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MICRONUTRIENTS, INFLAMMATION AND DEPRESSION AMONG WOMEN OF REPRODUCTIVE AGE FROM THE NATIONAL HEALTH AND NUTRITION EXAMINATION SURVEY 2005-2008

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A Dissertation Presented

by

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DEDICATION

To my dad, who is watching over me from heaven.

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ABSTRACT

MICRONUTRIENTS, INFLAMMATION AND DEPRESSION AMONG WOMEN OF

REPRODUCTIVE AGE FROM THE NATIONAL HEALTH AND NUTRITION

EXAMINATION SURVEY 2005-2008

MAY 2017

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Depression is the leading cause of disease burden among women of childbearing age, rendering female mental health a major public health problem in the U.S. Recent evidence indicates that inflammation is associated with depression, and many of the factors that contribute to inflammation can be addressed through nutritional and lifestyle interventions. There are two micronutrients that have been linked previously with depression and have established roles in inflammation: vitamins B_6 and D. There is scarce research on the potential association between these micronutrients, inflammation, and depression. The purpose of this research was to investigate how high sensitivity C-reactive protein (hs-CRP), a biomarker of inflammation, may be contributing to different dimensions of depression and to determine to what degree inflammation or lifestyle factors may be affecting the association between vitamins B_6 and D and depression symptoms among women of reproductive age. We carried out a secondary data analysis of non-pregnant women

ages 18-44 from the National Health and Nutrition Examination Survey (NHANES) 2005-2008. All analyses were weighted using NHANES sample weights to account for the complex survey design, survey non-response, and post-stratification. Depression scores were calculated based on the Patient Health Questionnaire-9 (PHQ-9) and categorized into total depression, somatic depression and non-somatic depression. High depression score (PHQ-9$\geq$10) use was also used as an outcome, as well as individual symptoms of depression. Overall, 7.5% of our population experienced high depression symptoms.

In the first study, hs-CRP was positively associated with sum depression scores after adjustments for demographics and behavioral characteristics, but prior to adjustment by body mass index (BMI), which significantly attenuated this association. In stratified analysis, hs-CRP was associated with higher depression scores after adjustments among underweight women ($p < 0.05$). With regards to individual depression symptoms, hs-CRP was associated with sleep disturbance (OR: 1.31, 95% CI 1.01-1.71) and changes in appetite.

In the second study, vitamin B_6, as serum pyridoxal-5'phosphate (PLP), was categorized as deficient (<20 nmol/L), insufficient (20-29.9 nmol/L) or normal ($\geq$30 nmol/L). We found the prevalence of depression to vary by CRP level and by vitamin B_6 concentration. Among those with moderate inflammation, the prevalence of depression was highest among women with vitamin B_6 deficiency (20%), followed by those with vitamin B_6 insufficiency (10%), and those with normal vitamin B_6 status (5%) (p= 0.02). In multivariable models including individual depression symptoms, vitamin B_6 levels below 30 nmol/L were associated with higher odds of

experiencing suicidal ideation (OR: 7.33, p=0.01 and OR: 3.5, p=0.06 for B_6 deficiency and insufficiency, respectively) and depressed mood (OR: 3.12, p=0.004 for B_6 insufficiency). Among those with moderate CRP levels, vitamin B_6 deficiency was associated with a higher score for psychomotor abnormalities (β: 0.14, p=0.02). In our third study, suboptimal vitamin D levels were significantly associated with higher odds for depression among women who had elevated CRP (OR: 6.55, p=0.02 and OR: 9.54, p=0.001 for 25-OHD<75nmol/L and 25-OHD<50nmol/L, respectively). Among women who reported sleeping 7 or more hours per night, vitamin D was inversely associated with depression, whereas the opposite was true among women who slept less than 7 hours per night (p-interaction: 0.01). Together, the results of these studies suggest that inflammation may be interacting with BMI, sleep, or micronutrient deficiencies, and altogether contributing to different symptoms or severities of depression. Lifestyle factors, such as BMI or sleep could be used together with nutritional interventions, to aid in efforts to prevent or help treat depression among women of reproductive age.

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CHAPTER 1

INTRODUCTION

Depression is the leading cause of disability world-wide, affecting 350 million individuals. [1] Within developed countries, major depression is second only to heart disease as the leading source of disease burden, and for women, it is already the primary cause of disease burden. [1] Women in their childbearing years make up the largest group of Americans with depression and women are 2 to 3 times more likely than men to experience depression. [2,3] This high prevalence of depression among women renders female mental health a major public health problem in the U.S., especially since not all depressed individuals respond to drug treatment and additional therapies are urgently needed. [4]

The complexity of depression is unmistakable, yet a key to its prevention and treatment may be a factor so fundamental that it has been broadly overlooked: overall nutritional status. [5] Recent evidence indicates that inflammation is associated with depression [6] and many of the factors that contribute to inflammation can be addressed through nutritional interventions. [7] Two micronutrients – Vitamins D and B_6 – have potential roles in both inflammation [8,9] and depression, [10,11] but to date, no studies have examined the potential association between these nutrients, inflammation, and depression, which is the main objective of my dissertation.

Depression is a multifaceted construct with a large range of symptoms. Research into the etiology of depression needs to account for the heterogenous nature of the condition and requires developing methods to characterize and objectively assess the

severity of depression and to classify depression into its different subtypes. [12] In order to address the issue of symptom diversity, subtypes of depressive symptoms have been proposed based on specific combinations of symptoms. [13] Somatic depression, characterized by sleep disturbance, fatigue, or changes in appetite, has been found to be more prevalent in females compared to males. [14] Evidence suggests that gender differences may account for heterogeneity in depression symptoms and the involvement of inflammation in the etiology of depression may be affected by lifestyle factors. [15] To the best of our knowledge, prior literature has not assessed how inflammation or nutritional deficiencies may be contributing to different sub-types of depression or individual depression symptoms among women of childbearing age. This lack of knowledge is problematic because an understanding of the modifiable nutritional and lifestyle factors that may be contributing to depressive symptoms, especially in the context of inflammation, is important for the prevention and early treatment of depression. This dissertation examined cross-sectional data from 1,489 non-pregnant women ages 18-44 years from the National Health and Nutrition Examination Survey (NHANES) to evaluate the extent to which inflammation, suboptimal vitamin B_6 and D concentrations, and lifestyle factors are associated with different dimensions of depression in women representative of the U.S. population.

CHAPTER 2

DEPRESSION

2.1 Epidemiology of Depression

Depression is a commonly occurring, seriously impairing, and often recurring psychiatric disorder.[4,5] The World Health Organization identified depression as the leading cause of disability world-wide, affecting 350 million individuals.[1] In the US, recent samples estimate a lifetime depression prevalence of 16.2% (approximately 33 million adults) and a 12-month prevalence of 6.6% or 13.5 million adults.[6] It is also the leading cause of disability for people ages 15-44 years old, resulting in almost 400 million disability days per year.[19] With a quarter of women all ages reporting depression symptoms according to recent national estimates,[7] they are 2 to 3 times more likely than men to experience depression.[2,3] Not only is depression the most common mental illness experienced by women, but rates of depression are on the rise.[8] The chance of suffering from depression is ten times greater for women today than it was for their grandmothers.[9]

2.2 Definition of Depression

Major depressive disorder goes beyond the normal bounds of sadness and is marked by hopelessness, loss of mood reactivity, inability to experience pleasure, suicidal thoughts, and psychotic symptoms such as delusions or hallucinations.[10] The actual diagnosis of major depressive disorder is based on criteria established in the *Diagnostic and Statistical Manual of Mental Disorders, fifth edition* (DSM-V) by the American Psychiatric Association (APA).[11] Major depressive disorder is characterized by discrete episodes of at least 2 week

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duration involving clear-cut changes in affect, cognition, and neuro-vegetative functions and inter-episode remissions.[11] Common symptoms of major depressive disorder or unipolar depression include a persistent sad mood, loss of interest or pleasure in activities that were once enjoyed, changes in appetite or weight, difficulty sleeping or oversleeping, physical slowing or agitation, energy loss, feeling worthlessness or inappropriate guilt, difficulty thinking or concentrating, and recurrent thoughts of death or suicide.[11] A diagnosis of major depressive disorder is made if a person has five or more of these symptoms and experiences impairment in usual functioning nearly every day during the same 2-week period and at least one of the symptoms must be either depressed mood or anhedonia (loss of interest of pleasure).[11]

Other manifestations of depressive disorders include: dysthymic disorder (or dysthymia), which is characterized by long-term (2 years or longer) symptoms that may not be severe enough to disable a person but can prevent normal functioning or feeling well; and minor depression, which is characterized by having symptoms for 2 weeks or longer that do not meet full criteria for major depression.[12] Those with minor depression are at increased risk for developing major depressive disorder.[12] Other forms of depression vary slightly or may develop under different circumstances, such as postpartum depression or seasonal affective disorder.[12]

2.2.1 Subtypes of Depression

Major depressive disorder patients vary considerably in clinical presentation, cause, treatment response, genetics, and neurobiology.[13,14] One explanation for this diversity is the *polyethic* definition of major depressive disorder, meaning that a patient needs to only satisfy some but not all symptoms to be classified as depressed.[14] This approach suggests there are 227 possible combinations of symptoms leading to a diagnosis of depression.[14] In order to tackle the issue of symptom diversity, subtypes of depressive disorders have been proposed based on specific combination of symptoms (i.e. melancholic depression, psychotic depression), onset (seasonal affective disorder postpartum, early versus late in life), course (single, recurrent, chronic), or severity.[15] Several subtypes of depression such as atypical, psychotic, cognitive, somatic and melancholic depression have been found in theoretical frameworks.[14] Of these, somatic symptomology has been found to be more prevalent in females compared to males.[16] Research into the etiology of depression needs to account for the heterogenous nature of the condition and requires developing methods to characterize and objectively assess the severity of depression and to classify depression into its different subtypes.[5] Investigating risk factors for specific symptoms offers opportunities to discover potential biological factors that may be related to specific syndromes but may be masked by aggregate scoring; individuals with similar total depression scores often have drastically different clinical conditions.[16] In conclusion, depression is a heterogeneous disorder with a highly variable course, [30] an inconsistent response to treatment, [4] and no established mechanism.[13] Therefore, further attempts to characterize depression and find new potential treatments are warranted.

2.3 Public Health Challenge

Currently, depression is one of the three leading causes of global disease burden, but it is predicted to become the leading cause by 2030.[17] This poses an important public health challenge because depression is often undiagnosed and untreated by physicians in primary care settings.[18] Despite heightened awareness and treatment of depression in primary care settings, the prevalence of depressive symptoms remains high, treatment levels remain low, and control of depressive symptoms are suboptima.[7] A recent study of a nationally representative sample of adults from NHANES found that even among individuals with severe depressive symptoms, a large proportion (36.9%) did not receive treatment for depression.[7] Furthermore, not all depressed individuals respond to drug treatment and additional therapies are urgently needed.[4] Among U.S. adults taking antidepressants, 26% reported feeling mild depressive symptoms and 19% reported feeling moderate, moderately severe, or severe depressive symptoms.[7] This suggests that antidepressants alone may not be the optimal treatment for depression, and further avenues, such as nutritional therapy, could be beneficial in treating depression.

Among women, maternal depression is a major public health problem in the United States[20] encompassing a range of emotional and physical changes that mothers can experience especially during pregnancy (prenatal) or in the year after giving birth (postnatal).[21] Ante-, peri- and postpartum depression occur in 10-20% of women, with rates of depression increasing in the last 2 trimesters of pregnancy up to 51% in the general population of pregnant women.[21] These estimates are likely conservative due to under-reporting and under-diagnosing maternal depression. Indeed, as many as half of all cases of perinatal depression go undetected, in part because many of the symptoms can be

wrongly attributed to the hormonal and physiological changes characteristic of

pregnancy.[22] Furthermore, studies have shown that depression in the postpartum period

can last for months or even years after giving birth.[23,24]

2.4 Public Health Impact of Depression

Depression has a major impact on functioning and quality of life, on public health and

on the economy. In 2000, the estimated costs of depression totaled $83.1 billion, including

$26.1 billion for direct medical costs, $5.4 billion for suicide-related mortality costs, and

$51.5 billion for workplace costs, including absenteeism and disability.[25] Based on a

nationally representative sample of U.S. workers, Kessler and colleagues (2006) estimated

that major depression resulted in 27.2 lost workdays per ill worker each year.[26]

Additionally, they found that the major workplace impact of depression is more likely to be

due to presenteeism (being physically present at work, but unable to function) than

absenteeism, which can be several times the cost of work loss time alone.[26]

From an interpersonal and domestic perspective, depression can have devastating

effects on a child's development and family health. Parents represent the earliest, most

constant, and most proximal influence on child development. Their psychological health, in

addition to parenting behavior, serves as an important environmental risk or protective

factor in the developmental outcomes for children, especially with regard to emergence

and course of behavior problems.[27] Depression among mothers is increasingly recognized

as a common and devastating public health problem affecting not only women but also the

children in their care. Maternal depression can have devastating effects on a child's health,

starting in pregnancy. Depressed mothers are more likely to have poor nutrition and to

smoke, drink, and/or use illicit drugs compared to non-depressed women; they are also more likely to engage in risky behavior, including suicidal thoughts, as well as underutilization of prenatal care services.[28] Maternal depression is one of the most common complications during pregnancy and it can lead to increased obstetrical complications, such as pre-eclampsia, excessive bleeding, placental rupture, and preterm birth and low birth weight babies.[29] The effects of maternal postpartum depression on infants can be long-lasting, affecting a child's development and well-being. Infancy is a vital developmental period, covering the time-frame when children develop social interaction skills, experience parental attachment and bonding, and learn how to communicate. If a mother's ability to interact with her child is impaired due to her own depression, her infant may fail to develop these important skills. Infants of depressed mothers often suffer from developmental delays in their emotional, social, cognitive, and linguistic abilities.[30,31] Furthermore, even mild or unrecognized maternal depressive symptoms in the first 4 months postpartum may have a significant impact on bonding with the infant.[32] In addition to emotional and development issues, maternal depression during the first years postpartum is a also a strong predictor of child overweight and obesity.[39] Long-term effects of maternal depression include increased risk of depression and anxiety in childhood and adolescence.[40] This is especially important because the earlier onset of depression in life is associated with greater severity of illness and higher risk of suicide than later onset depression.[40]

2.5 Risk factors for Depression in Women

Depression is a multifactorial condition involving genetic and environmental risk factors.[41] Even though depression may have a large genetics component including polymorphisms of the serotonin transporter promoter gene (5-HTTLPT),[213] studies on mood disorders have not provided consistent evidence regarding the roles of specific susceptibility genes or their pattern of inheritance. [50,51] A genetic predisposition, in combination with several potential environmental influences, is likely involved in the development of depression.[22 54,55] Environmental factors associated with depression in women include stress (e.g. physical, mental, and emotional trauma); viral infections; hormonal disorders; chronic diseases; and some medications (e.g. sedatives). [22,42] However, environmental factors do not appear to act alone, but can increase the risk for depression in those with a genetic susceptibility for the condition.[22]

In addition to genetic predisposition and environmental risk factors, several social and biological factors are associated with an increased risk for developing depression for women. Social risk factors include a history of depression/anxiety, lack of marital partner or marital difficulties,[44] low socioeconomic status,[45] lack of social support or social isolation,[46] major life events as well as family violence,[47] increased life stress, and substance abuse.[21] Psychological factors include history of depression [48] and history of psychiatric illness, such as premenstrual dysphoric disorder,[46] and depressive symptoms during pregnancy.[49]

Biological risk factors associated with depression in women are more challenging to determine. Some biological factors that may contribute to the pathophysiology of depression include hormonal fluctuations,[50] neurotransmitter function, [62-64] metabolic

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syndrome,[54] increased inflammation,[55] and nutrient deficiencies from malnutrition or poor diet quality.[56]

In summary, depression is a multifaceted construct whose etiology remains a mystery, and this may partly be due to the many potential risk factors that may be playing a role in its development and severity.

CHAPTER 3

INFLAMMATION

3.1 What is the Immune System and How Does it Function?

The immune system includes two major components: specialized cells that carry out the wide array of immune processes, and the chemical messengers that enable communication among immune cells, and between immune cells and other cells and tissues within the body.[57] The specialized cells and the messengers are coordinated to carry out two forms of protection, innate and acquired immunity, which also work together in a very complex manner to protect the body from foreign pathogens. *Innate* immunity is a general, non-specific first line of defense comprised of anatomic, physiologic, endocytic and phagocytic, and inflammatory barriers.[58] Innate immunity is moderated and enhanced by the acquired immune system, a specific form of defense that targets and labels pathogens for elimination.[59] Innate and acquired immunity operate in cooperative and interdependent ways. The activation of innate immune responses produces signals that stimulate and direct subsequent acquired immune responses, which involves cell-mediated responses.[58] The innate immune system continuously surveys the body for presence of invaders, and when activated, it sets in motion a localized inflammatory response to fight most pathogenic threats.[60]

3.2 What is Inflammation?

Inflammation is an essential immune response that enables survival during infection or injury and maintains tissue homeostasis under a variety of conditions.[61] The inflammatory response was first characterized in the 1st century AD by Cornelius Celsus, who coined the four cardinal symptoms of inflammation: *rubor et tumor cum calore et dolore* (redness and swelling with heat and pain).[62] The physiology behind these symptoms was discovered in the 1800s, when Augustus Waller (1846) and Julius Cohnheim (1867) discovered leukocyte emigration from blood vessels and other vascular changes characteristic of an acute inflammatory response. Upon microscopic observation of living tissue, Cohnheim noticed vasodilation, leakage of plasma, and migration of leukocytes out of blood vessels and into the surrounding tissue.[62] The 5th cardinal symptom of inflammation, *function laesa* (disturbance of function), was added in 1858 by Rudolph Virchow in this book Cellularpathologie.[62]

Currently, it is known that inflammation may be of different forms and modalities, which are directed by different mechanisms of induction, regulation, and resolution.[61] In the past few decades, the spectrum of inflammatory conditions has shifted from acute inflammatory reactions in response to wounds and infections to chronic inflammatory states that accompany chronic diseases such as type 2 diabetes, atherosclerosis, asthma, cancer, and neurodegenerative diseases.[61]

3.2.1 Acute Inflammatory Response

A typical acute inflammatory response consists of four components: inflammatory inducers, the sensors that detect them, the inflammatory mediators induced by the sensors, and the target tissues that are affected by the inflammatory mediators.[61]

Each component comes in various forms, and their combinations function in distinct inflammatory pathways. The type of pathway induced under given circumstances depends on the source of the inflammatory stimulus. Thus, bacterial pathogens are detected by receptors of the innate immune system expressed on tissue-resident macrophages and induce the production of inflammatory cytokines and chemokines, as well as prostaglandins.[61] These inflammatory mediators then act on target tissues, including local blood vessels, to induce vasodilation, extravasation of neutrophils, and leakage of plasma into the infected tissue. Depending on the type of infection (bacterial, viral, or parasitic), the sensors, mediators, and target tissues vary such that the most adequate type of inflammatory response is induced.[61] The acute inflammatory response is normally terminated once the trigger insult is eliminated, the infection is cleared, and damaged tissue is repaired. This is known as the resolution of inflammation.

3.2.2 Chronic Inflammation

Many chronic inflammatory conditions have been described where the initiating trigger is not well defined but does not seem to involve infection or tissue damage. These inflammatory conditions accompany many chronic diseases, including obesity and type 2 diabetes, atherosclerosis, neurodegenerative diseases, and cancer.[61] Several inflammatory

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mediators, such as acute-phase proteins, cytokines or chemokines, can be used to assess inflammation.

3.2.3 Inflammatory Mediators: Cytokines and Acute-Phase Reactants

As part of the inflammatory response, damaged or infected cells secrete chemical messengers or mediators called cytokines, which act as chemoattractants. The term "cytokines" encompasses a large family of proteins, including interleukins (IL), interferons (IFN) and colony-stimulating factors, as well as various growth factors and eicosanoids, including prostaglandins.[63] To differentiate among cytokines' biological activity, they are often described as either *pro-inflammatory* or *anti-inflammatory*. Following damage to or infection of cells and tissues, pro-inflammatory cytokines are produced and secreted to stimulate the activation of the immune system.[64] Anti-inflammatory cytokines, such as IL-10, are also secreted during inflammation in order to terminate pro-inflammatory cytokine activity.[63]

Cytokine expression normally occurs in a step-wise manner, with the expression of certain cytokines dependent upon the prior expression of others; for example, IL-1 is necessary to induce production of IL-2, IL-6 and tumor necrosis factor (TNF).[63] Cytokines are mainly produced by immune cells, but are also produced by a variety of other cell types, such as brain cells and adipose cells.[63] Peripheral cytokine production depends to a great degree on the state of immune activation. In pathological conditions of acute or chronic inflammation and tissue damage, the immune system is activated and macrophage activity increases, accounting for increased production of cytokines, such as IL-1, IL-6, and TNF-α. In the case of a viral infection, IFNs, released by activated T-cells, play a major role.[59] These

mediators, when secreted in sufficient amounts, can have systemic effects and can induce liver cells to produce acute phase proteins such as C-reactive protein (CRP) and coagulation factors, including pro-inflammatory prostaglandins.[61]

3.3 C-Reactive Protein: The Main Acute-Phase Reactant

C-reactive protein (CRP) is the prototypical acute phase protein in humans.[65] CRP is a serum protein that is massively induced as part of the innate immune response to infection and tissue injury.[66] CRP is composed of five 23-kDa subunits, and was named for its capacity to precipitate the somatic C-polysaccharide of *Streptococcus* penumoniae.[65] Even though it was the first acute-phase protein to be described and traditionally was viewed only as an acute phase reactant, CRP is currently considered the "best" systemic marker of non-specific inflammation and tissue damage.[67]

3.3.1 Biosynthesis of CRP

CRP was traditionally thought to be produced only by hepatocytes, but recent studies showed that CRP can be produced by non-hepatic tissues such as the renal epithelium and respiratory tract.[68,69] CRP synthesis is stimulated by inflammatory cytokines, such as IL-6 [70] and this synthesis is enhanced synergistically by IL-1β.[71] De novo hepatic synthesis initiates rapidly after a single stimulus, leading to serum concentrations above 5mg/L for approximately 6 hours and peaking at 48 hours.[72] The plasma half-life of CRP is roughly 19 hours and is constant under all conditions of health and disease, so that the sole determinant of circulating CPR levels is the synthesis rate, which is a direct reflection of the intensity of the pathological process stimulating CRP production.[67] When

the stimulus for increased production ceases, the circulating CRP level falls rapidly, at almost the rate of plasma CRP clearance.[67]

3.3.2 Circulating CRP Concentration

Healthy individuals in the general population tends to have stable CRP concentration, apart from occasional spikes thought to be related to minor or subclinical infections, inflammation, or trauma. There is no significant seasonal variation in baseline CRP concentration, and, remarkably, the self-correlation coefficient of measurements repeated years apart is approximately 0.5, which is comparable to that of cholesterol.[67] Acute-phase CRP values also show no diurnal variation and are unaffected by the fed state. Liver failure impairs CRP production, but no other pathologies and very few drugs reduce CRP values unless they also affect the underlying pathology providing the acute-phase stimulus.[67] The CRP concentration is a useful nonspecific biochemical marker of inflammation, measurement of which contributes importantly to screening for organic disease, monitoring of the response to treatment of inflammation and infection, and detection of intercurrent infection in immunocompromised individuals.[67]

In healthy young adults, the median concentration of CRP is 0.8 mg/L, the 90th percentile is 3.0 mg/L and the 99th percentile is 10 mg/L.[73] However, CRP is part of the acute phase response, and following injury, CRP levels increase dramatically, up to 10,000-fold from less than 50 ug/L to more than 500 mg/L.[67] This increase is rapid, but not immediate: circulating concentrations reach 5 mg/L by approximately 6 hours and peak at 48 hours.[72] CRP is clearance occurs at a constant rate, rendering CRP levels a reliable index of ongoing inflammation.[72] Dramatic elevations in CRP levels indicate ongoing

infection, and in the absence of infection, CRP reflects the degree of background inflammation characteristic of an individual.[67]

3.3.3 Role of CRP in the Inflammation Process

There are several mechanisms through which CRP enhances inflammation and activates the immune response. Human CRP binds with high affinity to phosphocholine residues, but it also binds to a variety of other autologous and extrinsic ligands, and it aggregates or precipitates the cellular, particular, or molecular structures bearing these ligands.[67] Autologous ligands include native and modified plasma proteins,[74] damaged cell membranes,[75] several different phospholipids and related compounds as well as small nuclear ribonucleoprotein particles,[76] and apoptotic cells.[77] Extrinsic ligands include many glycan, phospholipid, and other constituents of microorganisms, such as components of bacteria, fungi and parasites, as well as plant products. When aggregated or bound to macromolecular ligands, human CRP is resembles some of the key properties of antibodies, suggesting that under various circumstances CRP may contribute to host defense against infection, function as a pro-inflammatory mediator, and participate in physiological and pathophysiological handling of autologous constituents.[65]

3.4 Lifestyle Factors that can Impact Inflammation

3.4.1 Obesity and Inflammation

Increasing evidence indicates that obesity causes chronic low-grade inflammation, which contributes to the development of obesity-associated disorders, mainly

characterized by metabolic dysfunction.[78,79] Excess adipose mass is associated with increased levels of the pro-inflammatory marker CRP in the blood in children and adults.[80] Furthermore, weight loss interventions in those who were previously obese lead to reductions in the levels of pro-inflammatory proteins, including CRP.[81–83]

Adipose tissue is traditionally considered a long-term energy storage organ, but it also functions as a key endocrine organ by releasing multiple bioactive substances, known as adipose-derived secreted factors or *adipokines*, that have pro-inflammatory or anti-inflammatory activities.[78,84] The expression of adipokines varies depending on whether the fat is stored in visceral or subcutaneous adipose tissues, as each of these sites produces a unique profile of adipokines [85]. The production of most adipokines is upregulated in the obese state, and these pro-inflammatory proteins include: leptin, TNF, IL-6, resistin, retinol-binding protein 4, lipocalin 2, IL-18, angiopoietin-like protein 2, CC-chemokine ligand 2, CXC-chemokine ligand 5, and nicotinamide phosphor-ribosyltransferase.[78] Among these, leptin upregulates production of TNF and IL-6 by monocytes and increases the production of the T_H1-type cytokines IL-2 and IFN-γ, therefore acting as a pro-inflammatory adipokine.[86] Tumor-necrosis factor is another pro-inflammatory cytokine, mainly produced by monocytes and macrophages in visceral depots whose levels are increased in adipose tissue and plasma of obese individuals, and reduced following weight loss.[87,88] Interleukin-6 increases transcription of CRP, and is positively associated with adiposity in human populations.[89] Similarly to CRP and TNF, increased levels of IL-6 are seen in obese subjects, with weight loss leading to reduced IL-6 levels.[83] Furthermore, approximately one third of total circulating IL-6 is produced by adipose tissue.[89]

Interleukin-18 is another pro-inflammatory adipokine produced by adipose tissue, whose levels fall after obese subjects undergo weight loss.[90]

3.4.2 Estrogens and Inflammation

Studies conducted among users of exogenous hormones suggest that oral contraceptive (OC) use [91,92] and orally administered hormone replacement therapy (HRT) consistently elevate CRP, whether the preparation includes progesterone and estrogen or estrogen alone.[91,93] A study by Dreon (2003) comparing CRP levels in 18 OC users vs 12 non-OC users found that plasma CRP levels were twice as high among OC users compared to non-OC users (2 ± 0.2 vs 0.9 ± 0.3 mg/L; $p<0.001$) independent of phase of menstrual cycle. Furthermore, they found that in this group, OC use predicted 32% of the variance in CRP levels ($p<0.001$).[92] Even though exogenous estrogen has been demonstrated to increase CRP, dose and delivery method appear to mediate this increase. Prestwood et al (2004) found significantly higher CRP among women taking a higher estrogen HRT preparation and significantly lower levels among women taking a lower estrogen preparation; transdermally administered HRT has no observable effect on CRP.[94,95]

Studies examining the effects of endogenous hormones on CRP are inconsistent with the trends shown for exogenous hormones. Jilma et al (1997) examined CRP changes in 18 women during the follicular phase, at mid-cycle, and in the luteal phase of one menstrual cycle, and found that median CRP levels increased by 44% ($p<0001$) at mid-cycle and by 31% ($p=0.002$) in the luteal phase compared to the follicular phase.[96] Additionally, they found a significant association between an individual's relative increase in CRP and progesterone concentration from the follicular phase to mid-cycle ($r=0.60$, $p=0.01$) and

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from the follicular phase to the luteal phase (r=0.71, p=0.001).[96] In a second study of

endogenous hormones, Blum et al (2005) examined 15 measures from 15 women across

one menstrual cycle, and found an inverse association between estrogen and CRP (β=-0.23,

p<0.001) such that a 10-fold increase in estrogen was associated with a 41% decrease in

CRP.[97]   A third study of the effects of endogenous hormones on CPR found a 10-fold

increase in progesterone to be associated with a 23% increase in CRP (p=0.01), a ten-fold

increase in estrogen was associated with a 29% decrease in CRP (p=0.05).[98]

Thus the effects of exogenous and endogenous hormones on CRP appear to differ:

exogenous estrogen at most doses is associated with elevations in CRP,[91–93] whereas

endogenous estrogen seems to be correlated with lower CRP,[97,98] conversely, exogenous

progesterone may be associated with decreases in CRP,[99–101] whereas endogenous

progesterone seems to be associated with increases in CRP.[96,98]

3.4.3 Smoking and Inflammation

Environmental factors, such as smoking, have been reported to modify

inflammation progression, severity and outcome.[102] Chronic inhalation of cigarette smoke

alters a wide range of immunological functions, including innate and adaptive immune

responses.[103] Inflammatory mediators have been detected at higher levels in smokers in

several studies.[104,105] A large scale analysis with 2,920 healthy men ages 60-79 found that

current cigarette smokers showed significantly higher levels of CRP (2.53 vs 1.35 mg/L)

compared with never-smokers.[104] In women, Bermudez et al (2002) found higher levels of

plasma CRP in smokers enrolled in the Women's Health Study compared to never smokers

(3.80 vs 3.0 mg/L, p=0.032). [105] Therefore, the systemic inflammatory process appears heightened in those who smoke compared to nonsmoking subjects.

3.4.4 Physical Activity and Inflammation

Cross-sectional studies show an inverse association between regular physical activity and the serum concentration of inflammatory markers, suggesting that physical activity may have anti-inflammatory properties. Pitsavos et al found that CRP levels decreased with increasing physical activity in the ATTICA study, as sedentary subjects had the highest CRP concentration (1.47±1.4 mg/dL), followed by those who reported light-moderate physical activity (1.01±1.5 mg/dL); finally those with highest level of physical activity had the lowest serum CRP (0.91±1.3 mg/dL; p=0.020).[106]   A study in older adults measuring weekly caloric expenditure and inflammation found a dose-response relationship between physical activity and CRP in the Cardiovascular Health Study.[107] The results from several cross-sectional studies suggest that physical activity and fitness are associated with lower CRP levels.[108] Furthermore, recent longitudinal studies suggest that physical activity reduces CRP levels. A 2-year dietary and lifestyle intervention study in postmenopausal women showed that serum CRP was reduced by 34% in obese postmenopausal women; however, it is unclear as to whether the reduction in CRP was due to weight loss, the physical activity or a combination of both.[83] In a second longitudinal study, Obisesan et al found a 15% reduction in CRP following a six-month exercise and diet intervention, which did not result in weight loss.[109]

3.4.4.1 Effect of type, duration, and intensity of physical activity on CRP levels

Despite limited evidence on the relationship between physical activity and CRP, several studies have tried to ascertain the effect of type, duration, and intensity of physical activity on inflammatory markers in healthy adults. For the mode of physical activity that may most impact CRP, King et al (2003) found that activities such as jogging, swimming, cycling, aerobic dance and weight lifting performed over 12 times per month were associated with a lower likelihood of elevated CRP in adults ages 17-65 years old when compared to non-exercisers using data from NHANES III.[110] In particular, frequent jogging and aerobic dance were associated with the lowest proportion of exercising individuals with high CRP.[110] This analysis was limited, however, by the inability to assess differences in duration or intensity of each activity. Albert and colleagues assessed the baseline frequency of physical activity in adults participating in the Pravastatin Inflammation/CRP Evaluation (PRINCE) study, a multicenter community-based study.[111] They created 4 groups of physical activity based solely on frequency: less than once per week, once per week, 2-3 times per week, and 4 or more times per week; they found that CRP levels decreased progressively with increasing levels of physical activity frequency (p-trend <0.001). [111] In order to assess the relationship between intensity of physical activity and CRP levels, Pitsavos et al (2003) studied leisure-time activities such as walking, cycling, running, swimming, etc, in healthy adults ages 18 and older from the ATTICA study.[106] They found that among individuals who claimed to exercise at least once a week, a high exercise intensity (7 kcal/minute or more) was associated with a 38% decrease in CRP levels compared with light exercise intensity (<4 kcal/min. [106] With regards to overall duration of physical activity, the minimum length of time observed associated with lower CRP levels in

longitudinal studies has been 2 months.[108,112] Geffken et al (2001) observed that 3-4 days

of physical activity per week, for 40-80 minutes per day at an intensity of 70-80% of

V0$_2$max can lower CRP levels in as little as 2 months in a healthy elderly population.[107]

Despite all the limitations encountered, there is sufficient evidence to suggest that physical

activity lowers inflammatory biomarkers such as CRP in a variety of settings and

populations.

3.4.5 Sleep and Inflammation

Inadequate sleep and sleep deprivation cause several neurobehavioral and

physiological changes, including increases in a range of inflammatory markers.[216] Chronic

sleep disruption is regarded as a physiological stressor that can impair brain function[217]

and raises pro-inflammatory cytokines by inducing a functional alteration of the

proinflammatory cytokine response in monocytes, which is greater among females

compared to males.[218]

3.4.6 Other correlates of CRP

Among individuals not experiencing infection, CRP concentrations may vary with

many additional factors.[98] In adults, CRP has been found to be positively associated with

age[113] and negatively associated with birth weight.[114] Adult females have higher CRP than

males[113,115] Furthermore, some component of variation in circulating levels of CRP may be

heritable: estimates of heritability between 30% and 50% have been suggested.[116,117]

3.5 Physiology of Inflammation and Depression

Depression is a complex condition, and it is likely that alterations in multiple interacting systems underlie its pathogenesis.[118] Therefore, numerous hypotheses have been proposed to elucidate its origins. One of these is the inflammatory hypothesis, initially called the *macrophage theory of depression,*[119] presently also known as the malaise or cytokine theory of depression.[120,121] This inflammatory hypothesis emphasizes the role of psycho-neuroimmunological dysfunctions, which is based on several observations. The first one of these observations noted is that subsets of patients with major depression have an altered peripheral immune system, with impaired cellular immunity and increased levels of pro-inflammatory cytokines, including IL-6 and CRP.[122,123] Furthermore, postmortem gene analyses in the prefrontal cortex of patients who had major depression have suggested up-regulation of various pro and anti-inflammatory cytokines.[124] It is now known that cytokines can cross the blood-brain barrier and induce inflammation in the central nervous system and can influence neurotransmitter metabolism, neuroendocrine function and regional brain activity, all of which are relevant to depression.[63]

3.5.1 Cytokine Administration Induces Depression

It has been noted that chronic therapeutic administration of cytokines, mainly interferon-alpha (INF-α) for hepatitis C virus infection or cancer, can lead to depressive symptomatology in up to 50% of patients who will meet the criteria for major depression within 3 months of starting therapy;[125] up to 90% receiving this therapy will display at least one or two significant depressive symptoms.[126] Therefore, INF-α-induced depression has been used as a model to identify the specific changes in the immune system that may

play a role in the development of behavioral changes leading to depression.[127] INF-α is a cytokine released by the innate immune system in response to viral infections; it has been shown to induce IL-6 production, which stimulates CRP production. Additionally, some evidence indicates that INF-α induced depression can be prevented and/or treated using selective serotonin reuptake inhibitor (SSRIs).[125] Therefore, there is evidence supporting the directionality of inflammation leading to depression.

