THE RELATIONSHIP OF 5-AMINOLEVULINIC ACID ON MOOD AND COPING ABILITY IN PREDIABETIC ADULTS

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ABSTRACT

In the U.S., diabetes affects 25.8 million Americans (CDC 2011). The most effective diabetes prevention method in prediabetic individuals is lifestyle modification (Tabak, Herder et al. 2012). Despite the benefits of lifestyle change, diabetes prevalence continues to increase (CDC 2012). This may be because of the many emotional barriers that a prediabetic individual faces when it comes to starting an exercise routine and a healthy eating plan. A potential treatment for the emotional obstacles that prediabetic adults may face is a natural supplement called 5-aminolevulinic acid (5-ALA). In the current study, the group included 154 participants, both men and women, ranging between the ages of 41 to 71 years old. The study design was a double-blind, randomized parallel-group study. The Psychosocial Depression Symptoms Questionnaire (PDS) and the Perceived Stress Scale (PSS) were used to examine the relationship of two doses of 5-ALA (15mg and 50mg) with mood and coping ability. Using SAS software, an ordered logistic regression model was used to analyze the association between the dose groups (control, 15mg, and 50 mg) and responses to the two questionnaires. An integrative literature review, using the PubMed database, of the relationship between 5-ALA administrations and mood and coping ability was performed. First, our literature review resulted in zero published articles. Second, utilizing the two questionnaires, the intake of 5-ALA was found to be associated with significantly improve self-perception of effort spent ($p = 0.002$) and coping ability ($p = 0.004$). Finally, a significant dose-dependent relationship was found for the association of 5-ALA intake on measures of effort ($p = 0.003$), loneliness ($p = 0.006$), and coping ability ($p = 0.003$). The 50mg dose was more effective than the 15mg dose in improving these measures. In conclusion, we report that after 12 weeks of taking 5-ALA, self-perception of effort spent, loneliness, and coping ability were associated with improvements in a group of prediabetic adults. Improved mood and coping ability may enable prediabetic individuals to overcome the emotional obstacles preventing them from maintaining a healthy lifestyle and avoiding the development of diabetes.

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List of Abbreviations

5-ALA: 5-Aminolevulinic Acid

ADA: American Diabetes Association

ANOVA: Analysis of Variance

BMI: Body Mass Index

CBC: Complete Blood Count Test

IRB: Institutional Review Board

OGTT: Oral Glucose Tolerance Test

PASS: Prediabetes and Supplement Study

PDS: Psychosocial Depression Symptoms Questionnaire

PSS: Perceived Stress Scale

SAS: Statistical Analysis System

SBI: Strategic Business Innovations

YWCA: Young Women's Christian Association

INTRODUCTION

The overall objective of this proposal is to examine the relationship between administration of 5-aminolevulinic acid (5-ALA) and mood in a population of prediabetic adults. We need to have a better understanding of 5-ALA and its potential to help prediabetic individuals overcome the emotional health risks impeding their path to recovery. The central hypothesis motivating this research is that healthy lifestyle modifications are the best course of action for prediabetic individuals to prevent the development of diabetes. However, there are emotional barriers and health risks common to many individuals with prediabetes. 5-ALA has been shown to improve one of these health risks, sleep, and it has been hypothesized that it may improve other health risks common to prediabetic individuals such as mood and coping ability. The rationale for the proposed research is that understanding the potential applications of 5-ALA, something that is currently unknown, is critical to designing the most effective wellness and diabetes prevention program.

In the U.S., diabetes affects 25.8 million Americans (CDC 2011). That is, more than 1 in 10 adults have diabetes and the prevalence of diabetes continues to rise in both adults and children (CDC 2011; CDC 2012; May, Kurlina, and Yoon 2012). In fact, over the last 30 years the prevalence has increased by 176% (CDC 2012). In addition, in the year 2007, the estimated cost of diabetes was $174 billion dollars (ADA 2008). Due to the rising prevalence and astounding cost of diabetes, it is imperative that health care *prevention* strategies are optimized to reduce the economic and social burden of this disease.

Prediabetes or intermediate hyperglycemia is a high-risk state for the development of diabetes (Tabak, Herder et al. 2012). According to an American Diabetes Association (ADA) panel of experts, up to 70% of prediabetic individuals will ultimately develop diabetes (Tabak, Herder et al. 2012). Prediabetes is defined by glycemic variables (e.g., blood glucose concentrations) that are higher than normal limits, but lower than the diagnostic criteria for diabetes (Tabak, Herder et al. 2012). The most effective diabetes prevention method in prediabetic individuals is lifestyle modification (Tabak, Herder et al. 2012). This finding is supported by evidence showing a 40-70% relative-risk

reduction for diabetes development (Tabak, Herder et al. 2012). Lifestyle modifications include reducing body weight and moderate daily exercise (ADA 2013).

Despite the benefits of lifestyle change, diabetes prevalence continues to increase (CDC 2012). This may be because of the many physical and emotional barriers that a prediabetic individual faces when it comes to starting an exercise routine and a healthy eating plan. Some of the physical barriers to exercise and healthy eating include the rising costs of child-care, transportation, and fresh fruits and vegetables (YWCA 2012). Fortunately, there are community programs available, such as the YWCA's Diabetes Wellness and Prevention Program that may help eliminate physical barriers to exercise and healthy eating (YWCA 2013).

However, additionally, there are many emotional barriers that prediabetic individuals encounter. These emotional barriers also happen to be some of the health risks associated with prediabetes (Challem 2007). They include fatigue, poor sleep, mood swings, irritability, anxiety and depression (Challem 2007; Holt and Kalra 2013). Finding a solution to address and treat these prediabetic health risks will aid in initiating and maintaining the healthy lifestyle modifications necessary to prevent the development of diabetes.

