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## The effect of vitamin B-12 supplementation on visual reaction time in young adult females

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The effect of vitamin B-12 supplementation on visual reaction time in young adult females

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for the Degree of Master of Science

in Nutrition

in the Department of Food Science, Nutrition and Health Promotion

Mississippi State, Mississippi

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2023

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This study investigated the effect of vitamin B-12 supplementation on visual response time (VRT). Menstrual cycle phase (MCP) and 24-hour dietary intake of B-12 were also observed as dependent variables. This blind, placebo-controlled trial observed 14 young adult females in four treatment groups: control, 500mcg (low dose), 2000mcg (moderate dose), and 3000mcg (high dose). The study lasted 28 days, with participants treated weekly, randomly assigned to each of the treatment groups. When comparing B-12 dose treatment groups to VRT baseline, there was significant improvements in VRT. Compared to the placebo, there were no significant differences among treatment groups. Significant differences were observed among MCP in two of the four treatment groups. No significant differences were observed in 24-hour intake of B-12 prior to treatment.

## TABLE OF CONTENTS

LIST OF TABLES .....	iv
LIST OF FIGURES .....	v
CHAPTER	
I. INTRODUCTION .....	1
1.1 Research Rationale .....	1
1.2 Importance of Research .....	1
II. LITERATURE REVIEW: .....	3
2.1 Vitamin B-12 .....	3
2.1.1 Background .....	3
2.1.2 Digestion, Bioavailability, Biochemistry of Vitamin B-12 .....	3
2.1.3 Deficiency of Vitamin B-12 .....	5
2.1.4 Vitamin B-12 Deficiency Among Women .....	7
2.1.5 Evaluation of Vitamin B-12 .....	8
2.1.6 Recommended Intake of B-12 .....	9
2.1.7 B-12 Dosage .....	10
2.1.8 Commercial Dosage Vitamin B-12 .....	10
2.2 Human Response Time .....	11
2.2.1 Background .....	11
2.2.2 Visual vs. Auditory Response Time .....	11
2.2.3 Athletic Performance and Visual Response Time .....	12
2.2.4 Effect of Vitamin B-12 on Acute Visual Response Time .....	13
2.2.5 Visual Response Time as Method of Perceived Energy Levels .....	14
2.2.6 FitLight System .....	15
2.3 Menstrual Cycle and Response Time .....	16
2.3.1 Overview .....	16
2.3.2 The Menstrual Cycle Phases .....	17
2.3.3 Menstrual Cycle and Athletic Performance .....	18
2.3.4 Menstrual Cycle Hormones and Visual Response .....	19
2.3.5 Other Nutrients with Visual Response Time Considerations .....	20
III. METHODS .....	22
3.1 Participants .....	22

3.2	Treatment Groups .....	22
3.3	Procedures .....	23
3.4	Instruments .....	24
3.4.1	FitLight System .....	24
3.4.2	Menstrual Cycle Questionnaire .....	25
3.4.3	The Automated Self-Administered 24-hour (ASA24) Dietary Assessment <sup>62</sup> .....	25
3.5	Statistical Procedures.....	26
IV.	RESULTS.....	27
4.1	Demographic Analysis .....	27
4.1.1	Participants .....	27
4.1.2	Frequency Distribution for Menstrual Cycle Phase .....	27
4.1.3	Impact of Treatment Dose of B-12 supplementation on Visual Response Time.....	28
4.1.4	Impact of Menstrual Cycle Phase on Visual Response Time.....	29
4.1.5	Impact of Dietary B-12 Intake 24 hours before test on Visual Response Time .....	29
4.1.6	Linear Progression of VRT from Baseline.....	30
V.	DISCUSSION.....	31
5.1	Limitations.....	32
5.2	Future Implications.....	33
VI.	SUMMARY AND CONCLUSIONS.....	34
	REFERENCES .....	35
	APPENDIX	
A.	DATA TABLES, FIGURES, AND SURVEYS .....	41
A.1	DATA TABLES .....	42
A.2	FIGURES .....	52
A.3	SURVEYS.....	53
A.3.1	Menstrual Cycle Questionnaire .....	53

## LIST OF TABLES

Table 4.1	Frequency distribution of Menstrual Cycle Phase.....	27
Table 4.2	Frequency distribution of Treatment Dose of B-12 supplement. ....	28
Table A.1	Compiled Visual Response Time Scores with Treatment Group.....	42
Table A.2	Compiled VRT Results with Dietary B-12 and Menstrual Phases.....	43
Table A.3	Compiled 24-Intake of B-12 vs. RDA.....	47
Table A.4	Progression of Tests Table in Baseline VRT Differential.....	50
Table A.5	Demographic Information Table .....	51

## LIST OF FIGURES

Figure 2.1	FitLight system being used to evaluate motor movement time in response to a visual stimulus.....	15
Figure 3.1	A timeline of the procedures done in this study.....	24
Figure 3.2	FitLight system formation for this study.....	25
Figure A.1	FitLight System.....	52
Figure A.2	FitLight System Formation.....	52



# CHAPTER I

## INTRODUCTION

### **1.1 Research Rationale**

Individuals of all activity levels and ages have a desire for “natural” energy boosts to get through their day. Vitamin B-12 supplementation has been utilized to boost energy, acutely, due to the claims of the companies selling B-12 containing products commercially. These claims have trickled into the athletic community where many dietitians, coaches, strength coaches, athletic trainers, and athletes utilize these supplementations from those same assertions. This study seeks to explore the effect of commercial doses of B-12 on energy levels, measured in visual response time, as well as smaller and larger doses of the same B-12. This study will be blind to participants to observe any possible placebo effect of the supplementation. To mimic the athletic application of B-12 in pre-game or halftime scenarios, supplementation and response time measurements will be done acutely.

### **1.2 Importance of Research**

The importance of researching the effects of vitamin B-12 supplementation on visual response time lies in the widespread use of B-12 as a natural energy booster among individuals of all activity levels and ages. Despite the claims made by commercial companies regarding the benefits of B-12 supplementation, there is a lack of scientific evidence supporting these claims. Therefore, it is essential to explore the effects of different doses of B-12 on energy levels, particularly as measured by visual response time, to understand the potential benefits and

limitations of B-12 supplementation. This study aims to investigate the efficacy of B-12 supplementation on visual response time, both in smaller and larger doses. Furthermore, it seeks to identify any possible placebo effects of supplementation by conducting the study in a blind fashion. By identifying the menstrual cycle phase and 24-hour intake prior to supplementation, other variables can be accounted for that may be influencing its effects.

