ORIGINAL RESEARCH

A Plant-Based Dietary Supplement Improves Measures of Metabolic Detoxification and the Quality of Life: A Phase II Multicenter Randomized, Blinded, Placebo-Controlled Clinical Trial

Bassem F. El-Khodor, PhD; Wei Zang, PhD, MS; Heather Gorby, PhD, MA; Ashley Dominique, MSPH; Meghan Hamrock, MS, MPH; Brandon Metzger, PhD; Alessandra Pecorelli, PhD, PharmD; Saradhadevi Varadharaj, PhD; Giuseppe Valacchi, PhD

Abstract

Background • Persistent accumulation and hindered clearance of toxins from tissues over time may promote the development and exacerbation of several diseases. Hepatic metabolic detoxification is a key physiological process responsible for the clearance of toxic substances from the body. A healthy diet with nutritional dietary supplementation may support metabolic detoxification and help mitigate the negative effects of toxin burden.

Methods • A multicenter, randomized, single-blind, controlled trial was conducted to test the effects of a dietary detoxification product (detox; n = 20) versus an active dietary control product (active control; n = 20) on selected biomarkers of metabolic detoxification, general health, and well-being following 28 days of dietary supplementation. Study participants displayed multiple symptoms commonly associated with elevated toxin burden, but otherwise healthy.

Results • The detox group displayed significantly decreased levels of red blood cell total toxic metals, decreased urine total porphyrins, and decreased urine mutagenicity potency compared with baseline. Both the detox and active control groups showed improvements in the symptoms attributed to elevated toxin burden. Fatigue and sleep disruption scores were significantly reduced in the detox group compared with baseline. No significant differences in anthropometric measures and vital signs, and no adverse events or side effects were detected in either group over the study period.

Conclusions • This study demonstrates the benefit of nutritional intervention for supporting metabolic detoxification, evidenced by significant changes in multiple detoxification biomarkers and improvement in questionnaire scores related to quality of life, general health, and well-being.

Bassem F. El-Khodor, PhD; Wei Zhang, PhD, MS; Ashley Dominique, MSPH; Meghan Hamrock, MS, MPH; Brandon Metzger, PhD; Saradhadevi Varadharaj, PhD; Nutrition Innovation Center, Standard Process Inc, Kannapolis, NC. **Heather Gorby, PhD, MA,** Gorby Consulting, LLC, Washington, DC. **Alessandra Pecorelli, PhD, PharmD,** Plants for Human Health Institute, North Carolina State University, Kannapolis, NC. **Giuseppe Valacchi, PhD,** Plants for Human Health Institute, North Carolina State University, Kannapolis, NC; Department of Environmental Sciences and Prevention, University of Ferrara, Ferrara, Italy.

Corresponding author: Bassem F. El-Khodor, PhD
E-mail address: belkhodor@standardprocess.com

Introduction

On a daily basis, the human body is exposed to a variety of endogenous and environmental toxins that put pressure on the body's natural metabolic detoxification capacity.[1] Improper clearance and subsequent accumulation of toxins over time may play a role in the development and exacerbation of several diseases, such as obesity and diabetes,[2] cardiovascular diseases,[3,4] central nervous system disorders,[5,6] immune dysfunction and autoimmune diseases,[7,8] chemical intolerance, and reproductive and developmental concerns.[8-16] Some of these toxins include inorganic substances, such as heavy metals, arsenic, and mercury, as well as organochlorine pesticides, phthalates, bisphenol A, and polybrominated diphenyl ethers, among others.[13,17] The majority of toxins are lipophilic with long half-lives and can therefore penetrate lipid cell membranes and accumulate in the cells of various tissues, especially those rich in fat content, such as adipose tissue, the liver, and the nervous system.[18-23]

Metabolic detoxification is a key physiological process responsible for the clearance of toxic substances from the body. Detoxification occurs primarily in the liver and can be divided into 3 phases: phase I and II (biotransformation), and phase III (elimination) – all mediated by enzymatic pathways.[1,14,18,24-26] The primary role of biotransformation enzymes of phases I and II is to transform the toxins that are lipophilic into more water-soluble compounds that can

be more easily excreted from the body. In phase I, toxic substances are activated by phase I enzymes—primarily a family of cytochrome P450 enzymes.[27] Phase I activation results in the generation of free radicals and reactive intermediaries, which can themselves be toxic, and in some cases are even more toxic than the parent compounds.[18,25,26] These activated toxins (reactive intermediates) are either directly excreted (e.g., caffeine, which undergoes only phase I activation before elimination) or require phase II (conjugation) involving enzymes that conjugate a large, more water-soluble moiety to toxins, effectively altering their lipophilic characteristics, and facilitating elimination.[18] In phase III, transmembrane-spanning proteins transport the substrates out of the cells and these substances are eliminated via urine, sweat, and bile routes.[26] Metabolic detoxification processes are heavily dependent on energy and nutrition. Deficiency in key nutrients and cofactors essential for phase I/II biotransformation enzymes stalls, alters, or slows down the process of detoxification, leading to an increased toxin burden.[14,28,29] Therefore, a healthy diet with customized nutritional dietary supplementation is likely to support the detoxification process and decrease the negative effect of toxin burden on the body.

The association of toxin burden with negative health outcomes has spurred interest in research that focuses on lowering toxin intake/absorption and enhancing the detoxification process. However, to date, there has been little research on the impact of nutritional supplementation on metabolic detoxification. Studies conducting randomized, blind, well-controlled trials assessing the role of nutritional supplementation on reducing toxin load are lacking.[17-19,30,31] Additionally, methods and biomarkers to quantify and evaluate the efficiency of metabolic detoxification have not been adequately investigated. Recently, an open label pilot study (n = 12) included participants with a body mass index (BMI) >30 who underwent a 12-week therapeutic lifestyle change program composed of individual dietary modification, exercise and behavioral support, and supplemented with a commercial 30-day dietary detoxification intervention.[30] This multi-factorial intervention improved body composition and functional fitness; reduced levels of lipopolysaccharide, zonulin and leptin, and decreased measures of pain.[30] However, an open label study design that lacks a placebo arm is not ideal to demonstrate the effect of a detoxification program on the quality of life and other biomarkers of detoxification.

Detoxification enhancement approaches, including the intake of phytonutrients, typically target phase I and II detoxification pathways.[18,30] Some detoxification-promoting dietary strategies previously evaluated include increased intake of high-fiber phytonutrients, cruciferous vegetables, berries, soy, garlic, spices (i.e., turmeric), omega-3 poly-unsaturated fatty acids, berberine, and pre- and probiotics.[18,30,32] Direct measures of toxin exposure is not always feasible due to fast metabolism of some chemicals, their sequestration into fatty tissues, lack of suitable assay methods, and because exposures often involve complex mixtures of toxic compounds.[33] In these cases, indirect measurements of exposure can be useful and informative. Such biomarkers include glucaric acid (end-product of glucuronidation pathway),[34] mercapturic acid (end-product of glutathione reaction with electrophilic or alkylating compounds),[35] porphyrin pattern (altered with disruption in heme biosynthesis),[36-38] and Ames mutagenicity test.[39-41] All these biomarkers can be evaluated in urine samples.