A potential treatment for prediabetic emotional health risks, such as sleep and mood, is a natural compound called 5-aminolevulinic acid (5-ALA). Figure 1 below describes the structure of 5 ALA.

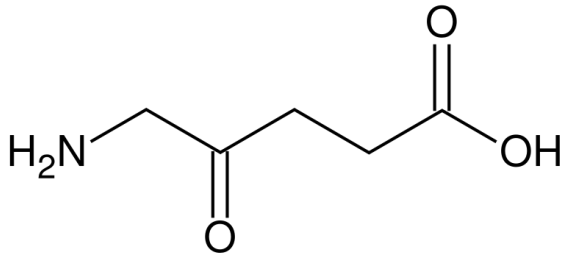

Figure 1. The chemical structure of 5-ALA

This compound is a heme precursor in mammals and a chlorophyll precursor in plants (Takahashi, Misawa et al., 2013; Beale 1990). Many common foods contains 5-ALA such as spinach, green peppers, tomatoes, bananas, and ground beef (Perez, Rodriguez et

al. 2013). A recent double-blind, randomized parallel-group study conducted by Perez and colleagues (2013) reported an improvement in sleep as a result of the administration of a dietary supplement containing 5-ALA (Perez, Shintani et al. 2013).

To the best of our knowledge, there are no published studies evaluating the relationship between 5-ALA administration and improvement in mood and coping ability. However, there are a few possible mechanisms for improvement in mood as a result of 5-ALA administration. In a previous study using a murine model, researchers found that regular administration of 5-ALA raised brain serotonin levels (Monti, Pandi-Perumal et al. 2008). Another study also suggested that 5-ALA might improve mood through its influence on neuroactive substances such as tryptophan, serotonin and melatonin (Perez, Shintani et al. 2013). Researchers hypothesized that an increase in serotonin levels may correspond with improvements in mood, calmness, irritability, and coping abilities (Monti, Pandi-Perumal et al. 2008).

The following specific aims summarize the goals of this investigation.

Specific Aims

Aim 1: To perform an integrative literature review of the relationship between administrations of 5-ALA with mood and coping ability.

Aim 2: **To determine the relationship between 15mg and 50mg 5-ALA and mood and coping ability using the Psychosocial Depressive Symptoms (PDS) and Perceived Stress Scale (PSS) Questionnaires.**

Aim 3: To determine if a dose-dependent relationship exists between 5-ALA, mood, and coping ability.

METHODS

Study Design

This research study uses previously collected data from the Prediabetes and Supplement Study (PASS). The PASS is a double-blinded randomized parallel-group

comparison prospective study using participants residing on Oahu, Hawaii. Recruitment of study participants utilized IRB-approved local newspaper advertisements, flyers, television and radio advertising, referrals from local physicians, and local seminars and community programs. The study group includes 154 participants, both men and women, between the ages of 40 to 70 years old. All enrolled participants signed the IRB-approved informed consent. The selection of study participants was based on the strict inclusion and exclusion criteria provided in Table 1.

Table 1. Inclusion and Exclusion Criteria

Inclusion Criteria:
1.) Healthy adults living in Oahu, Hawaii or able to attend five on-site one hour appointments over a 10 week period
2.) No medication or supplements currently taken for improvement in sleep or mood
3.) Body weight between 110 and 250 pounds
4.) Normal CBC and ferritin laboratory analysis done at initial screening
5.) Self report of insomnia, nocturnal awakening, difficulty falling and/or staying asleep, and report of mood imbalances
6.) Hemoglobin A1c level range from 5.8 to 7
7.) Fasting blood sugar level range from 110 to 125 (Impaired Glucose Tolerance range from 140 to 199 at 2 hours post load)
Exclusion Criteria:
1.) History of hepatitis, porphyria, hemochromatosis, and iron sensitivity
2.) Active liver disease
3.) Laboratory analysis with elevated ferritin levels above 125% normal levels obtained at initial screening visit
4.) Current participation in another clinical research study
5.) For women, pregnancy and breastfeeding
6.) Body Mass Index value of more than 30 and a body weights of <110 or >250 pounds
7.) Medical prescriptions that affect blood sugar level

Procedures

Potential participants were first screened and if eligible, consented at the initial visit. If eligible, participants were enrolled and asked to take a nutritional supplement (5-ALA) once a day for 90 days. The participants were randomly allocated to the following three study groups described in Table 2: control group (n = 51), 15 mg low dose of 5-ALA (n = 50), and 50 mg high dose of 5-ALA (n = 53).

Table 2. Group and Supplement Description

Supplement Content	Control Group (mg)	Low Dose Group (mg)	High Dose Group (mg)
5-ALA Phosphate	0 mg	15 mg	50 mg
Sodium Ferrous Citrate (SFCi)	0 mg	17.2 mg (1.82 mg as Iron)	57.4 mg (6.08 mg as Iron)
Other	Alpha starch, silicon dioxide	Alpha starch, silicon dioxide	Alpha starch, silicon dioxide

As described in Table 2, the control group was given a placebo capsule of identical size and color. Table 3 describes the administration of the 5-ALA supplement and participant compensation.

Table 3. Supplement Schedule and Participant Compensation

Visit Number	Visit Description	Timeline	Compensation
1	Screening	Screening	$0
2	Intervention period	Day-0	$100
3	Intervention period	Week-4	$50
4	Intervention period	Week-8	$50
5	Intervention period	Week-12	$100
6	Follow-up	Week-16	$100

Participants were required to report for periodic physical exams the initial screening visit and every four weeks thereafter (Week 4, 8, 12, and 16). During these periodic check-ups, blood samples were collected. Blood samples were sent to the Diagnostic Laboratory Services of Hawaii for analysis. Lastly, the participants received monetary compensation for their time and effort.

Data Collection Tools

Table 4 and 5 describes the study procedures.