CHAPTER II  
LITERATURE REVIEW:

**2.1 Vitamin B-12**

**2.1.1 Background**

Vitamin B-12, also known as cobalamin, is an essential nutrient that is necessary for the proper function of the brain, nerves, and blood cells.<sup>1,3</sup> It is involved in the metabolism of every cell in the body and is necessary for the synthesis of DNA, the genetic material present in all cells.<sup>2</sup> Vitamin B-12 is found naturally in a variety of animal-derived foods, such as meat, fish, and dairy products, and is also available as a dietary supplement.<sup>2</sup> There are different forms of vitamin B-12, including cyanocobalamin, methylcobalamin, and adenosylcobalamin.<sup>1</sup> These forms differ in their chemical structure and the way they are metabolized in the body.<sup>1,2</sup> Cyanocobalamin is the most common form found in supplements, while methylcobalamin and adenosylcobalamin are found more naturally in foods.<sup>2,57</sup>

**2.1.2 Digestion, Bioavailability, Biochemistry of Vitamin B-12**

Vitamin B-12 is not easily absorbed by the body and requires the presence of a protein called intrinsic factor, which is produced by the stomach, to facilitate its absorption.<sup>14</sup> Inadequate production of intrinsic factor, as occurs in pernicious anemia, can lead to vitamin B-12 deficiency.<sup>14</sup> Supplementation with vitamin B-12 in the form of tablets or injections can be effective in preventing and treating vitamin B-12 deficiency.<sup>14</sup> However, the bioavailability of vitamin B-12 supplements can vary depending on the form in which they are taken. For example,

studies have shown that the bioavailability of vitamin B-12 from tablets is lower than that from injections.<sup>15,14</sup> The bioavailability of vitamin B-12 from animal-based (methylcobalamin and adenosylcobalamin) products is higher than from fortified foods or supplements (cyanocobalamin). For example, the bioavailability of vitamin B-12 from milk is approximately 50%, while the bioavailability from supplements ranges from 0.5% to 4.0%.<sup>56</sup>

One study reported that oral supplementation with methylcobalamin resulted in a greater increase in plasma vitamin B-12 levels than supplementation with cyanocobalamin in healthy individuals.<sup>58</sup> Another study reported that sublingual administration of methylcobalamin was more effective than oral cyanocobalamin in improving vitamin B-12 status in individuals with vitamin B-12 deficiency.<sup>59</sup>

Estimating the uptake of vitamin B-12 at high levels based on the RDA is challenging due to variations in bioavailability and individual differences in absorption and metabolism. The Schilling test is one method used to evaluate vitamin B-12 absorption and excretion.<sup>59</sup> This test involves administering a radioactive form of vitamin B-12 and measuring its excretion in the urine. However, the Schilling test is invasive and not routinely used.<sup>59</sup>

The digestion and absorption of vitamin B-12 occurs in the small intestine. The first step in the process is the release of hydrochloric acid and pepsin from the stomach substances help to break down the food and release vitamin B-12 from its protein-bound form.<sup>17</sup> Once vitamin B-12 is released from its protein-bound form, it is bound to a protein called intrinsic factor, which is produced by the parietal cells in the stomach. The vitamin B-12-intrinsic factor complex is then absorbed by the small intestine through a process known as passive diffusion.<sup>17</sup> However, for vitamin B-12 to be effectively absorbed, the small intestine must have sufficient levels of

intrinsic factor. Without enough intrinsic factor, vitamin B-12 will not be absorbed and will instead be excreted in the feces.<sup>17</sup>

The biochemistry of vitamin B-12 in the body after ingestion involves several steps. Firstly, vitamin B-12 is released from the dietary protein by the action of protease enzymes in the stomach. Next, it binds to R protein, a glycoprotein produced in the salivary glands and stomach, which protects it from degradation by stomach acid. In the small intestine, pancreatic proteases degrade the R protein, releasing vitamin B-12.<sup>60,61</sup> Vitamin B-12 then binds to intrinsic factor, a glycoprotein produced by the parietal cells of the stomach, which facilitates its absorption. The vitamin B-12-intrinsic factor complex then binds to receptors on the surface of intestinal cells in the ileum, the final segment of the small intestine. This facilitates the transport of vitamin B-12 into the portal vein, which carries it to the liver for storage. The liver can store up to several years' worth of vitamin B-12. When the body requires vitamin B-12, it is released from the liver and transported to the cells that require it.<sup>60</sup>

Methylcobalamin is involved in the conversion of homocysteine to methionine, an essential amino acid, and in the synthesis of DNA and RNA. Adenosylcobalamin is involved in the conversion of methylmalonyl-CoA to succinyl-CoA, an important step in the metabolism of fats and proteins. Cyanocobalamin must be converted to the active forms of vitamin B-12, methylcobalamin and adenosylcobalamin, before it can be used by the body. Cyanocobalamin is converted to hydroxocobalamin in the liver, which is then converted to either methylcobalamin or adenosylcobalamin.<sup>61</sup>

### **2.1.3 Deficiency of Vitamin B-12**

Vitamin B-12 deficiency is a common condition that can lead to a variety of health problems, including anemia, nerve damage, and cognitive decline.<sup>5</sup> There are several causes of

vitamin B-12 deficiency, including dietary deficiency, malabsorption, and decreased production.<sup>6</sup> The most common cause of dietary deficiency is a vegan or vegetarian diet, as vitamin B-12 is found almost exclusively in animal-derived foods.<sup>6</sup> Malabsorption can be caused by conditions such as pernicious anemia, Crohn's disease, and celiac disease, which interfere with the body's ability to absorb vitamin B-12 from the gastrointestinal tract.<sup>5</sup> Decreased production can occur in people with conditions that affect the production of intrinsic factor, a protein necessary for the absorption of vitamin B-12.<sup>7</sup>

The prevalence of vitamin B-12 deficiency varies widely depending on the population being studied. In developed countries, the prevalence is generally low, but it is more common in older adults and people with certain risk factors, such as a vegan or vegetarian diet, pernicious anemia, or a history of gastric surgery.<sup>6</sup> In developing countries, vitamin B-12 deficiency is more prevalent due to a lack of animal-derived foods in the diet.<sup>4</sup>

The pathophysiology of vitamin B-12 deficiency involves a complex series of events that can affect multiple systems in the body. In the nervous system, vitamin B-12 deficiency can lead to the demyelination of nerves, resulting in symptoms such as numbness, tingling, and weakness.<sup>5</sup> In the hematopoietic system, it can cause megaloblastic anemia, a type of anemia characterized by the production of large, abnormal red blood cells.<sup>7</sup> Vitamin B-12 deficiency can also interfere with DNA synthesis and the metabolism of fats and proteins, leading to a variety of other health problems.<sup>4</sup>

