
7   1142,
8   New Zealand; E-Mail: l.ferguson@auckland.ac.nz
9 [2] Auckland Cancer Society Research Centre, FM&HS, University of Auckland, Private Bag
10   92019, Auckland 1142, New Zealand; E-Mail: k.bishop@auckland.ac.nz
11 [3] Nutrigenomics New Zealand, University of Auckland, Private Bag 92019, Auckland 1142,
12   New Zealand; E-Mails:n.karunasinghe@auckland.ac.nz;dy.han@auckland.ac.nz

13 * Author to whom correspondence should be addressed: E-Mail: sharon@houseofhealth.co.nz;
14   Tel.: +64-9-377-7070; Fax: +64-9-820-6498.

Introduction

17   On a global scale, prostate cancer is an important health consideration. It is the fourth most
18   common cancer internationally, and in men ranks second only to lung cancer(Ferlay et al., 2012).
19   Prostate cancer incidence is highest in developed nations, compared to less-developed countries,
20   a difference that is only partially explained by the higher use of prostate specific antigen (PSA)
21   as a screening tool in developed nations(Center et al., 2012). In New Zealand, cancer of the
22   prostate is the third most common cause of cancer-related mortality, with death rates
23   disproportionately higher in Māori men (28.7 c.f. 16.7 deaths per 100,000 (age-standardised rates
24   per 100,000 male population, standardised to the WHO world standard population)(MOH, 2013).
25   Worldwide patterns of prostate cancer incidence and mortality support the hypothesis that diet
26   and lifestyle are likely contributors to both development and progression of this
27   malignancy.Furthermore, alterations in risk associated with migratory patterns and the
28   westernisation of dietary patterns associated with globalisation (Baade, Youlden & Krnjacki,
29   2009; Melnik, John & Schmitz, 2011)give added credence to this theory.
30   Chronic inflammation and infection have been implicated in the development of around one-
31   fifth of all cancers(Greene et al., 2011), including prostate cancer (Gurel et al., 2014).Other
32   influences include genetic and epigenetic factors(Pantuck et al., 2006), imbalances between
33   reactive oxygen species and antioxidants, and DNA damage (Waris & Ahsan, 2006)(Figure 1).
34   The role of inflammation in prostate cancer is unclear.Elevated levels of inflammatory
35   markers have been associated with high-grade prostate cancer in some studies(Shariat et al.,
36   2001; Platz & De Marzo, 2004; Huffman et al., 2006; Gurel et al., 2014)but not in
37   others(Il'yasova et al., 2005; Stark et al., 2009).Systemic inflammation has been associated with
38   earlier cancer mortality(McArdle, Qayyum & McMillan, 2010; Shafique et al., 2012), which
39   adds weight to an argument for a detrimental effect of chronic inflammation and the potential
40   benefit of alow-inflammatory diet.
41   Increased levels of free radicals and pro-oxidant compounds (Arsova-Sarafinovska et al.,
42   2009; Qu et al., 2013; Kanwal et al., 2014), and decreased levels of antioxidant enzymes have
43   been found in prostate tissue (Kanwal et al., 2014), and in association with prostate
44   cancer(Arsova-Sarafinovska et al., 2009). Both prostatic cancer cells and high-grade prostatic
45   intraepithelial neoplasia are notably deficient in the important endogenous antioxidant,
46   glutathione *S*-transferase(Platz & De Marzo, 2004). This may be due to inflammation-induced
47   inactivation of genes that have roles in cellular protection and restoration of damaged DNA
48   (Kundu & Surh, 2012). Reduction in the ability of cells to produce protective antioxidants may
49   occur due to alterations in cell morphology, leaving the prostate vulnerable to damage by
50   carcinogenic compounds that might otherwise be neutralised by antioxidants. Indeed,
51   proliferative inflammatory atrophy, a precursor to development of prostate cancer, is a cellular
52   change that is postulated to be the result of cell damage(Platz & De Marzo, 2004; Brawer, 2005).

53   The Mediterranean diet has been extensively examined and its benefits in terms of reductions
54   in oxidative stress and inflammation are generally well-accepted (Urpi-Sarda et al., 2012;
55   Viscogliosi et al., 2013). This dietary style is centered on consumption of high amounts of extra
56   virgin olive oil, fruit and vegetables, pulses and legumes, whole-grains, and poultry, along with
57   some fish and seafood. Intake of red meat, dairy products and processed or refined foods is
58   traditionally low (Couto et al., 2011). Deviation away from such a pattern, towards a more
59   western-style diet, has been associated with increased prostate cancer incidence (Ambrosini et
60   al., 2008; Stott-Miller, Neuhouser & Stanford, 2013). Coutoet al.(Couto et al., 2011) examined
61   data from the large European Prospective Investigation into Cancer and Nutrition (EPIC) study
62   and concluded that a Mediterranean diet is particularly beneficial in protecting against breast,
63   colon, and prostate cancer.
64   The brassica family is not emphasised in general Mediterranean diet guidelines. However, this
65   family of vegetables has attracted much interest in recent years, primarily on account of the
66   levels of glucosinolates, which are particularly high in broccoli (Moreno et al., 2006).
67   Nutrigenomic effects of broccoli are discussed in detail in Ferguson andSchlothauer(Ferguson &
68   Schlothauer, 2012).
69   Pomegranate, and its juice, has received a great deal of attention related to potential chemo-
70   protective effects, including beneficial effects in slowing of prostate-specific antigen (PSA)
71   doubling time in men with prostate cancer (Pantuck et al., 2006, 2009; Paller et al., 2012). This
72   benefit is attributed to high levels of polyphenols (particularly punicalagin, an
73   elligatannin(Koyama et al., 2010)), that contribute to pomegranate's overall antioxidant capacity,
74   which is greater than either red wine or green tea (Gil et al., 2000). Polyphenols are also high in
75   extra virgin olive oil, red wine, and green tea (Tuck & Hayball, 2002). These phytochemicals
76   have demonstrable epigenetic effects(Joven et al., 2013), which may account for at least some of
77   the benefits attributed to their consumption. As pomegranate is found throughout the
78   Mediterranean area, it is logical to assume it would be commonly consumed in the region.
79   However, this has not been reported in nutritional research which focuses on Mediterranean
80   dietary patterns. It is quite possible that pomegranate may be a contributor to documented
81   advantages associated with diets in the Mediterranean region.
82   High fibre diets, including consumption of legumes and whole grains, have been linked to a
83   wide range of health benefits. Legumesare an important food group in the Mediterranean diet
84   (Ferrís-Tortajada et al., 2012), and have been associated with reduced prostate cancer risk (Chan,
85   Lok & Woo, 2009). Consumption of legumes (particularly soy) is significant in Asian diets
86   where prostate cancer incidence is also low (Chan, Lok & Woo, 2009). Mechanisms for benefit
87   are numerous and include: high fibre content, which may be advantageous by reduction of post-
88   prandial glycaemia (Gropper, Stepnick & Smith, 2013), lower levels of insulin-like growth factor
89   (Landberg et al., 2010), and increases in sex hormone binding globulin (Tymchuk et al., 1998).
90   The minimisation of post-prandial rises in blood sugar level is desirable in men with prostate