Table 4. Study Procedures by Assessment

Assessment Type	**Assessment Description**
Lifestyle-related questionnaires	Self-reported sleep history, food consumption, nutrient intake, drug history including prescription, illicit, alcohol, nicotine, etc.
Clinical examination	Medical history, body weight, height, BMI, systolic/diastolic blood pressure, heart rate, waist circumference, Oral Glucose Tolerance Test (OGTT)
Laboratory tests	Hepatic function (ALT, AST, LDH, γ-GTP, T-Bill, Alb, TP, urine bilirubin), Renal function (BUN, creatinine, uric acid, urinary protein, occult blood), Inflammation/Infection (WBC count, fibrinogen), Hyperlipidemia (total cholesterol, HDL-chr, LDL-chr, triglyceride), Anemia (RBC count, hemoglobin level, hematocrit, blood platelet count, serum iron level, ferritin, transferrin, UIBC, TIBC, MCH, MCV, MCHC), Glucose metabolism (Blood glucose, urinary glucose, urinary pH, HbA1c), Electrolyte metabolism (Na, K, Cl)
Dietary inquiry	Study diary, self-reported symptoms, use of dietary supplements
Psychiatric questionnaires	Perceived stress scale, psychosocial depressive symptoms

Table 5. Study Procedures by Study Task

Study Task	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6
Lifestyle, PDS, PSS, & Food Questionnaires		X			X	
Clinical Exam	X	X	X	X	X	X
Lab Tests	X	X	X	X	X	X
Oral glucose tolerance test (OGTT)		X			X	
Intake of Supplements (5-ALA or placebo)		X	X	X	X	X
Study Diary	X	X	X	X	X	X

Study participants were interviewed about diet and energy expenditure. The Prediabetes and Supplement Study (PASS) assessment tool, adapted from the Honolulu Heart Study (Rodriguez, Lau et al. 1999), was used to assess lifestyle and physical activity. The Perceived Stress Scale (PSS) was used to measure stress (Cohen, Kamarck et al. 1983). A calibrated digital precision scale and a stadiometer were used to measure the participant's weight and height, respectively. Body mass index (BMI) was calculated using weight (kg) divided by height (m^2). A calibrated digital sphygmomanometer was used to measure blood pressure. The average of two measurements was used to estimate systolic and diastolic pressures. Finally, blood was collected at the initial visit and at the 12-week visit. The oral glucose tolerance test (OGTT) was also performed at the 12-week visit. For this test, participants fasted for 10 hours prior to blood collection. The Diagnostic Laboratory Services of Hawaii performed the standardized blood analyses.

Questionnaires

The two questionnaires used in this study were the Psychological Depression Symptoms (PDS) Questionnaire and the Perceived Stress Scale (PSS) (see appendices). These questionnaires are included in the supplemental information section. The PDS questionnaire is a part of the Prediabetes and Supplement Study (PASS) assessment tool, adapted from the Honolulu Heart Study (Rodriguez, Lau et al. 1999). The PDS was

designed to measure self-perception of mood. The PSS, designed by Dr. Sheldon Cohen, is the most widely used psychological tool to measure self-perception of stress (Cohen, Kamarck et al. 1983). The questions in the PSS ask the respondent about his or her feelings during the last month regarding certain situations in one's life (Cohen, Kamarck et al. 1983). For each situation, the PSS is designed to measure the degree to which a situation is considered stressful (Cohen, Kamarck et al. 1983). Furthermore, since the questions of both the PDS and the PSS are written in a general nature, they are relatively free of content specific to any sub-population group (Cohen, Kamarck et al. 1983). For both questionnaires, participants were asked about their thoughts and feelings during the last month towards each question. The answer choice options for both questionnaires were: "never," "almost never," "sometimes," "fairly often," and "very often."

Literature Review Procedures

This literature review was focused on clinical trials, cross-sectional, case-control, and prospective cohort studies on the relationship between administrations of 5-ALA with mood and coping ability. A systematic search was conducted up to September 2014 using the PubMed database (MEDLINE, National Library of Medicine, Bethesda, MD, USA). To build a better query in PubMed, MeSH (Medical Subheadings) was utilized. MeSH is the National Library of Medicine controlled vocabulary thesaurus used for indexing articles for PubMed. In PubMed, the MeSH terms used were "5-ALA" or "5 5-aminolevulinic acid" along with other key words: "Prediabetes", "Hyperglycemia", "Type 2 Diabetes", "Mood", "Coping", "Emotional Health", and "Psychological Health". The search included both animal and human studies. The search was restricted to articles in English. All full-text studies were considered. This initial search resulted in zero articles. A further search was done using a list of relevant publications provided by SBI Pharmaceuticals Co., Ltd. This search resulted in zero articles. A final search using the above search terms was done using the Google Internet search engine and this also resulted in zero articles.

Statistical Analyses

Descriptive and inferential statistics were used to evaluate relationships between and among variables. The statistical programs utilized were SAS software, version 10 (SAS Institute Inc.) and GraphPad Prism software, version 6 (GraphPad Software, Inc.). For the inferential tests, statistical significance was defined as $p < 0.05$.

Using Prism software, a two-way ANOVA was performed to compare age and gender in the three groups. Using SAS software, an ordered logistic regression or proportional odds model was used to analyze the association between the dose groups (control, 15mg, and 50 mg) and responses to the Psychosocial Depressive Symptoms (PDS) Questionnaire and Perceived Stress Scale (PSS). The appropriateness of the proportional odds model was tested using scored p-values.