The present study was a multicenter, randomized, single-blind, placebo-controlled trial that tested the effectiveness of a metabolic detoxification-enhancing product, consisting of a variety of nutritional ingredients with diverse potential roles in supporting metabolic detoxification, compared with active control nutrition.

Materials and Methods

Ethical aspects

Forty participants received detailed information about all the procedures involved and signed the informed consent form. The study protocol was approved by the Ethics Committee of the Institutional Review Board (IRB) at WCG IRB (Formerly Western IRB [WIRB]; IRB No. 20191783). The study was conducted in accordance with the Declaration of Helsinki and all other applicable regulatory requirements.

Study participants

Participants were healthy adults between the ages of 18 and 75 years who met the inclusion criteria of elevated toxin burden with eight or more of the Living Matrix

Box 1

In the past 4 weeks, have you experienced any of the following symptoms (mark "X")?[a]	
Fatigue	Acne
Headache	Eczema
Feeling depressed	Rash
Anxiety	Hives or urticaria
Vision problems	Joint pain
Concentration or memory problems	Calf cramps
Distorted taste or smell	Joint or muscle stiffness
Mood swings	Muscle pain
Irritability	Foot cramps
Poor libido-low sex drive	Muscle weakness
Light-headedness	Muscle spasms
Low body temperature/cold hands & feet	Muscle twitches-arms or legs
	Nasal stuffiness
Dizziness or spinning	Sensitivity to auto exhaust fume
Ear ringing or buzzing	Sensitivity to perfume or cologne
Can't lose weight	Sensitivity to cigarette smoke
Hot flashes	
Breast tenderness	

[a]Participants who checked 8 or more of these symptoms were included in the study

Patient's Symptoms. Exclusion criteria were pregnancy or lactation; intake of lipid-lowering drugs or anticoagulant medications in the preceding 4 weeks and for the duration of the trial; serious medical illness; untreated endocrine, neurological, or infectious disease; HIV infection or AIDS; or history of significant liver or kidney disease. Participants were screened through Living Matrix database and recruited in equal numbers at two health clinics (n = 20 each, 40 total): Living Well Dallas (Dallas, TX, USA) and VIDA Integrative Medicine (Sunrise, FL, USA). Participants at each health clinic were randomized using simple 1:1 randomization into detox (n = 10) or active control (n = 10) groups and were blind to the treatment. Supplement bottles were labeled as either A or B, with odd numbers assigned to detox group and even numbers to an active control group.

Study design

This was a multicenter, randomized, single-blind, placebo-controlled clinical trial with a duration of 44 days and conducted in three stages: baseline (day 1, visit 1), treatment (28 days; week 4), and follow-up (14 days after the last serving of detox product or active control; week 6). Figure 1 shows the enrollment flow chart at the two clinical sites. The Living Matrix patients' database was used for preliminary screening and selection of potential candidates. Healthy participants meeting eight or more of the Living Matrix Patient's Symptoms (using Living Matrix Questionnaire, see Box 1) were invited to participate in this study. This cluster of symptoms, as reported by the participants in the Living Matrix database, was selected based on the literature reports of their association with environmental toxicant exposures[42,-45] and symptom clustering across multiple organ and physiological systems.

Participants were randomly assigned (1:1) to the detox or an active control (placebo) supplement group. The detox and active control supplements are commercially available and were obtained from Standard Process Inc. (Palmyra, WI). Both groups were asked to follow the detoxification program dietary and lifestyle guidelines during the four-week intervention phase. The guidelines included increasing water intake, inclusion of exercise into the daily routine, reducing unnecessary chemical exposures in the home environment (i.e., reducing the use of scented candles, air fresheners, toxic cleaning agents, and plastic containers), increasing the consumption of vegetables and reducing the consumption of processed and refined foods, sodas, sports drinks, fruit juices, sugar, caffeinated drinks, alcohol, and artificial food additives. The dietary supplement was incorporated into the meal pattern assisted by clinical staff to ensure that it was a part of a meal or snack within overall energy intake goals (see Table 1 for details of the 28-day program).

There was a total of 63 servings of the dietary supplement consumed over the 28-day period. The dietary supplement products were administered as follows: detox product at

Figure 1. Enrollment Flow Chart at the Two Clinical Sites. Flow Chart Showing the Number, Sex, and Average Age of Patients (± SD) in the Separate Phases of the Study.

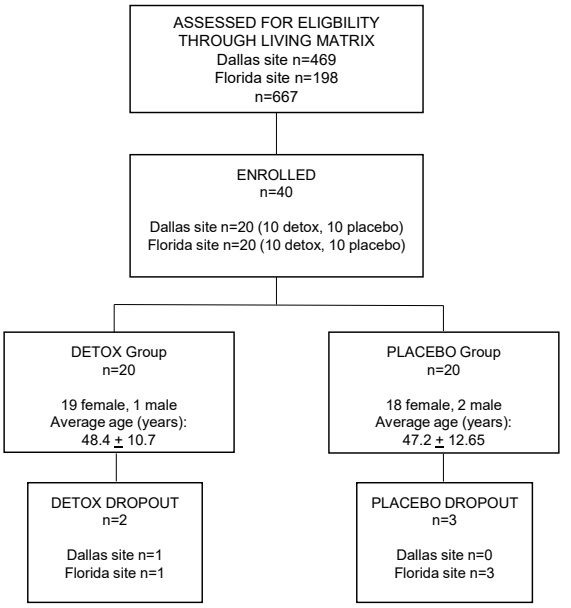


Table 1. 28-Day Nutritional Supplementation Program

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Week 1	1	1	1	2	2	2	2
Week 2	3	3	3	3	3	3	3
Week 3	3	3	3	3	3	3	3
Week 4	2	2	2	1	1	1	1

Note: Number of servings per day

Figure 2. Active Control Supplement Facts Label

Supplement Facts

Serving Size: 4 heaping tablespoons (scoops) (approx. 34 g)
Servings per Container: 18

	Amount per Serving	% Daily Value
Calories	130	
Total Fat	2.5 g	3% *
Total Carbohydrate	7 g	3% *
Dietary Fiber	2 g	7% *
Total Sugars	4 g	†
Includes 4 g Added Sugars		8% *
Protein	15 g	30% *
Calcium	20 mg	2%
Iron	4 mg	22%
Sodium	280 mg	12%
Potassium	200 mg	4%
Proprietary Blend	26 g	†

Organic pea protein, organic pumpkin seed protein, and organic sesame seed protein.

*Percent Daily Values based on a 2,000 calorie diet.
†Daily Value not established.