91 cancer, due to glycaemia-associated increases in markers of inflammation and oxidative
92 stress(Rytter et al., 2009). Additionally, foods that promote a high glycaemic response induce
93 insulin and insulin-like growth factor (Melnik, John & Schmitz, 2011), which may contribute to
94 prostate cancer progression(Chan et al., 2002).
95   In some studies (Gao, LaValley & Tucker, 2005; Koh et al., 2006; Allen et al., 2008), but not
96 all (Huncharek, Muscat & Kupelnick, 2008; Pettersson et al., 2012), consumption of dairy foods
97 has been linked to higher risk of developing prostate cancer. Risk has been associated with the
98 quantity consumed (Allen et al., 2008) and dairy intake during adolescence (Torfadottir et al.,
99 2012). Prostate cancer mortality has also been correlated to milk consumption(Ganmaa et al.,
100 2002). While these data are inconclusive, it does, nonetheless, raise concerns over the
101 recommendation of dairy as a source of calcium for men at risk of developing osteoporosis,
102 including those with prostate cancer who have had hormonal ablation treatment (Malcolm et al.,
103 2007).
104   Fish is not considered a major component of Mediterranean diets (Trichopoulou et al., 2005),
105 but is generally considered preferential to red meat for men with prostate cancer (Terry et al.,
106 2001; Chavarro et al., 2008; Bosire et al., 2013). Fish is regarded as a good source of omega-3
107 polyunsaturated fatty acids, promoting anti-inflammatory pathways (Jain et al., 2008), which has
108 been considered to be desirable in men for whom lowering inflammation is a goal. However, the
109 benefit of omega-3 polyunsaturated fatty acids in prostate cancer has recently been
110 challenged(Brasky et al., 2013). The role of dietary fats in prostate cancer is discussed in more
111 detail by Bishop,et al(Bishop et al., 2015).
112   Green tea is not a dietary feature in the Mediterranean region, but is a common beverage in
113 East Asian countries, where mortality rates from prostate cancer are the lowest, globally (Ferlay
114 et al., 2012). A mounting volume of evidence supports the recommendation of consumption of
115 green tea, due to its antioxidant potential, largely from polyphenols, in particular
116 epigallocatechin-3-gallate (EGCG) (Du et al., 2012). EGCG has documented anti-proliferative
117 properties (Du et al., 2012)and affords protection to DNA in prostate cells (Kanwal et al., 2014).
118 Furthermore, green tea consumption has been associated with lower prostate cancer incidence
119 (Zheng et al., 2011) and reduced risk of progression to advanced disease (Kurahashi et al., 2008).
120   This pilot study was undertaken to establish bothfeasibility and likely benefit of three months
121 of adherence to a Mediterranean dietary pattern, with specific modifications, on DNA damage
122 and inflammation in New Zealand men with prostate cancer.

123 **Materials and Methods**

124   Written approval for this pilot study was granted by the Northern Y Regional Ethics
125 Committee (New Zealand), study reference NTY/11/11/109.

126   Study volunteers with Gleason score 6 − 7 (3+3 or 3+4) who had participated in an earlier
127   study investigating DNA damage and genotypes (Karunasinghe et al., 2012)were invited to enrol
128   in this dietary intervention. Men with lower Gleason scores were selected preferentially to
129   minimise the possible confounding effect of advanced disease. Other criteria for inclusion were:
130   under 75 years of age, no diagnosis of diabetes, no evidence of progressionand not currently
131   receiving treatment for prostate cancer (hormonal therapyexcepted), and with no history of other
132   cancer except treated skin carcinomas.
133   The dietary guidelines were in accordance with general Mediterranean patterns as per a
134   validated tool (Martinez-Gonzalez et al., 2012) with specific adaptations.
135   Men were given nutritional counselling at enrolmentby nutritional specialistson the study
136   team and coached regarding the dietary guidelines. Both hard and soft copies of a comprehensive
137   compilation of recipes incorporating the main foods and principles of the diet were provided.
138   Additional support and clarification was provided on an as-needed basis. As a goal of this study
139   was to determine the degree of acceptance of this pattern of eating in the lifestyle of New
140   Zealand men, the intervention was based around general principles with specific
141   recommendations, as summarised in Table 1, rather than a prescriptive diet.
142   All participants were provided withNew Zealand-produced extra virgin olive oil with a
143   polyphenol level of 233 mg/kg (1 L/month), fresh frozen salmon (200 g/week), unsweetened
144   pure pomegranate juice (1 L/week) and samples of a variety of canned legumes. These were
145   donatedby New Zealand distributors: Oil Seed Extractions Ltd., Ashburton;Aoraki Smokehouse
146   Salmon, Twizel; Life Juices, Auckland, and Delmaine Fine Foods, Auckland, respectively, and
147   given to the volunteers at enrolment.
148   The validated 14-point Mediterranean diet adherence questionnaire published by Martinez-
149   Gonzalez(Martinez-Gonzalez et al., 2012)was adapted to be consistent with the
150   recommendations. Thequestionnaire was completed bystudy participants, along with four-day
151   diet diaries, at the beginning and conclusion of the study (Table 2). Adherence questionnaires
152   were scored out of 20. Serving sizes were estimated and recorded both in diet diaries and the
153   dietary adherence questionnaire.Diet diaries were analysed using FoodWorks®7 (Xyris 2012,
154   Xyris Software (Australia)Pty Ltd, Brisbane, Australia).
155   Blood samples were collected at baseline, and again at three months of follow-up, (plain,
156   EDTA, Heparin and SST II Advance Vaccutainers), and processed within 2 hours of collection.
157   C-reactive protein and PSA were tested using enzyme immunoassaysperformed by LabTests,
158   Auckland, New Zealand.
159   Comet assays were performed on both fresh blood and hydrogen-peroxide challenged samples
160   as described by Karunasingheet aland Fergusonet al(Karunasinghe et al., 2004; Ferguson et al.,
161   2010). As the data were skewed, figures for percentage tail DNA were log-transformed and the
162   back-transformed mean was used for the analysis.