Results

Participant Demographics

The study group includes 154 participants, both men and women, ranging between the ages of 41 to 71 years old. The participants were randomly allocated to the following three study groups described in Table 2: control group (n = 51), 15 mg low dose of 5-ALA (n = 50), and 50 mg high dose of 5-ALA (n = 53). The control group included 30 women and 21 men, the low dose group included 30 women and 20 men, and the high dose group included 33 women and 20 men. There were no significant differences in age and gender for any of the three groups (Group, $p = 0.10$; Gender, $p = 0.59$; Interaction, $p = 0.76$). Table 6 summarizes age and gender for each of the three groups.

Table 6. Age and Gender by Group with Means and Standard Errors

Group	Men			Women		
	Mean	**SEM**	**N**	**Mean**	**SEM**	**N**
High Dose Group	58.0	2.1	20	57.3	1.3	33
Low Dose Group	57.3	2.0	20	57.7	1.4	30
Control	61.5	1.4	21	59.6	1.3	30
*A Two-way ANOVA resulted in no significant differences: Group, p = 0.10; Gender, p = 0.59; Interaction, p = 0.76						

Group Comparisons

Intake of 5-ALA and the Psychological Depression Symptoms (PDS) Questionnaire

In the analysis of the association of 5-ALA on responses to the PDS Questionnaire, only the outcome "effort" was found to be significant (p = 0.002). Table 7 and Graph 1 summarize the results of the effect on 5-ALA intake on each outcome variable from the PDS Questionnaire. The proportional odds model was appropriate for this analysis since all of the scored p-values were not significant.

Table 7. Intake of 5-ALA and the Psychological Depression Symptoms Questionnaire

Outcome	Odd's Ratio	Lower CL*	Upper CL*	P value	Scored P Value	Predictor
Bothered	1.71	0.72	4.06	0.23	0.54	ALA
Poor Appetite	3.86	0.85	17.67	0.08	0.90	ALA
Poor Concentration	1.46	0.65	3.29	0.36	0.09	ALA
Effort	5.36	1.87	15.38	0.002	0.61	ALA
Depressed	1.55	0.54	4.49	0.42	0.89	ALA
Hopeful Future	1.39	0.46	4.17	0.56	0.58	ALA
Fearful	1.42	0.59	3.41	0.44	0.41	ALA
Restless Sleep	1.06	0.51	2.19	0.88	0.16	ALA
Happy	0.61	0.16	2.33	0.47	0.27	ALA
Lonely	2.92	0.89	9.58	0.08	0.41	ALA
Unmotivated	1.83	0.83	4.06	0.14	0.69	ALA
*95% confidence interval						

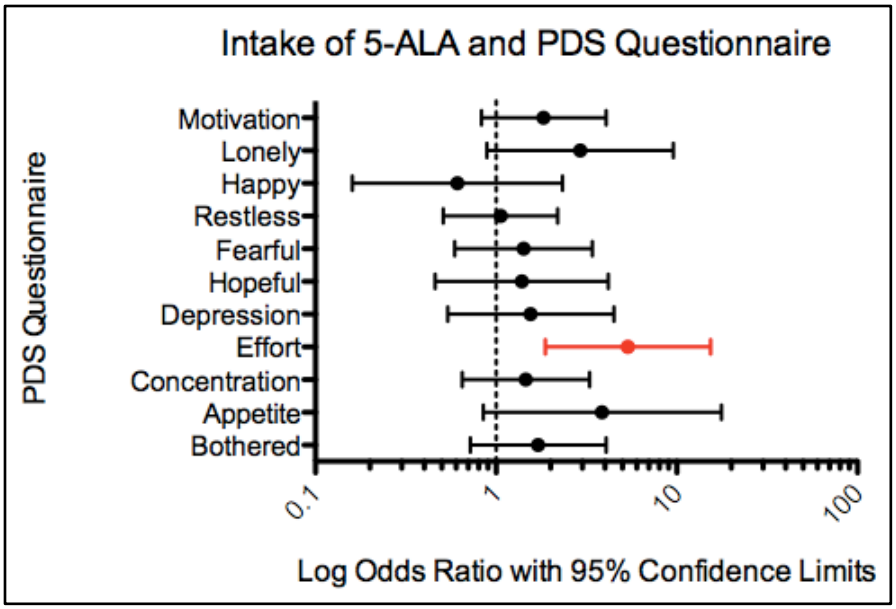

Figure 2. Intake of 5-ALA and PDS Questionnaire. Significant odds ratios are highlighted in red, $p < 0.05$.

Intake of 5-ALA and the Perceived Stress Scale (PSS)

In the analysis of the effect of 5-ALA on responses to the PSS Questionnaire, only the outcome "cope" was found to be significant ($p = 0.004$). Table 8 and Graph 2 summarize the results of the effect of 5-ALA intake on each outcome variable from the PSS Questionnaire. The proportional odds model was appropriate for this analysis since all of the scored p-values were not significant.

Table 8. Intake of 5-ALA and the Perceived Stress Scale

Outcome	Odd's Ratio	Lower CL*	Upper CL*	P value	Scored P Value	Predictor
Upset	1.20	0.73	3.32	0.26	0.44	ALA
Important	0.93	0.24	2.06	0.55	0.58	ALA
Stressed	1.03	0.70	2.11	0.67	0.24	ALA
Confident	0.60	0.26	2.63	0.54	0.59	ALA
Going Your Way	1.63	0.51	4.35	0.44	0.89	ALA
Cope	4.32	2.64	7.16	0.004	0.50	ALA
Irritation	1.45	0.58	3.32	0.63	0.66	ALA
On Top Of Things	0.51	0.13	2.10	0.57	0.45	ALA
Angered	1.75	0.74	3.78	0.16	0.56	ALA
Piling Up	1.35	0.67	4.12	0.51	0.71	ALA
*95% Confidence Interval						