Figure 3. Detox Supplement Facts Label

Supplement Facts

Serving Size: 2 Scoops (37 grams)
Servings per Container: 21

	Amount per Serving	% Daily Value
Calories	160	
Total Fat	5 g	6% *
Saturated Fat	0.5 g	3% *
Total Carbohydrate	11 g	4% *
Dietary Fiber	4 g	14% *
Total Sugars	1 g	†
Protein	17 g	34% *
Vitamin K1	4 mcg	3%
Choline	100 mg	18%
Calcium	70 mg	5%
Iron	4 mg	22%
Magnesium	70 mg	17%
Sodium	150 mg	7%
Potassium	230 mg	5%
Arginine	1300 mg	†
Glycine	600 mg	†
L-isoleucine	850 mg	†
L-leucine	1600 mg	†
DL-methionine	300 mg	†
L-valine	900 mg	†
Creatine	600 mg	†
Proprietary Blend	34.4 g	†

Organic pea protein, flax meal, oat flour, organic pumpkin seed protein, organic buckwheat flour, organic beet (leaf) juice powder, organic buckwheat (aerial parts), apple pectin, juniper (berry) powder, organic spanish black radish (root), burdock (root) powder, organic beet (root), calcium citrate, organic barley (grass), dandelion (leaf), broccoli (aerial parts), inositol, organic alfalfa (aerial parts) juice powder, oregon grape (root) powder, globe artichoke (leaf), sunflower lecithin powder, milk thistle extract (80% silymarins), organic cordyceps mushroom powder, organic carrot, organic sweet potato, and red wine extract

*Percent Daily Values based on a 2,000 calorie diet.
†Daily Value not established.

the recommended label dose (1 serving equals 2 heaping scoops [37 g] in 10-12 ounces of water, see Table 1), active control at half of the recommended label dose (participants assigned to the active control group were instructed to take 2 heaping scoops, instead of the recommended label serving size of 4 heaping scoops, in 4-6 ounces of water). Figures 2 and 3 describe the nutritional fact labels for each product. Detoxification product contained plant-derived ingredients that were reported to aid in toxin elimination.[46-50] Active control product contained a general panel of plant-derived extracts devoid of ingredients that may help with toxin elimination. Participants were instructed to make no changes to non-prescription/over-the-counter medications use or nutritional supplement intake during the study and to report any changes in prescription medication. Short-form Food Frequency Questionnaire (FFQ)[51] was used to monitor any significant deviations in dietary habits during the study.

Clinical evaluations

The following measurements were recorded at baseline and at every visit: anthropometric (body weight, height, and BMI), vital signs (pulse rate and blood pressure), and medication/supplement usage. Urine (morning first void) collection occurred at each visit and overnight fasted blood (morning) collection occurred at baseline and following the end of the dietary supplement (week 4). Participants also completed the following questionnaires at each visit: Metabolic Screening Questionnaire (MSQ), Patient Health Questionnaire (PHQ),[52] Athens Insomnia Scale (AIS),[53] the Functional Assessment of Chronic Illness Therapy – Fatigue Scale (FACIT-F Fatigue scale),[54] Short-form FFQ,[51] the Magnesium Status Questionnaire,[55] and the 10-point pain scale during each blood draw session.

Urine samples were analyzed for the general biomarkers of metabolic detoxification that included D-glucaric acid,[34,56] mercapturic acid,[35] porphyrin panel,[57-60] and DNA oxidative damage assay (8-hydroxy 2 deoxyguanosine). Analysis was conducted by Doctor's Data Specialty Testing Clinical Laboratory (St. Charles, IL, USA).[33,35,56,59-61] Blood samples were analyzed with a methylation panel for methionine, cysteine, S-adenosylmethionine (SAM), S-adenosylhomocysteine (SAH), homocysteine, cystathionine, as well as red blood cell (RBC) minerals (including calcium, magnesium, potassium, phosphorus, copper, zinc, iron, manganese, selenium, boron, molybdenum, arsenic, cadmium, cesium, chromium, lead, mercury, and thallium) by Doctor's Data Specialty Testing Clinical Laboratory (St. Charles, IL, USA).

Urine mutagenicity with the Ames test

Urine samples were analyzed for mutagenicity potency at the Nutrition Innovation Center, Standard Process Inc. (Kannapolis, NC, USA) and Plants for Human Health Institute, North Carolina State University (Kannapolis, NC, USA), as described by Yamasaki and Ames (1977) and Smith, McKarns et. al. (1996) with slight modifications.[62,63] Briefly, urine samples stored at -80°C were thawed, mixed thoroughly, and the urothelial cells were removed by centrifugation at 3000 g for 5 min. Urine creatinine analysis was performed by creatinine assay using Architect (Abbott Core Laboratory, Abbott Park, IL, USA). Urine samples were then extracted and concentrated.[62,63] The samples were stored at -80°C until the mutagenicity test was conducted.

Urine mutagenicity was evaluated using a liquid microplate format modification of the classic Ames test according to the manufacturer's guidance (Ames MPF™ Penta 1, XENOMETRIX AG, Allschwil, Switzerland) in a blinded fashion. Tester strains TA98, TA100, TA1535, and TA1537 without S9-mix metabolic activation were used in this test. DMSO (100% biological grade) was used as a negative control to determine spontaneous reversion activity. Each urine sample dilution and DMSO were evaluated in triplicates.

To assess the mutagenicity potency, the average number of revertants from each urine sample was plotted as a function of urine dosing volume after subtracting the average number of spontaneous revertants in DMSO conditions, and linear regression analysis was performed over the linear portions of dose-response curves to determine the

Table 2. Body composition and vitals at baseline, week 4, and week 6.

Parameter	Group	n	Baseline Mean ± SD	Week 4 Mean ± SD	Week 6 Mean ± SD	Baseline vs. Week 4 Paired Test *P* value	Baseline vs. Week 6 Paired Test *P* value
Weight, lb.	Active Control	13	172.7 ± 43.3	170.5 ± 40.1	170.8 ± 39.3	.06812	.1909
	Detox	14	165.4 ± 30.9	164.7 ± 30.7	165.8 ± 31.0	.6698	.5416
Height, in	Active Control	14	64.6 ± 2.7	64.6 ± 2.7	64.6 ± 2.6	nd	1
	Detox	14	65.6 ± 3.2	65.8 ± 3.4	65.5 ± 3.3	.3458	.3458
Body mass index (BMI), kg/m^2	Active Control	14	30.2 ± 7.3	29.9 ± 7.2	29.8 ± 6.8	.2201	.1075
	Detox	14	27.1 ± 5.2	26.8 ± 5.2	27.3 ± 5.4	.4209	.362
Pulse rate, bpm	Active Control	14	75 ± 15	76 ± 9	74 ± 10	.7063	.6371
	Detox	13	75 ± 16	74 ± 16	69 ± 7	.8139	.3452
Systolic blood pressure, mm/Hg	Active Control	13	130 ± 21	126 ± 12	124 ± 14	.7528	.2481
	Detox	14	122 ± 14	119 ± 12	118 ± 14	.5297	.3058
Diastolic blood pressure, m/Hg	Active Control	13	82 ± 13	79 ± 8	79 ± 6	.552	.4231
	Detox	14	77 ± 8	75 ± 10	73 ± 11	.5487	.07345

