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100% Watermelon Juice as a Food-First Intervention to Improve Cognitive Function: Ancillary Findings from a Randomized Controlled Trial

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Abstract

Lycopene exhibits neuroprotective properties due to its antioxidant and anti-inflammatory functionality. As watermelon is a rich source of lycopene, pasteurized watermelon juice provides lycopene in its most bioavailable form. This study examined relationships between circulating lycopene, cognitive performance, and biomarkers of oxidative stress and inflammation in response to pasteurized 100% watermelon juice supplementation. A placebo-controlled, randomized, double-blind, crossover trial was conducted with postmenopausal women (n = 16, 60 + 4.1y). Participants consumed two 360mL servings of pasteurized 100% watermelon juice or a placebo beverage for four weeks. Fasting blood samples were collected, and cognitive tests were administered to assess various neurocognitive domains. Statistical analyses included mixed models and Spearman correlations. Serum lycopene exhibited a significant treatment effect (p=0.002) with a mean increase of 81%. However, this increase was not associated with changes in oxidative stress, inflammation, or cognitive function. Additional research is warranted to determine dose-durational effects for promoting cognition.

Keywords

Lycopene; watermelon; postmenopausal women; cognitive function

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Introduction

Cognition demands high rates of energy production. Unfortunately, this demand is accompanied by free radical generation as a result of electron leakage within the mitochondria.[1] Thus, the brain represents a dynamic battleground for redox balance. In context of aging, accumulation of reactive oxygen species (ROS) over the lifespan leaves the older adult brain extremely vulnerable to oxidative insults and subsequent upregulation of inflammatory cytokines. Acknowledging the growing prevalence of Alzheimer's disease and related dementias, the role of specific nutrients and dietary patterns for neuroprotection has attracted much research attention.

In animal models, supplementation of lycopene has been shown to prevent cognitive deficits through attenuation of oxidative stress and inflammation [2 3–5], and a recent functional MRI study reported improved cognitive function among older adults with higher levels of plasma lycopene. [6] Second only to tomatoes, watermelon is a rich food source of lycopene. [7] Thus, 100% watermelon juice may represent a convenient, shelf-stable means of conferring cardiovascular benefits of watermelon. Pasteurized watermelon juice may be particularly efficacious from a food safety standpoint, but pasteurization also provides lycopene in its most bioavailable form of cis-lycopene. [8]

This study was ancillary to a larger randomized controlled trial that aimed to examine the effect of 100% watermelon juice on robust measures of vascular function (NCT03626168). [9] The current study objectives were to examine the effect of 100% watermelon juice on circulating lycopene, cognitive performance, and biomarkers of oxidative stress and inflammation.

Materials and Methods

By study design, all participants were ambulatory, had a body mass index (BMI) of 18–29.9 kg/m^2, and were in overall good health without cognitive impairment at baseline as determined by a score of ≥ 24 on the Mini-Mental State Examination (MMSE). Exclusion criteria included food allergy to watermelon, terminal illness, history of hypotension or hypertension, chronic kidney disease, diabetes, previous cardiac events and procedures, smoking or other tobacco use within the past six months, use of anticoagulant, cholesterol-lowering, or blood-pressure medications, vasodilatory or antioxidant dietary supplements (garlic, fish oil), dietary supplements containing lycopene, ascorbic acid, L-glutamine, L-arginine, or L-citrulline, or weight change of ≥ 10% within the previous six months. If prescribed by a physician, participants were allowed to continue calcium and vitamin D supplementation. Prior to study initiation, all protocols were approved by the Institutional Review Board at The University of Alabama.