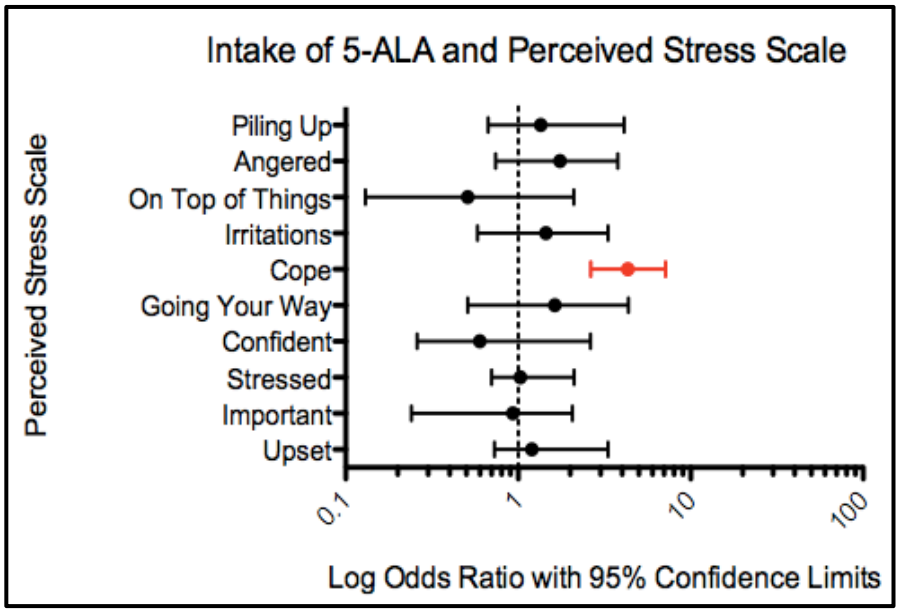

Figure 3. Intake of 5-ALA and the Perceived Stress Scale. Significant odds ratios are highlighted in red, $p < 0.05$.

Intake of 15mg and 50mg 5-ALA and the PDS Questionnaire

In the analysis of the effect of 5-ALA (either 15mg and 50mg dose) on responses to the PDS Questionnaire, the outcomes "effort" and "lonely" were found to be significant (effort, $p = 0.003$; lonely, $p = 0.006$). Table 9, Graph 3, and Graph 4 summarizes the results of the effect for both 15mg and 50mg 5-ALA intake on each outcome variable from the PDS Questionnaire. The proportional odds model was appropriate for this analysis since all of the scored p-values were not significant.

Table 9. Intake of 15mg and 50mg 5-ALA and the PDS Questionnaire

Outcome	Odd's Ratio	Upper CL*	Lower CL*	P value	Scored P Value	Predictor
Bothered – Low Dose	1.31	1.72	1.00	0.05	0.54	ALA 15mg
Bothered – High Dose	2.49	6.08	1.02	0.05	0.54	ALA 50mg
Poor Appetite – Low Dose	1.32	1.94	0.90	0.15	0.90	ALA 15mg
Poor Appetite – High Dose	2.55	9.11	0.71	0.15	0.90	ALA 50mg
Poor Concentration – Low Dose	1.06	1.37	0.82	0.68	0.09	ALA 15mg
Poor Concentration – High Dose	1.2	2.84	0.51	0.68	0.09	ALA 50mg
Effort – Low Dose	1.52	2.00	1.16	0.003	0.61	ALA 15mg
Effort – High Dose	4.01	9.88	1.63	0.003	0.61	ALA 50mg
Depressed – Low Dose	1.04	1.44	0.75	0.83	0.89	ALA 15mg
Depressed – High Low	1.13	3.39	0.37	0.83	0.89	ALA 50mg
Hopeful Future – Low Dose	0.93	1.36	0.64	0.72	0.58	ALA 15mg
Hopeful Future – High Dose	0.8	2.77	0.23	0.72	0.58	ALA 50mg
Fearful – Low Dose	1.22	1.60	0.92	0.16	0.41	ALA 15mg
Fearful – High Dose	1.92	4.78	0.77	0.16	0.41	ALA 50mg
Restless Sleep – Low Dose	0.97	1.24	0.76	0.8	0.16	ALA 15mg
Restless Sleep – High Dose	0.9	2.03	0.40	0.8	0.16	ALA 50mg
Happy – Low Dose	0.84	1.25	0.56	0.39	0.27	ALA 15mg
Happy – High Dose	0.56	2.12	0.15	0.39	0.27	ALA 50mg
Lonely – Low Dose	1.63	2.30	1.15	0.006	0.41	ALA 15mg
Lonely – High Dose	5.05	16.00	1.60	0.006	0.41	ALA 50mg
Unmotivated – Low Dose	1.11	1.42	0.87	0.4	0.69	ALA 15mg
Unmotivated – High Dose	1.43	3.25	0.62	0.4	0.69	ALA 50mg
*95% Confidence Interval						

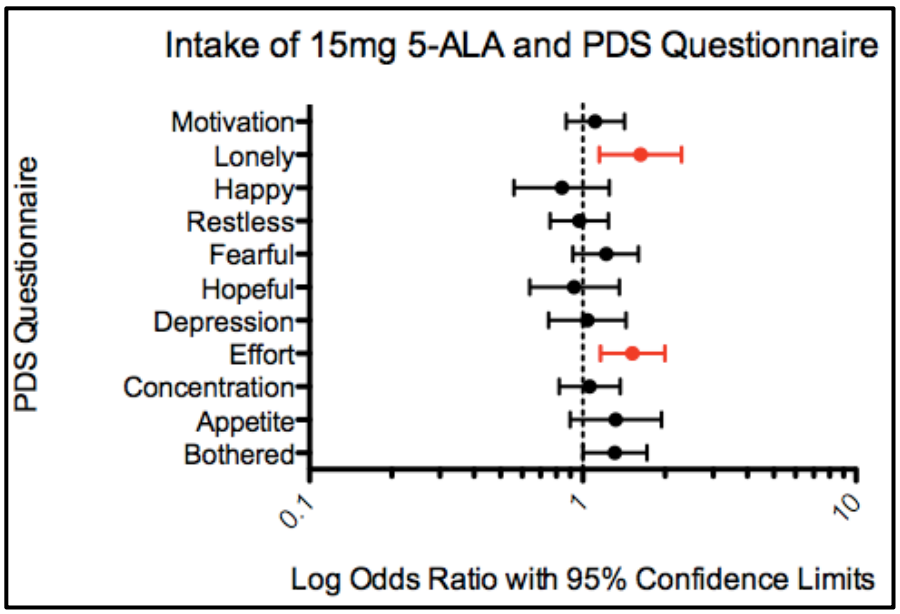

Figure 4. Intake of 15mg 5-ALA and the PDS Questionnaire. Significant odds ratios are highlighted in red, $p < 0.05$.