163   Statistical evaluation was performed using SAS (v9.2 SAS Institute, Cary, NC, USA).
164   Correlations were examined using Spearman's rho. The Student's paired *t*-Test was used to
165   evaluate differences between baseline and study-end.

166   **Results**

167   A total of twenty-eight men were enrolled in the study. While we sought to enrol men on
168   active surveillance protocols, a low response rate resulted in the recruitment of those who had
169   received treatment for PCa but within the Gleason score range specified.Eight men were not
170   included in the final analysis: four were lost to follow-up for a variety of reasons, two dropped
171   out of the study due to life stresses, one had difficulty conforming to the diet and one was
172   eliminated due to unreliability of dietary information provided. The characteristics for the
173   remaining twenty participants are presented in Table 3.
174   Data on tumour staging was available for half of the cohort. Using the Tumour (T), Node (N),
175   Metastasis (M) grading system, grades were T1-T3, N0 and M0.
176   Of the twenty participants in the final analysis, none were current smokers. Mean time since
177   smoking cessation was 31 years (SD=13). Past history of smoking of less than one pack-year
178   was regarded as a "never-smoker". Pack-year history was positively associated with the use of
179   Aspirin and/or Diclofenac (*p*=0.007).
180   Over the course of the study two men ceased taking and one commenced low dose
181   Aspirin.Another participant discontinued use of Diclofenac during the study period.
182   Of this somewhat sedentary group of men, 70% were overweight or obese (BMI >25 kg/m^2).
183   Mean body weight reduced by 2.3 kg (95% CI 1.11–3.49, *p*<0.001) over the course of the
184   study.There was a mean reduction in body mass index of 0.85 kg/m^2 (95% CI 0.52–1.18,
185   *p*<0.001).
186   Men who were less active tended to have higher levels of C-reactive protein (*p*=0.003).This
187   association remained at study end, albeit slightly weaker (*p*=0.055).
188   At baseline, dietary scores for the targeted Mediterranean-style pattern were low.Mean
189   adherence was 6.3 (SE 0.69), with individual scores ranging from 2 to 13 (of a maximum of
190   20).At three months of follow-up, mean adherence was 13.63 (SE 0.49), range 9 to 17.The mean
191   change in dietary adherence from baseline to study end was +7.33 (95% CI 5.76–8.89), which
192   was highly significant (*p* <0.001).
193   There were no statistically significant relationships between dietary adherence and either C-
194   reactive protein or PSA at either baseline or three months.
195   Improvements were noted in all areas evaluated on the adherence questionnaire, with the
196   exception of servings of fruit, vegetables, use of *sofrito*(tomato-based sauce prepared with garlic
197   and/or onion), the intake of sweetened beverages, and wine. Pooled group adherence scores are
198   presented in Table 4.

199   Estimated energy requirements and reported energy intakewere calculated using
200   FoodWorks®7 software, from recorded body weights, reported energy expenditure and diet
201   diaries (Table 5). There was a tendency to under-report energy intake. This was not statistically
202   significant at baseline (SE 280, 95% CI 108–1063, $p=0.10$). At the end of the study period, the
203   difference reached statistical significance, with reported energy intake a mean 720 kilojoules
204   lower than estimated requirements (SE 194, 95% CI 314–1125, $p=0.007$).
205   Energy obtained from saturated fat, as % of total energy intake, decreased significantly
206   ($p<0.001$). Other sources of energy did not alter significantly over the course of the study
207   (Figure 2).
208   Increases in intake of broccoli, *sofrito*, and pomegranate juice were statistically significant, as
209   was a decrease in refined carbohydrate intake (per reported intakes of sweetened beverages and
210   baked goods) (Table 6). The reduction in carbohydrate intake was not significant. No change was
211   observed in regards to intake of fruit, vegetables, dietary fibre, or total sugar. It was apparent that
212   the source of dietary sugars shifted away from sucrose and lactose, towards fructose and glucose.
213   Participants significantly reduced their consumption of red meat ($p< 0.001$), and increased
214   their intake of fish ($p< 0.001$), and legumes ($p = 0.005$), with no net change in protein
215   intake(Table 7).
216   Alterations in dietary fats and associated relationships are discussed in detail in Bishop, et
217   al(Bishop et al., 2015).
218   Reductions in DNA damage were noted after three months of the dietary intervention. This
219   did not reach significance for basal (fresh-blood) DNA damage ($p=0.075$), but was highly
220   significant for peroxide-induced DNA damage ($p=0.009$).
221   Spearman bivariate correlation was used to identify relationships between DNA damage at
222   study end and intake of the items specified on the adherence questionnaire and data generated
223   from diet diaries. Overall, following the dietary pattern was inversely associated with DNA
224   damage ($p= 0.013$). DNA damage was also inversely associated with consumption of green tea
225   and intake of legumes ($p = 0.002$ and $p = 0.004$ respectively), and positively associated with red
226   meat intake ($p= 0.007$). Intake of dairy products and margarine/butter/cream was also correlated
227   with DNA damage at study end. These results are discussed in Bishopet al.(Bishop et al., 2015).
228   No significant relationships were noted between DNA damage and vegetable, fruit, or
229   pomegranate intake.
230   An inverse association between DNA damage and vitamin C intake was apparent. This was
231   weak at baseline ($p=0.098$), and became significant at studyend ($p=0.007$). Dietary folate intake
232   at three months was inversely associated with hydrogen peroxide-induced DNA damage
233   ($p=0.023$). Vitamin E intake, which increased significantly (Table 4), was inversely associated
234   with both basal and peroxide-induced DNA damage at the end of the study. However, with *p*-
235   values of 0.175 for each, these did not attain statistical significance.

236   There were no significant relationships between C-reactive protein, PSA and DNA damage.A

237   non-significant trend towards a correlation between C-reactive protein and peroxide-induced

238   DNA damage was observed (p=0.156 and p=0.223 at baseline and three months, respectively).

239   **Discussion**

240   The primary goal of this pilot study was to establish both feasibility and likelihood of benefit,

241   as determined by a reduction in inflammation and DNA damage, for New Zealand men with

242   prostate cancer following a modified Mediterranean diet. We sought to enrol men with untreated

243   prostate cancer; however, due to low numbers of volunteers, men with low Gleason (3 + 3 or 3 +

244   4) who had previously had treatment were included. It was anticipated that as our subjects had

245   volunteered to participate in this intervention, they would be motivated, and thus amenable to

246   changes that might be seen as advantageous in delaying disease progression. This was observed,

247   and is consistent with what has been previously demonstrated in regards to cancer, motivation

248   and dietary changes (Allen et al., 2008; Avery et al., 2013). It was anticipated that the dietary

249   intervention would have demonstrable effects on DNA damage,PSA and C-reactive protein.