During the study, participants were asked to consume their typical diet apart from foods high in lycopene. Participants were provided with a list of lycopene-rich foods and were asked to limit these to two servings per day for study duration. Adherence to the low-lycopene diet was assessed by unannounced 24-hr recalls throughout the study. Following a one-week run-in period, participants were randomized to consume either two 360 mL servings of

pasteurized 100% watermelon juice or an isocalorically-matched placebo beverage for four weeks. The mean daily provision of lycopene from the intervention juice was 14.4 ± 0.34 mg. The duration of the intervention/placebo arms as well as the two-week washout period was determined based on the approximate 5 day half-life of lycopene. [10] Thus, it is plausible that mechanistic influence of lycopene on cognition can be observed within the four-week intervention arm and cleared within the two-week washout period. Following the washout period, participants received the opposite beverage for an additional four weeks. Compliance was assessed by log forms with check-off boxes for each dose and random 24-hour diet recalls. Watermelon juice was supplied by Frey Farms (Keenes, IL) from Estrella variety melons. Prior to juice distribution, replicate juice samples were extracted and analyzed for lycopene concentrations. Pre and post each intervention arm, fasting blood and urine samples were collected, and cognitive tests were administered by one trained study personnel to assess various neurocognitive domains.

Cognitive Testing

Selection of cognitive tests was guided by two full Professors of Clinical Geropsychology and the International Life Sciences Institute (ILSI) Europe Marker Initiative which identified the evidence-base for domain-specific cognitive tests with established sensitivity to nutrition interventions. [11] The following tests included in this study assessed cognitive function falling into five of the six neurocognitive domains: 5-item Delayed Recall, a Letter Fluency test, Digit Span Forward and Backward, and the Trail Making Test Part A and B.

Quantification of Bioactive Compounds in Serum

Serum was stored at −80°C until analysis at which time it was thawed and handled under dim light. Lycopene extraction was conducted according to a previously validated procedure. [12] Chromatographic separation was carried out using an ACQUITY ultra-high performance liquid chromatography system with a photodiode array detector and ACQUITY BEH Shield RP18 column (2.1 × 100mm, 1.7μm) (Waters, Milford, MA) according to a previously described method for fat-soluble micronutrients. [13] Lycopene and β-apo-8'-carotenal were sourced from Sigma-Aldrich (St. Louis, MO), and β-apo-8'-carotenal served as the internal standard. The limit of quantitation for lycopene was 0.039 μM. Samples were extracted in duplicate and run in random order.

Assessment of Biomarkers of Oxidative Stress and Inflammation

Malondialdehyde (MDA), a product of lipid peroxidation, is a biomarker of oxidative stress. Urine lipid peroxides were quantified according to the thiobarbituric acid reactive substances assay with results expressed as mM MDA. [14]

Assessment of inflammatory biomarkers included high sensitivity C-reactive protein (hs-CRP) measured on a Stanbio Sirrus (Boerne, TX) automated analyzer using Pointe Scientific (Canton, MI) turbidometric reagent. Other inflammatory biomarkers included, tumor necrosis factor-α (TNF- α), interleukin-6 (IL-6), interleukin-8 (IL-8), and interleukin-10 (IL-10) measured with a MesoScale Discovery (Rockville, MD) human pro-inflammatory panel 1 kit using chemiluminescence.

Statistical Analysis

Sample size considerations for the parent feasibility study were based on published data regarding the influence of lycopene on inflammation. In short, significant reduction in hs-CRP, an acute phase inflammatory biomarker, was observed among 24 healthy young adults consuming 2c/d tomato juice for two weeks. [15] Based on similar juice provision and an average change in serum lycopene of 0.1 μM with a standard deviation of change of 0.025 μM and a significance level of 5%, there is an 80% power to detect a significant change in lycopene with 15 participants. Although the study was powered to detect the aforementioned differences in circulating lycopene, the investigation of changes in cognitive function represents an exploratory analysis.

Generalized linear mixed models were conducted to assess changes in circulating lycopene, changes in cognitive performance, and biomarkers of oxidative stress and inflammation. [16] Partial spearman correlational analyses were conducted to assess the relationship between circulating lycopene and changes in cognition, oxidative stress, and inflammation. These correlations were computed adjusting for age and BMI. The false discovery rate was computed using associated p-values. The statistical plan determined *a priori* was not adjusted for age, ethnicity, or educational status due to the participant homogeneity. Statistical analysis was conducted using SAS software, Version 9.4. Copyright © 2002–2012 SAS Institute Inc. (Cary, NC, USA). Given the exploratory nature of these analyses, exact p-values are presented along with associations and estimates that have p-values less than 0.05.