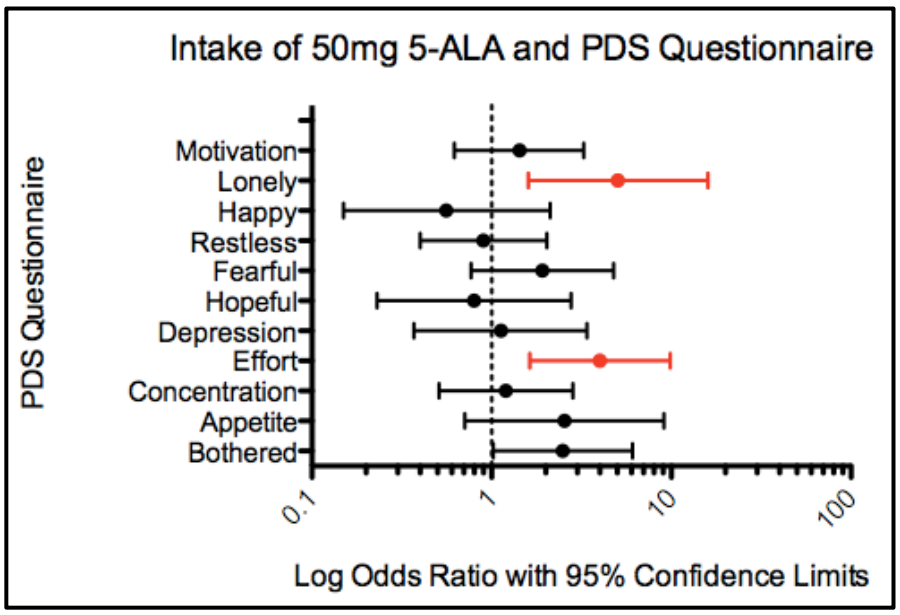

Figure 5. Intake of 50mg 5-ALA and the PDS Questionnaire. Significant odds ratios are highlighted in red, $p < 0.05$.

Intake of 15mg and 50mg 5-ALA and the Perceived Stress Scale

In the analysis of the effect of 5-ALA (either 15mg and 50mg dose) on responses to the PSS Questionnaire, only the outcome "cope" was found to be significant (cope, $p = 0.004$). Table 10, Graph 5, and Graph 6 summarizes the results of the effect for both 15mg and 50mg 5-ALA intake on each outcome variable from the PSS Questionnaire. The proportional odds model was appropriate for this analysis since all of the scored p-values were not significant.

Table 10. Intake of 15mg and 50mg 5-ALA and the Perceived Stress Scale

Outcome	Odd's Ratio	Lower CL*	Upper CL*	P value	Scored P Value	Predictor
Upset – Low Dose	0.84	0.54	1.23	0.45	0.44	ALA 15mg
Upset – High Dose	0.71	0.36	2.44	0.45	0.44	ALA 50mg
Important – Low Dose	1.12	0.76	1.66	0.37	0.58	ALA 15mg
Important – High Dose	0.86	0.21	2.30	0.37	0.58	ALA 50mg
Stressed – Low Dose	0.95	0.60	1.34	0.81	0.24	ALA 15mg
Stressed – High Dose	0.83	0.59	2.11	0.81	0.24	ALA 50mg
Confident – Low Dose	0.97	0.50	1.47	0.56	0.59	ALA 15mg
Confident – High Dose	0.51	0.23	2.48	0.56	0.59	ALA 50mg
Going Your Way – Low Dose	1.02	0.68	1.67	0.93	0.89	ALA 15mg
Going Your Way – High Dose	0.93	0.26	3.32	0.93	0.89	ALA 50mg
Cope – Low Dose	1.49	1.14	2.21	0.003	0.50	ALA 15mg
Cope – High Dose	3.32	1.43	6.54	0.003	0.50	ALA 50mg
Irritation – Low Dose	1.03	0.52	1.67	0.78	0.66	ALA 15mg
Irritation – High Dose	1.21	0.44	3.17	0.78	0.66	ALA 50mg
On Top Of Things – Low Dose	0.56	0.23	2.33	0.50	0.45	ALA 15mg
On Top Of Things – High Dose	0.88	0.54	1.32	0.50	0.45	ALA 50mg
Angered – Low Dose	1.22	1.00	1.86	0.65	0.56	ALA 15mg
Angered – High Dose	1.65	1.39	4.18	0.65	0.56	ALA 50mg
Piling Up – Low Dose	1.03	0.83	1.56	0.23	0.71	ALA 15mg
Piling Up – High Dose	1.87	0.67	4.33	0.23	0.71	ALA 50mg
*95% Confidence Interval						