Abbreviations: nd, not determined; SD, standard deviation

Table 3. Questionnaire results

Questionnaire	Group	n	Baseline Mean ± SD	Week 4 Mean ± SD	Week 6 Mean ± SD	Baseline vs. Week 4 Paired Test *P* value	Baseline vs. Week 6 Paired Test *P* value
MSQ	Active Control	17	53 ± 32	35 ± 19	28 ± 15	.01378[a] ↓	.004502[a] ↓
	Detox	16	36 ± 16	22 ± 12	18 ± 13	.004767[a] ↓	.001467[a] ↓
FACIT_F total scores	Active Control	17	109.3 ± 26.8	118.1 ± 23.5	118.3 ± 26.0	.01125[a] ↑	.1549
	Detox	15	131.2 ± 15.4	140.5 ± 12.9	133.2 ± 14.8	.01347[a] ↑	.5509
FACIT_G Physical WB score	Active Control	16	21.6 ± 5.6	23.4 ± 4.4	22.7 ± 5.4	.05837	.1541
	Detox	15	25.2 ± 2.0	25.7 ± 1.7	26.0 ± 1.4	.2468	.1151
FACIT_G Social/Family WB scores	Active Control	17	16.8 ± 6.9	19.1 ± 5.8	18.0 ± 6.1	.02867[a] ↑	.2846
	Detox	17	20.7 ± 5.7	21.0 ± 6.5	19.4 ± 5.6	.9175	.3151
FACIT_G Emotional WB scores	Active Control	16	18.1 ± 4.3	19.7 ± 4.1	20.3 ± 2.6	.03683[a] ↑	.03999[a] ↑
	Detox	13	21.3 ± 2.3	21.8 ± 2.2	21.3 ± 2.5	.4718	1
FACIT_G Functional WB scores	Active Control	17	18.2 ± 5.8	19.9 ± 5.5	19.7 ± 4.6	.1045	.145
	Detox	14	20.9 ± 4.7	23.2 ± 4.2	21.3 ± 4.5	.05886	1
FACIT_F Fatigue subscale scores	Active Control	15	34.3 ± 13.2	36.9 ± 12.4	37.6 ± 12.6	.1975	.4131
	Detox	14	43.2 ± 4.2	47.3 ± 3.7	47.0 ± 2.5	.02048[a] ↑	.00862[a] ↑
Athens Insomnia scores	Active Control	17	9 ± 4	7 ± 5	7 ± 4	.1057	.1989
	Detox	16	7 ± 4	5 ± 5	5 ± 3	.1248	.03028[a] ↓
PHQ9 scores	Active Control	16	5 ± 4	3 ± 3	3 ± 2	.03212[a] ↓	.00801[a] ↓
	Detox	16	3 ± 1	2 ± 1	2 ± 2	.001712[a] ↓	.01384[a] ↓
PHQ15 scores	Active Control	17	8 ± 3	6 ± 3	6 ± 3	.04366[a] ↓	.02712 ↓
	Detox	17	6 ± 3	6 ± 4	5 ± 3	.03982[a] ↓	.01782[a] ↓
GAD7 scores	Active Control	17	6 ± 4	4 ± 3	4 ± 2	.01807[a] ↓	.05397
	Detox	16	2 ± 1	1 ± 1	2 ± 2	.4911	.6148
PHQ-SADS scores	Active Control	17	19 ± 9	13 ± 7	14 ± 7	.004014[a] ↓	.006311[a] ↓
	Detox	16	11 ± 4	8 ± 5	8 ± 5	.0129[a] ↓	.01412[a] ↓
Mg (A) Diet and Lifestyle scores	Active Control	17	7 ± 3	5 ± 3	5 ± 3	.01663[a] ↓	.01068[a] ↓
	Detox	17	5 ± 3	4 ± 4	3 ± 3	.2043	.0958
Mg (B) Health Conditions scores	Active Control	17	9 ± 8	8 ± 8	7 ± 5	.3434	.07676
	Detox	17	7 ± 4	3 ± 3	4 ± 5	.001597[a] ↓	.02769[a] ↓
Mg (C) Treatments, Medications, Supplements scores	Active Control	13	2 ± 2	1 ± 2	0 ± 0	.5862	.05791
	Detox	13	1 ± 1	0 ± 1	0 ± 0	.8501	.1736
Mg (D) Nervous Systems scores	Active Control	17	11 ± 6	9 ± 8	7 ± 6	.09949	.0009502[a]↓
	Detox	17	7 ± 6	5 ± 5	4 ± 4	.09753	.03575[a] ↓
Mg Total scores	Active Control	17	31 ± 14	24 ± 16	20 ± 12	.009157[a] ↓	.0003173[a] ↓
	Detox	17	19 ± 8	13 ± 8	12 ± 10	.003174[a] ↓	.009851[a] ↓
Blood draw pain scores	Active Control	14	2 ± 1	3 ± 3	NA	.02897[a] ↑	NA
	Detox	13	1 ± 0	1 ± 0	NA	.1489	NA

[a]denotes statistical significance at $P < .05$

Abbreviations: NA, not available; SD, standard deviation

mutagenicity potency. To remove the variation due to hydration levels of participants, mutagenicity potency was represented as the number of revertants per mg creatinine. Since the specific mutagenic compounds present in the urine were not identified in this study, and due to distinct toxin sensitivities between the tested strains, a sum of mutagenicity potency (total number of revertants across all tested bacterial strains) was obtained to express the urine mutagenicity for each participant at each visit.[39,64-66]

Safety and compliance

Participants were interviewed at each visit to determine if any adverse events (AEs) or serious AEs occurred since the previous visit. At the final visit, participants met with the study coordinator to review the supplement worksheet and any unused product collected.

Statistical analysis

Two-tailed, paired Student *t* tests or Wilcoxon tests were performed to compare changes between baseline and post-intervention (baseline vs. week 4, baseline vs. week 6) within each group. Two-tailed unpaired Student t-tests or Wilcoxon tests were used to assess changes between active control and detox groups at the same timepoint, where indicated. Simple linear regression analysis was performed to examine the relationship between baseline total porphyrins and baseline RBC toxic metals, D-glucaric acid, and mercapturic acid. No significant violation of model assumptions was observed. Results are reported as mean ± standard deviation (SD) in the tables and as mean ± standard error of the mean (SEM) for results presented in Figures 4 to 9. Statistical significance was set at $P < .05$. Outliers were removed based on 3.0 × interquartile range (IQR) test. All statistical evaluations were performed using R Statistical Software Package Version 3.6.0. (R Core Team, 2019) for Microsoft Windows.

