Metering Mood with Veggies: Association of skin carotenoid levels and depressive symptoms or mood disturbances

By

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Metering Mood with Veggies: Association of skin carotenoid levels and depressive symptoms or mood disturbances

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Abstract

Background: Mood disturbances and depressive symptoms are a common occurrence among those with Alzheimer's disease (AD). They are associated with poor quality of life for the person with AD, as well as increased caregiver burden which can result in earlier institutionalization. In the search for more effective therapeutic interventions, nutritional interventions are being explored to target specific pathways in the body thought to be associated with mood disturbances and depression.

Objective: The purpose of this thesis project was to determine if there is an association between skin carotenoid status and depressive symptoms or mood disturbances in cognitively normal older adults.

Design: Baseline data were collected from 109 cognitively normal men and women ages 65 years and older from the Nutrition Interventions for Cognitive Enhancement (NICE) cohort study. Skin carotenoid status was measured by the Veggie Meter®. Mood disturbances and depressive symptoms were measured by the Geriatric Depression Scale – Short Form and subscales and composite scores from the RAND 36-Item Health Survey.

Results: Participants were predominantly white, non-Hispanic females (89%, 91.7%, 79.8%, respectively) and had a mean BMI of 28.3 ± 4.6 kg/m^2. Participants had a mean Veggie Meter® score of 269.99 ± 84.25. Significant correlations were found between Veggie Meter® score and the physical functioning, social functioning, and general health RAND subscales ($p<0.05$), but not mood or depressive symptoms.

Conclusions: The data suggest no association between skin carotenoid status and mood disturbances or depressive symptoms. Further research with less exclusion criteria, multiple data

collection points, and more specific tools for mood disturbances and depressive symptoms is needed.

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Chapter 1: Introduction

1.1 Background

Mood disturbances and depressive symptoms are a common occurrence among those with Alzheimer's disease (AD). Apathy, depression, aggression, anxiety, and sleep disorder are the most prevalent types of disturbances in AD (1). Nearly 77% of AD participants experienced at least one mood disturbance (2). The combination of mood disturbances and AD is associated with poor quality of life for the person, as well as increased caregiver burden which can result in earlier institutionalization (3). Currently there is no cure for AD, so intervention for alleviating the burden of the disease is needed. Non-pharmacologic treatments for mood disturbances include light therapy, cognitive stimulation, music-based therapy, and cognitive behavioral therapy (4). Despite these options, there is a need for more effective therapeutic interventions. Nutritional interventions are being explored to target specific pathways in the body thought to be associated with mood disturbances. Carotenoids are one nutritional approach that is being researched.

1.2 Reasons for Further Investigation

Limited research exists on the relationship between carotenoids and mood disturbances in AD, but the research that does exist suggests a need for further investigation. Milaneschi and colleagues reported a significantly lower risk of depressed mood in older adults with higher plasma carotenoid levels ($p=0.04$)(5). When these participants were assessed at the 6-year follow-up, the rate of depressed mood was lower in those with higher plasma levels ($p=0.04$)(5). Repeated studies of this nature are needed to strengthen the evidence for this association. Identifying an association would open the door to investigation of a carotenoid supplement or diet intervention to alleviate depressive symptoms and mood disturbances in this population.

1.3 Purpose Statement

The purpose of this study was to determine if there is an association between skin carotenoid status and depressive symptoms or mood disturbances in cognitively normal older adults.

1.4 Research Question

Is there a relationship between skin carotenoid status and depressive symptoms or mood disturbances in cognitively normal older adults? Skin carotenoid status was measured by the Veggie Meter®. Mood disturbances and depressive symptoms were measured by the Geriatric Depression Scale – Short Form and subscales and composite scores from the RAND 36-Item Health Survey.

Chapter 2: Literature Review

2.1 What is Alzheimer's disease?

Alzheimer's disease (AD) is a progressive and fatal brain disease that damages and destroys neurons (nerve cells) that control thinking, learning, and memory. This disease is responsible for 60-80% of dementia cases making it the most common cause of dementia (6). Researchers do not fully understand the cause of the disease and no cure currently exists. AD is characterized by neuron death that causes brain tissue damage (6). The neuron death is currently understood to be caused by a buildup of beta-amyloid plaques outside of the neuron and tau tangles inside the neuron (6). Other hypothesized pathologies for AD are impaired glucose metabolism in the brain, chronic inflammation from overworked microglia that clear the plaques and tangles, and cell loss that causes atrophy of the brain (6). In addition to dementia, AD leads to major complications such as immobility, infections, swallowing disorders, and malnutrition (6). These complications can lead to the two main causes of death in this population which are pneumonia and circulatory system disease (7). Alzheimer's disease is a devastating disease that is still very unknown.

2.1.1 Diagnosis and progression

Currently, there is no singular diagnostic test for AD, so a variety of methods are used for making a diagnosis. A diagnosis is made utilizing a combination of medical and family history, brain imaging, blood tests, physical and neurologic examinations, input from family, and various cognitive tests (6). The disease is best described by a continuum of three phases defined by symptom severity. In the preclinical stage, the person does not experience symptoms and the disease is often unnoticeable (6). The next phase is described as mild cognitive impairment (MCI) due to AD and the person will begin to experience very mild symptoms (6). Lastly, the

third phase is described as dementia due to AD and is categorized into mild, moderate, and severe (6). Hallmark symptoms of AD "include impaired communication, disorientation, confusion, poor judgment, behavioral changes, and difficulty speaking, swallowing, and walking" (6). Although similar, each person's diagnosis and disease progression are unique.