There are several forms of vitamin B-12 medication available, including oral tablets, capsules, and liquids, as well as intramuscular and subcutaneous injections.<sup>2</sup>

Oral forms of vitamin B-12 are the most convenient and widely available and can be taken as a supplement or as a prescription medication.<sup>20</sup> They are typically well-tolerated and

effective at raising vitamin B-12 levels in the body<sup>2</sup>, although they may not be suitable for everyone. For example, people with gastrointestinal disorders, such as celiac disease or Crohn's disease, may have difficulty absorbing vitamin B-12 from oral forms.<sup>20</sup>

Injections of vitamin B-12 are another option for people who have difficulty absorbing the nutrient from oral forms.<sup>2</sup> They are usually administered by a healthcare provider and can be given intramuscularly (into a muscle) or subcutaneously (under the skin).<sup>20</sup> Injections are generally more effective at raising vitamin B-12 levels than oral forms<sup>2</sup> and are often used to treat vitamin B-12 deficiency.<sup>20</sup>

#### **2.1.4 Vitamin B-12 Deficiency Among Women**

There is some evidence that females may be at risk for vitamin B-12 deficiency due to various factors, including vegetarian or vegan diets, gastrointestinal disorders, and certain medications. A research study from 2010 reported that vegan and vegetarian women may be at increased risk for vitamin B-12 deficiency due to their exclusion of animal-derived foods from their diets.<sup>8</sup> The review also concluded that older women may be at higher risk due to decreased stomach acid production, which can lead to impaired absorption of vitamin B-12 from food.<sup>9</sup>

Another study, from 2013, reported that gastrointestinal disorders, like their male counterparts, can lead to vitamin B-12 deficiency in females due to impaired absorption of the nutrient.<sup>11</sup> Certain medications, such as proton pump inhibitors (PPIs) and histamine-2 receptor antagonists (H2RAs), may also interfere with the absorption of vitamin B-12.<sup>12</sup> A review published in 2016 reported that long-term use of PPIs was associated with an increased risk of vitamin B-12 deficiency.<sup>13</sup>

### **2.1.5 Evaluation of Vitamin B-12**

There are several methods for evaluating vitamin B-12 status in the body, including serum vitamin B-12 concentration, serum methylmalonic acid (MMA) and homocysteine concentrations, and red blood cell (RBC) vitamin B-12 concentration.<sup>17</sup> Serum vitamin B-12 concentration is the most used method for evaluating vitamin B-12 status.<sup>18</sup> Normal serum vitamin B-12 concentrations are generally considered to be above 200 ng/L, while concentrations below this value may indicate vitamin B-12 deficiency.<sup>19</sup> However, it is important to note that the cutoff values for normal and abnormal serum vitamin B-12 concentrations may vary depending on the laboratory and the specific assay used.

Serum MMA and homocysteine concentrations are also used to evaluate vitamin B-12 status, as elevated levels of these metabolites may indicate a deficiency in vitamin B-12.<sup>17</sup> Normal values for serum MMA and homocysteine concentrations may vary depending on the specific assay and the reference range used by the laboratory.

RBC vitamin B-12 concentration is another method for evaluating vitamin B-12 status, and normal values are generally considered to be above 300 pg/mL.<sup>18</sup> However, like serum vitamin B-12 concentration, the cutoff values for normal and abnormal RBC vitamin B-12 concentrations may vary depending on the laboratory and the specific assay used.

It is important to note that the evaluation of vitamin B-12 status should not be based on a single laboratory test, as the results of these tests may be influenced by a variety of factors such as diet, medication use, and other underlying health conditions.<sup>17</sup> Therefore, the diagnosis of vitamin B-12 deficiency should be based on a combination of clinical and laboratory findings.



### **2.1.6 Recommended Intake of B-12**

The recommended dietary allowance (RDA) for vitamin B-12 varies by age and life stage.<sup>20</sup>

The RDAs for adults are as follows:

- 2.4 micrograms/day for men and women aged 19 and over<sup>20</sup>
- 2.6 micrograms/day for pregnant women<sup>20</sup>
- 2.8 micrograms/day for breastfeeding women<sup>20</sup>

The RDAs for children and adolescents are as follows:

- 0.9 micrograms/day for children aged 4-8<sup>20</sup>
- 1.2 micrograms/day for children aged 9-13<sup>20</sup>
- 1.8 micrograms/day for adolescents aged 14-18<sup>20</sup>

To ensure adequate intake of vitamin B-12, it is recommended to consume a varied diet that includes foods rich in this nutrient.<sup>20</sup> Some good dietary sources of vitamin B-12 include:

- Meat, poultry, and fish: Beef, liver, chicken, turkey, salmon, and cod are all good sources of vitamin B-12.
- Dairy products: Milk, cheese, and yogurt are all good sources of vitamin B-12.
- Eggs: Eggs are a good source of vitamin B-12, with one large egg providing approximately 0.6 micrograms.
- Fortified foods: Many breakfast cereals, plant-based milks, and other fortified foods are enriched with vitamin B-12.
- Fermented foods: Some fermented foods, such as tempeh and miso, contain small amounts of vitamin B-12 due to the presence of bacteria that synthesize the nutrient

### **2.1.7 B-12 Dosage**

Low doses of vitamin B-12 supplementation are typically defined as those that provide less than the Recommended Dietary Allowance (RDA) for vitamin B-12. The RDA for vitamin B-12 varies depending on age and gender, with adults aged 19-70 years old requiring 2.4 micrograms (mcg) per day.<sup>22</sup>

High doses of vitamin B-12 supplementation are generally considered to be those that exceed the RDA. A 1000 mcg per day dose of B-12 is recommended for adults aged 19-70 years, but is still considered to be on the higher end of daily intake by the institute of medicine.<sup>23</sup> There is no known upper limit for vitamin B-12 intake, as the body can excrete any excess through urine.<sup>24</sup> However, high doses of vitamin B-12 supplements (above the RDA) have been associated with some side effects, such as acne, dizziness, and gastrointestinal distress.<sup>24</sup>

It is important to note that while high doses of vitamin B-12 are generally considered to be safe, there is a lack of research on the long-term effects of very high doses of vitamin B-12.<sup>23</sup> Because of the water-soluble nature of the vitamin, commercial doses of B-12 are generally considered safe when taken as directed as well. Most people can meet their vitamin B-12 needs through a balanced diet and do not need to take supplements.<sup>24</sup>

