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FOOD & FUNCTION

Suppression of DNA damage in human peripheral blood lymphocytes by a juice concentrate: A randomized, double-blind, placebo-controlled trial

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Chronic inflammation contributes to many prevalent diseases worldwide, and it is widely accepted that inflammatory molecules contribute to DNA damage. In this ancillary study, we investigated the influence of an encapsulated fruit and vegetable juice powder concentrate on peripheral blood lymphocytes (PBL) DNA damage. Using a double-blind, placebo-controlled approach, subjects were randomly assigned capsules containing placebo, or one of two formulations of the juice powder. Blood was drawn at baseline and after 60 days of capsule consumption. We found DNA damage in isolated PBL is suppressed after consumption of the encapsulated juice powder, and damage was correlated with the level of systemic inflammation. These data suggest a potential health benefit by consuming the juice concentrate capsules through their ability to suppress DNA damage as measured in surrogate tissues (PBL).

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Chronic inflammation contributes to prevalent diseases worldwide, and reduces human lifespan [1]. Therefore, mechanisms of suppressing chronic inflammation would likely be beneficial to human health. The ability of fruits and vegetables to suppress chronic inflammation [2] and protect against many chronic diseases is well documented [3]. However, changing dietary habits remain a challenge, and therefore, it is reasonable to identify nutritional supplements that are readily available in capsules and consumed with little effort.

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Abbreviations: **CI**, confidence interval; **FV**, fruit and vegetable; **FVB**, fruit, vegetable, berry; **MCP-1**, monocyte chemotactic protein-1; **PBL**, peripheral blood lymphocytes; **RANTES**, regulated upon activation, normal T cell expressed and secreted; **RONS**, reactive oxygen and nitrogen species; **SOD**, superoxide dismutase

In addition, there are many mechanisms that link chronic inflammation and disease [4]. One well accepted hypothesis is that reactive oxygen and nitrogen species (RONS) are released by inflammatory cells (leukocytes and lymphocytes) and damage themselves, as well as otherwise healthy surrounding cells [5]. To this end, many fruit and vegetable constituents suppress RONS and other inflammatory species [6]. This led us to hypothesize that phytonutrients in fruits and vegetables can suppress DNA damage in humans. Because studies examining DNA damage in cells directly from tissues are difficult to carry out, and the process is highly invasive, the use of surrogate tissues such as peripheral blood lymphocytes (PBL) to monitor general DNA damage is an attractive alternative. We carried out a double-blind, placebo-controlled study to examine the effects of an encapsulated fruit and vegetable concentrate on inflammatory markers [2], then performed this ancillary study to assess PBL DNA damage.

We evaluated PBL samples from 106 of the 117 participants in the primary trial [2]. The study was approved by the University of South Carolina Institutional Review Board (HSA-3470). Subjects were assessed at baseline and again

after the 60-day intervention period. Assessment included questionnaires (these were collected only at baseline, and included information on demographics, nutrition, and physical activity), body mass index (BMI), and a fasting blood sample. Demographics are described in Supporting Information Table S1. Briefly, there was no significant difference at baseline between all three groups [Placebo, fruit/vegetable (FV) and the fruit/vegetable/berry (FVB)] with regards to age, gender, race, BMI, education, employment status, marital status, and smoking status. Details of contents of the Placebo, FV (Juice Plus^{+}® Orchard and Garden), and FVB (Juice Plus^{+}® Vineyard in addition to Orchard and Garden blend, both NSA, LLC, Collierville, TN) capsules consumed by the subjects participating in the study are described in Supporting Information and have been described by our group previously [2]. Briefly six capsules daily (three in the morning and three in the evening after meals) of the FV provided approximately 7.5 mg β-carotene, 234 mg vitamin C, 30 mg vitamin E, 420 μg folate, 60 mg calcium, and about 42 kJ; and six capsules daily of FVB provided approximately 7.5 mg β-carotene, 276 mg vitamin C, 72 mg vitamin E, 780 μg folate, 80 mg calcium, and about 63 kJ. Consistent with many previous studies (21 published studies to date; Supporting Information), there were no attributed adverse effects with these preparations.

