Secondary outcomes of a randomized controlled crossover trial to explore the effects of a high chlorophyll dietary intervention to reduce colon cancer risk in adults: The Meat and Three Greens (M3G) Feasibility Trial

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BACKGROUND AND METHODS

High red meat (RM) intake is associated with increased colon cancer (CC) risk. Pre-clinical and epidemiological evidence indicates green leafy vegetables (GLV) may mitigate the oxidative damage and inflammation induced by RM; we sought to investigate circulating biomarkers under high and low GLV intake.

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

Table 1. Baseline characteristics of participants in a high dietary green leafy vegetable crossover trial

	Total	Immediate	Delayed	
	(n=50)	(n=26)	(n=24)	
	Mean (SD)			*p*
RM servings per week	10.39 (5.03)	10.5 (4.8)	10.2 (5.4)	0.846
GLV servings per week	0.21 (0.25)	0.20 (0.26)	0.22 (0.23)	0.852
Age (years)	48 (13.1)	47 (13)	49(13)	0.649
Body Mass Index (kg/m2)	36.2 (4.7)	35.2 (4.6)	37.3 (4.8)	0.123
	-------- N (%) --------			*p*
Gender				1.000
Male	19 (38)	10 (39)	9 (38)	
Female	31 (62)	16 (61)	15 (62)	
Race				0.035
African-American	10 (20)	2 (8)	8 (33)	
White	40 (80)	24 (92)	16 (67)	
Education				0.332
>High school graduate	1 (2)	0 (0)	1 (4)	
High school graduate	4 (8)	0 (0)	4 (17)	
Some College/Technical	2 (4)	1 (4)	1 (4)	
Associate degree	19 (38)	11 (42)	8 (33)	
Bachelor's degree	13 (26)	7 (27)	6 (25)	
Professional degree	1 (2)	1 (4)	0 (0)	
Doctorate	10 (20)	6 (23)	4 (17)	
Marital Status				0.498
Single	12 (24)	6 (23)	6 (25)	
Married	29 (58)	16 (62)	13 (54)	
Widowed	2 (4)	0 (0)	2 (8)	
Separated	7 (14)	4 (15)	3 (13)	

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 ORM1, plasma 8OHdG, serum CRP, serum LBP and fecal 8OHdG were significantly reduced during the intervention period in the delayed group.

RESULTS

Table 2. Cytokine values measured in biological samples during a randomized controlled crossover high green leafy vegetable dietary intervention

	T0	T4	Change (T4-T0)	p-value[1]	T8	T12	Change (T12-T8)	p-value[1]
Immediate Group	Mean (SD)				Mean (SD)			
TNFa (pg/mL)	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)	0.117
IL6 (pg/mL)	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)	0.046
ORM1 (pg/mL)	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)	0.449
8OHdG (ng/mL)	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)	0.090
CRP (pg/mL)	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)	0.220
LBP (ng/mL)	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)	0.306
Fecal 8OHdG (µg/mL)	38.33 (73.85)	13.41 (30.24)	-24.92 (53.23)	**0.031**	29.42 (73.83)	23.01 (47.9)	-6.41 (39.24)	0.432
Vitamin K1 (ng/mL)	0.06 (0.18)	0.87 (0.96)	0.79 (0.97)	**0.001**	0.50 (0.96)	0.40 (0.56)	-0.10 (0.75)	0.550
Delayed Group	Mean (SD)				Mean (SD)			
TNFa (pg/mL)	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)	**0.011**
IL6 (pg/mL)	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)	**0.106**
ORM1 (pg/mL)	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)	**0.828**
8OHdG (ng/mL)	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)	**0.079**
CRP (pg/mL)	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)	**0.867**
LBP (ng/mL)	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)	**0.414**
Fecal 8OHdG (µg/mL)	10.29 (14.51)	6.13 (6.34)	-4.16 (14.99)	0.187	6.3 (7.65)	7.1 (6.53)	0.8 (5.92)	**0.514**
Vitamin K1 (ng/mL)	0.15 (0.34)	0.36 (0.52)	0.20 (0.67)	0.231	0.14 (0.19)	0.29 (0.39)	0.14 (0.33)	**0.072**

[1]P-values were computed using paired sample t-test. Bold indicates p-values across the intervention period.

- 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)
- Plasma Vitamin K levels significantly increased in the immediate group during the intervention period (p=0.001)

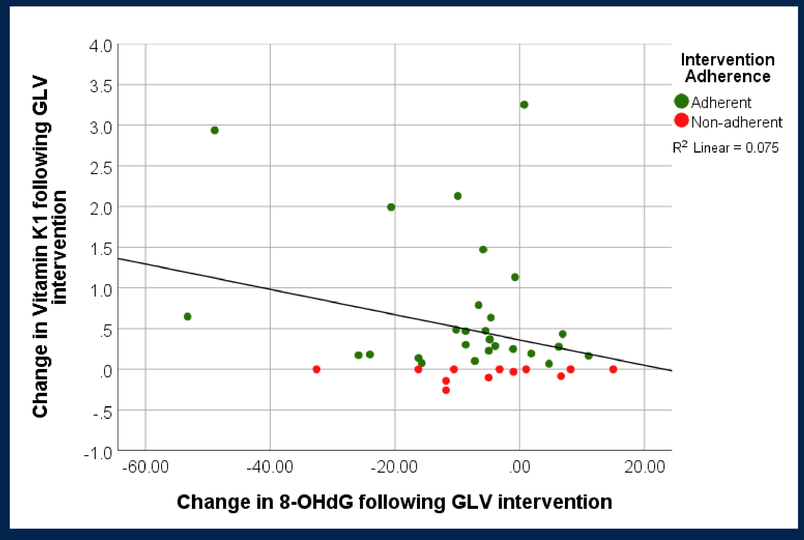

Figure 1. Change in plasma vitamin K levels plotted against plasma oxidative DNA damage. Participants are grouped based on adherence to high GLV dietary intervention determined by changes in plasma phylloquinone levels following intervention period.

CONCLUSION

This 12-week crossover RCT aimed to increase GLV consumption in order to reduce CC risk in high-risk individuals. This dietary intervention met 2 of 3 feasibility targets.

Circulating oxidative DNA damage was significantly reduced post-intervention in all participants. Additionally, fecal oxidative DNA damage was significantly reduced in both groups following the high GLV intervention. This suggests colonocyte protection from oxidative species via mucin layer preservation after consumption of high chlorophyll-containing GLV.

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.

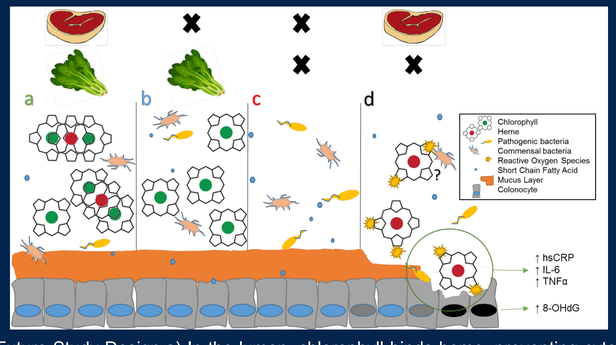

Figure 3 Future Study Design.a) In the lumen, chlorophyll binds heme, preventing cytotoxicity. b) Absence of heme reduces pathogenic bacteria and increases SCFA productions. c) Normal lumen in absence of heme and chlorophyll d) Heme is easily oxidized in the absence of chlorophyll; it is unknown if microbes, 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.