The Effects of Vitamin B6 Supplementation on Mood States in College Women Taking Oral Contraceptives

by

Anne Curtin

A Thesis Presented in Partial Fulfillment
of the Requirements for the Degree
Master of Science

Approved November 2019 by the
Graduate Supervisory Committee:

Carol Johnston, Chair
Corrie Whisner
Shauna Grant

ARIZONA STATE UNIVERSITY

May 2020

ABSTRACT

Oral contraceptives are one of the most frequently used forms of birth control among young women. However, research has shown that this type of medication can contribute to negative changes in mood and diminished vitamin status. In particular, women taking oral contraceptives are at an increased risk of vitamin B6 deficiency due to changes in enzyme activity with estrogen intake. Depressed mood is one of the known symptoms of vitamin B6 deficiency as this vitamin acts as an essential cofactor in converting tryptophan to the neurotransmitter, serotonin. Lack of adequate levels of vitamin B6 therefore contribute to decreased production of serotonin and subsequent changes in mood, including symptoms of depression. With vitamin B6 being the most common nutrient deficiency, and the ever increasing prevalence of depression in the United States, especially among young adults, it is crucial that researchers investigate ways to mitigate both of these undesirable side effects. Current research on the topic fails to directly connect supplementation of vitamin B6 to positive changes in mood in oral contraceptive users.

This 12-week long double-blinded, placebo-controlled crossover trial examined the effects of daily supplementation of vitamin B6 as 100 mg of pyridoxine hydrochloride, on mood states in 8 healthy college women (18-25 y) that use combined oral contraceptives. Vitamin status was assessed via plasma pyridoxal 5'-phosphate (PLP). Plasma PLP levels significantly increased by >193% ($p=0.003$) with daily supplementation of 100 mg B6 over a four week period. Mood changes with supplementation were assessed using the Profile of Mood States (POMS). Although a small improvement in the POMS depression sub score was observed after 4 weeks of

vitamin B6 supplementation (14.7%), the changes were insignificant ($p>0.05$). Furthermore, total mood disturbance scores did not significantly change with either the placebo or supplement periods. While mood states were not improved, a significant decrease in the presence of depressive symptoms as measured by the Beck Depression Inventory was observed after vitamin B6 supplementation, compared to placebo ($p=0.047$). The results of this study necessitate further investigation into the use of B6 supplementation as a means of reducing negative mood changes in oral contraceptive users.

ACKNOWLEDGMENTS

The completion of this thesis project would not have been possible without the tremendous support from my family, friends, and the academic community at ASU. This study was also supported by ASU's Office of Knowledge Enterprise Development, the Graduate College, and the Graduate and Professional Student Association. Materials for analysis of blood samples and participant incentives were covered in part by this funding.

I would like to thank all of my committee members for their time, support and guidance throughout this process. I would like to thank my chair, Dr. Carol Johnston, for her invaluable input, direction, and constant encouragement. I would also like to thank my committee members, Shauna Grant and Dr. Corrie Whisner for their willingness to be a part of this project and pushing me to do my best.

I am also grateful for the efforts of Veronica Zamora, Ginger Hook, Cathie Nguyen and Justin Le for their assistance in completing blood draws, processing samples and the many hours spent in the lab. Furthermore, I would like to thank the participants that made this study possibly by committing their valuable time to this study.

Lastly, I would like to thank all of my friends and family that have supported me over the past year. Thank you to my MSDI cohort including Sara Lolley, Bethany Weigand, April Incollingo, and Emily Pelham for empowering me and inspiring me to continue working. Special thanks to Raymond Cooley, Allison Bramanti, Britney Ater, Andra Yung, and my mom for always being there to encourage me and brighten my day.

TABLE OF CONTENTS

Page

APPENDIX Page

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Vitamin B6 deficiency is the most common nutrient deficiency in the United States, with approximately 15-27% of adults having suboptimal levels [1]. Furthermore, some subpopulations are at a particularly high risk of this nutrient deficiency due to interacts with medications or lifestyle choices such as smoking [2,3]. Multiple studies have shown that women who use oral contraceptives are at a notably higher risk of lowered vitamin B6 status [4,5,6]. According to NHANES data, as many as 40% of women in this population have low plasma pyridoxal phosphate levels, the primary indicator of vitamin B6 status [1]. This presents issues for this population as vitamin B6 is an essential coenzyme for hundreds of biochemical reactions [7].

One of the primary functions of vitamin B6 includes acting as a cofactor in the synthesis of neurotransmitters [7]. Pyridoxal phosphate (PLP), the most active form of vitamin B6, is particularly important for the conversion of tryptophan to serotonin [8]. Serotonin, along with other neurotransmitters, are important chemicals in the brain that help regulate mood. When vitamin B6 levels are low, the synthesis of serotonin cannot occur, as plasma PLP is not available to assist the enzyme, L-amino acid decarboxylase, which is needed for serotonin synthesis from tryptophan [8]. This lack of plasma PLP contributes to less serotonin in the brain, which leads to symptoms of depression and depressed mood [7,8]. Depressive symptoms are a well-known sign of vitamin B6 deficiency and should be appropriately monitored when assessing factors that impact the risk of vitamin deficiency.

Although literature has confirmed the link between vitamin B6 deficiency with symptoms of depression, as well as the risk of decreased vitamin status from oral contraceptive use, the current literature fails to identify a direct connection between decreased plasma B6 levels and diminished mood states related specifically to the use of oral contraceptives in this potentially vulnerable population. Investigating this direct link could help determine possible steps that could be taken to minimize the risk of depressed mood states. Moreover, examining the effect of vitamin B6 supplementation on mood states in women that use oral contraceptives could help establish a possible treatment for this undesirable side effect of oral contraceptive use.

Purpose of Study

The purpose of this crossover study was to determine how vitamin B6 supplementation impacts mood states in college age (18-25 years) women that use oral contraceptives, in comparison to a placebo treatment. Participants also were expected to have marginal B6 status based on the results of a food frequency questionnaire. The intervention group received 100 mg of B6 daily for four weeks while the control group received a control pill (vinegar pill) to blind participants to which group they were in. After four weeks of intervention, and a four week washout period, participants were assigned to the opposite treatment for four weeks following a crossover study design to control for variations between participants.

Research Aim and Hypothesis

This crossover study aimed to examine the effects of daily vitamin B6 supplementation (100 mg) over a 4-week period on mood states in college age (18-25 y) women that have used oral contraceptives consistently for at least a year. The women in

this study also were expected have a low B6 status based on a food frequency questionnaire.

H1: Daily supplementation of vitamin B6 (100 mg) over a 4-week period will improve mood states in college age women (18-25 y) with marginal vitamin B6 status that use oral contraceptives, compared to the placebo treatment.

Definition of Terms

- Mood state: an emotional state of an individual at a particular point in time, as measured by the Profile of Moods States (POMS) and Beck assessment. POMS measures mood through six domains including vigor-activity, anger-hostility, tension-anxiety, fatigue-inertia, depression-dejection, and confusion-bewilderment. A Likert scale is used to determine how well a particular feeling aligns with an individual.
- Marginal B6 status: Plasma PLP below 30 nmol/L.
- Oral contraceptive: Daily pills taken by mouth that use a 28-day cycle and are intended to prevent pregnancy. For this particular study, participants only took a form that contained both estrogen and progestin.
- Oral contraceptive user: a women that has consistently used oral contraceptives for at least one year prior to the start of the study.
- Vitamer: different forms of a vitamin with a similar chemical structure and vitamin activity.
- Pyridoxal phosphate: A vitamer of vitamin B6, the active coenzyme form of B6.
- Coenzyme: a component needed to help an enzyme function.

Delimitations and Limitations

Delimitations:

- Female oral contraceptive users between the ages of 18-25 years old were recruited for this study.
- Participants, on average, consumed less than or equal to 2.6 mg B6 daily, or 200% of the RDA (1.3 mg).
- Participants were non-smokers that were not on medications that could alter mood states, such as antidepressants. Participants were also all students from Arizona State University.

Limitations:

- Participants were instructed to continue their normal eating patterns; however, it is difficult to determine adherence to this instruction.
- Participant adherence to treatment, either vitamin supplement or placebo vinegar pill, over the 4-week trial is not guaranteed. Subjects were instructed to keep track of days that they followed the treatment and compliance was recorded in results.
- Since all participants were ASU students, this study may not be as generalizable to other populations.
- Multiple factors including significant life events and illness can contribute to changes in mood state. These issues were addressed though a questionnaire taken after the administration of the mood assessment and controlled for during analysis.

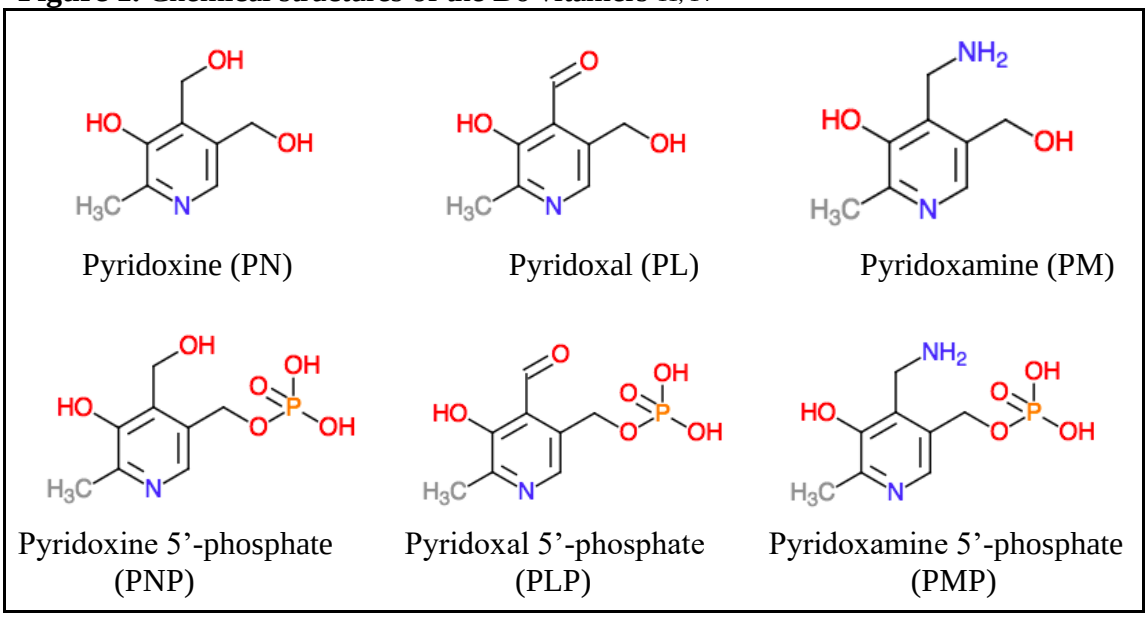
CHAPTER 2

REVIEW OF LITERATURE

Structure of Vitamin B6

Vitamin B6 was first discovered in 1934 as a factor that helped prevent a skin condition known as acrodynia in rats [9]. By the end of the 1930s, the vitamin had been successfully isolated and named "pyridoxine" [10]. Since then, the structure and functions of vitamin B6 have been studied extensively due to its involvement in a plethora of reactions within the human body. Vitamin B6 can be found in nature as six different vitamers: pyridoxine (PN), pyridoxal (PL), pyridoxamine (PM), pyridoxine phosphate (PNP), pyridoxal phosphate (PLP), and pyridoxamine phosphate (PMP) (Figure 1) [11]. All forms of vitamin B6 are characterized by the presence of a pyridine ring (nitrogen containing), along with a methyl group in the 1' position and hydroxyl group in the 2' position [11]. The most stable of the vitamers is pyridoxine, which contains an additional alcohol group as part of its structure (Figure 1). The unphosphorylated vitamers also contain a hydroxyl group at the 5' position, while the phosphorylate vitamers have a phosphate group at the 5' position [11]. While all of these forms can be active, pyridoxal phosphate, the phosphorylated form of PL, is the main form needed for B6 to act as a coenzyme in a myriad of reactions. This active form, PLP, is primarily produced in the liver, as the enzymes that are needed for conversion are found in this organ [12,13].

Figure 1. Chemical structures of the B6 vitamers [11, 14]



Absorption

In order for absorption of B6 vitamers from foods to occur, the vitamin must be in one of the dephosphorylated forms. If B6 is ingested in the form of PLP, PMP or PNP, hydrolysis of the phosphate group occurs at the brush border of the intestines to allow for absorption to occur [15]. This process is generally done with help from the zinc-dependent enzyme, alkaline phosphatase, which removes the phosphate group to permit absorption [16, 17]. Free PN, PL and PM from dietary sources are absorbed via passive diffusion in the jejunum of the small intestine [17]. Absorption can be impeded with certain forms of B6 due to interference from the food matrix and conjugation of vitamers. In the case of many plant foods, B6 is available as PN [18]. However, this source of B6 can be difficult to absorb as PN is often conjugated to glucose molecules, forming a pyridoxine glucoside [18]. Glucosides are generally too large to be passively absorbed. The presence of these glucosidic bonds has been found to reduce bioavailability up to 75-80% [19]. In a study by

Gregory et al. examining the bioavailability of PN and PN-glucosides using isotopic labeling, the PN-glucosides were found to have only 58% bioavailability compared to the PN alone [20]. With the reduced bioavailability of PN-glucosides, in order for PN to be absorbed appropriately, the glucosidic bond needs to be broken via the enzyme, glucosidase to release free PN [18]. The Gregory et al. study indicates that this enzyme can be found in the intestinal mucosa and potentially microflora to help release PN from the glucoside for better absorption [20]. Once absorbed, PN, PL and PM can be released into portal blood for utilization and conversion to other vitamer forms [15,18].