3.5.2 Cytokines Alter Neurotransmitter Metabolism

Numerous human and laboratory animal studies have demonstrated that multiple neurotransmitter systems, including monoamines, serotonin, dopamine, and glutamate, are affected by acute and chronic administration of cytokines.[121] Serotonin has been the most studied neurotransmitter with regards to inflammation and depression. INF-α has been shown to attenuate the expression of 5HT1A, a serotonin receptor.[128] Patients treated with INF-α have decreased serum serotonin, and this has been more pronounced in those who developed depression.[129] Moreover, INF-α -associated increases in cerebrospinal fluid (CSF) levels of IL-6 have been inversely associated with the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA), indicating lowered brain serotonergic activity.[130] The exact pathway affected is not clear, but it is know that the enzyme indoleamine 2,3-dioxygenase (IDO) is activated by various cytokines alone or in combination.[131] IDO catabolizes tryptophan, the primary amino acid precursor of serotonin, into kynurenine. Evidence of a role of IDO in cytokine-induced depression comes from several studies that demonstrated correlations between INF-α-induced depression and decreases in tryptophan accompanied by an increase in kynurenine and/or the kynurenine to

tryptophan ratio.[131] Although much of the attention regarding IDO was initially focused on

the depletion of tryptophan and therefore, of serotonin, several lines of evidence have

started to challenge this pathway, and have focused more on the presence of increased

kynurenine and its metabolites for immune-induced depression.[131]

3.5.3 Effects of Inflammation on Neurogenesis and Neurotrophic Factors

Pro-inflammatory cytokines can influence neuronal functioning through changes in

apoptosis, oxidative stress, and metabolic derangement, as well as by impairing processes

of synaptic plasticity and neurogenesis.[120,132] In humans, treatment with INF-α has been

associated with reduced levels of peripheral brain-derived neurotrophic factor (BDNF),

which are known to correlate well with BDNF availability in the CNS.[133,134] Furthermore

reduced BDNF during INF-α treatment has been found to correlate with increased

depression.[134]

Figure 1: Potential mechanisms linking inflammation to depression.
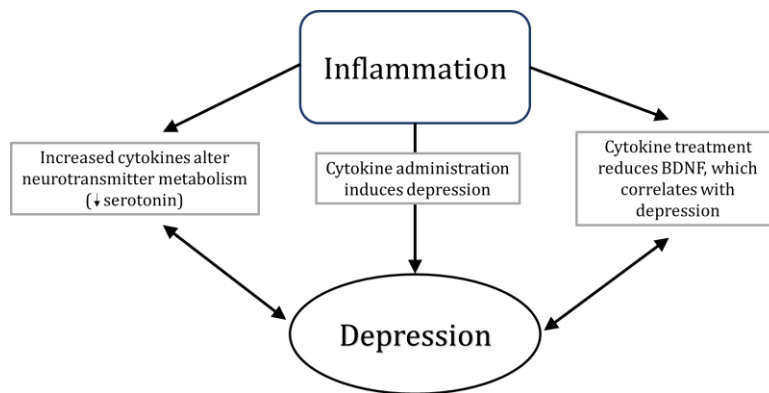
3.6 Epidemiology of Inflammation and Depression in Women

Research on the association between inflammation and depression has mainly

focused in elderly populations, but recent studies have included younger adults, children,

and different at-risk populations, such as pregnant or post-partum women. The first large

cross-sectional study to analyze the association between CRP and depression in a

nationally representative sample using NHANES included 6,914 non-institutionalized men

and women ages 18-39 years old (women sample size: 3,760). [55] Employing the Diagnostic

Interview Schedule (DIS) depression questionnaire to assess depression, which was

categorized into recurrent depression and severe depression within the last year and

within their lifetime. Ford and colleagues found a higher proportion of women

experiencing lifetime depression (11.7% vs 5.7%) compared to men. CRP was

dichotomized at the limit of detection (0.21 mg/dL), and labelled as "low CRP" and "high

CRP". Almost a third (27.3%) of women were found to have high CRP levels compared to

only 13% of men. A history of major depression was associated with elevated CRP level

(OR: 1.64, 95%CI 1.20, 2.24) in both men and women. When stratified by gender, no

significant associations were found between depression and inflammation in women (past

year depression OR=0.76, p=0.33; history of depression OR= 1.39, p=0.24). Among men,

CRP levels were significantly higher in those experiencing a recent episode of depression

(OR=3.00, 95% CI 1.39-6.48) and those with recurrent depression (OR= 3.55, 95% CI 1.55-

8.14) compared to their non-depressed counterparts.[55] Despite having a large, nationally

representative sample size, this study was limited by the low sensitivity of the CRP

concentration detected by their assay, as 74.1% of the population fell below the limit of

detection (0.21 mg/dL), therefore the study was forced to categorize CRP levels into

low/high based on the limit of detection (low CRP: <0.21 mg/dL and high: >0.21 mg/dL). In addition to the cross-sectional limitation of the study design, the severity of depression was not determined since the depression questionnaire only assessed the presence of major depression disorder in a yes/no manner, limiting the potential to assess the degree to which inflammation may be contributing to depression.

In another study, Matthews et al. (2010) assessed the bi-directionality of the relationship between depression and CRP in the prospective Study of Women's Health Across the Nation (SWAN) over a 7-year period.[135] The Center for Epidemiological Studies Depression scale (CES-D) was used to assess depressive symptoms during the previous week in 1,781 women ages 42-52 years old on a yearly basis. Using multivariable longitudinal linear mixed regression model (woman-specific), they found that initial higher depression scores at time X predicted higher subsequent CRP levels and vice-versa over a 7-year period. After adjustment for BMI, physical activity, medications, and health conditions, higher CRP levels at year X significantly predicted higher depression scores in the subsequent year (p=0.03). Higher depressive symptoms also predicted higher CRP in the following year, but this association was not significant (p=0.10). In other words, they found that CRP remained a modest, yet statistically significant, predictor of subsequent depressive symptoms one year later, whereas the reverse association (depression as predictor of inflammation) only approached significance. Despite it being a strong study with multiple measurements of each exposure and outcome, and an ethnically-diverse population, this study was limited by its inclusion of only an older sample population of women. This is an important study because it sheds light on the directionality of this

association, suggesting that among women, inflammation is a predictor of depression, and not the other way around.

Gimeno et al. (2008) assessed whether CRP predicted cognitive symptoms of depression or whether the symptoms of depression predicted inflammatory markers in the Whitehall II study in London.[136] Participants consisted of office staff ages 35-55 from 20 different London-based civil service departments. Baseline measurements took place in 1991-1993, and follow-up assessment took place in 2002-2004 (mean follow-up was 11.8 years). They excluded those with CRP >10 mg/L and those who reported a cold or flu in the 2 weeks prior to assessments. The remaining sample size included 5,978 participants (1,803 women) for baseline and 3,339 for follow-up (951 women). At baseline, women had significantly higher levels of both CRP and cognitive symptoms of depression compared to men (CRP: 0.91±3.2 vs 0.77±3.0 mg/L; $p<0.001$; Depression score: 1.17±1.9 vs 0.95±1.7; $p<0.001$, respectively). However, in the cross-sectional analyses at baseline of inflammation and cognitive symptoms of depression, CRP ($\beta=-0.067$, $p=0.011$) was negatively associated with cognitive symptoms of depression among women, but not among men (p for sex interaction=0.025). In longitudinal analyses of CRP and cognitive depression score, the association was statistically significant for the whole group ($\beta=0.046$, $p=0.004$). When the analysis was stratified by gender, men had a slight positive and significant association between CRP and depression ($\beta=0.058$, $p=0.002$), but this was no longer significant in women ($\beta=0.02$, $p=0.522$). It must be noted that 2,388 men were included in this analyses, whereas only 951 women were included. Additionally, they showed that cognitive symptoms of depression at baseline did not predict CRP levels at follow-up ($\beta= -0.013$, $p=0.341$) for either gender.[136]

Some of the limitations of this study include an older sample population (mean age for women was 50.4±6.1 years) and no mention of menopausal status of the women, allowing for the possibility that menopausal state could bias the results towards the null, as younger women (including women with menstrual cycles) tend to have higher depression rates. Furthermore, for the longitudinal analysis, the long follow-up time (11.8 years) could actually be too long to be able to detect a true predictive association between inflammation at baseline and depression at follow-up.

Overall, Ford et al found a significant association between lifetime history of major depression and elevated CRP levels in a nationally representative sample of US adults with a cross-sectional analysis.[55] In longitudinal analysis, two studies found that inflammation predicts depression, but that depression does not predict inflammation.[135,136] There is growing evidence that nutrition could be playing a very important role in both inflammation and the development or severity of depression, and none of the previous studies accounted for any biomarkers of nutritional status.

CHAPTER 4

NUTRITIONAL STATUS: EMPHASIS ON VITAMINS D AND B6

4.1 Micronutrients of Interest - Introduction

Micronutrient status plays an important role in maternal and child health. Both vitamin D and B_6 are considered micronutrients with a surprisingly large prevalence of insufficiency and deficiency in the female U.S. population, which can lead to increased risk of disease. This chapter will review the epidemiology of each micronutrient in the U.S. with an emphasis on women of reproductive age, followed by background information on metabolism, transport, food sources, and assessment of each. The association between each micronutrient and inflammation as well as depression will also be reviewed.

4.1.2 Epidemiology of Vitamin D Status in the US

According to the National Center for Health Statistics, in 2001-2006, one third of individuals aged 1 year were at risk for vitamin D inadequacy or deficiency.[141] Furthermore, a recent publication using NHANES data shows that mean serum 25-hydroxy vitamin D (25-OHD) was lower in 2000-2004 compared to 1988-1994, suggesting a decline in either consumption of vitamin-D containing foods, cutaneous vitamin D synthesis, or both.[141]

For women of childbearing age, maternal vitamin D malnutrition imposes multiple health impacts on both women and their offspring.[142] Women with suboptimal vitamin D status are at increased risk for pre-eclampsia and eclampsia, gestational diabetes, and bacterial vaginosis.[142] Furthermore, vitamin D deficiency during pregnancy or in the postpartum period has been associated with increased risk of rickets, type 1 diabetes,

allergies, asthma, and schizophrenia in the offspring as well as maternal depression.[143] The

potential consequences of vitamin D deficiency in women of reproductive age is

particularly troublesome considering that a large proportion of this population within the

U.S. has suboptimal levels of circulating vitamin D. Recent estimates found that

approximately 78% of women have vitamin D insufficiency and 42% have vitamin D

deficiency.[144] Additionally, these proportions vary by ethnicity: vitamin D levels are much

lower in non-Hispanic Blacks, followed by Hispanics, and non-Hispanic White females.

Eighty-six percent of African American women have vitamin D deficiency, followed by 60%

of Hispanic and 26% of non-Hispanic White women. With regards to insufficiency, 99%,

93% and 69% of non-Hispanic Blacks, Hispanics, and White women, respectively, have

suboptimal levels of circulating vitamin D. [159,219]

4.2 Background on Vitamin D

Vitamin D is both a hormone and a vitamin, known to have many functions in health

maintenance and disease prevention. The term vitamin D refers collectively to the two

major nutritionally relevant forms: vitamin D_3 or cholecalciferol, which is formed in the

skin following exposure to sunlight or ultraviolet light, and ergocalciferol or vitamin D_2

which is obtained by irradiation of plants, phytoplankton, yeast, invertebrates, and fungi

containing ergosterol.[145]

4.2.1 Uptake of Dietary Vitamin D

Due to the fat-soluble nature of vitamin D, it is absorbed with other lipids in the

intestine, a process facilitated by bile and pancreatic lipase. Following absorption, vitamin

D is integrated into chylomicrons and enters the lymphatic system, which drains into the

main circulation. Lipoprotein lipase, particularly in adipose tissue, acts upon chylomicron lipids and may result in a portion of the vitamin D being taken up by fat cells. This observation suggests a mechanism whereby increased adiposity causes sequestering of vitamin D and is related to lower vitamin D status.[220] Vitamin D sequestration in adipose tissue is a nonspecific process, and these stores may not be actively used in periods of need.[220] After depletion of triacylglycerols, the cholesterol-rich chylomicron remnants, which still contain a significant fraction of the absorbed vitamin D, are taken up by the liver.

4.2.2 Endogenous Synthesis of Vitamin D

Most vitamin D in serum comes from endogenous production in human skin, from the precursor, 7-Dehydrocholesterol (7-DHC). Following exposure to sunlight, 7-DHC in the skin absorbs ultraviolet B (UVB) photons between the wavelengths of 290-320nm and is converted to pre-vitamin D_3.[221] At normal body temperature, pre-vitamin D_3 undergoes thermal isomerization to form vitamin D_3, which is more easily translocated from the skin cells to the bloodstream. In circulation, vitamin D_3 travels bound to a specific $\alpha1$-globulin known as vitamin D-binding protein (DBP), the major plasma carrier of all vitamin D metabolites. This complex then travels to the liver, where vitamin D metabolism starts. Toxic levels of vitamin D do not occur from prolonged sun exposure, which degrades into many inactive forms, such such as lumisterol and tachysterol.[222]

4.2.3 Metabolism of Vitamin D

Vitamin D, as either D_2 or D_3, is considered biologically inactive until it undergoes two enzymatic hydroxylation reactions. Circulating vitamin D_2 or D_3 in chylomicron

remnants, as well as vitamin DBP-bound vitamin D_3 from endogenous synthesis, are first taken up by the liver and hydroxylated by hepatic 25-hydroxylase (25-OH-ase) from the CYP27A gene to form 25-hydroxycholecalciferol (25-OHD). 25-OHD then circulates in the blood stream as a complex with DBP, and when the active form of vitamin D is required due to decrease in serum calcium or phosphate, 25-OHD is further hydroxylated by 1α-hydroxylase (CYP27B1) in the kidneys. [223] This second hydroxylation converts 25-OHD to the biologically active form: 1,25-dihydroxyvitamin D (1,25-[OH]$_2$D), also known as calcitriol. The 1α-hydroxylase gene is also expressed in several extra-renal tissues, but its contribution to the formation of the active form of vitamin D in these tissues is unknown. [224] The enzymatic reaction producing 1,25-(OH)$_2$D is tightly regulated by two counteracting hormones: up-regulation by parathyroid hormone (PTH) and down-regulation via fibroblast-like growth factor-23 (FGF23).[225] This control of vitamin D activation constitutes the basis of the vitamin D endocrine system that is central to maintaining calcium and phosphate homeostasis. Following its synthesis in the kidney, calcitriol binds to DBP to be transported to target organs. Catabolism of 1,25(OH)$_2$D to more polar and readily excretable inactive metabolites, 24,25(OH)$_2$D and 1α, 24,25(OH)$_2$D, begins with the 24-hydroxylation of either 25-OHD or 1,25(OH)$_2$D, respectively. Calcitriol induces its own destruction by stimulating these enzymes.[226]

4.2.4 Assessment of Vitamin D Status

Both 25-OHD and 1,25(OH)$_2$D levels can be measured in serum or plasma.[227] However, circulating 25-OHD concentration is the best biomarker of vitamin D status because it reflects both increases in vitamin D intake and cutaneous production of vitamin

D3. Serum 25-OHD also has a longer half-life of approximately 15 days compared to the shorter half-life of 4-6 hours for 1,25(OH)2D.[168] Furthermore, serum 1,25(OH)$_2$D concentration is tightly controlled by PTH, calcium, and phosphate,[168] and levels do not typically decrease until vitamin D deficiency is severe. [228]

The serum vitamin 25-OHD concentrations used to define deficiency, marginal deficiency, normal ranges, and toxic levels are somewhat controversial. Table 1 shows the Institute of Medicine (IOM)-proposed cut-offs for assessing vitamin D sufficiency, deficiency, and toxicity using serum 25-OHD concentrations. While these cutoffs serve as the more "traditional" reference guidelines, literature on the topic suggests that the optimal serum concentration of 25-OHD is probably much higher than the recommended concentration for adults. Table 2 shows the proposed alternate 25-OHD reference limits considered "acceptable" by leading researchers in the field. [229,230]

Table 1: Institute of Medicine (IOM) Recommended Serum 25-Hydroxyvitamin D (25-OHD)		
25-OHD level (ng/mL)*	25-OHD Level (nmol/L)**	Health Status
<12	<30	Associated with vitamin D deficiency, leading to rickets in children and osteomalacia in adults
12 to 20	30 to 50	Generally considered inadequate for bone and overall health in healthy individuals
≥20	≥50	
>50	<125	Emerging evidence links potential adverse effect to such high levels, particularly >150 nmol/L (>60 ng/mL)

Source: Institute of Medicine, Food and Nutrition Board. Dietary Reference Intakes for Calcium and Vitamin D. Washington, DC. National Academy Press, 2010.
*Serum concentrations of 25-OHD are reported in both nanomoles per liter (nmol/L)
**1ng/mL = 2.496 nmol/L

Table 2: Alternate Recommendation of Different Levels of Serum 25-OHD		
25-OHD Level (ng/mL)*	25-OHD Level (nmol/L)**	Health Implication
<20	50	Deficiency
20-29.9	50-75	Insufficiency
≥30	>75	Sufficiency
>150	>374	Intoxication

Source: Holick, Michael F. Vitamin D Deficiency. N Engl J Med 2007; 357:266-281
*Serum concentrations of 25-OHD are reported in both nanomoles per liter (nmol/L) and nanograms per milliliter (ng/mL).
**1ng/mL = 2.496 nmol/L

4.2.5 Factors Affecting Vitamin D Status

Many variables can affect cutaneous synthesis of vitamin D, making it a challenge to estimate average 25-OHD levels produced by sun exposure in North America.[231] Cutaneous synthesis of vitamin D is affected by the amount of solar UVB radiation that reaches the earth's surface, which depends on latitude, season of the year, and time of the day of sun exposure. At latitudes above 40° N below 40° S, the amount of vitamin D synthesized between November and February is extremely low because vitamin-D producing UVB photons pass through the ozone layer at an oblique angle, causing many to be absorbed by the ozone. More UVB photons penetrate the ozone layer in the spring, summer, and fall months because the sun is directly overhead. Studies suggest a seasonal change in serum 25-OHD concentrations between the winter nadir and the summer zenith of approximately 25 nmol/L.[231]

The cutaneous synthesis of vitamin D, and its contribution to serum vitamin D levels, also depends upon the presence of 7-DHC in the skin. Aging, melanin pigmentation,

clothing, and use of sunscreen also affect cutaneous synthesis. [232] Aging decreases the

synthesis of 7-DHC in skin, thereby reducing the production of vitamin D_3 by approximately

75% by age 70 years compared to younger adults. [232] The skin pigment melanin is a

natural sunblock that competes with 7-DHC for UVB photons, therefore, darker-skinned

persons require a much longer sun exposure in order to make the same amount of vitamin

D as lighter-skinned individuals.[233] Using sunscreen and wearing clothing that completely

covers the body can also block UVB photons, reducing vitamin D synthesis. [240]

Obesity can affect the amount of dietary vitamin D that reaches the liver. While in

chylomicrons, a portion of dietary vitamin D is sequestered in adipose tissue in a

nonspecific manner, and these stores may not be actively used in periods of need. This

suggests a mechanism whereby increased adiposity causes sequestering of vitamin D and is

related to lower vitamin D status. [220] Therefore, obese individuals may require higher

intakes of vitamin D to achieve serum concentrations of 25-OHD comparable to those

observed among lean individuals. For instance, Blum et al (2008) supplemented elderly

subjects with 700 IU daily for a year, and found that serum levels of 25-OHD were

approximately 10 nmol/L lower for every additional 15 kg of weight above "normal". [235]

Available literature demonstrates that serum 25-OHD levels increase in response to

increased vitamin D intake, although overall, it can be concluded that the relationship is

non-linear rather than linear. A summary of 16 human trials suggested the following

association between dietary vitamin D and 25-OHD concentration: for each additional 100

IU of vitamin D_3, serum 25-OHD concentrations increased by 1 to 2 nmol/L.[236] However,

this increase of serum 25-OHD differs based on the starting 25-OHD concentrations. Chung

et al (2009) confirmed the association between increasing doses of vitamin D and

increasing net change in serum 25-OHD concentration in both adults and children, but concluded that the dose-response relationships differ depending on the subjects' baseline serum 25-OHD levels (≤40 vs >40 nmol/L) and duration of the supplementation (≤3 vs >3 months).[237] There is evidence that increasing serum 25-OHD level above approximately 50 nmol/L requires more vitamin D intake than does increasing serum 25-OHD levels when the starting point is less than 50 nmol/L.[238] Furthermore, there have been reports that the rise in serum 25-OHD level for a given dose tends to stabilize by week 6 and does not vary with age at least up to 80 years of age. [239]

4.2.6 Dietary Sources & Recommended Dietary Allowance for Vitamin D

The dietary sources of vitamin D include food and dietary supplements. Very few foods naturally contain vitamin D_3. These include egg yolks and oily fish, such as salmon, mackerel and herring and liver oils from cod, tuna and shark. [173] Sun-dried mushrooms also contain some vitamin D_2. In the United States, the foods fortified with vitamin D (D_2 or D_3) include milk, breads and cereals, margarine, yogurts and cheeses, and some brands of orange juice.[173]

In recent years, dietary supplements containing vitamin D have become more common and have been more frequently consumed. [173] Traditionally, many supplements have contained 400 IU per daily dose, but levels in supplements have been increasing and can be found in the range of 1,000 to 5,000 IU of vitamin D_3 per dose and even up to 50,000 IU of vitamin D_2 per dose.[173] Recent findings suggest that vitamin D_3 may be more efficient at increasing serum 25-OHD levels compared to vitamin D_2.[240]

The current recommended dietary allowance (RDA) is the daily dose that should meet the nutritional needs of 98% of healthy individuals in the US for a specific nutrient. The RDA for vitamin D represents a daily intake that is sufficient to maintain normal calcium homeostasis and bone health in healthy individuals with minimal sun exposure. RDAs for vitamin D vary by age and gender. For women of reproductive age (14-50yrs), the RDA is set at 600 International Units (IUs) (15micrograms) per day.[173]

4.2.7 Biological Activities of Vitamin D

$1,25(OH)_2D$ is considered the biologically active hormonal form of vitamin D responsible for carrying out most, if not all, of the biological functions of vitamin D. The biological actions of $1,25(OH)_2D$ involve regulation of gene expression at the transcriptional levels and are mediated through binding to a vitamin D receptor (VDR), located primarily in the nuclei of target cells.[241] Until recently, the main known role of vitamin D was to control bone metabolism and calcium and phosphorus homeostasis. But in the past decade, the VDR has been discovered in many tissues throughout the body (e.g. gut, kidney, gonads, heart, muscle, brain, and skin), implying that $1,25(OH)_2D$ may play a role in these organs. Furthermore, the specific vitamin D-responsive elements (VDREs) considered the hallmark of vitamin D action are present in a large number of human genes involved in a wide range of roles, such as modulation of cell growth,[242] neuromuscular[243] and immune function, including and reduction of inflammation,[244] to mention a few.

4.3 Vitamin B$_6$

4.3.1 Epidemiology of Vitamin B$_6$ Status in the US

A recent cross-sectional study on vitamin B$_6$ status in the US population found that regardless of vitamin B$_6$ supplementation, plasma Pyridoxal-5'phosphate (PLP) – the principal biomarker of vitamin B$_6$– of women of childbearing age was significantly lower than those of comparably aged men. A fourth of women in the U.S. have insufficient vitamin B$_6$ levels as defined plasma PLP levels <20 nmol/L.[137] Among oral contraceptive users, seventy-five percent were found to have suboptimal vitamin B$_6$ levels (Morris et al, 2008). These statistics are worry-some considering that maternal vitamin B$_6$ levels may affect the neurological development of their offspring.[138] Furthermore, clinical studies have shown that suboptimal vitamin B$_6$ status decreases probability of conception[139] and increases risk of pregnancy complications, including spontaneous abortions.[140]

4.3.2 Vitamin B$_6$: Background

Vitamin B$_6$ is a water-soluble vitamin that functions as a coenzyme in over 100 enzymatic reactions involved in the metabolism of amino acids, carbohydrates, neurotransmitters and lipids.[146] As of recently, it has also been coined a potent antioxidant that effectively quenches reactive oxygen species.[147] Vitamin B$_6$ currently refers to a collective of six biologically interconvertible 3-hydroxy-2-methylpyridine compounds: pyridoxine (PN), pyridoxal (PL), pyridoxamine (PM) and their respective 5'-phosphates: PNP, PLP and PM.[148] In addition to the 6 forms of vitamin B$_6$, there also is a glucoside form of pyridoxine that exists in variable amounts in plant foods.[149]

The most abundant and biologically active form of vitamin B_6 is PLP, serving as a coenzyme in numerous biological processes that include tryptophan-niacin conversion, heme synthesis, gluconeogenesis, neurotransmitter synthesis, and amino acid metabolism.[150] Given PLP's wide range of functions, deficiency of vitamin B_6 can have serious and diverse physiologic effects, including microcytic anemia, seborrheic dermatitis, epileptiform convulsions, depression, and confusion.[148]

Although vitamin B_6 is widely available in food such as fortified cereal products, fruits, vegetables, nuts, beans, legumes, and animal products, recent literature suggests that a larger portion of the population than previously thought may be at risk for low vitamin B_6 status, specifically women of childbearing age.[137] This review will provide background on vitamin B_6, its sources, metabolism, assessment in humans, and its connection with inflammation.

4.3.3 Dietary Sources, Intake & DRI for Vitamin B_6

Vitamin B_6 is widely available in foods such as whole grain products, meat, fish, poultry and non-citrus fruits in the American diet. Other rich sources include highly fortified cereals, beef liver, and other organ meats.[148] The major forms in animal tissue are the coenzyme species PLP and PMP. In most fruits, vegetables and cereal grains, a significant fraction of vitamin B_6 is pyridoxine-5'-β-D-glucoside, a form that is partially available as a source of vitamin B_6.[146] Vitamin B compounds, particularly PL and PLP, are light sensitive; furthermore large losses of food vitamin B_6 can occur during heating and leaching of the vitamin during food preparation.[148]

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The adequacy of vitamin B_6 nutrition depends on the quantity of dietary B_6 consumed as well as its bioavailability, defined as the extent of intestinal absorption and metabolic utilization.[146] Vitamin B_6 is absorbed in the small intestine by a non-saturable passive diffusion mechanism.[148] The bioavailability of B_6 supplements or fortified foods in the form of pyridoxine is roughly 90%, whereas in the rest of non-fortified sources, it depends on the form of B_6; bioavailability of non-glucoside forms of the vitamin in food is greater than 75% and the bioavailability of pyridoxine-5'-β-D-glucoside in food is about half as much.[146]

4.3.4 Dietary Reference Intakes for Vitamin B_6

The main criterion used in setting the estimated average requirement (EAR) for vitamin B_6 is plasma PLP value of at least 20 nmol/L.[151] The EAR for vitamin B_6 is 1.1 mg per day for adults ages 19-50 years old, and the RDA is 1.3 mg/day or 20% more than EAR to allow for variance in need.[148] Data from NHANES 2001-2002 showed the median vitamin B_6 dietary intake of U.S. women (19 years or older) is 1.45 mg/day. Intake of women ranges (5th to 95th percentile) from 0.79 to 2.51 mg/day. Recent evidence shows that a higher RDA may be prudent, especially for women. Data from NHANES 2003-2004 suggests that a daily vitamin B_6 intake of 3.0 to 4.9 mg/day is generally adequate to maintain an acceptable plasma PLP concentration, but a large portion of women of childbearing age, do not maintain adequate vitamin B_6 status at that intake level.[137]

4.3.5 Vitamin B_6 Metabolism

After consumption, the phosphorylated vitamers are dephosphorylated in the intestinal mucosa, and are absorbed rapidly by carrier-mediated diffusion.[152] Most of the

absorbed non-phosphorylated B_6 is taken up by the liver and metabolized to PLP, the major co-enzymatic form.[148]   PN, PL, and PM are converted to their respective 5'-phosphate derivatives by the enzyme PL kinase, which is present in all tissues, including erythrocytes. Non-phosphorylated forms of vitamin B_6 can move freely in and out of tissues, whereas phosphorylation ensures metabolic trapping. Tissue concentrations of pyridoxal phosphate are controlled by the balance between phosphorylation and de-phosphorylation.[152] Free pyridoxal either leaves the cells or is oxidized to 4-pyridoxic acid by aldehyde dehydrogenase (which is present in all tissues) and also by hepatic and renal aldehyde oxigenases.[152] 4-Pyridoxic acid is the inactive excretory metabolite, which is actively secreted by the renal tubules.

At high intakes of vitamin B_6, tissue-binding capacity may be exceeded and the free PLP is rapidly hydrolyzed to the non-phosphorylated PL, which is released by the liver and other tissues into circulation. However, product inhibition of the PNP oxidase by PLP renders the relationship between PN intake and PLP concentration in tissues and plasma non-linear.[148]

4.3.6 Vitamin B_6 Transport and Turnover

Under normal conditions, most circulating vitamin B_6 is present as PLP that is mainly Schiff base-linked to proteins, primarily albumin in plasma and hemoglobin in erythrocytes. Although 90% of vitamin B_6 is plasma is present as PLP, the total is less than 0.1% of the total body vitamin B_6.[153] Estimates of total body vitamin B_6 stores in healthy adults range from approximately 400 μmol to 1,000 μmol (60 to 170 mg), and 80% to 90% of this is in muscle, primarily bound to glycogen phosphorylase.[148] The overall body half-

life of vitamin B_6 is estimated to be roughly 25 days, with an estimated daily fractional

turnover rate of less than 3%. However, this half-life could be longer given the slow

turnover of the large pool of B_6 bound to glycogen phosphorylase.[148]

4.3.7 Assessment of Vitamin B_6 Status

A variety of biochemical indicators have been used to assess vitamin B_6 status. Even

though vitamin B_6 status is most appropriately evaluated using a combination of direct (i.e.,

vitamin concentration in plasma or cells) and indirect (i.e., erythrocyte amino transferase

saturation by PLP or tryptophan metabolites),[153] plasma or serum PLP may be the best

single indicator because it is a reflection of liver PLP and thus tissue stores.[154] Circulating

PLP concentration is normally measured by an enzymatic assay using the apoenzyme form

of tyrosine decarboxylase or by high performance liquid chromatography.[148]

A plasma or serum PLP level of less than 20 nmol/L is considered to reflect

inadequate vitamin status in adults, although some support a threshold of 30 nmol/L for

assessing sufficiency.[148] However, there is no definite consensus for a value that

unquestionably defines a deficient state of this nutrient.[155] Plasma PLP increases

approximately 12 nmol/L for each 1 mg/day increase in vitamin B_6 intake.[137] Indicators

for assessing vitamin B_6 status are shown in the table below (table 3).[153]

Table 3: Vitamin B$_6$ Biomarkers.

Indicator	Assessment
Plasma PLP	Major vitamin B-6 form in tissue and reflects liver PLP; changes fairly slowly in response to vitamin intake
Urinary vitamin B-6 catabolite excretion	Excretion rate of vitamin and particularly 4-pyridoxate reflect intake; 5-pyridoxate appears with excess intake
Erythrocyte aminotransferases activity coefficients	Enzymes for aspartate and alanine reflect PLP levels; large variation in activity coefficients
Tryptophan catabolites	Urinary xanthurenate excretion, especially after a tryptophan load test, but can be nonspecific test
Other	Erythrocyte and whole blood PLP, plasma homocysteine

Source: McCormick, D. B. in Present Knowledge in Nutrition **1,** 269–277 (International Life Sciences Institute, 2006).

4.3.8 Factors that Can Affect Vitamin B$_6$ Status

There are several factors that have been shown to decrease plasma PLP status, including smoking, certain medications, oral contraceptives or hormone replacement therapy, pregnancy, renal failure, alcoholism and inflammation.[197,198] Vitamin B$_6$ deficiency my result from the prolonged use of drugs that are carbonyl-trapping reagents, and can form biologically inactive adducts with pyridoxal and pyridoxal phosphate.[152] Such compounds include penicillamine, the antituberculosis drug isoniazid, and the anti-Parkinsonian drugs benserazide and carbidopa.

Several studies have found smokers to have lower plasma PLP levels presumably linked to smoker's increased inflammation levels.[137,157,158] With regards to alcohol intake, despite alcoholism being associated with nutritional deficiency of B-vitamins, a recent study found alcohol intake to be positively associated with higher plasma PLP in the Boston Puerto Rican Health Study.[157]

Plasma PLP decreases during pregnancy more than can be accounted for by increased blood volume, with the most significant drop in the third trimester.[159] This

reduction in plasma PLP may represent a normal physiological change in pregnant women as there have been no reports of clinical symptoms of deficiency in pregnant women with decreased status.[159] There may be several reasons for this decrease, including fetal sequestration of plasma PLP or a shift in maternal distribution in favor of erythrocytes over plasma.[153,160] However, Barnard and colleagues (1987) have shown that, despite the fall in plasma PLP in pregnancy, the plasma concentration of PLP plus pyridoxal is unchanged.[161] Renal failure and other conditions in which plasma alkaline phosphatase is elevated (i.e., pregnancy and metabolic bone disease) are associated with low plasma PLP and a corresponding increase in pyridoxal.[153]

Obesity has also been noted to affect vitamin B_6 status. In a cross-sectional study of morbidly obese adults in Norway, several vitamins including plasma B_6, vitamin C, vitamin D and vitamin E, were found to be lower in morbidly obese men and women compared to healthy controls.[162] Given that obesity is a state of chronic low-grade inflammation, it could be contributing to compromised B_6 status via inflammation. Plasma PLP is also affected by acute phase responses, such as in inflammation and infection.[163] This association will be further discussed in the following sections.

4.3.8.1 Estrogens and Vitamin B_6 Status

The connection between vitamin B_6 status and oral contraceptive use was first noted by Rose in 1966, when increased urinary excretion of xanthurenic acid, kynurenine, and 3-OH-kyurenine was observed following a tryptophan load test.[164] Several studies followed with similar findings, where supplementation with high doses of vitamin B_6 in the form of pyridoxine hydrochloride corrected the abnormal tryptophan metabolism in

women taking OCs.[165–167]   Although these studies may appear as evidence of estrogen-induced vitamin B_6 deficiency or depletion, when other indices of vitamin B_6 nutritional status were measured, they seemed to be unaffected by contraceptive use, suggesting an effect on tryptophan metabolism, rather than on vitamin B_6 nutritional status.[152]   These studies led to a general consensus that estrogen disrupts tryptophan metabolism independent of vitamin B_6 status, rendering the tryptophan load test a poor indicator of vitamin B_6 status in OC users.[168]

Although the association between OC use and tryptophan metabolism is well established, the potential effect of OC use on vitamin B_6 status and requirements remains controversial.[169]   In a recent large-scale population-based study performed in the U.S. using the NHANES 2003-2004 cycle, plasma PLP concentration was found to be significantly lower (<20 nmol/L) in 75% of OC users who were not supplementing with vitamin B_6.[137] Morris and colleagues also assessed current vitamin B_6 intake and suggested that intakes higher than the current RDA may be necessary to maintain adequate vitamin B_6 status in OC users.[137]   In another cross-sectional study in Italy, women who had not been taking OCs for at least 12 months had significantly higher vitamin B_6 levels compared to current OC users.[170]   In a prospective studies of OC use and vitamin B_6 status, an initial lowering of plasma PLP levels was observed three months after start of OC use; however, plasma PLP levels returned to pre-treatment levels by six months of OC use.[171]   These findings suggest that the effects of OC use on vitamin B_6 concentrations may be transient.

Although OC use may be associated with a reduction in plasma PLP levels, the mechanism by which OC use may lower plasma PLP concentration is not well defined, but some suggest that plasma PLP may redistribute into tissue in response to OC use.[172]

Another possible explanation could be that OC use induces certain PLP-dependent enzymes, such as tryptophan oxygenase, causing an increased need for the vitamin in metabolism.[172] A third explanation for the low plasma PLP concentrations observed in OC users involves inflammation.[169] OC use is associated with increased serum CRP concentrations.[67] Krintus and colleagues (2009) found three-fold higher CRP levels among OC users compared to non-OC users.[173] Furthermore, Williams and colleagues found similar results in their study, which indicated that combined OC use was associated with increased CRP levels in women independent of other factors, such as obesity.[174]

4.3.9 Biological Activities of Vitamin B$_6$

The metabolically active vitamer is pyridoxal phosphate, which serves as a coenzyme for over 100 reactions, primarily in amino acid metabolism, such as aminotransferases, decarboxylases, aldolases, racemases and dehydratases.[148] The catabolism of nearly all amino acids involves transfer of their amino group to α-keto acids, which requires PMP. Furthermore, decarboxylases are essential in the formation of many hormones and neurotransmitters, such as serotonin, epinephrine, and dopamine, rendering vitamin B$_6$ essential for their synthesis. PLP is also a cofactor for mitochondrial δ-aminolevulinate synthase which catalyzes the first and rate-limiting step in heme biosynthesis in the liver.[148] The role of PLP in one-carbon metabolism may be especially important in nucleic acid biosynthesis and immune system function.[168] In addition, PLP enzymes are also involved in lipid and carbohydrate metabolism, including in muscle glycogen phosphorylase and sphingolipid synthesis and may modulate glucocorticoid action.[148] Figure 3 below shows a summary of the main metabolic actions of vitamin B$_6$.[153]

4.4 Nutrients related to Depression

4.4.1 Physiology of Vitamin D and Depression

Several biological mechanisms may explain the link between vitamin D deficiency and depression. These proposed mechanisms are based on the presence of VDR and 1,α-hydroxylase in the brain. First of all, calcitriol can cross the blood-brain barrier and is also synthesized in the brain.[246] Secondly, VDRs have been found in many areas of the human brain, including the prefrontal cortex, hippocampus, cingulated gyrus, thalamus, hypothalamus, and substantia nigra – all of which have been previously linked to the physiology of depression. However, current experimental evidence does not fully satisfy criteria to establish causality.[247]

Vitamin D has the potential to regulate expression of many genes, including the expression of neurotrophins, which regulate neural development and also function in the adult brain.[248] Gene expression of three of the four neurotrophins present in mammalian brains (NGF, BDNF, NT-3, and NT4/5), are affected by vitamin D.[247] Vitamin D could be playing an important role in the regulation of neurotransmission, neuroprotection, neuroimmodulation, and nerve growth factor synthesis.[250] Therefore, a vitamin D deficiency may be contributing to neuronal decline and disruption in neural transmission.[249]

Another mechanism linking vitamin D to depression is vitamin D's involvement in the neuroendocrinological system by modulating glucocorticoid signaling.[251] Glucocorticoid levels have been found to be higher in patients suffering from major depressive disorder. A malfunction in glucocorticoid signaling and the release of cortisol

may be a result of low vitamin D levels.[251] However, the exact mechanism linking

glucocorticoids, vitamin D and depression remains unknown.