### **2.1.8 Commercial Dosage Vitamin B-12**

The average commercial dose available for Vitamin B-12 supplements can vary depending on the product and intended use.<sup>61</sup> Common doses of Vitamin B-12 supplements available on the market include 500 micrograms per tablet or capsule, 1000 micrograms per tablet or capsule, 2500 micrograms per lozenge or sublingual tablet, and 5000 micrograms per injection or high-dose oral supplement.<sup>60</sup>

## **2.2 Human Response Time**

### **2.2.1 Background**

Human response time refers to the amount of time it takes for an individual to respond to a particular stimulus.<sup>25</sup> It is a fundamental element of human cognition and has been the subject of numerous scientific studies. One of the earliest studies on response time was conducted by Francis Galton in the late 19th century. Galton reported that the average response time for a simple task, such as pressing a button in response to a visual stimulus, was around 215 milliseconds. He also reported that response times varied significantly among individuals and were influenced by factors such as age, gender, and physical fitness.<sup>25</sup>

Since then, numerous studies have been conducted to investigate the factors that influence response time and to understand the underlying cognitive processes involved. One influential study on response time was conducted by psychologists John S. Leigh and David S. Poulton in the 1970s.<sup>26</sup> They reported that response times were faster for stimuli that were presented in the center of an individual's visual field, compared to stimuli presented at the periphery. They also reported that response times were faster for stimuli that were presented briefly, as opposed to those that were presented for longer periods of time.<sup>26</sup>

Response time is an important element of human cognition that has been the subject of these studies. Some of these studies have identified several factors that influence response time, including visual field location, stimulus duration, attention, and motivation.<sup>26,27</sup>

### **2.2.2 Visual vs. Auditory Response Time**

Researchers have primarily focused on two modalities of response time: auditory and visual. Auditory response time refers to the time it takes for an individual to respond to an

auditory stimulus, such as a sound or a voice. Visual response time refers to the time it takes for an individual to respond to a visual stimulus, such as a light or an image.

Studies have consistently shown that visual response time is generally faster than auditory response time. For example, a study from 2009 reported that visual response time was approximately 100 milliseconds faster than auditory response time.<sup>28</sup> Other studies have also reported similar results, with visual response time ranging from 70 to 120 milliseconds faster than auditory response time.<sup>28,30,31</sup>

One potential reason for this difference in response time is the different pathways that auditory and visual stimuli follow in the brain. Auditory stimuli are processed in the auditory cortex, which is in the temporal lobe. This processing involves multiple stages, including sound localization and identification, which may contribute to longer response times. In contrast, visual stimuli are processed in the primary visual cortex, which is in the occipital lobe. This processing is generally faster, which may explain the faster response times observed in visual tasks.

Another factor that may contribute to the difference in auditory and visual response time is the availability of attentional resources. Visual stimuli are generally more attention-grabbing than auditory stimuli, which may make it easier for individuals to attend to and respond to visual stimuli. This effect has been supported by studies showing that dividing attention between auditory and visual tasks leads to longer response times in both modalities.<sup>29</sup>

### **2.2.3 Athletic Performance and Visual Response Time**

Visual response time has been reported to be an important factor in athletic performance.<sup>32</sup> Many studies have investigated the relationship between visual response time and athletic performance in various sports, including soccer<sup>33</sup>, basketball<sup>34</sup>, and tennis.<sup>32</sup>

One study reported that soccer players with faster visual response times had a higher number of successful tackles and interceptions compared to those with slower response times.<sup>33</sup> Another reported that basketball players with faster visual response times had a higher number of successful passes and a lower number of turnovers.<sup>34</sup>

A review of literature reported that visual response time is an important predictor of performance in tennis, with faster response times being associated with a higher number of successful shots and a lower number of errors.<sup>32</sup> In addition to the specific sports mentioned above, visual response time has also been reported to be an important factor in the performance of other sports, including cricket, rugby, and track and field.<sup>32</sup>

#### **2.2.4 Effect of Vitamin B-12 on Acute Visual Response Time**

One area of research on vitamin B-12 is its effects on acute visual response time and perceived energy levels. Acute visual response time is the amount of time it takes for an individual to respond to a visual stimulus and is often used as a measure of cognitive function.<sup>48</sup> Perceived energy levels refer to an individual's subjective experience of their energy and alertness.<sup>49</sup>

Several studies have investigated the relationship between vitamin B-12 intake and acute visual response time. A 2014 study reported that higher levels of vitamin B-12 were associated with faster visual response times in elderly individuals.<sup>50</sup> Another study from 2011 concluded that vitamin B-12 supplementation improved visual response time in young adults.<sup>48</sup>

There is also evidence to suggest that vitamin B-12 may be able to improve perceived energy levels. A study in 2007 reported that vitamin B-12 supplementation was associated with improved energy levels and decreased fatigue in individuals with vitamin B-12 deficiency.<sup>49</sup> Another study in 1988 reported that vitamin B-12 supplementation improved energy levels and

decreased fatigue in individuals with pernicious anemia, a condition in which the body is unable to absorb vitamin B-12 due to a lack of intrinsic factor.<sup>51</sup>

Overall, the evidence suggests that vitamin B-12 intake may have a positive effect on both acute visual response time and perceived energy levels. However, more research is needed to fully understand the relationship between vitamin B-12 and these outcomes.

### **2.2.5 Visual Response Time as Method of Perceived Energy Levels**

Visual response time has been shown to be sensitive to changes in mental workload, fatigue, and even acute changes in energy levels. There is evidence that VRT can be affected by acute changes in energy levels, such as those caused by caffeine consumption. A study from 1998 reported that participants who consumed caffeine demonstrated faster VRT compared to a control group.<sup>54</sup> Similarly, it was reported that caffeine consumption resulted in significantly faster VRT and improved performance on a visual tracking task.<sup>53</sup>

In addition to caffeine, other factors such as sleep deprivation<sup>52</sup> and glucose levels<sup>55</sup> have also been reported to impact VRT. In 1997, a study reported that sleep deprivation was associated with slower VRT, while another reported that low glucose levels were associated with slower VRT.<sup>55</sup>

Overall, the research suggests that VRT is a useful measure of acute changes in energy levels and can provide insight into the effects of factors such as caffeine<sup>53,54</sup>, sleep deprivation<sup>52</sup>, and glucose levels<sup>55</sup> on cognitive function. This information validates that VRT can also be used to investigate perceived acute energy changes or lack thereof with vitamin B-12 supplementation.