Metabolism

Once B6 vitamers have been absorbed and released into portal blood, they travel to the liver to be metabolized. Vitamers are taken up by the liver via passive diffusion, as they are already in one of the dephosphorylated forms from being absorbed into the portal blood from the intestines [18]. Once in the liver, vitamers can be interconverted by action of kinases and oxidases [21]. These interconversions allow for the formation of more PLP. This action is necessary as PLP is the primary active coenzyme form of B6 and more PLP is needed for metabolic processes than is available from dietary sources alone. Kinases utilize adenosine triphosphate (ATP) to help add a phosphate group to the unphosphorylated vitamers [21]. Once in the phosphorylated form, PNP and PMP can be converted to PLP by riboflavin (FMN)-dependent enzymes [13]. This type of oxidase is primarily found in the liver and intestines [13]. If needed, the phosphorylated vitamers can be converted back to PN, PM or PL with help from phosphatases that remove a phosphate group from the structure [21]. PLP and PL are the main vitamers that are released from hepatic tissue into the bloodstream.

Many factors, including smoking and liver disease may interfere with adequate metabolism of the vitamin. In a study conducted by Vermaak et al. B6 status was assessed in 268 healthy males that were either smokers, ex-smokers, or non-smokers [3]. This study found that participants that were current smokers had significantly lower plasma PLP concentrations compared to both the non-smokers and ex-smokers [3]. A proposed explanation of these findings is that smoking may contribute to oxidative stress which interferes with enzyme activity and in turn could limit B6 conversion to PLP [3,22,23].

PLP concentrations have also been found to be lowered in individuals with liver disease. A study published in the American Journal of Clinical Nutrition that examined vitamin B6 metabolism in cirrhosis patients confirmed that these individuals had significantly lower levels of plasma PLP [13]. This study explained that liver enzyme activity is decreased with cirrhosis due to liver damage. The observed decrease in enzymatic activity prevents proper conversion of B6 vitamers to PLP, thus lowering plasma levels [13].

Older individuals have also been found to have diminished vitamin B6 status as assessed by plasma PLP. In a study by Fonda and Eggers, the mechanism for these lower observed levels was examined in mice [24]. They found that the older mice were less able to appropriately convert B6 vitamers to PLP to be utilized by the body, compared to the young mice[24]. This study also found that more of the B6 vitamers in the livers of the older mice were being converted to pyridoxic acid, and as a result were being excreted from the body [24]. This mechanism explains the decreased vitamin B6 status in older adults.

Transport

B6 vitamers are transported between organs and tissues primarily as one of the unphosphorylated forms. When in these forms, vitamers can be passively diffused. Additionally, vitamers are often found bound to binding proteins via Schiff bases [25]. In tissues, the unphosphorylated vitamers are taken up from circulation into the cell. Once in the cell the vitamers are phosphorylated by a kinase (most often PL to PLP). When phosphorylated vitamers are bound to proteins, they cannot be transported in and out of cells [25]. Protein binding also helps prevent dephosphorylation, which allows PLP to be kept in tissues to be used as a coenzyme [26]. Furthermore, when PLP is bound, it cannot be hydrolyzed and excreted. In plasma, PLP binds to proteins including albumin and hemoglobin [26,27]. In the case of red blood cells, PL that has been taken up is converted to PLP then bound to hemoglobin [27].

The action of other vitamins is important in the process of interconverting and transporting B6 vitamers. Zinc is a required component of the zinc-ATP complex which is needed by pyridoxal kinase to add a phosphate group to the unphosphorylated vitamers [28]. This process helps create PNP, PLP and PMP [28]. Riboflavin is necessary for the FMN-dependent oxidases found in the liver that allow for interconversions between PNP and PMP to PLP [13]. Magnesium is also a cofactor in these enzymatic reaction and is involved with zinc-ATP complex [21,29].

Storage

Vitamin B6 is stored in variable amounts within the body. Studies suggest that body stores of vitamin B6 range from approximately 60 mg to 170 mg [30,31]. B6 is primarily stored within muscles tissues, with approximately 75-80% of storage in these

tissues [30]. In muscle tissue, B6 is primarily stored as PLP and is bound to glycogen phosphorylase [30]. The phosphorylation of PL after it has entered the muscle tissue prevents PLP from leaving cells via diffusion. Protein binding, as described previously, ensures that hydrolysis of PLP does not occur. B6 is also stored in smaller amounts, approximately 5-10%, in the liver [32] . Small amounts of vitamin B6 may also be stored in organs such as the brain, kidneys and spleen [32].

Excretion

As a water soluble vitamin, B6 can be excreted from the body in multiple forms, primarily through urine. People receiving adequate amounts of the vitamin via diet, will excrete the vitamin mainly as 4-pyridoxic acid [33]. 4-Pyridoxic acid is the principal urinary metabolite and makes up approximately half of the B6 that has been excreted through urine [33]. This metabolite is formed when PL is oxidized. In the liver and kidneys, this oxidation can be done by FAD-dependent aldehyde oxidases or NAD-dependent aldehyde dehydrogenase, which can be found in all tissues [33,34]. When B6 intake is high, for example in individuals that take vitamin B6 supplements, it may be excreted in urine as unchanged PN or as 5-pyridoxic acid [35]. Excessive dietary or supplement intakes promotes excretion in these forms as there are not enough binding proteins to help retain the vitamin in the body [34].

Primary Functions

B6 is a crucial component in a variety of chemical processes within the body. Specifically, B6 has been identified as an essential cofactor for over 100 reactions [16]. Some of these reactions include decarboxylation, elimination and deamination, dehydration, transulfhydration, transelenation, cleavage, racemization, and glycogen

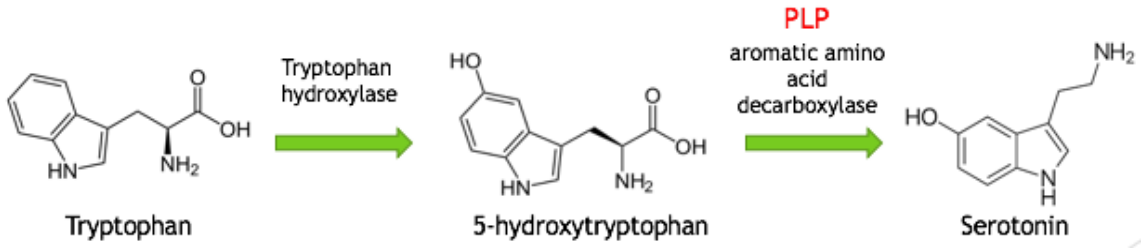
degradation [36]. B6 is also a necessary component for amino acid metabolism. B6 vitamers create a Schiff base that labilizes bonds on the amino acid which destabilizing the bonds [37]. This action effectively marks the bond, making it easier for enzymes to act on and degrade the amino acids as they are already in an unstable form. Other functions of vitamin B6 include assisting enzymes involved in glucose and lipid metabolism, gene expression, and heme synthesis [7,38].

This vitamin also interacts with a myriad of other vitamins and nutrients within the body to carry out biochemical reactions. One of the critical functions of B6 is as a cofactor is the synthesis of multiple neurotransmitters, including dopamine, serotonin and epinephrine [7]. Without this vitamin present in adequate amounts, the neurotransmitters cannot be synthesized appropriately, which may contribute to changes in psychological function and mood [7].

Tryptophan to Serotonin

In the synthesis of serotonin (5-hydroxytryptamine) from tryptophan, vitamin B6 acts as an essential cofactor for the enzyme, aromatic L-amino acid decarboxylase [8]. A brief illustration of this mechanism can be seen in Figure 2. Serotonin is a neurotransmitter that is important for regulation of mood and is popularly known as the happy chemical [39-41]. When plasma PLP levels are lowered, tryptophan may not be appropriately converted to serotonin, contributing to diminished serotonin levels and therefore worsened mood or depressive symptoms. Individuals that are at risk for lower levels of B6, such as with oral contraceptive users, smokers, and those with liver disease, may be at an increased risk of altered mood related to this mechanism being interrupted [3,12,13].

Figure 2. Tryptophan conversion to serotonin with PLP as a cofactor for the enzyme aromatic amino acid decarboxylase.



Recommended Intake

The recommended intake for vitamin B6 varies by both age group and gender. For infants 0-12 months old, an adequate intake (AI) is used rather than a recommended dietary allowance (RDA). The AI is based on the amount of B6 that can be found in human breast milk. According to a study conducted by West and Kirksey, the average B6 content in breastmilk varied according to the intake of the mother [42]. In women that consumed <2.5 mg of B6 daily, milk contained approximately 0.13 mg/L, while those that consumed 2.5-5 mg had an average of 0.24 mg [42]. Other studies have found consistent data suggesting that the mean B6 content is between 0.15-0.21 mg/L in breastfeeding women that consume 2.5 mg daily [43]. Based on these studies and the mean breastmilk intake of infants, the AI for infants 0-6 months was set at 0.1 mg/day and 0.3 mg/day for infants 7-12 months old [16].

As individuals get older, the RDA for vitamin B6 increases. Table 1 shows a full list of RDAs based on age and gender. The RDA for vitamin B6 is 120% of the estimated average requirement (EAR) [16]. The EAR for each age group/ and gender was established based on studies focused on B6 requirements. In a study using depletion-repletion diets to assess B6 requirements, Huang et al. found that women between the age of 28-34 years that had been provided with a depletion diet of only 0.45 mg B6 from food sources, had

plasma PLP levels of just above 20 nmol/L [44]. Furthermore, during the repletion phase, women that consumed 1.26 mg B6 daily had an adequate plasma PLP of 38 nmol/L [44]. This suggests that the EAR for women is somewhere between 0.45 mg and 1.26 mg [44]. Another study looking at how protein impacts B6 status found that women between the ages of 19-38 years old that consumed 1.25 mg B6, along with various levels of protein, had normal plasma PLP concentrations (above 30 nmol/L) [45]. However, women that consumed the highest amount of protein (2 g/kg body weight) did have slightly lower plasma PLP levels but did not drop below 20 nmol/L[45]. The results of this study confirms that the EAR would be less than 1.25 mg for women.

Multiple studies have also examined the requirements for males in the 19-50 age group, however these studies primarily have assessed B6 status using a tryptophan load test. One of these studies conducted by Yess et al. found that after receiving 100 g of protein and 0.16 mg PN daily for nearly 2 months, men were able to normalize tryptophan catabolites after supplementing with 0.9 mg of PN [46]. This indicates that the EAR would be around 1.0 mg of B6 from food [46]. Based on the information from these studies, the RDA for both men and women between the ages of 19 to 50 years old was set at 1.3 mg/day [16].

Studies have also shown that older adults require higher intakes of B6 as their ability to adequately absorb B6 diminishes. In a cross-sectional study, Kjeldby et al. examined dietary habits as well as PLP levels in 61 nursing home residents with a mean age of 85.3 year old [47]. This study found the median level of plasma PLP to be 20.7 nmol/L [47]. With this, 30 of the 61 participants were classified as having vitamin B6 deficiency. Furthermore, of those that participated in the study, 14 of them used vitamin

B6 supplements and all of these individuals had sufficient plasma PLP levels [47]. Another study published in the American Journal of Clinical Nutrition investigated riboflavin and plasma PLP levels in participants between the ages of 65-91 years old. Of 41 participants in this study, 12 (29%) had suboptimal plasma PLP levels at baseline [48]. These results confirm that B6 deficiency is especially prominent in elderly individuals [48]. Due to the lower observed levels as well as decreased ability to absorb B6, the RDA for individuals over the age of 51 years old is 1.5 mg/day for females and 1.7 mg/day for males (Table 1) [16].

Vitamin B6 needs are also increased in women that are pregnant and lactating. In women that are pregnant, plasma PLP levels have been shown to decrease significantly as blood volume increases and PLP concentrations increase in cord blood to provide the fetus with nutrients [49]. During lactation, needs are increased as B6 intake by the mother impacts the concentration in breastmilk [42]. For these reasons, the RDA during pregnancy and lactation are 1.9 and 2.0 mg/day, respectively [16].

Table 1. Recommended Dietary Reference intakes for Vitamin B6 according to age and gender.

Age (years)	RDA for males (mg)	RDA for females (mg)
0-6 months (AI)	0.1	0.1
6-12 months (AI)	0.3	0.3
1-3	0.5	0.5
4-8	0.6	0.6
9-13	1.0	1.0
14-18	1.3	1.2
19-50	1.3	1.3
51+	1.7	1.5
Pregnancy (14-50)	n/a	1.9
Lactation (14-50)	n/a	2.0

Adapted from the Institute of Medicine DRI tables [16].

Dietary Sources

All forms of vitamin B6 can be found in a variety foods. PM, PL, PMP and PLP are mostly found in animal products, while PN and PNP are primarily in plant foods [16]. Foods containing relatively high amounts of B6 include beef liver (0.9 mg/3oz), bananas (0.4 mg/banana), tuna (0.9 mg/3oz), chickpeas (1.1 mg/cup), potatoes (0.4 mg/cup) and Cheerios (0.5 mg/cup) [50]. Table 1 includes a more comprehensive list of food items and the amount of B6 in each item.

While some plants may contain significant amounts of B6, PN can become conjugated when a glucosidic bond forms and creates a glucoside [51]. This process in which PN presents as a glucoside, inhibits appropriate absorption of the vitamin as the form has been altered. PN glucosides are only about half as bioavailable compared to the non-glucoside form [51]. B6 vitamers that are found in meats and grains can also have lower than expected levels of B6, as the vitamin can be lost during processing, such as

milling for grains, and cooking and freezing for meats [52]. Therefore, the bioavailability of vitamin B6 varies from approximately 60-90% depending on the specific vitamer and the factors discussed above [52]. Yet another factor that can limit vitamin B6 availability and content is processing of foods prior to consumption. As discussed in an article by JM Dietz and JW Erdman, some cooking methods can destroy vitamin B6 and cause lower levels in food [53]. Cooking using hot water, such as with blanching, may destroy up to 21% of B6 in foods [53,54]. Other factors that may reduce vitamin B6 content include exposure to light and alkaline environments [53].

Table 2. Amounts of vitamin B6 found in various foods.