250   Adherence to a low inflammatory diet such as that used in this study may help to mitigate

251   inflammation-associated increases in oxidative stress, genomic instability and damage to DNA

252   (Kundu & Surh, 2012).

253   Inflammation was evaluated by measuring C-reactive protein,a non-specific acute phase

254   protein that serves as a surrogate for interleukin 6, an inflammatory cytokine associated with

255   angiogenesis, tumour growth and metastases. Within 6 hours of an inflammatory assault, C-

256   reactive protein increases. Constant levels are maintained commensurate with inflammatory

257   processes and rapid clearance occurs when the stimulus is removed (Pepys & Hirschfield, 2003).

258   No change in C-reactive protein was observed in our cohort afterthree months of the dietary

259   intervention, which is in contrast to other studies using a similar dietary regimen (Marlow et al.,

260   2013; Ellett et al., 2013). Noteworthy in this regard is that baseline C-reactive protein in this

261   group was low, with 95% of the cohort within the normal range of <5 mg/L, leaving little room

262   for improvement. An inverse association between C-reactive protein and physical activity levels

263   that was noted at the outset of this study was not evident at study end. Medication did not change

264   in the group, with the exception of one participant who ceased taking non-steroidal anti-

265   inflammatory drugs during the study period. There was no resultant increase in C-reactive

266   protein in this participant.

267   The effect of excess adiposity is an important consideration on the results seen. Obesity is a

268   chronic, low-grade inflammatory state, which has been associated with both incidence and

269   progression of prostate cancer (Ho et al., 2012). It was expected that study participants,four of

270   whom were obese at the beginning of the study, might lose weight by following the dietary

271   recommendations, (Martinez-Gonzalez et al., 2012). This did occur(mean weight loss2.3 kg),

272 and while desirable in overweight men, may have masked anti-inflammatory attributes of the
273 diet. When stored adipose tissue is catabolised, the pro-inflammatory omega-6 fatty acid,
274 arachidonic acid, tends to be liberated (Phinney et al., 1991). Thus true benefit in terms of
275 lowering of inflammation might be better observed once body weight has stabilised. This would
276 be best assessed following a longer intervention. No significant associations between body
277 weight, BMI (or changes thereof) and DNA damage were noted in this study.C-reactive protein
278 responds to a number of factors. Participants were not evaluated for injury or opportunistic
279 infection at either the beginning or end of the study. While men retrospectively reported that they
280 were "well" at the time of both blood draws, minor injuries or low-grade infections have the
281 potential to increase acute phase inflammatory markers, C-reactive protein included. Similarly,
282 study participants were not assessed for other factors that might impact C-reactive protein, such
283 as sleep disturbances (Meier-Ewert et al., 2004)and food intake the day of sample
284 collection(Margioris, 2009; Farnetti et al., 2011).
285   Levels of PSA were largely unchanged over the course of the study. However, PSA was
286 below the level of detection in the majority of study participants, asis associated with successful
287 treatment of prostate cancer. The lack of change in PSA, particularly in those men who had not
288 undergone treatment for prostate cancer (n=6), may indicate benefit in terms of PSA doubling
289 time. While this is best evaluated over a longer period of time, mitigation of PSA increases have
290 been demonstrated after 3 months of a dietary shift towards a more plant-based pattern (Saxe et
291 al., 2008).
292   There are many individual components of the Mediterranean diet that have been studied in
293 regards to their effect on a number of health outcomes.The health advantages of a diet that is
294 high in fruit and vegetables, ostensibly due to the diversity of nutrients, with high levels of
295 antioxidants and fibre associated with such dietary patterns is generally accepted.Indeed, lower
296 levels of inflammation and increases in antioxidants have been correlated to fruit and vegetable
297 intake (Root et al., 2012).While not all studies concur (Ambrosini et al., 2007; Boffetta et al.,
298 2010), there is evidence suggesting benefit from vegetable and fruit intake in regards to prostate
299 cancer (Riso et al., 1999; Hardin, Cheng & Witte, 2011; Shahar et al., 2011).
300   The main benefit (weight loss aside) associated with this dietary intervention was reduction in
301 DNA damage after three months, when compared to baseline data. This outcome was inversely
302 associated with dietary adherence(p=0.013).
303   Three months is considered sufficient time to determine the impact of diet on DNA repair in
304 lymphocytes.As part of the circulatory system lymphocytes are constantly exposed to the
305 positive and negative effects of diet and lifestyle.Therefore, they are an ideal target cell to assess
306 the nutritional or chemical effect on DNA damage, regardless of their age.Other studies have
307 demonstrated the effect of dietary on DNA repair in lymphocytes in as little as 21 (Riso et al.,
308 1999)and 24 days(Guarnieri et al., 2008).