Endpoints examined here were DNA damage; micronutrients (β-carotene, ascorbic acid, and α-tocopherol); monocyte chemotactic protein-1 (MCP-1); regulated upon activation, normal T cell expressed and secreted (RANTES); and superoxide dismutase (SOD). Because age, BMI, body fat, physical activity, and percent calories from fat and daily servings of fruits and vegetables can potentially cause changes in inflammatory load, and be confounding factors, these variables were compared between the groups at baseline. Supporting Information Table S2 shows there was no significant difference between the groups on these factors. After 60 days, serum micronutrients in both the FV and FVB groups were significantly increased, but these parameters were unchanged in the placebo group.

Using an overlapping similar data set to that used here, we have recently shown that FV and FVB capsule supplementation suppressed inflammatory load [2], referred to in molecular epidemiology as a 'biologically effective dose' [7]. To better understand the impact of FV and FVB capsule supplementation, we have now measured outcomes on genetic material (i.e. DNA); to assess what, in molecular epidemiology, is referred to as an 'early genotoxic effect' [7]. To this end, a key aim of this study was to investigate whether the 60-day addition of the placebo, FV or FVB capsules to the habitual diet could impact observed DNA strand breaks in PBL from healthy adults using the Alkaline Comet Assay. This test measures both single and double strand breaks. Table 1 shows there was no significant difference in Olive Tail Moment (DNA damage) between the three groups at baseline. Importantly, Table 1 also indicates a trend towards decreasing DNA damage with the FV group, this trend was not statistically significant. However, FVB supplementation for 60 days did result in a statistically significant decrease in single and double strand breaks.

Table 2. Correlations between Comet Tail Moment and inflammation marker levels, combining treatment and placebo groups[a)]

Inflammation marker	*r*	*p*-value
Superoxide dismutase	−0.31[b)]	0.001
Monocyte chemotactic protein-1	0.24[c)]	0.02
RANTES	0.27[c)]	0.006

a) Calculated as the post-measure − the pre-measure for all analytes.
b) Pearson product moment correlation.
c) Spearman's rank correlation.

Because we had previously collected data on the levels of MCP-1, RANTES, and SOD, we could investigate any correlation to DNA damage. Table 2 indicates MCP-1 and RANTES levels were significantly positively correlated with DNA damage (Comet Tail Moment), while SOD levels were significantly negatively correlated with DNA damage.

Here, we show that dietary supplementation with FVB results in a reduction in PBL DNA damage, consistent with previous findings by independent groups. For example, Nantz et al. [8] found a 39% reduction in PBL DNA damage (DNA fragmentation assay) following 77 days supplementation with a similar formulation to the FVB used here. Kim et al. [9] found a 36% decrease in PBL Comet Tail Moment in healthy males following 8 weeks supplementation with 15 mg lycopene. Gill et al. [10] found a 17% reduction in the

Table 1. Results for Comet Tail Moment[a)] comparing the change from baseline to follow-up measurements for FV and FVB groups relative to placebo

Treatment	Baseline LS Mean (95%CI)[b)]	Follow-up LS Mean (95%CI)[b)]	Intervention vs. placebo[c)]	*p*-value[d)]
Placebo	14.9 (13.3, 16.4)	14.6 (13.1, 16.1)		
FV	15.6 (13.9, 17.2)	14.4 (12.7, 16.0)	−1.0 (−2.4, 0.3)	0.12
FVB	14.4 (12.8, 16.0)	12.6 (11.0, 14.1)	−1.6 (−2.8, −0.4)	0.009

a) Comet Tail Moment is measured in Arbitrary Units (see supporting information for details).
b) Least square means with its 95% Confidence Interval after adjusting for age and gender.
c) Calculated as (Treatment post − Treatment pre) − (placebo post-placebo pre).
d) The *p*-value was derived from a *t*-test comparing the change in treatment to change in placebo using Proc Mixed (SAS).