Food (serving size)	**B6 content per serving (mg)**
Chickpeas (1 cup)	1.1
Beef liver (3 oz)	0.9
Tuna (3 oz)	0.9
Chicken breast (3 oz)	0.5
Fortified cereals, Cheerios (1 cup)	0.5
Potatoes (1 cup)	0.4
Turkey meat (3 oz)	0.4
Bananas (1 medium)	0.4
Ground beef, 85% lean (3 oz)	0.3
Bulgar (1 cup)	0.2
1% low fat cottage cheese (1 cup)	0.2
Squash (½ cup)	0.2
Onion (½ cup)	0.1
Spinach (½ cup)	0.1
Nuts, mixed (1 oz)	0.1
Cooked rice, white (1 cup)	0.1

Adapted from USDA factsheet [50]

Assessment

B6 status is directly assessed by measuring plasma pyridoxal phosphate (PLP) levels [55]. Plasma PLP most accurately reflects B6 status in the body as it accounts for B6 stores in tissues, while other potential measures, such as total B6 concentration in blood or urinary B6 concentrations, do not [56]. A plasma PLP >30 nmol/L is considered adequate in both men and women. Plasma levels of PLP can be accurately measured through an apo tyrosine decarboxylase assay [16]. Excessive protein intake has been suggested to interfere with B6 status when plasma PLP is measured [57,58]. Multiple studies have shown that increased dietary protein intakes contribute to decreased levels of vitamin B6 in the body [58]. This is likely due to an increased need for PLP to help break down amino acids [37,57]. Furthermore, this issue has been shown to be especially prominent in females [45,57].

One study by Hansen et al. investigated vitamin B6 status in women based on three different levels of protein intake. For this study, 9 women were given diets containing either 0.5, 1.0 or 2.0 g protein/kg body weight along with a consistent 1.25 mg B6/day for two weeks [45]. Analysis of blood and urine measures of B6 confirmed that higher protein intakes were correlated with increased excretion of B6 metabolites and lower plasma PLP levels [45]. For this study, the vitamin B6 to protein ratio for maintaining a sufficient B6 status was determined to be 0.02 mg B6/g protein [45]. A later study conducted by the same group studied the effect of varying levels of vitamin B6 intake with a constant 85 g/day protein intake in a total of 16 women[59]. For this study, a vitamin to protein ratio of >0.016 mg B6/ g protein was needed to maintain an optimal B6 status

in women [59]. Based on these findings, those that have high dietary intakes of protein may need to increase vitamin B6 consumption to support amino acid metabolism.

B6 levels can also be assessed functionally by conducting a tryptophan load test, in which xanthurenic acid excretion in urine is measured. High levels of xanthurenic acid in the urine indicate a B6 deficiency as B6 is needed to remove the alanine moiety from 3-hydroxykynurenine, which is an intermediate in niacin synthesis from tryptophan [21]. When B6 is lacking, xanthurenic acid is produced, rather than 3-hydroxyanthranilic acid [21,34]. This product is then excreted through urine. While 4-PA is also excreted in urine, this measure is not appropriate for assessing vitamin B6 status because it can change frequently and depends primarily on recent dietary intake of B6 [35]. Furthermore, measurement of total B6 concentration in blood is an inadequate measure of B6 status as this measure can fluctuate greatly. Fluctuations are especially in women, as this measurement can be impacted by a women's menstrual cycle [60].

Deficiency and Toxicity

Vitamin B6 deficiency is the most common nutrient deficiency in the United States. According to NHANES data collected from 2003-2004, 15-27% of adults between 21-44 years old had deficient B6 levels, with plasma PLP levels lower than 20 nmol/L [1]. Possible symptoms of deficiency include depression, rashes, seizures and confusion. Since B6 is needed to produce heme in red blood cells, the lack of this vitamin also leads to hypochromic microcytic anemia [16,38].

B6 is necessary for multiple neurological processes to occur. As noted earlier, B6 is an essential cofactor in neurotransmitter synthesis. Diminished levels of B6 in the body can lead to various issues with cognitive functions [7]. Symptoms of deficiency related to

impaired synthesis of neurotransmitters include confusion, tiredness, depressed mood and seizures [7].

As previously discussed, smokers, individuals with liver disease, and oral contraceptive users may be at an increased risk of deficiency [3,4,13]. Additionally, alcoholics are at a higher risk as important B6 converting enzymes are located in the liver, and excessive alcohol intake can damage this organ [61]. Alcohol consumption may also contribute to decreased plasma PLP levels due to competition for protein binding with some alcohol metabolites [61]. With this, PLP is more quickly degraded and excreted from the body [62]. Therefore, individuals that are heavy consumers should be aware of possible deficiency.

According to the Institute of Medicine's Dietary Reference Intakes, the upper limit (UL) for vitamin B6 is 100 mg/day for adults older than 19 years old [16]. This safe upper limit was established through multiple studies that examined the effects of mega dosing the vitamin. The UL was determined though studies to be lower for individuals under the age of 19, compared to adults. For individuals 18 and younger, the UL ranges from 30-80 mg depending on age. Children 1-3 years old should not have more than 30 mg/day [16]. An upper limit for children under 12 months old has not been established as children in this age range should be receiving vitamin B6 through breast milk(Table 3) [16].

In many of the studies involving mega dosing of vitamin B6, those consuming excessive amounts of B6 in the form of supplements began experiencing severe symptoms such as ataxia and neuropathy [63,64]. In a case study discussed by Gdynia et al., a 75 year old individual had been consuming 9.6 grams of pyridoxine/day for three years [63]. At this level, the individual began experiencing symptoms of toxicity including severe

neuropathy, reduced or absent reflexes, and muscle weakness to the point of becoming wheelchair bound [63]. However, with cessation of supplement consumption, the individual's symptoms were alleviated over time to the point that they no longer required use of a wheelchair. While this level of consumption is extremely high compared to the current UL of 100 mg for B6, this showed how vitamin B6 can be detrimental when taken in such high concentrations. In another study with patients that had been receiving between 2-7 grams of B6 in the form of pyridoxine, similar neuropathy symptoms were observed [64]. Likewise, Berger et al. also noticed the development of neuropathy in healthy individuals that had been given either 1 g or 3 g of pyridoxine [65]. These symptoms continued for weeks after participants stopped use of the supplements [65]. However, no studies have shown symptoms of neurological damage with daily intakes below 200 mg [66]. Due to the negative symptoms observed with high B6 intakes, specifically with extreme supplement use, the UL was set at a point much lower than that which causes these severe symptoms to promote safe consumption levels.

Table 3. Daily Upper Limit for Vitamin B6 according to age.

Age (years)	**Upper Limit (mg)**
0-12 months	n/a
1-3	30
4-8	40
9-13	60
14-18	80
19+	100
Pregnancy & Lactation	
14-18	80
19+	100

Adapted from the Institute of Medicine DRI tables [16].

Oral Contraceptives

With oral contraceptive use at approximately 34-46% in college women, it is important to evaluate all potential risks involved with use of this type of medication [67]. National surveys have been conducted on the different types of contraceptives that are used among women in the United States. Based on data from the National Survey of Family Growth, oral contraceptives (OC) are the most commonly used form of contraception in the United States [68]. This includes a variety of different OC brands, however only two primary forms of OCs exist: a combined estrogen and progestin form, and a progestin only form [69]. There are currently more types of combined OCs available, but progestin only options are also obtainable. Use of progestin only pills is also much less common, with only 0.4% of U.S. women that are of reproductive age using this form [69].

Most studies examining the effects of OCs on nutrients have primarily looked at the use of combined forms of the pill. Although multiple studies have been conducted on whether or not OC use impacts mood, these studies have shown contradictory results related to differences in the specific populations studied. In a longitudinal study exploring the effects of OCs on mood in healthy women (18-35 y) that did not have premenstrual syndrome, those that used OCs were found to have improved mood compared to those that do not use the medication [70]. However, a double-blinded, randomized trial looking at the effects of oral contraceptives on mood found that mental health status played a role in mood changes related to OC use [71]. Of 202 women that were given either an oral contraceptive or a placebo, women that had preestablished mental health disorders had more negative changes in mood when given a combined OC [71]. These results indicate that

previous mental health status could be another factor that determines interactions with OCs. Analysis of data from the same study found that the use of OCs resulted in a significant increase in anxiety and irritability for participants that were given the combined oral contraceptives. Moreover, these symptoms were the primary reason for discontinuation of the medication [72]. This further demonstrates that oral contraceptive use may negatively impact mood.

In the study that found that OCs had a negative effect on mood, a suggested reason for the diminished mood state was the negative effect that OCs have on vitamin and nutrient status. A handful of studies have shown that using oral contraceptives that contain both estrogen and progestin can decrease the nutrient status of multiple vitamins. In one such study by Prasad et al., vitamin status was examined before and after 3 months use of one of two types of combine oral contraceptives [73]. Results were compared to a control group of women that did not use any forms of hormonal contraception. Women that had been on either of the combined oral contraceptives were found to have significantly lower levels of vitamin B6, B12 and folate, as well as had more clinical signs of deficiencies compared to the control group [73]. Yet, Wilson et al explain that with newer forms of oral contraceptives, dosages are much lower than when OCs first became available to consumers. With these lower dose forms of OCs, the incidence of low levels of folate and B12 in users has decreased [4]. However, even with the decreased dosages, low levels of plasma PLP have still been observed in women taking oral contraceptives [4]. Less research has been done on the effects of progestin only oral contraceptives as these are relatively newer forms of the medication.

The diminished nutrient status related to OC use may contribute to negative side effects possibly including depressed mood. Depression and confusion are known symptoms of both B6 and B12 deficiency [16]. Furthermore, studies have confirmed that low levels of vitamin B6 contributes to symptoms of depression as B6 is needed to help synthesize neurotransmitters [74]. This nutrient is specifically needed for the conversion of tryptophan to serotonin, a neurotransmitter that assists in mood regulation[74]. Based on the potential risk for depressive mood states related to OC use, and the lack of definitive information on the topic, further research is needed to address the prevalence of these depressed mood states in women that use oral medications as a means of contraception.

The exact mechanism for how OCs interfere with plasma PLP levels has yet to be examined and described in detail [4]. One theory for the observed decreased levels is that the estrogen component found in many oral contraceptive pills contributes to an increase in synthesis and activity of enzymes, such as those involved in tryptophan metabolism [75-77]. Vitamin B6 in the form of PLP is an essential cofactor for enzymes involved in this mechanism. Therefore, when activity of enzymes is increased, plasma PLP levels are diminished, contributing to a vitamin B6 deficiency [75,77]. This mechanism needs further examination but has been proposed by multiple investigators examining the observed lowered level of plasma PLP in OC users [75,78].

Mood States and Depression

A mood state is how a person feels at a specified point in time. While mood states can include feeling down or sad, this differs from a clinical diagnosis of depression in that depression is an ongoing state of depressed mood [79]. Furthermore, mood states

encompass all a person is feeling at a particular point in time, rather than focusing on negative aspects of mood.

Depression is a growing issue in the United States. According to CDC data collected from 2013-2016, 8.1% of people in the U.S. have symptoms of depression [79]. This data also suggests that the prevalence of depression in women aged 20-39 is nearly double of that in men, with 10.1% of women showing signs of depression and 5.5% of men [79]. This significant difference in symptoms of depression in women emphasizes a need to identify causes and treatments of depression for this population. Furthermore, due to the widespread impact that depression has on society, the issue needs to be examined in depth to help minimize the risks for all people. Depression can be different for everyone and reviewing potential factors that could contribute to an increased risk of this disorder is necessary.

A variety of factors can contribute to the development of depression, but the symptoms of clinical depression are most often directly caused by chemical imbalances within the brain [80]. In a 2002 article published in the Journal of Clinical Psychopharmacology, Blardi et al. examined the effects of a selective serotonin reuptake inhibitor on platelet and plasma serotonin levels [81]. All participants in this study had previously been diagnosed with depression. The depressed subjects in this study presented with lower than normal levels of both plasma serotonin and platelet serotonin at baseline [81]. This finding confirmed that serotonin measures are decreased in depressed individuals. Muck-Seler et al. also examined serotonin levels by focusing specifically on platelet levels of serotonin in 166 depressed individuals compared to 175 healthy control subjects [82]. The lowest levels of platelet serotonin were observed in depressed women,

while healthy men had the highest levels. Furthermore, this study found that the lowest platelet serotonin concentrations were associated with higher incidences of suicide attempts and suicidal behavior [82]. Multiple studies have confirmed these finding in depressed individuals, supporting a relationship between depressed mood and low serotonin levels [83,84]. While these observed decreased levels of neurotransmitters contribute to observable symptoms of depression, many factors can impact these chemical imbalances.

In a study assessing mental health among undergraduate students from 108 colleges across the U.S., high levels of stress were found to be strongly associated with depression and likelihood of attempting suicide [85]. Other factors such as sexuality, gender and race were also found to impact like likelihood of an individual having mental health challenges [85]. Although clinical depression is clearly an issue in a variety of subpopulations, the presence of depressive mood states is also cause for concern. Depressive mood states differ from clinical depression as mood states are temporary and can change, while depression is an ongoing condition characterized by overall lack of interest, in addition to a combination of symptoms such as weight gain or loss, insomnia, confusion and fatigue [86]. Despite these differences in definition, the presence of depressive mood states is important to address as prolonged negative mood states may contribute to depression. In a study conducted by Nery et al. on personality traits and characteristics of those with Major Depressive disorder (MDD) compared to healthy subjects, reoccurring negative mood states were associated with personality traits of those with MDD [87].

The symptoms that accompany depression also influence mood state. The symptoms of depression include feeling of irritation, sadness, and emptiness [88]. These signs can also be present with depressive mood states. Many factors can contribute to the symptoms related to depression and negative mood states. Some determinants of mood states include sleep quality, significant life events, stress, physical activity, and diet [89,90]. A longitudinal study conducted by Brown et al. looked to examine various factors that contribute to the onset and progression of depression in working-class women [89]. This study found that severe life events, and low self-esteem were associated with symptoms of depression [89]. Another study looking at the impact of sleep on mood in men and women, found that females that were sleep deprived had significantly higher depression and anxiety scores than when they were able to sleep longer hours [90]. Additionally, both males and female subjects had higher confusion sub scores with sleep deprivation, compared to baseline [90]. This study confirms that inadequate sleep can negatively impact mood.