And lastly, since vitamin D is associated with higher levels of inflammatory

cytokines, and inflammation has been linked with depression, compromised vitamin D

status may be contributing to an inflammatory-induced depression. There is supporting

evidence for a modulatory effect of vitamin D both *in vivo* and *in vitro* on proinflammatory

cytokines. Firstly, treatment with vitamin D has resulted in decreased production of

proinflammatory cytokines (or increased production of anti-inflammatory cytokines) in a

variety of cell types including monocytes, microglia (an important source of

proinflammatory cytokines in the brain), keratinocytes, and endothelial cells, among

others.[247] Furthermore several studies have found increased proinflammatory cytokine

levels in individuals with compromised serum vitamin D.

In summary, there are several mechanisms through which low vitamin D can be

contributing to depression and there is ample biological evidence to suggest an important

role for vitamin D in brain development and function, but the current experimental

evidence base does not yet fully satisfy causal criteria. [247]

4.4.2 Epidemiology of Vitamin D and Depression

The largest cross-sectional study of vitamin D concentrations and depression in

adults in the US included 7,970 adults representing over 90 million US non-

institutionalized, civilian population, aged 15-39 from the NHANES III (1988-1994).[252]

Vitamin D was categorized into deficient (<50 nmol/L), insufficient (50-75 nmol/L), and

sufficient (>75 nmol/L). Depression was assessed using the Diagnostic Interview Schedule

(DIS), and categorized into major depression, depression episodes longer than two years, and current depression. The proportion of individuals with major depression did not vary by vitamin D status (p=0.86). However, the proportion of individuals with vitamin D deficiency was significantly higher among those who had experienced depression for over two years compared to those who had not experienced depression for over two years (24.7% vs 19.1%, p=0.039), and those with current depression compared to those without depression (27.4% vs 14.5%, p=0.003). In multivariable logistic model, they found that those with vitamin D deficiency have 1.85 times the odds of having current depression, compared to individuals with sufficient vitamin D levels (OR=1.85, p=0.021), after adjusting for age, gender, ethnicity, poverty income ratio, nutritional supplements, medication use, geographical location, BMI and serum creatinine. Additionally, they observed a higher prevalence of vitamin D deficiency in women, non-Hispanic Blacks, persons living below poverty, non-supplement consumers, those with higher BMI, and in those with current depression compared to their counterparts.[252] A more recent secondary analysis of NHANES 2005-2006 focused solely on women ages 20 and over to assess the relationship between vitamin D and depression, using the same vitamin D assessment method, but the depression assessment tool used in this cycle was the Patient Health Questionnaire 9 (PHQ-9). None-the-less, they found a linear statistically significant relationship between circulating 25-OHD and depression score (p=0.002) after controlling for age, race, and education.[175]

Even though most of the available epidemiological studies have been unable to establish causality due to their cross-sectional design, a randomized double-blind trial of vitamin D supplementation and depression by Jorde et al (2008) provided important

evidence for a possible causal relationship between vitamin D and depression. Participants in the randomized clinical trial included 334 overweight and obese men and women ages 21 to 70 years old who were randomized to receive one of two doses of vitamin D (40,000 IU/week, 20,000 IU/week) or a weekly placebo. Serum vitamin D and depression (using the Beck Depression Inventory) were assessed at baseline and at the end of the one year period. Even though they did not find continuous 25-OHD to be significantly correlated with depression scores at baseline, after participants were stratified by vitamin D status (sufficient vs insufficient, or <40 vs ≥40 nmol/L), those with insufficient 25-OHD had significantly higher total depression scores (6.0 vs 4.5, p<0.05) compared to those with sufficient vitamin D concentration. [253] There was also a modest, yet significant decrease in median total depression scores after 1 year of vitamin D supplementation with 40,000 IU (median score: 4.5 to 3.0, p<0.01), with 20,000 IU/week (median score: 5.0 vs 4.0, p<0.01). The placebo group did not have a significant decrease in depression score after 1 year (median score: 4.0 vs 3.8, p>0.05). However, this study was limited to overweight and/or obese individuals, and it is not clear whether these findings could be generalized to a normal-weight population; furthermore, the median baseline depression scores were low in this population, suggesting a very low number of clinically depressed participants. And finally, the effect of vitamin D supplementation with 400 IU/day on depression was evaluated in the Women's Health Initiative, a large prospective study that included 36,282 post-menopausal women. They employed the Burnham scale and current antidepressant use to assess depression status, which was assessed every two years.[254] After 2 years follow-up, those who were randomized to consume daily vitamin D/calcium supplements

had an odds ratio of 1.16 (95% CI 0.86-1.56), suggesting that among this population, this level of supplementation had no beneficial effects on depression.

Most epidemiological studies to date have been either cross-sectional or have had a small range of depression scores, as the number of individuals with clinically-significant depression have been small. The two studies that focused on a large female population did not limit their study to females of reproductive age, which are known to have higher rates of depression. Furthermore, very few have accounted for inflammation, which may be a strong confounder, as vitamin D affects inflammation status, and that, in turn, is contributing to the development of depression. To date, two studies have examined if the association between vitamin D and depression is partially affected by CRP levels.[222,223] Although both studies found an inverse association between circulating vitamin D levels and depression, they did not find a significant difference in risk of depression among subjects with low vitamin D and elevated CRP,[222] nor did they find CRP to mediate the association between vitamin D and depression. [223] However, these studies did not assess whether the association between vitamin D and depression was symptom specific, nor did they stratify by gender given that vitamin D and depression affect women disproportionately.

4.4.3 Physiology of Vitamin B_6 and Depression

Even though the exact pathway linking vitamin B_6 to depression is not entirely understood, two main theories/mechanisms have been proposed for the etiology of depression involving vitamin B_6 and tryptophan metabolism. The first mechanism involves pyridoxal 5'-phosphate as a coenzyme in the tryptophan-serotonin pathway, and a lack of

B_6 may theoretically cause depression by reducing serotonin levels.[257] The second mechanism involves vitamin B_6 as a key player in the kynurenine pathway which is thought to play an important role in the pathogenesis of inflammation-dependent depression. [258]

A link between tryptophan metabolism and depression first became evident with the development of serotonin reuptake inhibitor drugs, leading to the first theory, the *Monoamine/serotonin (5-HT) Theory of Depression*, which involves an alteration in tryptophan and other monoamine activities in the etiology of depression.[259] Nevertheless, a direct connection between changes in monoaminergic neurotransmission such as serotonin and depression has not been definitely proven.[258]

The alternative hypothesis is based on a shift in the tryptophan metabolism from serotonin synthesis to the generation of neuroactive metabolites, such as quinolinic acid and kynurenic acid, by the kynurenine pathway. The balance between serotonergic and kynurenine pathways is controlled by the expression of three rate-limiting enzymes that convert tryptophan to kynurenine: indolamine 2,3 dioxygenase (IDO1), IDO2, and tryptophan 2,3 dioxygenase (TDO2).[258] The expression of these enzymes is tightly regulated by the innate immune system. The *Macrophage Theory of Depression,* first proposed in 1990, implicated elevated pro-inflammatory cytokines as a cause of depression. [260] With the discovery that several pro-inflammatory cytokines induce the expression of IDO1, the two theories were joined to form the new *Kynurenine Theory of Depression*, which ties activation of the immune system to a shift in tryptophan metabolism. [261]

4.4.4 Epidemiology of Vitamin B$_6$ and Depression

After it was first discovered that women taking oral contraceptives had compromised vitamin B$_6$ status in the early 1970's, studies sought to explore possible effects of vitamin B$_6$ deficiency on female health. The link between vitamin B$_6$ status and depression was first observed in the late 1970's when an inverse association was found between pure pregnancy depression and serum B$_6$ levels in 15 pregnant women. [262] This was thought to be due to the high levels of estrogen during pregnancy, which changed the brain metabolism increasing the need for pyridoxal 5'-phosphate. Furthermore, fetal sequestration of vitamin B$_6$ was thought to contribute to vitamin B$_6$ deficiency in this population, leading to depression in the perinatal period. That same year, Livingston and colleagues (1978) carried out a more complex study where they assessed the vitamin B$_6$ status of 40 non-pregnant women of reproductive age (control group), 30 pregnant women, 20 postpartum non-depressed, and 24 postpartum depressed women, to see whether vitamin B$_6$ status differed by depression state and by pregnancy status. However, they found no evidence for vitamin B$_6$ deficiency in women suffering from postpartum depression. [263]

Several studies have looked at the association between vitamin B$_6$ and depression in the past decade, but the results have varied depending on whether vitamin B$_6$ is measured in plasma or in diet. Hvas and colleagues (2004) examined the association between depression and vitamin B$_6$ status using the Major Depression Inventory (MDI) and plasma PLP in a small cross-sectional analysis of 140 individuals aged 19-92 years old.[11] In this study of mostly an elderly population (mean age= 75 years old), they found plasma PLP concentration to be inversely associated with depression score after adjusting for age and

gender (r= -0.25, p=0.002). Individuals with PLP concentration below 30 nmol/L were also found to have higher depression scores compared to those with normal PLP levels after adjustment for age and sex (p=0.02; 95%CI 1.2-9.4); however, this study was limited by small sample size, older age of participants despite the range including adults aged 19 and older, and limited number of participants with depression.

A study by Tucker and colleagues (2008) examined the association between both dietary vitamin B_6 and plasma PLP in 618 participants from the Massachusetts Hispanic Elderly Study and a neighborhood based comparison group of 251 non-Hispanic Whites living in Massachusetts.[264] Depressive symptoms were assessed using the Center for Epidemiologic Studies Depression Scale (CES-D) with the cutoff denoting depression set at 16 or higher. Dietary vitamin B_6 was assessed using a semi-quantitative food frequency questionnaire designed for this population. After adjusting for age, sex and ethnicity, they found plasma PLP to be significantly associated with lower CES-D score in the total sample (β= -0.12, p<0.05) and in non-supplement users (β= -0.14, p<0.05) as well as with depression in the total sample (OR=0.74, 95% CI 0.56, 0.99, after adjustment for folate) and in non-supplement users (OR= 0.71, 95% CI 0.52, 0.98). Furthermore, those who had plasma PLP levels below 20 nmol/L had almost twice the likelihood of having high depression score, compared to those with PLP levels above 20 nmol/L in the total sample (OR= 1.74, 95%CI 1.02, 2.89) and in non-supplement users (OR= 1.78, 95% CI 1.03, 3.09). However, even though total intake of vitamin B_6 (dietary + supplements) was not associated with depression score, dietary intake of vitamin B_6 alone was inversely associated with depression score in the total sample and in non-supplement users (p<0.001).[264] In this particular study, dietary intake of vitamin B_6 was more strongly

associated with depressive symptoms in an older cohort than total B_6 intake or plasma PLP. They determined that for each additional log unit of dietary vitamin B_6 intake, there was a 40% reduction of high depressive symptomatology in this cohort. In the most recent study, higher plasma PLP was significantly associated with a decreased prevalence of depression (p-trend: 0.03) among 422 adults ages 21-67 years old in Japan.[232] In cross-sectional analyses, they found that the odds of depression were 46% lower among those in tertiles two and three (PLP >25.5 nmol/L; OR: 0.54, 95% CI 0.31, 0.94 and OR: 0.54, 95% CI 0.30, 0.96, respectively) compared to those in the lowest tertile of PLP (range: 8.1 to 25.5 nmol/L) after adjusting for age, gender, office, smoking, alcohol intake, physical activity, job position, marital status, history of cancer, cardiovascular disease, or mental disease, dietary vitamin B_{12} intake, serum folate and homocysteine concentration, and depression score at baseline. However, this association was no longer significant in longitudinal analyses.

Intervention studies with vitamin B_6 supplementation on depression have been hard to interpret because most have either focused on supplementation with vitamin B complex.[265,266] or have studied the effect of supplemental vitamin B_6 on other aspects of mental health, such as pre-menstrual syndrome[267] or anxiety or depression in schizophrenic patients[268] or in post-stroke patients. [238] A more recent study by Almeida and colleagues (2014) found that even though supplemental B vitamins (including 25 mg of vitamin B_6/day) did not increase anti-depressant efficacy in adults taking citalopram, it did enhance and sustain antidepressant response over 1 year, suggesting a potential for the a change in treatment guidelines of depression to incorporate B-vitamins.[269] Little is known about the role of vitamin B_6 in the development and severity of depression in adults, especially in women of reproductive age, who are at increased risk of depression.

The studies examining dietary vitamin B_6 alone have yielded controversial results regarding the association between vitamin B_6 intake and depression.[270,271] However, those assessing plasma vitamin B_6 and depression have consistently shown an inverse association between plasma PLP and depression scores[11,232,240] Studies on vitamin B_6 and depression to date have been mostly limited by small sample size, age of population (mostly elderly), heterogeneity in the measure of depression, lack of assessment of possible confounders and none have taken inflammation into account.

4.5 Nutrients Related to Inflammation

The influence of infection-related inflammation on micronutrient status was first recognized in the 1960s by Scrimshaw et al, when changes in concentrations of circulating minerals and vitamins, including vitamin D, were observed following infection.[176] Several decades later, the association between nutrition and inflammation remains an important research topic. A recent study attempting to shed light on the association between inflammation and nutritional status examined the relationship between c-reactive protein and micronutrient concentrations cross-sectionally in a cohort of 2,000 persons from the Scottish Trace Element and Micronutrient Reference Laboratory between 2001 and 2011.[177] C-reactive protein was categorized into 6 groups ranging from the lower limit of detection to over 80 mg/L. With the exception of vitamin E and copper, all micronutrients tested (vitamins A, D, B_6, and C) showed similar significant trends of decreasing plasma concentrations as CRP concentrations increased.[177] However, due to the cross-sectional nature of this study, directionality could not be demonstrated. Even though the mechanisms for each of these associations may differ, the effects of inflammation on

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micronutrient status can be, in part, mediated by pro-inflammatory cytokines that suppress hepatic production of many carrier proteins, increasing capillary permeability, and promoting sequestration of some micronutrients into the liver and other organs.[178] There is also the possibility of reverse causation if individuals are sick and have systemic inflammation, their appetite may be diminished[274] leading to limited dietary intake, and consequently, compromised micronutrient status.

Many different micronutrient biomarkers have been studied with regards to inflammation, such as iron, vitamin A, vitamin E, selenium, copper, omega-3 fatty acids among others, but vitamin B_6 and vitamin D are of special interest in light of inflammation and depression. Serum and plasma biomarkers of both of these vitamins are reduced in an inflammatory state, and a deficiency of both micronutrients has been associated with depression. It is of special interest to study these biomarkers in a female population of reproductive age, because both vitamin D and B_6 deficiencies are highly prevalent in this group, and they are essential for healthy pregnancy outcomes. The following sections will provide a review of the literature on each of these vitamins with regards to inflammation.

4.5.1 Physiology of Vitamin D and Inflammation

There is considerable evidence for a modulatory effect of vitamin D treatment on proinflammatory cytokines both *in vitro* and *in vivo*. The vitamin D receptor has been identified in peripheral lymphocytes, macrophages, and thymus tissue.[179] The biological actions of vitamin D are mediated by its binding to a high-affinity vitamin D receptor that acts as a ligand-activated transcription factor. Cell-based and animal studies have suggested that vitamin D influences inflammation by modulating the expression of several

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cytokine genes controlled by the VDR. Vitamin D and its analogues inhibit production of proinflammatory cytokines (or increase production of anti-inflammatory cytokines) in a variety of cell types, including monocytes,[180] microglia,[181] keratinocytes,[182] endothelial cells,[183] and human benign prostatic hyperplastic cells.[184] In addition to mediating the release of specific cytokines, vitamin D also increases the expression of cytokine receptors.[275] Vitamin D promotes proliferation of immunosuppressive regulatory T cells towards a T-helper 2 type profile, which has an anti-inflammatory effect.[185] VDR knock-out mice have higher levels of proinflammatory cytokines and are more susceptible to inflammatory diseases such as inflammatory bowel disease compared to normal mice.[186] In humans, vitamin D studies conducted in subjects with a wider range of serum vitamin D levels have shown variable results regarding the association of vitamin D and inflammatory cytokines. The following section summarizes recent studies on human populations.

4.5.2 Epidemiology of vitamin D and Inflammation

Low vitamin D status has been associated with increased risk for chronic disease in which inflammation is an underlying component.[187] Even though most studies have examined the association between vitamin D status and inflammation in adults with acute or chronic illnesses, a few recent studies have examined the association between vitamin D status and CRP in healthy adults.[188] Three recent cross-sectional studies evaluating the association between serum vitamin D and c-reactive protein found an inverse association in adults without acute illness.[189–191] However, the available literature is conflicting. Three recent cross-sectional studies found no association between serum vitamin D and CRP[192–194] and two recent randomized clinical trials also found no association between vitamin D

supplementation and circulating CRP levels.[195,196] However, a meta-analysis of ten

randomized clinical trials of vitamin D supplementation found a significant inverse

association between serum vitamin D levels and circulating hs-CRP.[197]

Given the inconsistent results in the literature, Amer and Qayyam employed single-

knot spline regression at the median of serum vitamin D concentration to evaluate the

shape of the association between inflammation and both low and high vitamin D levels in

NHANES adults. Study participants (15,167 men and women) were divided into two

groups: low vitamin D group (those with 25-OHD < 21 ng/mL) and high vitamin D group

(those with 25-OHD ≥ 21 ng/mL). Mean CRP values in the lower and higher 25-OHD were

2.2 and 1.7 mg/L, respectively.[189] In univariate spline linear regression model among those

in the low vitamin D group, CRP decreased as the serum 25-OHD level increased (p<0.001).

However, in the high vitamin D group, an increase in 25-OHD was not associated with any

statistically significant decrease in the CRP level (95%CI -0.11, 0.001; p=0.07). In

multivariable linear regression, this inverse association seen between vitamin D and CRP in

the low vitamin D group (< 21ng/mL) remained unchanged (p<0.001). However, in the

high vitamin D group, the association between CRP and vitamin D reversed in multivariable

regression, and CRP was positively associated with vitamin D levels.[189] These results shed

light on the potential interactions and mechanisms at play in the association between

circulating vitamin D levels and inflammation. It is possible that vitamin D supplementation

among healthy subjects with baseline vitamin D levels ≥21 ng/mL might not have

additional effects on systemic inflammation. A second study using restricted spline

regression [86] found a U-shaped association between 25(OH)-vitamin D and CRP among

healthy adults, with a nadir between 52-62 nmol/L (21-25 ng/mL), suggesting that below

these levels, 25(OH)-vitamin D may have an anti-inflammatory effect, but above these levels vitamin D may have a pro-inflammatory effect.

In order to discern the association between vitamin D status, supplementation, and its effect on inflammation, Chen et al (2014) completed a meta-analysis of ten randomized controlled trials including a total of 924 participants. They found that vitamin D supplementation significantly decreased the circulating CRP level by 1.08 mg/L (95%CI -2.13, -0.03). Furthermore, after subgroup analysis, they found a higher reduction of CRP by 2.21 mg/L (95%CI -3.50,-0.92) among participants with baseline CRP level ≥5mg/L. Additionally, their analysis revealed that baseline CRP level, supplemental dose of vitamin D and intervention duration together may be attributed to the heterogeneity across studies.[197] They concluded that vitamin D supplementation was indeed beneficial for the reduction of circulating CRP, but the results of studies should be interpreted with caution.

The current literature suggests the mechanism by which vitamin D may influence inflammation may be more complex, and perhaps only those with very low vitamin D status may benefit from vitamin D supplementation. However, more research is needed to shed light on dosage required and vitamin D status adequate for improving inflammatory state.

4.5.3 Physiology of Vitamin B_6 and Inflammation

In addition to functioning as a cofactor in a wide variety of enzymes involved in macronutrient metabolism and in neurotransmitter synthesis, vitamin B_6 plays a critical role in both the innate and adaptive immune responses.[198] Vitamin B_6 is required for the production of cytokines,[199] which are among the primary mediators of inflammation, as

well as for the proliferation and activation of lymphocytes that characterize the inflammatory response.[200,201]

There are several hypotheses regarding the underlying mechanism for the association between low plasma PLP and inflammation. One hypothesis suggests that low dietary intake of vitamin B_6 confers increased risk of inflammation; however, in most studies of inflammatory conditions such as rheumatoid arthritis (RA) where plasma PLP levels are low, vitamin B_6 intakes correlate poorly with plasma PLP concentration.[202] Despite lower plasma PLP levels, RA patients have normal measures of other indicators of vitamin B_6 status, such as erythrocyte aspartate transaminase (EAST) activity coefficient and erythrocyte PLP concentration,[202] suggesting a functional deficiency rather than a frank deficiency.

A second hypothesis suggests increased vitamin B_6 requirements during inflammation are due to catabolism of vitamin B_6. In the study by Chiang et al (2003), urinary excretion of 4-pyridoxic acid, which is a measure of vitamin B_6 metabolism, was not correlated with plasma PLP in RA patients, suggesting that low plasma PLP in this condition is not due to increased metabolism of vitamin B_6.[202] However, this idea remains in debate, as a new study by Ulvik and colleagues revealed that there was an increase in vitamin B_6 catabolism during inflammation by assessing the substrate product ratio in plasma (pyridoxic acid to the addition of PL + PLP).[277] This new approach to assess vitamin B_6 catabolism may provide new insights into the potential mechanisms underlying the association between plasma vitamin B_6 and inflammation.

It appears the lower plasma PLP observed in inflammatory conditions is not linked to dietary inadequacy or to vitamin B_6 deficiency in most cases, but rather is due to

metabolic phenomena inherent to inflammation.[271]   Among NHANES participants, the

prevalence of low plasma PLP (<20nmol/L) is approximately 2.5 times higher in

individuals with higher CPR (>10 mg/L) compared to those with CPR levels ≤3mg/L. [278]

These data suggest there is a higher need tor plasma PLP by the immune system during

active inflammation.

Current evidence points to the third hypothesis: that the inverse association

between plasma PLP and inflammation is the result of mobilization of plasma PLP to sites

of active inflammation for use by the PLP-dependent enzymes that play a role in the

inflammatory response. [279] Because the PLP in muscle is phosphorylated, it is not easily

released; thus, when there is an increased demand for PLP during inflammation or when

intake is low, PLP is supplied by liver and plasma stores. This evidence comes from a study

with an animal model of RA, which found that during bouts of inflammation, PLP levels fell

in liver and plasma, but not in other tissues. [205] Hence, plasma PLP concentration would be

highly susceptible to a sudden increase in PLP demand during an immune response.[271]

This third hypothesis proposes that the lower plasma PLP levels observed during

inflammation are due to the mobilization of PLP to the site of inflammation for degradation

of tryptophan via the kynurenine pathway, metabolism of immunomodulatory

sphingolipids, and the proliferation of immune cells.[271]   Degradation of tryptophan

through the indoleamine 2,3-dioxygenase (IDO) pathway is a hallmark of inflammation.[271]

Tryptophan metabolism through the kynurenine pathway includes two vitamin B_6-

dependent enzymes. These studies suggest that impaired vitamin B_6 status in inflammation

is not solely caused by either lower intake, malabsorption or excessive catabolism of the

vitamin, but may be a result of metabolism mechanisms inherent to the inflammatory

process. The sequestration of PLP in the sites of inflammation may appear to be the most convincing hypothesis to date, but further studies are needed to rule out the remaining hypotheses and confirm the latter.

4.5.4 Epidemiology of Vitamin B_6 and Inflammation

A number of population studies have shown that low plasma vitamin B_6 levels are associated with typical chronic inflammatory diseases, such as rheumatoid arthritis and inflammatory bowel diseases,[203] and are inversely related to markers of inflammation.[163] However, this association seems to be affected by several factors, such as OC use, age, and dietary supplementation.

The first large study to investigate this association was carried out over a decade ago. Using the Framingham Heart Study cohort, Friso and colleagues examined the association between plasma PLP levels and CRP in 891 older adults.[156] They found those with high CRP levels (≥ 6 mg/L) had significantly lower PLP levels (36.5 vs 55.8 nmol/L, p-value<0.001) than individuals with low CRP values. Only 6.4% (n=57) of the study population had high CRP as defined by the cut-point used. Vitamin B_6 intake did not differ between the CRP groups (2.4 vs 2.1 mg/day, p-value>0.2), therefore the lower PLP levels in one group were not due to diminished intake.[156]

A larger, more recent cross-sectional study using the National Health and Nutrition Examination survey evaluated the relationship between both vitamin B_6 intake and plasma PLP concentrations and serum CRP in 2,686 adults representative of the adult U.S. population.[204] They investigated whether the vitamin B_6 requirement is affected by inflammation by determining the vitamin B_6 intake associated with minimal prevalence of

vitamin B_6 inadequacy in different serum CRP categories. Vitamin B_6 intake was assessed using two 24-hour diet recalls as well as from supplement use data. Those who were diabetic, pregnant, lactating, taking hormones or steroidal anti-inflammatory drugs were excluded. They found female gender, current smoking, and low albumin to be positively associated with higher inflammation and vitamin B_6 deficiency (<20 nmol/L). After multivariable adjustment for demographics, smoking, BMI, alcohol use, antioxidant vitamin status, intakes of protein and energy, and serum concentrations of creatinine and albumin, high vitamin B_6 intake was associated with protection against high CRP levels (>10 mg/L) compared with those with low CRP (≤3 mg/L). However, plasma PLP ≥20 nmol/L compared with <20 nmol/L was inversely related to serum CRP independently of B_6 intake (p<0.001). Higher vitamin B_6 intakes were found to be protective against inflammation, with higher vitamin B_6 intakes associated with maximum protection against vitamin B_6 inadequacy in the presence of inflammation.[204] This study confirmed the association between inflammation and low plasma PLP concentration, but challenged the notion that vitamin B_6 intake is unrelated to inflammation. From these studies, it appears that suboptimal vitamin B_6 status can be corrected via supplementation, but the amount of vitamin B_6 intake needed to protect against vitamin B_6 inadequacy may be elevated in the presence of inflammation.[272] Vitamin B_6 supplementation with 50 mg/day of pyridoxine for 30 days may correct vitamin B_6 deficiency,[275] but higher levels (100mg/day) may be needed to improve inflammation among individuals with high inflammation levels, such as rheumatoid arthritis patients.[276]

CHAPTER 5

RESEARCH GAP & PURPOSE OF THE STUDY

Depression is the leading cause of disease burden among women of reproductive age. This population is at increased risk for both vitamin D and vitamin B_6 deficiencies compared to men, as has been shown by previous NHANES analyses. Additionally, this population has been repeatedly shown to have higher inflammation levels compared to men. This is particularly interesting given that inflammation has been linked to the development and severity of depression since the early 1990s. Given all the evidence linking vitamin D and B_6 to inflammation in numerous animal and population-based studies, it is surprising to see how little is known about how these factors may be connected, and how they may be contributing to depression severity or symptomatology. It is of special interest to find new avenues for depression prevention and treatment due to the low response rate of antidepressants.

What was once coined the "macrophage theory of depression" may now be the basis for how low-grade inflammation could be contributing to compromised micronutrient status, and how the interaction between these factors could be an underlying cause for the depression seen in women of reproductive age. This knowledge gap prevents our understanding to what extent correcting micronutrient status and inflammation might be a simple, economic, and effective component of a prevention strategy aimed at reducing the risk of developing depression.

The primary objective in the proposed study was to assess how inflammation is related to depression, while accounting for micronutrients numerous lifestyle and

behavioral factors, such as body mass index, leisure-time physical activity, sleep, and oral

contraceptive use. Our study also considered different types of depressive symptoms, such

as affective and somatic, in addition to individual depression symptoms, to determine how

inflammation, vitamin D and B_6 concentrations may be interacting and contributing to

different dimensions of depression. The study findings shed light on potential mechanisms

linking nutritional status and inflammation to different aspects of depression and

depression severity. Findings may be used to create new approaches that include targeting

nutritional status to prevent, treat, and help manage depression among women of

reproductive age.

5.1 Specific Aims and Hypotheses by Manuscript Title

5.1.2 Study #1: Inflammation is associated with depression symptomatology among women of reproductive age from NHANES 2005-2008

Our *working hypothesis* for this aim is that elevated inflammation is positively associated with depression in women of reproductive age. Our *experimental approach* was to investigate the association between high-sensitivity C-reactive protein (hs-CRP) and sum depression scores, as well as individual symptoms and depression subtypes measured with the PHQ-9 to determine the extent to which inflammation is associated with increased depression. We also explored which lifestyle and behavioral factors affected this association through moderation or mediation pathways in post-hoc analysis.

5.1.2.1 Specific Aim #1:

To assess the association hs-CRP and different dimensions of depression among non-pregnant women of reproductive age from NHANES 2005-2008.

5.1.2.2 Hypothesis #1a:

Among women of reproductive age, hs-CRP will be positively associated with depression score and depression severity as determined by the PHQ-9.

5.1.2.3 Hypothesis #1b:

Among women of reproductive age, the association between inflammation and depression will depend on the nature of the symptoms.

5.1.3 Study #2. Does inflammation influence the association between vitamin B_6 and depression in non-pregnant women ages 15-44 from NHANES 2005-2008

Our *working hypothesis* for this aim is that elevated hs-CRP is modifying the association between vitamin B_6 levels and depression in women of reproductive age. Our *experimental approach* was to investigate the association between vitamin B_6 levels and depression scores among women with high and low inflammation levels to determine the extent to which inflammation influences the association between vitamin B_6 status and different dimensions of depression.

5.1.3.1 Specific Aim #2:

To assess the association between vitamin B_6 concentrations and different dimensions of depression, stratified by CRP categories, in women of reproductive age from NHANES 2005-2008.

5.1.3.2 Hypothesis #2a:

Among women of reproductive age, hs-CRP will be inversely associated with serum vitamin B_6 status.

5.1.3.3 Hypothesis #2a:

Among women of reproductive age, depression will be inversely associated serum vitamin B_6 status, and this association will be strongest among women with elevated hs-CRP concentrations.

5.1.4 Study #3. Do inflammation or lifestyle factors influence the association between vitamin D status and depression in non-pregnant women ages 15-44 from NHANES 2005-2008

Our *working hypothesis* for this aim is that inflammation modifies the association between vitamin D status and depression in women of reproductive age. Our *experimental approach* was to investigate the association between vitamin D categories and depression scores measured with the PHQ-9 among women with high and low CRP levels to determine the extent to which hs-CRP influences the association between vitamin D and different dimensions of depression symptomology. We also explored which lifestyle and behavioral

factors affected this association through moderation or mediation pathways in post-hoc analysis.

5.1.4.1 Specific Aim #3:

To assess whether inflammation modifies the association between vitamin D and depression in non-pregnant women of reproductive age from NHANES 2005-2008.

5.1.4.2 Hypothesis #3a:

Among women of reproductive age, serum vitamin D status will be inversely association with depression.

5.1.4.3 Hypothesis #3b:

Among women of reproductive age, the association between vitamin D status and depression depends on inflammation.

CHAPTER 6

STUDY DESIGN AND METHODS

6.1 Study Design and Population

We examined cross-sectional data from the 2005-2008 National Health and Nutrition Examination Survey (NHANES), which is national survey from the National Center for Health Statistics of the Centers for Disease Control and Prevention (CDC), designed to assess the health and nutritional status of the U.S. population. NHANES employs a complex, multistage, probability sampling design to select individuals representative of the civilian, non-institutionalized population, with oversampling of special sub-populations, such as racial and ethnic minorities. Detailed descriptions of the survey design and procedures are available at the study website.[277]

Briefly, approximately 5,000 people are surveyed each year via in-person interviews and physical examinations. The interview, which is conducted in participants' homes, includes demographic, socioeconomic, dietary, and health-related questions; the examination component is comprised of medical, dental, and physiological measurements, as well as laboratory tests, which take place in specially-designed and equipped mobile examination centers (MEC) that travel throughout the country. The National Center for Health Statistic's Institutional Review Board has reviewed and approved the NHANES protocol. [282]

Participants were eligible for our study if they were women of reproductive age (15-44 years old) who were not pregnant. From the initial sample of 2,609 eligible women,

we excluded those who were taking anti-inflammatory medications, including NSAIDs (n=70), those with acute inflammation denoted by CRP levels above 10 mg/L (n=201)[277] and those with missing data for either depression (n=506) or inflammation (n=104). Depression data for those younger than 18 years was not publicly available; therefore, our age range was restricted to women ages 18-44 years old. Women who reported current antidepressant use were additionally excluded (n=135).

6.2 Exposure Assessment

Fasting whole blood samples were collected during the medical examination. Venipuncture was performed using standard phlebotomy techniques by NHANES-trained personnel. Serum specimens were frozen at -20 degrees Celsius until biomarker assessment at varying laboratories designated by the Center for Disease Control. Further detailed instructions on specimen collection and processing can be found at NHANES website.[21] A brief description for each biomarker assessment is provided below.

Serum hs-CRP was assayed at the University of Washington Medical Center. Hs-CRP was quantified by latex-enhanced nephelometry using a Dade Behring Nephelometer II analyzer System (Dade Behring Diagnostics Inc, Somerville NJ). Monoclonal antibodies were used to detect serum CRP, and N rheumatology standard SL was used to standardize against the World Health Organization's International Reference Preparation of CRP. This high-sensitivity assay allowed for a minimum detection of 0.2 mg/L and does not have a maximum reportable limit. Further details can be found on the website. [277] CRP was modeled as three categories: no inflammation (CRP<1mg/L), low inflammation (CRP between 1-3 mg/L) and mid-high inflammation (CRP between 3-10 mg/L), following

guidelines for increased risk of cardiovascular disease set forth by the American Heart Association. [278]

Serum specimens for pyridoxal 5'-phosphate (PLP) were processed, stored and shipped to the Division of Environmental Health Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention in Atlanta, Georgia. Briefly, serum PLP, the main bioactive form of vitamin B_6, was measured using reverse-phase high performance liquid chromatography (HPLC) analysis using fluorometric detection at 325nm excitation and 425nm emission. Quantitation of PLP was based on analyte peak area interpolated against a five-point calibration curve from aqueous standards. This HPLC assay has a lower limit of detection compared to the enzymatic assay used in previous NHANES cycles (0.3 vs 10 nmol/L). [277] Serum PLP was also divided into categories indicating vitamin B_6 deficiency (PLP <20nmol/L), insufficiency (PLP 20 to 29.9 nmol/L) and sufficiency (PLP ≥30 nmol/L) following previously used cut-offs for vitamin B_6 status. [277]

Measurements of serum 25(OH) vitamin D were performed with the DiaSorin RIA kit (Stillwater, MN) for NHANES 2001-2006, and with liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for NHANES 2007-2008. [279] In order to use vitamin D data from these two different methods, NHANES converted the vitamin D data from 2001-2006 assayed with the RIA method to equivalent 25(OH)D measurements from a standardized LC-MS/MS method using regression analysis. This was done to allow researchers to use vitamin D data that are equivalent to 25(OH)D measurements assayed with LC-MS/MS method. [279] Serum vitamin D was primarily categorized into variables

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indicating vitamin D deficiency (<50nmol/L), insufficiency (50 to 75 nmol/L) and sufficiency (>75 nmol/L) following previously used cut-offs for vitamin D status.[165,280]

6.2.1 Laboratory Quality Assurance and Monitoring

The NHANES quality control and quality assurance protocols meet the 1988 Clinical Laboratory Improvement Act mandates at all the locations. Quality control measures and device calibrations were performed daily for all laboratory procedures.[277]

6.3 Depression Assessment

The Patient Health Questionnaire-9 (PHQ-9)[281] was administered during the MEC interview to assess depressive symptom severity over the past two weeks. Based on the diagnostic criteria for major depressive disorder in the Diagnostic and Statistical Manual Fourth Edition (DSM-IV), the PHQ-9 employs a 4-point scale (0=not at all, 1= several days, 2= more than half the days, 3= nearly every day) to determine the frequency with which respondents experienced the following nine symptoms of major depressive disorder: anhedonia, depressed mood, sleep disturbance, fatigue, appetite changes, low self-esteem, concentration problems, psychomotor retardation/agitation, and suicidal ideation. Total depression scores range from 0 to 27, and scores of 10 or higher indicative of clinical depression.[282] The PHQ-9 has been shown to be a reliable and valid questionnaire as indicated by its high internal consistency and good sensitivity (88%) and specificity (88%) for identifying cases of major depressive disorder in community samples.[283–285]

In addition to the total depression score, we calculated somatic and cognitive-affective depression subscale scores. The somatic depression subscale was calculated by adding the scores for frequency of somatic depression symptom from the PHQ-9 (sleep disturbance,

fatigue, appetite changes, and psychomotor retardation/agitation) and the cognitive-affective subscale score was calculated adding the scores for non-somatic symptoms (anhedonia, depressed mood, low self-esteem, concentration problems and suicidal ideation).[287] While previous factor analyses supported a one-factor model with all nine items,[288,289] recent confirmatory factor analyses have found that subscales of depression dimensions may provide a better fit to the data.[290,291] It has also been argued that given the heterogeneity of symptoms for depression, individual symptoms should be analyzed.[29] We included a separate analysis for individual depressive symptoms, coded as continuous variables (scores ranging from 0-3) and as binary variables. To dichotomize the variable, those items with responses "more than half the days" and "nearly every day" indicated the presence of the symptom.[292,293]

6.4 Covariate Assessment

We considered as possible covariates a selected set of demographic, lifestyle, and socioeconomic factors available through data collected during the demographic or household section of the NHANES questionnaire. The National Center for Health Statistics (NCHS) standard definitions for ethnicities were used, and ethnicities were categorized as non-Hispanic whites, non-Hispanic blacks, Mexican-Hispanics, other Hispanics, and other race. Educational attainment was measured as the highest completed grade of school regardless of age, and categorized into four levels: less than high school, high school equivalent, some college, and college graduate or above. The smoking assessment for respondents aged 18-19 years (MEC) was not identical to that for respondents aged 20+ years (household interview). For respondents aged 20+ years, we classified as current

smokers those who reported smoking at least 100 cigarettes during their lifetime and reported that they now smoke cigarettes every day or some days. For respondents ages 18-19 years, we classified as current smokers those who reported smoking 15 or more day in the last 30 days. To determine a smoking dose from current smokers, we classified those who reported smoking <15 days per month or "some days" as medium dose smoking, and those who smoked more than 15 days per month or "every day" as high dose smokers. To create the alcohol groups, we used data obtained during the alcohol use interview at the MEC and calculated the drinks per day as suggested by Case and Stewart.[294] Alcohol consumption, which was only available for participants of legal drinking age (21 and older), was categorized into abstainer, one or fewer drinks/day and more than one drink per day. Leisure-time physical activity (LTPA) was assessed by asking participants about their involvement in 48 specific recreation activities of moderate or vigorous intensity. Frequency was first multiplied by the duration in hours and divided by 4.3 to obtain hours of LTPA per week. To calculate MET hrs/week, each activity was multiple by its MET level per NHANES physical activity codes.[295] Participants were then categorized into quartiles of physical activity. Sleep duration was assessed by asking participants how many hours of sleep they usually get on a weekday/workday, and those who reported sleeping 12 or more hours were labeled as sleeping 12 hours/night. Oral contraceptive use was determined by self-report of current use, with "past" or "never" users classified as not currently using oral contraceptives (OC). Body mass index (BMI) (calculated as kg/m^2) was computed from height and weight measurements and categorized into four standard categories: underweight (BMI<18.5 kg/m^2), normal weight (18.5 ≤ BMI<25), overweight (25≤ BMI<30) and obese (BMI≥30 kg/m^2).