2.1.2 Prevalence

Alzheimer's disease is a prevalent disease that continues to grow in prevalence. An estimated 5.8 million Americans age 65 and older currently are living with AD (8). By 2025, this number is expected to increase by 22% to 7.1 million (8). The mortality rate of AD is similarly high. In 2018, AD was the 6th leading cause of death in the United States (9). The three main risk factors associated with the disease are increased age, genetics, and family history of AD (6, 8), but having one or more of these risk factors does not mean a guaranteed diagnosis. Having the apolipoprotein E4 (APOE-e4) gene is associated with AD, but not all people with AD have it. In a sample of 1,770 people with AD, 65% had at least one copy of the apolipoprotein E4 (APOE-e4) gene (10). Fifty-six percent of Americans with AD had one copy of the APOE-e4 gene (11). As prevalence of the disease expands, more research is needed to understand why AD occurs.

2.1.3 Economic and public health impact

The time and costs associated with AD rival those of other major diseases. In 2016, AD was the 6th most burdensome disease or injury as measured by disability-adjusted life-years (12). An estimated 16 million Americans are unpaid caregivers for someone with AD or other dementias (6). This amounts to 18.6 billion hours of unpaid caregiving which would cost approximately $244 billion (6). The yearly cost per person of unpaid care is $41,689 and healthcare costs are $33,329 (13). The cost of healthcare is calculated by the cost of nursing home care, out of pocket

spending, paid home care, and Medicare (13). Alzheimer's disease is a burden to the person, caregivers, and the healthcare system. One of the many burdens involves mood disturbances observed in individuals with AD.

2.2 What are mood disturbances?

Identifying those with AD is further complicated by the presence of mood disturbances. Mood disturbances refer to the affective, non-cognitive symptoms displayed in people. They are also referred to as neuropsychiatric symptoms (NPS). Neuropsychiatric symptoms can hide or overshadow clinical signs that would trigger an assessment for AD. The most common neuropsychiatric symptoms include anxiety, insomnia, regular headaches, excessive sleepiness, attention deficits, memory loss, and depression (14). These symptoms are experienced by 36.6% of adults living in the United States, which amounts to an estimated 81.6 million people (14). The prevalence of mood disturbances in the general population masks whether mood disturbances increase the risk of AD or are comorbidities of the disease. "Baseline agitation, nighttime behaviors, depression, and apathy" in subjects with mild cognitive impairment were associated with risk of incident dementia (15). The subtlety of this association between AD and mood disturbances continues to be researched.

2.2.1 Methods of measuring

Mood disturbances in AD are measured in various ways. The Neuropsychiatric Inventory (NPI) measures frequency and severity of symptoms by obtaining information from a caregiver and is a common assessment in this population (16). The shortened five-item Geriatric Depression Scale (GDS) is used in combination with questions from the NPI to create an overall behavioral score (3). Seven different assessments have been used to assess 12 common neuropsychiatric

symptoms (1). They include the five-item GDS (17), NPI (16), Behavioral Pathology in Alzheimer's Disease Scale (18), Consortium to Establish a Registry for Alzheimer's Disease Behavior Rating Scale for Dementia (19), Cornell Scale for Depression in Dementia (20), and the Hamilton Rating Scale for Depression (1). Despite validated assessment tools for mood disturbances, limitations occur in these assessments.

2.2.2 Biases and limitations in measuring mood disturbances

There are many biases and limitations in studies that measured mood disturbances. Elderly subjects and their research partners' responses showed differences in the assessment of irritability and apathy of the subject ($p<0.015$) (3). The research partners rated the subjects as more irritable and apathetic than the subjects viewed themselves (3). It is unclear if this is from self-unawareness of the elderly subjects or biases of the partners. This limitation exists in non-demented elderly populations and is further exacerbated when the subject is cognitively impaired. Additional limitations identified in prior published research included lack of heterogeneity between study designs and results, some neuropsychiatric symptoms missing on assessments, questionable quality of the studies due to attrition reporting, cross-sectional and clinic-based studies, and the inability to rule out publication bias of the studies (1). Beyond limitations of the tools themselves, difficulties arise when measuring mood disturbances in elderly or cognitively impaired individuals.

2.2.3 Measuring in cognitively normal versus cognitively impaired

Differences in cognitively normal versus cognitively impaired populations present challenges in how mood disturbances are assessed. Cognitive decline in those with dementia warrants the need for a caregiver to be involved in the assessment (2, 21). The subjective nature of the assessments

creates the problem of ensuring caregiver assessments are valid, reliable, and free from bias. Differences were found in depression assessments as reported by the AD patient and the nurse caregiver, suggesting the need for reports from both parties, despite the cognitive impairments of the patient (22). It is also important to choose an assessment that is validated in a demented population. The Cornell Scale for Depression in Dementia is a valid and specific assessment tool in a demented population, whereas the Geriatric Depression Scale lacks validity in this population (23). The validity of assessments is a vital aspect of researching mood disturbances.

2.3 Mood disturbances in Alzheimer's disease

Mood disturbances are a common occurrence among those with AD. In this population, they are also referred to as Behavioral and Psychological Symptoms of Dementia. Twelve symptoms categorized into four sub-syndromes have been identified (1). The sub-syndromes are "hyperactivity (aggression, disinhibition, irritability, aberrant motor behavior, and euphoria), psychosis (delusion, hallucination, and sleep disorder), affective (depression and anxiety), and apathy (apathy and appetite disorder)" (1). This appears to be the first meta-analysis that specifically studies the prevalence of mood disturbances in AD. Among the different mood disturbances, certain types are more prevalent among those with AD. Apathy, depression, aggression, anxiety, and sleep disorder are the most prevalent types of disturbances in AD (1). Individual studies had wide ranges of results, but the pooled prevalence percentages showed that nine of the types were prevalent in over 30% of participants (1). Apathy, the most prevalent disturbance, was reported in 25 studies and had an overall pooled prevalence of 49% (95% CI 41-57%) (1). Depression had a prevalence of 42% (95% CI 37–46%) and aggression a prevalence of 40% (95% CI 33–46%) (1). Nearly 77% of AD participants experienced at least

one mood disturbance (2). Apathy and depression are the most common mood disturbances in those with AD.