Results

Clinical evaluations

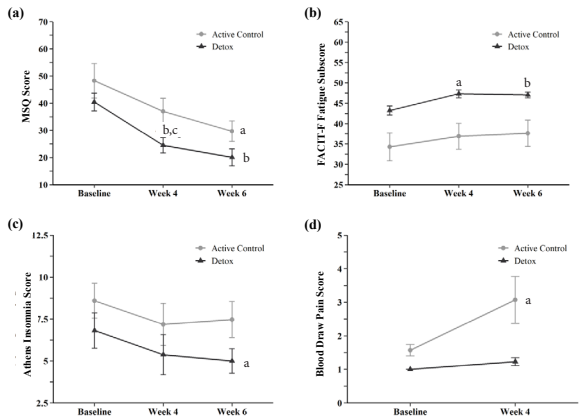
Forty adult participants (18-75 years of age) were randomly assigned to detox or active control (n = 20 per treatment arm), after meeting inclusion criteria indicative of elevated toxin burden via the Living Matrix Questionnaire.

Anthropometrics (body weight, height, and BMI) and vital signs (pulse rate and blood pressure) at baseline, 4 weeks, and 6 weeks are shown in Table 2. There were no statistically significant differences for any of these parameters over the treatment period.

Results from the administered questionnaires are presented in Table 3. Key findings from the MSQ, Athens Insomnia Scale, FACIT-F Fatigue scale, and the Blood Draw Pain Scale are presented as graphs in Figure 4. All participants showed significant improvement in their total MSQ scores following intervention ($P < .05$); see Table 3. Except for the FACIT-F questionnaire, higher scores on the behavioral questionnaires signify worse outcomes.

However, when classifying the MSQ score at baseline as low (<10), mild (10-50), moderate (50-100), and high (>100), participants with mild or moderate scores on the MSQ at baseline in the active control group had significant improvement at week 6 compared with baseline ($P < .05$) but not at week 4 ($P = .05$); see Figure 4a. Participants in the detoxification program had significantly improved MSQ scores at both time points - week 4 ($P < .01$) and week 6 ($P < .01$) compared with baseline (Figure 4a). The detoxification group had significantly improved FACIT-F fatigue subscale scores (Figure 4b) at both week 4 ($P < .05$) and week 6 ($P < .01$), compared with baseline (higher score indicates less fatigue). There was no significant improvement in FACIT-F fatigue subscale scores in the active control group at weeks 4 or 6. The detoxification group also had a significant improvement in the Athens Insomnia Scale at week 6 ($P < .05$) compared with baseline, but there was no difference at week 4 ($P = .13$); see Figure 4c. The blood drawing score increased in the active control group from baseline to week 4 ($P < .05$), while there were no changes in the detoxification group (Figure 4d). This interesting observation of altered perception of pain to a seemingly innocuous procedure (blood draw) between these two groups may indicate differences in emotional state.[67] All participants showed significant improvement in their PHQ9, PHQ15, PHQ-SADS, and the Mg status total scores (Table 3). No AEs were reported during the study.

Figure 4. The Effect of Detox on (**a**) MSQ (Participants with Mild and Moderate Scores at Baseline), (**b**) Fatigue Subscore, (**c**) Athens Insomnia Scale, and (**d**) Blood Draw Pain Questionnaires



[a]$P < .05$ - Significant Difference from Baseline
[b]$P < .01$ - Significant Difference from Baseline
[c]Significant Difference Between the Detox and the Active Control Groups, $P < .05$.

Abbreviations: FACIT-F, Functional Assessment of Chronic Illness Therapy; MSQ, Metabolic Screening Questionnaire.

Table 4. Detoxification panels.

Parameter	Group	n	Baseline Mean ± SD	Week 4 Mean ± SD	Week 6 Mean ± SD	Baseline vs. Week 4 Paired Test *P* value	Baseline vs. Week 6 Paired Test *P* value
Hepatic Detox Panel							
D-Glucaric acids, phase I, nM/mg creatinine	Active Control	15	115.3 ± 71.3	126.7 ± 65.3	153.0 ± 99.6	.2584	.1026
	Detox	16	226.9 ± 181.5	225.6 ± 166.0	217.1 ± 162.2	.8361	.816
Mercapturic acids, phase II, µM/mM creatinine	Active Control	15	61.7 ± 30.8	51.1 ± 20.6	65.5 ± 30.0	.32	.268
	Detox	16	60.9 ± 26.5	62.5 ± 18.5	65.4 ± 37.8	.7547	.6603
Creatinine, mg/dL	Active Control	16	90.4 ± 57.7	76.3 ± 31.2	105.8 ± 51.1	.3934	.05768
	Detox	15	77.3 ± 34.3	70.7 ± 35.7	79.6 ± 46.3	.1552	.978
Porphyrin Panel							
Uroporphyrins, nmol/g creatinine	Active Control	16	15.3 ± 5.5	16.2 ± 5.1	14.0 ± 3.8	.5092	.7546
	Detox	15	14.8 ± 6.0	13.7 ± 3.3	14.5 ± 4.9	.5523	.8202
Heptacarboxylporphyrins, nmol/g creatinine	Active Control	15	1.7 ± 0.7	1.6 ± 0.5	1.4 ± 0.6	.5887	.2453
	Detox	16	2.0 ± 0.9	1.9 ± 0.6	2.0 ± 0.8	.7547	.9547
Hexacarboxylporphyrins, nmol/g creatinine	Active Control	17	0.84 ± 0.37	0.85 ± 0.32	0.56 ± 0.31	.6025	.08865
	Detox	15	0.94 ± 0.48	1.02 ± 0.65	0.86 ± 0.49	.9341	.9773
Pentacarboxylporphyrins, nmol/g creatinine	Active Control	16	0.99 ± 0.37	1.22 ± 0.49	1.38 ± 0.61	.1728	.009186[a] ↑
	Detox	15	1.40 ± 0.63	1.25 ± 0.38	0.93 ± 0.23	.5717	.005866[a] ↓
Coproporphyrin I, nmol/g creatinine	Active Control	17	22.4 ± 8.1	27.4 ± 12.2	24.1 ± 9.1	.1086	.5346
	Detox	15	23.3 ± 6.9	25.5 ± 7.7	25.5 ± 9.3	.2995	.5082
Coproporphyrin III, nmol/g creatinine	Active Control	17	67.2 ± 19.2	81.2 ± 38.1	79.8 ± 26.2	.301	.08389
	Detox	16	79.9 ± 36.3	83.1 ± 30.9	76.9 ± 31.5	.8752	.4262
Coproporphyrin I to Coproporphyrin III	Active Control	17	0.34 ± 0.11	0.35 ± 0.12	0.31 ± 0.10	.5932	.03382[a] ↓
	Detox	16	0.35 ± 0.14	0.33 ± 0.09	0.37 ± 0.10	.9321	.3941
Total Porphyrins, nmol/g creatinine	Active Control	16	108.4 ± 27.7	118.6 ± 36.1	116.0 ± 27.8	.3785	.4422
	Detox	16	127.7 ± 55.4	127.9 ± 40.0	122.5 ± 44.1	1	.5699
Precoproporphyrin I, nmol/g creatinine	Active Control	17	0.55 ± 0.23	0.76 ± 0.33	0.71 ± 0.31	.006653[a] ↑	.1089
	Detox	15	0.60 ± 0.22	0.63 ± 0.18	0.67 ± 0.29	.5509	.826
Precoproporphyrin II, nmol/g creatinine	Active Control	17	0.95 ± 0.41	1.21 ± 0.64	1.01 ± 0.43	.2342	.6293
	Detox	15	1.13 ± 0.56	1.10 ± 0.39	1.07 ± 0.43	1	.9001
Precoproporphyrin III, nmol/g creatinine	Active Control	11	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	NA	NA
	Detox	13	0.00 ± 0.00	0.00 ± 0.00	0.015 ± 0.053	NA	1
Total Precoproporphyrins, nmol/g creatinine	Active Control	17	1.5 ± 0.5	2.0 ± 0.9	1.8 ± 0.7	.06125	.1404
	Detox	15	1.8 ± 0.8	1.9 ± 0.6	1.8 ± 0.8	.6292	.7006
Precoproporphyrins to Uroporphyrins	Active Control	16	0.11 ± 0.05	0.13 ± 0.05	0.13 ± 0.07	.3484	.3654
	Detox	14	0.13 ± 0.06	0.13 ± 0.03	0.13 ± 0.04	.8015	.7775
Creatinine, mg/dL	Active Control	17	86.2 ± 59.9	71.1 ± 32.8	95.4 ± 48.9	.4038	.07141
	Detox	14	69.9 ± 29.8	60.3 ± 21.9	73.1 ± 41.7	.1353	.9515