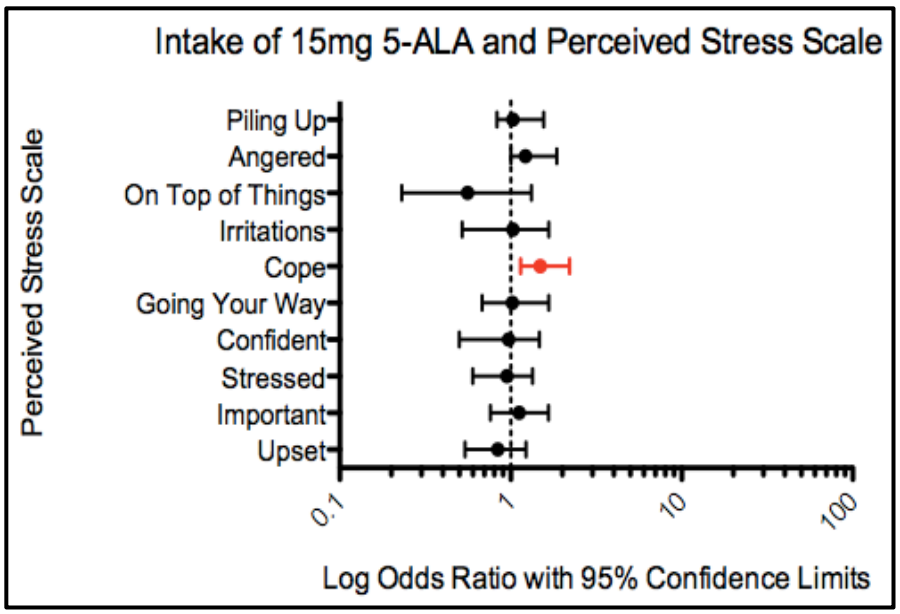

Figure 6. Intake of 15mg 5-ALA and the Perceived Stress Scale. Significant odds ratios are highlighted in red, $p < 0.05$.

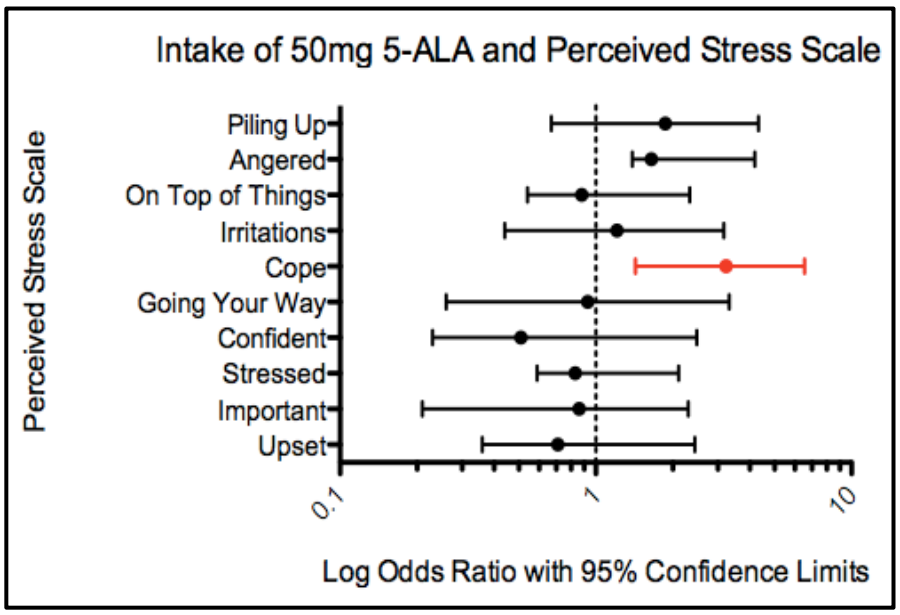

Figure 7. Intake of 50mg 5-ALA and the Perceived Stress Scale. Significant odds ratios are highlighted in red, $p < 0.05$.

Discussion

To summarize, the three specific aims of this study were met. First, an integrative literary review searching for previous publications on the relationship between administrations of 5-ALA and mood and coping ability resulted in zero published articles. To the best of our knowledge, this is the first study to report the effect of 5-ALA intake on mood and coping ability. Second, utilizing the Psychological Depression Symptoms (PDS) and Perceived Stress Scale (PSS) questionnaires, the intake of 5-ALA was found to improve self-perception of effort spent and coping ability. Finally, a dose-dependent relationship was found for the effect of 5-ALA intake on measures of effort, loneliness, and coping ability. The 50mg dose was more effective than the 15mg dose in improving these measures. Overall, the intake of 5-ALA improved self-perception of mood and coping ability in a population of prediabetic adults.

According to the Psychological Depression Symptoms (PDS) questionnaire, the intake of 5-ALA after 12 weeks improved self-perception of effort exerted (PDS Question: "I felt that everything I did was an effort"). Compared to the participants' answers at Week 1 (no 5-ALA), the intake of the 15mg and 50mg of 5-ALA significantly improved their perception of effort exerted by 1.5 and 4.0 times, respectively. Improvement in self-perception of effort exerted is important in helping prevent the development of diabetes in prediabetic adults because maintaining a healthy lifestyle is not always an easy task. While the most effective preventive strategy is lifestyle modification (Tabak, Herder et al. 2012), for some individuals this task may feel like an enormous effort. Supplementing a healthy diet with 5-ALA in prediabetic adults may be the extra factor needed to boost energy and motivation for healthy lifestyle change.

The PDS questionnaire also revealed that the intake of 5-ALA significantly improved self-perception of loneliness (PDS Question: "I felt lonely"). Compared to the participants' answers at Week 1 (no 5-ALA), 15mg and 50mg of 5-ALA significantly improved their perception of feeling alone by 1.6 and 5.1 times, respectively. Interestingly, it has been shown that loneliness depends on perception rather than social situation (Kurina, Knutson et al. 2011). Often patients withdraw from family and friend support because they feel isolated or different from other people ("Loneliness and

Diabetes" 2012). Some diabetic patients contribute the feeling of isolation with the responsibilities that come with their condition (i.e, diet restriction, checking blood glucose, insulin shots, etc.) ("Loneliness and Diabetes" 2012). Improving self-perception of loneliness is an important first step in preventing diabetes because prediabetic adults who have help from family, friends, and a community of similar patients are more likely to maintain a healthier lifestyle ("Staying Motivated" 2014).