### 2.2.6 FitLight System

A FitLight® system is a tool used in athletic training, rehabilitation, and performance enhancement. It consists of a series of small, portable, wireless light-emitting diodes (LEDs) that can be placed on a floor or wall and activated in various sequences to create specific training tasks or drills. The system is designed to improve response time, agility, coordination, and overall athletic performance by requiring individuals to respond to the changing patterns of light quickly and accurately.



Figure 2.1 FitLight system being used to evaluate motor movement time in response to a visual stimulus.

One study reported that using a FitLight® system in soccer training resulted in significant improvements in players' response time, sprint speed, and change of direction ability.<sup>36</sup> Another study reported that using a FitLight® system as part of a plyometric training program significantly improved vertical jump height and agility in female basketball players.<sup>35</sup>

In addition to its use in training, the FitLight® system has also been used in rehabilitation settings to improve balance, gait, and functional mobility in individuals with neurological conditions such as stroke and traumatic brain injury.<sup>37,38</sup> A review of the literature reported that the FitLight® system was effective in improving balance, response time, and functional mobility in older adults.<sup>37</sup>

A FitLight® system consists of 8 lights that can be placed on multiple surfaces that allow whoever is participating to react as quickly as possible to the lights when illuminated. After performing a set time with the machine, the FitLight® records the statistics of the participant including average response time to the visual stimulus. Figure 2.1 shows an example of a set-up of the FitLight® system.

## **2.3 Menstrual Cycle and Response Time**

### **2.3.1 Overview**

The menstrual cycle is a regular natural bodily process that occurs in women of reproductive age.<sup>40</sup> It involves the shedding of the uterine lining (endometrium) and the release of an egg from the ovaries, known as ovulation. The menstrual cycle is an important aspect of female reproductive health and is influenced by various hormonal and physiological factors.<sup>39</sup>

One of the key hormones involved in the menstrual cycle is estrogen, which plays a role in the growth and thickening of the endometrium.<sup>40</sup> Another important hormone is progesterone, which helps to maintain the endometrium and prepare the uterus for pregnancy.<sup>39</sup> The levels of these hormones fluctuate throughout the menstrual cycle and can affect the timing and characteristics of menstrual bleeding.



The menstrual cycle typically lasts about 28 days, although the length can vary from person to person and can be affected by factors such as stress, illness, and medications.<sup>40</sup> The first day of bleeding is considered the first day of the cycle.<sup>39</sup> The bleeding, known as menstruation, typically lasts for 3-7 days and is caused by the shedding of the endometrium.

Ovulation typically occurs around the midpoint of the menstrual cycle and is marked by the release of an egg from the ovary.<sup>39</sup> The release of the egg is triggered by a surge in luteinizing hormone (LH) and is accompanied by an increase in estrogen levels.<sup>40</sup> The egg travels through the fallopian tube. If the egg is not fertilized, the levels of estrogen and progesterone decrease, leading to the shedding of the endometrium and the start of the next menstrual cycle.<sup>39</sup>

### 2.3.2 The Menstrual Cycle Phases

The menstrual cycle can be divided into four main phases: the menstrual phase, the follicular phase, the ovulatory phase, and the luteal phase.

1. The **menstrual phase**, also known as the menstrual period, is the first phase of the menstrual cycle. It is marked by the shedding of the uterine lining, known as the endometrium, and the release of menstrual blood. This phase typically lasts 3-7 days and is caused by the decrease in level of the hormones estrogen and progesterone.<sup>40</sup> Menstrual cycle is often looped in with the follicular phase.
2. The **follicular phase** is the second phase of the menstrual cycle. It begins after the menstrual phase and lasts until ovulation. During this phase, the ovaries prepare for the release of an egg by producing follicle-stimulating hormone (FSH). This hormone stimulates the growth of a group of follicles, each containing an egg. One of these follicles will eventually mature and release an egg during ovulation.<sup>40</sup>

3. **The ovulatory phase** is the third phase of the menstrual cycle. It is marked by the release of an egg from the ovary, known as ovulation. Ovulation is triggered by a surge in luteinizing hormone (LH), which causes the mature follicle to rupture and release the egg. Ovulation typically occurs around the midpoint of the menstrual cycle, around day 14 in a 28-day cycle.<sup>40</sup>
4. The **luteal phase** is the final phase of the menstrual cycle. It begins after ovulation and lasts until the start of the next menstrual period. During this phase, the ruptured follicle becomes the corpus luteum, which produces progesterone to help maintain the endometrium in preparation for pregnancy. If the egg is not fertilized, the corpus luteum will eventually break down, leading to a decline in progesterone levels and the shedding of the endometrium, marking the start of the next menstrual cycle.<sup>40</sup>

### 2.3.3 Menstrual Cycle and Athletic Performance

The menstrual cycle has long been a subject of debate and research within the field of sports and exercise science.<sup>42</sup> Specifically, there has been an interest in understanding whether and how the menstrual cycle might affect athletic performance.

Several studies have investigated the relationship between the menstrual cycle and athletic performance in various sports, including running, cycling, and swimming.<sup>41</sup> In general, these studies have reported that the menstrual cycle does not significantly impact athletic performance in most women. For example, a study of elite runners reported no significant differences in running times or endurance between the follicular and luteal phases of the menstrual cycle.<sup>42</sup> Similarly, a study of elite swimmers reported no significant differences in swim times or endurance between the two phases of the menstrual cycle.<sup>41</sup>

However, some studies have reported small but statistically significant differences in certain aspects of athletic performance during different phases of the menstrual cycle. For example, one study reported that women had slightly higher  $VO_{2max}$  (a measure of aerobic capacity) and lower heart rates during exercise in the follicular phase compared to the luteal phase.<sup>42</sup> Another study reported that women had slightly lower strength and power output in the luteal phase compared to the follicular phase.<sup>41</sup>

### **2.3.4 Menstrual Cycle Hormones and Visual Response**

Female menstrual cycle hormones, particularly estrogen and progesterone, have been shown to have an impact on visual response time and perceived energy levels.<sup>44,45,46,47</sup>

One study reported that estrogen levels were significantly correlated with faster response times in a visual search task.<sup>45</sup> Similarly, another study reported that estrogen levels were positively correlated with response time in a visual discrimination task.<sup>46</sup> These findings suggest that estrogen may play a role in enhancing visual processing speed.

Progesterone has also been reported to influence visual response time. A study concluded that women in the luteal phase of their menstrual cycle, when progesterone levels are highest, had slower response times compared to women in the follicular phase, when progesterone levels are lower.<sup>45</sup> This suggests that progesterone may have a negative effect on visual response time.