2.3.1 Negative impacts

Mood disturbances and behavioral symptoms have negative impacts on the patient, as well as caregivers. An association between apathy and severity of cognitive impairment has been reported (1). Further research is needed to determine the association of mood disturbances and the cognitive impairment seen in AD. Behavioral symptoms are associated with a poor quality of life and a decreased ability to perform activities of daily living (ADL) (3). Mood disturbances are associated with a poor quality of life ($p<0.0001$) (3). Decline in autonomy due to decreased ability to perform ADL, contributes to an increase in caregiver burden. Increases in agitation, aggression, and irritability, not only presents a risk of injury to the patient, but also a need for earlier institutionalization (2). Caregivers are tasked with managing the behavioral and emotional aspects of the disease, on top of many other tasks.

2.3.2 Current therapeutic interventions

Limited therapeutic interventions currently exist for mood disturbances in AD. Hallucinations, aggression, and agitation are treated with antipsychotic medications, but this comes with an increased risk of stroke and death warranting a “black box warning” (24, 25). Non-pharmacologic treatments are more effective than pharmacologic treatments when targeting agitation and aggression in dementia (4). Non-pharmacologic treatments include light therapy, cognitive stimulation, music-based therapy, and cognitive behavioral therapy (4). With a need for more effective therapeutic interventions, nutritional interventions are being explored to target

specific pathways in the body thought to be associated with mood disturbances. Consumption and levels of carotenoids are one nutritional approach that is being researched.

2.4 What are carotenoids?

Carotenoids are phytochemicals primarily found in orange pigmented fruits and vegetables, as well as eggs and dairy foods. The major types include beta-carotene, alpha-carotene, lycopene, lutein, and xanthophylls (26). Many carotenoids are precursors for Vitamin A in the body and all are believed to play a role in reducing oxidative stress. Consumption of vegetables and fruits, such as those containing carotenoids, is associated with reduced incidence of chronic diseases, such as diabetes, hypertension, coronary heart diseases, and cancers (27). Carotenoids, among other antioxidants, are well known for their protective role in chronic diseases.

2.4.1 Methods of measuring

Carotenoid status is assessed using plasma concentrations of blood samples or concentrations of skin or retinal pigmentation. Serum concentration of beta-carotene is commonly assessed using high-performance liquid chromatography from a blood sample (21, 28-30). This method is validated, but a need for a quicker and non-invasive method led to the assessment of skin pigmentation. The Veggie Meter® is a tool used to assess skin carotenoid concentration by measuring the yellow coloration of the skin which is correlated with consumption of carotenoid containing foods and supplements (26). Both the blood and skin assessment techniques utilize spectroscopy to assess carotenoid status.

2.4.2 Correlation of dietary intake and carotenoid status

Carotenoid status is associated with consumption of carotenoid containing foods and supplements. A positive correlation was found between carotenoid skin score and weekly consumption of dark green leafy vegetables ($p<0.001$) and carrots or pumpkin ($p<0.001$) (26). High carotenoid containing diet patterns, such as the Mediterranean Diet (Med Diet), have impacted carotenoid status. An association was found between high adherence to the Med Diet (Med Diet Score ≥ 6) and increased plasma carotenoid levels (28). An increase in plasma concentrations of beta-carotene ($p<0.001$) and lutein ($p<0.001$) after a 12-week Mediterranean style diet intervention was detected (29). Carotenoid status is used as a biomarker for fruit and vegetable intake.

2.5 Connection between carotenoids and brain health

Carotenoids and brain health are associated, specifically in the prevention of neurodegenerative diseases, but the exact association is still being researched. The brains of five elderly cadavers were dissected and the carotenoid concentrations of brain tissue found in the frontal lobe cortex and occipital cortex were reported (31). They identified at least 16 carotenoids in the brain tissues, of which xanthophylls accounted for 66-77% of all carotenoids that were identified in the tissues (31). This was the first report to assess the concentrations of carotenoids in the brain. Accumulating studies found the dietary intake of beta-carotene was associated with a lower risk of AD (95% CI: 0.73, 1.03) (32). However, other studies did not find any associations between beta-carotene consumption and risk of AD (33, 34). A recent pilot study found participants with AD had lower plasma beta-carotene levels compared to healthy controls ($p=0.001$) (30) and a positive correlation between plasma beta-carotene and telomerase activity ($p=0.008$) (30). This

suggests that beta-carotene may have a modulating effect on telomerase activity and may be protective for the effect on telomeres and age-related disease (30).

2.5.1 Carotenoids and cognitive function

Limited evidence exists for the relationship between carotenoids and cognitive function of those already diagnosed with AD. The plasma carotenoid levels of those with MCI and AD were assessed and depletions were found (35). Compared to controls, participants with AD had depleted levels of lutein and zeaxanthin ($p<0.0001$) and beta-cryptoxanthin and alpha-carotene ($p< 0.001$) (35). Improvements in verbal fluency scores ($p<0.03$) were reported following a lutein or combination lutein and docosahexaenoic acid (DHA) supplement (36). Improvements in memory score and rate of learning were observed in those taking the combination supplement ($p<0.03$) (36). More research is needed to determine the association between carotenoids and cognitive function.

2.5.2 Carotenoids and mood disturbances

Limited research exists on the relationship between carotenoids and mood disturbances in AD. One study examined the impact of lutein supplementation alone or combined with docosahexaenoic acid (DHA). Both were found to not have a significant effect on mood after four months of supplementation (36). In a different study, a lower risk of depressed mood was found in participants with higher plasma carotenoid levels at baseline ($p= 0.04$)(5). When these participants were assessed at the 6-year follow-up, the rate of depressed mood was lower in those with higher plasma levels ($p=0.04$) (5). Repeated studies of this nature are needed to strengthen the evidence for an association.