Research has also been conducted on the association between exercise and mood. In one study examining how different levels of exercise impacted mood in women with Major Depressive Disorder, all types of exercise were shown to improve mood states as defined by the Profile of Mood States assessment [91]. Furthermore, there were no differences found between levels of exercise, indicating that even light or moderate exercise can be helpful in improving mood.

Assessing Mood and Symptoms of Depression

Multiple methods have been found as accurate and valid measures for assessing an individual's mood. The Profile of Mood States is one commonly used assessment.

This method was developed in 1971 by McNair, Lorr & Droppleman as a means to specifically measure mood state as opposed to symptoms of depression [92]. The POMS assessment consists of 65 terms and covers six different components of mood including depression-dejection, anger-hostility, fatigue, tension-anxiety, confusion-bewilderment and vigor [92]. Individuals taking the assessment rate each term on a 5 point Likert scale from "not at all" to "extremely" to indicate how they have felt over the past week or day depending on the version [92]. An individual's total mood disturbance is then calculated from adding the subcategories together, with the exception of vigor, in which the score is subtracted from the total [92]. Although this assessment was originally developed nearly 50 years ago, it is still commonly used in research as a valid assessment tool.

The POMS assessment has been utilized and validated for use with a variety of populations. Early versions of the POMS assessment were primarily tested in men and in clinical setting including through the Veteran's Administration clinics [93]. The participants in the first studies to test this assessment were all psychiatric patients. In one of these studies, 150 VA psychiatric outpatients were administered the POMS assessment before and after receiving four weeks of treatment [94]. This data displayed that the POMS assessment could reliably be used to assess the six mood categories with a high retest consistency of 0.61-0.69 [94]. Furthermore, in analyzing the internal consistency of the tool as it was used in assessment of 1,000 male and female outpatients from the Boston University Medical Center Psychiatric clinic, McNair, Lorr & Droppleman found reliabilities for all of the 6 mood factors to be close to 0.90, or higher in some cases [93]. The POMS has also been used and validated for use in cancer patient, drug trails, and in sports and exercise research [95-97]. These studies helped to confirm that the Profile of

Mood States has a high internal consistency and can be used in wide variety of populations.

With the multitude of studies that have utilized POMS, normative data for various groups has been collects. In a study by Killen et al. that used the POMS to evaluate 2,360 adults in a program to stop smoking, the total mood disturbance score for this population was found to be 48.4 (33.6) [98]. Normative data has also been examined in college students. In looking at the six sub scores of the POMS, a study examining mood in healthy female college students from a large northwestern university showed that the mean and standard deviation for the tension and depression sub scores were 14.8 (11.4) and 13.9 (7.4), respectively [99]. However, this study did not specify total mood disturbance scores for the population. Based on the scores listed for each of the sub scores, total mood disturbance would have a mean of approximately 44.8 for this population of female college students [99]. In yet another study by Nyenhuis et al. normative POMS scores for adult populations were assessed [100]. These data, which consisted of 400 adult men and women, found the mean and standard deviation of POMS scores in adult women to be 20.3 (33.1) [100]. In breaking down POMS scores by age group, Nyenhuis et al. also found that college students had a much higher mean (SD) POMS scores of 39.9 (37.1) [100]. The data from these studies suggests that average POMS scores for college students is significantly higher than that of the average adult population, suggesting greater mood disturbances.

An abbreviated form of the POMS assessment, known as the Profile of Mood States-short form (POMS-SF) has also been developed as a validated tool for determining mood states. The POMS-SF was developed by Shacham in 1983 [101]. This version only

contains 37 of the 65 descriptive terms from the original, but still covers all six subcategories that are examined with the full length POMS assessment. Shacham developed this version by administering the assessment to 83 cancer patients and eliminating questions that compromised the internal consistency of each subscale [101]. The resulting 37 item assessment was shown to be highly correlated (r= 0.81 to 0.95) with all subscales of the original POMS, as well as the total mood disturbance score (r= 0.92) [101, 102]. The high correlations found between the POMS and POMS-SF confirms the validity of the POMS-SF as an accurate tool for determining an individual's mood. Furthermore, this shortened version can be more quickly completed, making in a slightly faster and easier alternative [102]. While this form of the assessment has been proven as a valid measurement in comparison to the original, the POMS assessment is still preferred as it includes a broader range of questions to assess mood and still can be completed relatively quickly in approximately 3-7 minutes by health individuals [102].

Beck Depression Inventory

The Beck Depression inventory (BDI) is a tool specifically used as a diagnostic test to assess the severity of symptoms of clinical depression. The BDI assessment was developed by Beck et al. in 1961[103]. The BDI determines the presence of depressive symptoms through 21 self-reported items which are given a score of 0-3 for each item. For this assessment, scores range from 0-63, where a higher score indicates that symptoms of depression are present [103]. A score of 0-10 indicates that the person being assessed is likely experiencing normal ups and downs. Scores from 11-16 may indicate that a person is experiencing mild disturbances in mood slightly outside of the normal range [103]. If a person receives a score of 17-20, they are considered to be borderline

clinically depressed**.** Individuals with scores of 21-30 are diagnosed with moderate depression, while scores of 31-40 are considered severely depressed. Any scores above 40 correspond to extreme depression [103].

This assessment has since been updated multiple times but is still commonly used to identify the presence and magnitude of depression. Since the development of this diagnostic assessment, a multitude of studies have been conducted that have confirmed the validity of the BDI. The BDI was first validated for use in psychiatric populations when it was developed over 50 years ago. One such study, reported by Beck et al. used this assessment for analyzing the depth of depression in 187 psychiatric patients and found a biserial correlation of 0.67 [103]. This study along with one other helped to initially confirm the validity of the assessment, promoting its use in future studies.

Although the Beck Depression Inventory was initially used for psychiatric patients, it has since been validated for use with other populations. In a 1978 study, Bumberry, Oliver and McClure examined the validity of the BDI for use in university students [104]. For this study, the original version of the BDI was administered to a total of 56 male and female university students from two different schools in the midwestern region of the United States. In addition to receiving the BDI, participants were assessed by board certified psychiatrists to assess depth of depression [104]. The results of the self-administered BDI were compared to results of psychiatric assessment. The study found a biserial correlation coefficient of 0.77 [104]. The results of this study suggested that the BDI is a valid assessment for use in university students.

While other validated methods exist for determining mood state and depression, the POMS and Beck Depression Inventory are simple and effective tools that can be used in a wide variety of populations.

After analysis of the current literature, there is an apparent lack of research connecting oral contraceptive use to diminished vitamin B6 status and the resulting effect on mood and symptoms of depression. Moreover, the question of whether or not vitamin B6 supplementation acts as a method of improving mood in those that use modern oral contraceptives has yet to be answered. The present study was therefore designed to address this paucity of research and determine if and to what degree supplementation of vitamin B6 impacts mood and symptoms of depression in oral contraceptive users.

CHAPTER 3

METHODS

Recruitment

Approval from the Arizona State University (ASU) Institutional Review Board was obtained prior to recruitment for the study (appendix A). Recruitment began in January 2019. Information about the study was sent to students via ASU Listservs. Flyers were also posted on the ASU Downtown Campus to increase reach. Potential participants were provided with a link to complete an online questionnaire designed using Qualtrics. Those that completed the survey and qualified for an in-person prescreening visit to further assess participant eligibility were contacted via email. During the initial in-person screening visit, participants that did not meet the inclusion criteria were excluded from the study. The remaining respondents that met inclusion criteria were notified of their eligibility and were invited begin the study.

Participants

Healthy, non-smoking females between the ages of 18-25 years old, who have taken a combined oral contraceptive (estrogen with progestin) consistently for at least one year prior to the start of the study, were recruited for this research study. Participants were non-vegetarians without food allergies and did not use prescription medicine, with the exception of oral contraceptives. Participants also did not use any type of supplements including mineral, herbal or B vitamin supplements prior to starting the study. Individuals that consumed at or below 200% of the RDA, or 2.6 mg vitamin B6 based on a food frequency questionnaire were eligible for the study. All participants provided written

voluntary consent after being informed about the purpose and requirements of the study. In total, 8 individuals were included in the study.

Study Design

This study was a 12-week, randomized, double-blinded crossover trial. After eligibility was confirmed through prescreening assessments, participants were stratified by age and BMI. Appendix B shows the extent of participation required for each visit. Once stratified, participants were randomized by a coin flip into the experimental group (B6 supplement) or control group (low does vinegar pill). Participants were instructed to take the provided pills once daily for four weeks. After a four week period, participants were instructed to continue their normal eating patterns and complete a four week washout period in which they did not receive any pills. After the four week washout period, participants were assigned to the opposite intervention/ placebo group. Therefore, participants that had initially been placed in the experimental group would now take a placebo vinegar pill once daily for four weeks, while those that had been in the control group at the start of the study would take the B6 supplement once daily for four weeks. Hence, participants acted as their own control in this crossover trial.

All participants were instructed to keep exercise and eating patterns consistent throughout the duration of the study. Additionally, participants were instructed not to start any new medications or supplements. To ensure compliance, participants were provided calendars and were instructed to check off each day that the pills were consumed during the treatment periods.

Anthropometric data was collected and blood draws were performed at weeks 0, 4, 8 and 12. Participants completed a health history questionnaire which included the

Godin Leisure-Time Exercise questionnaire to assess physical activity levels at week zero. A vitamin B6 food frequency questionnaire was also completed at all five visits. Additionally, mood assessments, including the Profile of Mood States and the Beck Depression Inventory were administered at weeks 0, 4, 8 and 12 (appendix B).

Study Variables

The independent variable for this study was supplementation of vitamin B6. During the experimental period of this study, participants received 100 mg of B6 in the form of pyridoxine hydrochloride (Vitamin B6 100 mg Tablets, Nature Made, Northridge, CA) [105]. Participants were instructed to take one pill daily for four weeks (28 days). During the control period, participants received a low dose vinegar pill that contained less than 23 mg of acetic acid per tablet (Apple Cider Vinegar Tablets, NOW Foods, Bloomingdale, IL) [106]. Detailed supplement information can be found in appendix C. Participants were instructed to take one pill once daily for four weeks.

The dependent variable examined in this study was mood state. Mood state was quantified using the Profile of Mood States assessment (POMS). The presence of depressive symptoms was also assessed through administration of the Beck Depression Inventory. The POMS and BDI are both validated assessments for use in a variety of populations [93-97, 101-104]. B6 status in terms of plasma PLP levels was examined as a secondary dependent variable to identify the changes in body levels of vitamin B6 with supplementation.

Protocol Procedures

Prescreening: Survey and Visit 1

Potential participants were prescreened to assess for eligibility through a Qualtrics survey sent out via ASU Listservs. Individuals that met inclusion criteria from the prescreening survey were asked to come in to be further assessed for eligibility using a health history questionnaire, anthropometric data and food frequency questionnaire to assess baseline B6 status. Anthropometric data was collected by a trained staff member. Those meeting all requirements and consumed at or below 2.6 mg B6 based on the food frequency questionnaire were included in the study (n=8). Potential participants were informed of the length of study and study details including participation requirements and number of visits. Possible risks and benefits involved with participation were explicitly outlined. Visits 2-5 were scheduled for those that were found to meet all requirements.

Start of Study (Week 1-4)

Participants came in for their second visit on day 1 of the study. Prior to the second visit, eligible participants were stratified by BMI and age, then randomized by coin flip into either the experimental or control group to begin the study. Basic anthropometric data, including weight, was collected and recorded first for all participants by a trained staff member. Next, blood draws were performed to measure baseline plasma PLP status. Blood draws were completed by a phlebotomist.

Next, the Profile of Mood States assessment was administered to all participants to address their level of mood disturbance, as well as the subcategories: anger-hostility, fatigue, tension-anxiety, depression-dejection, confusion-bewilderment, and vigor [92]. A Likert scale from “not at all” to “extremely” was used for each term in the assessment [92].

If participants were unfamiliar with any terms in the list, they were provided a definition of the term by a trained staff member. Participants were then instructed to complete the Beck Depression Inventory (BDI). After completion of the BDI, participants completed a questionnaire to address any potential factors that could impact mood including significant life events, conflicts with peers, perceived sleep quality from the night prior to assessment, and recent illness. Answers to questions were quantified to assess risk of altered mood based on these factors. Lastly, participants filled out a food frequency questionnaire based on their approximate intake over the previous 7 days.

At the end of the visit, participants were provided with their appropriate supplement or control treatment based on their assigned group. Both groups were instructed to take one tablet (B6 supplement or vinegar pill) daily for four weeks. Additionally, participants were provided with a compliance calendar to assist in tracking of the days that they took the supplement (or placebo). Participants were also instructed to continue their normal eating patterns and exercise regimes throughout the entire 12 weeks. Email reminders were sent out weekly to participants to encourage compliance.

At the end of week 4, participants came in for their third visit. At this visit, anthropometric data was collected and a blood draw was performed by a phlebotomist to assess for changes in plasma PLP status. Participants completed the POMS assessment and Beck Depression Inventory at this time as well to assess any changes in mood state. The questionnaires on mood influencing factors and food frequency were also obtained. Participants also turned in their compliance calendars that they had been provided with at the start of week one. Participants were given instructions for weeks 5-8 of the study at the end of this visit.

Week 5-8

During weeks 5-8, participants were instructed to continue their normal eating and exercise patterns. Participants did not receive either the treatment or placebo tablets during this time period as this was a washout period. Tracking compliance was not necessary as no treatment was provided in this time period. A reminder email was sent out during week 6 and at the beginning of week 8 to remind participants of their fourth visit at the end of week 8.