[a]denotes statistical significance at $P < .05$

Abbreviations: NA, not available; SD, standard deviation.

Urine analysis

At baseline, total porphyrins in urine were significantly correlated with RBC total toxic metals ($P < 0.01$, $r^2 = 0.21$) and urine D-glucaric acid ($P < 0.01$, r2 = 0.23), and no correlation was observed between total porphyrins and urine mercapturic acid or urine mutagenicity potency (Figure 5).

D-glucaric acid test. Numerical values for the detoxification panels are described in Table 4. In participants with elevated porphyrins (defined as urine total porphyrins >90 nmol/g creatine) at baseline, there was a trend towards a numerically greater increase in urine D-glucaric acid at week 6 compared with baseline ($P = .08$); see Figure 6a. This indicates that participants in the detox group with elevated urine total porphyrins tend to excrete more D-glucaric acid in urine, which suggests that the elevated levels are induced by the detoxification program and may be indicative of higher activity of detoxification enzymes.

Mercapturic acid test. No correlation was detected between baseline urine mercapturic acid levels and baseline urine total porphyrin levels ($P = .75$); see Figure 5c. There were no differences between baseline, week 4, and week 6 in the levels of urine mercapturic acid for either treatment group (Table 4); and this held true for participants with elevated porphyrins at baseline ($P = .31$); see Figure 6b.

Porphyrin test. Recent evidence from animal and human studies suggests that increased concentrations of porphyrins in the urine may indicate high-level exposure to heavy metals and other toxic substances.[60,68,69]

No significant changes between the detox and active control groups were detected with respect to urine total porphyrins. However, the detoxification program, but not

Table 5. DNA oxidative damage assay.

Parameter	Group	n	Baseline Mean ± SD	Week 4 Mean ± SD	Week 6 Mean ± SD	Baseline vs. Week 4 Paired Test *P* value	Baseline vs. Week 6 Paired Test *P* value
8-hydroxy-2'deoxyguanosine (8-OHdG), ng/mg creatinine	Active Control	15	10.9 ± 7.3	12.7 ± 6.2	11.4 ± 5.0	.2524	.3795
	Detox	15	9.6 ± 5.4	9.7 ± 5.7	9.3 ± 6.0	.6387	.3303
Creatinine, mg/dL	Active Control	16	91.9 ± 59.6	75.4 ± 33.5	103.3 ± 53.9	.3755	.2312
	Detox	15	77.5 ± 34.6	70.0 ± 35.4	79.2 ± 45.8	.1205	.9341

Figure 5. At Baseline, there was a Significant Correlation Between (**a**) Total Urine Porphyrins and RBC Total Toxic Metals ($P = .035$), and (**b**) Total Urine Porphyrins and Urine D-Glucaric Acid Levels ($P = .0026$). No Correlation was Found Between (**c**) Total Urine Porphyrins and Urine Mercapturic Acid Levels at Baseline, or (**d**) Between Total Urine Porphyrins and Urine Mutagenicity Potency at Baseline.

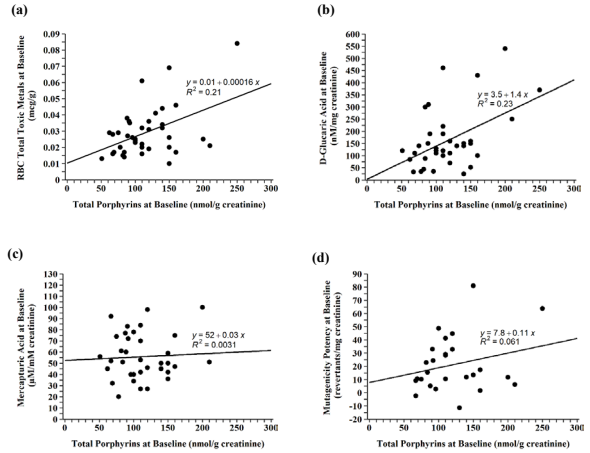


Figure 6. The Effects of Detox on (**a**) Urine D-Glucaric Acid and (**b**) Mercapturic Acid in Participants with Elevated Total Porphyrins (> 90 nmol/g Creatinine) at Baseline

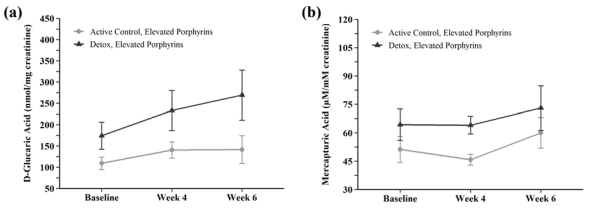
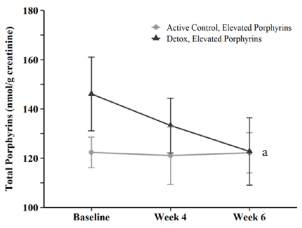
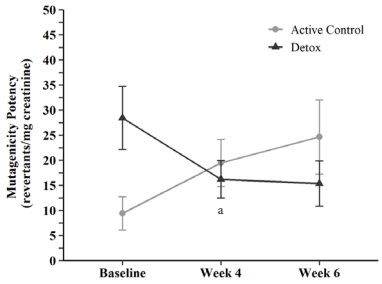


Figure 7. The Effects of Detox on Urine Total Porphyrins Levels in Participants with Total Porphyrins > 90 nmol/g Creatinine at Baseline



[a]$P < .05$ - Significant Difference from Baseline.