309   DNA damage has been positively associated with prostate cancer risk(Lockett et al., 2006),
310   hence increased DNA protection and repair is a highly desirable outcome, further supporting the
311   benefit of a diet high in antioxidants and low in saturated fat. Specific foods and nutrients,
312   particularly antioxidants and polyphenol compounds, can positively affect DNA repair(Duthie et
313   al., 1996; Giovannelli et al., 2002; Machowetz et al., 2007; Guarnieri et al., 2008). For example,
314   consumption of green tea (Kanwal et al., 2014), broccoli (Riso et al., 2010) and vitamin C
315   intake(Fraga et al., 1991) have been associated with increased DNA repair in previous studies,
316   while increases in DNA damage have been attributed to oxidative stress (Freitas et al., 2012;
317   Kundu & Surh, 2012) and peroxidation of fatty acids (Gropper, Stepnick & Smith, 2013). The
318   benefit of a Mediterranean diet on markers of DNA damage has been reported in women with the
319   metabolic syndrome (Mitjavila et al., 2013). As far as the authors are aware, this has not
320   previously been reported in men with prostate cancer.
321   Diet is a complex interaction of a wide range of foods and numerous individual compounds.
322   Genetic and epigenetic modifications can be affected by dietary phytonutrients, which modulate
323   DNA methylation and may induce or enhance DNA repair, the isolation of these compounds is a
324   commonly used, but reductionist approach, to nutritional research. In this study, dietary
325   adherence scores informed a comparison of the adoption of the diet as a whole, as well as the
326   integration of various aspects of it. The aspects of this diet that study participants found the most
327   acceptable were the incorporation of whole grains, olive oil, pomegranate juice, substitution of
328   red meat for chicken, and reducing consumption of sweetened beverages. Each of these achieved
329   >85% compliance overall. On the other hand, the least embraced components were inclusion of
330   *sofrito,* green tea, vegetables, broccoli, and adoption of recommended guidelines for alcohol (in
331   particular red wine). While compliance on these latter items was less than 60%, changes in most
332   did reach statistical significance (Tables 4, 6and7).
333   It is apparent from the inverse association of adherence to the recommended diet with DNA
334   damage that this overall dietary pattern could be of benefit for men with prostate cancer. While
335   the possibility exists that reduced damage to DNA may be attributable to other characteristics of
336   this cohort, no parameter reported here suggests that this was the case. Other factors associated
337   with the Mediterranean diet, when compared to a typical western diet, include alterations in the
338   type of dietary fat and resultant changes in blood fatty acids. Such changes did occur in this
339   study, as is evident by the decrease in energy from saturated fat (Figure 2). Relationships
340   between DNA damage, dietary fats and blood fatty acids are explored elsewhere (Bishop et al.,
341   2015).
342   An inquiry into relationships between individual food items and the benefits seen on this diet
343   aids in justification of continued inclusion or otherwise in future studies. Such data is particularly
344   useful when recommending the incorporation of foods that may be considered unusual and to
345   support the development of strategies to aid increased compliance with less accepted
346   recommendations.

347   While participants indicated that they were more inclined to choose chicken over red meat,
348   overall complicity to the criteria for restriction of red meat consumption (to less than once
349   weekly) did not indicate that this had actually occurred. In general, New Zealand consumption of
350   beef and lamb is high (FAO, 2013), which is reflected in the baseline data.At the beginning of
351   the study participants were consuming almost 4 servings of red and/or processed meat per
352   week.Hence, while the reduction noted was significant ($p<0.001$), intake was still higher than
353   requested, suggesting that minimising red meat was a difficult change for participants to
354   make.This may have been caused or compounded by the fact that this study was conducted over
355   the summer period.The New Zealand summer coincides with the festive season, during which
356   barbeques, as social events, are a common aspect of the culture.Thus avoidance of red meat may
357   have been challenging for some participants.If indeed this was a major factor, it also raises the
358   possibility that consumption of pro-carcinogenic heterocyclic amines, as is associated with meat
359   cooked at high temperatures (Norrish et al., 1999), may have negated some of the benefits of the
360   overall diet.
361   Green tea was not well accepted, with palatability being the reported obstacle. Nevertheless,
362   green tea intake was associated with significant reduction in DNA damage ($p=0.002$). This is in
363   alignment with the remarkable antioxidant and anti-inflammatory properties associated with
364   green tea, which contribute to its effect on DNA methylation (Julie & Tim, 2012). Green tea
365   consumption was reported by 25% of participants at baseline and 60% at study end. However,
366   less than half were consuming the recommended intake of 2 cups daily. This relatively low
367   uptake, but notable benefit, suggests that the incorporation of a beneficial dietary component
368   may result in substantial gains, even if target levels are not attained.
369   No associations were noted for broccoli intake, which is in contrast to other reports (Latté,
370   Appel & Lampen, 2011; Ferguson & Schlothauer, 2012). However, the inverse correlation noted
371   between folate intake and DNA damage is consistent with current understanding of the role of
372   folate in DNA methylation (Gropper, Stepnick & Smith, 2013), and concurs with a recent study
373   in which folate's role in DNA stability was demonstrated (Ong, Moreno & Ross, 2011). In the
374   current study, folate intake was calculated from diet diaries, and broccoli intake was determined
375   from an adherence questionnaire, which was not sensitive to other sources of either folate or
376   other cruciferous vegetables.
377   The role of vitamin C in regards to cancer is conflicting (Fraga et al., 1991; Herbert et al.,
378   2006) making the finding of this study difficult to compare with other research. A dose-response
379   relationship has been observed in regards to protection from DNA damage, with increasing
380   vitamin C benefiting those with baseline low levels (Herbert et al., 2006; Freitas et al., 2012). In
381   our study a significant inverse association was apparent between vitamin C intake and DNA
382   damage ($p=0.007$) at study end. Dietary vitamin C in the cohort was not low (Table 6).
383   Examination of the data indicated that the source of this water soluble antioxidant shifted from
384   fruit at baseline to vegetables at the end of the study.However, overall vegetable intake did not

385   change.Furthermore, vegetables are more likely to be consumed cooked, which reduces vitamin

386   C content(Gropper, Stepnick & Smith, 2013).Together, this suggests that vitamin C was not

387   solely associated with the effect noted, but rather, that a nutrient-nutrient interaction, such as

388   with vitamin E, may have occurred.Vitamin E intake increased over the course of the study

389   (p=0.005) (Table 6).This is congruent with current understanding of the synergistic relationship

390   that exists between these two antioxidant vitamins (Gropper, Stepnick & Smith, 2013). A large

391   prospective study by Wrightet al.(Wright et al., 2007) suggested that dietary (but not

392   supplemental) vitamin E may be an important nutrient in reducing the risk of advanced prostate

393   cancer.

394   Diet is a complex interaction of phytonutrients and it is an accumulative, and/or synergistic

395   effect of these that confers overall benefit.In other words, overall dietary patterns are possibly

396   more important than individual components – a concept that has been proposed elsewhere

397   (Viscogliosi et al., 2013).

398   From the results, it appears that the adoption of such a dietary pattern is feasible in motivated

399   men. Not only were the changes, for the most part, embraced, but feedback from individual

400   participants was encouragingly positive. While not part of the study design, at the end of the

401   study and 3 months later, informal feedback was gathered from the participants. The information

402   provided indicated that participants were heartened by their weight loss and the majority

403   continued with the new diet. Study participants also reported improvements in overall energy and

404   well-being, as well as positive effects on a range of other factors, including serum cholesterol,

405   arthritic pain, and nocturia. Formal collection and evaluation of such data in future studies would

406   contribute to strategies for supporting dietary change in men with prostate cancer.