6.5 Statistical Analysis Plan

The complex survey design used for NHANES data collection was incorporated into all data analyses using the "svy" command with appropriate weighting in Stata 12.0 (StataCorp LP, College Station, TX). Detailed information on the procedures for taking into account survey sampling weights have been described elsewhere.[296]

Descriptive statistics were calculated for the overall sample using frequency distributions for categorical variables and means and standard errors for continuous variables. Natural logarithmic transformations were performed on hs-CRP, PLP, and vitamin D to normalize the distribution, and their geometric means and 95% confidence intervals are presented. Bivariate analyses were carried out for each covariate and each exposure as well as outcome using weighted ANOVAs, Chi square tests and 2-sample t-test as appropriate. In linear regression models, all covariates that changed the estimated coefficient for the primary predictor of interest by more than 10% were retained in the multivariable model. In addition, known risk factors for depression were retained in the model as well as those that were associated with both the predictor and the outcome.

To examine whether each exposure was more strongly related with certain depression symptoms, we conducted a series of linear regression analyses involving total depression score, somatic depression score, and non-somatic depression score as well as each individual depression symptom as outcome. A similar series of logistic regression analyses was also completed for dichotomous individual depressive symptoms. For all the individual depression symptoms, we also adjusted for the remaining depression symptom scores to account for any potential overlap between different symptoms and reduce the probability of Type I error. Logistic regression analyses were carried out for depression

PHQ-9 score of 10 or greater. The command "mfpigen" was used to assess all possible interactions among variables in the full models.

For each study, several lifestyle variables were assessed as potential mediators by running Sobel mediation tests and the Preacher and Hayes test of indirect effects to quantify their effects on any observed relationships between exposure and depression.[297,298] Effect modification was assessed by stratified regression analyses and by including multiplicative interaction terms in the multivariable analysis. Final model fit was assessed using goodness of fit tests. All linear and logistic regression estimates were weighted using NHANES sample weights, which account for the complex survey design, survey nonresponse, and post-stratification. All analyses were considered significant at an alpha of 0.05.

CHAPTER 7

"THE ASSOCIATION BETWEEN C-REACTIVE PROTEIN AND DEPRESSIVE SYMPTOMS AMONG WOMEN OF REPRODUCTIVE AGE IS MODERATED BY BODY MASS INDEX"

7.1 Abstract

Depression is the leading cause of disability world-wide, and women in their childbearing years make up the largest group of Americans with depression. Recent evidence indicates that inflammation is associated with depression, and this relationship may differ by gender and by depression symptoms. We conducted a secondary data analysis to evaluate the association between c-reactive protein (CRP) and different dimensions of depressive symptomatology in non-pregnant women ages 18-44 years from the cross-sectional National Health and Nutrition Examination Survey (NHANES) 2005-2008. Depression scores were calculated based on the Patient Health Questionnaire-9 (PHQ-9) and categorized into total depression, somatic and non-somatic depression. High depression score (PHQ-9≥10) was also used as an outcome, as were individual symptoms of depression. High-sensitivity CRP was quantified by latex-enhanced nephelometry. To account for NHANES' complex survey design sample, we incorporated the sampling weights to analyze 1,489 observations. CRP was positively related to total (p=0.006), somatic (p=0.01) and non-somatic depression scores (p=0.01) in unadjusted models. However, in the multivariable adjusted models, CRP was positively associated with depression scores (total depression, p=0.001; somatic depression, p=0.001; non-somatic depression, p=0.007) only among those who were underweight (BMI<18.5 kg/m^2). BMI accounted for 44.8% of the association between CRP and total depression scores (p<0.05).

When individual depression symptoms were examined, BMI was negatively associated with abnormal appetite score among women with no inflammation (CRP<1mg/L), but BMI was positively associated with appetite score among those with moderate inflammation (CRP 3-10mg/L). BMI appears to play a role in the association between inflammation and depression symptoms among women. Further research is needed determine whether changes in biomarkers of inflammation predict changes in specific dimensions of depression in this population, and whether lifestyle modification can be used to prevent depression or as adjuvant treatment for depression among women.

7.2 Introduction

Depression is common in the United States, with approximately 16% of adults experiencing clinical depression at some point in their lives.[1] Women are two- to three-times more likely than men to experience depression.[2,3] Depression is a multifaceted phenomenon with a large range of symptoms, and different subtypes of depression have been proposed based on specific combinations of symptoms.[4] Research into the etiology of depression needs to account for the heterogeneous nature of the condition and requires developing methods to characterize and objectively assess the severity of depression and to classify depression into its different subtypes.[5] In order to address the issue of symptom diversity, subtypes of depressive symptoms have been proposed based on specific combination of symptoms. Somatic depression, characterized by sleep disturbance, fatigue, or changes in appetite, has been found to be more prevalent in females compared to males,[6] and other evidence suggests that gender differences may account for heterogeneity

in depression symptoms and that the involvement of inflammation in the etiology of depression may be affected by lifestyle factors.[7]

Several observational studies reported that increases in inflammatory biomarkers are associated with increased risk for depression; however, conflicting findings exist regarding the directionality of the association, and the underlying mechanisms are not fully understood. Recent research has specifically focused on the association between high sensitivity c-reactive protein (hs-CRP), interleukin-6 (IL-6), tumor necrosis factor α (TNF-α), and other markers of systemic inflammation and depression.[8,9] However, very few studies have investigated depression subtypes, including somatic and cognitive/affective,[6,10–14] and no studies have examined whether the association between inflammation and depression among women is symptom-specific. Individual depression symptoms differ in their biological correlates, which underscores the heterogeneous nature of depression and may explain the lack of progress in validating depression diagnoses with biomarkers.[15] Assessing the association between inflammation and specific symptoms offers opportunities to investigate potential biological factors that may be related to specific syndromes but may be masked by aggregate scoring; individuals with similar total depression scores often have drastically different clinical conditions.[16] The analysis of individual symptoms could reveal patterns that are currently missed.

The purpose of this study was to further explore the relationship between inflammation and depression among U.S. reproductive-age women and to determine whether CRP, a marker of inflammation, is associated with depression subtypes or individual symptoms of depression among this population. Our goal was to determine whether serum CRP levels in the low-grade inflammation range are associated with

particular subtypes of depressive symptoms among 1,489 women, aged 18-44 years, from the National Health and Nutrition Examination Survey (NHANES) 2005-2008 and to assess whether body mass index (BMI) and other lifestyle factors potentially modify this association.

7.3 Methods

7.3.1 Study Design and Study Population

We analyzed cross-sectional data from the 2005-2008 National Health and Nutrition Examination Survey (NHANES), which is a national survey from the National Center for Health Statistics of the Centers for Disease Control and Prevention (CDC), designed to assess the health and nutritional status of the U.S. population. NHANES employs a complex, multistage, probability sampling design to select persons representative of the civilian, non-institutionalized population, with oversampling of special sub-populations, such as racial and ethnic minorities. Detailed descriptions of the survey design and procedures are available at the NHANES website.[16]

Briefly, approximately 5,000 people are surveyed each year via in-person interviews and physical examinations. The interview, which is conducted in participants' homes, includes demographic, socioeconomic, dietary, and health-related questions; the examination component is comprised of medical, dental, and physiological measurements, as well as laboratory tests, which take place in specially-designed and equipped mobile examination centers (MEC) that travel throughout the country. The National Center for Health Statistic's Institutional Review Board has reviewed and approved the NHANES protocol.

Participants were eligible for our study if they were women of reproductive age (15-44 years old) who were not pregnant. From the initial sample of 2,609 eligible women, we excluded those who were taking anti-inflammatory medications, including NSAIDs (n=70); those with acute inflammation denoted by CRP levels above 10 mg/L (n=201);[17] those reporting antidepressant use (n=135) and those with missing data for either depression (n=506) or inflammation (n=104). Depression data for those younger than 18 years was not publicly available; therefore, our age range was restricted to women ages 18-44 years old. The remaining sample contained 1,489 observations.

7.3.2 Assessment of Depression Status

The Patient Health Questionnaire-9 (PHQ-9)[18] was administered during the MEC interview to assess depressive symptom severity over the past two weeks. Based on the diagnostic criteria for major depressive disorder in the Diagnostic and Statistical Manual Fourth Edition (DSM-IV), the PHQ-9 employs a 4-point scale (0=not at all, 1= several days, 2= more than half the days, 3= nearly every day) to determine the frequency with which respondents experienced the following nine symptoms of major depressive disorder: anhedonia, depressed mood, sleep disturbance, fatigue, appetite changes, low self-esteem, concentration problems, psychomotor retardation/agitation, and suicidal ideation. Total depression scores range from 0 to 27, and scores ≥10 represent clinically significant depressive symptoms.[18] The PHQ-9 has been shown to be a reliable and valid questionnaire as indicated by its high internal consistency and good sensitivity (88%) and specificity (88%) for identifying cases of major depressive disorder in community samples.[19–21]

In addition to the total depression score, we calculated somatic and cognitive-affective depression subscale scores. The somatic depression subscale was calculated by adding the scores for frequency of somatic depression symptom from the PHQ-9 (sleep disturbance, fatigue, appetite changes, and psychomotor retardation/agitation), and the cognitive-affective subscale score was calculated adding the scores for non-somatic symptoms (anhedonia, depressed mood, low self-esteem, concentration problems and suicidal ideation).[10] While previous factor analyses supported a one-factor model with all nine items,[22,23] recent confirmatory factor analyses have found that subscales of depression dimensions may provide a better fit to the data.[24,25] Some also argue that given the heterogeneity of symptoms for depression, individual symptoms should be analyzed.[15] We also included a separate analysis for individual depressive symptoms, coded as continuous variables (scores ranging from 0-3) and as binary variables. To dichotomize the variable, those items with responses "more than half the days" and "nearly every day" indicated the presence of the symptom.[26,27]

7.3.3 C-Reactive Protein

Fasting whole blood samples were collected during the medical examination. Venipuncture was performed using standard phlebotomy techniques by NHANES-trained personnel. Serum specimens were frozen at -20 degrees Celsius until biomarker assessment at the University of Washington Medical Center. Serum high-sensitivity CRP was quantified by latex-enhanced nephelometry using a Dade Behring Nephelometer II analyzer System (Dade Behring Diagnostics Inc, Somerville NJ). Monoclonal antibodies were used to detect serum CRP, and N rheumatology standard SL was used to standardize

against the World Health Organization's International Reference Preparation of CRP. This high-sensitivity assay allowed for a minimum detection of 0.2 mg/L and does not have a maximum reportable limit. Further details can be found on the website.[16] Given that hs-CRP was not normally distributed, it was converted to its natural logarithm to improve normality. In additional analyses, CRP was modeled as three categories: no inflammation (CRP<1mg/L), low inflammation (CRP between 1-3 mg/L) and moderate inflammation (CRP between 3-10 mg/L), following guidelines for increased risk of cardiovascular disease set forth by the American Heart Association.[17]

7.3.4 Covariates and Confounders

We considered as possible covariates a selected set of demographic, lifestyle, and socioeconomic factors available through data collected during the demographic or household section of the NHANES questionnaire. The National Center for Health Statistics (NCHS) standard definitions for ethnicities were used, and ethnicities were categorized as non-Hispanic whites, non-Hispanic blacks, Mexican-Hispanics, other Hispanics, and other race. Educational attainment was measured as the highest completed grade of school regardless of age, and categorized into four levels: less than high school, high school equivalent, some college, and college graduate or above. The smoking assessment for respondents aged 18-19 years (MEC) was not identical to that for respondents aged 20+ years (household interview). For respondents aged 20+ years, we classified those who reported smoking at least 100 cigarettes during their lifetime and reported that they now smoke cigarettes every day or some days as current smokers. For respondents ages 18-19 years, we classified those who reported smoking 15 or more day in the last 30 days as

current smokers. To create the alcohol groups, we used data obtained during the alcohol use interview at the MEC and calculated the drinks per day as suggested by Case and Stewart.[13] Alcohol consumption, which was only available for participants of legal drinking age (21 and older), was categorized into abstainer, one or fewer drinks/day and more than one drink per day. Oral contraceptive use was determined by self-report of current use, with "past" or "never" users classified as not currently using oral contraceptives (OC).

Body mass index (BMI), leisure-time physical activity (LTPA), and sleep duration have been associated with both C-reactive protein and depression[7,28,29] and were examined as potential mediators/confounders of the relationship between CRP and depressive symptoms. BMI (kg/m^2) was computed from height and weight measurements and categorized into four standard categories: underweight (BMI<18.5 kg/m^2), normal weight (18.5 ≤ BMI<25), overweight (25≤ BMI<30) and obese (BMI≥30 kg/m^2). LTPA was assessed by asking participants about their involvement in 48 specific recreation activities of moderate or vigorous intensity. Frequency was first multiplied by the duration in hours and divided by 4.3 to obtain hours of LTPA per week. To calculate MET hrs/week, each activity was multiplied by its MET level according to NHANES physical activity codes.[30] Participants were then categorized into quartiles of physical activity. Sleep duration was assessed by asking participants how many hours of sleep they usually get on a weekday/workday; those who reported sleeping 12 or more hours were labeled as sleeping 12 hours/night.

7.3.5 Data Analysis

The complex survey design used for NHANES data collection was incorporated into all data analyses using the "svy" command with appropriate weighting in Stata 12.0 (StataCorp LP, College Station, TX). Detailed information on the procedures for taking into account survey sampling weights have been described elsewhere.[31]

Descriptive statistics were calculated for the overall sample using frequency distributions for categorical variables and means and standard errors for continuous variables. Because hs-CRP was logarithmically transformed when analyzed as a continuous variable, geometric means (antilogarithms of the transformed means) and their 95% confidence intervals are presented. Bivariate analyses were carried out for each covariate and log-transformed hs-CRP and CRP categories as well as for covariates and total depression, somatic/non-somatic depression and binary depression variables, using weighted ANOVAs, Chi square tests and 2-sample t-tests, as appropriate. In linear regression analyses, all covariates that changed the estimated coefficient for the primary predictor of interest by more than 10% were retained in the multivariable model. In addition, known risk factors for depression, such as smoking and education levels, were retained in the model as were risk factors were associated with both the predictor and the outcome (e.g. BMI, LTPA, OC use).

To examine whether hs-CRP was more strongly related to certain depression symptoms, we conducted a series of linear regression analyses involving total depression score, somatic depression score, and non-somatic depression score as well as each individual depression symptom as outcome. Individual symptom analysis was repeated via logistic regression for dichotomous symptoms. For all the individual depression symptoms,

we also adjusted for the remaining depression symptom scores to account for any potential

overlap between different symptoms. Logistic regression analyses were carried out for

depression PHQ-9 score of 10 or greater. Model adequacy was assessed using goodness of

fit tests.

BMI, LTPA and sleep were assessed as potential mediators by running Sobel

mediation tests and the Preacher and Hayes test of indirect effects to quantify the effects of

BMI, LTPA, and sleep on any observed relationships between hs-CRP and depression.[32,33]

Effect modification was assessed by stratified regression analyses and by including

multiplicative interaction terms in the multivariable analyses.

7.4 Results

Description of Study Cohort. The mean age of the 1,489 women included in our

sample was 31.7 years. The majority of women were white (63.5%), married or living with

a partner (60.4%,) did not use oral contraceptives (80.8%), reported an average of seven

hours of sleep per night, and were nonsmokers (76.3%). Almost eight percent (n=132) of

our sample reported moderate to severe depression symptoms indicative of clinically

significant depression, denoted by a score of 10 or higher on the PHQ-9 (Table 4).

In bivariate analysis, depression scores were positively associated with BMI (p-

trend <0.02 for all) and negatively associated with education (p<0.001) and with LTPA

(p<0.001). Depression score was higher for both somatic and non-somatic subtypes for

women who were not currently taking oral contraceptives (p=0.05 and p=0.01,

respectively) and for smokers (p<0.001 for all). Women with high depression scores slept

less (6.1 vs 7.0 hours/night, p<0.001) compared to those with low depression scores. The

prevalence of depression was significantly higher among women who were separated or divorced, who smoked, and those who reported the lowest levels of LTPA. The prevalence of a high depression score was lower among women who reported current oral contraceptive use compared to non-OC users (4.5% vs 8.0%, p=0.09; Table 4).

Just over a quarter of women (27.8%) had hs-CRP levels between 3-10 mg/L, indicative of moderate inflammation. CRP was positively associated with BMI, with moderate inflammation being much more prevalent among obese (55.4%) or overweight (27.0%) women compared to normal weight (13.8%) or underweight women (6.6%; p<0.001). We found a negative association between educational attainment and hs-CRP (p<0.001). Hs-CRP levels were higher in oral contraceptive users compared to non-users (2.1 vs 1.1 mg/L, p<0.001), and only 21% of current users had hs-CRP levels <1mg/L compared to 43.3% of non-contraceptive users (p<0.001). Those in the lower quartile of LTPA had higher circulating hs-CRP concentration compared to the rest of the quartiles (p=0.05), with some evidence of an LTPA dose response (p for trend=0.02). CRP concentration did not vary by age, smoking status or marital status (Table 5).

Association of hs-CRP with depression. In univariate linear regression, hs-CRP was positively associated with total depression score (β: 0.32, p=0.006), somatic depression score (β:0.17, p=0.01) and non-somatic depression score (β:0.14, p=0.01). After adjustment for demographics and behavioral characteristics, hs-CRP remained statistically significantly associated with all three depression scores, although the betas and p values were somewhat attenuated. Upon further adjustment for sleep duration, BMI and LTPA, the association of hs-CRP with total depression and depression subtypes was attenuated and no longer significant (Table 6).

To determine which depression symptoms were associated with hs-CRP, we first estimated bivariate associations, and found that hs-CRP was significantly higher among those experiencing trouble sleeping (1.70 vs 1.24 mg/L, p=0.009) or those with abnormal appetite (1.85 vs 1.24, p<0.001) (Table 8). We also estimated linear and logistics regression models for continuous and dichotomized symptoms. In linear regression analyses of individual depression symptoms, hs-CRP was significantly associated with sleep disturbance, abnormal appetite, psychomotor abnormalities (somatic symptoms) and with anhedonia, depressed mood, and trouble concentrating (non-somatic symptoms). However, after adjustment for age, race/ethnicity, marital status, education, smoking, sleep, BMI and LTPA, as well as mutual adjustment for remaining depression symptoms, these associations were no longer significant (Table 9). In univariate logistic regression, hs-CRP was significantly associated with sleep disturbances (OR:1.32 p=0.01) and abnormal appetite (OR:1.42, p<0.001) (Table 10). After adjustment for age, ethnicity, education, marital status, smoking, OC use, LTPA, sleep duration, and BMI, as well as mutual adjustment for the remaining depressive symptom score, a one standard deviation increase in log-transformed CRP was significantly associated with a 31% higher odds of sleep disturbance (OR: 1.31, p=0.05), and marginally associated with an 82% higher odds of suicidal ideation (OR: 1.82%, p=0.06). No other significant associations remained.

To examine BMI as a potential modifier of the hs-CRP-depression association, we first ran the linear regression model adjusting for BMI, which attenuated the association between hs-CRP and total depression as well as the association with subtypes of depression (Table 7). This addition did not change the direction of the association. Adjustment for BMI did not change the direction of the association. Addition of an

interaction term between continuous BMI and continuous hs-CRP into the multivariable model did not result in any significant changes (not shown). To further assess how BMI may be influencing the association between hs-CRP and depression, we carried out a series of linear regressions using each one of the nine depression symptoms from the PHQ-9 questionnaire as the main outcome (Table 9). This analysis revealed the following symptoms of somatic depression to be affected by addition of the interaction term for BMI and CRP: fatigue (β:-0.26, p=0.07) and changes in appetite (β: -0.23, p=0.02). The interaction terms for continuous BMI x CRP for these two symptoms were significant (p=0.04 and p=0.02, respectively). To better understand how the outcome of changes in appetite may relate to BMI and CRP, we plotted this interaction with predictive margins to visualize the score for abnormal appetite for those with no inflammation, low inflammation, and mid-high inflammation (using the mean CRP for each category) based on BMI levels. We found that among those with CRP levels below 1 mg/L, BMI was negatively associated with abnormal appetite score, but among those with CRP levels between 3-10 mg/L, BMI was positively associated with predicted appetite score (Figure 3).

Furthermore, we stratified our analysis by BMI category and found that after adjustment in our final model, an increase in one standard deviation of log transformed hs-CRP was associated with an increase in total depression score (β:1.60, p=0.001), somatic (β:1.07, p=0.001), and non-somatic depression score (β:0.49, p=0.007) only among those who were underweight. This association was not significant in any other BMI category (Table 7).

To assess whether BMI, LTPA or sleep were potential mediators or confounders of the association between hs-CRP and depression, we ran a series of Sobel tests and Preacher

and Hayes tests of indirect effects with total depression score, somatic depression score and non-somatic depression score as the main outcomes (Figure 2). For total depression, 44.8% of the total effect was significantly mediated by BMI (p<0.05); for somatic depression, 43.8% of the total effect was mediated by BMI (p<0.05) and for non-somatic depression, 45.8% of the total effect was mediated by BMI (p=0.09). LTPA also attenuated the association between hs-CRP and depression variables, but these indirect effects were not statistically significant (all p>0.09). For total depression, the standardized CRP effect size was reduced by 16.9% (p=0.03) upon addition of sleep to the model; for somatic depression, the effect size was reduced by 20.7% (p=0.03), and for non-somatic depression, it was reduced by 12.4% (p=0.05). We also conducted analyses to test hs-CRP x sleep (p>0.16 for all) and hs-CRP x LTPA (p>0.20 for all) interactions for the full models for total depression, somatic and non-somatic depression, all of which were not significant.

In univariate logistic regression, an increase in one standard deviation in log-transformed hs-CRP was associated with an 18% increase in the odds of having depression, and this was borderline significant (OR: 1.18, 95% CI 0.98, 1.41, p=0.07) (data not shown). No significant interactions were evident in logistic regression analyses.
Association between Sleep and Physical Activity and Depression. Sleep duration was inversely associated with total depression score (β:-0.41, p<0.001), somatic depression score (β:-0.31, p<0.001) and non-somatic depression score (β:-0.11, p=0.04) in the final, multivariable linear regression model (data not shown). In multivariable logistic regression, sleeping more than seven hours was associated with a 53% lower odds of having high depression score (OR:0.47, p=0.005). LTPA was inversely associated with total depression, somatic and non-somatic depression in multivariable linear regression as well

in multivariable logistic regression (data not shown). Being in the lowest quartile of LTPA was significantly associated with higher total depression score (β:1.4, p<0.001), somatic depression score (β:0.82, p=0.001) and non-somatic depression score (β:0.58, p=0.001). In multivariable logistic regression, the relative odds of a high depression score (OR: 2.8, p=0.01) was significantly higher among women in the lowest quartile of LTPA compared to those with more activity.

7.5 Discussion

In this large representative sample of reproductive age US women, we found that hs-CRP was positively associated with total depression, somatic depression, and non-somatic depression after adjustment for demographic and behavioral characteristics. However, these associations were attenuated and were no longer significant after adjustment for BMI, sleep, and LTPA, suggesting that the association between inflammation and depression may be mediated or confounded by these lifestyle factors. Although previous studies have also reported an association between higher levels of inflammatory biomarkers such as hs-CRP and increased depressive symptoms,[8,14,34–37] our study is the first to investigate how these lifestyle factors may influence the association between inflammation and various dimensions of depression among women of reproductive age.

Body mass index significantly attenuated the association between CRP and depression among women in our analysis, and this finding is consistent with, and expands upon the result of a recent analysis by Liu et al (2014), where CRP was significantly associated with depression among men, but not among women after adjustment for BMI.[7] We observed a similar pattern among our sample of women of reproductive age, suggesting that BMI, a

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proxy for adiposity, partially explained the association observed between CRP and total depression score in unadjusted models. Adipose tissue functions as a key endocrine gland, releasing adipokines, which can have pro- or anti-inflammatory activities, depending on the adipose storage site.[38] Pro-inflammatory adipokines, such as leptin and IL-6, are upregulated in proportion to adipose tissue and increase transcription of hs-CRP.[39] Compared to men, women generally have greater body fat content,[40] which is associated with higher BMI and higher leptin levels. BMI may be masking the association between CRP and depression given that adiposity is associated with higher inflammation and, in some studies, predicts future depression.[41] However, given that our study is cross-sectional, and depression has also been found to predict weight gain through emotional eating and weight gain,[42] we cannot rule out the possibility that BMI may be operating as a mediator instead of a confounder.

Although the association between obesity and depression seems clearly established, conflicting evidence remains regarding the shape of the relationship between body weight and depression when considering the full range of BMI, including those who are underweight.[43–45] Some suggest the relationship between body weight and depression is U-shaped, with those who are underweight and overweight or obese having higher odds of depression.[7] We observed a positive association between CRP and depression scores, evident only among those who were underweight (BMI <18.5 kg/m^2), suggesting that BMI modifies the CRP-depression association. It is possible that inflammation originating from non-adipose tissue is strongly related to depression among those with lower levels of adiposity. Among those with higher levels of adiposity, adipokine-derived inflammation may be attenuating this association. These results are consistent with recent findings from

a cross-sectional analysis of the relationship between log-transformed CRP and depression scores among an elderly Chinese population, where Qin et al. (2017) found that CRP was significantly associated with total depression score, somatic depression score, and non-somatic depression score among underweight individuals after adjustment for age, sex, education, marital status, smoking, and alcohol consumption.[46] Similarly, they did not observe any significant association between CRP and any type of depression in their full sample after adjustments or in the normal, overweight, or obese BMI categories. Additionally, they found a lower prevalence of depression among those in the higher BMI categories, whereas we found that the prevalence of depression increased with increasing BMI. This inverse association between BMI and depression is not a new phenomenon among Asian populations, as obesity may be thought to protect middle-aged and elderly Chinese against depression.[47] Given the demographic and cultural differences between these two populations, the similarities in results need to be examined further to determine whether different mechanisms are involved.

Previous studies indicate that inflammation is more strongly related to somatic symptoms of depression.[6,11,13,14,25] We did not see a significant difference between CRP and somatic vs non-somatic depression scores. However, when analyzing individual symptoms of depression, CRP was significantly associated with sleep disturbances and changes in appetite. Previous studies have also reported that CRP is significantly associated with sleep disturbances[26,27] and appetite changes,[27] in addition to other somatic symptoms (fatigue and low energy) after adjustment for age, gender, ethnicity and the sum of remaining depression symptoms. In some cases,[26,27] the associations between CRP and additional symptoms of depression could be due to including participants with acute

inflammation (CRP>10 mg/L) as well as including men and women in the analysis. One of

the studies[27] found that, relative to the lowest quartiles, those in the highest quartile of CRP

(range: 3.31-157.50 mg/L) have higher odds of all the somatic symptoms they assessed;

these relationships were not significant among lower quartiles of CRP. In support of

immune-to-brain-pathways, sleep disturbance is one of the sickness symptoms found to be

mediated by increases in circulating pro-inflammatory cytokines.[48] Therefore, our finding

that inflammation was associated with higher odds of sleep disturbance, even after

adjustment for sleep and other potential confounders, is consistent with previous

studies,[28,49] and adds to the evidence that sleep disturbances may be one of the primary

manifestations of inflammation-associated depression, which may be more evident among

women even at lower inflammation levels.

The occurrence of appetite change was the only other somatic symptom associated with

inflammation, although this relationship showed evidence of effect modification by BMI.

BMI was negatively associated with abnormal appetite score among those with CRP levels

below 1mg/L, but was positively associated with predicted appetite score among those

with CRP levels between 3-10mg/L. These findings suggest that baseline inflammation

levels can affect how BMI is associated with appetite changes, and BMI can either be

protective or increase the risk of experiencing this somatic symptom of depression.

However, interpretation can be challenging due to the ambiguity in the question used to

assess changes in appetite (*"Over the past two weeks, how often have you been bothered by

poor appetite or overeating?"*), which asks about both negative or positive changes in

appetite that could relate to either melancholic/typical depression (characterized by

decreased appetite and weight loss) or to atypical depression (characterized by overeating

and weight gain). Some evidence indicates that atypical depression is more strongly associated with inflammation and BMI than is melancholic depression.[50]

From a physiologic perspective, endocrine changes in adipose-related hormones can partially explain how inflammation may be related to changes in appetite among individuals with different BMI levels. Leptin, an adipocyte-derived hormone also serves as a feedback signal to control energy intake and regulate hunger.[51] Increased leptin signaling associated with weight gain or excess adipose tissue increases energy expenditure and suppresses food intake, whereas decreased leptin signaling elicits anabolic activities by reducing energy expenditure and increasing food intake.[51] Leptin levels are proportional to fat mass, but sustained high levels can lead to leptin resistance, which is a hallmark of obesity. Elevated CRP levels can also lead to leptin resistance.[52] Therefore, leptin may play a more influential role in controlling changes in appetite and weight, but among those with elevated CRP, hunger and appetite regulation through leptin may be compromised if leptin resistance is present. A recent study[53] found that rats treated with a leptin receptor antagonist showed depressive-like symptoms accompanied by peripheral inflammation, demonstrating that leptin resistance can induce an obesity phenotype in healthy rats that is characterized by immune changes and depressive like behaviors. These observations support the idea that comorbid obesity and depressive illness develop are a joint response to changes in peripheral endocrine and metabolic environment.

Our results are consistent with findings that show an inverse association between regular physical activity and serum concentration of inflammatory markers, suggesting that physical activity may have anti-inflammatory properties.[54,55] The most sedentary women in our analysis had slightly higher mean hs-CRP levels, and LTPA was inversely

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associated with total depression. These findings agree with previous suggestions of physical activity as a prevention or treatment for depression.[56,57] Although little to no LTPA appeared to increase the odds of depression in this cross-sectional study, we cannot rule out a possible reverse causation, where those with high depression scores are more sedentary. Suarez et al (2013) found that high depression scores may inhibit the anti-inflammatory effects of LTPA.[29]

Our study has important strengths, including a large, diverse sample of women representative of the US population, the use of a validated, multidimensional depressive symptom measure, and the testing of multiple candidate mediators such as BMI, physical activity, and sleep. We also adjusted for sleep duration to avoid potential confounding by conditions such as sleep apnea or insomnia. Furthermore, we excluded those who are currently taking antidepressants to prevent nondifferential misclassification of outcome, given that some of the common side effects of antidepressants reported by patients are the very symptoms that are used to measure depression, such as insomnia, hypersomnia, agitation, restlessness, fatigue, somnolence, weight gain or weight loss, and decreased or decreased appetite.[58] This overlap of antidepressant side-effects and depression symptoms provides a compelling reason for analyzing symptoms separately from total depression scores.[15]

Our study also has important limitations. First, due to the cross-sectional nature of NHANES study design, we were unable to determine the directionality of the association between hs-CRP and depression symptoms. It is reasonable to speculate that the impact of depression may affect inflammation-reducing activities, such as healthy eating or exercise, and may lead to increased adiposity through weight gain. Prospective studies are

warranted to establish directionality and experimental models are needed to understand causality. The lack of additional markers of inflammation can also be considered a limitation; however, CRP is considered the best marker of systemic inflammation.[59] In healthy young adults CRP is relatively stable, with a median concentration of around 0.8 mg/L, a 90th percentile of around 3 mg/L and a 99th percentile of approximately 10 mg/L.[60] We excluded those with CRP levels higher than 10 mg/L, in order to avoid spurious associations between depression and acute inflammation due to current infections. Although CRP levels tend to remain stable in the general population, they can vary significantly across the menstrual cycle, as reported by Gaskins et al (2012), who observed a larger portion of women with CRP levels above 3 mg/L during menses compared to other phases of the menstrual cycle.[61] There is evidence that inflammation markers are associated with menstrual symptoms and PMS,[62] so it may also help to standardize CRP measurements to menstrual cycle phase in reproductive aged women. In addition, depending on the phase of the menstrual cycle our participants were currently in when they were interviewed, their symptoms of depression as measured by the PHQ-9 may have been higher or lower if they experienced pre-menstrual syndrome. These issues of misclassification of exposure or outcome may have biased our results towards the null. Data on menstrual cycle phase would have allowed us to discern whether depression symptoms were related to hormonal changes or PMS. And finally, this survey from a non-institutionalized population may represent only less severe depression, because those who may be experiencing severe depression may either be institutionalized or disproportionately chose not to participate in the survey. Therefore, our results might not be generalizable to women with severe depression or major depressive disorder.

7.6 Conclusion

In summary, we found that the relationship between CRP and depressive symptoms varies by BMI; among those who are underweight, CRP was positively associated with depressive symptoms. Furthermore, the relationship between inflammation and changes in appetite and sleep disturbance may underlie previous findings that somatic depression is more prevalent among women. The current study expands our understanding of how systemic inflammation may contribute to depression symptoms among women of reproductive age. Understanding how inflammation and BMI may affect depression symptoms among women can help identify potential novel treatment avenues that may benefit from changes in lifestyle to promote greater efficacy and remission.