Figure 8. The Effect of Detox on Mutagenicity Potency (Ames Test for all TA Strains)



[a]$P < .001$ - Significant Difference from Baseline.

the active control, significantly reduced urine total porphyrins ($P < .05$) in participants that had elevated porphyrins at baseline (defined as urine total porphyrins >90 nmol/g creatine) at week 6 compared to baseline (Figure 7).

Ames test. Mutagenicity potency was significantly reduced in the detoxification program group at week 4 in comparison to the baseline ($P < .001$). Although the mutagenicity potency remained low at week 6 in the detox group, this did not reach statistical significance in comparison to the baseline ($P > .05$); see Figure 8. There were no significant differences in the urine mutagenicity potency in the active control group. The Ames test is widely accepted as a measure of toxin burden,[39,64,66,70] and these results demonstrate that the detoxification program decreased the urine toxin content.

Oxidative DNA damage assay

No differences were observed in the 8-hydroxy-2-deoxyguanosine assay, detecting oxidative DNA damage associated with toxic environmental exposures,[61,71] in either group when compared with the baseline (Table 5).

Blood analysis

Methylation panel. Results from the methylation panel are described in (Table 6). The level of methionine decreased and cystathionine increased in the detox group at week 4 compared with the baseline values, while at the same time point there were no changes in the active control group.

Analysis of toxic metals. The detox group showed a significant reduction in RBC toxic metals by 10% ($P < .01$) at week 4 in comparison to the baseline (Figure 9). We defined total RBC toxic metals as the sum of RBC levels of

Table 6. Methylation panel.

Parameter	Group	n	Baseline Mean ± SD	Week 4 Mean ± SD	Paired Test *P* value
Methionine, µmol/dL	Active Control	14	2.5 ± 0.4	2.3 ± 0.3	.1074
	Detox	16	2.7 ± 1.0	2.3 ± 0.6	.02369[a] ↓
Cysteine, µmol/dL	Active Control	14	30 ± 7	30 ± 5	.833
	Detox	16	28 ± 3	27 ± 3	.1455
SAM, nmol/L	Active Control	14	101 ± 23	98 ± 21	.53
	Detox	16	91 ± 17	90 ± 25	.2239
SAH, nmol/L	Active Control	14	21.3 ± 9.3	17.9 ± 4.7	.2718
	Detox	16	18.2 ± 6.5	20.1 ± 7.7	.605
Homocysteine, µmol/L	Active Control	14	7.8 ± 2.1	7.1 ± 1.5	.09612
	Detox	16	7.2 ± 1.9	6.8 ± 1.7	.3387
Cystathionine, µmol/dL	Active Control	14	0.02 ± 0.01	0.02 ± 0.01	.2561
	Detox	16	0.01 ± 0.01	0.05 ± 0.05	.002468[a] ↑
SAM, SAH ratio	Active Control	14	5.8 ± 2.8	5.8 ± 1.6	.8139
	Detox	16	5.6 ± 1.9	5.2 ± 2.3	.2932

[a]denotes statistical significance at $P < .05$

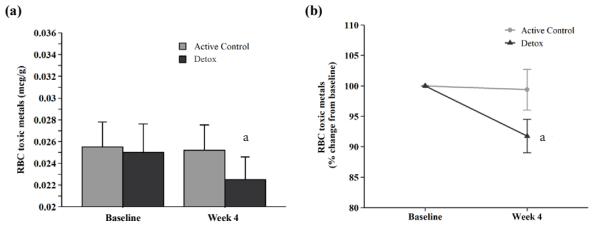
Table 7. RBC minerals panel.

Minerals	Group	n	Baseline Mean ± SD	Week 4 Mean ± SD	Paired Test *P* value
Calcium, µg/g	Active Control	14	10.6 ± 1.6	11.4 ± 3.2	.6998
	Detox	15	11.3 ± 2.1	11.3 ± 1.6	.9771
Magnesium, µg/g	Active Control	14	49.0 ± 4.5	48.8 ± 3.9	.4685
	Detox	16	49.0 ± 5.1	48.7 ± 4.8	.6219
Potassium, mEq/L	Active Control	14	88.8 ± 3.4	88.3 ± 2.6	.6228
	Detox	16	89.4 ± 1.8	88.3 ± 2.8	.2973
Phosphorus, µg/g	Active Control	14	600.7 ± 39.4	588.3 ± 38.7	.2329
	Detox	15	604.3 ± 42.3	600.5 ± 25.7	.6494
Copper, µg/g	Active Control	13	0.626 ± 0.058	0.612 ± 0.032	.3396
	Detox	14	0.637 ± 0.042	0.633 ± 0.051	.9499
Zinc, µg/g	Active Control	14	11.4 ± 1.5	11.2 ± 1.6	.3137
	Detox	16	11.3 ± 1.5	11.2 ± 1.7	.6596
Iron, µg/g	Active Control	13	943.3 ± 28.5	948.2 ± 37.3	.576
	Detox	16	948.3 ± 42.0	944.0 ± 38.8	.8971
Manganese, µg/g	Active Control	14	0.015 ± 0.004	0.015 ± 0.005	1
	Detox	16	0.017 ± 0.006	0.017 ± 0.006	.105
Selenium, µg/g	Active Control	10	0.260 ± 0.012	0.260 ± 0.013	.8457
	Detox	16	0.263 ± 0.063	0.255 ± 0.062	.1626
Boron, µg/g	Active Control	13	0.035 ± 0.009	0.045 ± 0.018	.2439
	Detox	15	0.030 ± 0.020	0.030 ± 0.021	.7197
Molybdenum, µg/g	Active Control	14	0.0003 ± 0.0001	0.0003 ± 0.0001	.6632
	Detox	15	0.0003 ± 0.0001	0.0003 ± 0.0001	.6471
Arsenic, µg/g	Active Control	14	0.0016 ± 0.0015	0.0014 ± 0.0009	.255
	Detox	15	0.0014 ± 0.0013	0.0012 ± 0.0012	.3304
Cadmium, µg/g	Active Control	14	0.0004 ± 0.0003	0.0003 ± 0.0003	.4098
	Detox	16	0.0004 ± 0.0004	0.0005 ± 0.0004	.5728
Cesium, µg/g	Active Control	14	0.0063 ± 0.0013	0.0063 ± 0.0012	.9165
	Detox	16	0.0072 ± 0.0030	0.0066 ± 0.0028	.007141[a] ↓
Chromium, µg/g	Active Control	11	0.0002 ± 0.0001	0.0003 ± 0.0000	.1427
	Detox	16	0.0003 ± 0.0001	0.0003 ± 0.0001	.4732
Lead, µg/g	Active Control	14	0.0145 ± 0.0085	0.0141 ± 0.0083	.5736
	Detox	14	0.0141 ± 0.0067	0.0129 ± 0.0056	.01189[a] ↓
Mercury, µg/g	Active Control	14	0.0026 ± 0.0020	0.0028 ± 0.0027	.8612
	Detox	16	0.0032 ± 0.0031	0.0025 ± 0.0023	.02758[a] ↓
Thallium, µg/g	Active Control	9	0	0	nd
	Detox	13	0	0	nd

[a]denotes statistical significance at $P < .05$

Abbreviations: nd, not determined.