In addition to visual response time, menstrual cycle hormones have also been reported to affect perceived energy levels. One study concluded that women reported higher levels of fatigue and lower levels of physical and mental energy during the premenstrual and menstrual phases of their cycle, when estrogen and progesterone levels are lower.<sup>47</sup> Another study reported that women in the luteal phase of their cycle reported higher levels of fatigue compared to those in

the follicular phase.<sup>44</sup> These findings suggest that changes in estrogen and progesterone levels may be associated with changes in energy levels as well.

Further research is needed to fully understand the mechanisms underlying these effects and to determine the clinical implications of these findings.

### **2.3.5 Other Nutrients with Visual Response Time Considerations**

There are other vitamins and minerals that have been studied for their potential to improve visual response time. Some of these are: vitamin A, vitamin C, vitamin E, zinc, and magnesium.

Studies have suggested that vitamin A supplementation may improve visual response time. A study conducted on healthy young adults found that supplementing with vitamin A improved their visual response time by 17.4% compared to a placebo group.<sup>66</sup> Another study on elderly subjects with low vitamin A levels showed that supplementation improved visual reaction time by 11.4%.<sup>67</sup>

Vitamin C has also been looked at in relation to VRT. Studies have suggested that vitamin C supplementation may improve visual response time.<sup>66,67</sup> A study conducted on healthy young adults reported that supplementing with vitamin C improved their visual response time by 7.5% compared to a placebo group.<sup>66</sup> Another study on elderly subjects reported that supplementation with vitamin C improved visual reaction time by 10.6%.<sup>67</sup>

Studies have also suggested that vitamin E supplementation may improve visual response time.<sup>67</sup> A study conducted on healthy young adults found that supplementing with vitamin E improved their visual response time by 11.8% compared to a placebo group.<sup>67</sup>

Zinc supplementation has also been studied as a possible factor to improve visual response time. A study conducted on healthy young adults found that supplementing with zinc

improved their visual response time by 7.3% compared to a placebo group.<sup>66</sup> Another study on elderly subjects found that supplementation with zinc improved visual reaction time by 5.8%.<sup>67</sup>

Lastly, magnesium supplementation has been reported in studies to potentially improve visual response time. A study conducted on healthy young adults found that supplementing with magnesium improved their visual response time by 8.9% compared to a placebo group.<sup>66</sup>

In the studies forementioned, an improvement was found in those with insufficient or deficient nutrient levels. More research is needed to fully understand the effects of these nutrients on visual response time and in these studies.

## CHAPTER III

### METHODS

#### **3.1 Participants**

The sample for this study consisted of 14 female college students who self-reported as being moderately to very active daily (at least 30 minutes of exercise 5 days or more per week). The ages of the participants ranged from 19 to 22 years old. All the participants were non-vegetarian and reported having a normal duration menstrual cycle. Participants were recruited through the distribution of recruitment fliers and class announcements. Inclusion criteria for the study included being a female college student, self-reported moderate to high levels of daily physical activity, and a non-vegetarian diet. Exclusion criteria included being a vegan or vegetarian, being sedentary or less active than moderately active, and having an abnormal menstrual cycle.

#### **3.2 Treatment Groups**

In this study, four treatments are being compared. The first treatment is the control or placebo group, which receives a drink that is flavored with citric acid but contains no added cobalamin (vitamin B-12). The second group receives a lower dose of cobalamin (500 micrograms) compared to the typical commercial supplement. The third group receives a dose of cobalamin (2,000 micrograms) that is similar to a commercial dose and application. The fourth group receives a higher dose of cobalamin (3,000 micrograms) than the commercial dose, to determine if the commercial dose is sufficient to produce improvements in response time. By

comparing the effects of these different treatment groups, the study aims to understand the optimal dosage and potential benefits of cobalamin supplementation.

### **3.3 Procedures**

Participants in this study underwent familiarization trials over the course of one week, during which they performed a response time test using the FitLight System with a day in between each trial (Monday, Wednesday, Friday). These trials eliminated user bias and were not formally recorded. Following the familiarization week, a baseline score for visual response time was obtained on the system.

Over the course of 28 days, each participant was tested four times at random, once in each of the four treatment groups. The treatment group was determined by an online random number generator. Testing of VRT took place at the same time on the same day for the participants, 7 days apart. Prior to each VRT recording, participants completed a 24-hour dietary recall on the Automated Self-Administered 24-hour (ASA24) Dietary Assessment Tool to determine vitamin B-12 intake leading up to testing. Dietary vitamin B-12 from their 24-hour recall nutrient analysis, were used to compare intake levels with VRT results. Participants also filled out a menstrual cycle questionnaire prior to each recording to determine which menstrual cycle phase they were in during each treatment group assignment. Participants consumed their assigned treatment drink and performed a single response time test 15 minutes later. The test featured a randomized and different pattern of lights each time in order to prevent memorization or familiarization with the test. The same number of lights were lit each test and each light lit the same number of times as previous tests, regardless of the randomized nature. The 15-minute timing of the testing from administration of B-12 is to mimic and examine the acute effect nature

in question. Results from each FitLight test were not discussed with participants until after the conclusion of the study.

The study design aimed to observe the relationship and any significance between the following:

- Vitamin B-12 supplementation doses and VRT results.
- Acute dietary vitamin B-12 intake (24-hour) and VRT results.
- Menstrual cycle phase and VRT results.

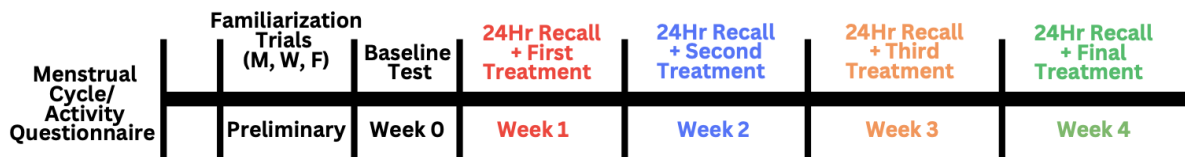


Figure 3.1 A timeline of the procedures done in this study.

### 3.4 Instruments

#### 3.4.1 FitLight System

The FitLight system consists of a series of light-emitting diodes (LEDs) that can be mounted on stands or attached to walls or other surfaces. The LEDs can be programmed to flash in various patterns, and users are typically required to react to the flashing lights by touching the illuminated LED with a hand-held sensor. In this study, the diodes were mounted on a wall and arranged in a half circle shape like seen in image 2.