Week 9-12

When participants came in for their fourth visit at the end of week 8, the same procedure as their second visit was followed. Once anthropometric data, questionnaires, assessments, and blood draws were completed, participants were provided with the opposite treatment from their original assignment. Those that had received the control treatment during weeks 1- 4, were now given B6 supplements for weeks 9-12, while those that had been given the supplement during weeks 1- 4, were now given the vinegar tablets to take during weeks 9-12. Participants were unaware of whether they were receiving the vinegar tablets (placebo) or the B6 supplements (treatment). Again, both groups were instructed to take one of the provided tablets daily for four weeks. Compliance calendars were sent home to help participants remember to take their assigned tablet. Participants were again reminded to continue their normal exercise and eating patterns.

At the end of week 12, participants came in for their fifth and final visit. The same procedure as the third visit was followed. Participants completed all assessments and final blood draws were performed. Compliance calendars were collected from all

individuals to help determine overall compliance throughout the duration of the study. Participants were compensated for their participation with a $50 Amazon gift card once the trial was complete.

Laboratory Analyses

Participant blood samples were collected at visits 2 - 5 to assess vitamin B6 status of participants and changes with supplementation. Plasma PLP levels were quantified using a B6 Enzymatic Assay (KK-VB6-U, Bühlmann, Amherst, NH) [107]. The protocol for this assay and the mechanism of action can be found in appendices D and E. Blood samples were tested in duplicate to ensure accuracy of measurements.

Statistical Analysis

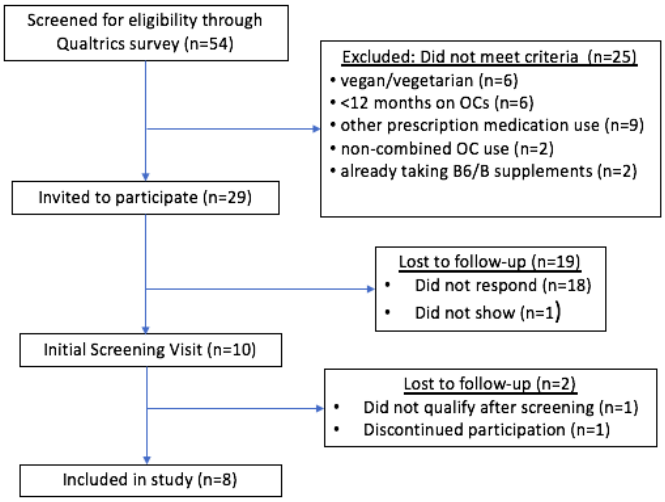
In analyzing the data, both questionnaire and laboratory data were assessed. Results are expressed as the mean ± the standard deviation. A significance level of $p \leq 0.05$ was set for this study. A sample size of approximately 25 participants was calculated to provide 80% power to observe differences in POMS scores between the supplement and placebo treatments [108-113] (appendix F). All data were analyzed using Statistical Package for Social Sciences (SPSS) version 25 for Mac. Descriptive statistics were performed using the data collected to find the mean and standard deviations during the control and treatment portions of the study. All variables were tested for normality using the Shapiro-Wilk test for normality. Data that was not normally distributed was log transformed to create normal curves. Additionally, data were checked for outliers (3 standard deviations from the mean). No outliers were identified. Dietary B6 intake and life stressor values were analyzed using repeated measures ANOVA. Change data were calculated for the outcome variables and analyzed using a paired t-test.

CHAPTER 4

RESULTS

In recruiting for the study, 54 individuals completed the Qualtrics screening survey (Figure 3). Of these individuals, 25 did not qualify based on their survey responses. Six did not qualify as they were either vegetarian or vegan; six did not qualify as they had not been taking oral contraceptives for at least 12 months; two were on oral contraceptives that were non-combined forms; nine were on prescription medications other than oral contraceptives; and two did not qualify because they were already taking either B6 or B complex supplements. Qualifying respondents were sent invitation emails on three occasions. Of the 29 individuals that did qualify, only 11 responded to invitations to participate in the study. One person did not attend the initial screening visit. One individual did not qualify based on initial screening assessment due to clinical diagnosis of depression, and one individual did not continue the study after the initial visit. The 8 remaining participants attended all 5 visits and completed the study (Figure 3).

Figure 3. Recruitment of study participants from initial n=54 screened via Qualtrics survey, to n=8 included in the study.



The 8 participants that were included in the study were stratified by age and BMI and randomly assigned to receive either the B6 supplement or control pill for a 4-week period. All participants completed a 4-week washout period before receiving the alternate treatment for the last four weeks of the study. Thus, all participants received the vitamin B6 treatment and served as their own control. All participants were between the ages of 18-24 years old with an average age of 21.0 ± 2.0 years (Table 4). At baseline, the mean and standard deviation of the BMI of participants was 21.4 ± 2.5 kg/m2. Activity level was also assessed at baseline to ensure that participants were not exercising excessively in their day to day routines. Using the Godin Leisure-Time Exercise questionnaire to assess activity levels of participants, the mean score was 52.3 ± 30.7 metabolic equivalents (MEQ)/week. Of the eight women that completed the survey, seven (87.5%) were Caucasian and one was Hispanic. All participants were undergraduate or graduate students at Arizona State University and were primarily in nutrition (37.5%) or other health related majors (50.0%); 12.5% were in non-health related majors. Three (37.5%) of the participants were in graduate school, 25% were sophomores, 12.5% were juniors, and 25% were seniors (Table 4).

Table 4. Description of participant characteristics at baseline.

	Participant Characteristics
Characteristic	n (%)
Ethnicity	
Caucasian	7 (87.5%)
Hispanic	1(12.5%)
Major	
Health Sciences	1 (12.5%)
Kinesiology	1 (12.5%)
Medical Studies	1 (12.5%)
Nursing	1 (12.5%)
Nutrition	3 (37.5%)
Architecture	1 (12.5%)
Grade level	
Freshman	-
Sophomore	2 (25.0%)
Junior	1 (12.5%)
Senior	2 (25.0%)
Graduate	3 (37.5%)
	N=8, M (SD)
Age (years)	21.0 (2.0)
Weight (kg)	55.6 (6.0)
Height (cm)	161.5 (4.8)
BMI (kg/m_2)	21.4 (2.5)
Activity level (MEQ/week)	52.3 (30.7)

Based on compliance calendars completed by participants, all participants took their assigned control pill or supplements every day throughout the two 4-week intervention periods. All participants completed vitamin B6 focused food frequency questionnaires (FFQ) at each of the five study visits. At baseline, the mean intake of vitamin B6 based on the questionnaires was 1.46 ± 0.75 mg/day (Table 5). The intake of B6 from foods did not change significantly throughout the duration of the study ($p= 0.317$).

Table 5. Intake of vitamin B6 based on FFQs throughout at baseline, pre- and post-control, and pre- and post-B6 supplementation (p= 0.317, repeated measures ANOVA).

	FFQ B6 intake (mg/day)				
Statistic	Baseline	Pre Control	Post Control	Pre B6	Post B6
Mean	1.46	1.31	1.29	1.15	1.38
SD	0.75	0.74	0.97	0.66	1.02

Participants completed a brief questionnaire to assess for the presence of stressors at each of the 5 visits. For this assessment, each "yes" answer was quantified as a value of 1, while "no" responses were given a value of 0. Responses were totaled at each visit and had a possible score range of 0-17. The presence of stressors based on this assessment did not significantly differ between treatment periods (p= 0.634) (Table 6).

Table 6. The perceived presence of stressors in participant lives pre- and post- control vs pre- and post- vitamin B6 supplementation. Data presented as mean and standard deviation; means did not differ significantly during the study (p=0.634; repeated measures ANOVA).

	Life Stressors Score			
Statistic	Pre Control	Post Control	Pre B6	Post B6
Mean	6.6	7.1	6.1	5.1
SD	3.6	3.6	2.8	3.2

Plasma PLP was measured to assess vitamin B6 status in participants before and after each supplement period. For the periods before each treatment, the plasma PLP levels of participants did not differ significantly. After four-week supplementation of 100 mg of vitamin B6 daily, the plasma PLP levels of participants increased by more than 193% (Table 7). This increase differed significantly from the change noted after supplementation with the control pill (+29%; p=0.003). The measured values of plasma PLP post B6 supplementation were capped at 209 nmol/L as these values were higher than the range that the B6 assay could detect.

Table 7. Plasma PLP values of participants pre and post control or B6 supplementation periods (p=0.003 for the change by treatment; paired T-test). *All plasma PLP levels were above 209.0 nmol/L after consumption of 100 mg B6 supplements.

Statistic	Plasma PLP levels pre and post interventions (nmol/L)					
	Pre Control	Post Control	% change	Pre B6	Post B6	% change
Mean	59.9	77.4	29.3	71.3	>209.0*	193.3
SD	21.6	48.5		40.3	-	

Total mood disturbance (TMD) scores assessed using the POMS assessment did not significantly change with intervention (p=0.720). While the sub scores of Anger and Depression decreased by 10.3% and 14.7%, respectively, from pre-intervention, these values were not statistically significant (Table 8). With B6 supplementation, the Vigor sub score did increase by 8.4%, however this increase was also not significant (p>0.05). The mean ± SD for the TMD scores pre control and pre B6 intervention were 43.5 ± 40.6 and 30.1 ± 31.3, respectively. During the period of the control, TMD scores increased by 9.8%. TMD scores for the B6 supplementation period were also slightly worse with a 6.2% increase. There was no significant change in TMD scores throughout the duration of the study.

Table 8. Profile of Mood States sub scores and total mood disturbance before and after control and intervention periods. Higher scores, with the exception of vigor, are unfavorable.

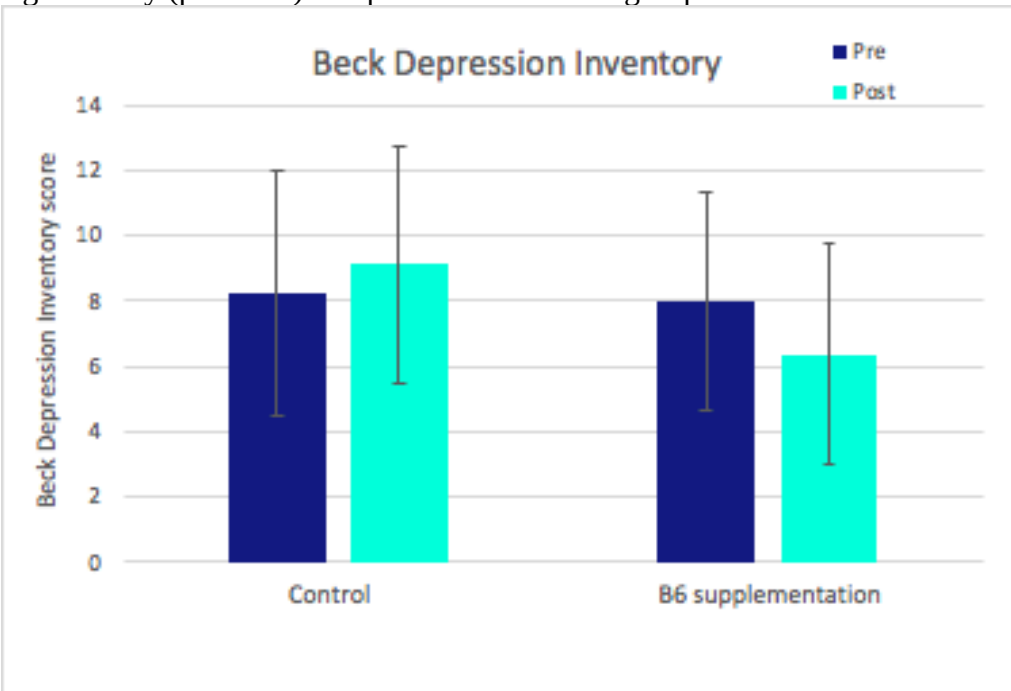
POMS Total Mood Disturbance and sub score means and standard deviations: M (SD)							
Sub score	Pre Control	Post Control	% change	Pre B6	Post B6	% change	P value
Anger	8.9 (9.1)	9.0 (6.9)	1.4	6.0 (6.1)	5.4 (4.9)	-10.3	0.627
Fatigue	13.3 (7.7)	11.6 (6.0)	-12.2	8.6 (4.5)	9.5 (5.9)	10.1	0.307
Tension	12.5 (10.4)	17.5 (8.5)	40.0	10.9 (7.0)	14.5 (6.1)	33.3	0.668
Confusion	9.5 (5.3)	9.8 (4.2)	2.6	7.1 (3.6)	8.6 (3.1)	21.0	0.272
Depression	9.6 (11.0)	10.1 (10.6)	5.2	9.4 (15.0)	8.0 (8.3)	-14.7	0.566
Vigor	10.3 (6.8)	10.3 (6.3)	0.0	11.9 (6.7)	12.9 (4.1)	8.4	0.474
TMD	43.5 (40.7)	47.8 (33.3)	9.8	30.1 (31.3)	32 .0 (24.1)	6.2	0.720

Presence of symptoms of clinical depression was also assessed using the Beck Depression Inventory. After log transformation of data to normalize, a significant decrease in presence of depressive symptoms was observed after periods in which participants had been consuming the vitamin B6 supplements compared to placebo treatment ($p = .047$) (Table 9). The mean BDI score post B6 intervention decreased by 20.3%, while the mean BDI score increased by 10.7% in during periods in which participants consumed the control pill.

Table 9. Beck Depression Inventory scores of participants pre and post 4 week control and intervention periods. Higher scores are unfavorable. The p value represents paired T-test.

Beck Depression Inventory means and standard deviations							
Statistic	Pre Control	Post Control	% change	Pre B6	Post B6	% change	P value
Mean	8.3	9.1	10.7	8.0	6.4	-20.3	.047
SD	10.7	9.4		10.2	9.5		

Figure 4. Error bars use standard error. The Beck Depression Inventory scores decreased significantly (p= 0.047) compared to the control group.



CHAPTER 5

DISCUSSION

This study was conducted to identify the impact that vitamin B6 supplementation has on mood states in college age women that are more likely to have lower plasma PLP levels due to their use of oral contraceptives [4,73]. Much of the current literature on this topic has primarily focused on either the link between low vitamin B6 status and depressed mood or on the diminished vitamin B6 status with oral contraceptive use [4-7,73,74]. However, little research has discussed the link between low vitamin B6 status related to oral contraceptive use and the relationship that has to depressed mood.