Results

Demographic data and baseline characteristics are presented in Table 1. Adherence to the beverage consumption was 92%, thus exceeding the minimal adherence threshold of 70%. Serum lycopene exhibited a significant treatment effect (p=0.002) with a mean increase of 81%. However, changes in serum lycopene were not associated with changes in cognitive performance, and no changes in biomarkers of oxidative stress or inflammation were observed (Table 2).

Discussion

In light of recent evidence suggesting that lycopene in 100% watermelon juice may offer neuroprotection [3–5], this study examined the effect of 100% watermelon juice supplementation on cognitive performance and biomarkers of oxidative stress and inflammation using a placebo-controlled, randomized, double-blind, crossover design. Although lycopene was significantly increased in serum post-supplementation, this increase was not associated with cognitive test scores or changes in biomarkers of oxidative stress or inflammation.

Antioxidant and anti-inflammatory properties of lycopene in 100% watermelon juice underpinned the hypothesized benefits for the juice intervention to improve cognitive function. [17] However, two daily 360 mL doses of 100% watermelon juice for a four-week period did not show appreciable effects on measures of cognition, oxidative stress, or

systemic inflammation. These findings are in contrast to a recent systematic review of 10 clinical trials suggesting improvements in biomarkers of oxidative stress post-consumption of 100% fruit juice. [18] Factors that may account for result discrepancies include dose and duration of intervention as well as juice type with more highly pigmented juices shown to contain greater amounts of antioxidants. [19] Although not observed in this study, recent research suggests that sustained increases in circulating lycopene attenuate neuroinflammation and oxidative stress resulting in enhanced cognitive performance as evidenced by functional MRI. [6] Despite largely null findings reported herein, 100% watermelon juice is indeed an effective vehicle for bolstering serum lycopene. It is plausible, however, that the study duration was not long enough to assess cognitive changes.

Although our study provides insightful results, the homogeneity of participants limits generalizability. Furthermore, despite using five cognitive tests with established sensitivity to nutrition interventions [11], participants were all without cognitive impairment by study design, thus potentially precluding gross changes in cognitive function. Nevertheless, it is imperative that interventions are conducted with individuals with and without cognitive impairment to better characterize the magnitude of effect and timing. Also with regard to cognitive testing, the study findings may be limited by a potential carryover effect of changes in cognition that occurred in the first arm for participants randomized to receive the intervention first; however, with no significant improvements in cognitive performance at the start of the second arm, it is unlikely that such a effect impacted study results. It should be noted that while this study focused solely on the impact of supplemental lycopene, participants were asked to maintain their usual diets and limit lycopene-rich foods to prevent dietary confounding. Furthermore, all participants were screened for supplement intake, and any supplement that may influence cognition or other outcomes of interest was stopped during the two-week run-in through study completion to further limit confounding of results. Another strength of the study is that the dose and duration of the intervention was adequate for significantly improving serum lycopene. To confirm that the intervention beverage was the primary contributor to increasing serum lycopene, 24-hour recalls were incorporated throughout the study to randomly assess adherence to the low-lycopene diet. As previously published, the average lycopene intake across the study was 1.78 ± 3.24mg/d. [20] It should be noted that this intake is far lower than the average daily intake of lycopene, owing to the fact that participants adhered to the low-lycopene diet with some participants not consuming any dietary lycopene. [21] Lastly, this study was strengthened by the variety of cognitive tests across multiple neurocognitive domains and robust methodology for assessing circulating bioactive compounds as well as inflammatory and oxidative stress biomarkers.

Conclusion

A recent systematic review highlighted the relationship between circulating levels of lycopene and cognition [22]; however, available studies were cross-sectional or longitudinal in design, thus limiting the ability to ascribe causality. This study is the first of its kind to investigate the relationship between consumption of 100% watermelon juice and changes in cognitive performance, oxidative stress, and inflammation. As consumption of 100% watermelon juice is an effective strategy for improving circulating lycopene, additional

research is warranted to further investigate dose-durational effects and optimum timing of intervention initiation for improving or maintaining cognition.