2.6 Nutrition intervention for mood disturbances

Nutrition interventions for mood disturbances in MCI or AD are lacking. A 1-month pilot intervention study of daily apple juice consumption was associated with improvement in caregiver reports of mood and behavior (37). A 27% improvement ($p<0.001$) from baseline in mood and behavior was reported in the cohort (37). The carotenoid profile of apple juice is relatively low, so this study did not contribute to the pool of research on carotenoid interventions. This study appears to be the only study of a single food consumption and the effect of mood disturbances in AD.

2.6.1 Supplementation intervention

Research on carotenoid containing dietary supplements for mood disturbances is also very limited. The association of a nutraceutical formulation (NF) and the effect on Behavioral and Psychological Symptoms of Dementia has been studied (38-41). The NF was not carotenoid containing as it consisted of folate, vitamin B6, alpha-tocopherol, S-adenosyl methionine, N-acetyl cysteine, and acetyl-L-carnitine (38-41). The effectiveness of the NF varied across studies. The only carotenoid supplement intervention for mood disturbances to date supplemented subjects with a xanthophyll carotenoid only formulation or a formulation that also contained fish oil (21). Positive anecdotal improvements of mood were recorded by caregivers in those receiving the combination supplement (21). The strength of this study was limited due to mood being anecdotally reported by caregivers instead of using a validated assessment tool.

2.6.2 Mediterranean or modified Mediterranean diet intervention

The Mediterranean diet is a dietary pattern reflecting eating patterns of those who live near the Mediterranean Sea. The plant-based nature of the dietary pattern and modified versions of the pattern are associated with beneficial impacts on chronic conditions. The association between adherence to the Med Diet and the risk of stroke, depression, cognitive impairment, and Parkinson's disease has been reported (42). Decreased risk of depression (RR = 0.68, 95% CI: 0.54–0.86) and cognitive impairment (RR = 0.60, 95% CI: 0.43–0.83) was associated with high adherence to the diet (42). The association of a Med Diet intervention, carotenoid status, and mood disturbances in AD has not been researched.

2.7 Conclusion

Mood disturbances are prevalent in those with mild cognitive impairment and Alzheimer's disease and cause significant impacts on the person and caregivers. A lack of effective treatment presents a need for further research in interventions. Research is needed to further the understanding of the relationship between carotenoid status and mood disturbances in older adults as a means of preliminary research for the treatment of mood disturbances in AD. Conducting research in populations with AD presents many challenges, so research often begins with cognitively normal adults. Additional research on the effects of a Mediterranean style diet intervention on carotenoid status and mood disturbances will contribute to the pool of research on nutrition interventions for mood disturbances in AD.

Chapter 3: Methods

3.1 Overview

This study was a secondary data analysis using data from the Nutrition Interventions for Cognitive Enhancement (NICE) study (KUMC IRB # STUDY00142752; NCT03841539). The NICE study is a randomized clinical trial intervention study that follows cohorts of cognitively normal older adults that have been randomized to a Mediterranean diet or low-fat diet. To assess the relationship between skin carotenoid status and depressive symptoms and mood disturbances, baseline data from the NICE study was used. Baseline data included Veggie Meter® score, Geriatric Depression Scale- Short Form score, RAND 36-Item Health Survey subscale and composite scores, body weight, height, waist circumference, health history questionnaire responses, and demographics.

3.2 Subject Criteria

Inclusion and exclusion criteria were the same as the NICE study. See Table 1 Subject Criteria for detailed criteria. Since only baseline data were used, no interventions took place which eliminated the need for a control group. 109 subjects from four cohorts were analyzed. The cohorts were recruited at ~3-month intervals.

Table 1. Subject Criteria

Inclusion Criteria
1. Cognitively normal older adults ≥65 years. This is defined by a Mini-Mental State Examination (MMSE) score ≥ 25 (43); and AD dementia screening (AD8) score of 2 or less (44); no prior diagnosis of mild cognitive decline, AD or dementia; and not medically treated for cognitive impairment or dementia)
2. Speak English as primary language
3. Be able to read and write in English
4. Live in the Greater Kansas City, Metropolitan Area
5. Body Mass Index (BMI) range between 20 - 40 kg/m^2
Exclusion Criteria
1. Serious medical risk such as type 1 diabetes mellitus, cancer, recent cardiac event (e.g. heart attack, angioplasty)
2. Taking the prescription drug Warfarin
3. Taking a prescription fish oil, such as Lovaza, Omtryg, Vascepa, Epanova, etc, or prescribed a dose of over the counter (OTC) fish oil containing ≥300 mg Docosahexaenoic Acid (DHA) that cannot be adjusted to a lower dose.
4. Tree Nut allergy or fish allergy (not including shellfish)
5. Adherence to specialized diet regimes (e.g., vegan, bariatric, renal, etc.) that make following either of the dietary plans impossible or unsafe.
6. Unwilling to be randomized to one of two diets
7. Does not have access to or independence over grocery shopping and meal preparation (i.e. those in military, retirement community with reliance on dining facilities for meals, etc.)
8. Already consuming a Mediterranean diet as determined using the 14-item Mediterranean Diet Assessment Tool (score of ≥8) (45)
9. Already consuming a low-fat diet as determined by the National Cancer Institute (NCI) Percentage Energy from Fat Screener (≤ 15% of calories from fat) (46)
10. Evidence of severe major depression (Geriatric Depression Scale-Short Form (GDS) ≥9) (47) or presence of a major psychiatric disorder that in the investigator's opinion, could interfere with adherence to research assessments or procedures