Table 4: Distribution of covariates by depression in women of reproductive age from NHANES 2005-2008 (N=1489)														
	Total Population		Total PHQ-9 Score (range 0-27)			Somatic Depression Score (range 0-12)			Non-Somatic Depression Score (range 0-15)			High Depression Score (PHQ-9 score ≥10)			
												No		Yes	
	N	%	Mean	SE	p-value	Mean	SE	p-value	Mean	SE	p-value	N	%	N	%	p-value
Total population sample	1489		2.9	0.1	--	1.8	0.1	--	1.1	0.1	--	1357	92.5	132	7.5	--
C-reactive protein (mg/L)*	1.3	1.2, 1.4		--			--			--		1.3	1.2, 1.4	1.6	1.3, 1.9	0.09
Age, years (mean, SE)	31.7	0.3		--			--			--		31.7	0.3	32	0.8	0.48
Sleep per night, hours (mean, SE)	6.9	0.1		--			--			--		7.0	0.1	6.1	0.2	<0.01
Body Mass Index (kg/m2)					0.06			0.13			0.08					0.43
less than 18.5	49	3.5	2.5	0.5		1.6	0.3		0.9	0.2		47	97.1	2	2.9	
18.5 to 24.9	593	44.4	2.6	0.1		1.7	0.1		1.0	0.1		543	93.3	50	6.7	
25-29.9	423	26.3	3.0	0.3		1.9	0.2		1.1	0.1		385	91.5	38	8.5	
30 or greater	422	25.9	3.4	0.3		2.1	0.1		1.4	0.1		380	91.4	42	8.6	
p-trend					0.01			0.02			0.01					
Marital Status					0.12			0.15			0.08					2E-04
Single/Never Married	553	29.2	3.0	0.2		1.9	0.1		1.0	0.1		508	93.6	45	6.4	
Married/with partner	783	60.4	2.7	0.1		1.7	0.1		1.0	0.1		718	93.5	65	6.5	
Divorced/Separated/Widowed	153	10.2	4.0	0.5		2.4	0.3		1.7	0.3		131	83.5	22	16.5	
Education					<0.001			<0.001			<0.001					<0.01
Less than Highschool	369	15.6	4.1	0.3		2.4	0.2		1.7	0.1		317	86.0	51	14.0	
Highschool or GED	354	20.6	3.5	0.3		2.1	0.2		1.4	0.2		317	89.1	37	10.9	
Some college	482	36.0	2.9	0.2		1.8	0.1		1.1	0.1		446	93.0	36	7.0	
College graduate	284	27.8	2.0	0.2		1.4	0.1		0.6	0.1		276	97.9	8	2.1	
p-trend					<0.01			<0.001			<0.001					
Race/Ethnicity					0.27			0.37			0.27					0.26
Non-Hispanic White	557	63.5	2.8	0.2		1.8	0.1		1	0.1		508	92.9	49	7.1	
Mexican Hispanic	364	10.2	3.2	0.2		1.9	0.1		1.3	0.1		336	92.5	28	7.5	
Other Hispanic	141	6.6	3.6	0.4		2.1	0.2		1.5	0.2		122	89.4	19	10.6	
Non-Hispanic Black	348	12.6	3.4	0.3		2.1	0.1		1.3	0.1		315	89.7	33	10.3	
Other Race	79	7.2	2.9	0.3		1.7	0.2		1.2	0.1		76	96.2	3	3.8	
Oral Contraceptive use					0.01			0.05			0.01					0.09
Not currently using	1231	80.8	3.0	0.1		1.9	0.1		1.2	0.1		1118	92.0	113	8.0	
Currently using	213	19.2	2.3	0.3		1.5	0.2		0.8	0.2		201	95.5	12	4.5	
Smoke					<0.001			<0.001			<0.001					<0.01
Not current smokers	1126	76.3	2.5	0.1		1.6	0.1		0.9	0.1		1057	95.3	69	4.7	

	n	%			p			p			p	n	%	n	%	p
Current smokers	324	23.7	4.4	0.4		2.7	0.2		1.8	0.2		263	83.4	61	16.6	
Alcohol Consumption					0.57			0.63			0.81					0.24
none	386	24.3	2.8	0.2		1.8	0.1		1.1	0.1		351	92.5	35	7.6	
<1 drink/day	901	75.0	3.0	0.2		1.9	0.1		1.1	0.1		818	92.4	83	7.6	
>1 drink/day	10	0.7	4.4	2.0		2.4	1.0		2.0	1.0		6	75.8	4	24.2	
p-trend					0.47			*0.43*			*0.56*					
Leisure Time Physical Activity					<0.001			<0.001			<0.001					<0.01
Q1 (0-64 MET minutes/week)	386	25.1	4.2	0.3		2.5	0.2		1.7	0.2		321	84.5	65	15.5	
Q2 (65-594 MET min/week)	302	24.8	2.5	0.2		1.6	0.1		0.9	0.1		283	95.2	19	4.9	
Q3 (595-1560 MET min/week)	270	23.9	2.3	0.2		1.6	0.1		0.8	0.1		258	96.3	12	3.7	
Q4 (>1560 MET min/week)	321	26.3	2.2	0.3		1.4	0.2		0.8	0.1		306	95.9	15	4.1	
p-trend					<0.001			<0.001			<0.001					

Weighted regression and t-tests for continuous depression scores and design-based Pearson chi square for categorical depression variables. *Geometric mean and 95% CI for ln-transformed CRP.

Table 5: Distribution of covariates by inflammation (hs-CRP) in women of reproductive age from NHANES 2005-2008 (N=1,489)										
	hs-CRP*			hs-CRP Category						
				CRP<1mg/L		1<CRP<3mg/L		3<CRP<10mg/L		
	Geometric mean	95% CI	p-value	N	%	N	%	N	%	p-value
Total population sample	1.3	1.16, 1.42	--	553	38.7	492	33.6	444	27.8	--
Depression Score (PHQ-9)		--		2.6	0.2	2.9	0.2	3.4	0.3	<0.05
Age, years (mean, SE)		--		31.9	0.4	31.9	0.6	32.6	0.4	0.42
Sleep per night, hours (mean, SE)		--		7.1	0.1	6.9	0.1	6.8	0.1	0.05
Body Mass Index (kg/m²)			<0.001							<0.001
less than 18.5	0.5	0.33, 0.78		32	72.6	12	20.9	5	6.5	
18.5-24.9	0.8	0.66, 0.87		336	56.6	172	29.6	85	13.8	
25-29.9	1.6	1.9, 1.75		137	31.6	171	41.3	115	27.0	
30 or greater	3.0	2.63, 3.40		48	10.5	135	34.1	239	55.4	
p-trend			<0.001							
Marital Status			0.10							0.66
Single/Never Married	1.3	1.14, 1.47		221	38.8	175	34.3	157	29.2	
Married/Living with partner	1.3	1.10, 1.43		283	39.6	260	32.4	240	27.8	
Divorced/Separated/Widowed	1.5	1.27, 1.79		49	32.6	57	38.2	47	29.2	
Education			0.005							0.002
Less than High school	1.6	1.35, 1.95		122	30.5	117	34.3	129	35.2	
High school or GED	1.5	1.24, 1.71		128	36.5	119	32.2	107	31.4	
Some college	1.3	1.12, 1.47		176	36.1	165	35.9	141	28.0	
College graduate	1.0	0.88, 1.23		126	48.00	91	31.3	67	20.7	
p-trend			<0.001							
Race/Ethnicity			0.009							0.05
Non-Hispanic White	1.3	1.10, 1.44		216	39.3	195	34.7	146	26.1	
Mexican Hispanic	1.6	1.33, 1.99		118	31.6	121	34.2	125	34.2	
Other Hispanic	1.4	0.99, 2.09		52	36.1	47	30.3	42	33.7	
Non-Hispanic Black	1.5	1.33, 1.71		126	34.8	105	30.3	117	34.9	
Other Race	0.8	0.58, 1.10		41	52.6	24	31.6	14	15.8	
Oral Contraceptive use			<0.001							<0.001
Not current user	1.1	1.02, 1.27		501	43.3	394	31.7	336	25.0	
Current user	2.1	1.73, 2.49		42	21.0	82	40.8	89	38.2	

	Mean	95% CI	p	n	%	n	%	n	%	p
Smoke			0.13							0.49
Not a current smoker	1.3	1.12, 1.40		425	39.5	369	32.9	332	27.7	
Current smoker	1.4	1.22, 1.62		109	35.7	114	36.1	101	28.2	
Alcohol Consumption			0.09							0.02
None	1.6	1.29, 1.91		121	33.9	120	29.8	145	36.3	
<1 drink/day	1.2	1.09, 1.37		339	39.8	318	34.8	244	25.3	
>1 drink/day	1.4	0.73, 2.64		3	33.2	5	52.2	2	14.6	
p-trend			*0.04*							
Leisure Time Physical Activity			0.05							0.03
Q1 (0-64 MET min/week)	1.5	1.33, 1.78		116	29.4	144	38.7	126	32.0	
Q2 (65-594 MET min/week)	1.1	0.88, 1.42		126	44.7	90	30.0	86	25.3	
Q3 (595-1560 MET min/week)	1.2	1.01, 1.39		113	43.3	84	33.3	73	23.4	
Q4 (>1560 MET min/week)	1.2	1.05, 1.37		125	40.8	110	34.5	86	24.8	
p-trend			*0.02*							

*ln-transformed hs-CRP, with geometric means and 95% confidence intervals. Weighted regression and t-test for continuous hs-CRP and chi square test for categorical CRP.

	Depression defined as:					
	Total PHQ-9 score (range 0-27)		**Somatic Depression score (range 0-12)**		**Non-somatic Depression score (range 0-15)**	
Per 1 S.D. increase in hs-CRP:	**β (95% CI)**	**p-value**	**β (95% CI)**	**p-value**	**β (95% CI)**	**p-value**
Unadjusted Model	0.32 (0.10, 0.54)	0.006	0.17 (0.04, 0.30)	0.01	0.14 (0.04, 0.25)	0.01
Model 1	0.24 (0.02, 0.46)	0.03	0.13 (0.006, 0.26)	0.04	0.11 (0.002, 0.22)	0.05
Model 2	0.11 (-0.16, 0.37)	0.43	0.06 (-0.11, 0.22)	0.48	0.05 (-0.08, 0.18)	0.42

Table 6: Association of total depression score and depression subtypes with inflammation in women of reproductive age from NHANES 2005-2008 (n=1489)

Model 1 adjusted for age, race/ethnicity, education, marital status, smoking and oral contraceptive use. Model 2 includes those variables adjusted in Model 1 plus sleep, leisure-time physical activity and body mass index categories.

Figure 1. Linear regression analysis assessing mediators/confounders of the relationship between hs-CRP and (a) total depression score, (b) somatic depression score and (c) non-somatic depression score among 1489 women of reproductive age from NHANES 2005-2008. The y-axis represents the percent change in the effect of hs-CRP upon inclusion of potential mediators into model. Unadjusted β for total depression (0.32, p=0.006), somatic (0.17, p=0.01) and non-somatic (0.14, p=0.01).

Portion of total effect of hs-CRP on (a) total depression scores, (b) somatic depression depression, and (c) nonsomatic depression that is mediated by the following:

BMI: -44.8% -43.8% -45.8%
LTPA: -11.4% -10.7% -12.3%
Sleep: -16.9% -20.7% -12.4%

■ Total depression ■ Somatic depression □ Nonsomatic depression
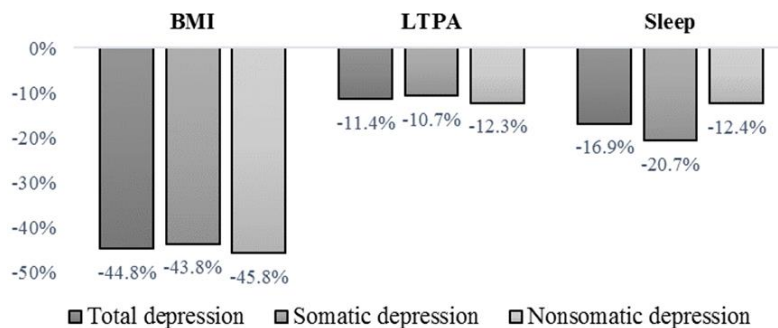
β for hs-CRP z-score for:	Crude Model	Including BMI	Including LTPA	Including Sleep
(a) Total Depression:	0.32 (p=0.006)	0.17 (p=0.19)*	0.19 (p=0.11)	0.26 (p=0.02)*
(b) Somatic depression:	0.17 (p=0.01)	0.10 (p=0.25)*	0.11 (p=0.09)	0.14 (p=0.03)*
(c) Non-somatic Depression	0.14 (p=0.01)	0.08 (p=0.21)	0.08 (p=0.20)	0.13 (p=0.02)*

*p<0.05 for Preacher and Hayes test of indirect effects.

Table 7: Association of total depression score and depression subtypes with inflammation in women of reproductive age from NHANES 2005-2008 stratified by Body Mass Index categories

Per 1 S.D. increase in hs-CRP:	Depression defined as:					
	Total PHQ-9 score (range 0-27)		Somatic Depression score (range 0-12)		Non-somatic Depression score (range 0-15)	
	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value
BMI <18.5 kg/m²						
Unadjusted	0.51 (-0.08, 1.10)	0.09	0.27 (-0.18, 0.73)	0.23	0.24 (-0.10, 0.57)	0.16
Adjusted	1.60 (0.74, 2.47)	0.001	1.07 (0.47, 1.66)	0.001	0.49 (0.15, 0.84)	0.007
BMI 18.5-24.9 kg/m²						
Unadjusted	0.15 (-0.18, 0.49)	0.36	0.08 (-0.16, 0.32)	0.51	0.08 (-0.06, 0.21)	0.27
Adjusted	0.01 (-0.34, 0.36)	0.95	-0.03 (-0.23, 0.16)	0.72	0.05 (-0.14, 0.24)	0.60
BMI 25-29.9 kg/m²						
Unadjusted	0.13 (-0.39, 0.64)	0.62	0.06 (-0.22, 0.35)	0.65	0.06 (-0.20, 0.32)	0.64
Adjusted	0.15 (-0.55, 0.86)	0.66	0.06 (-0.35, 0.47)	0.77	0.09 (-0.24, 0.43)	0.58
BMI ≥30 kg/m²						
Unadjusted	0.20 (-0.41, 0.80)	0.51	0.12 (-0.25, 0.49)	0.52	0.08 (-0.23, 0.39)	0.60
Adjusted	0.29 (-0.40, 0.98)	0.40	0.24 (-0.12, 0.60)	0.19	0.03 (-0.34, 0.41)	0.85

Adjusted models include age, race/ethnicity, marital status, education, smoking, oral contraceptive use, physical activity, sleep and continuous body mass index.

Table 8: Distribution of c-reactive protein and CRP categories by dichotomized individual depression symptoms among women										
	Serum hs-CRP*			**C-Reactive Protein Categories**						
				<1 mg/L		**1-3mg/L**		**3-10 mg/L**		
	Mean	**95% CI**	**p-value**	**N**	**%**	**N**	**%**	**N**	**%**	**p-value**
Trouble sleeping**			0.009							0.07
No	1.24	(1.1, 1.4)		492	91.4	421	86.1	380	86.3	
Yes	1.70	(1.4, 2.1)		61	8.7	71	13.9	64	13.7	
Fatigue			0.31							0.56
No	1.27	(1.1, 1.4)		464	85.1	418	85.4	360	82.4	
Yes	1.41	(1.2, 1.7)		89	14.9	74	14.6	84	17.6	
Abnormal appetite**			<0.001							<0.001
No	1.24	(1.1, 1.4)		508	93.2	454	93.2	388	86.3	
Yes	1.85	(1.6, 2.2)		45	6.8	38	6.8	56	13.7	
Moving or speaking too slow or too fast			0.09							0.35
No	1.28	(1.2, 1.4)		539	98.2	475	96.9	427	96.6	
Yes	1.75	(1.3, 2.5)		14	1.8	17	3.1	17	3.4	
Anhedonia			0.35							0.10
No	1.28	(1.2, 1.4)		526	96.4	459	94.2	418	95.3	
Yes	1.4	(1.1, 1.8)		27	3.6	33	5.8	26	4.7	
Depressed mood			0.17							0.39
No	1.27	(1.1, 1.4)		516	95.3	463	94.2	407	92.7	
Yes	1.56	(1.2, 2.0)		37	4.7	29	5.8	37	7.3	
Low self-esteem			0.27							0.48
No	1.27	(1.2, 1.4)		527	96.0	467	94.7	414	94.1	
Yes	1.56	(1.1, 2.2)		26	4.0	25	5.3	30	5.9	
Trouble concentrating			0.18							0.19
No	1.27	(1.2, 1.4)		530	96.2	472	96.7	418	93.9	
Yes	1.66	(1.1, 2.4)		23	3.8	20	3.3	26	6.1	
Suicidal ideation			0.29							0.31
No	1.28	(1.2, 1.4)		545	99.4	488	99.6	436	98.8	
Yes	1.83	(0.9, 3.7)		8	0.6	4	0.4	8	1.2	

Weighted t-tests and design-based Pearson chi square for categories of CRP. *Natural log transformed hs-CRP. **Significant trend test for weighted proportions.

Table 9: Linear regression of total depression score and individual depression symptoms with hs-CRP in women of reproductive age from NHANES 2005-2008

Per 1 S.D. increase in hs-CRP:	Total PHQ-9 score		Somatic Depression Symptoms							
			Sleep disturbance		Fatigue*		Abnormal appetite*		Psychomotor abnormalities	
	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value
Unadjusted	0.38 (0.14, 0.62)	0.003	0.06 (0.002, 0.11)	0.04	0.02 (-0.04, 0.08)	0.51	0.07 (0.03, 0.11)	0.001	0.03 (0.003, 0.05)	0.03
Model 1	0.15 (-0.11, 0.42)	0.25	0.04 (-0.02, 0.10)	0.21	0.01 (-0.06, 0.09)	0.72	-0.009 (-0.06, 0.04)	0.73	0.01 (-0.02, 0.04)	0.44
Model 2	-0.27 (-0.99, 0.46)	0.46	0.09 (-0.16, 0.34)	0.46	-0.27 (-0.55, 0.02)	0.07	-0.23 (-0.41, -0.04)	0.02	0.03 (-0.07, 0.14)	0.52
Model 3	--		0.03 (-0.02, 0.08)	0.27	-0.0004 (-0.06, 0.06)	0.99	-0.02 (-0.07, 0.02)	0.25	0.007 (-0.03, 0.04)	0.69
Per 1 S.D. increase in hs-CRP:	Non-somatic Depression Symptoms									
	Anhedonia		Depressed mood		Low Sef-esteem		Trouble concentrating		Suicidal Ideation	
	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value
Unadjusted	0.03 (0.006, 0.06)	0.02	0.04 (0.001, 0.07)	0.05	0.03 (-0.01, 0.07)	0.17	0.04 (0.009, 0.07)	0.01	0.008 (-0.004, 0.02)	0.18
Model 1	0.01 (-0.02, 0.04)	0.63	0.002 (-0.04, 0.05)	0.92	0.01 (-0.04, 0.06)	0.66	0.02 (-0.01, 0.05)	0.26	0.01 (-0.005, 0.03)	0.17
Model 2	-0.008 (-0.14, 0.13)	0.91	0.06 (-0.13, 0.24)	0.53	-0.08 (-0.26, 0.11)	0.41	-0.07 (-0.20, 0.06)	0.30	0.04 (-0.02, 0.09)	0.16
Model 3	-0.003 (-0.03, 0.02)	0.83	-0.01 (-0.04, 0.02)	0.56	-0.001 (-0.04, 0.04)	0.96	0.01 (-0.02, 0.04)	0.47	0.01 (-0.006, 0.03)	0.22

β= standardized beta coefficient for increase in 1 standard deviation in log-transformed hs-CRP. Model 1 is adjusted for age, race/ethnicity, marital status, education, smoking, oral contraceptive use, sleep, antidepressant use, leisure-time physical activity, and body mass index. Model 2 is model 1 plus an interaction term for BMIxCRP. Model 3 is additionally adjusted for the sum of the remaining depression items (mutually adjusted associations). *Interaction term for BMIxCRP is statistically significant

Table 10: Logistic regression of association of total depression score and individual depression symptoms with hs-CRP in reproductive-aged women from NHANES 2005-2008

Per 1 S.D. increase in hs-CRP:	High depression score		Somatic Depression Symptoms							
			Sleep disturbance		Fatigue		Abnormal appetite		Psychomotor abnormalities	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Unadjusted	1.18 (0.98, 1.41)	0.07	1.32 (1.07, 1.62)	0.01	1.1 (0.91, 1.32)	0.31	1.42 (1.21, 1.67)	<0.001	1.31 (0.94, 1.85)	0.11
Model 1	--		1.23 (0.98, 1.5)	0.07	0.94 (0.73, 1.22)	0.64	1.36 (1.12, 1.64)	0.002	1.12 (0.72, 1.73)	0.61
Model 2	--		1.31 (1.01, 1.71)	0.05	1.03 (0.71, 1.48)	0.88	0.91 (0.68, 1.22)	0.53	1.03 (0.62, 1.73)	0.90

Per 1 S.D. increase in hs-CRP:	Non-somatic Depression Symptoms									
	Anhedonia		Depressed mood		Low Self-esteem		Trouble concentrating		Suicidal Ideation	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Unadjusted	1.10 (0.90, 1.34)	0.36	1.19 (0.92, 1.54)	0.18	1.19 (0.86, 1.64)	0.29	1.25 (0.88, 1.79)	0.20	1.37 (0.72, 2.61)	0.33
Model 1	0.88 (0.70, 1.11)	0.28	1.02 (0.74, 1.40)	0.90	0.99 (0.67, 1.48)	0.97	1.11 (0.74, 1.66)	0.60	1.13 (0.54, 2.34)	0.74
Model 2	0.91 (0.67, 1.24)	0.55	0.99 (0.69, 1.42)	0.94	1.19 (0.74, 1.92)	0.46	1.14 (0.78, 1.66)	0.49	1.82 (0.97, 3.40)	0.06

Model 1 is mutually adjusted for the sum of other depressive symptoms. Model 2 is model 1 plus age, race/ethnicity, marital status, education, smoking, oral contraceptive use, sleep duration, leisure-time physical activity, and body mass index.

Figure 3: The effect of BMI on abnormal appetite score (item 5 of the PHQ-9) for women with no inflammation (CRP=0.4 mg/L), low and moderate inflammation (CRP=1.9mg/L and CRP=5.6mg/L, respectively).
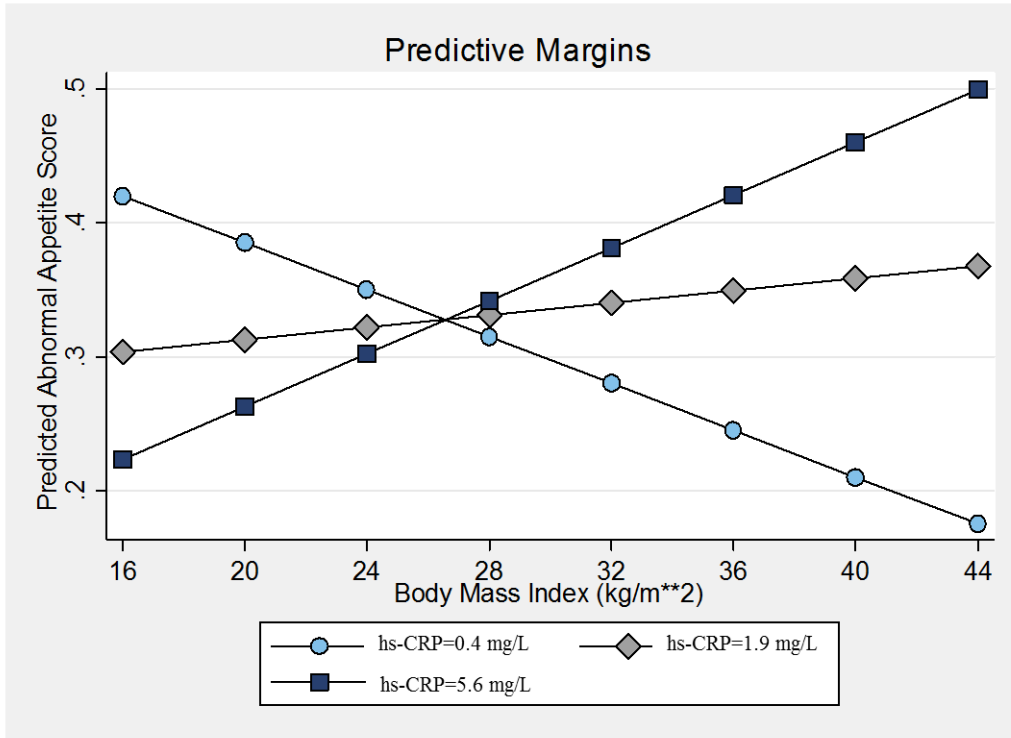
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"THE ASSOCIATION BETWEEN SERUM PYRIDOXAL 5'-PHOSPHATE AND DEPRESSION VARIES BY INFLAMMATION AND DEPRESSION SYMPTOMS AMONG WOMEN OF REPRODUCTIVE AGE FROM NHANES 2005-2008"

8.1 Abstract

Depression is the leading cause of disease burden in U.S. women of reproductive age. Inflammation is associated with depression, yet the mechanism for this association remains unclear. Vitamin B_6 status is reportedly compromised during inflammation and has been linked to depression. In secondary data analyses, we evaluated the association between vitamin B_6 status, inflammation, and depression in 1,489 non-pregnant women (18-44 yrs) from NHANES 2005-2008. Depression scores were calculated based on the Patient Health Questionnaire-9 (PHQ-9) and categorized into total depression, somatic and non-somatic depression. High depression score (PHQ-9 ≥10) was also used as an outcome, as were individual symptoms of depression. Vitamin B_6, as serum pyridoxal-5'phosphate (PLP), was categorized as deficient (<20 nmol/L), insufficient (20-29.9 nmol/L) or normal (≥30 nmol/L). Low inflammation was defined as a C-reactive protein (CRP) concentrations between 1-3 mg/L and moderate inflammation as CRP between 3-10 mg/L. Overall, 7.5% of women had a high depression score, but this proportion varied by inflammation and vitamin B_6 status. When stratified by CRP categories, the prevalence of depression was higher among those with vitamin B_6 deficiency (21%) than among those with vitamin B_6 insufficiency (10%) or those with normal vitamin B_6 status (5%), although this trend was only significant among those with moderate inflammation (p-trend=0.02). A similar trend

of increased depression with lower vitamin B_6 concentration was observed among women with CRP levels between 1-3 mg/L, but not among those with CRP <1 mg/L. In multivariable models including individual depression symptoms, PLP levels below 30 nmol/L were associated with higher odds of experiencing suicidal ideation (OR: 7.33, p=0.01 and OR: 3.5, p=0.06 for B_6 deficiency and insufficiency, respectively) and depressed mood (OR: 3.12, p=0.004 for B_6 insufficiency). Among those with elevated CRP, vitamin B_6 deficiency was associated with a higher score for psychomotor abnormalities (p=0.02). Understanding the mechanism linking inflammation and vitamin B_6 to various dimensions of depression could lead to new approaches to prevent and treat depression.

8.2 Introduction

Depression is common in the United States; approximately 16% of adults experience clinical depression at some point in their lives.[1] Women are two- to three-times more likely than men to experience depression,[2,3] and among women, depression is the leading source of disease burden.[2-4] Numerous risk factors for depression have been identified, among them vitamin B_6 deficiency.[5-8] Population-based studies estimate that a quarter of U.S. women of childbearing age have suboptimal vitamin B_6 concentrations, and the prevalence may be even higher among women currently taking oral contraceptives (78%).[9] In addition to a potential link with depression, these numbers are alarming considering that suboptimal maternal vitamin B_6 status has been shown to adversely affect other health outcomes, including conception rates[10] and pregnancy complications such as spontaneous abortions,[11] and may affect the neurological development of the offspring.[12]

Serum pyridoxal 5'-phosphate (PLP), the bioactive form of vitamin B_6, serves as a coenzyme in numerous metabolic reactions, including the tryptophan-serotonin pathway, and a lack of vitamin B_6 may lead to depression by reducing serotonin levels.[13] Studies examining dietary vitamin B_6 alone have yielded conflicting results regarding the association between vitamin B_6 intake and depression.[14,15] However, studies assessing serum PLP and depression have consistently shown an inverse association.[6-8] To date, most studies of vitamin B_6 and depression have been limited by small sample size, use of an elderly population, heterogeneity in the measures of depression, or failure to account for inflammatory biomarkers. Inflammation is an important consideration because, in addition to functioning as a cofactor in neurotransmitter synthesis, vitamin B_6 plays an important role in the production of cytokines, the primary mediators of inflammation.[16] Lower serum PLP concentrations are associated with inflammation,[17,18] which may not necessarily be linked to dietary vitamin B_6 inadequacy in some cases, but rather could be due to metabolic phenomena inherent to inflammation.[19] During inflammation, tryptophan metabolism shifts from serotonin synthesis to generation of neuroactive metabolites, such as quinolinic acid and kynurenic acid, through the kynurenine pathway.[20] This shift in utilization of vitamin B_6 could potentially contribute to depression. To date, no studies have investigated how inflammation may affect the association between vitamin B_6 status and depression.

The purpose of this study was to further explore the relationship between vitamin B_6 status and depression among U.S. reproductive-age women and to determine whether high sensitivity C-reactive protein (hs-CRP), a marker of inflammation, modifies the association between vitamin B_6 and depression subtypes or individual symptoms of depression among reproductive-age women.

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8.3 Methods

8.3.1 Study Design and Study Population

We analyzed cross-sectional data from the 2005-2008 National Health and Nutrition Examination Survey (NHANES), which is national survey from the National Center for Health Statistics of the Centers for Disease Control and Prevention (CDC), designed to assess the health and nutritional status of the U.S. population. The NHANES employs a complex, multistage, probability sampling design to select persons representative of the civilian, non-institutionalized US population, with oversampling of special sub-populations, such as racial and ethnic minorities. Detailed descriptions of the survey design and procedures are available at the NHANES website.[21]

Briefly, approximately 5,000 people are surveyed each year via in-person interviews and physical examinations. The interview, which is conducted in participants' homes, includes demographic, socioeconomic, dietary, and health-related questions; the examination component is comprised of medical, dental, and physiological measurements, as well as laboratory tests, which take place in specially-designed and equipped mobile examination centers (MEC) that travel throughout the country. The National Center for Health Statistic's Institutional Review Board has reviewed and approved the NHANES protocol.

Participants were eligible for our study if they were women of reproductive age (15-44 years old) who were not pregnant. From the initial sample of 2,609 eligible women, we excluded those with missing data for either depression (n=506) or serum pyridoxal 5'-phosphate (n=7). Depression data for individuals younger than 18 years old was not publicly available; therefore, our age range was restricted to women ages 18-44 years old.

Also excluded were women with acute inflammation denoted by CRP levels above 10 mg/L

(n=201),[22] those who were taking anti-inflammatory medications, including NSAIDs

(n=70), and those who reported current antidepressant use (n=135). The remaining

sample contained 1,489 women.

8.3.2 Assessment of Depression Status

The Patient Health Questionnaire-9 (PHQ-9)[23] was administered during the MEC

interview to assess depressive symptom severity over the past two weeks. Based on the

diagnostic criteria for major depressive disorder in the Diagnostic and Statistical Manual

Fourth Edition (DSM-IV), the PHQ-9 employs a 4-point scale (0=not at all, 1= several days,

2= more than half the days, 3= nearly every day) to determine the frequency with which

respondents experienced the following nine symptoms of major depressive disorder:

anhedonia, depressed mood, sleep disturbance, fatigue, appetite changes, low self-esteem,

concentration problems, psychomotor retardation/agitation, and suicidal ideation. Total

depression scores range from 0 to 27, with scores of 10 or higher indicative of clinical

depression.[24] The PHQ-9 has been shown to be a reliable and valid questionnaire as

indicated by its high internal consistency and good sensitivity (88%) and specificity (88%)

for identifying cases of major depressive disorder in community samples.[25–27]

In addition to the total depression score, we calculated somatic and cognitive-

affective depression subscale scores. The somatic depression subscale was calculated by

adding the scores for frequency of somatic depression symptom from the PHQ-9 (sleep

disturbance, fatigue, appetite changes, and psychomotor retardation/agitation) and the

cognitive-affective subscale score was calculated adding the scores for non-somatic

symptoms (anhedonia, depressed mood, low self-esteem, concentration problems and suicidal ideation).[28] While previous factor analyses supported a one-factor model with all nine items,[29,30] recent confirmatory factor analyses have found that subscales of depression dimensions may provide a better fit to the data.[31,32] Some have also argued that given the heterogeneity of symptoms for depression, individual symptoms should be analyzed.[33] We included a separate analysis for individual depressive symptoms, coded as continuous variables (scores ranging from 0-3) and as binary variables. To dichotomize the variable, those items with responses "more than half the days" and "nearly every day" indicated the presence of the symptom.[34,35]

8.3.3 Serum Pyridoxal 5'-Phosphate

Fasting whole blood samples were collected during the medical examination. Venipuncture was performed using standard phlebotomy techniques by NHANES trained personnel. Serum specimens were processed, stored and shipped to the Division of Environmental Health Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention in Atlanta, Georgia. Further detailed instructions on specimen collection and processing can be found at NHANES website.[21] Briefly, serum PLP, the main bioactive form of vitamin B_6, was measured using reverse-phase high performance liquid chromatography (HPLC) analysis using fluorometric detection at 325nm excitation and 425nm emission. Quantitation of PLP was based on analyte peak area interpolated against a five-point calibration curve from aqueous standards. This HPLC assay has a lower limit of detection compared to the enzymatic assay used in previous NHANES cycles (0.3 vs 10 nmol/L).[21] Given that PLP was not normally

distributed, PLP measures were converted to their natural logarithm to improve normality. Serum PLP was also divided into categories indicating vitamin B_6 deficiency (PLP <20nmol/L), insufficiency (PLP 20 to 29.9 nmol/L) and sufficiency (PLP ≥30 nmol/L) following previously used cut-offs for vitamin B_6 status.[36–38]

8.3.4 Covariates and Confounders

We considered as possible covariates a selected set of demographic, lifestyle, and socioeconomic factors using responses to the demographic and household sections of the NHANES questionnaire. The National Center for Health Statistics (NCHS) standard definitions for ethnicities were used; ethnicities were categorized as non-Hispanic whites, non-Hispanic blacks, Mexican-Hispanics, other Hispanics, and other race. Educational attainment was measured as the highest grade of school completed, and categorized into four levels: less than high school, high school equivalent, some college, and college graduate or above. The smoking assessment for respondents aged 18-19 years (MEC) was not identical to that for respondents aged 20+ years (household interview). For respondents aged 20+ years, we classified those who reported smoking at least 100 cigarettes during their lifetime and reported that they now smoke cigarettes every day or some days as current smokers. For respondents ages 18-19 years, we classified as current smokers those who reported smoking 15 or more day in the last 30 days. To create the alcohol groups, we used responses to the alcohol use interview at the MEC and calculated the drinks per day as suggested by Case and Stewart.[39] Alcohol consumption, which was only available for participants of legal drinking age (21 and older), was categorized into abstainer, one or fewer drinks/day and more than one drink per day. Leisure-time physical activity (LTPA)

was assessed by asking participants about their involvement in 48 specific recreation activities of moderate or vigorous intensity. Frequency was first multiplied by the duration in hours and divided by 4.3 to obtain hours of LTPA per week. To calculate MET hrs/week, each activity was multiplied by its MET level per NHANES physical activity codes.[40] Participants were then categorized into quartiles of physical activity. Sleep duration was assessed by asking participants how many hours of sleep they usually get on a weekday/workday, and those who reported sleeping 12 or more hours were labeled as sleeping 12 hours/night. Oral contraceptive use was determined by self-report of current use, with "past" or "never" users classified as not currently using oral contraceptives (OC).

Body mass index (BMI) and high-sensitivity C-reactive protein (CRP) have been associated with both vitamin B_6 levels and depression[9,17,41,42] and were examined as potential confounders/mediators of the relationship between PLP and depression. BMI (kg/m^2) was computed from height and weight measurements and grouped into four standard categories: underweight (BMI<18.5 kg/m^2), normal weight (18.5 ≤ BMI<25), overweight (25≤ BMI<30) and obese (BMI≥30 kg/m^2). High-sensitivity c-reactive protein was quantified using latex-enhanced nephelometry using a Dade Behring Nephelometer (Dade Behring Diagnostics Inc, Somerville, NJ). CRP was classified into three categories: those with no inflammation (CRP <1 mg/L), low inflammation (CRP 1-3 mg/L) and moderate inflammation (CRP 3-10 mg/L) following previously used guidelines.[22]

8.3.5 Data Analysis

The complex survey design used for NHANES data collection was incorporated into all data analyses using the "svy" command with appropriate weighting in Stata 12.0 (StataCorp LP, College Station, TX). Detailed information on the procedures for taking into account sampling weights have been described elsewhere.[43]

Descriptive statistics were calculated for the overall sample using frequency distributions for categorical variables and means and standard errors for continuous variables. Because both PLP and hs-CRP were logarithmically transformed when analyzed as continuous variables, geometric means (antilogarithms of the transformed means) and their 95% confidence intervals are presented for serum PLP and hs-CRP. Bivariate analyses were carried out for each covariate and ln-transformed PLP and vitamin B_6 categories as well as for covariates and total depression, somatic/non-somatic depression and binary depression variables, using weighted ANOVAs, Chi square tests and 2-sample t-test as appropriate. T-tests and chi-square tests were performed to assess crude differences in PLP and vitamin B_6 status by individual depressive symptom. Adjusted analyses were performed using multivariable linear regression. In linear regression models, all covariates that changed the estimated coefficient for the primary predictor of interest by more than 10% were retained in the multivariable model. In addition, known risk factors for depression were retained in the model as well as those that were associated with both the predictor and the outcome.

To explore variation in the association of vitamin B_6 status with dimensions of depression, we conducted a series of linear regression analyses in which total depression score, somatic depression score, non-somatic depression score and each individual

depression symptom served as an outcome variables. A similar series of logistic regression analyses was also completed for dichotomized individual depressive symptoms. For all the individual depression symptoms, we adjusted for the remaining depression symptoms to account for any potential overlap between different symptoms and to reduce the probability of type I error. Logistic regression analyses were also carried out for depression PHQ-9 score of 10 or greater. The command "mfpigen" was used to assess all possible interactions among variables in the full models.