The Perceived Stress Scale (PSS) revealed that coping ability was improved in a group of prediabetic adults (PSS Question: "How often have you found that you could not cope with all the things that you had to do?"). Successful coping ability includes all the necessary skills to minimize and manage stress related to personal problems. Compared to the participants' answers at Week 1 (no 5-ALA), 15mg and 50mg of 5-ALA after 12 weeks significantly improved coping ability by 1.5 and 3.2 times, respectively. Diabetes is a stressful, incurable disease that is associated with a higher incidence of depression and anxiety (Sobol-Pacyniak, Szymczak et al. 2014). For prediabetic adults, the ability to cope with the threat of developing diabetes is important to minimize stress so they can focus on changing their lifestyle.

The mechanism for how 5-ALA improved mood and coping ability is beyond the scope of this project. However, there are a few possible mechanisms for improvement in mood and coping ability as a result of 5-ALA intake. In a previous study using a murine model, researchers found that regular administration of 5-ALA raised brain serotonin levels (Monti, Pandi-Perumal et al. 2008). Another study also suggested that 5-ALA might improve mood through its influence on neuroactive substances such as tryptophan, serotonin and melatonin (Perez, Shintani et al. 2013). Researchers hypothesized that an increase in serotonin levels may correspond with improvements in mood, calmness, irritability, and coping abilities (Monti, Pandi-Perumal et al. 2008). Also, a study conducted by Perez and colleagues (2013) reported an improvement in sleep as a result of 5-ALA intake (Perez, Shintani et al. 2013). The authors suggest that improvement in sleep as a result of 5-ALA intake may be related to a boost in cellular metabolism, such that circadian rhythms are better defined (Perez, Shintani et al. 2013). It is well accepted that sleep and mood are closely linked (Epstein 2008). Poor sleep is associated with irritability and stress, while adequate sleep is associated with enhanced well-being

(Epstein 2008). Improvement in sleep in prediabetic adults may increase the energy needed to cope with the possibility of being diagnosed with diabetes. Also, improved sleep may help prediabetic adults feel less alone. A previous study found that people who feel lonely experience more disruptions while they sleep than those who don't feel lonely (Kurina, Knutson et al. 2011).

In conclusion, we report that after 12 weeks of taking 5-ALA, self-perception of effort spent, loneliness, and coping ability were improved in a group of prediabetic adults. Improved mood and coping ability may enable prediabetic individuals to overcome the emotional obstacles preventing them from maintaining a healthy lifestyle and avoiding the development of diabetes.

APPENDIX A. PSYCHOSOCIAL DEPRESSION SYMPTOMS QUESTIONNAIRE
(See next page.)

PSYCHOSOCIAL - DEPRESSIVE SYMPTOMS

Participant is unable or unwilling to complete this section.	No 0	Yes 1

If "Yes", specify reasons and skip this page.

Below is a list of the ways you might have felt or behaved.
Please indicate how often you have felt this way during the past week.

Would you say in the last week	Rarely or none of time (less than 1 day)	Some or little of the time (1 - 2 days)	Occasionally or a moderate amount of the time (3 - 4 days)	Most of the time	Don't know or refused
1. I was bothered by things that usually don't bother me.	0	1	2	3	9
2. I did not feel like eating, my appetite was poor.	0	1	2	3	9
3. I had trouble keeping my mind on what I was doing.	0	1	2	3	9
4. I felt that everything I did was an effort.	0	1	2	3	9
5. I felt depressed	0	1	2	3	9
6. I felt hopeful about the future.	0	1	2	3	9
7. I felt fearful.	0	1	2	3	9
8. My sleep was restless.	0	1	2	3	9
9. I was happy.	0	1	2	3	9
10. I felt lonely.	0	1	2	3	9
11. I could not get going.	0	1	2	3	9

APPENDIX B. PERCEIVED STRESS SCALE
(See next page.)

Instructions: The following questions ask you about your feelings and thoughts during the last month. In each case, please indicate with a check how often you felt or thought a certain way.

1. In the last month, how often have you been upset because of something that happened unexpectedly?

___0=never ___1=almost never ___ 2=sometimes ___3=fairly often ___4=very often

2. In the last month, how often have you felt that you were unable to control the important things in your life?

___0=never ___1=almost never ___ 2=sometimes ___3=fairly often ___4=very often

3. In the last month, how often have you felt nervous or "stressed"?

___0=never ___1=almost never ___ 2=sometimes ___3=fairly often ___4=very often

4. In the last month, how often have you felt confident about your ability to handle your personal problems?

___0=never ___1=almost never ___ 2=sometimes ___3=fairly often ___4=very often

5. In the last month, how often have you felt that thinks were going your way?

___0=never ___1=almost never ___ 2=sometimes ___3=fairly often ___4=very often

6. In the last month, how often have you found that you could not cope with all the things that you had to do?

___0=never ___1=almost never ___ 2=sometimes ___3=fairly often ___4=very often

7. In the last month, how often have you been able to control irritations in your life?

___0=never ___1=almost never ___ 2=sometimes ___3=fairly often ___4=very often

8. In the last month, how often have you felt that you were on top of things?

___0=never ___1=almost never ___ 2=sometimes ___3=fairly often ___4=very often

9. In the last month, how often have you been angered because of things that were outside of your control?

___0=never ___1=almost never ___ 2=sometimes ___3=fairly often ___4=very often

10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?

___0=never ___1=almost never ___ 2=sometimes ___3=fairly often ___4=very often

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