11. Alcohol (over 3 drinks per day or total of 18 per week) or drug abuse, defined as the use of chemical substances (prescription, OTC, or illegal drugs) in a pattern that can lead to an increased risk of problems and an inability to control the use of the substance 12. Already participating in another research study 13. Another member of household is already participating in this study 14. Have a visual impairment that greatly diminishes ability to read or write 15. Currently attempting to lose weight
Participation in other research studies
A participant may not participate in another research study while participating in this study

3.2.1 Recruitment

Recruitment is ongoing. Recruitment is supported by the KU Alzheimer's Disease Center Recruitment Division, which is a part of the KU Alzheimer's Disease Center Outreach and Recruitment Core (P30AG035982). The Recruitment Database contains over 6500 older adults with and without cognitive change who have expressed interest in participating in research. A listing on the Alzheimer's Prevention Registry website, as well as in their newsletter, email and Facebook account, was also utilized for recruitment. All interested individuals were referred to the Recruitment Division of the KU ADC for further information and to initiate the screening and enrollment process. Additional recruitment efforts included emails and flyers posted within the University of Kansas Medical Center community, flyers posted in the local Kansas City Metro community, website advertisements, including the Pioneers Research website, the Frontiers recruitment registry, HERON database, or community posts through sites like Facebook or Nextdoor. Potential subjects were then screened by NICE study staff for eligibility with their information from the database. This information includes, but is not limited to: age, gender, ethnicity, and race. Additional screening was performed at the baseline visit, after obtaining written consent.

3.3 Setting

All research was conducted at the University of Kansas Medical Center. Participants had baseline data obtained in-person at the KU Clinical Research Center in Fairway, KS. Records are kept in the NICE Study Coordinator's office, G031 Center for Child Health and Development, in a locked filed cabinet. Approximate time frames for baseline data collection of the four cohorts used were Fall 2018, Spring 2019, Fall 2019, and Spring 2020.

3.4 Ethics

This thesis project was covered under the existing approved KUMC Human Subjects Committee protocol for the NICE study. Written consent forms were obtained from all subjects prior to baseline data collection.

3.5 Procedures

Only selected parts of the NICE study were included in this study, so only those being used for this student thesis project were included here even though subjects underwent further testing. This was an observational study using only baseline data, so no nutrition interventions were included in the procedures. All procedures and tests were for research purposes only. Participants, their families, or third-party payers were not billed for research assessments. Specific individual results were not provided to the participants.

Subjects underwent the following:

a. Phone screener for inclusion/exclusion criteria check completed prior to scheduling potential subject for their first in-person visit. A verbal consent was administered.

b. Informed consent process.

c. Then the following measures were obtained as outcome data at baseline.

Health History was collected using a brief questionnaire regarding medical and surgical history, prescription and over the counter medication use, alcohol and tobacco habits, weight history, marital status, and socioeconomic questions. For this thesis project, only marital status was used from this survey.

Depression was evaluated by the Geriatric Depression Scale – Short Form (GDS) (47) to determine eligibility for the NICE study. The GDS contains 15 questions and is a validated tool to screen for depression in older adults (47). Scores range from 0 to 15, with a higher score indicating more depressive symptoms (48). A score of 0-4 is considered within normal range, a score of 5-9 indicates mild depression, and a score of 10 or more indicates moderate to severe depression (48). For the NICE study, severe major depression or presence of a major psychiatric disorder were exclusionary criteria. This was identified either by a previous diagnosis or a GDS score of $\geq$ 9. For this thesis project, scores were analyzed as a primary outcome.

Body Composition (body weight, height, and waist circumference) were measured and recorded. Participants reported to the Clinical Research Center (CRC) between 7 and 10 a.m. after an overnight fast. Weight was obtained by trained research staff using a digital scale (± 0.1 kg; Befour Inc model #PS6600, Saukville, WI) and height was measured using a stadiometer (Model PE-WM-60-84, Perspective Enterprises, Portage, MI). Body mass index (kg/m^2) was calculated. Waist circumference was obtained using a calibrated tape (49). All anthropometric measurements were completed in about 15 minutes.

Quality of life was assessed using the RAND 36-Item Health Survey (SF-36) (50). The SF-36 assesses physical, mental, and social health. The full survey is divided into eight subscales:

physical functioning, social functioning, role limitations (physical), role limitations (emotional), mental health, vitality, pain, and general health perception (51). The scaled scores are calculated by taking responses and converting them to a 0 to 100 scale where 100 represents better quality of life (51). A physical and mental composite T score were then determined using the subscales and previous data, as described in previous published work (52). The full 36-item survey was given to participants and was analyzed, but for this thesis project the primary interest was on the mental composite score and the subscales role limitations (emotional), emotional well-being, and energy/fatigue as those are the ones that most pertain to the specific research question.

Skin carotenoids were measured using a non-invasive optical biomarker assessment tool, the Veggie Meter® (Longevity Link Corporation). This is a tool that uses reflection spectroscopy to measure skin carotenoid levels which can then be associated with fruit and vegetable consumption. The participant placed an index finger in the probe for about 10-15 seconds while the skin tissue was being measured. A total of three measurements were taken and averaged. Veggie Meter® scores can range from 0-800 with a higher score representing a higher concentration of carotenoids in the skin. No exact score interpretation is currently available.

Baseline testing for the NICE study was completed in 2-3 visits, however all assessments and procedures being analyzed in this study were conducted in the initial assessment visit.