C-reactive protein and BMI were assessed as potential mediators or effect modifiers by running mediation tests, stratifying regression analyses, and by including multiplicative interaction terms in the multivariable analysis. To quantify the effects of CRP and BMI on any observed relationships between vitamin B_6 status and depression, we carried out Sobel mediation tests and Preacher and Hayes bootstrap tests of indirect effects.[44,45] All linear and logistic regression estimates were weighted using NHANES sample weights, which account for the complex survey design, survey nonresponse and post-stratification.

8.4 Results

Description of Study Cohort. The characteristics of the 1,489 women who comprised our final sample are shown in table 11. The mean age of our sample was 31.7 years. The majority of women were white (63.5%), married or living with a partner (60.4%,), did not use oral contraceptives (80.8%), reported an average of seven hours of sleep per night, and were nonsmokers (76.3%). Almost eight percent (n=132) of women in our sample reported moderate to severe depression symptoms indicative of clinically significant depression, denoted by a score of 10 or higher on the PHQ-9 (Table 11).

In bivariate analysis, depression scores were positively associated with BMI (p-trend <0.02) and hs-CRP (p-trend<0.02) and negatively associated with education (p<0.001) and LTPA (p<0.001) (Table 12). Depression scores were higher for both somatic and non-somatic subtypes among women who were not currently taking oral contraceptives (p=0.05 and p=0.01, respectively) compared to OC users and for smokers (p<0.001) compared to non-smokers. Women with high depression scores slept less (6.1 vs 7.0 hours/night, p<0.001) compared to those with low depression scores. The prevalence of depression defined as a PHQ-9 score of 10 or greater was significantly higher among women who were separated or divorced compared to women who were married/living together with partner or who were single/not married; for those who smoked compared to non-smokers; and those who reported the lowest levels of LTPA compared to the rest of the LTPA quartiles. The prevalence of a high depression score was lower among women who reported current oral contraceptive use compared to non-OC users (4.5% vs 8.0%, p=0.09).

Approximately one third of women (32%) had suboptimal vitamin B_6 levels and 10% percent had biochemical evidence of vitamin B_6 deficiency (PLP<20 nmol/L). Serum PLP was negatively associated with BMI (p-trend <0.001), CRP levels (p-trend <0.001), and smoking dose (p-trend<0.001), and positively associated with LTPA (p-trend <0.001) and education (p-trend<0.001). Those with vitamin B_6 deficiency slept less than those with insufficient and sufficient vitamin B_6 levels (p=0.003). Vitamin B_6 concentration did not vary by age or oral contraceptive use (Table 12).

Association of PLP with depression scores. Mean serum PLP was lower among women who had high depression scores compared to those who had low depression scores (34.4 vs

47.1 nmol/L, p=0.004), and total depression score was higher for women who had vitamin B_6 deficiency compared to those who had insufficiency or sufficiency (4.4 vs 3.2 vs 2.6 points, respectively; p=0.003). In univariate analyses, vitamin B_6 deficiency was associated with a higher total depression score (β: 1.7, p=0.001), somatic depression score (β: 0.99, p<0.001), and non-somatic depression score (β: 0.72, p=0.02). These associations were attenuated and no longer significant after adjustment for demographics, lifestyle and behavioral factors (data not shown).

To assess whether inflammation affects the association between vitamin B_6 status and depression, we first visualized the relationship using bar graphs (Figure 4). Depression scores tended to increase with increasing CRP level, especially among those with vitamin B_6 deficiency (p-trend=0.04). When stratified by CRP categories (<1 mg/L, 1-3 mg/L and 3-10 mg/L), the prevalence of depression was highest among those with vitamin B_6 deficiency, followed by those with vitamin B_6 insufficiency; however, this trend was only significant among those with moderate inflammation (CRP levels 3-10 mg/L ; p-trend=0.02), and borderline significant among those with low inflammation (p-trend=0.07) (Figure 5). A similar pattern was found in linear regression models stratified by CRP levels. Vitamin B_6 deficiency was associated with higher total depression score (β: 1.48, p=0.01 and β:2.52, p=0.01) and somatic depression score (β:0.93, p=0.008; β:1.33, p=0.004) among those with low or moderate inflammation, but not among those with CRP levels below 1 mg/L in unadjusted analyses. Once again, adjustment for covariates completely attenuated these results (data not shown).

To assess whether BMI or CRP were potential mediators or confounders of the association between PLP and depression, we ran a series of Sobel mediation tests followed

by bootstrap analysis for the Preacher and Hayes test of indirect effect.[44,45] For total

depression score, 7.9% of the total effect was significantly mediated by CRP (p=0.045)

(Figure 6); BMI also attenuated the association between PLP and depression variables. For

total depression, the standardized PLP effect size was reduced by 10.8% upon addition of

BMI to the model (p=0.02); the effect of PLP on somatic and non-somatic depression was

also reduced by 9% and 13.5%, and these mediations were significant (p=0.03 and p-0.04,

respectively).

To determine which depression symptoms were associated with vitamin B_6 status,

we estimated the bivariate associations, and then fit a series of linear and logistic

multivariable models for continuous and dichotomized symptoms. In bivariate analysis,

PLP was significantly lower among those experiencing most symptoms of depression,

except for moving or speaking too slow or too fast (p=0.13). The prevalence of vitamin B_6

deficiency was significantly higher among those experiencing most individual depression

symptoms, except for low self-esteem (p=0.25) and trouble concentrating (p=0.17) (Table

13). In linear regression with individual depression symptoms, vitamin B_6 deficiency was

significantly associated with a higher score for most of the symptoms of depression

(sleeping problems β: 0.30, p=0.001; fatigue β: 0.33, 0.001, abnormal appetite β: 0.27,

0.002; anhedonia β:0.18, p=0.03; depressed mood β: 0.22, p=0.008; low self-esteem β: 0.12,

p=0.05; suicidal ideation β: 0.07, p=0.05), but these associations were attenuated after

mutual adjustment and adjustment for demographics, behavioral, and lifestyle factors.

Compared to somatic symptoms, vitamin B_6 deficiency was associated with fewer non-

somatic symptoms in unadjusted analysis among those with low inflammation levels

(anhedonia, low self-esteem) and moderate inflammation (depressed mood and suicidal

ideation) in unadjusted analysis (data not shown). In univariate logistic regression with dichotomized individual symptoms of depression, vitamin B_6 deficiency was significantly associated with all somatic symptoms (Table 14), and some non-somatic symptoms (Table 15). In the fully adjusted models, those with vitamin B_6 deficiency had much higher odds of experiencing suicidal thoughts (OR: 7.33, 95% CI 1.59-33.9), and those with vitamin B_6 insufficiency had three times the odds of experiencing depressed mood (OR: 3.12, 95% CI 1.49-6.52) after adjustments.

High depression score was only associated with vitamin B_6 deficiency in unadjusted analyses (data not shown). There were no significant interactions between any variables and vitamin B_6. When stratified by CRP level, vitamin B_6 deficiency was only crudely associated with higher odds of high depression score among those with CRP levels between 1-10 mg/L (data not shown).

8.5 Discussion

In this large representative sample of reproductive age women, we found that suboptimal vitamin B_6 levels were associated with several dimensions of non-somatic depression, including depressed mood and suicidal ideation. When considering inflammation, the association of vitamin B_6 deficiency with a higher score for the somatic symptom of psychomotor agitation was observed among women with moderate inflammation. Although previous studies have reported an association between lower vitamin B_6 status and increased depressive symptoms,[6–8,15] our study is the first to examine how inflammation may influence the association between vitamin B_6 status and various dimensions of depression among women of reproductive age.

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A role for suboptimal vitamin B_6 levels in depression is supported by three previous studies. The first, conducted in an elderly Danish population, found that plasma PLP concentration was inversely associated with depression score after adjusting for age and gender.[6] A second study examined the association between plasma PLP in an elderly population from the Boston Puerto Rican Study. They found that plasma PLP was inversely associated with depression scores (β: -0.12, p<0.05), with vitamin B_6 deficiency (PLP< 20 nmol/L) associated with an almost doubling of the odds of depression in multivariable models (OR: 1.74, 95% CI 1.02, 2.89).[7] In the most recent study, higher plasma PLP was significantly associated with a decreased prevalence of depression (p-trend: 0.03) among 422 adults ages 21-67 years old in Japan.[8] In cross-sectional analyses, they found that the odds of depression were 46% lower among those in tertiles two and three (PLP >25.5 nmol/L; OR: 0.54, 95% CI 0.31-0.94 and OR: 0.54, 95% CI 0.30-0.96, respectively) compared to those in the lowest tertile of PLP (range: 8.1 to 25.5 nmol/L), after adjusting for numerous covariates (age, gender, office, smoking, alcohol intake, physical activity, job position, marital status, history of cancer, cardiovascular disease, or mental disease, dietary vitamin B_{12} intake, serum folate and homocysteine concentration, and depression score at baseline). However, the association between PLP and depression was no longer significant in longitudinal analyses.[8]

Our results for total depression scores and high depression were only significant prior to adjustments for sociodemographic, behavioral, and lifestyle factors, suggesting that these covariates play a role in the relationship between vitamin B_6 and depression. Because of the cross-sectional design of NHANES, we cannot determine whether higher PLP levels

are protective against depression or whether depression somehow leads to lower vitamin B_6 levels.

When investigating the association between PLP and different subtypes of depression, we found that vitamin B_6 deficiency (PLP <20 nmol/L), but not B_6 insufficiency (20 <PLP <30 nmol/L), was significantly associated with both higher somatic and non-somatic depression scores in unadjusted analysis. This grouping of depression symptoms into subtypes, although shown to be a better fit for data in factor analysis than the total sum score of depression symptoms,[28] may still be masking the association between vitamin B_6 deficiency and specific symptoms of depression. Previously, vitamin B_6 was associated with depression among a Japanese population, but the association only reached statistical significance when using a higher depression cut-off point of 19 instead of the usual cut-off point of 16 in the Center for Epidemiological Studies Depression screener (CES-D).[8] It is possible that low vitamin B_6 levels are only associated with more severe cases of depression. In our analysis using dichotomized symptoms of depression, vitamin B_6 deficiency was associated with higher odds of experiencing suicidal thoughts, a symptom typically experienced by those with severe cases of depression (with PHQ-9 score of 15 or higher).[46] Furthermore, compared to women without suicidal thoughts, those experiencing suicidal ideation also had the lowest mean serum PLP level of all the groups (21 vs 46.3 nmol/L, p<0.001). However, given the wide confidence intervals in regression analyses, these results should be interpreted with caution, and a larger study with more cases of depression may be necessary to get a more precise estimate of effect of vitamin B_6 deficiency on symptoms of severe depression.

Although the pathway linking vitamin B_6 to depression is not entirely understood, a role of vitamin B_6 in tryptophan metabolism has been suggested. Vitamin B_6, as PLP, serves as a coenzyme in the tryptophan-serotonin pathway, and some suggest that a lack of vitamin B_6 may cause depression by reducing serotonin levels.[13] In addition, vitamin B_6 also serves as a key player in the kynurenine pathway, which is thought to play an important role in the pathogenesis of inflammation-dependent depression.[20] The shift in PLP metabolism to the kynurenine pathway from its role in tryptophan-to-serotonin would result in lowered serotonin levels, but the second pathway would also coincide with heightened circulating inflammatory biomarkers. After stratifying by different levels of CRP, in unadjusted models, vitamin B_6 deficiency was associated with most dimensions of depression among those with some degree of inflammation (denoted by CRP >1 mg/L), but not among those with no inflammation (CRP <1mg/L). However, after adjustment for demographic, behavioral and lifestyle factors, most of these associations were attenuated and no longer significant, except for psychomotor retardation/agitation. Vitamin B_6 deficiency was associated with a higher score for psychomotor abnormalities (β: 0.14, p=0.02) among those with elevated CRP (3-10mg/L) compared to those with normal PLP levels. Some evidence indicates that inflammation may be related more to somatic symptoms of depression,[47] which are more pronounced in women than in men, rather than to non-somatic (affective) symptoms.[48] These findings suggest that PLP may play a role in the activation of muscle synergy, which is presumed to be modulated by neural command signals.[49] Pyridoxine is a cofactor in the metabolism and production of many neurotransmitters involved in regulation of mobility, including serotonin, dopamine, and gamma-aminobutyric acid (GABA).[50] Vitamin B_6 deficiency may contribute to impaired motor performance given PLP's indirect role in

neural control of movement. It is possible that this motor impairment may be heightened in the context of very low PLP levels and an additional trigger, such as the presence of inflammation, but no studies to date have examined this possibility. Studies are needed to investigate the kinetics and regulation of vitamin B_6 vitamers and enzymes in different compartments in the presence of inflammation.[51]

Our results suggest that CRP may act as an effect modifier in the association between vitamin B_6 status and depression. This conclusion is supported by our finding that the crude association between vitamin B_6 deficiency and depression was only evident among those who have CRP levels above 1 mg/L, and it was more pronounced among those with CRP levels above 3 mg/L. The question remains as to whether inflammation leads to low PLP levels or whether low PLP levels enhance inflammation, but both scenarios would probably be associated with higher depression scores.

There is evidence supporting an inflammation-to-vitamin B_6 deficiency pathway, since induced vitamin B_6 deficiency did not lead to subsequent inflammation among a group of healthy adults who underwent a 28-day vitamin B_6 restriction study.[52] Although we cannot determine directionality of depression with either vitamin B_6 or inflammation in this cross-sectional study, we found the prevalence of depression (PHQ-9 score of 10 or higher) did not vary significantly based on CRP levels (p=0.15). However, the prevalence of depression did vary by vitamin B_6 status, with a higher prevalence among those with lower B_6 levels (15.4% among those with B_6 deficiency, 8.8% for B_6 insufficiency and 5.9% among those with normal B_6 status, respectively, p=0.004). Although events of depression did not vary significantly by CRP in this population, when stratified by CRP, the prevalence of depression was mostly evident among those with higher levels of CRP and suboptimal B_6

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status. If inflammation depletes circulating PLP levels due to increased demand for B_6 for the kynurenine pathway, then inflammation-reducing treatments may result in improved vitamin B_6 levels, and consequently, improved serotonin levels, which would theoretically translate to lowered rates of depression. Many selective serotonin reuptake inhibitors (SSRI) possess anti-inflammatory properties, and this is thought to be one of the mechanisms by which SSRIs alleviate depression symptoms.[53]

We limited our study to biomarkers of vitamin B_6 because studies examining dietary vitamin B_6 have yielded controversial findings on the association between vitamin B_6 and depression.[14,15,54] However, those assessing circulating PLP concentrations and depression have consistently shown an inverse association between plasma PLP and depression scores. A possible reason for these inconsistencies may be the low correlation between dietary vitamin B_6 intake and plasma PLP that has been consistently observed in various studies.[7,8,55–58] Several non-dietary factors can affect circulating PLP concentration, including body mass index, inflammation, smoking, and oral contraceptive use.[9,56,59,60] In our study, we controlled for all of these variables. Vitamin B_6 supplements, on the other hand, are positively correlated with circulating PLP levels,[9,56,58] confirming that suboptimal vitamin B_6 status can be corrected via supplementation, but the amount of vitamin B_6 intake needed to protect against vitamin B_6 inadequacy may be elevated in the presence of inflammation.[17] Vitamin B_6 supplementation with 50 mg/day of pyridoxine for 30 days may correct vitamin B_6 deficiency,[61] but higher levels (100 mg/day) may be needed to resolve inflammation among individuals with high inflammation levels, such as rheumatoid arthritis patients.[62] Longitudinal studies are needed to determine whether vitamin B_6 supplementation alters inflammation and affects depression symptomatology or severity.

Understanding these relationships will help to determine to what degree vitamin B_6 supplementation could protect against inflammation-associated depression.

Almost a fifth of our sample reported current OC use, and contrary to previous studies,[9,63] oral contraceptive use was not related to a higher prevalence of vitamin B_6 deficiency or depression in our sample. Current OC users had a slightly lower mean depression score and lower prevalence of depression, which is also contrary to recent findings from a large population-based study in the Netherlands, where OC use was associated with increased risk of depression.[63] Our findings are in agreement with a recent randomized controlled trial that found OCs were associated with improvements in depression among young women in the premenstrual phase.[64] The prevalence of vitamin B_6 deficiency was 8.3% among OC users, compared to 11% among non-users (p=0.53), and this is much lower than the calculations from Morris et al (2008) from NHANES 2003-2004 where up to 78% of current OC users had vitamin B_6 deficiency.[9] This could be due to more routine vitamin B_6 supplementation in OC users or analytical changes in PLP assessment between the NHANES 2003-2004 and more recent cycles, where the previously used enzymatic assay was changed to an HPLC method, which has a higher sensitivity.[21] Two more recent studies also failed to observe higher rates of vitamin B_6 deficiency among OC users.[58,65]

Our study has important strengths, including a large, diverse sample of women representative of the US population, the use of a validated, multidimensional depressive symptom measure, and the testing of multiple candidate mediators/confounders. We also adjusted for sleep duration to avoid potential confounding by conditions such as sleep apnea or insomnia. Furthermore, we excluded those who were currently taking

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antidepressants to prevent nondifferential misclassification of outcome, given that some of the common side effects of antidepressants reported by patients are the very symptoms that are used to measure depression, such as insomnia, hypersomnia, agitation, restlessness, fatigue, somnolence, weight gain or weight loss, and decreased or decreased appetite.[66] This overlap of antidepressant side effects and depression symptoms provides a compelling reason for analyzing symptoms separately from total depression scores.[33]

Our study also has important limitations. First, due to the cross-sectional nature of the NHANES study design, we were unable to determine the directionality of the association between PLP and depression symptoms. Both directions are plausible. Of the three previous studies on PLP and depression, no significant prospective associations were found between PLP and depression. One study,[8] however, observed lower depression scores after three years among those with better baseline vitamin B_6 status (both PLP and dietary), suggesting that vitamin B_6 may decrease the risk of depression, rather than low vitamin B_6 level is the result of depression. Additionally, vitamin B_6 can also act as an antioxidant,[67] so it is possible that adequate nutritional status and optimal vitamin B_6 levels could be protective against depression by reducing oxidative stress that may be associated with inflammation-associated depression. And finally, this survey from a non-institutionalized population may only capture those with less severe depression, because those with severe depression may either be institutionalized or disproportionately unlikely to participate in the survey. Therefore, our results might not be generalizable to women with severe depression or major depressive disorder.

8.6 Conclusion

In summary, vitamin B$_6$ deficiency may be associated with symptoms experienced among those with more severe cases of depression in this population, such as suicidal ideation. When stratified by inflammation, we observed a pattern of increasing effect size for vitamin B$_6$ deficiency on individual somatic symptom scores with increasing CRP levels, which was not evident for non-somatic symptoms. Although these patterns were only observed in crude analyses, it remains possible nonetheless that inflammation may affect vitamin B$_6$ levels via the shift in PLP from the tryptophan-serotonin pathway to the kynurenine pathway, and these two coexisting conditions may be contributing to somatic symptoms rather than cognitive/affective symptoms of depression. Inflammation and compromised vitamin B$_6$ status may play important roles in the manifestation of depression, but longitudinal studies are needed to better understand how vitamin B$_6$ or inflammation may affect the development or severity of depression symptoms. The heterogeneity of depression also warrants further research based on symptoms in order to identify potential treatments that may focus on vitamin B$_6$ status for patients with different dimensions of depression.

Table 11: Distribution of covariates by depression in women of reproductive age from NHANES 2005-2008 (N=1,489)															
	Total Population		Total PHQ-9 Score (range 0-27)			Somatic Depression Score (range 0-12)			Non-Somatic Depression Score (range 0-15)			High Depression Score (PHQ-9 score ≥10)			
												No		Yes		
	N	%	Mean	SE	p-value	Mean	SE	p-value	Mean	SE	p-value	N	%	N	%	p-value
Total population sample	1489		2.9	0.1	--	1.8	0.1	--	1.1	0.1	--	1357	92.5	132	7.5	--
Serum PLP (nmol/L)**	46.3	43.2, 49.6		--			--			--		47.1	44.0, 50.5	34.4	27.8, 42.5	0.004
Age, years (mean, SE)	31.7	0.3		--			--			--		31.7	0.3	32.3	0.8	0.48
Sleep per night, hrs (mean, SE)	6.9	0.1		--			--			--		7.0	0.1	6.1	0.2	<0.001
Body Mass Index (kg/m2)					0.06			0.13			0.08					0.43
less than 18.5	49	3.5	2.5	0.5		1.6	0.3		0.9	0.2		47	97.1	2	2.9	
18.5 to 24.9	593	44.4	2.6	0.1		1.7	0.1		1.0	0.1		543	93.3	50	6.7	
25-29.9	423	26.3	3.0	0.3		1.9	0.2		1.1	0.1		385	91.5	38	8.5	
30 or greater	422	25.9	3.4	0.3		2.1	0.1		1.4	0.1		380	91.4	42	8.6	
p-trend					0.005			*0.02*			*0.009*					
Inflammation Categories					0.05			0.08			0.05					0.15
hs-CRP <1 mg/L	553	38.7	2.6	0.2		1.7	0.1		1.0	0.1		509	94.2	44	5.8	
hs-CRP 1-2.99 mg/L	492	33.6	2.9	0.2		1.8	0.1		1.1	0.1		446	91.6	46	8.5	
hs-CRP 3-10 mg/L	444	27.8	3.4	0.3		2.1	0.1		1.4	0.1		402	91.3	42	8.7	
p-trend					0.001			0.02			0.01					
Marital Status					0.12			0.15			0.08					<0.001
Single/Never Married	553	29.2	3.0	0.2		1.9	0.1		1.0	0.1		508	93.6	45	6.4	
Married/with partner	783	60.4	2.7	0.1		1.7	0.1		1.0	0.1		718	93.5	65	6.5	
Divorced/Separated/Widowed	153	10.2	4.0	0.5		2.4	0.3		1.7	0.3		131	83.5	22	16.5	
Education					<0.001			<0.001			<0.001					<0.001
Less than High school	369	15.6	4.1	0.3		2.4	0.2		1.7	0.1		317	86.0	51	14.0	
High school or GED	354	20.6	3.5	0.3		2.1	0.2		1.4	0.2		317	89.1	37	10.9	
Some college	482	36.0	2.9	0.2		1.8	0.1		1.1	0.1		446	93.0	36	7.0	
College graduate	284	27.8	2.0	0.2		1.4	0.1		0.6	0.1		276	97.9	8	2.1	
p-trend					<0.001			*<0.001*			*<0.001*					
Race/Ethnicity					0.27			0.37			0.27					0.26
Non-Hispanic White	557	63.5	2.8	0.2		1.8	0.1		1	0.1		508	92.9	49	7.1	
Mexican Hispanic	364	10.2	3.2	0.2		1.9	0.1		1.3	0.1		336	92.5	28	7.5	
Other Hispanic	141	6.6	3.6	0.4		2.1	0.2		1.5	0.2		122	89.4	19	10.6	

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	N	%			p			p			p	N	%	N	%	p
Non-Hispanic Black	348	12.6	3.4	0.3		2.1	0.1		1.3	0.1		315	89.7	33	10.3	
Other Race	79	7.2	2.9	0.3		1.7	0.2		1.2	0.1		76	96.2	3	3.8	
Smoke					<0.001			<0.001			<0.001					<0.001
Not current smokers	1126	76.3	2.5	0.1		1.6	0.1		0.9	0.1		1057	95.3	69	4.7	
Current smokers	324	23.7	4.4	0.4		2.7	0.2		1.8	0.2		263	83.4	61	16.6	
Leisure Time Physical Activity					<0.001			<0.001			<0.001					<0.001
Q1 (0-64 MET min/week)	386	25.1	4.2	0.3		2.5	0.2		1.7	0.2		321	84.5	65	15.5	
Q2 (65-594 MET min/wk)	302	24.8	2.5	0.2		1.6	0.1		0.9	0.1		283	95.2	19	4.9	
Q3 (595-1560 MET min/wk)	270	23.9	2.3	0.2		1.6	0.1		0.8	0.1		258	96.3	12	3.7	
Q4 (>1560 MET min/wk)	321	26.3	2.2	0.3		1.4	0.2		0.8	0.1		306	95.9	15	4.1	
p-trend					<0.001			*<0.001*			*<0.001*					
Oral Contraceptive use					0.01			0.05			0.01					0.09
Not currently using	1231	80.8	3.0	0.1		1.9	0.1		1.2	0.1		1118	92.0	113	8.0	
Currently using	213	19.2	2.3	0.3		1.5	0.2		0.8	0.2		201	95.5	12	4.5	

Weighted regression and t-tests for continuous depression scores and design-based Pearson chi square for categorical depression variables

* ln-transformed PLP, presenting geometric means and

95% CI.

Table 12:Distribution of covariates by vitamin B_6 (Pyridoxal 5'-Phosphate) variables in women of reproductive age from NHANES 2005-2008 (N=1,489)										
				Serum PLP Category						
	Serum PLP*			**Deficiency (PLP<20 nmol/L)**		**Insufficiency (PLP 20-29.9)**		**Sufficiency (PLP≥30 nmol/L)**		
	Mean	**95% CI**	**p-value**	**N**	**%**	**N**	**%**	**N**	**%**	**p-value**
Total population	46.0	42.9, 49.4	--	183	10.7	327	21.3	972	68.0	---
Depression Score (PHQ-9)		--		4.4	0.4	3.2	0.3	2.6	0.2	0.003
Sleep duration(hr), mean(SE)		--		6.6	0.1	6.8	0.1	7.0	0.1	0.003
Body Mass Index (kg/m²)			<0.001							0.003
less than 18.5	44.9	33.5, 60.1		6	11.5	11	26.9	32	61.6	
18.5 -24.9	53.4	48.1, 59.3		58	8.8	110	17.7	421	73.5	
25-29.9	44.8	40.7, 49.3		53	11.1	84	17.7	285	71.2	
30 or greater	36.8	33.7, 40.3		66	13.6	121	30.3	233	56.1	
p-trend			<0.001							
Inflammation Categories			<0.001							0.0001
hs-CRP <1 mg/L	54.6	50.0, 59.6		40	6.3	94	16.5	415	77.2	
hs-CRP 1-3 mg/L	42.7	30.0, 47.9		69	12.1	114	23.4	307	64.5	
hs-CRP 3-10 mg/L	39.8	35.3, 44.8		74	15.1	119	25.5	250	59.4	
p-trend			<0.001							
Education			<0.001							0.0007
Less than Highschool	36.5	32.7, 40.8		57	16.9	82	23.0	229	60.2	
Highschool or GED	42.1	38.7, 45.8		49	13.6	87	24.8	214	61.7	
Some college	46.0	42.8, 49.4		52	9.3	108	21.9	320	68.9	
College graduate	56.3	42.8, 49.4		24	6.8	50	17.1	209	76.2	
p-trend			<0.001							
Race/Ethnicity			0.008							0.07
Non-Hispanic White	48.1	43.0, 53.9		75	10.7	118	21.0	361	68.3	
Mexican Hispanic	41.3	38.7, 44.0		34	9.5	79	23.1	251	67.4	
Other Hispanic	47.6	42.9, 52.8		11	5.3	30	23.4	98	71.3	
Non-Hispanic Black	38.3	35.0, 42.0		55	16.1	88	24.6	204	59.4	
Other Race	48.8	41.7, 57.2		8	8.1	12	13.6	58	78.3	
Smoke			<0.001							<0.001
Not a current smoker	50.4	46.7, 54.4		107	7.7	238	19.8	775	72.5	
Yes	34.6	30.6, 39.1		75	20.6	78	26.0	170	53.4	
Leisure Time Physical Activity			<0.001							0.0003
Q1 (0-64 MET minutes/week)	39.5	35.4, 43.9		59	13.9	92	24.6	231	61.5	
Q2 (65-594 MET minutes/week)	45.1	41.0, 49.5		37	12.0	54	6.5	211	71.5	
Q3 (595-1560 MET minutes/wk)	48.8	42.8, 55.6		27	7.4	58	22.1	184	70.5	
Q4 (>1560 MET minutes/week)	60.3	52.9, 68.7		20	3.9	68	19.1	231	77.1	
p-trend			<0.001							
Oral Contraceptive use			0.10							0.53
not a current user	45.6	42.6, 48.8		152	11.0	266	21.3	807	67.8	
current user	52.9	44.4, 62.9		23	8.3	48	20.2	141	71.5	

*natural log transformed serum PLP and 95% Confidence Intervals. Weighted ANOVA and linear regression for p-trend for continuous serum PLP; Weighted Pearson chi-square for categorical PLP.

Figure 4: Higher depression scores are evident among those with vitamin B_6 deficiency, and an increasing trend is statistically significant as CRP levels increase. P-trend is significant for increasing depression score as CRP increases only among those with vitamin B_6 deficiency (p-trend=0.04)

Depression Score by Vitamin B_6 Status and C-Reactive Protein levels
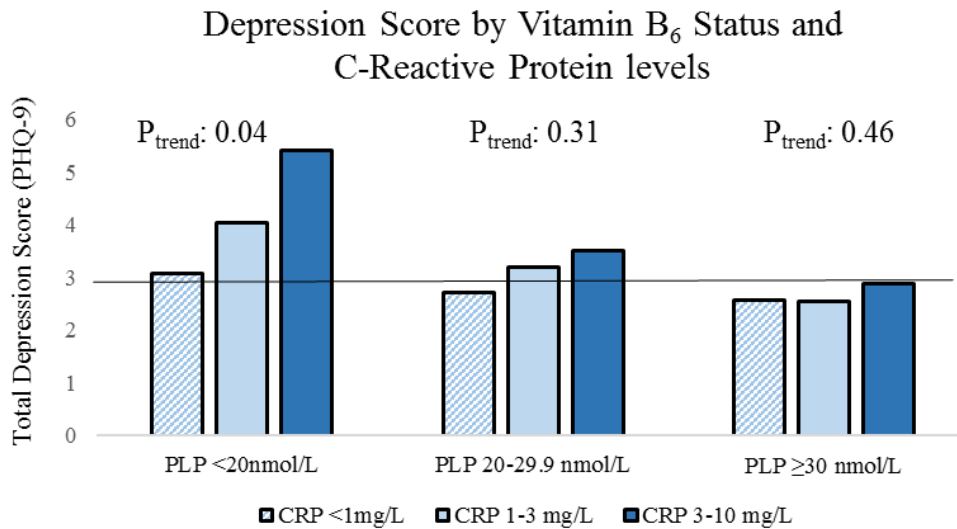
Figure 5: Prevalence of depression is highest among those with higher CRP levels, and decreases with improving vitamin B_6 status. Chi square results are only significantly different for higher prevalence of depression with decreasing vitamin B_6 concentrations among women with elevated CRP (p=0.02), but not among women with CRP 1-3mg/L (p=0.07) or CRP <1mg/L (p=0.92)

Prevalence of high depression score (PHQ-9≥10) by vitamin B_6 concentration and CRP level
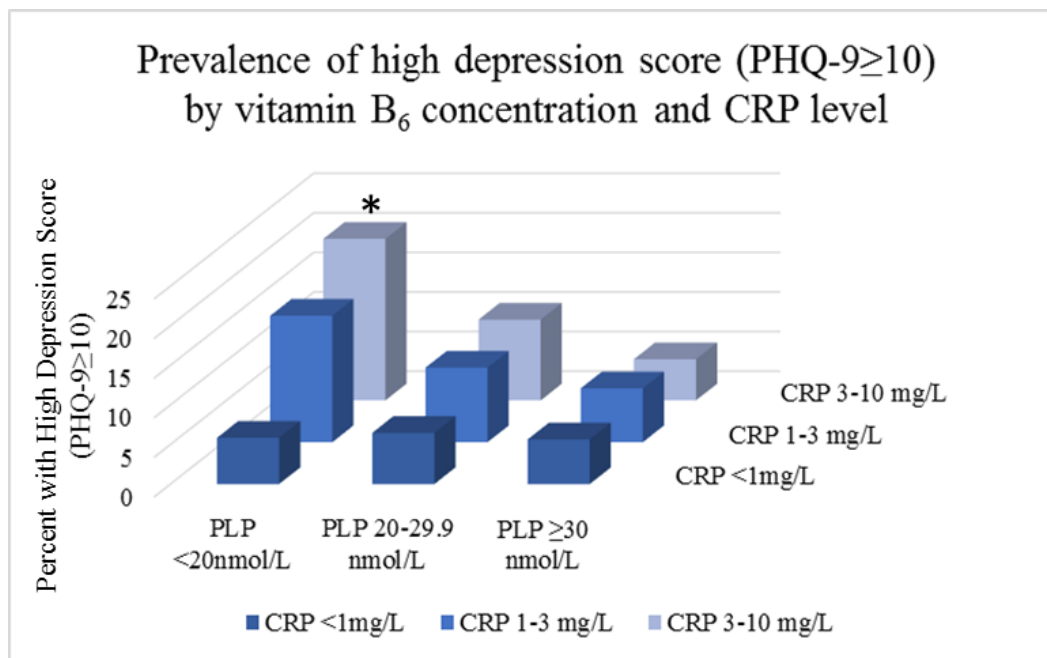
Figure 6. Linear regression analysis assessing mediators/confounders of the relationship between PLP and (a) total depression score, (b) somatic depression score and (c) non-somatic depression score among women of reproductive age from NHANES 2005-2008. The y-axis represents the percent change in the effect of PLP upon inclusion of potential mediators into model. Unadjusted β for total depression (-0.49, p=0.003), somatic (-0.29, p=0.001) and non-somatic (-0.19, p=0.03).

Portion of total effect of PLP on (a) total depression score, (b) somatic depression, and (c) non-somatic depression that is mediated by the following:

CRP | BMI

0%

-5%

-6.65%

-7.89%

-10%

-9.80%

-9.06%

-10.80%

-15%

-13.47%

□ Total depression score   ■ Somatic depression score   □ Non-somatic depression score
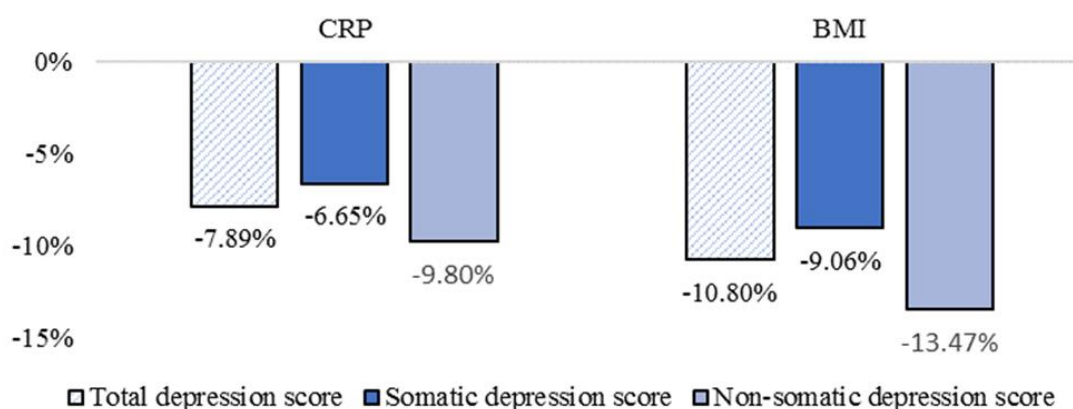
β for PLP z-score for:	Crude Model	Including CRP	Including BMI
(a) Total Depression:	-0.49 (p=0.003)	-0.45 (p=0.007)*	-0.44 (p=0.008)*
(b) Somatic depression:	-0.29 (p=0.001)	-0.28 (p=0.002)	-0.27 (p=0.002)*
(c) Non-somatic Depression	-0.19 (p=0.03)	-0.18 (p=0.053)	-0.17 (p=0.053)*

*p<0.05 for Preacher and Hayes test of indirect effects.

Table 13:Distribution of serum pyridoxal 5'-phosphate and vitamin B6 categories by individual depression symptoms among women of reproductive age from NHANES 2005-2008 (N=1,489)										
				Serum PLP Category						
	Serum PLP*			Deficiency (PLP<20 nmol/L)		Insufficiency (PLP 20-29.9)		Sufficiency (PLP≥30 nmol/L)		
	Mean	95% CI	p-value	N	%	N	%	N	%	p-value
Trouble sleeping**			<0.001							0.0005
No	47.9	44.5, 51.5		148	77.3	280	86.2	860	90.6	
Yes	34.2	28.9, 40.3		35	22.7	47	13.2	112	9.5	
Fatigue**			0.03							0.04
No	47.3	43.9, 51.1		140	76.1	271	83.5	826	86.2	
Yes	39.6	34.1, 46.0		43	23.9	56	16.5	146	13.8	
Abnormal appetite**			0.003							0.002
No	47.2	44.2, 50.4		156	82.2	297	89.9	891	93.1	
Yes	35.6	29.2, 43.4		27	17.8	30	10.1	81	6.9	
Moving or speaking too slow or too fast			0.13							0.03
No	46.4	43.2, 49.8		171	93.9	319	98.3	945	97.6	
Yes	36.1	26.3, 49.6		12	6.1	8	1.7	27	2.4	
Anhedonia			0.04							0.02
No	46.6	43.4, 50.0		166	90.1	309	95.7	922	96.1	
Yes	36.4	28.7, 46.2		17	9.9	18	4.3	50	3.9	
Depressed mood**			<0.001							0.0001
No	47.2	43.9, 50.7		161	88.2	297	90.4	922	96.3	
Yes	30.7	27.1, 34.8		22	11.8	30	9.6	50	3.7	
Low self-esteem			0.006							0.25
No	46.6	43.6, 49.8		169	92.7	308	94.2	925	95.7	
Yes	36.1	29.4, 44.3		14	7.3	19	5.8	47	4.3	
Trouble concentrating			0.04							0.17
No	46.6	43.3, 50.1		172	92.1	310	96.1	932	96.3	
Yes	35.8	28.6, 44.8		11	7.9	17	3.9	40	3.7	
Suicidal ideation			<0.001							0.002
No	46.3	43.3, 49.5		178	97.4	321	99.0	963	99.7	
Yes	21.0	14.8, 29.8		5	2.6	6	1.0	9	0.3	

Weighted t-tests and design-based Pearson chi square for categories of plasma PLP. *ln-transformed PLP. **Significant trend test for weighted proportions.