407   The ethnic homogeneity of the group in the current study meant that the influence of

408   variances in genetic expression was reduced. However, a number of other confounders, including

409   genetic influences, were not controlled for as this would have rendered the group too small for

410   meaningful analysis. The authors recognise the importance of gene-diet interactions and

411   acknowledge that any testing related to genetic expression requires a larger study to demonstrate

412   relevance. Despite the fact that this was a small feasibility study, positive outcomes were noted

413   within a short time frame. In order to apply the findings to a general population, it is necessary to

414   confirm the results with a largercohort. Such a study would allow stratification to control for a

415   variety of confounding factors, such asadiposity, baseline dietary patterns, co-morbidities and

416   lifestyle habits. The inclusion of a control group of men without prostate cancer would also be

417   advantageous.

418   **Conclusions**

419   We demonstrated that dietary change towards a Mediterranean-style pattern is both achievable

420   and beneficial for men with prostate cancer in New Zealand, albeit in a small and motivated

421 group. While there have been numerous studies evaluating the impact of a Mediterranean diet on
422 clinical indicators of disease (Giovannelli et al., 2002; Menendez & Lupu, 2006; Fernández-Real
423 et al., 2012; Kontogianni et al., 2012; Marlow et al., 2013; Mitjavila et al., 2013; Viscogliosi et
424 al., 2013; Ellett et al., 2013) including prostate cancer(Ferrís-Tortajada et al., 2012; Kenfield et
425 al., 2014), we believe this is the first time such a study has been conducted in regards to prostate
426 cancer in New Zealand.This pilotstudy shows that a holistic approach to diet may contribute to
427 modulation of DNA damage in spite of low baseline levels of inflammation. This is quite
428 possibly due to an undeterminable synergistic effect of dietary components and associated
429 phytonutrients.
430   Reduction in DNA damage was significantly associated with overall conformity to the general
431 dietary pattern as well as intake of green tea, legumes, dietary vitamin C and folate. While no
432 effect on inflammatory markers was demonstrated, baseline inflammation in this small cohort
433 was low. Nonetheless, the results obtained add weight to the notion that a low-inflammatory,
434 high antioxidant diet may be of benefit for men with prostate cancer.
435   Certain aspects of the diet were more acceptable to participants than others. An exploration of
436 the challenges faced in integration of specific dietary components would inform strategies to
437 encourage ongoing compliance, and ultimately, long-term benefit for men with prostate cancer,
438 including those with more advanced disease.

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446 **Author Contributions**

447   KSB planned and initiated the study. SE and KSB carried out the study, planned, and edited
448 the manuscript.SE carried out the Comet assays and wrote the manuscript.SE and NK interpreted
449 the resultsand edited drafts of the manuscript.DYH carried out the statistical analysis.LRFedited
450 drafts of the manuscript.

451 **Conflicts of Interest**

452 The authors declare no conflict of interest. Companies who donated food items for the study
453 were not involved (financially or otherwise) in the study design, approval, writing or publication
454 of the manuscript.

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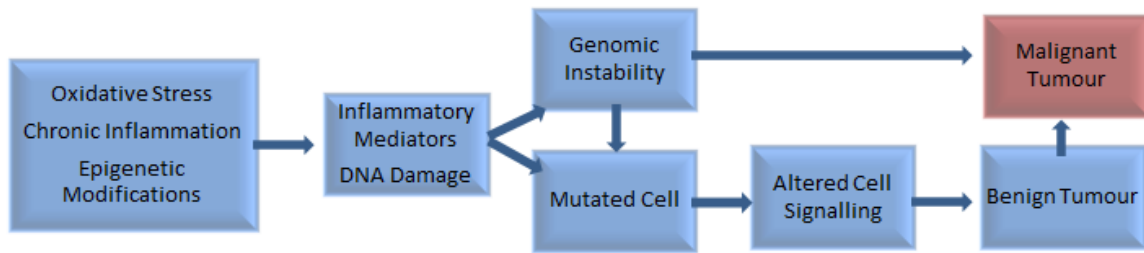

A brief outline of food items/groups that study-participants were asked to consume or avoid.

*Unless contraindicated due to health problems, allergies or intolerances **Preferred items

3   Table 1. A brief outline of food items/groups that study-participants were asked to consume or

4   avoid.

Foods to consume	Recommendation*	Foods to avoid/limit	Quantity
Oily fish	⩾2 servings weekly	Red meat	≤1 serving weekly
Olive oil	⩾1 tblsp daily	Butter (high fat dairy)	Avoid/reduce
Chicken (skinless)	≤2 servings weekly	Processed meats	Avoid
Fresh vegetables (broccoli**, dark leafy vegetables, cooked tomatoes, sweet potato & salads)	⩾5 servings daily	Eggs	1 - 4 per week
Whole grains	Daily as required	Fruit (berries**)	Up to 2 portions daily
Red wine	≤2 glasses daily	Refined foods (white bread, crisps, pies)	Reduce or avoid
Green tea	≥2 cups daily	Other alcohol	Avoid
Pomegranate juice	150 mL daily	Fruit juice	Avoid
Fats and oils (avocado, olives, nuts and seeds)	Daily as required	Sugar (biscuits, cakes, sweets)	Avoid

5   *Unless contraindicated due to health problems, allergies or intolerances

6   **Preferred items

Dietary Adherence Questionnaire

Adapted from Martinez-Gonzalez, M. A., Garcia-Arellano, A., Toledo, E., Salas-Salvado, J., Buil-Cosiales, P., Corella, D., . . . Investigators, P. S. (2012). A 14-item Mediterranean diet assessment tool and obesity indexes among high-risk subjects: the PREDIMED trial. PLoS ONE [Electronic Resource], 7(8), e43134. (Martinez-Gonzalez et al., 2012)