3.6 Materials

Table 2. Data and Instruments

Instrument	Data Being Collected
Alzheimer's Disease Center Recruitment Database	Age, gender, ethnicity, and race
Health History Survey	Marital status
Geriatric Depression Scale – Short Form	Depression score (0-15)
Fasting Anthropometrics	Weight, height, waist circumference, calculated BMI
RAND 36-Item Health Survey	Eight subscale scores, physical composite score, mental composite score
Veggie Meter®	Skin carotenoid level as measured by Veggie Meter® score (0-800)

3.7 Analysis of Data

The statistical analysis for this thesis project included descriptive statistics, Pearson correlation coefficient, and Spearman's rank correlation coefficient. SPSS software version 26 was used for descriptive statistics and Spearman's rank correlation coefficients. JMP Pro software version 15.2.0 was used for Pearson correlation coefficients. Descriptive statistics (mean, standard deviations, and frequencies) were used for demographics, marital status, anthropometrics, Veggie Meter® score, GDS score, SF-36 subscale scores, and SF-36 composite scores. Spearman's rank correlation coefficient was used for Veggie Meter® score and the SF-36 subscales and composite scores. Pearson correlations coefficient was used for Veggie Meter® score and the SF-36 subscales and composite scores, as well as Veggie Meter® score and GDS score. All correlation variables were analyzed continuously.

Chapter 4: Results

The purpose of this thesis project was to determine the association between skin carotenoid status and depressive symptoms or mood disturbances. An association would be shown as a correlation between high Veggie Meter® scores (closer to 800) and lower scores on the Geriatric Depression Scale – Short Form (closer to 0). The association would also be shown as a correlation between high Veggie Meter® scores and high means for the subscale scores and composite scores on the RAND 36-Item Health Survey (closer to 100).

4.1 Demographics, Marital Status, and Anthropometrics

109 participants were recruited across four cohorts. The mean age at baseline for the sample was 71.76 ± 5.12 years. The sample was predominantly female (79.8%) and White/Caucasian (89.0%). Approximately half of the sample was married (50.5%). For a full overview of the subject demographics and anthropometrics, see Tables 3 and 4, respectively.

Table 3. Subject demographics of the sample

	All Participants *n=109*
Age, *mean ± SD*	71.76 ± 5.12
Gender, *n (%)*	
Male	22 (20.2)
Female	87 (79.8)
Race, *n (%)*	
White/Caucasian	97 (89.0)
Black/African American	9 (8.3)
Other	1 (0.9)
Unknown	2 (1.8)
Ethnicity, *n (%)*	
Non-Hispanic	100 (91.7)
Hispanic	2 (1.8)
Unknown	7 (6.4)
Marital Status, *n (%)*	
Single	15 (13.8)
Married	55 (50.5)
Divorced	19 (17.4)
Widowed	13 (11.9)
Unknown	6 (5.5)

Table 4. Subject anthropometrics of the sample

	All Participants *n=109* *Mean ± SD*
Height (cm)	165.59 ± 8.36
Weight (kg)	78.10 ± 16.03
BMI (kg/m2)	28.35 ± 4.64
Waist Circumference (cm)*	97.67 ± 14.49

*Missing waist circumference data for 3 participants

4.2 Veggie Meter®

The mean Veggie Meter® score was 269.99 ± 84.25. The minimum score was 72 and maximum was 484.

4.3 Geriatric Depression Scale – Short Form (GDS)

The mean GDS score for the sample was 0.98 ± 1.43. The minimum score was 0 and the maximum score was 5. 3.67% of the sample has a score of 5 and 55.96% of the sample had a score of 0.

4.4 RAND 36-Item Health Survey (SF-36)

The sample scored high across all subscales of the SF-36 with the highest means belonging to the subscales of role limitation due to emotional problems (90.83 ± 23.07) and social functioning (90.37 ±15.83). The lowest scored subscale was energy and fatigue (67.54 ± 20.08). All subscales had a maximum score of 100. The sample size for both composite scores were reduced to 102 participants, due to missing values in subscales. The mean physical composite score for the sample was 47.88 ± 8.95. The mean mental composite score was 55.35 ± 6.78.

Table 5. Means of primary outcome variables

	All Participants *n=109* *Mean ± SD*
Veggie Meter® Score	269.99 ± 84.25
GDS Score	0.98 ± 1.43
SF-36 Subscales	
Physical Functioning	79.76 ± 22.98
Role Limitations due to Physical Health	78.67 ± 33.82
Role Limitations due to Emotional Health	90.83 ± 23.07
Energy/Fatigue	67.54 ± 20.08
Emotional Well-Being	83.52 ± 13.13
Social Functioning	90.37 ± 15.83
Pain	76.56 ± 19.34
General Health	75.92 ± 15.70
SF-36 Composite Scores*	
Physical Composite Score	47.88 ± 8.95
Mental Composite Score	55.35 ± 6.78

*n=102 due to missing values in subscales that could not be used in composite score calculations (52), GDS= Geriatric Depression Scale-Short Form, SF-36= RAND 36-Item Health Survey

4.5 Correlations of Primary Variables

Veggie Meter® scores and GDS scores were negatively correlated using the Pearson correlation, but the correlation was not significant (p=0.09). The SF-36 subscales physical functioning, social functioning, and general health were each significantly positively correlated with Veggie Meter® score using the Spearman's correlation coefficient ($p<0.05$). When the Pearson correlation was used, those three subscales again were significantly correlated ($p<0.05$). Physical and mental composite SF-36 scores were not significantly correlated with Veggie Meter® scores using either statistical analysis method. See Tables 6 and 7 for full Pearson and Spearman's correlations, respectively.

