High green leafy vegetable dietary intervention decreases plasma oxidative DNA damage: secondary outcomes analysis of the Meat and Three Greens (M3G) Feasibility Trial

Aaron J. Riviere, Kristen S. Smith MS, Megan Schaberg, Andrew D. Frugé PhD, RD
Department of Nutrition, Dietetics and Hospitality Management, Auburn University

BACKGROUND AND METHODS

Methods and preliminary outcomes of this crossover RCT are published and can be found at this URL: https://www.ncbi.nlm.nih.gov/pubmed/31581743

RESULTS

Primary Outcomes

Accrual target was met with 50 adults being recruited in 44 days
Retention targets were met with 48 participants completing the study
Adherence:

- Participants achieved 73.2% adherence of daily goal of 1 cup GLV
- Participants consumed any amount of GLV 88.8% of days
- Average daily intake was 0.91 cup GLV per day

Secondary Outcomes:

Plasma 8OHdG was significantly reduced in all participants after administration of GLV intervention by an average of 8.05 ng/mL (SD=13.9; p=0.001)
Plasma and fecal 8OHdG were significantly reduced in the immediate group following the high GLV intervention
Serum AGP, plasma 8OHdG, serum CRP, serum LBP and fecal 8OHdG were significantly reduced during the intervention period in the delayed group.
The delayed group significantly increased red meat intake during to the intervention period (p = 0.026)
Daily dark green vegetable intake increased from ~0.25 cup to 0.85 cups during intervention period in both immediate and delayed groups (p = 0.011, p = 0.006, respectively)
Increase in GLV intake resulted in significantly increased vitamin K intake in both immediate and delayed groups (p = 0.009, p = 0.001, respectively)

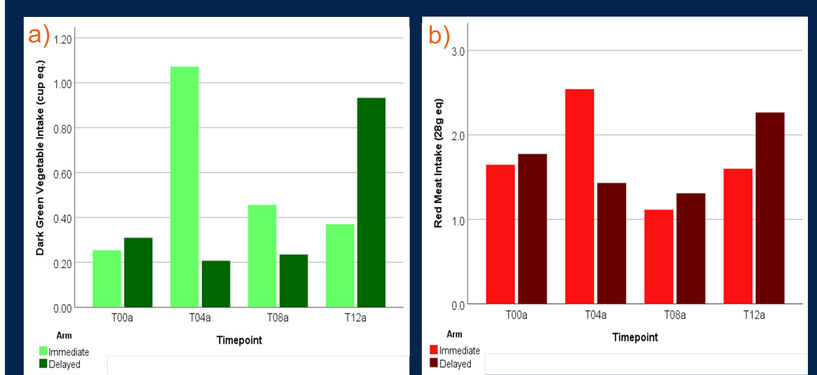

Figure 1. a) Adherence to dietary intervention was measured by dark green vegetable consumption generated from obtained 24-hour recalls and analysis of outputs from Automated Self-Administered 24-hour Dietary Assessment tool (ASA24). GLV intake was significantly increased during the intervention period for both groups (p=0.011, p=0.006). b) Average red meat intake obtained from ASA24 output throughout duration of study by intervention group. Red meat intake significantly increased in the delayed group from T8 to T12 (p=0.026).

RESULTS

Table 1. Cytokine values measured in biological samples during a randomized controlled crossover high green leafy vegetable dietary intervention

	T0	T4	Change (T4-T0)		T8	T12	Change (T12-T8)	
Immediate Group	Mean (SD)			p [1]	Mean (SD)			p [1]
TNFa	166.48 (56.68)	145.43 (6.01)	-22.54 (57.58)	**0.088**	147.43 (11.54)	132.58 (43.71)	-14.85 (41.51)	0.161
IL6	4.56 (2.09)	5.35 (2.84)	0.7 (3.67)	**0.395**	4.09 (1.83)	5.51 (2.79)	1.42 (3.06)	0.454
AGP	1504.75 (1161.6)	1734.12 (1854.19)	180.97 (1346.96)	**0.545**	1798.49 (1691.17)	1640.89 (1478.65)	-157.6 (935.52)	<0.001*
8OHdG	45.56 (22.02)	35.11 (10.89)	-11.23 (16.25)	**0.005***	36.09 (9.13)	40.83 (14.6)	4.74 (12.18)	0.009*
CRP	3542.95 (4656.78)	3541.05 (4592.57)	-203.62 (4980.55)	**0.853**	4117.86 (5193.53)	2868.95 (4052.84)	-1248.9 (4516.29)	0.010*
LBP	3.85 (1.75)	4.69 (4.55)	0.84 (4.74)	**0.428**	4.41 (2.54)	3.86 (1.47)	-0.55 (2.41)	0.093
Fecal 8OHdG	38333.4 (73853.5)	13410.5 (30244.9)	-24922.9 (53228.3)	**0.031***	29422.5 (73831)	23009.2 (47896.2)	-6413.3 (39240.3)	<0.001*
Delayed Group								
TNFa	143.53 (8.1)	149.94 (18.21)	6.69 (21.36)	0.215	145.5 (8.66)	123.07 (33.59)	-22.42 (34.42)	**0.898**
IL6	5.68 (4.11)	5.88 (5.29)	0.26 (6.95)	0.877	3.59 (2.33)	4.87 (2.38)	1.28 (3.27)	**0.884**
AGP	1119.94 (928.13)	1194.55 (1058.4)	244.64 (867.32)	0.262	1416.12 (1200.87)	1451.22 (1116.35)	35.09 (692.41)	**<0.001***
8OHdG	36.95 (10.2)	39.94 (24.26)	-3.06 (9.2)	0.189	37.13 (12.46)	32.59 (6.33)	-4.54 (10.64)	**0.022***
CRP	2893.33 (3004.98)	3156.78 (2898.98)	200.06 (2135.91)	0.704	4503.84 (5941.1)	4604.84 (6808.31)	101 (2598.74)	**<0.001***
LBP	3.38 (2.84)	2.79 (1.67)	-0.41 (2.7)	0.536	3.83 (2.28)	4.11 (1.83)	0.28 (1.45)	**<0.001***
Fecal 8OHdG	10293.6 (14508.8)	6135 (6344.9)	-4158.7 (14993.3)	0.187	6302.7 (7652.8)	7104.1 (6529.3)	801.4 (5919.9)	**<0.001***