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Kristi M. Crowe-White and Amy C. Ellis are Co-Principal Investigators who contributed equally to the design and management of this study. As such, they should be considered co-anchor authors on this article. The Principal Investigators would like to thank study participants as well as the outstanding graduate and undergraduate students who contributed greatly to the study, Frey Farms (Keenes, IL) for donating the pasteurized 100% watermelon juice, and the Alabama Research Institute on Aging for clinical guidance in shaping this study.

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Biographical Notes

Kristi Crowe-White, PhD, RD is an Associate Professor and Department Chair at the University of Alabama. Dr. Crowe-White's research focuses on redox theory and understanding the interplay between bioactive food compounds, redox and inflammatory balance, and clinical outcomes related to obesity, cardiometabolic disease, and aging. Her prevention-oriented research employs the use of genetic testing to identify genetic variants underlying responsiveness or non-responsiveness to nutrition interventions for the purpose of optimizing precision nutrition. As a result of her research, she has co-authored a position paper on functional foods, serves on several editorial boards, and holds leadership positions in the Academy of Nutrition and Dietetics, the American Society for Nutrition, as well as the Institute of Food Technologists.

Vinoth Aryan Nagabooshanam earned a B.Tech in Information Technology from Anna University followed by M.S in Data Science from Indiana University, where he investigated how data analytical methodology can be used to improve the quality of life for individuals with various disabilities. He also learned more about the capturing data from various electronic devices and various machine learning and statistical method.

Vinoth joined as statistician at University of Alabama at Birmingham in 2018, where he started working on various clinical trails in which he collaborated with investigators to identify potential statistical issues as well as to examine modeling assumption and propose resolutions and insightful findings with statistical interpretations. Vinoth also developed various voice call and text application which are used to monitor participants' performance while in research studies.

Dr. Tanja Dudenbostel, MD, FASH, FAHA is an Assistant Professor in the Vascular Biology and Hypertension Program, Division of Cardiology, Department of Medicine, at the University of Alabama at Birmingham. As an Internist and board-certified Specialist in Clinical Hypertension, she has more than 15 years of experience as a successful biomedical researcher. Her research focus is primary and secondary prevention and has been largely patient-based in evaluating mechanisms and novel treatment modalities in cardiovascular disease. Specifically, her research focuses on mechanisms of hypertension and vascular

aging and treatment options such as lifestyle, nutrition, pharmaceutical and interventional treatments to improve blood pressure, vascular function, and cardiovascular outcomes.

Dr. Julie Locher is a Medical Sociologist and Health Services Researcher. Her primary area of research focuses on social and environmental factors, including the role of social support on influencing health-related behaviors and outcomes, especially those related to eating. Her research as Principal Investigator has been consistently funded by the National Institutes of Health since 2001 on multiple mechanisms (K07, R01, R21, and R03). All of her work over the past more than twenty-five years has involved collaboration with investigators representing diverse, multidisciplinary backgrounds within and outside UAB. She has much experience in mentoring junior faculty and pre- and post-doctoral fellows. In 2011, she received the Graduate Dean's Award for Excellence in Mentorship of pre-doctoral trainees. She serves as the Co-Director for Enrichment within the UAB Nutrition Obesity Research Center (P30) and Training Director for the UAB Strategically Focused Obesity Center. The majority of her current activities focus on supporting translational and nutrition and aging research initiatives and enhancing training activities for emerging scientists across disciplines and supporting junior faculty in becoming strong investigators and mentors. In 2016, she was recognized university-wide for supporting the scientific careers of female trainees and colleagues (Becky Trigg Outstanding Woman UAB Faculty Member) and nationally for making significant contributions to gerontological research (M. Powell Lawton Award).