Table 6. Pearson pairwise correlations by Veggie Meter® score

	All Participants *n=109* *Correlation*	*p-value*
GDS Score	-0.16	0.09
SF-36 Subscales		
Physical Functioning	0.21	**0.03**
Role Limitations due to Physical Health	0.07	0.47
Role Limitations due to Emotional Health	0.13	0.18
Energy/Fatigue	0.12	0.21
Emotional Well-being	0.03	0.77
Social Functioning	0.23	**0.01**
Pain	0.11	0.26
General Health	0.22	**0.02**
SF-36 Composite Scores*		
Physical Composite Score	0.15	0.14
Mental Composite Score	0.05	0.65

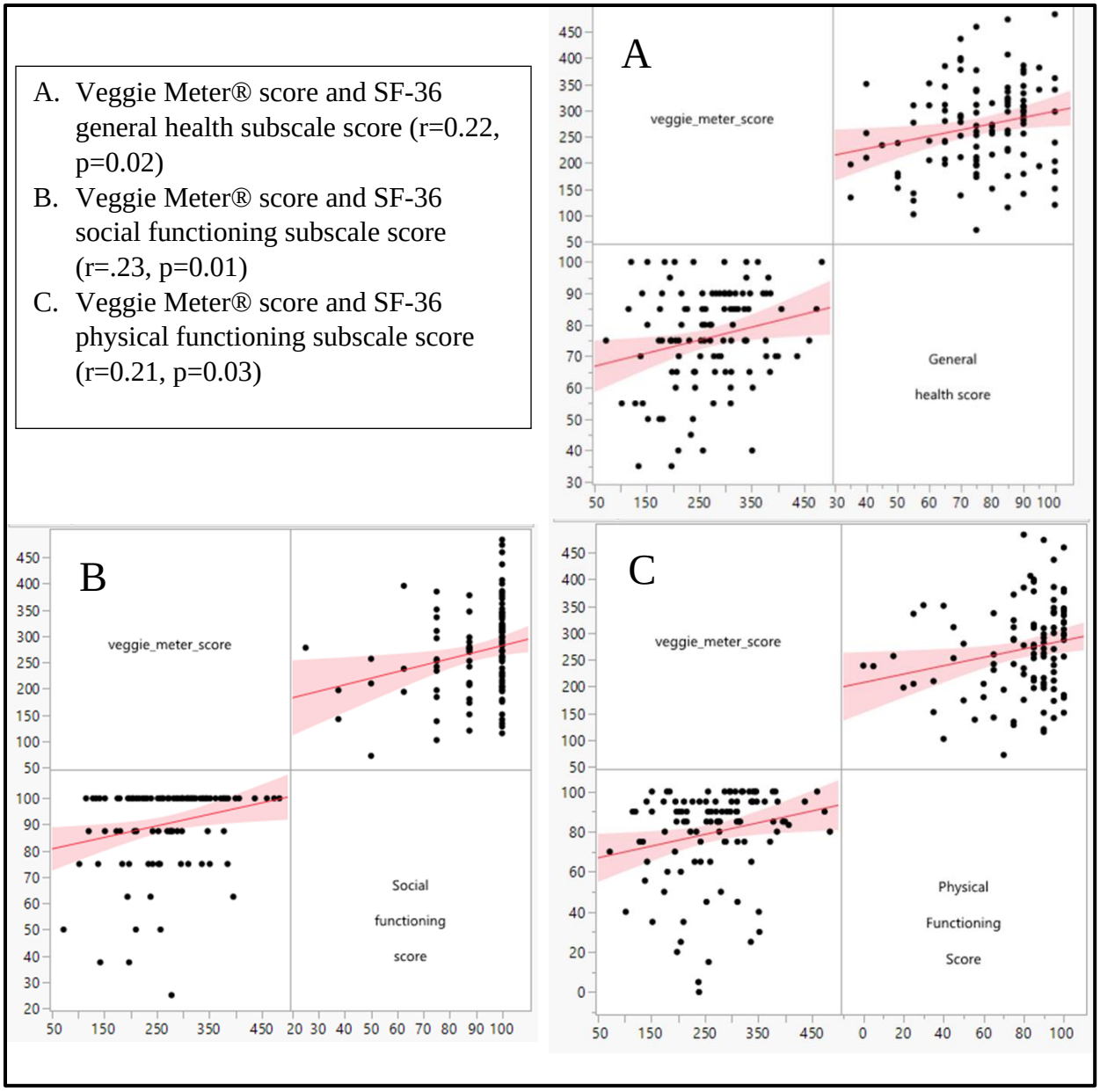
*n=102 due to missing values in subscales that could not be used in composite score calculations (52), GDS= Geriatric Depression Scale-Short Form, SF-36= RAND 36-Item Health Survey

Table 7. Spearman's rho rank correlation of Veggie Meter® score and SF-36 scores

		Veggie Meter® score *n=109*
Veggie Meter® Score	Correlation Coefficient	1.000
	Sig. (2-tailed)	.
Energy/Fatigue	Correlation Coefficient	.151
	Sig. (2-tailed)	.117
Emotional Well-Being	Correlation Coefficient	.017
	Sig. (2-tailed)	.859
Role Limitation due to Emotional Health	Correlation Coefficient	.133
	Sig. (2-tailed)	.169
Physical Functioning	Correlation Coefficient	.232
	Sig. (2-tailed)	**.015**
Role Limitations due to Physical Health	Correlation Coefficient	.078
	Sig. (2-tailed)	.423
Social Functioning	Correlation Coefficient	.228
	Sig. (2-tailed)	**.017**
Pain	Correlation Coefficient	.090
	Sig. (2-tailed)	.352
General Health	Correlation Coefficient	.195
	Sig. (2-tailed)	**.042**
Physical Composite Score*	Correlation Coefficient	.181
	Sig. (2-tailed)	.068
Mental Composite Score*	Correlation Coefficient	.055
	Sig. (2-tailed)	.586

*n=102 due to missing values in subscales that could not be used in composite score calculations (52), GDS= Geriatric Depression Scale-Short Form, SF-36= RAND 36-Item Health Survey

Figure 1. Scatterplot matrices for Veggie Meter® score and SF-36 subscale scores



GDS= Geriatric Depression Scale-Short Form, SF-36= RAND 36-Item Health Survey

Chapter 5: Discussion

To our knowledge this was the first study to evaluate the relationship between skin carotenoid status and depressive symptoms or mood disturbances in older adults. While the sample for this study did not show signs of Alzheimer's disease, this thesis project adds to the growing pool of Alzheimer's disease related research.