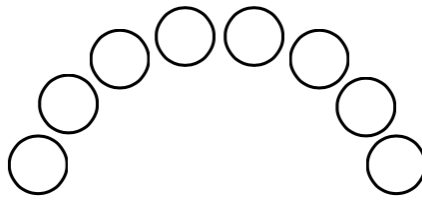


Figure 3.2 FitLight system formation for this study.

### **3.4.2 Menstrual Cycle Questionnaire**

Each participant was asked to complete a questionnaire regarding their last menstruation date, length of cycle and their use of contraception (or lack thereof). Their activity level was also asked on this questionnaire.

### **3.4.3 The Automated Self-Administered 24-hour (ASA24) Dietary Assessment<sup>62</sup>**

The Automated Self-Administered 24-hour (ASA24) Dietary Assessment<sup>62</sup> is a tool that is used to collect dietary intake data from study participants. It is a web-based application that allows participants to enter information about the foods and beverages they consumed over a 24-hour period.

Participants were asked to enter detailed information about the foods and beverages they consumed, including portion sizes and specific brands or types. The website also includes a food and beverage database that allowed participants to search for and select items from a list of common foods and beverages. Their intake was compared against the RDA for their age and sex, which is 2.4 mcg/day, and utilized to observe any relationship between dietary intake and VRT results after the various treatments.

### **3.5 Statistical Procedures**

To assess the statistical significance of the treatment groups compared to the placebo and the baseline, a series of t-tests were conducted. Within each treatment group, an ANOVA was performed to evaluate the significance across menstrual cycle phases. Additionally, a series of T-tests were utilized to investigate the significance of B-12 intake prior to treatment. IBM® SPSS Statistics system was used to analyze the statistics of this study.

## CHAPTER IV

### RESULTS

#### 4.1 Demographic Analysis

##### 4.1.1 Participants

Most of the participants in this study are between the ages of 19 and 21, with 10 out of 14 participants falling within this age range. The range of ages is 19-22, the mean age is 20.5, with a standard deviation of 0.91. The range of height for the participants 157.5-175.3 cm and the mean height is 165.1 cm. The range of weights is 52.3-68.2 kg, the mean weight is 59.2 kg, with a standard deviation of 7.27.

The participants are evenly split between "Moderately" and "Very" active. Many of the participants exercise between 4-8 hours per week, with 8 out of 14 participants falling within this range. The range of hours per week of exercise is 4-10, and the mean hours per week exercise is 6, and the standard deviation was 1.76. The range of average length of cycle is 28-34 and the mean length of cycle is 30.4, with a standard deviation of 2.12.

##### 4.1.2 Frequency Distribution for Menstrual Cycle Phase

Table 4.1 shows the frequency distribution of Menstrual Cycle Phases. Among the total number of respondents, Luteal (n= 23, 41.4%) which was greater than other subcategories. Table 4.2 shows the frequency distribution of Treatment Dose of B-12 supplementation groups.

Table 4.1 Frequency distribution of Menstrual Cycle Phase

Sub – category	Frequency	Percentage
Follicular	16	28.6
Luteal	23	41.1
Ovulation	17	30.4
Total	56	100.0

Table 4.2 Frequency distribution of Treatment Dose of B-12 supplement.

Sub – category	Frequency	Percentage
High	14	25.0
Low	14	25.0
Moderate	14	25.0
Placebo	14	25.0
Total	56	100.0

**4.1.3 Impact of Treatment Dose of B-12 supplementation on Visual Response Time**

This study investigated the relationship between treatment dose of vitamin B-12 supplementation and acute visual response time. When comparing the difference in acute visual response time from baseline, the results improved visual response time in all four treatment groups. The placebo group scores improved by an average of 52.03 ms ( $p = .003$ ), the low dose group scores improved by an average of 57.93 ms ( $p = .006$ ), the moderate dose group scores improved by an average of 50.40 ms ( $p = .022$ ) and the high dose scores increased by an average of 44.24 ms ( $p = .005$ ).

When comparing the impact of treatment doses on the visual response time differences vs. the placebo group, no significant improvement was observed. The  $p$  value of the low dose treatment group vs. placebo was 0.256, the  $p$  value of the moderate dose treatment group vs. placebo was 0.461, and the  $p$  value of the high dose treatment group was 0.235.

#### **4.1.4 Impact of Menstrual Cycle Phase on Visual Response Time**

This study also aimed to investigate the relationship between menstrual cycle phase and visual response time. There was no significant difference among the three observed menstrual cycle phases within the placebo treatment group ( $p = .296$ ) or the high dose treatment group ( $p = .426$ ). There was a significant difference among the three menstrual cycle phases in the low dose treatment group ( $p = .039$ ) and in the moderate dose treatment group ( $p = .044$ ). In the low dose treatment groups, follicular and ovulatory phases displayed the biggest improvement in VRT scores while the ovulatory phase provided the biggest improvement in VRT in the moderate dose group.

#### **4.1.5 Impact of Dietary B-12 Intake 24 hours before test on Visual Response Time**

This study also investigated the influence of dietary B-12 intake 24 hours, measured as the difference from the RDA intake of 2.4 mcg, before a test on visual response time. In each treatment group, the change in VRT was compared against B-12 intake. For the placebo treatment group, there was no significant improvement in VRT scores ( $p = .148$ ). There was also no significant improvement in the low dose treatment group ( $p = .162$ ), the moderate dose treatment group ( $p = .162$ ), or the high dose treatment group ( $p = .860$ ).

Two groups, one of VRT results from those who consumed below the RDA 24-hours prior to testing and a group of those who consumed above the RDA 24-hours prior to testing,

were compared. There was no significant improvement in either group when comparing VRT ( $p = 0.163$ )

#### **4.1.6 Linear Progression of VRT from Baseline**

The average VRT scores of participants through chronological tests, regardless of treatment group assigned, was measured. Test 1 was the first random treatment group assigned with the test 2, 3, and 4 being the second, third, and fourth test and treatment completed. From test 1 to test 2, there was no significant improvement, with an average improvement of 34.47 ms ( $p = 0.135$ ). However, test 1 compared to test 3 and test 4 showed a significant improvement in VRT scores ( $p = 0.005$ ,  $p = 0.001$ ). From test 1 to 3, scores improved by an average of 58.73 ms and improved by 68.02 ms from score 1 to 4. Significant improvement in VRT scores were also observed in test 2 compared to test 3 (14.07 ms,  $p = 0.039$ ), test 2 to test 4 (23.36 ms,  $p = 0.005$ ), and test 3 to test 4 (9.29 ms,  $p = 0.003$ ).