2   Table 2. Dietary Adherence Questionnaire

3   Assessing Your "Mediterranean Diet"

4   Please indicate your answers to the questions in the space provided.

Question		**Answer**
1.	Do you use olive oil as a culinary fat?	
2.	How much olive oil do you use in a given day (including oil used for frying, salads, out-of-house meals, etc.)?	
3.	How many vegetable servings do you consume per day? **Cooked:** (1 serving : ½ cup cooked vegetables or 1 cup raw vegetables) [consider side-dishes as half a serving] **Raw / salad:**	
4.	How many fruit units (including natural fruit juices) do you consume per day?	
5.	How many servings of red meat, hamburger, or meat products (ham, sausage, etc.) do you consume per week? (1 serving : 100 - 150 g)	
6.	How many servings of butter, margarine or cream do you consume per day? (1 serving : 12 g / approx. 2 tsp)	
7.	How many sweet or carbonated beverages do you drink per day?	
8.	How much wine do you drink per week?	
9.	How many servings of legumes do you consume per week? (1 serving : 150 g)	
10.	How many servings of fish or shellfish do you consume per week? (1 serving : 100 - 150 g of fish, or 200 g / 4 - 5 units of shellfish)	
11.	How many times per week do you consume sweets or pastries, such as cakes, cookies, biscuits or custard?	
12.	How many servings of nuts (including peanuts) do you consume per week? (1 serving : 30 g)	
13.	Do you preferentially consume chicken, turkey or rabbit meat instead of veal, pork, hamburger or sausage?	
14.	How many times per week do you consume vegetables, pasta, rice or other dishes seasoned with sofrito (sauce made with tomato and onion, leek or garlic and simmered with olive oil)?	
15.	How many servings of pomegranate fruit (1 serving : 1 piece), pomegranate juice (1 serving : 200 mL), or of pomegranate molasses (1 serving : ½ tblsp) do you have per day?	
16.	How much alcohol (other than wine) do you drink per week? (1 serving : 1 nip spirits, 300 mL beer)	
17.	How many cups of green tea do you drink per day?	
18.	How many servings of broccoli do you consume per week?	
19.	How many servings of dairy products do you have per week? (1 serving : 30 g cheese, 250 mL milk, 100 mL yoghurt)	
20.	Do you preferentially use wholegrain bread and crackers instead of white / refined bread and crackers?	

5   Adapted from Martinez-Gonzalez, M. A., Garcia-Arellano, A., Toledo, E., Salas-Salvado, J., Buil-Cosiales, P., Corella, D., . . . Investigators, P. S.
6   (2012). A 14-item Mediterranean diet assessment tool and obesity indexes among high-risk subjects: the PREDIMED trial. *PLoS ONE [Electronic*
7   *Resource], 7*(8), e43134. (Martinez-Gonzalez et al., 2012)

Characteristics of study participants at baseline.

2   **Table 3.** Characteristics of study participants at baseline.

Characteristic	*n*	Characteristic	*n*
Age 52–74 Years		BMI 23–33 kg/m^2	
50–59	3	≥20–≤25	4
60–69	12	>25–≤30	12
>70	5	>30	4
Marital Status		Family History of PCa	
Married or with Partner	17	1° relative with PCa	3
Single or Widowed	3	Other relative with PCa	2
Ethnicity		Gleason Score	
Caucasian	20	3 + 3	14
Smoking Status		3 + 4	6
Never	7	Time since diagnosis (years)	1-10
Former	13	Treatment type	
Current	0	None	6
Activity Level *		Prostatectomy	10
Heavy	1	Prostatectomy + Hormones + DxR	1
Moderate	2	Prostatectomy + DxR	1
Light-Moderate	1	Hormones + DxR	1
Light	5	Brachytherapy	1
Sedentary	10	Medication	
Very Sedentary	1	Statins	6
Alcohol Intake		Aspirin	5
Non-Drinkers	4	Diclofenac	4
≤2 Standard Drinks Per Day	10	Anti-hypertensive	4
>2 Standard Drinks Per Day	6	TNF-antagonist	1
PSA (mean, µg/L)	1.53	Allopurinol	1
CRP (mean, mg/L)	1.55	Other	6

3   BMI, body mass index; 1°, first degree; PCa, prostate cancer; DxR, radiotherapy; PSA, prostate-
4   specific antigen; CRP, C-reactive protein; * Physical activity level as defined by FoodWorks®7.
5

Pooled dietary adherence scores from 20 participants at baseline and three months. A maximum of 1 point for each item per participant was possible. 20 points reflects complete adherence by the whole cohort.

* Half-points were allocated wherever a shift towards improved adherence of ≥30% was

evident

tblsp, tablespoon; incl, including; excl, excluding.

2   **Table** 4 Pooled dietary adherence scores from 20 participants at baseline and three months. A

3   maximum of 1 point for each item per participant was possible. 20 points reflects complete adherence

4   by the whole cohort.

Dietary Component	Criteria for One Point	Baseline	Three Months *	p
1. Olive Oil As Culinary Fat	Yes	13	20.0	**0.021**
2. Olive Oil Used Daily	≥1 tblsp	8	17.0	**0.003**
3. Servings of Vegetables/Day	≥4	6	8.5	0.0563
4. Servings of Fruit/Day (Incl. Pomegranate)	≤2	9	11.5	0.449
5. Servings of Red Meat, Hamburger, *etc.*/Week	<1	1	11.5	**<0.001**
6. Servings of Butter, Margarine or Cream/Day	<1	3	14.5	**<0.001**
7. Sweet or Carbonated Beverages/Day	<1	16	20.0	0.083
8. Wine Consumed/Week (Glasses)	7–14	5	5.5	0.577
9. Servings of Legumes/Week	≥5	6	16.0	**<0.001**
10. Servings of Fish or Shellfish/Week	≥3	4	14.0	**<0.001**
11. Sweets or Pastries/Week	<3	7	16.0	**<0.001**
12. Servings of Nuts/Week	≥5	5	16.0	**<0.001**
13. Preference for Chicken, *etc.*	Yes	7	18.0	**<0.001**
14. Use of *Sofrito* Sauce/Week	≥2	6	9.5	0.297
15. Servings of Pomegranate/Day	≥1	1	19.5	**<0.001**
16. Units of Other Alcohol (Excl. Wine)/Week	0	5	7.5	**0.025**
17. Cups of Green Tea/Day	≥2	2	9.0	**0.008**
18. Servings of Broccoli/Week	≥5	0	6.5	**0.004**
19. Servings of Dairy Products/Week	≤5	5	13.0	**0.003**
20. Use of Whole Grains	Yes	16	20.0	**0.042**

5   * Half-points were allocated wherever a shift towards improved adherence of ≥30% was evident;

6   tblsp, tablespoon; incl, including; excl, excluding.