The present study connected these ideas and aimed to determine a method of minimizing negative mood changes in oral contraceptive users through supplementation of vitamin B6. With this focus, the primary outcome investigated in this study was mood states. Participant mood states were quantified using the Profile of Mood States assessment. The results of the study indicated that mood states were not significantly changed with daily supplementation of 100 mg of vitamin B6 over a four week period. Therefore, the hypothesis was rejected. However, while mood states did not significantly improve, the presence of depressive symptoms in participants declined during the periods of vitamin B6 supplementation. Symptoms of depression were assessed with the Beck Depression Inventory (BDI), which is a validated measure used as a diagnostic test for clinical depression [103,104]. For this assessment, a significant improvement in mean BDI score was observed after the vitamin B6 supplementation period ($p= 0.047$). This improvement suggests that supplementation of 100 mg of vitamin B6 may mitigate depressive symptoms in women that are taking a combined form of oral contraceptives.

This information is a crucial finding as it indicates that vitamin B6 supplementation may alleviate a major negative side effect that oral contraceptive users experience. The results of this study also provide valuable information for future researchers examining the impact that vitamin B6 has on depressive symptoms in various populations.

Although the BDI scores of participants decreased significantly with the supplementation period, it is important to note that the mean BDI scores pre- and post-intervention and control periods were all considered to be in the "normal" category, as the means were within the 0-10 score range [103]. However, the post control mean BDI score was 9.1, which is on the high end of normal, while the post intervention (B6 supplementation) mean was only 6.4, which is near the middle of the normal range [103]. If this study were to be repeated, having greater variation, or higher BDI scores at baseline could potentially show more of an improvement with B6 supplementation.

A possible explanation for the mean BDI scores being in the normal range throughout the course of the study is that nearly all participants had an adequate plasma PLP status of above 30 nmol/L [16]. While plasma PLP levels did significantly increase with supplementation of B6 by more than 193% ($p=0.003$), participants were not at marginal or deficient levels prior to supplementation and during the control periods. This lack of marginal vitamin B6 status likely contributed to the observed normal ranges for BDI scores as the participants were not at a high risk of B6 deficiency-related mood changes.

Based on the literature, as many as 40% of oral contraceptive users have marginal B6 status[1]. However, this was not the case for the study sample. Only one participant (12.5%) was found to have a marginal B6 status with a plasma PLP level of <30 nmol/L

throughout the study, with the exception of the period post B6 supplementation. For all participants, the mean plasma PLP level prior to the B6 supplementation period was 71.3 ± 40.3 nmol/L and was 59.9 ± 21.6 nmol/L prior to placebo intake. These levels are consistent with adequate plasma PLP status as they are >30 nmol/L [16]. The plasma PLP levels found in this study are also similar to the plasma PLP levels found in comparable samples. In an article by Deac et al. examining the differences in vitamin B6 status among various populations, the mean plasma PLP of 385 oral contraceptive users between 20-25 years old was 68.7 nmol/L [114]. This suggests that the vitamin B6 status of the study sample is comparable to other oral contraceptive users.

The normal plasma PLP levels of participants that were observed could have potentially been related to participant intake of foods containing vitamin B6. During the initial screening, participants completed a self-reported vitamin B6 food frequency questionnaire. For this study a FFQ cutoff of <200% of the RDA for vitamin B6 was chosen as an inclusion criteria, as studies have shown that women on oral contraceptives need as much as 20-25 mg B6/day to support proper tryptophan metabolism and adequate B6 status [5,115]. Therefore, in setting the criteria for this study, it was presumed that women on oral contraceptives that were consuming less than 2.6 mg B6/day would still have a marginal B6 status. Moreover, the RDA of 1.3 mg B6/day was not used as it further restricted the eligible sample group. If resources permit, future studies on the topic should assess plasma PLP status prior to study completion to ensure that participants have marginal or deficient status at baseline. This could also help ensure that observed changes in mood or symptoms of depression are related directly to vitamin B6 supplementation and improvement in plasma PLP status.

Limitations

This study had multiple limitations that could have impacted the significance of study findings. Some of the limitations include the lack of regulation over participant diets and exercise patterns. Participants were instructed to keep diet and exercise consistent throughout the study however this was only controlled through self-reported food frequency questionnaires and interviewing participants at visits. Additionally, this study was limited to female students from Arizona State University which could limit the application of results to this geographical region.

Perhaps the most significant limitation of this study was the small sample size. The calculated sample size for observable differences to provide 80% power was 25 participants [108-113] (appendix F). Difficulties in recruiting individuals that met all inclusion criteria and that were willing to complete all visits contributed to a limited sample size of 8 individuals. Many of the individuals that expressed interest in the study by completing the screening survey did not qualify as they followed a vegan or vegetarian diet, were taking other medications or supplements, or had not been on a combined form of oral contraceptives (Figure 1). Another large portion of potential participants did not respond to email invitations to be screened in person. The small sample size that was obtained makes it difficult to apply study outcomes to the general population. However, although the sample size was small, study adherence was 100% based on compliance calendars that were completed by participants throughout the course of the study.

Generalizability

The results of this study are primarily generalizable to young college women that are on a form of oral contraceptives that contains both estrogen and progestin. Women over the age of 25 were not included in this study so these results may not accurately reflect mood changes and depressive symptoms in oral contraceptive users that are outside of the specified 18-25 year old age range. This study also excluded individuals that are vegan or vegetarian and therefore results may not be as generalizable to these individuals.

CHAPTER 6

CONCLUSION AND APPLICATIONS

With the results of the present study indicating a significant decrease in the presence of depressive symptoms but not an improvement in mood states, this study may act as a starting point for future research on the relationship between vitamin B6 supplementation and symptoms of depression in oral contraceptive users. Further, the results of this study provide valuable information for both future researchers and those taking oral contraceptives as depressive symptoms in this population is one of the primary reasons that women choose to discontinue use of the medication [72]. With the possibility that supplementation could mitigate these unfavorable symptoms, further research should be conducted to confirm the validity of these results and investigate the use of vitamin B6 supplementation with larger sample sizes.

With the large proportion of college women using oral contraceptives and the common side effect of depressed mood, it is imperative that researchers continue to examine methods for minimizing the associated risks. Further studies could continue to investigate the effectiveness of vitamin B6 supplementation in reducing symptoms of depression in this susceptible population. Additionally, research on appropriate dosage for vitamin B6 supplementation would also be appropriate and beneficial to the scientific community as this could help determine the optimal dose for mood and symptom improvement with oral contraceptive use. Researchers could additionally examine the impact that various types of contraceptives have on the severity of mood changes.

This study confirms that there is a potential for vitamin B6 supplementation to alleviate mental health side effects of oral contraceptives and provides a framework for

future studies to advance on the topic. The outcomes of this study also necessitate a focus on minimizing the risks of depression in college women to support mental health and the utilization of safe and effective forms of contraceptives.

REFERENCES

1. Morris MS, Picciano MF, Jacques PF, Selhub J. Plasma pyridoxal 5-phosphate in the US population: the National Health and Nutrition Examination Survey, 2003–2004 *Am J Clin Nutr.* 2008;87(5):1446–54.

2. Bhagavan HN. Interaction between vitamin B6 and drugs. *Vitamin B6: Its role in Health and Disease.* New York, NY: Liss;1985.

3. Vermaak WJ, Ubbink JB, Barnard HC, Potgieter GM, van Jaarsveld H, Groenewald AJ. Vitamin B-6 nutrition status and cigarette smoking. *Am J Clin Nutr.* 1990;51(6):1058-61.

4. Wilson SMC, Bivins BN, Russell KA, and Bailey LB. Oral contraceptive use: impact on folate, vitamin B_6, and vitamin B_{12} status. *Nutr Res.* 2011;69(10):572–583. doi:10.1111/j.1753-4887.2011.00419.x

5. Webb JL. Nutritional effects of oral contraceptive use: a review. *J Reprod Med*. 1980; 25(4):150-6.

6. Lussana F, Zighetti ML, Bucciarelli P, Cugno M, Cattaneo M. Blood levels of homocysteine, folate, vitamin B6 and B12 in women using oral contraceptives compared to non-users. *Thromb Res*. 2003;112(1-2):37-41.

7. Mikkelsen K, Stojanovska L, Apostolopoulos V. The Effects of Vitamin B in Depression. *Curr Med Chem.* 2016;23(38):4317-4337.

8. Allen GFG, Neergheen V, Oppenheim M, et al. Pyridoxal 5′-phosphate deficiency causes a loss of aromatic l-amino acid decarboxylase in patients and human neuroblastoma cells, implications for aromatic l-amino acid decarboxylase and vitamin B6 deficiency states. *J Neurochem*. 2010;114(1):87-96. doi:10.1111/j.1471-4159.2010.06742.x

9. György P. Vitamin B2 and the Pellagra-like Dermatitis in Rats. *Nature*. 1934;133:498.

10. Snell EE. Vitamin B6. *Compr Biochem.* 1963;11:48-58. https://doi.org/10.1016/B978-1-4831-9711-1.50012-0

11. Stiller ET, Keresztesy JC, Stevens JR. The Structure of Vitamin B6. I. *J Am Chem Soc*. 1939;61(5):1237-1242. doi:10.1021/ja01874a067

12. Merrill AH, Henderson JM, Wang E, McDonald BW, Millikan WJ. Metabolism of Vitamin B-6 by Human Liver. *J Nutr*. 1984;114(9):1664-1674. doi:10.1093/jn/114.9.1664

13. Merrill AH, Henderson JM, Wang E, Codner MA, Hollins B, Millikan WJ. Activities of the hepatic enzymes of vitamin B6 metabolism for patients with cirrhosis. *Am J Clin Nutr*. 1986;44(4):461-467. doi:10.1093/ajcn/44.4.461

14. Bachmann T, Rychlik M. Synthesis of [$_{13}C_3$]-B6 vitamers labelled at the three consecutive positions starting from [$_{13}C_3$]-Propionic acid. *Molecules.* 2018;23(9):2117. https://doi.org/10.3390/molecules23092117

15. Albersen M, Bosma M, Knoers NVVAM, et al. The intestine plays a substantial role in human vitamin B6 metabolism: a Caco-2 cell model. *PLoS ONE*. 2013;8(1):e54113. doi:10.1371/journal.pone.0054113

16. Institute of Medicine. Food and Nutrition Board. Dietary Reference Intakes: Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline. Washington, DC: National Academy Press; 1998.

17. Hamm MW, Mehansho H, Henderson LM. Transport and metabolism of pyridoxamine and pyridoxamine phosphate in the small intestine of the rat. *J Nutr.* 1979;109:1552-1559.

18. Nakano H, Gregory JF. Pyridoxine and pyridoxine 5'-beta-D-glucosidase activity in rats. *J Nutr*. 1997;125:2751-2762.

19. Reynolds RD. Bioavailability of vitamin B-6 from plant foods. *Am J Clin Nutr*. 1988;48(3 Suppl):863-867. doi:10.1093/ajcn/48.3.863

20. Gregory JF, Trumbo PR, Bailey LB, Toth JP, Baumgartner TG, Cerda JJ. Bioavailability of Pyridoxine-5′-β-D-Glucoside Determined in Humans by Stable-Isotopic Methods. *J Nutr*. 1991;121(2):177-186. doi:10.1093/jn/121.2.177

21. Wilson RG, Davis RE. Clinical Chemistry of Vitamin B6. *Adv Clin Chem.* 1983;23:1-68

22. Yanbaeva DG, Dentener MA, Creutzberg EC, Wesseling G, Wouters EFM. Systemic Effects of Smoking. *Chest*. 2007;131(5):1557-1566. doi:10.1378/chest.06-2179

23. Ulvik A, Ebbing M, Hustad S, et al. Long- and short-term effects of tobacco smoking on circulating concentrations of B vitamins. *Clin Chem*. 2010;56(5):755-763. doi:10.1373/clinchem.2009.137513

24. Fonda ML, Eggers DK. Vitamin B-6 metabolism in the blood of young adult and senescent mice. *Experimental Gerontology*. 1980;15(5):465-472. doi:10.1016/0531-5565(80)90054-6

25. Lumeng L, Brashear RE, Li T-K. Pyridoxal 5′-phosphate in plasma: source, protein-binding, and cellular transport. *J Lab Clin Med*. 1974;84(3):334-343. doi:10.5555/uri:pii:0022214374901243

26. Fonda ML, Trauss C, Guempel UM. The binding of pyridoxal 5′-phosphate to human serum albumin. *Arch Biochem Biophys*. 1991;288(1):79-86. doi:10.1016/0003-9861(91)90167-H

27. Ink SL, Henderson LM. Vitamin B_6 Metabolism. *J Biol Chem.* 1984; 259(9):5833-5837.

28. Combs GF. *The Vitamins: Fundamental aspects in nutrition and health.* 5th ed. Cambridge, MA: Academic Press; 2013.

29. Huskisson E, Maggini S, Ruf M. The role of vitamins and minerals in energy metabolism and well-being. *J Int Med Res*. 2007;35(3):277-289. doi:10.1177/147323000703500301

30. Coburn SP, Lewis DL, Fink WJ, Mahuren JD, Schaltenbrand WE, Costill DL. Human vitamin B6 pools estimated through muscle biopsies. *Am J Clin Nutr.* 1988;48(2):291-294.

31. Johansson S, Lindstedt S, Register U, Wadstrom L. Studies on the metabolism of labeled pyridoxine in man. *Am J Clin Nutr.* 1966;18:185-196.

32. Coburn SP, Mahuren JD, Kennedy MS, Schaltenbrand WE, Sampson DA, O'Connor DK, Snyder DL, Wostmann BS. B6 vitamin content of rat tissues measured by isotope tracer and chromatographic methods. *Biofactors.* 1988;1(4):307-312.