Tinsley Phillips Chavers, MS is a Registered Dietitian practicing in Mobile, Alabama. She completed her Master of Science in Human Nutrition at the University of Alabama. Her graduate research focused on the role of specific bioactive compounds on cognitive function and longevity. She currently uses this knowledge base in her treatment recommendations to adults with diabetes to contribute to better disease control and overall well-being.

Amy Ellis, PhD, RD is a Registered Dietitian and Associate Professor at the University of Alabama where she teaches graduate and undergraduate courses in clinical nutrition and translational nutrition science. Her research interests include changes in body composition with aging and influences of genetic variation on individual responses to diet interventions.

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Take Away Points

Dietary supplementation of lycopene may improve cognition as a result of its antioxidant and anti-inflammatory functionality. As pasteurized watermelon juice provides lycopene in its most bioavailable form, results demonstrated significant increases (81%) in serum lycopene. However, this increase was not associated with changes in oxidative stress, inflammation, or cognitive function. Additional research is warranted to determine dose-durational effects of supplementation for promoting cognition.

Table 1.

Baseline Characteristics

Demographics	**Mean + SD**
Age (y)	59.5 ± 3.9
BMI (kg/m^2)	25.1 ± 3.7
Ethnicity (%)	
Hispanic or Latino	0
Not Hispanic or Latino	100
Education Level	
High School	2
College/Post-graduate	14
Serum Measures	
Lycopene (uM)	1.45 ± 0.81
TBARS (um MDA)	0.47 ± 0.47
hsCRP (mg/L)	2.17 ± 3.43
IL-6 (pg/mL)	0.73± 0.75
IL-8 (pg/mL)	14.13 ± 5.37
IL-10 (pg/mL)	0.30 ± 0.17
TNF-α (pg/mL)	2.43 ± 0.40
Cognitive Testing [a]	
Letter Fluency[a]	13.5 ± 5.25
Digit Span Forward[b]	10.9 ± 2.5
Digit Span Backwards[c]	6.3 ± 2.7
Delayed Recall[d]	14.1 ± 1.1
Trail Making Test A[e]	73.7 ± 16.3
Trail Making Test B[e]	127.7 ± 50.4

N = 16 participants who completed the ancillary study

BMI = body mass index, WJ = 100% watermelon juice, TBARS = thiobarbituric acid reactive substances, hsCRP = high-sensitivity C-reactive protein, IL = interleukins, TNF-α = tumor necrosis factor-α

[a]Mean number of original words given for three different letters over a one-minute span each.

[b]Mean number of correct digit spans repeated forwards.

[c]Mean number of correct digit spans repeated backwards.

[d]Mean score for the five-item delayed recall among three attempts.

[e]Mean number of correct forward digit spans repeated.

Table 2.

Changes in Serum Measures and Cognitive Testing in Response to 100% Watermelon Juice Consumption

Serum Measures	Coefficients + SEM
Lycopene (uM)	0.548 ± 0.175 *
TBARS (um MDA)	−0.014+0.182
hsCRP (mg/L)	−0.091 ± 0.157
IL-6 (pg/mL)	−0.158 ± 0.112
IL-8 (pg/mL)	−0.100 ± 0.098
IL-10 (pg/mL)	−0.017 ± 0.092
TNF-α (pg/mL)	−0.052 ± 0.083
Cognitive Testing [a]	
Letter Fluency[a]	0.023 ± 0.058
Digit Span Forward[b]	0.039 ± 0.038
Digit Span Backwards[c]	0.126 ± 0.074
Delayed Recall[d]	−0.019 ± 0.027
Trail Making Test A[e]	0.024 ± 0.104
Trail Making Test B[e]	0.117 ± 0.095

BMI = body mass index, TBARS = thiobarbituric acid reactive substances, hsCRP = high-sensitivity C-reactive protein, IL = interleukins, TNF-α = tumor necrosis factor-α

[a] Mean number of original words given for three different letters over a one-minute span each.

[b] Mean number of correct digit spans repeated forwards.

[c] Mean number of correct digit spans repeated backwards.

[d] Mean score for the five-item delayed recall among three attempts.

[e] Mean number of correct forward digit spans repeated.

* $p < 0.01$.