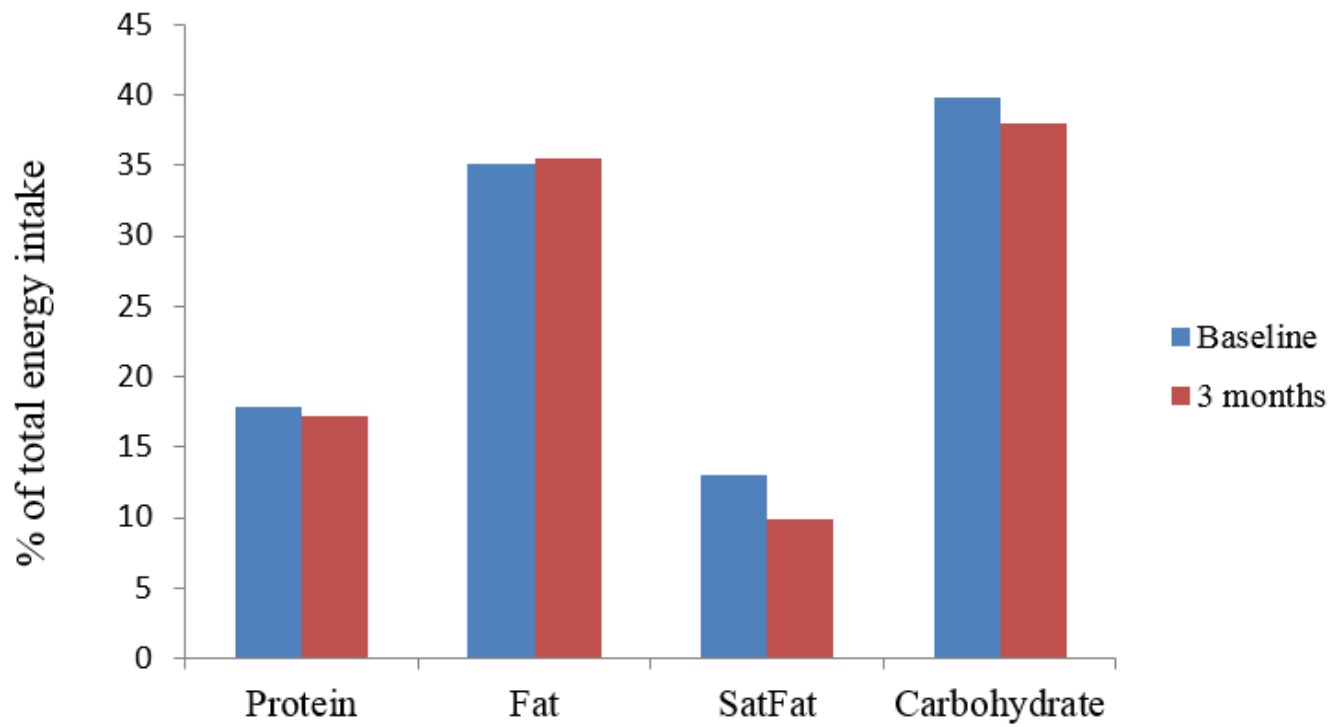
Comparison of estimated energy requirements and reported energy intake.

2   **Table** 5. Comparison of estimated energy requirements and reported energy intake.

Energy Intake	Baseline Mean (SE)	Three Months Mean (SE)	Mean Difference (95% CI)	*p-value*
EER /kJ	10,675.6 (305.43)	10,476.05 (292.00)	−199.6 (−427.40–28.27)	0.083
Reported energy intake /kJ	10,197.7 (341.64)	9,756.43 (267.09)	−441.3 (−970.60–88.05)	0.098

3   EER, estimated energy requirement; kJ, kilojoules; SE, standard error; CI, confidence interval.

Change in sources of energy expressed as % of total energy intake at baseline and three months.

SatFat = saturated fat; * p < 0.001.

Changes in intake of dietary items and nutrients, from baseline to three months.

2   **Table 6.** Changes in intake of dietary items and nutrients, from baseline to three months.

Dietary Component	Baseline Mean (SE)	3 Months Mean (SE)	Mean Difference (95% CI)	*p*
Carbohydrate (total) (g/day)	246.53 (11.20)	234.91 (11.00)	−11.63 (−30.47–7.22)	0.212
Dietary fibre (total) (g/day)	31.23 (1.86)	32.28 (1.60)	1.04 (−1.83–3.92)	0.456
Sugar (total) (g/day)	108.14 (7.90)	110.86 (7.96)	2.72 (−11.32–16.75)	0.690
Glucose (g/day)	19.00 (2.28)	32.30 (2.97)	13.22 (7.30–19.20)	**<0.001**
Fructose (g/day)	20.80 (2.40)	28.80 (2.80)	8.55 (2.80–14.30)	**0.006**
Sucrose (g/day)	35.30 (4.80)	24.8(3.50)	−10.56 (−17.75–1.37)	**0.026**
Lactose (g/day)	12.80 (1.53)	6.53 (1.17)	−6.28 (−9.70–2.80)	**0.001**
Folate (total) (µg/day)	537.00 (43.50)	564.00 (40.40)	27.00 (−31.00–85.40)	0.340
Vitamin C (mg/day)	133.60 (13.20)	169.40 (22.40)	35.90 (−1.03–72.80)	0.056
Vitamin E (mg/day)	18.60 (4.90)	26.53 (5.46)	7.94 (3.18–12.70)	**0.005**
Vegetables (servings/day)	2.80 (0.28)	2.63(0.31)	−0.18(−1.13–0.78)	0.705
Broccoli (servings/week)	1.58 (0.27)	2.42 (0.45)	0.84 (−0.19–1.49)	**0.014**
Sofrito sauce (servings/week)	1.53 (0.45)	2.40 (0.47)	0.88 (0.28–1.47)	**0.006**
Fruit * (servings/day)	2.78 (0.49)	2.50 (0.31)	−0.28 (−1.01–0.46)	0.440
Pomegranate (servings/day)	0.05 (0.05)	1.28 (0.16)	1.23 (0.86–1.59)	**<0.001**
Sweetened beverages (servings/week)	0.51 (0.20)	0.15 (0.06)	−0.35 (−0.70–0.01)	**0.046**
Cakes and biscuits (servings/week)	4.38 (1.01)	2.05 (0.55)	−2.33 (−3.82–0.83)	**0.004**
Green tea (cups/day)	0.35 (0.17)	1.13 (0.30)	0.78 (−0.28–1.84)	**0.004**

3   g, grams; SE, standard error; CI, confidence interval; * All fruit and fruit juice, including pomegranate.

Changes in protein intake from baseline to three months.

2   **Table 7.** Changes in protein intake from baseline to three months.

Dietary Component	Baseline Mean (SE)	3 Months Mean (SE)	Mean Difference (95% CI)	p
Protein (g/day)	106.73 (5.52)	99.49 (4.99)	−7.24 (−17.32-2.85)	0.149
Red & processed meat (servings/week)	3.89 (0.48)	1.94 (0.36)	−1.95 (−2.59-1.32)	**<0.001**
Fish (servings/week)	1.65 (0.20)	3.48 (0.46)	1.83 (0.91-2.74)	**<0.001**
Legumes (servings/week)	2.37 (0.58)	3.78 (0.46)	1.41 (0.48-2.34)	**0.005**

3   SE, standard error: CI, confidence interval.