33. Shultz TD, Leklem JE. Urinary 4-pyridoxic acid, urinary vitamin B6, and plasma pyridoxal phosphate as measures of vitamin B6 status and dietary intake in adults. Methods in Vitamin B6 Nutrition. New York, NY: Plenum Press; 1981:389-392.

34. Lepkovsky S, Roboz E, Haagen-Smit A J. Xanthurenic acid and its role in the tryptophan metabolism of pyridoxine-deficient rats. *J. Biol. Chem.* 1943;140:195-201.

35. Lui A, Lumeng L, Aronoff GR, Li T-K. Relationship between body store of vitamin B6 and plasma pyridoxal-P clearance: metabolic balance studies in humans. *J Lab Clin Med.* 1985;106:491-497.

36. Mooney S, Leuendorf JE, Henderickson C, Hellman H. Vitamin B6: A long known compound of surprising complexity. *Molecules.* 2009;14(1):329-351. doi:10.3390/molecules14010329

37. Parra M, Stahl S, Hellmann H. Vitamin B_6 and its role in cell metabolism and physiology. *Cells*. 2018;7(7):84. doi:10.3390/cells7070084.

38. Suzuki M, Nakagawa M, Shimizu Y, Suemura M, Sato B. Therapy-resistant microcytic hypochromic anemia from malabsorption-related vitamin B6 deficiency after a gastrointestinal operation. *Rinsho Ketsueki*. 2005;46(9):1044-1048.

39. Kiser D, Steemer S B, Branchi I, Homberg JR. The reciprocal interaction between serotonin and social behaviour. *Neurosci Biobehav Rev*. 2012;36(2):786-798. doi:10.1016/j.neubiorev.2011.12.009

40. Strasser B, Gostner JM, Fuchs D. Mood, food, and cognition. *Curr Opin Clin Nutr Metab Care*. 2016;19(1):55–61. doi: 10.1097/MCO.0000000000000237.

41. Burke SM, van de Giessen E, de Win M, et al. Serotonin and dopamine transporters in relation to neuropsychological functioning, personality traits and mood in young adult healthy subjects. *Psychol Med*. 2011;41(2):419-429. doi:10.1017/S0033291710000486

42. West KD, Kirksey A. Influence of vitamin B6 intake on the content of the vitamin in human milk. *Am J Clin Nutr.* 1976;29:961-969.

43. Borschel MW, Kirksey A, Hanneman RE. Effects of vitamin B6 intake on nutriture and growth of young infants. *Am J Clin Nutr.* 1986;43:7-15.

44. Huang Y-C, Chen W, Evans MA, Mitchell ME, Shultz TD. Vitamin B6 requirements and status assessment of young women fed a high-protein diet with various levels of vitamin B6. *Am J Clin Nutr.* 1998;67:208-220.

45. Hansen CM, Leklem JE, Miller LT. Vitamin B-6 status of women with a constant intake of vitamin B-6 changes with three levels of dietary protein. *J Nutr.* 1996;126:1891-1901.

46. Yess N, Price JM, Brown RR, Swan PB, Linkswiler H. Vitamin B6 depletion in man: Urinary excretion of tryptophan metabolites. *J Nutr.* 1964;84:229-236.

47. Kjeldby IK, Fosnes GS, Ligaarden SC, Farup PG. Vitamin B6 deficiency and diseases in elderly people – a study in nursing homes. *BMC Geriatr*. 2013;13(1):13. doi:10.1186/1471-2318-13-13

48. Madigan SM, Tracey F, McNulty H, et al. Riboflavin and vitamin B-6 intakes and status and biochemical response to riboflavin supplementation in free-living elderly people. *Am J Clin Nutr*. 1998;68(2):389-395. doi:10.1093/ajcn/68.2.389

49. Hamfelt A, Tuvemo T. Pyridoxal phosphate and folic acid concentration in blood and erythrocyte aspartate aminotransferase activity during pregnancy. *Clin Chim Acta.* 1972; 41:287-298.

50. U.S. Department of Agriculture and U.S. Department of Health and Human Services. National Institutes of Health. Vitamin B6. https://ods.od.nih.gov/factsheets/VitaminB6-HealthProfessional/. Accessed September 6, 2018.

51. Gregory JF. Bioavailability of vitamin B6. *Eur J Clin Nutr*. 1997;51:S43-S48.

52. Gregory JF, Kirk JR. The Bioavailability of Vitamin B6 in Foods. *Nutr Res*, 1981;39(1):1–8.

53. Dietz JM, Erdman JW. Effects of Thermal Processing upon Vitamins and Proteins in Foods. *Nutr Today*. 1989;24(4):6.

54. Lund D. Effects of Heat Processing on Nutrients. In: Karmas E, Harris RS, eds. *Nutritional Evaluation of Food Processing*. Dordrecht: Springer Netherlands; 1988:319-354. doi:10.1007/978-94-011-7030-7_12

55. Ueland PM, Ulvik A, Rios-Avila L, Midttun O, Gregory JF. Direct and functional biomarkers of vitamin B6 status. *Annu Rev Nutr.* 2015;35: 33–70. doi:10.1146/annurev-nutr-071714-034330.

56. Lumeng L, Ryan MP, Li TK. Validation of the diagnostic value of plasma pyridoxal 5'-phosphate measurements in vitamin B6 nutrition of the rat. *J Nutr*. 1978;108:545-553.

57. Miller LT, Leklem JE, Shultz TD. The effect of dietary protein on the metabolism of vitamin B6 in humans. *J Nutr*. 1985;115:1663-1672.

58. Baker EM, Canham JE, Nunes WT, Sauberlich HE, Mcdowell ME. Vitamin B6 Requirement for Adult Men. *Am J Clin Nutr*. 1964;15:59-66. doi:10.1093/ajcn/15.2.59

59. Hansen CM, Leklem JE, Miller LT. Changes in vitamin B-6 status indicators of women fed a constant protein diet with varying levels of vitamin B-6. *Am J Clin Nutr*. 1997;66(6):1379-1387. doi:10.1093/ajcn/66.6.1379

60. Contractor SF, Shane B. Estimation of vitamin B6 compounds in human blood and urine. *Clin Chim Acta.*1968;21:71-77.

61. Vech RL, Lumeng L, Li TK. Vitamin B6 metabolism in chronic alcohol abuse The effect of ethanol oxidation on hepatic pyridoxal 5'-phosphate metabolism. *J Clin Invest.* 1975;55(5):1026-1032.

62. Labadarios D, Rossouw JE, McConnell JB, Davis M, Williams R. Vitamin B6 deficiency in chronic liver disease--evidence for increased degradation of pyridoxal-5'-phosphate. *Gut.* 1977;18(1):23-27. doi:10.1136/gut.18.1.23

63. Gdynia H-J, Müller T, Sperfeld A-D, et al. Severe sensorimotor neuropathy after intake of highest dosages of vitamin B6. *Neuromuscular Disord.* 2008;18(2):156-158. doi:10.1016/j.nmd.2007.09.009

64. Schaumburg H, Kaplan J, Windebank AJ, et al. Sensory neuropathy from pyridoxine abuse. A new megavitamin syndrome. *N Engl J Med*. 1983;309(8):445-448.

65. Berger AR, Schaumburg HH, Schroeder C, Apfel S, Reynolds R. Dose response, coasting, and differential fiber vulnerability in human toxic neuropathy: a prospective study of pyridoxine neurotoxicity. *Neurology*. 1992;42(7):1367-1370. doi:10.1212/wnl.42.7.1367

66. Bender DA. Non-nutritional uses of vitamin B6. *Br J Nutr*. 1999;81(1):7-20.

67. Gregory ST, Hall K, Quast T, Gatto A, Bleck J, Storch EA, DeBate R. Hormonal contraception, depression, and academic performance among females attending college in the United States. *Psychiatry Res.* 2018;15(270):111-116. doi: 10.1016/j.psychres.2018.09.029. [Epub ahead of print]

68. Mosher WD, Jones J. Use of contraception in the Unites States: 1982-2008. *Vital and health statistics. Series 23, Data from the National Survey of Family Growth*. 2010;29:1-44.

69. Hall KS, Trussell J, Schwarz EB. Progestin-only contraceptive pill use among women in the United States. *Contraception*. 2012;86(6):653-658. doi:10.1016/j.contraception.2012.05.003

70. Hamstra DA, de Kloet ER, de Rover M, Van der Does W. Oral contraceptives positively affect mood in healthy PMS-free women: A longitudinal study. *J Psychosom Res.* 2017;103:119-126. doi: 10.1016/j.jpsychores.2017.10.011. Epub 2017 Oct 20.

71. Bengtsdotter H, Lundin C, Gemzell Danielsson K, Bixo M, Baumgart J, Marions L, Brynhildsen J, Malmborg A, Lindh I, Sundström Poromaa I. Ongoing or previous mental disorders predispose to adverse mood reporting during combined oral contraceptive use. *Eur J Contracept Reprod Health Ca*re. 2018;23(1):45-51. doi: 10.1080/13625187.2017.1422239. Epub 2018 Jan 11.

72. Lundin C, Danielsson KG, Bixo M, Moby L, Bengtsdotter H, Jawad I, Marions L, Brynhildsen J, Malmborg A, Lindh I, Sundström Poromaa I. Combined oral contraceptive use is associated with both improvement and worsening of mood in the different phases of the treatment cycle-A double-blind, placebo-controlled randomized trial. *Psychoneuroendocrinology*. 2017;76:135-143. doi: 10.1016/j.psyneuen.2016.11.033. Epub 2016 Nov 29.

73. Prasad AS, Lei KY, Moghissi KS, Stryker JC, Oberleas D. Effect of oral contraceptives on nutrients. III. Vitamins B6, B12, and folic acid. *Am J Obstet Gynecol.* 1976;125(8):1063-9.

74. Hvas AM, Juul S, Bech P, Nexø E. Vitamin B6 level is associated with symptoms of depression. *Psychother Psychosom*. 2004;73(6):340-3.

75. Price JM, Thornton MJ, Mueller LM. Tryptophan metabolism in women using steroid hormones for ovulation control. *Am J Clin Nutr*. 1967;20(5):452-456. doi:10.1093/ajcn/20.5.452

76. Dante G et al. *Int J Reprod Contracept Obstet Gynecol*. 2014 Mar;3(1):1-10.

77. Coates PM, Paul MC, Blackman M, et al. *Encyclopedia of Dietary Supplements (Print)*. CRC Press; 2004.

78. Rose DP. Effect of ovarian hormones and oral contraceptives on tryptophan metabolism. *J Clin Pathol Suppl (Assoc Clin Pathol)*. 1969;3:37-42.

79. Brody DJ, Pratt LA, Hughes JP. Prevalence of depression among adults aged 20 and over: United States, 2013-2016. US Department of Health and Human Services. https://permanent.access.gpo.gov/gpo89902/db303.pdf. Published February 2018. Accessed November 20, 2018.

80. Khalsa SR, McCarthy KS, Sharpless BA, Barrett MS, Barber JP. Beliefs about the causes of depression and treatment preferences. *J Clin Psychol*. 2011;67(6):539-549. doi:10.1002/jclp.20785

81. Blardi P, De Lalla A, Leo A, et al. Serotonin and Fluoxetine Levels in Plasma and Platelets After Fluoxetine Treatment in Depressive Patients. Journal of Clinical Psychopharmacology. 2002;22(2):131-136.

82. Mück-Šeler D, Jakovljević M, Pivac N. Platelet 5-HT concentrations and suicidal behaviour in recurrent major depression. *J Affect Disord*. 1996;39(1):73-80. doi:10.1016/0165-0327(96)00024-9

83. Blardi P, de Lalla A, Urso R, et al. Activity of Citalopram on Adenosine and Serotonin Circulating Levels in Depressed Patients. *J Clin Psychopharm*. 2005;25(3):262-266. doi: 10.1097/01.jcp.0000161500.58266.90.

84. Tuomisto J, Tukiainen E. Decreased uptake of 5-hydroxytryptamine in blood platelets from depressed patients. *Nature*. 1976;262:596-598.

85. Liu CH, Stevens C, Wong SHM, Yasui M, Chen JA. The prevalence and predictors of mental health diagnoses and suicide among U.S. college students: Implications for addressing disparities in service use. *Depress Anxiety*. 2018. doi: 10.1002/da.22830. [Epub ahead of print]

86. Rosenström T, Jokela M. Reconsidering the definition of major depression based on collaborative psychiatric epidemiology surveys. *J Affect Disord*. 2017;207:38-46. doi:10.1016/j.jad.2016.09.014

87. Nery FG, Hatch JP, Nicoletti MA, et al. Temperament and character traits in major depressive disorder: influence of mood state and recurrence of episodes. *Depress Anxiety*. 2009;26(4):382-388. doi:10.1002/da.20478

88. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. 5th ed. Arlington, VA: American Psychiatric Publishing; 2013.

89. Brown GW, Bifulco A, Harris T, Bridge L. Life stress, chronic subclinical symptoms and vulnerability to clinical depression. *J Affect Disord*. 1986;11:1-19.

90. Short MA, Louca M. Sleep deprivation leads to mood deficits in healthy adolescents. *Sleep Med*. 2015;16(8):987-993. doi:10.1016/j.sleep.2015.03.007

91. Meyer JD, Koltyn KF, Stegner AJ, Kim J-S, Cook DB. Influence of exercise intensity for improving depressed mood in depression: A dose-response study. *Behav Ther*. 2016;47(4):527-537. doi:10.1016/j.beth.2016.04.003

92. McNair DM, Lon M, Droppelman LF. *Manual for the profile of mood states*. San Diego, CA: Educational and Industrial Testing Service; 1971.

93. McNair DM, Lorr M, Droppleman LF. *Edits Manual for the profile of mood states, Revised 1992*. San Diego, CA: Educational and Industrial Testing Service; 1992.

94. McNair DM, Lorr M. An analysis of mood in neurotics. *J Abnorm Soc Psychol.* 1964;69:620-627.

95. Taylor SE, Litchman RR, Wood JV. Attributions, beliefs about control and adjustment to breast cancer. *J Pers Soc Psychol.* 1984;46:489-502.