[1] P-values were computed using paired sample t-test. Bold indicates p-values across the intervention period for that group.
*indicates significance at 0.05 level; colors represent **expected** vs. **unexpected** results

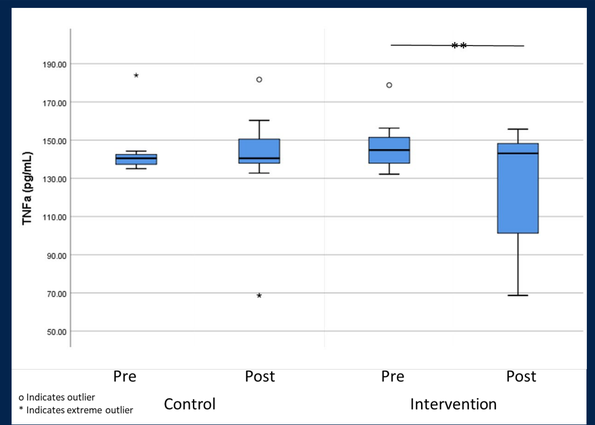

Figure 2. Serum TNFα in men measured at all time points. Data from participants in both study arms were pooled and analyzed before and after the control and intervention periods. **TNFα decreased during the intervention period (p= 0.047),

Table 2. Gender-controlled correlations between change in serum inflammatory cytokines during high green leafy vegetable dietary intervention

		Change in IL6 (pg/mL)	Change in AGP (pg/mL)	Change in 8OHdG (ng/mL)	Change in CRP (pg/mL)
Change in TNFa (pg/mL)	r	0.068	-0.072	-0.161	0.021
	p	0.681	0.664	0.327	0.900
	N	40	40	40	40
Change in IL6 (pg/mL)	r		-0.002	0.302	0.026
	p		0.991	0.062	0.876
	N		40	40	40
Change in AGP (pg/mL)	r			0.482	0.554
	p			0.002*	<0.001*
	N			40	40
Change in 8OHdG (ng/mL)	r				0.47
	p				0.003*
	N				40

*indicates significance at 0.05 level

CONCLUSION

Circulating oxidative DNA damage was significantly reduced during a high GLV dietary intervention in all participants. Additionally, fecal oxidative DNA damage was significantly reduced in both groups following the high GLV intervention. This suggests that consumption of high chlorophyll-containing GLV may provide chemopreventive benefits in the context of a high RM diet.

Analysis of stool samples will determine the effects of GLV on gut microbiota composition and diversity. Further research is needed to investigate quantity and frequency of GLV necessary for reducing deleterious effects of RM.

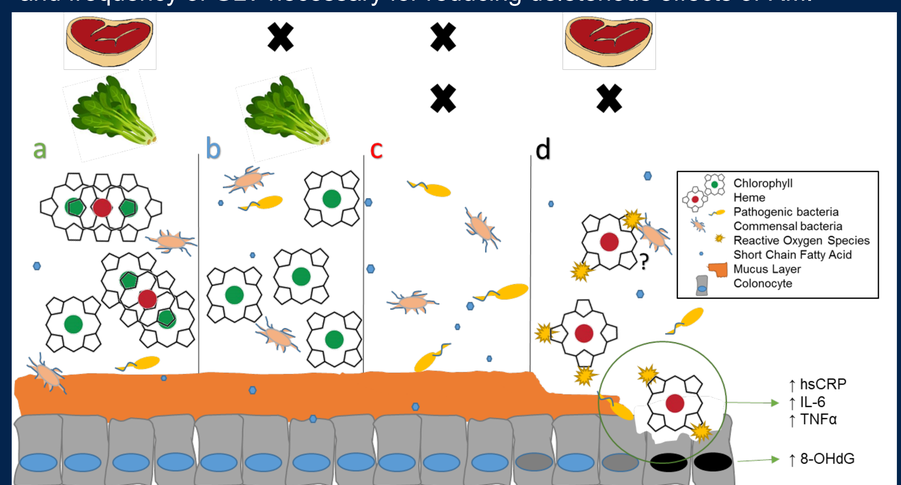

Figure 3. Future Study Design. a) In the lumen, chlorophyll binds heme, preventing cytotoxicity. b) Absence of heme reduces pathogenic bacteria and increases SCFA productions. c) Normal lumen in absence of heme and chlorophyll d) Heme is easily oxidized in the absence of chlorophyll; it is unknown if microbes, their metabolites, or both react with heme. Degradation of the mucin layer increases susceptibility of epithelial cells to pathogens and oxidative stress resulting in elevated systemic inflammation. Necrosis leads to compensatory hyperproliferation and DNA damage.