percentage of Comet Tail DNA following consumption of 85 g watercress daily for 8 weeks. Using a single gel electrophoresis assay with lesion specific enzymes (formamidopyrimidine DNA glycosylase), Misik et al. [11] showed a 13% reduction in PBL DNA migration following supplementation with 800 mL paper-filtered coffee daily for 5 days. Other clinical trials have also shown an impact of supplements (e.g. fruit juice, flavonoid glycosides from an onion meal, apples, brussel sprouts, almonds, blueberry juice, olive oil, multivitamins, kiwifruit, beta-carotene, vitamin E) on PBL DNA damage [12–25]. Although our results are somewhat modest (13% reduction in Comet Tail Moment with FVB supplementation) compared with some of these studies, direct comparison is difficult because of many factors, including the study population, the dose of supplement, the type of supplement, and duration of supplementation, PBL processing, the population demographics, and methodological considerations (e.g. the raw Comet Tail Moment depends on factors such as buffers used, and length of electrophoresis time, among others). The key finding in our current study is that there was a significant decrease in PBL Comet Tail Moment following 60 days supplementation with the FVB in this double-blind, placebo-controlled trial. This is important, because it is often difficult to change the eating habits of individuals that would otherwise result in health benefits.

The finding that there was a correlation between PBL DNA damage and other inflammatory markers is interesting, but difficult to interpret at this time. MCP-1 is a pro-inflammatory chemokine and is chemotactic for monocytes and T lymphocytes. It is believed to play a role in the pathogenesis of obesity and diabetes [26], is elevated in the epithelium and subepithelial tissues of bronchial biopsies from asthmatic subjects [27]. Serum MCP-1 levels also correlate with the degree of inflammation in patients with hepatitis [28] and with cancer stage [29]. RANTES induces migration and mediates the trafficking of lymphoid cells. RANTES is produced by CD8+ T cells, epithelial cells, fibroblasts, and platelets, and is elevated in a wide range of chronic inflammatory conditions, including atherosclerosis, arthritis, atopic dermatitis, asthma, glomerulonephritis, and endometriosis [30], where RANTES is thought to act by promoting leukocyte infiltration to sites of inflammation [31]. Because these particular functions of MCP-1 and RANTES do not appear to be interrelated with PBL DNA damage, we are unaware of a biological mechanism to explain the observed correlation between a decrease in these inflammatory markers and the decrease in PBL DNA damage. Future studies on the biological functions of MCP-1 and RANTES may expose reasons for this correlation, and help elucidate the connection. Alternatively, although correlative, the reduced pro-inflammatory marker load and DNA damage may be mutually exclusive. As indicated earlier, DNA damage is caused by many inflammatory mediators, including RONS. Although we did not measure such levels in this study, a finding of a reduction in RONS by the FVB formulation would help explain the suppression of PBL DNA damage. To this end, the negative correlation between erythrocyte SOD and PBL DNA may be explained in that SOD is an antioxidant enzyme which prevents the formation of the highly destructive hydroxyl radical by conversion of superoxide anion to hydrogen peroxide. Therefore, the observed elevation in SOD may explain, at least in part, a reduction in cellular DNA damage. It is also possible that future studies will reveal that overall health may require both a reduction in inflammatory load ('biologically effective') and reduction in DNA damage ('early genotoxic effect'). While the FV and FVB formulations did suppress inflammatory load in healthy adults [2], this ancillary study suggests the FVB formulation is more effective in reducing PBL DNA damage in our study population.

Overall, our results suggest that the encapsulated FVB blend suppresses PBL DNA damage. The long-term implications of such a finding have yet to be determined. However, there is increasing evidence that DNA damage in surrogate tissues (i.e. PBL) are elevated in chronic diseases [32], correlate with the onset of chronic diseases [33–35], are elevated in high-risk populations [36]) and lowered by healthy lifestyle factors (reviewed in [37]). This, in addition to the understanding that changing dietary habits is often difficult for much of the population, gives a rationale for the use of dietary phytonutrient supplements to suppress inflammation and its biological consequences (e.g. DNA damage). These observations warrant additional research to further elucidate the biological connections between these various markers and health promotion.

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