96. Fischman MW, Shuster CR, Hatano Y. A comparison of the subjective effects of cocaine and lidocaine in humans. *Pharmacol Biochem Behav.* 1983;18:123-127.

97. Thaxton L. Physiological and psychological effects of short time exercise in moods of ultramarathoners. *J Sport Exerc Phys.* 1982;4:73-80.

98. Killen JD, Fortmann SP, Telch MJ, Newman B. Are heavy smokers different from light smokers. *J Am Med Assoc.* 1988;260:1581-1585.

99. Gondola JC, Tuckman BW. Psychological mood states in "average" marathon runners. *Percept Motor Skill.* 1982;55:1295-1300.

100. Nyenhuis DL, Yamamoto C, Luchetta T, Terrien A, Parmentier A. Adult and geriatric normative data and validation of the profile of mood states. *J Clin Psych*. 1999;55(1):79-86. doi:10.1002/(SICI)1097-4679(199901)55:1<79::AID-JCLP8>3.0.CO;2-7

101. Shacham S. A shortened version of the profile of mood states. *J Pers Assess*. 1983;47(3):305–306

102. Curran SL, Andrykowski MA, Studts JL. Short form of the profile of mood states (POMS-SF): Psychmetric information. *Psychol Assess.* 1995;7(1):80-83. Doi: 10.1037/1040-3590.7.1.80

103. Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. An inventory for measuring depression. *Arch Gen Psychiatry.* 1961;4(6):561-571.

104. Bumberry W, Oliver JM, McClure JN. Validation of the Beck Depression Inventory in a university population using psychiatric estimate as the criterion. *J Consult Clin Psych*. 1978;46(1):150-155. Doi:http://dx.doi.org.ezproxy1.lib.asu.edu/10.1037/0022-006X.46.1.150

105. Vitamin B6 100 mg Tablets, Nature Made. Northridge, CA.

106. Apple Cider Vinegar Tablets, NOWFOODS. Bloomingdale, IL.

107. B6 Enzymatic Assay. #KK-VB6-U. Bühlmann, Amherst, NH.

108. Edwards MK, Loprinzi PD. Experimental effects of brief, single bouts of walking and meditation on mood profile in young adults. *Health Promot Perspect*. 2018;8(3):171-178. Doi:10.15171/hpp.2018.23

109. Stenbæk DS, Budtz-Jørgensen E, Pinborg A, Jensen PS, Frokjaer VG. Neuroticism modulates mood responses to pharmacological sex hormone manipulation in healthy women. *Psychoneuroendocrinology*. 2019;99:251-256. Doi:10.1016/j.psyneuen.2018.10.016

110. Herrlinger KA, Nieman KM, Sanoshy KD, et al. Spearmint Extract Improves Working Memory in Men and Women with Age-Associated Memory Impairment. *J Altern Complement Med*. 2018;24(1):37-47. Doi:10.1089/acm.2016.0379

111. Breymeyer KL, Lampe JW, McGregor BA, Neuhouser ML. Subjective mood and energy levels of healthy weight and overweight/obese healthy adults on high-and low-glycemic load experimental diets. *Appetite*. 2016;107:253-259. Doi:10.1016/j.appet.2016.08.008

112. Zhang M, Robitaille L, Eintracht S, Hoffer LJ. Vitamin C provision improves mood in acutely hospitalized patients. *Nutrition*. 2011;27(5):530-533. Doi:10.1016/j.nut.2010.05.016

113. Wang Y, Liu XJ, Robitaille L, Eintracht S, MacNamara E, Hoffer LJ. Effects of vitamin C and vitamin D administration on mood and distress in acutely hospitalized patients. *Am J Clin Nutr*. 2013;98(3):705-711. Doi:10.3945/ajcn.112.056366

114. Deac OM, Mills JL, Shane B, et al. Tryptophan Catabolism and Vitamin B-6 Status Are Affected by Gender and Lifestyle Factors in Healthy Young Adults. *J Nutr*. 2015;145(4):701-707. Doi:10.3945/jn.114.203091

115. Leklem JE, Brown RR, Rose DP, Linkswiler H, Arend RA. Metabolism of tryptophan and niacin in oral contraceptives users receiving controlled intakes of vitamin B6. *Am J Clin Nutr*. 1975;28(2):146-156. Doi:10.1093/ajcn/28.2.146

APPENDIX A

ARIZONA STATE UNIVERSITY INSTITUTIONAL REVIEW BOARD APPROVAL

ASU Knowledge Enterprise Development

APPROVAL: EXPEDITED REVIEW

Carol Johnston
Nutrition
602/496-2539
CAROL.JOHNSTON@asu.edu

Dear Carol Johnston:

On 12/31/2018 the ASU IRB reviewed the following protocol:

Type of Review:	Initial Study
Title:	Vitamin B6 Supplementation and Mood States in College Women Taking Oral Contraceptives
Investigator:	Carol Johnston
IRB ID:	STUDY00009386
Category of review:	(2)(a) Blood samples from healthy, non-pregnant adults, (4) Noninvasive procedures, (7)(b) Social science methods, (2)(b) Blood samples from others, (7)(a) Behavioral research
Funding:	Name: Graduate College (GRAD)
Grant Title:	
Grant ID:	
Documents Reviewed:	• POMS questionnaire, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • online survey, Category: Recruitment Materials; • Beck depression inventory, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • food questionnaire for dietary B6, Category: Screening forms; • protocol, Category: IRB Protocol; • ad and verbal script, Category: Recruitment Materials; • mood questionnaire, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • referal to ASU Counseling Services, Category: Participant materials (specific directions for them); • health history questionnaire, Category: Screening forms; • consent, Category: Consent Form;

The IRB approved the protocol from 12/31/2018 to 12/30/2019 inclusive. Three weeks before 12/30/2019 you are to submit a completed Continuing Review application and required attachments to request continuing approval or closure.

If continuing review approval is not granted before the expiration date of 12/30/2019 approval of this protocol expires on that date. When consent is appropriate, you must use final, watermarked versions available under the "Documents" tab in ERA-IRB.

In conducting this protocol you are required to follow the requirements listed in the INVESTIGATOR MANUAL (HRP-103).

Sincerely,

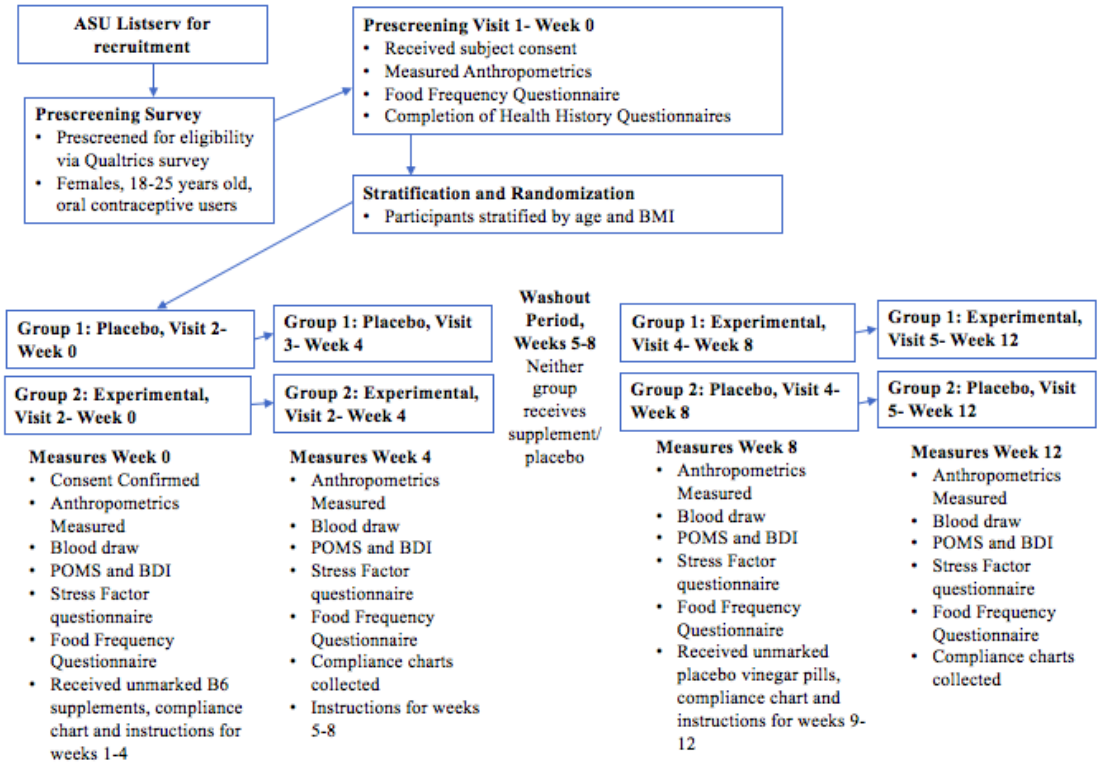
IRB Administrator

cc:
Anne Curtin

APPENDIX B

FLOW CHART OF STUDY DESIGN

Study Design Flow Chart



APPENDIX C

SUPPLEMENTS USED IN STUDY

Control treatment: Now Foods, vinegar tablets

Experimental treatment: Nature Made Vitamin B6 tablets

Tablet type	Picture of bottle	Supplement Facts
NOW Foods, Apple Cider Vinegar Tablets 450 mg. Bloomingdale, IL.		
Nature Made-Vitamin B6 100 mg Tablets. Northridge, CA.		

APPENDIX D

B6 ASSAY PROTCOL

Vitamin B6 Enzymatic Assay (#KK-VB6-U, Bühlmann, Amherst, NH)

Manual Procedure

Reagents have to be adjusted to 18-28°C .

Dilute EDTA plasma and Controls 1:40 in Dilution Buffer

Pipett 50 µl Substrate R1 into each well

Pipett 50 µl Calibrators 0, 20, 200 nmol/L
into the respective wells

Pipett 50 µl Control low and normal (diluted)
into the respective wells

Pipett 50 µl diluted sample into the subsequent wells.

Pipett 50 µl Apo-Enzyme R2 into each well.


shake and incubate for 30 + 5 min at 37°C in a plate incubator

Pipett 100 µl Enzyme R3 into each well


shake and incubate for 15 + 3 min at 37°C in a plate incubator

Read OD at 546 nm (alternatively at 520-595 nm)

Use endpoint mode with two calibrators (20 and 200 nmol/L). Calibrator 0 is used as Blank. Have a standard curve created by using linear curve-fitting.

Special Equipment

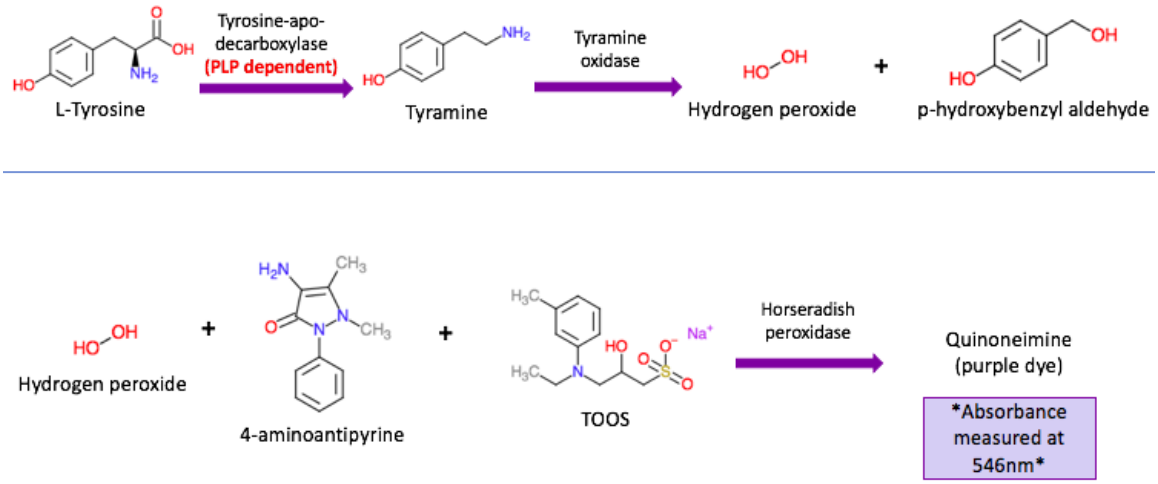
Manual procedure:
Microtiterplate reader with a filter at 546 nm, (520-595 nm) incubation chamber at 37°C and software suitable for endpoint measurements.

Microtiterplates, e.g. NUNC Maxisorb F8

APPENDIX E

B6 ASSAY SCHEMATIC

Mechanism of action of vitamin B6 assay



APPENDIX F

SAMPLE SIZE CALCULATION CHART

Sample Size Calculations

	Author	Year	Mood State Change	n per group	Calculated n per group	Age range	Location of Study	Test
1	Edward MK, Loprinzi PD	2018	5.2 ± 7.9	22	39	Females: 22.6 ± 3.2 years	Mississippi, USA	Effect of meditation on mood states assessed using POMS (parallel-arm)
2	Stenbæk et al.	2018	5.25 ± 1.8	12	5	Females: 28.3 ± 7.1 years	Copenhagen, Denmark	Effect of synarela (GnRHa spray) on mood
3	Herrlinger et al.	2018	5.56 ± 2.9	30	7	Males and Females (57%): 59.4 ± 0.6 years	Illinois, USA	Spearmint extract on cognitive performance
4	Breymeyer et al.	2016	3.56 ± 1.81	41	7	Males and Female (50%): 18-45 years	Washington, USA	Effect of high and low glycemic load diets on mood and energy
5	Zhang et al.	2011	9.8 ±2 0.6	15	72	Males and Females (46.7%): age range not listed	Montreal, Canada	Effect of vitamin C supplementation on mood in hospitalized patients
6	Wang et al.	2013	17.1 ± 16.3	26	17	Male and Female (46.6%): 65.5 ± 15.4	Canada	Effect of vitamin C supplementation on mood in acute care patients
Average				25	**24.5 (~25 people)**			