5.1 Demographics, Marital Status, and Anthropometrics

The sample was very homogenous in terms of gender, ethnicity, and race. However, the sample was diverse in age and body composition. The sample had a mean age of 71.76 $\pm$ 5.12 years, BMI of 28.35 $\pm$ 4.64 kg/m2, and waist circumference of 97.67 $\pm$ 14.49 cm. All participants were ages 65 years and older which is the population most susceptible to developing Alzheimer's disease.

5.2 Veggie Meter®

The results from this study add to the growing pool of data using the Veggie Meter®. The instrument was first developed in 1999 and was only patented in 2004 as the Veggie Meter®, so data with this instrument is limited, especially in this population (53).

5.3 Geriatric Depression Scale – Short Form (GDS)

Participants scored a mean score of 0.98 ± 1.43 reflecting that the majority of the sample was not experiencing depressive symptoms, as a score of 0-4 is considered within normal range (48). Scores ranged from 0-5 meaning that those who scored a 5 have indications of mild depression (48). At baseline screening, participants would have been excluded for the NICE study with a score of 9 or greater, but that scenario did not occur. The researcher believes the lack of scores ranging 6-8 was because those scoring higher on the GDS, which reflects worse depressive symptoms, were less likely to volunteer to participate in a yearlong nutrition intervention study.

5.4 RAND 36-Item Health Survey (SF-36)

Overall, scores were relatively high on the SF-36, likely due to the same reason the scores were low on the GDS. The lowest subscale score was energy and fatigue, which may be more due to age alone as opposed to mental well-being.

5.5 Correlations of Primary Variables

Limited research exists on the relationship between carotenoids and mood disturbances in AD, so this thesis adds to the pool of research. The subscales that were found as significantly correlated to Veggie Meter® score were subscales that were not specific to the research questions. The general health, physical functioning, and social functioning may be impacted by depressive symptoms and mood disturbances, but they present a better picture of overall quality of life. The SF-36 subscales and composite scores that most pertained to the research question were role limitations due to emotional problems, emotional well-being, energy/fatigue, and mental composite score. Additionally, the GDS score pertained specifically to the research question. None of these variables were significantly correlated with Veggie Meter® score; however, they all had slight positive correlations. The exception was the correlation of GDS score and Veggie Meter® score which was negative. However, this aligned with the other positive correlations since a low GDS represents better mental health as opposed to the SF-36 where a high score represents better mental health. A previous study found a significantly lower risk of depressed mood in those with higher plasma carotenoid levels at baseline ($p=0.04$) and again at the 6-year follow-up ($p=0.04$) (5). The findings of this thesis project neither refute nor support the findings of the previous study, but rather show that more research is needed to determine the association of these variables.

5.6 Limitations

A few limitations exist for this study. These limitations are related to Veggie Meter® scoring, exclusion criteria, the Geriatric Depression Scale- Short Form, and the COVID-19 pandemic.

There is not an exact score interpretation for Veggie Meter® scores, so scores were analyzed continuously. Since scores were only being analyzed at baseline, variation among subjects was not able to be compared to another score for that same person. This study only allowed for a one-time snapshot of a subject's Veggie Meter® score.

The exclusion criteria for the NICE study includes severe major depression or other major psychiatric disorders, as well as if the person is already consuming a Mediterranean diet as determined using the 14-item Mediterranean Diet Assessment Tool. Excluding those with a clinical diagnosis of severe major depression or other major psychiatric disorders eliminated the ability to analyze Veggie Meter® scores in those populations. Exclusion of those following a Mediterranean diet or close to a Mediterranean diet, limited the strength of this study. Higher Veggie Meter® scores are associated with higher adherence to a diet high in carotenoid containing foods (26), such as the Mediterranean diet, so scores may be skewed lower compared to a sample that did not have this population excluded.

The Geriatric Depression Scale- Short Form is mainly used as a screening tool and not a diagnostic tool. Use of a diagnostic tool would have been preferred for this thesis project, but that tool was not used in the NICE study since it was used primarily for exclusion criteria purposes only.

The COVID-19 pandemic began in March of 2020 and baseline data collection for cohort 4 occurred during Spring 2020. Although cohort 4 consisted of 26 participants which made it the

second largest cohort, it had the highest number of participants that were enrolled but did not follow through with baseline testing. Nine participants did this for cohort 4 comparted to only one for cohort 3 and none for cohorts 1 and 2. Aside from the reduce sample size, answers may be skewed due to effects of the pandemic. Surveys were reworded or had additional wording for this cohort to ask them to respond not considering the current pandemic to reduce the likelihood that responses reflect changes due to the pandemic. However, responses may have still been impacted.

5.7 Future Research

In the future, additional studies could be conducted with less exclusion criteria and with at least two data collection points. A repeat of this study without the exclusion criteria of a clinical diagnosis of depression and high Mediterranean diet adherence would allow for a study sample more representative of the actual population. A longitudinal cohort study would allow for analysis of intrapersonal variation of Veggie Meter® scores. Additionally, other validated instruments to assess depressive symptoms and mood disturbances could be used. Should studies of this nature show a more definitive correlation between skin carotenoid status and depressive symptoms or mood disturbances, further research that implements an intervention would be warranted. Implementing a carotenoid supplement or carotenoid containing randomized controlled diet intervention, such as the Mediterranean diet, could establish this causal relation.

5.8 Conclusion

The data suggest no association between skin carotenoid status and mood disturbances or depressive symptoms. However, further research with less exclusion criteria, multiple data

collection points, and more specific tools for mood disturbances and depressive symptoms is needed to confirm these findings.

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