Figure 9. The Effects of Detox on RBC Toxic Metals Levels: (**a**) RBC Toxic Metals Values Shown in mcg/g; (**b**) Percent Change in Toxic Metals Levels Compared to Baseline



[a]$P < .01$, Significant Difference from Baseline.

arsenic, cadmium, cesium, chromium, lead, mercury, and thallium. The baseline levels of the RBC toxic metals were significantly correlated with the baseline levels of porphyrins ($P < .01$). It is possible that the observed decrease in total RBC toxic metals could explain the decrease in urine total porphyrins in participants with elevated total porphyrins at baseline. The detoxification program significantly reduced cesium, lead, and mercury levels at week 4 compared with baseline ($P < .05$); see Table 7.

Discussion

This is the first randomized, single-blind, placebo-controlled trial that evaluates the effect of nutritional intervention on metabolic detoxification. The primary objectives of this study were to examine the changes in distinct biomarkers of metabolic detoxification and in scores from questionnaires assessing general health, psychological wellbeing, mood, and fatigue, at baseline compared with 4 weeks of intervention and after two weeks of a wash-out period. Secondary objectives were to examine the changes in anthropometrics and vital measures (body temperature, blood pressure, and heart rate). In this study, we enrolled patients who reported multiple symptoms associated with increased exposure to toxins or high toxin burden.

Although this is a small study, it provides compelling evidence for an important impact of nutrition on the metabolic detoxification process and improving health outcomes in participants with suspected elevated toxin burden. Given that the detoxification product used in this study is formulated to include a variety of nutritional ingredients known to promote detoxification, specific individual bioactive components that might explain the observed outcomes of this intervention are difficult to identify. In addition to dietary fiber (4.39 g per serving), which is known to aid in toxin elimination,[50,72,73] the tested product contained broccoli leaf,[48] Spanish black radish root powder,[47,74] beet root powder,[49] burdock root,[75] and milk thistle extract (80% silymarins) – all demonstrated to activate the initial phases of detoxification process. The combination of ingredients that support phases I-III of metabolic detoxification either directly or indirectly,[18,30] along with dietary and lifestyle guidelines, are likely to collectively promote a healthy detoxification process.[14,28,29]

Several notable findings were revealed by the participant questionnaires. The detox group participants with MSQ scores defined as mild to moderate at baseline, showed significant improvement in MSQ at weeks 4 and 6, which suggests that health concerns captured by the MSQ questionnaire may have been reduced by the detox program (Figure 4). Fatigue scores significantly improved in the detox group at both timepoints, weeks 4 and 6, compared with the baseline values. The detoxification group also had a significant improvement in the Athens Insomnia Scale scores at week 6, compared with the baseline values.

Importantly, we first showed that the participants with elevated total urine porphyrins had higher total RBC toxic metal and urine D-glucaric acid levels at baseline (Figure 5). Specifically, we found a significant correlation between urine porphyrin levels and RBC total toxic metals – participants with elevated RBC toxic metal levels displayed elevated urine porphyrin levels at the onset of the study. This agrees with published data demonstrating that toxic minerals, metals, and other toxic substances (i.e., arsenic and mercury) interfere with or inhibit enzymes involved in heme biosynthesis and increase the levels of porphyrins in the urine.[36,37,57,76,77] In addition, we detected a significant correlation between the levels of D-glucaric acid and porphyrins in the urine at baseline, with participants exhibiting elevated D-glucaric acid also showing elevated urine porphyrins.

At the end of the intervention period, detox group participants showed a significant reduction in RBC toxic metals, urine porphyrins and urine mutagenicity, while displaying an increase in D-glucaric acid, which together indicate an enhancement in efficiency of metabolic detoxification pathways and elimination and/or neutralization of sequestered toxins from the body. Specifically, RBC toxic metals were significantly reduced by 10% in the detoxification group at week 4. Urine total porphyrins were also significantly reduced at week 6 in the detox group participants who exhibited elevated porphyrins at baseline. Similarly, there was an increase in urine D-glucaric acid in participants in the detoxification program with elevated porphyrins at baseline when week 6 was compared with baseline, although this did not reach statistical significance. Remarkably, there was a clinically meaningful decrease in urine mutagenicity potency at weeks 4 and 6 in the detox group but not in the active control group. These findings are also in agreement with the literature and further confirm previous reports that the accumulation of toxins in cells interferes with enzymes involved in heme biosynthesis.[11,12,78-80] Removal of toxins inhibiting these enzymes within the cells would lower the accumulation of porphyrins and facilitate better heme biosynthesis. Among the enzymes involved in phase II detoxification are methyl transferases, such as catechol-O-

methyltransferase.[81-84] These enzymes are dependent on healthy cellular methylation capacity defined as a ratio of SAM/SAH > 4.85 The average SAM/SAH ratio for participants in this study was above 4 indicating that cellular methylation capacity was not an impeding factor for detoxification in this cohort. In summary, our findings of a decrease in RBC total toxic metals, elevation of urinary D-glucaric acid, decrease in urine total porphyrins, and decrease in urine mutagenicity potency provide compelling evidence for the positive effect of this intervention on supporting metabolic detoxification, mobilization, and removal of toxins at the cellular level.

Taken together, these results demonstrate clinically meaningful and beneficial effects of nutritional intervention on the measures of quality of life and the biomarkers of metabolic detoxification. The current study was not designed as a weight loss study, and we did not detect changes in participants' body weights. However, fasting is another beneficial dietary intervention that can potentially reduce toxin burden by eliminating accumulated toxins.[86] In summary, metabolic detoxification is a nutrition- and energy-dependent process, and a nutrient-rich diet and supplement support metabolic detoxification processes and avoid depletion of cofactors critical for phase I and II enzymes.

Data Availability

Data reported in this study will be available upon a reasonable request.

Author Disclosure Statement

B.F.El-K., N.S., W.Z., M.W., A.D., and J.R. are either current or former employees of Standard Process Inc.; the rest of the authors declare no conflicts of interest.

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