Table 14: Logistic regression analysis of the association of individual somatic depressive symptoms (dichotomous) with serum pyridoxal 5'-phosphate in women (n=1,489)

	Somatic Depression Symptoms							
	Sleeping problems		Fatigue		Abnormal appetite		Psychomotor abnormalities	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Unadjusted Model								
PLP <20nmol/L	2.82 (1.68, 4.72)	<0.001	1.96 (1.33, 2.90)	0.001	2.94 (1.68, 5.13)	<0.001	2.63 (1.04, 6.65)	0.04
PLP 20-29.9	1.45 (0.89, 2.37)	0.13	1.24 (0.73, 2.11)	0.42	1.53 (0.86, 2.72)	0.14	0.71 (0.27, 1.83)	0.46
PLP ≥30 nmol/L	ref		ref		ref		ref	
Adjusted Model								
PLP <20nmol/L	1.60 (0.52, 4.90)	0.40	0.96 (0.58, 1.58)	0.85	0.79 (0.25, 2.57)	0.69	2.02 (0.45, 9.09)	0.35
PLP 20-29.9	1.10 (0.63, 1.94)	0.73	0.68 (0.31, 1.48)	0.32	1.11 (0.60, 2.07)	0.73	0.21 (0.03, 1.34)	0.10
PLP ≥30 nmol/L	ref		ref		ref		ref	

Adjusted model includes age, ethnicity, smoking, oral contraceptive use, sleep duration, physical activity, body mass index, CRP and mutually adjusted for the sum of the remaining depression items.

Table 15: Logistic regression analysis of the association of individual non-somatic depressive symptoms (dichotomous) with serum pyridoxal 5'-phosphate in women (n=1,489)										
	Non-somatic Depression Symptoms									
	Anhedonia		Depressed mood		Low self-esteem		Trouble concentrating		Suicidal ideation*	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Unadjusted Model										
PLP <20nmol/L	2.71 (1.31, 5.61)	0.009	3.46 (1.78, 6.73)	0.001	1.74 (0.93, 3.25)	0.08	2.21 (0.92, 5.29)	0.07	8.46 (1.94, 36.86)	0.006
PLP 20-29.9	1.12 (0.49, 2.57)	0.79	2.76 (1.70, 4.48)	<0.001	1.36 (0.67, 2.75)	0.38	1.05 (0.45, 2.45)	0.91	3.32 (0.85, 12.92)	0.08
PLP ≥30 nmol/L	ref		ref		ref		ref		ref	
Adjusted Model										
PLP <20nmol/L	0.29 (0.07, 1.18)	0.08	1.22 (0.33, 4.52)	0.76	0.49 (0.11, 2.19)	0.34	0.68 (0.18, 2.60)	0.56	7.33 (1.59, 33.9)	0.01
PLP 20-29.9	0.53 (0.21, 1.32)	0.17	3.12 (1.49, 6.52)	0.004	1.05 (0.39, 2.83)	0.92	0.76 (0.24, 2.37)	0.62	3.50 (0.93, 13.1)	0.06
PLP ≥30 nmol/L	ref		ref		ref		ref		ref	

Adjusted model includes age, ethnicity, smoking, oral contraceptive use, sleep duration, physical activity, body mass index, CRP and mutually adjusted for the sum of the remaining depression items. *Suicidal ideation not adjusted for education due to collinearity.

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CHAPTER 9

"VITAMIN D, DEPRESSION, AND INFLAMMATION AMONG WOMEN OF REPRODUCTIVE AGE FROM NHANES 2005-2008"

9.1 Abstract

Depression is the leading cause of disability world-wide, and women in their childbearing years make up the largest group of Americans with depression. Recent evidence indicates that vitamin D deficiency, which is more prevalent among women, may be associated with mood and depression, but much remains unknown concerning how inflammation, lifestyle and behavioral factors may influence this association. We conducted a secondary data analysis to evaluate the effects of inflammation, sleep, oral contraceptive use and body mass index on the association between serum vitamin D [as 25(OH) vitamin D] with depression in non-pregnant women ages 18-44 from the cross-sectional National Health and Nutrition Examination Survey (NHANES) 2005-2008. Depression scores were calculated based on the Patient Health Questionnaire-9 (PHQ-9) and categorized into total depression, somatic and non-somatic depression. High depression score (PHQ-9 ≥ 10) use was also used as an outcome, as well as individual symptoms of depression. Serum 25(OH) vitamin D was categorized into deficiency (<50 nmol/L), insufficiency (50-75 nmol/L) and sufficiency (>75 nmol/L). To account for NHANES' complex survey design sample, we incorporated the sampling weights in the analysis of 1,397 observations. Mean 25(OH) vitamin D did not differ by depression

(56.7 vs 60 nmol/L, p=0.27), but suboptimal vitamin D levels were significantly

associated with higher odds for depression among women who had elevated CRP

($OR_{deficiency}$: 6.55, p=0.02 and $OR_{insufficiency}$: 9.54, p=0.001). We observed a significant

trend for decreasing depression score with increasing vitamin D level for total

depression (p-trend:0.02) and somatic depression (p-trend: 0.006). When stratified

by BMI, vitamin D was positively associated with depression scores (p=0.01) among

underweight women. Among women who reported sleeping 7 or more hours per

night, vitamin D was inversely associated with depression, whereas the opposite

was true among women who slept less than 7 hours/night (p-interaction=0.01).

Sleep appeared to significantly modify the association between vitamin D and

depression among women. Further research is needed to determine how changes in

inflammation or sleep may contribute to vitamin D deficiency, and how altogether,

these lifestyle and behavioral factors may be used to prevent or treat depression

among women.

9.2 Introduction

Depression is common in the United States, with approximately 16% of

adults experiencing clinical depression at some point in their lives.[1] Numerous

biological, psychological, and environmental risk factors for depression have been

proposed,[2] among them vitamin D deficiency.[3] Although vitamin D is mainly known

for its role in maintaining calcium homeostasis and bone health,[4] brain

development and function also depend on vitamin D; [5-8] vitamin D receptors found

in several regions of the brain may play a role in serotonin synthesis[9] and have been linked to mood regulation and affective disorders.[10–12]

Women are two- to three-times more likely than men to experience depression, and among women, it is the leading source of disease burden.[13–15] Recent national estimates indicate that suboptimal vitamin D status is common in women, affecting up to 78% of U.S. women of childbearing age, although the prevalence is even higher among non-Hispanic Black and Hispanic women.[16] Suboptimal maternal vitamin D status poses multiple health risks for both women and their offspring. Vitamin D deficiency during pregnancy or in the postpartum period has been associated with increased risk of rickets, type 1 diabetes, allergies, asthma, and schizophrenia in the offspring[17] and with preeclampsia, gestational diabetes, bacterial vaginosis and perinatal depression in the mother.[18–22] Better understanding of the role of vitamin D in depression among reproductive age women could lead to additional treatment options.

Most studies investigating vitamin D and depression have produced inconsistent results.[23] In part, this may have been due to differences in populations, heterogeneity of depression assessment, or the lack of inclusion of several lifestyle and behavioral factors that may affect the association between vitamin D and depression, such as inflammation, sleep, body mass index, and oral contraceptive (OC) use. Inflammation has been linked to vitamin D deficiency in numerous *in vitro* and *in vivo* studies[24] since vitamin D receptors were discovered in cells of the immune system. In humans, vitamin D supplementation decreases circulating C-reactive protein (CRP), a pro-inflammatory marker.[25] Inflammation has also been

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implicated as a potential risk factor for depression,[26-28] and given vitamin D's immunomodulatory potential, the association between vitamin D and depression may be affected by the presence of inflammation. To date, two studies have examined whether the association between vitamin D and depression is partially affected by CRP levels.[29,30] Although both studies found an inverse association between circulating vitamin D levels and depression, they did not find a significant difference in risk of depression among subjects with low vitamin D and elevated CRP,[29] nor did they find CRP to mediate the association between vitamin D and depression.[30] However, these studies did not assess whether the association between vitamin D and depression was symptom-specific, nor did they stratify by gender, although vitamin D deficiency and depression both affect women disproportionately.

Several lifestyle factors have been previously associated with inflammation, vitamin D, and depression separately. Sleep duration has also been associated with both vitamin D deficiency[31,32] and depression;[75] yet, to date, no studies have assessed how sleep may affect the association between vitamin D and depression. OC use is related to increased vitamin D levels,[33] yet OC use has been found to both increase[34] or decrease[35] risk of depression in different studies. And finally, BMI, a proxy for adiposity, is linked to lower vitamin D levels[36] and higher depression score[37,38] in several studies, but to date, its effect on the association between vitamin D and depression has not been examined thoroughly. Therefore, investigating the association between vitamin D, various lifestyle and behavioral factors that may be more common among women, and individual symptoms of

depression in addition to total depression scores offers an opportunity to explore

potential biological factors related to the etiology of depression that may not be

evident among mixed populations or when aggregate scoring is used.

The purpose of this study was to explore the relationship between vitamin D

status and depression among U.S. reproductive-age women and to determine

whether inflammation and other lifestyle factors modify the association between

circulating serum vitamin D levels and depression subtypes or individual symptoms

of depression among 1,397 women, aged 18-44 years, from the National Health and

Nutrition Examination Survey (NHANES) 2005-2008.

9.3 Methods

9.3.1 Study Design and Study Population

We analyzed cross-sectional data from the 2005-2008 National Health and

Nutrition Examination Survey (NHANES), which is national survey from the

National Center for Health Statistics of the Centers for Disease Control and

Prevention (CDC), designed to assess the health and nutritional status of the U.S.

population. NHANES employs a complex, multistage, probability sampling design to

select persons representative of the civilian, non-institutionalized US population,

with oversampling of special sub-populations, such as racial and ethnic minorities.

Detailed descriptions of the survey design and procedures are available at the study

website.[39]

Briefly, approximately 5,000 people are surveyed each year via in-person

interviews and physical examinations. The interview, which is conducted in

participants' homes, includes demographic, socioeconomic, dietary, and health-related questions; the examination component is comprised of medical, dental, and physiological measurements, as well as laboratory tests, which take place in specially-designed and equipped mobile examination centers (MEC) that travel throughout the country. The National Center for Health Statistic's Institutional Review Board has reviewed and approved the NHANES protocol.

Participants were eligible for our study if they were women of reproductive age (15-44 years old) who were not pregnant. From the initial sample of 2,609 eligible women, we excluded those with missing data for either depression (n=506) or serum vitamin D (n=92). Depression data for individuals younger than 18 years was not publicly available; therefore, our age range was restricted to women ages 18-44 years. We also excluded women with acute inflammation denoted by CRP levels above 10 mg/L (n=201),[40] those who were taking anti-inflammatory medications including NSAIDs (n=70) or reported current antidepressant use (n=135). The remaining sample contained 1,397 observations.

9.3.2 Assessment of Depression Status

The Patient Health Questionnaire-9 (PHQ-9)[41] was administered during the MEC interview and assessed depressive symptom severity over the past two weeks. Based on the diagnostic criteria for major depressive disorder in the Diagnostic and Statistical Manual Fourth Edition (DSM-IV), the PHQ-9 employs a 4-point scale (0=not at all, 1= several days, 2= more than half the days, 3= nearly every day) to determine the frequency with which respondents experienced the following nine

symptoms of major depressive disorder: anhedonia, depressed mood, sleep disturbance, fatigue, appetite changes, low self-esteem, concentration problems, psychomotor retardation/agitation, and suicidal ideation. Total depression scores range from 0 to 27, and scores of 10 or higher are classified as clinical depression.[42] The PHQ-9 has been shown to be a reliable and valid questionnaire as indicated by its high internal consistency and good sensitivity (88%) and specificity (88%) for identifying cases of major depressive disorder in community samples.[43–45]

In addition to the total depression score, we calculated somatic and cognitive-affective depression subscale scores. The somatic depression subscale was calculated by adding the scores for frequency of the four somatic depression symptom from the PHQ-9 (sleep disturbance, fatigue, appetite changes, and psychomotor retardation/agitation) and the cognitive-affective subscale score was calculated adding the scores for the five non-somatic symptoms (anhedonia, depressed mood, low self-esteem, concentration problems and suicidal ideation).[46] While previous factor analyses supported a one-factor model with all nine items,[47,48] recent confirmatory factor analyses have found that subscales of depression dimensions may provide a better fit to the data.[49,50] Some also argue that given the biological heterogeneity of symptoms for depression, individual symptoms should be analyzed separately.[51] Thus, we included a separate analysis for individual depressive symptoms, coded as continuous variables (scores ranging from 0-3) and as binary variables. To dichotomize the variable, those items with responses "more than half the days" and "nearly every day" indicated the presence of the symptom.[52,53]

9.3.3 Serum Vitamin D

Fasting whole blood samples were collected during the medical examination. Venipuncture was performed using standard phlebotomy techniques by NHANES trained personnel. Serum specimens were processed, stored and shipped to the Division of Environmental Health Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention in Atlanta, Georgia. Further detailed instructions on specimen collection and processing can be found at NHANES website.[39] Briefly, measurements of serum 25(OH) vitamin D, the biomarker of vitamin D status, were performed with the DiaSorin RIA kit (Stillwater, MN) for NHANES 2001-2006, and with liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for NHANES 2007-2008.[54] In order to use vitamin D data from these two different methods, NHANES converted the vitamin D data from 2001-2006 assayed with the RIA method to equivalent 25(OH)D measurements from a standardized LC-MS/MS method using regression analysis. This adjustment was completed to allow researchers to use vitamin D data that are equivalent to 25(OH)D measurements assayed with LC-MS/MS method.[54] Serum vitamin D was categorized into variables indicating vitamin D deficiency (<50nmol/L), insufficiency (50 to 75 nmol/L) and sufficiency (>75 nmol/L) following previously used cut-offs for vitamin D status.[4,55] Given that 25(OH) vitamin D was not normally distributed, it was converted to its natural logarithm to improve normality.

9.3.4 Covariates and Confounders

We considered as possible covariates a selected set of demographic, lifestyle, and socioeconomic factors available through data collected during the demographic or household section of the NHANES questionnaire. The National Center for Health Statistics (NCHS) standard definitions for ethnicities were used, and ethnicities were categorized as non-Hispanic whites, non-Hispanic blacks, Mexican-Hispanics, other Hispanics, and other race. Educational attainment was measured as the highest completed grade of school regardless of age, and categorized into four levels: less than high school, high school equivalent, some college, and college graduate or above. The smoking assessment for respondents aged 18-19 years (MEC) was not identical to that for respondents aged 20+ years (household interview). For respondents aged 20+ years, we classified as current smokers those who reported smoking at least 100 cigarettes during their lifetime and reported that they now smoke cigarettes every day or some days. For respondents ages 18-19 years, we classified as current smokers those who reported smoking 15 or more per day in the last 30 days. To create the alcohol groups, we used data obtained during the alcohol use interview at the MEC and calculated the drinks per day as suggested by Case and Stewart.[56]   Alcohol consumption, which was only available for participants of legal drinking age (21 and older), was categorized into abstainer, one or fewer drinks/day and more than one drink per day. Leisure-time physical activity (LTPA) was assessed by asking participants about their involvement in 48 specific recreation activities of moderate or vigorous intensity. Frequency was first multiplied by the duration in hours and divided by 4.3 to obtain hours of LTPA per

week. To calculate MET hrs/week, each activity was multiplied by its MET level per NHANES physical activity codes.[57]   Participants were then categorized into quartiles of physical activity.

Body mass index (BMI), OC use, sleep, and C-reactive protein (CRP) have been associated with both vitamin D levels and depression, and were examined as potential confounders/mediators of the relationship between vitamin D and depression. BMI (kg/m^2) was computed from height and weight measurements and categorized into four standard categories: underweight (BMI<18.5 kg/m^2), normal weight (18.5 ≤ BMI<25), overweight (25≤ BMI<30) and obese (BMI≥30 kg/m^2). OC use was determined by self-report of current use, and women were categorized into either "current", "past" or "never" users. Sleep duration was assessed by asking participants how many hours of sleep they usually get on a weekday/workday, and those who reported sleeping 12 or more hours were labeled as sleeping 12 hours/night. High sensitivity c-reactive protein was quantified using latex-enhanced nephelometry using a Behring Nephelometer (Dade Behring Diagnostics Inc, Somerville, NJ) and was classified into three categories: no inflammation (CRP <1mg/L), low inflammation (CRP 1-3mg/L) and moderate inflammation (CRP 3-10mg/L) following previously used guidelines.[40] CRP was also transformed to its natural logarithm due to its skewed distribution.

9.3.5 Data Analysis

The complex survey design used for NHANES data collection was incorporated into all data analyses using the "svy" command with appropriate

weighting in Stata 12.0 (StataCorp LP, College Station, TX). Detailed information on the procedures for taking into account survey sampling weights have been described elsewhere.[58]

Descriptive statistics were calculated for the overall sample using frequency distributions for categorical variables and means and standard errors for continuous variables. Because both serum 25(OH) D and hs-CRP were logarithmically transformed when analyzed as continuous variables, geometric means (antilogarithms of the transformed means) and their 95% confidence intervals are presented. Bivariate analyses were carried out for each covariate and ln-transformed vitamin D and vitamin D categories as well as for covariates and total depression, somatic/non-somatic depression and binary depression variables, using weighted ANOVAs, Chi square tests and 2-sample t-tests as appropriate. T-tests and chi-square tests were completed to assess group differences in mean ln-transformed serum 25(OH) D as a continuous variable and proportions of vitamin D categories by individual depressive symptom. In linear regression modeling of depression, all covariates that changed the estimated coefficient for the primary predictor of interest by more than 10% were retained in the multivariable model. In addition, known risk factors for depression, such as smoking, and education, were retained in the model as well as those that were associated with both the predictor and the outcome, such as LTPA and OC use. Model adequacy was assessed using goodness of fit tests.

To examine whether vitamin D status was more strongly related to certain dimensions of depression, we conducted a series of linear regression analyses in

which total depression score, somatic depression score, non-somatic depression score and each individual depression symptom served as outcome variables. A similar series of logistic regression analyses was also completed for dichotomous individual depressive symptoms. For all the individual depression symptoms, we adjusted for the remaining depression symptom to account for any potential overlap between different symptoms and to reduce the probability of type I error. Logistic regression analyses were also carried out for depression PHQ-9 score of 10 or greater.

C-reactive protein, OC use, sleep, and BMI were assessed as potential mediators or effect modifiers by running mediation tests, stratifying regression analyses, and by including interaction terms in the model estimates. To quantify the effects of these variables on any observed relationships between continuous vitamin D (ln-transformed) and depression, we carried out Sobel mediation tests and Preacher and Hayes bootstrap tests of indirect effects.[59,60] All linear and logistic regression estimates were weighted using NHANES sample weights, which account for the complex survey design, survey nonresponse, and post-stratification.

9.4 Results

Description of Study Cohort. The characteristics of the 1,397 women in our final sample are shown in table 16. The mean age of women in our sample was 31.7 years. The majority of women were white (63.5%), married or living with a partner (60.5%,), did not use OCs (77%), slept an average of seven hours per night, and were nonsmokers (75.9%). Almost eight percent (7.3%) of our sample population

reported moderate to severe depression symptoms, indicative of clinically significant depression denoted by a score of 10 or higher on the PHQ-9.

In bivariate analyses (Table 16), all three depression scores (total, somatic and non-somatic depression) were positively associated with BMI (p for trend <0.02 for all three depression scores) and CRP (p for trend<0.03 for all three depression scores) and negatively associated with education (p for trend<0.001) and LTPA (p for trend<0.001). Mean total depression score was lower for women currently taking OCs compares to non-and past-OC users (p=0.01) and higher for smokers compared to non-smokers (p<0.001 for all three depression scores). Women with high depression scores slept less (6.1 vs 7.0 hours/night, p<0.001) compared to those with low depression scores. The prevalence of depression was significantly higher among women who were separated or divorced compared to married or single women, among smokers compared to non-smokers, and among those who reported the lowest levels of LTPA compared to those who reported higher levels of LTPA.

Approximately two thirds of women (67.8%) were vitamin-D insufficient or deficient (Table 17). These proportions varied greatly by race/ethnicity. About half of non-Hispanic white females had optimal vitamin D levels, but only 2.5% and 6.3% of non-Hispanic Black and Mexican-Hispanic women, respectively, had optimal vitamin D levels (p<0.001). Serum vitamin D was negatively associated with BMI (p-trend <0.001), and positively associated with LTPA (p-trend: 0.001) and education (p-trend<0.001). Serum vitamin D concentration was much higher in current OC users compared to non-users (79.9 vs 55 nmol/L, p<0.001), and the percentage of

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women with sufficient vitamin D levels was much higher among current OC users compared to past or never users (62.8 vs 27.2 vs 18.5%, p<0.001 for current, past and never OC user, respectively). Women who provided blood samples between the months of November and April had twice the prevalence of vitamin D deficiency compared to those who donated blood between May and October (45.1 vs 21.3%, p<0.001). Vitamin D levels did not vary by age, sleep duration, CRP level or smoking status (Table 17).

Association of Vitamin D with depression scores. In univariate linear analyses, vitamin D deficiency was associated with a higher total depression score (β: 0.66, p=0.02) and somatic depression score (β: 0.39, p=0.005), but not a higher non-somatic depression score (β: 0.27, p=0.11). These associations were attenuated and were no longer significant after adjustment for demographics, lifestyle, behavioral factors and season of blood draw (Table 18).

To assess whether inflammation affects the association between vitamin D status and depression, we first visualized the relationship using bar graphs (Figure 7). Depression score tended to decrease with increasing vitamin D levels (p=0.03, p-trend:0.02) in the total population (Figure 7a). When stratified by CRP levels, however, these trends were less clear. Among women with elevated CRP (3-10mg/L), those with vitamin D sufficiency had lower depression scores (p=0.003, p-trend:0.004, Figure 7a) and lower prevalence of high depression score (p=0.02, Figure 7b). A similar pattern was found in linear regression models stratified by CRP levels (data not shown). In unadjusted analyses, vitamin D deficiency and insufficiency were associated with higher total depression score (β: 1.63, p=0.002

and β:1.78, p=0.008), somatic depression score (β:0.85, p=0.003; β:0.98, p=0.008), and non-somatic depression score (β:0.78, p=0.009; β:0.80, p=0.02), evident only among those with moderate inflammation (CRP 3-10 mg/L). Adjustments for age, race/ethnicity, education, BMI, sleep duration, LTPA, smoking, OC use, and continuous CRP attenuated these results, all of which became non-significant.

Association of Vitamin D with Individual Depression Symptoms. To determine which depression symptoms were associated with vitamin D status, we first looked at the bivariate associations, followed by a series of linear and logistics models for continuous and dichotomized symptoms. In bivariate analysis, women experiencing the somatic symptoms of fatigue and abnormal appetite had significantly lower mean serum vitamin D levels (54.7 vs 60.8 nmol/L, p=0.004 and 55.4 vs 60.3 nmol/L, p=0.03, respectively) compared to those without these symptoms (Table 19). In linear regression with individual depression symptoms, vitamin D deficiency was significantly associated with a higher score for fatigue (β: 0.21, p=0.001), changes in appetite (β: 0.13, p=0.008), psychomotor abnormalities (β:0.06, p=0.03), anhedonia (β:0.12, p=0.004) and suicidal ideation (β:0.03, p=0.05). These associations were completely attenuated after adjustment for demographics, behavioral, lifestyle factors, and season of blood draw (data not shown).

In stratified analysis, among women with moderate inflammation, vitamin D deficiency was associated with fatigue, abnormal appetite, psychomotor abnormalities, anhedonia, depressed mood, low self-esteem, and suicidal ideation in univariate analysis. In adjusted models, however, vitamin D deficiency was significantly associated only with a lower score for sleeping disturbances (β: -0.34,

p=0.01) (Table 20). Individual depression symptoms were not significantly associated with suboptimal vitamin D levels in multivariable logistic models (data not shown). Vitamin D insufficiency and deficiency were associated with higher odds of depression among those with moderate inflammation (CRP 3-10mg/L) in adjusted models ($OR_{deficiency}$: 6.55, p=0.02 and $OR_{insufficiency}$ 9.54, p=0.001) (Table 21).

Effects of potential modifiers or confounders. To examine BMI as a potential modifier of the vitamin D-depression association, we stratified our analysis by BMI category and found that after full adjustments a one standard-deviation increase in ln-transformed vitamin D was associated with a 1.7-point increase in total depression score (β: 1.71, p=0.01), almost a 1-point increase in somatic (β: 0.95, p=0.02), and 0.76-point increase in non-somatic depression score (β: 0.76, p=0.01) only among those who were underweight. This association was not significant for any other BMI group (Table 22). Despite this apparent interaction, addition of an interaction term between continuous BMI and vitamin D in the final model was not statistically significant (not shown).

To evaluate BMI as a potential mediator or confounder of the association between vitamin D and depression, we ran a series of Sobel mediation tests followed by bootstrap analysis for the Preacher and Hayes test of indirect effect.[59,60] For total depression score, 41.5% of the total effect was significantly mediated by BMI (p=0.01) (Figure 9). BMI also partially explained the association between vitamin D and both somatic (35.2%, p=0.01) and non-somatic depression (52%, p=0.03).

Influence of sleep duration on the association between vitamin D and depression. To determine whether sleep duration influences the association between vitamin D status and depression, we first assessed differences in sleep by vitamin D status. Mean sleep duration did not vary by vitamin D level (p=0.43), but mean vitamin D levels were slightly higher among women who reported sleeping more than 7 hours/night (60.9 vs 57.9, p=0.065). Adding an interaction term to the final model indicated that sleep is a strong effect modifier of the association between vitamin D and depression (p-interaction=0.01). When stratified by sleep (dichotomized at the median- less than 7 hours or 7 or more hours per night), vitamin D was inversely associated with odds of depression among women who slept 7 or more hours, but was positively associated with depression among women who slept less than 7 hours per night (Figure 9). We also ran logistic regressions using the "mfpigen" command to create a plot for the odds of depression at different hours of total sleep, and found a negative association between vitamin D and depression among women who slept 7-9 hours per night, but this association was positive among women who slept too little (5 hours/night) or too much (12 or more hours per night) (Figure 10).

Influence of oral contraceptive use on the association between vitamin D and depression. Mean vitamin D was much higher among current OC users (79.9 nmol/L), compared to past users (57.6 nmol/L) or never users (51.5 nmol/L; p<0.001). In multivariable logistic regression, there were no statistically significant associations observed between vitamin D and depression when stratified by OC use, nor was a significant interaction evident.

9.5 Discussion

In a large representative sample of reproductive age women, we found that depression score was inversely related to 25(OH)-vitamin D level, and among those with elevated CRP, suboptimal 25(OH)-vitamin D levels were associated with increased odds of depression. Furthermore, the association between vitamin D deficiency and depression was modified by sleep and by BMI. Although some studies have reported an association between lower vitamin D status and increased depressive symptoms among women,[61–65] our study is the first to examine whether inflammation and lifestyle factors influence the association between 25(OH)-vitamin D status and various dimensions of depression among reproductive-age women.

Although studies on vitamin D status and depression have yielded inconsistent findings, those that assessed the different association between 25(OH)-vitamin D and subtypes of depression have found that vitamin D deficiency is more strongly related to somatic symptoms of depression, rather than non-somatic symptoms.[99] In our sample of women, we found a significant trend of increasing total depression score and somatic depression score with decreasing 25(OH)-vitamin D level, which was not evident for non-somatic depression score or in adjusted models. Furthermore, women experiencing fatigue had significantly lower mean serum 25(OH)-vitamin D concentration, suggesting that 25(OH)-vitamin D may be involved in sleep regulation. Vitamin D receptors (VDR) have been found in brain areas that control the endocrine-autonomic system as well as the motor system.[10] Fatigue may be common to both vitamin D deficiency and depression,

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since muscle weakness and chronic fatigue are commonly experienced by persons with vitamin D deficiency.[66,67] Mechanistic evidence, therefore, suggests that 25(OH)-vitamin D levels may affect somatic symptoms related to motor function and sleep, and we found that suboptimal 25(OH)-vitamin D concentrations were associated with increased score for psychomotor retardation among women with elevated CRP levels, and these associations were borderline significant after full adjustments ($\beta_{deficiency}$: 0.15, p=0.06; $\beta_{insufficiency}$: 0.12, p=0.09). These findings suggest that in addition to vitamin D deficiency, a second trigger, such as inflammation, may exacerbate somatic depressive symptoms.

Previous studies reported inverse associations between sleep and depression[68] as well as sleep and vitamin D status.[31,32] When we stratified our analysis by sleep duration, vitamin D-deficient women who slept seven or more hours per night had higher depression scores and much higher odds of depression than women who slept less (p for vitamin D x sleep = 0.01). Adequate sleep is an indicator of health status[69] and may be a proxy for health-related behaviors,[70] so our findings suggest that among otherwise healthy women who regularly get enough sleep, vitamin D deficiency may be associated with increased odds of depression, but this is not the case among women who sleep less than 7 hours per night. The latter group may be experiencing insomnia, sleep apnea, stress, or other unmeasured comorbidity that could allow for residual confounding to affect the association between vitamin D and depression. When we adjusted for sleep, the effect size of serum 25(OH)-vitamin D on depression was somewhat attenuated, and mediation tests indicated significant indirect effects of 25(OH)-vitamin D on

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depression through sleep. Sleep may be acting as a mediator between vitamin D

deficiency and depression through the potential effects of vitamin D on neurons

thought to be involved in sleep regulation.[10,31] Some evidence suggests that

insomnia leads to depression in 69% of cases of comorbid insomnia and depression,

whereas prior depression does not lead to insomnia.[71] However, given the cross-

sectional nature of this analysis, we cannot determine whether sleep is acting as a

confounder or a mediator in the association between vitamin D and depression.

Adjustment for BMI significantly attenuated the association between vitamin

D and depression scores among women in our analysis, and this was especially true

for non-somatic depression, where inclusion of BMI in regression models reduced

the effect size of the association of vitamin D on depression by half. This finding

suggests that adiposity accounted for much of the association observed between

vitamin D and depression scores in crude analyses. From a physiologic perspective,

non-specific sequestering of vitamin D in adipose tissue[72] suggests a mechanism

whereby increased adiposity leads to lower vitamin D levels.[36] BMI has also

predicted future depression,[73] and therefore could potentially act as confounder or

effect modifier of the association between vitamin D and depression. Although tests

for an interaction between BMI and vitamin D status were nonsignificant, when

stratified by BMI in multivariable analysis, serum 25(OH)-vitamin D was positively

associated with depression scores, and this was only evident among underweight

women. Among the rest of the women with higher BMI, the association was

negative, but not significant, suggesting potential effect modification by adiposity on

the association between vitamin D levels and depression.

In our sample, underweight women had the highest mean 25(OH)-vitamin D (mean 67.1 nmol/L, 95% CI 59.2-76, p<0.001) and the lowest prevalence of vitamin D deficiency compared to women in the rest of the BMI categories. Two recent cross-sectional studies[74,75] found a positive association between 25(OH)-vitamin D and CRP at 25(OH)-vitamin D concentrations above 52-62 nmol/L, suggesting that below these levels, 25(OH)-vitamin D may have an anti-inflammatory effect, but above these levels it may have a pro-inflammatory effect. Inflammation has been positively associated with depression in numerous studies,[28] and could potentially explain why 25(OH)-vitamin D was positively associated with depression in this group. If this is a true association, then the underlying mechanisms for a potential pro-inflammatory role of vitamin D at higher concentrations still needs to be discovered.

Consistent with previous research, OC users had higher 25(OH)-vitamin D concentration compared to non-OC users.[33,76,77] Since OC use has also been associated with both higher and lower depression risk,[34,35] we considered OC use as a potential confounder. Including OC in the model significantly attenuated the association between 25(OH)-vitamin D and depression. Based on the Sobel mediation test, OC use can be considered a potential mediator of the association between vitamin D and non-somatic depression scores. However, from a biological perspective, mediation by oral contraceptive use seems unlikely, given that OC use affects both exposure and outcome, and not the other way around. Estrogen downregulates the main catabolic enzyme in the vitamin D pathway, 24-hydroxylase,[33,78,79] and may upregulate 1α-hydroxylase, the enzyme required to

activate vitamin D. OC use may also affect the expression of the VDR and other vitamin D binding proteins.[33,78,79] Together, these actions may lead to elevated circulating vitamin D levels, although the clinical significance of this effect remains unknown.[76] Although current OC users appeared to have lower odds of depression compared to non-users in our unadjusted analysis, we did not find significant evidence of effect modification by OC use.

Our study has important strengths, including use of a large, diverse sample of women representative of the U.S. population, the use of a validated, multidimensional depressive symptom measure, and the testing of multiple candidate mediators or confounders, such as CRP, sleep, BMI, and OC use. Furthermore, we excluded women who were currently taking antidepressants to prevent nondifferential misclassification of outcome, given that some of the common side effects of antidepressants reported by patients are the very symptoms that are used to measure depression, such as insomnia, hypersomnia, agitation, restlessness, fatigue, somnolence, weight gain or weight loss, and decreased or decreased appetite.[80] This overlap of antidepressant side-effects and depression symptoms provides a compelling reason for analyzing symptoms separately from total depression scores.[51]

Our study also has important limitations. First, due to the cross-sectional nature of the NHANES study design, we were unable to determine the directionality of the association between 25(OH)-vitamin D and depression symptoms. Although previous studies have found depression scores to improve upon supplementation with vitamin D in certain populations,[81,82] conflicting evidence remains concerning

the directionality of the association between vitamin D status and depression and inconclusive findings in the vast majority of supplementation studies.[83–85] Depression may affect vitamin D-enhancing activities, such as healthy eating, exercising, or outdoor activities, and may lead to lower 25(OH)-vitamin D levels through weight gain and sequestering of vitamin D in adipose tissue. Prospective studies are warranted to establish directionality, and randomized clinical trials are needed to understand the complexity of this association and to determine whether there is, in fact, a direct link between 25(OH)-vitamin D and depression. Secondly, we did not account for region of residence and latitude for the participants, but we did account for the six-month period of blood draw, which was the only data publicly available from NHANES that could be used to address seasonal variations in serum 25(OH)-vitamin D levels. We do not consider this a major limitation since surrogate markers of vitamin D concentrations, such as regional latitude, although somewhat correlated to serum 25(OH)-vitamin D, have not been found to adequately reflect circulating 25(OH)-vitamin D measures. In a large nested-case control study of the Women's Health initiative, mean annual solar irradiance only explained 1% of the variation in serum 25(OH)-vitamin D levels.[86] And finally, this survey from a non-institutionalized population may represent only those with less severe depression, because women experiencing severe depression may either be institutionalized or may have disproportionately chosen not to participate in the survey. Therefore, our results might not be generalizable to women with severe depression or major depressive disorder.

9.6 Conclusion

We found that suboptimal vitamin D status was associated with higher total depression score and somatic depression score and higher odds of depression in the presence of inflammation. We also found that among underweight women, 25(OH)-vitamin D was positively associated with depression scores. Previous research on vitamin D and depression has yielded inconsistent or null findings, which may be due in part to a failure to account for inflammation or lifestyle factors, such as sleep, that affect both vitamin D status and depression. Given the evidence linking inflammation to vitamin D status and depression, additional studies are needed to determine to what extent vitamin D influences depression through changes in adiposity, inflammation and sleeping patterns.

Table 16: Distribution of covariates by depression in women of reproductive age from NHANES 2005-2008 (N=1,397)															
	Total Population		Total PHQ-9 Score (range 0-27)			Somatic Depression Score (range 0-12)			Non-Somatic Depression Score (range 0-15)			High Depression Score (PHQ-9 score ≥10)			
												No		Yes	
	N	%	Mean	SE	p-value	Mean	SE	p-value	Mean	SE	p-value	N	%	N	%	p-value
Total population sample	1397		2.9	0.1	--	1.8	0.1	--	1.1	0.1	--	1276	92.7	121	7.3	--
Serum 25 (OH) D (nmol/L)*	59.8	57.1, 62.8		--			--			--		60.1	57.3, 60.3	56.7	50.6, 63.5	0.27
Age, years (mean, SE)	31.7	0.3		--			--			--		31.6	0.3	32.3	0.9	0.47
Sleep per night, hours (mean, SE)	6.9	0.1		--			--			--		7.0	0.1	6.1	0.2	<0.001
Body Mass Index (kg/m2)					0.06			0.13			0.10					0.42
less than 18.5	47	3.5	2.6	0.5		1.7	0.3		0.9	0.2		45	96.9	2	3.1	
18.5 to 24.9	552	44.4	2.6	0.1		1.7	0.1		0.9	0.1		507	93.6	45	6.5	
25-29.9	400	26.6	3.0	0.3		1.8	0.2		1.1	0.1		365	91.5	35	8.5	
30 or greater	396	25.7	3.4	0.2		2.1	0.1		1.3	0.1		357	91.9	39	8.1	
p-trend	--				*0.005*			*0.02*			*0.01*					
Inflammation Categories					0.05			0.09			0.06					0.07
hs-CRP <1 mg/L	510	38.1	2.6	0.2		1.7	0.1		0.9	0.1		473	94.7	37	5.3	
hs-CRP 1-2.99 mg/L	465	33.8	2.9	0.2		1.8	0.1		1.1	0.1		421	91.3	44	8.7	
hs-CRP 3-10 mg/L	422	28.1	3.3	0.2		2.0	0.1		1.3	0.1		382	91.6	40	8.4	
p-trend					*0.01*			*0.03*			*0.02*					
Marital Status					0.11			0.17			0.06					<0.001
Single/Never Married	526	29.4	2.9	0.2		1.9	0.1		1.0	0.1		487	94.5	39	5.5	
Married/with partner	731	60.5	2.7	0.1		1.7	0.1		1.0	0.1		670	93.4	61	6.6	
Divorced/Separated/Widowed	140	10.1	4.1	0.6		2.4	0.3		1.7	0.3		119	83.4	21	16.6	
Education					<0.001			<0.001			<0.001					<0.001
Less than Highschool	343	15.6	4.1	0.3		2.4	0.2		1.7	0.1		297	86.3	46	13.7	
Highschool or GED	336	20.7	3.5	0.4		2.1	0.2		1.4	0.2		301	88.9	35	11.1	
Some college	449	36.7	2.8	0.2		1.8	0.1		1.1	0.1		415	93.0	34	7.0	
College graduate	268	28.00	1.9	0.2		1.4	0.1		0.5	0.1		262	98.7	6	1.3	
p-trend					*<0.001*			*<0.001*			*<0.001*					

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	N	%	mean	SE	mean	SE	mean	SE	N	%	N	%	p
Race/Ethnicity				0.28		0.41		0.29					0.25
Non-Hispanic White	521	63.5	2.7	0.2	1.7	0.1	1.0	0.1	477	93.2	44	6.8	
Mexican Hispanic	130	6.6	3.1	0.3	1.8	0.2	1.2	0.1	317	93.1	25	6.9	
Other Hispanic	342	10.1	3.6	0.4	2.1	0.2	1.5	0.2	112	89.2	18	10.8	
Non-Hispanic Black	329	12.6	3.3	0.2	2.1	0.1	1.3	0.1	298	89.8	31	10.2	
Other Race	75	7.2	2.7	0.3	1.7	0.2	1.1	0.2	72	96.0	3	4.0	
Smoke				<0.001		<0.001		<0.001					<0.001
Not current smoker	1082	80.3	2.4	0.1	1.6	0.1	0.9	0.1	997	95.5	63	4.5	
Current smoker	246	19.7	4.4	0.4	2.7	0.2	1.7	0.2	242	83.6	56	16.4	
Leisure Time Physical Activity				<0.001		<0.001		<0.001					<0.001
Q1 (0-64 MET minutes/week)	343	23.9	4.2	0.3	2.5	0.2	1.7	0.2	286	84.7	57	15.3	
Q2 (65-594 MET minutes/week)	291	26	2.5	0.2	1.6	0.1	0.9	0.1	273	95.2	18	4.8	
Q3 (595-1560 MET minutes/week)	256	24.6	2.4	0.2	1.5	0.1	0.8	0.1	244	96.1	12	3.9	
Q4 (>1560 MET minutes/week)	297	25.5	2.10	0.2	1.3	0.2	0.7	0.1	284	96.4	13	3.6	
p-trend				<0.001		<0.001		<0.001					
Oral Contraceptive use				0.01		0.09		0.001					0.19
Never user	425	23.0	3.1	0.2	1.9	0.1	1.2	0.1	393	92.3	31	7.7	
Past user	728	58.2	3.0	0.1	1.8	0.1	1.1	0.1	657	92.1	71	7.9	
Current user	199	18.8	2.1	0.3	1.5	0.2	0.8	0.2	188	96.1	11	3.9	
p-trend				0.02		0.09		0.01					
Semester of blood draw				0.27		0.43		0.17					0.23
November 1 through April 30	669	41.6	2.7	0.2	1.7	0.1	1.0	0.1	627	93.9	42	6.1	
May 1 through October 31	728	58.4	3.0	0.2	1.9	0.1	1.2	0.1	649	91.8	79	8.2	

Weighted regression and t-tests for continuous depression scores and design-based pearson chi square for categorical depression variables. *Ln-transformed vitamin D presented as geometric means and 95% CI.

Table 17: Distribution of covariates by vitamin D variables in women of reproductive age from NHANES 2005-2008 (N=1,397)

| | Serum 25(OH) Vitamin D* | | | Serum Vitamin D Categories | | | | | |
| | | | | Deficiency (<50 nmol/L) | | Insufficiency (50-75 nmol/L) | | Sufficiency (>75 nmol/L) | | |
	Mean	95% CI	p-value	N	%	N	%	N	%	p-value
Total population	59.8	57.1, 62.8	--	643	31.2	459	36.7	295	32.2	--
Depression Score (PHQ-9)**		--		3.3	0.2	2.8	0.2	2.6	0.2	0.03
Age (years)**		--		31.7	30.9, 32.5	32.1	31.3, 32.8	31.2	29.9, 32.4	0.43
Sleep duration (hours)**		--		6.9	6.7, 7.0	6.9	6.8, 7.0	7.0	6.9, 7.2	0.19
Body Mass Index (kg/m²)			<0.001							<0.001
less than 18.5	67.1	59.2, 76.0		15	21.3	18	32.8	14	46.0	
18.5 -24.9	66.5	62.9, 70.4		193	22.7	196	36.6	163	40.7	
25-29.9	59.0	54.9, 63.4		196	33.2	127	35.7	77	31.1	
30 or greater	49.9	46.5, 53.4		239	45.2	116	38.1	41	16.8	
p-trend			<0.001							
Inflammation Categories			0.07							0.099
hs-CRP <1 mg/L	60.5	57.1, 64.1		222	30.4	177	38.6	111	31.0	
hs-CRP 1-2.99 mg/L	61.6	8.1, 65.4		200	26.9	160	37.8	105	35.3	
hs-CRP 3-10 mg/L	57.0	53.1, 61.1		221	37.4	122	32.7	79	29.9	
p-trend			0.11							
Marital Status			0.04							0.003
Single/Never Married	57.3	53.0, 61.9		298	38.7	131	28.5	97	32.9	
Married/Living with partner	61.6	58.4, 64.9		278	26.3	280	40.9	173	32.8	
Divorced/Separated/Widowed	57.4	52.8, 62.3		67	38.8	48	35.2	25	26.0	
Education			<0.001							<0.001
Less than Highschool	51.8	47.2, 56.8		198	48.6	105	33.0	40	18.5	
Highschool or GED	58.3	53.8, 63.2		178	37.3	94	34.0	64	28.7	
Some college	62.0	58.7, 65.6		178.0	25.4	158	37.8	113	36.8	
College graduate	63.3	60.1, 66.6		88	24.2	102	39.3	78	36.5	
p-trend			<0.001							
Race/Ethnicity			<0.001							<0.001
Non-Hispanic White	71.4	68.6, 74.3		78	14.4	207	39.8	236	45.8	
Mexican Hispanic	46.6	43.3, 50.2		208	56.8	114	36.9	20	6.3	
Other Hispanic	49.5	44.8, 54.8		69	56.7	38	26.2	23	17.1	
Non-Hispanic Black	36.2	33.9, 38.6		252	75.1	70	22.4	7	2.5	

	Mean*	95% CI	p-value	Count	%	Count	%	Count	%	p-value
Other Race	51.6	46.5, 57.4		36	42.7	30	42.9	9	14.3	
Smoke			0.31							0.89
No	59.3	56.1, 62.7		496	31.0	349	37.2	215	31.8	
Yes	62.1	57.7, 66.9		118	31.1	102	35.4	78	33.5	
Leisure Time Physical Activity			0.003							0.03
Q1 (0-64 MET minutes/week)	54.7	48.8, 61.4		176	38.3	104	32.6	63	29	
Q2 (65-594 MET minutes/week)	62.1	58.5, 66.0		127	29.7	96	35.8	68	34.4	
Q3 (595-1560 MET minutes/week)	61.2	57.5, 65.2		104	27.7	91	39.7	61	32.6	
Q4 (>1560 MET minutes/week)	67.2	63.4, 71.2		110	20.4	103	37.9	84	41.7	
p-trend		*0.001*								
Oral Contraceptive use			<0.001							<0.001
Never user	51.5	47.9, 55.3		248	44.8	132	32.8	45	18.5	
Past user	57.6	54.6, 60.7		329	32.8	258	39.9	141	27.2	
Current user	79.9	74.8, 85.3		50	10.9	51	27.2	98	62.8	
p-trend			<0.001							
Semester of blood draw			<0.001							<0.001
November 1 through April 30	51.7	47.7, 56.0		405	45.1	188	33.7	76	21.3	
May 1 through October 31	66.4	63.6, 69.4		238	21.3	271	38.8	219	39.9	

*ln-transformed hs-CRP, with geometric means and 95% confidence intervals. ** Mean(SE) for continuous variables.

Analysis of variance for continuous vitamin D and weighted, design-based pearson chi square for categories of vitamin D.

	Depression defined as:					
	Total PHQ-9 score (range: 0-27)		**Somatic Depression score (range: 0-12)**		**Non-somatic Depression score (range: 0-15)**	
	β (95% CI)	**p-value**	**β (95% CI)**	**p-value**	**β (95% CI)**	**p-value**
Unadjusted Model						
Vitamin D <50nmol/L	0.66 (0.11, 1.20)	0.02	0.39 (0.12, 0.65)	0.005	0.27 (-0.06, 0.60)	0.11
Vitamin D 50-75 nmol/L	0.15 (-0.53, 0.83)	0.67	0.06 (-0.36, 0.48)	0.78	0.09 (-0.23, 0.41)	0.58
Vitamin D >75 nmol/L	ref		ref		ref	
Adjusted Model						
Vitamin D <50nmol/L	-0.03 (-0.77, 0.72)	0.94	-0.03 (-0.36, 0.31)	0.88	-0.001 (-0.47, 0.47)	0.99
Vitamin D 50-75 nmol/L	-0.13 (0.86, 0.60)	0.72	-0.07 (-0.49, 0.35)	0.74	-0.06 (-0.44, 0.32)	0.74
Vitamin D >75 nmol/L	ref		ref		ref	

Table 18: Linear regression of the association of total depression score and depression subtypes with vitamin D in women (n=1,397)

Adjusted for age, race/ethnicity, education, smoking, oral contraceptive use, sleep duration, physical activity, body mass index, high-sensitivity CRP, and season of blood draw.

Figure 7: (a) Total depression score by CRP level and Vitamin D status. (b) Prevalence of high depression score varies by CRP level, evident mainly among women with CRP between 3-10mg/L.

Depression Score by Vitamin D Status, stratified by hs-CRP levels

(a)

Vit. D <50 nmol/L Vit D 50-75 nmol/L Vit D. >75 nmol/L

Prevalence of depression (PHQ-9 ≥10) by Vitamin D status, stratified by hs-CRP level

P-value: 0.84   P-value: 0.55   P-value: 0.02   P-value: 0.40
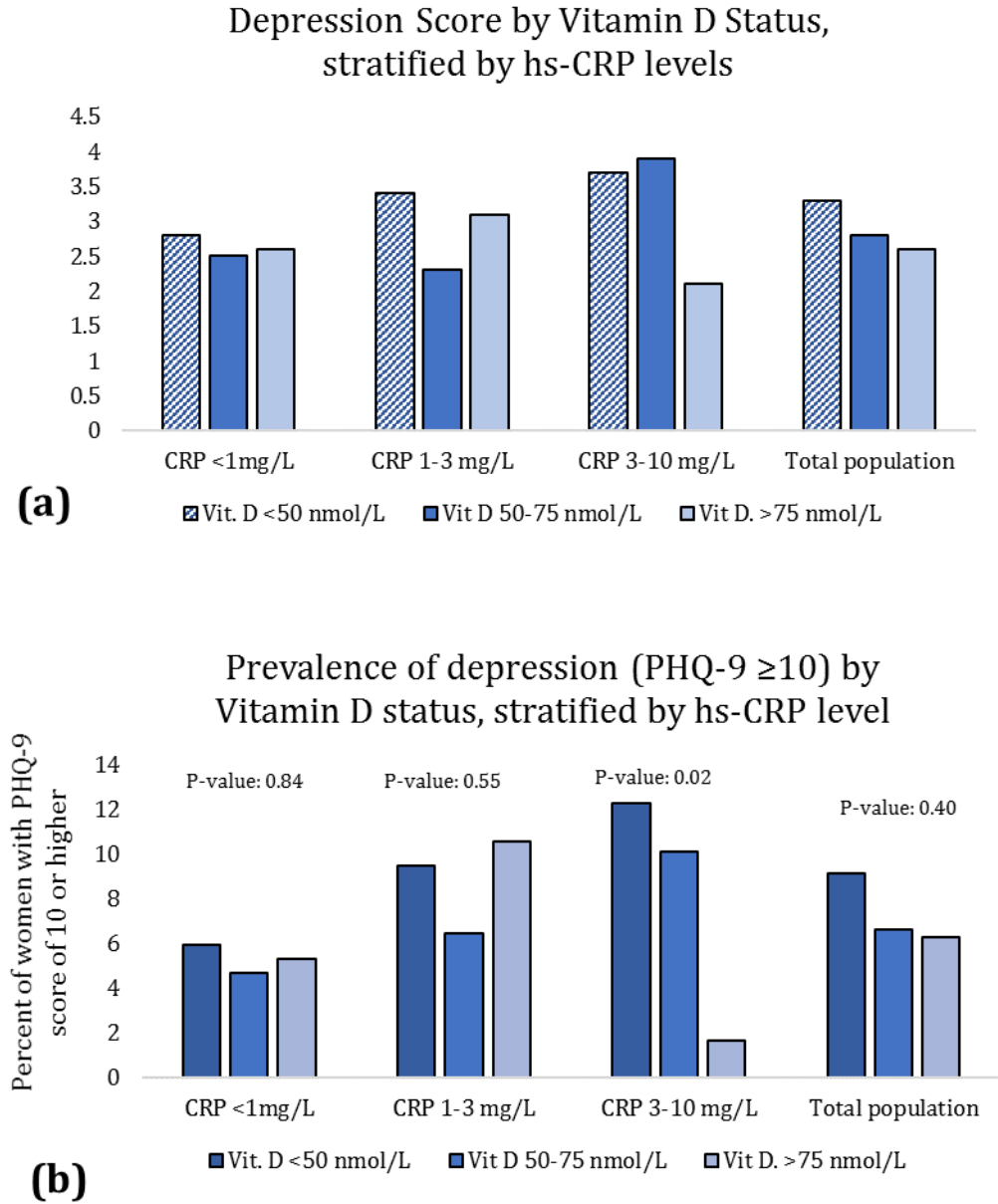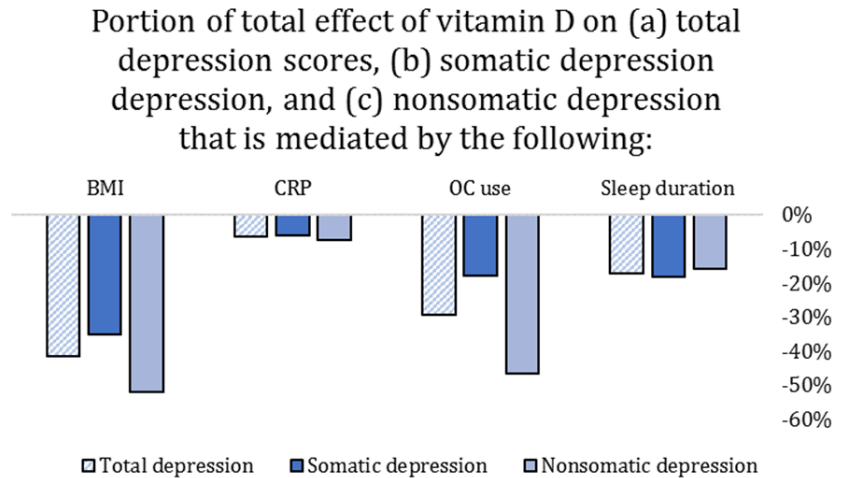
(b)

Vit. D <50 nmol/L Vit D 50-75 nmol/L Vit D. >75 nmol/L

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Table 19: Distribution of serum 25(OH)D and vitamin D categories by individual depression symptoms among women (N=1,397)									
	Serum Vitamin D*			Serum Vitamin D Categories					
				Deficiency (Vit D <50 nmol/L)		Insufficiency (50-75 nmol/L)		Sufficiency (≥75 nmol/L)	
	Mean	95% CI	p-value	N	%	N	%	N	%	p-value
Trouble sleeping			0.87							0.46
No	59.9	57.2, 62.7		554	87.3	409	89.9	253	87.5	
Yes	59.6	54.6, 64.9		89	12.7	50	10.1	42	12.5	
Fatigue**			0.004							0.07
No	60.8	58.2, 63.6		526	80.3	389	85.9	256	88.0	
Yes	54.7	49.9, 59.9		117	19.7	70	14.1	39	12.0	
Abnormal appetite**			0.03							0.17
No	60.3	57.5, 63.2		576	89.2	419	91.8	275	93.6	
Yes	55.4	50.9, 60.3		67	10.8	40	8.2	20	6.4	
Moving or speaking too slow or too fast			0.46							0.62
No	59.9	57.1, 62.9		623	96.7	447	97.6	283	97.6	
Yes	56.9	49.7, 65.3		20	3.3	12	2.4	12	2.4	
Anhedonia			0.21							0.46
No	60.1	57.3, 63.0		602	94.5	437	96.0	280	96.3	
Yes	55.6	48.8, 63.3		41	5.5	22	4.0	15	3.7	
Depressed mood			0.79							0.49
No	59.9	57.1, 62.9		595	93.2	432	95.4	274	94.0	
Yes	58.9	52.0, 66.7		48	6.8	27	4.6	21	6.0	
Low self-esteem			0.82							0.76
No	59.8	57.0, 62.8		611	95.9	433	94.8	279	95.1	
Yes	60.8	52.9, 69.7		32	4.1	26	5.2	16	4.9	
Trouble concentrating			0.89							0.70
No	59.8	57.0, 62.8		617	96.2	436	95.1	281	96.1	
Yes	60.4	53.1, 68.7		26	3.8	23	4.9	14	3.9	
Suicidal ideation			0.13							0.41
No	59.9	57.1, 62.9		634	98.9	452	99.3	291	99.5	
Yes	49.9	39.2, 63.5		9	1.1	7	0.7	4	0.5	

Weighted t-tests and design-based Pearson chi square for categories of plasma vitamin D. *ln-transformed vitamin D, reporting geometric mean and 95% CI. **Significant p-value for trend test of proportions.

Figure 8: Linear regression analysis assessing mediators/confounders of the relationship between log-transformed vitamin D and (a) total depression score, (b) somatic depression score and (c) non-somatic depression score among women of reproductive age from NHANES 2005-2008.

Portion of total effect of vitamin D on (a) total depression scores, (b) somatic depression depression, and (c) nonsomatic depression that is mediated by the following:

β for Vitamin D z-score for:	Crude Model	Including BMI	Including CRP	Including OC use	Including Sleep
(a) Total Depression:	-0.26 (p=0.03)	-0.15 (p=0.18)*	-0.24 (p=0.04)	-0.20 (p=0.09)	-0.21 (p=0.06)*
(b) Somatic depression:	-0.16 (p=0.005)	-0.11 (p=0.06)*	-0.15 (p=0.01)	-0.14 (p=0.02)	-0.13 (p=0.02)*
(c) Non-somatic Depression	-0.10 (p=0.16)	-0.05 (p=0.49)*	-0.09 (p=0.20)	-0.06 (p=0.40)*	-0.08 (p=0.22)*

*p<0.05 for Preacher and Hayes test of indirect effects.

Table 20: Association of individual somatic depressive symptoms with vitamin D - stratified by CRP levels								
	Somatic Depression Symptoms							
	Sleeping problems		**Fatigue**		**Abnormal appetite**		**Psychomotor abnormalities**	
	β (95% CI)	**p-value**	**β (95% CI)**	**p-value**	**β (95% CI)**	**p-value**	**β (95% CI)**	**p-value**
CRP <1 mg/L								
Unadjusted Model								
25(OH)D <50nmol/L	-0.01 (-0.25, 0.22)	0.91	0.14 (-0.16, 0.43)	0.36	0.08 (-0.08, 0.23)	0.33	0.02 (-0.07, 0.12)	0.64
25(OH)D 50-75 nmol/L	-0.11 (-0.36, 0.14)	0.39	0.06 (-0.20, 0.31)	0.66	0.08 (-0.14, 0.30)	0.46	-0.02 (-0.11, 0.07)	0.65
25(OH)D >75 nmol/L	ref		ref		ref		ref	
Adjusted Model								
25(OH)D <50nmol/L	0.00 (-0.22, 0.22)	0.99	0.08 (-0.17, 0.33)	0.53	-0.01 (-0.15, 0.13)	0.88	0.03 (-0.06, 0.12)	0.50
25(OH)D 50-75 nmol/L	-0.04 (-0.21, 0.14)	0.67	0.04 (-0.12, 0.19)	0.62	0.08 (-0.15, 0.13)	0.23	-0.01 (-0.10, 0.08)	0.78
25(OH)D >75 nmol/L	ref		ref		ref		ref	
CRP 1-3 mg/L								
Unadjusted Model								
25(OH)D <50nmol/L	-0.07 (-0.32, 0.17)	0.54	0.19 (-0.04, 0.42)	0.10	0.09 (-0.13, 0.31)	0.41	0.01 (-0.12, 0.14)	0.86
25(OH)D 50-75 nmol/L	-0.29 (-0.64, 0.05)	0.10	-0.06 (-0.28, 0.17)	0.61	-0.13 (-0.36, 0.09)	0.23	-0.05 (-0.18, 0.09)	0.49
25(OH)D >75 nmol/L	ref		ref		ref		ref	
Adjusted Model								
25(OH)D <50nmol/L	0.08 (-0.16, 0.33)	0.49	0.08 (-0.18, 0.34)	0.53	0.06 (-0.15, 0.28)	0.55	-0.01 (-0.14, 0.12)	0.84
25(OH)D 50-75 nmol/L	-0.10 (-0.27, 0.07)	0.25	-0.01 (-0.18, 0.15)	0.87	-0.01 (-0.19, 0.17)	0.91	0.01 (-0.11, 0.14)	0.82
25(OH)D >75 nmol/L	ref		ref		ref		ref	
CRP 3-10 mg/L								
Unadjusted Model								
25(OH)D <50nmol/L	0.09 (-0.10, 0.28)	0.35	0.32 (0.07, 0.58)	0.02	0.25 (0.06, 0.45)	0.01	0.18 (0.09, 0.28)	0.001
25(OH)D 50-75 nmol/L	0.19 (-0.08, 0.45)	0.17	0.29 (-0.005, 0.59)	0.05	0.32 (0.05, 0.59)	0.02	0.18 (0.05, 0.31)	0.01
25(OH)D >75 nmol/L	ref		ref		ref		ref	
Adjusted Model								
25(OH)D <50nmol/L	-0.34 (-0.60, -0.09)	0.01	-0.11 (-0.42, 0.20)	0.47	-0.14 (-0.46, 0.18)	0.38	0.15 (-0.003, 0.31)	0.06
25(OH)D 50-75 nmol/L	-0.14 (-0.44, 0.16)	0.35	0.008 (-0.21, 0.23)	0.94	0.06 (-0.18, 0.31)	0.60	0.12 (-0.02, 0.26)	0.09
25(OH)D >75 nmol/L	ref		ref		ref		ref	

Mutually adjusted model includes a variable sum of the remaining depressive symptoms. Adjusted model includes age, ethnicity, smoking, oral contraceptive use, sleep duration, physical activity, body mass index, hs-CRP and season of blood draw.

Table 21: Logistic regression analysis of the association of high depression score with vitamin D in women (n=1,397)		
	High Depression Score (PHQ-9≥10)	
	OR (95% CI)	**p-value**
CRP<1 mg/L		
Unadjusted Model		
Vitamin D <50nmol/L	1.13 (0.29, 4.47)	0.86
Vitamin D 50-75 nmol/L	0.87 (0.17, 4.40)	0.87
Vitamin D >75 nmol/L	ref	
Adjusted Model		
Vitamin D <50nmol/L	1.47 (0.20, 10.6)	0.69
Vitamin D 50-75 nmol/L	1.44 (0.28, 7.29)	0.65
Vitamin D >75 nmol/L	ref	
CRP 1-3 mg/L		
Unadjusted Model		
Vitamin D <50nmol/L	0.89 (0.34, 2.30)	0.80
Vitamin D 50-75 nmol/L	0.58 (0.18, 1.87)	0.35
Vitamin D >75 nmol/L	ref	
Adjusted Model		
Vitamin D <50nmol/L	0.53 (0.15, 1.85)	0.31
Vitamin D 50-75 nmol/L	0.59 (0.17, 2.01)	0.39
Vitamin D >75 nmol/L	ref	
CRP 3-10 mg/L		
Unadjusted Model		
Vitamin D <50nmol/L	8.15 (2.74, 24.3)	<0.001
Vitamin D 50-75 nmol/L	6.55 (2.16, 19.8)	0.002
Vitamin D >75 nmol/L	ref	
Adjusted Model		
Vitamin D <50nmol/L	6.55 (1.30, 32.8)	0.02
Vitamin D 50-75 nmol/L	9.54 (2.70, 33.6)	0.001
Vitamin D >75 nmol/L	ref	

Adjusted for age, race/ethnicity, education, smoking, oral contraceptive use, sleep duration, physical activity, body mass index, high-sensitivity CRP, and season of blood draw.

Figure 9: Probability of depression changes with vitamin D levels based on sleep duration (<7 hours vs 7 or more hours per night). Interaction term significant at p=0.02 after adjustments for age, race/ethnicity, education, BMI, OC use, LTPA, CRP, smoking, and season of blood draw.
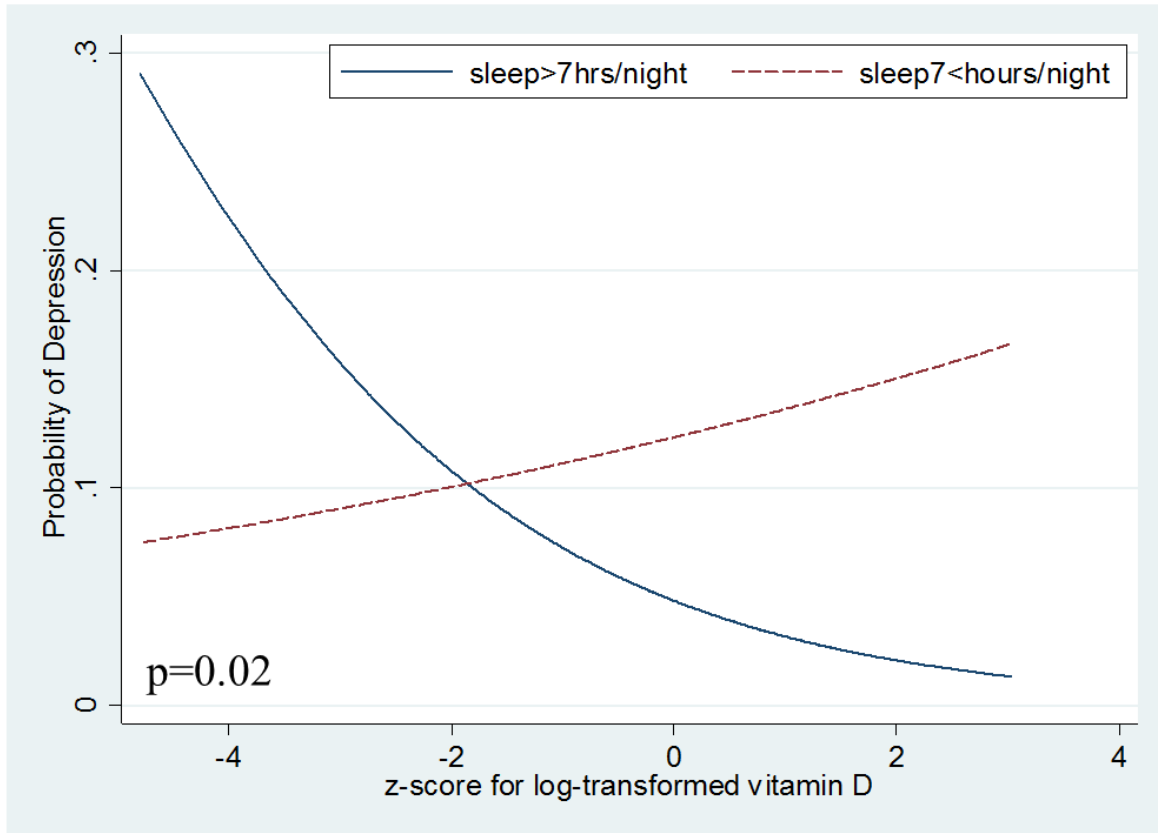
Figure 10: Probability of depression changes with vitamin D levels based on sleep duration. For women who sleep too little or too much (5 hours or 12+ hours per night), vitamin D is positively associated with odds of depression. Among women who sleep 7 or 9 hours per night, vitamin D is inversely related to odds of depression. Interaction term significant at p=0.01 after adjustments for age, race/ethnicity, education, BMI, OC use, LTPA, CRP, smoking, and season of blood draw.
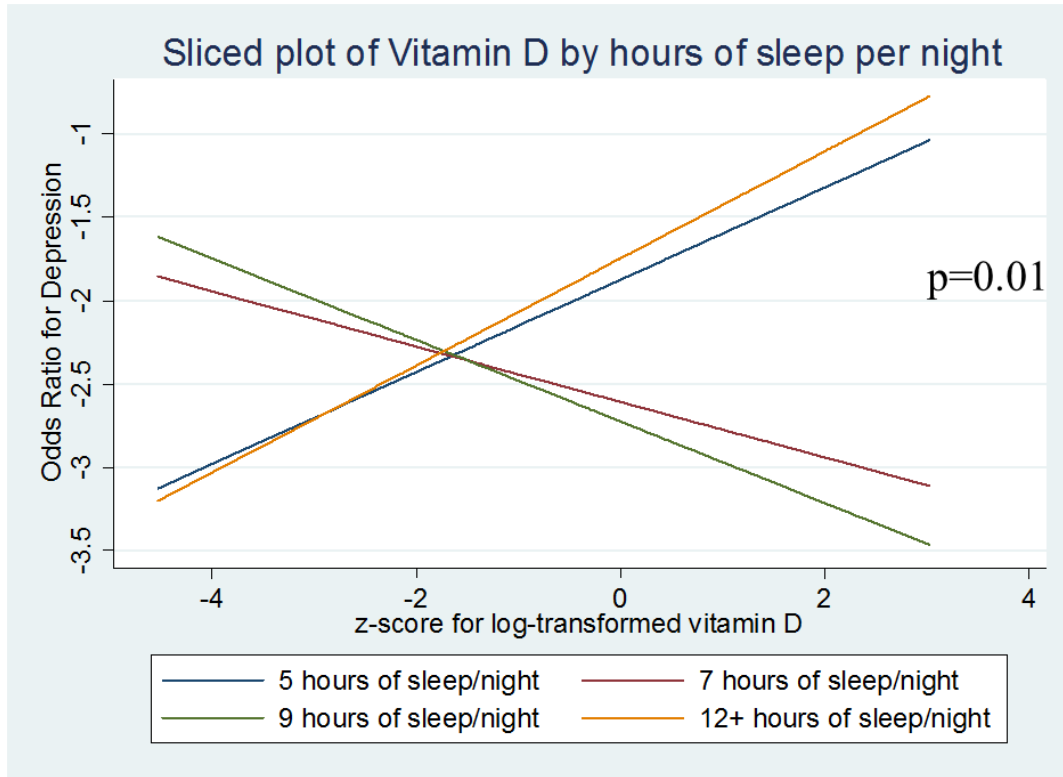
Table 22: Linear regression of the association of total depression score and depression subtypes with vitamin D in women of reproductive age from NHANES 2005-2008 stratified by Body Mass Index categories						
Per 1 S.D. increase in log-transformed 25(OH) vitamin D:	Depression defined as:					
	Total PHQ-9 score (range: 0-27)		Somatic Depression score (range: 0-12)		Non-somatic Depression score (range: 0-15)	
	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value
BMI <18.5 kg/m²						
Unadjusted	0.13 (-0.67, 0.94)	0.73	0.18 (-0.30, 0.66)	0.44	-0.05 (-0.43, 0.33)	0.80
Adjusted	1.71 (0.41, 2.99)	0.01	0.95 (0.16, 1.73)	0.02	0.76 (0.18, 1.33)	0.01
BMI 18.5-24.9 kg/m²						
Unadjusted	-0.27 (-0.60, 0.06)	0.11	-0.18 (-0.39, 0.04)	0.1	-0.09 (-0.28, 0.10)	0.35
Adjusted	0.07 (-0.43, 0.57)	0.78	-0.006 (-0.28, 0.26)	0.97	0.08 (-0.21, 0.36)	0.59
BMI 25-29.9 kg/m²						
Unadjusted	-0.14 (-0.61, 0.33)	0.55	-0.11 (-0.36, 0.14)	0.37	-0.03 (-0.28, 0.22)	0.81
Adjusted	0.11 (-0.44, 0.67)	0.69	0.05 (-0.24, 0.33)	0.74	0.06 (-0.23, 0.35)	0.67
BMI ≥30 kg/m²						
Unadjusted	-0.11 (-0.66, 0.44)	0.68	-0.09 (-0.40, 0.23)	0.59	-0.03 (-0.29, 0.23)	0.83
Adjusted	-0.22 (-0.83, 0.40)	0.48	-0.10 (-0.42, 0.23)	0.55	-0.12 (-0.49, 0.24)	0.50

Adjusted for age, race/ethnicity, education, smoking, sleep, physical activity, hs-CRP, continuous body mass index, OC use and season of blood draw.

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CHAPTER 10

CONCLUSION AND SIGNIFICANCE

Our work highlights the complexity of the relationship among micronutrient status, inflammation, and depression in women of reproductive age. The etiology of depression remains to be fully understood, and given the higher prevalence of depression among women, it is important to understand how nutrition, inflammation, and lifestyle factors may contribute to different dimensions of depression in this population. Body mass index, a proxy for adiposity, accounts for a large portion of the association between c-reactive protein and serum 25-hydroxyvitamin D with depression; vitamin B_6 deficiency was associated with increased odds of suicidal ideation, and sleep duration modified the association between vitamin D concentration and depression. These findings altogether indicate that the relationship between micronutrients and depression is multifaceted, and inflammation and lifestyle factors, such as sleep and body mass index, need to be considered when researching depression. Additionally, we provide increasing evidence in support of analyzing symptoms separately from total depression scores in order to shed light on the etiology of depression. This research expands our understanding of how deficiencies of vitamin B_6 or D, inflammation, and other lifestyle factors affect depression symptoms among women and may help identify potential prevention strategies or adjuvant treatments for depression that incorporate changes in nutrition or lifestyle factors.

CHAPTER 11

FUTURE DIRECTIONS

Our research has contributed to the field of nutrition and depression research by identifying how vitamin B_6 and vitamin D status may be related to various dimensions of depression among women, and how inflammation, sleep duration, and body mass index affect these associations. Our findings, which were mainly limited by the cross-sectional nature of the study design, provide scientific justification for future prospective studies where the directionality of the association between inflammation and micronutrient status can be examined over time to determine their contribution to each other and to the development of depression. In the context of low-grade inflammation, research is warranted to determine the optimal doses of vitamin B_6 to achieve concentrations that are associated with good health. It should be noted that future studies may lead to the reassessment of the current Recommended Dietary Allowance for vitamin B_6 among women of reproductive age. Furthermore, our findings emphasize the need for studies on vitamin D and depression to consider inflammatory status in order to determine whether those with inflammation would benefit from higher doses of vitamin D supplementation to alleviate depression symptoms.

Future studies can also assess how dietary inflammatory indices are related to micronutrient status and depression in this population while considering lifestyle factors. Although we did not assess dietary intake, ultimately, it is our goal as nutrition researchers to find evidence as to how nutritional changes promote better

health and reduce inflammation and to test whether these changes lead to improved

mental health and prevent or treat depression.

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