## CHAPTER V

### DISCUSSION

First, it's important to estimate the absorption of the treatment doses of vitamin B-12 from cyanocobalamin. Based on the available evidence, the estimated absorption rate of cyanocobalamin is around 1.5% to 2%.<sup>63,64</sup> Using this information, we can estimate the likely amount of absorption as follows:

- 500 mcg dose: 7.5 mcg to 10 mcg.
- 2000 mcg dose: 30 mcg to 40 mcg.
- 3000 mcg dose: 45 mcg to 60 mcg.

It's important to note that these are estimates based on the available evidence and individual factors can affect the absorption rate of cyanocobalamin.<sup>63,64,65</sup> These estimated amounts may be helpful in determining the intake of active B-12 needed to achieve similar results.

The results of this study showed a significant improvement in VRT among every treatment group. For this reason, the treatment groups were compared to the placebo group results in addition to the baseline results. Since there was no significant improvement between the treatment groups (low, moderate, high), a placebo effect may be taking place in the participant taking these doses of vitamin B-12. Another consideration is that the participants became more familiar and skilled at the VRT test. The significant improvements from the first tests completed through the final treatment confirms the possibility of this limitation in the study.

The relationship between menstrual cycle phase and visual response time was also investigated using a series of T-tests. The significant improvements observed in the low and moderate dose groups among the cycle phases suggest that menstrual cycle phase could possibly influence the response to B-12 acutely. However, it is difficult to draw conclusions with the sample sizes within these treatment groups ( $N = 3$ ), and due to the lack of consistency in which phase positive affected the VRT, due to all three phases being significant across the two treatment groups determined to have significant differences in VRT. There was no significant improvement in VRT when observing vitamin B-12 intake leading up to the treatment.

These findings suggest that taking higher doses of B-12 supplements may have an improving impact on visual response time, but further studies would need to be done to validate due to the small sample size of this study. This information could be useful for individuals or athletes who are considering taking B-12 supplements to improve their visual response time. The limitations in this study need to be further studied to confirm this vs. a trainability response from the participants.

## **5.1 Limitations**

One limitation of our study design is that familiarity and increased visual response time skill were likely developed by participants throughout the study, regardless of treatment. Considering these limitations, future research could benefit from a larger sample size, increasing the generalizability of the findings. Additionally, future studies could include a more diverse sample to increase external validity and test the generalizability of the findings to different populations. Furthermore, future research could also focus on long-term effects of B-12 intake levels and menstrual cycle to avoid acute dietary changes influenced by the study and to observe the effect of commercial doses on acute visual response time, auditory or other visual response



times using lower extremities or full body, as well as subjective alertness levels. Lastly, measuring serum B-12 levels would provide more concrete data on the participants' B-12 intake and could be included in future research to provide a more complete understanding of the relationship between B-12 intake and visual response time, while controlling for familiarity and increased VRT skill.

## **5.2 Future Implications**

Considering these limitations, future research could benefit from a more thorough familiarity trial to decrease the potential improvement throughout the study. Future studies could also benefit from a larger sample size, increasing the generalizability of the findings. Additionally, a more diverse sample to increase external validity and test the generalizability of the findings to different population would be beneficial. Furthermore, future research could also focus on long-term effects of B-12 intake levels and menstrual cycle to avoid acute dietary changes influenced by the study and to observe that effect of commercial doses on acute visual response time. Auditory or other visual response times using lower extremities or full body could be tested as well as subjective alertness levels. Lastly, measuring serum B-12 levels would provide more concrete data on the participants' B-12 intake and could be included in future research to provide a more complete understanding of the relationship between B-12 intake and visual response time.

## CHAPTER VI

### SUMMARY AND CONCLUSIONS

In conclusion, the results of this study suggest that taking higher doses of vitamin B-12 supplements may improve visual response time. However, this improvement is likely due to a placebo effect rather than the actual effect of the supplement. It's important to note that the estimated absorption rates of cyanocobalamin were used to determine the amount of active B-12 absorbed, but individual factors can affect absorption rates. Additionally, the study reported that menstrual cycle phase may influence the acute response to B-12, but the sample sizes within the treatment groups were too small to draw definitive conclusions. Finally, the 24-hour intake of B-12 prior to supplementation had no significant effect on the response times measured. The limitations of the study suggest that further research is needed to confirm whether the observed improvements are due to a placebo effect or a trainability response from the participants. These findings may be useful for individuals or athletes considering taking B-12 supplements to improve their visual response time.

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APPENDIX A  
DATA TABLES, FIGURES, AND SURVEYS

## A.1 DATA TABLES

Table A.1 Compiled Visual Response Time Scores with Treatment Group

Participant Number:	Baseline RT: (ms)	Placebo RT: (ms)	Low Dose RT: (ms)	Moderate Dose RT: (ms)	High Dose RT: (ms)
1	636.8	594.133	616.667	596.2	611.4
11	438.533	408	447.267	444.867	445.133
13	522.133	482.6	522.4	484.267	492.6
14	668.733	582.467	592.533	585.867	629.8
15	607.467	588.333	568	547.133	555
16	670.133	542.6	572.667	567.267	626.133
17	687.8	594.333	575.533	620.667	590.867
19	435.733	382.667	377.6	409.8	431.667
3	570.067	506.733	507.267	575.667	501
4	492.933	525.267	490.733	487.067	501.447
6	581.533	522.533	499.867	566.6	492.733
7	682.667	596.333	519.133	502.067	578.8
8	468.2	445.8	435	428.676	424.324
9	479.733	442.267	406.733	413.733	431.533

Table A.2 Compiled VRT Results with Dietary B-12 and Menstrual Phases

<b>Menstrual Cycle Phase:</b>	<b>Treatment Dose of B-12 supplement:</b>	<b>Visual Response Time (ms)</b>	<b>Dietary B-12 Intake 24 hours before test (mcg)</b>
Ovulation	High	431.533	9.82
Follicular	High	431.667	1.11
Luteal	High	445.133	2.7
Follicular	High	477	4.03
Follicular	High	492.6	5.29
Ovulation	High	492.733	4.49
Follicular	High	501	3.65
Luteal	High	501.447	7.19
Luteal	High	555	5.66
Ovulation	High	578.8	3.4
Luteal	High	590.867	6.03
Ovulation	High	611.4	3.89
Luteal	High	626.133	15.22
Luteal	High	629.8	5.27
Ovulation	Low	406.733	3.84
Luteal	Low	377.6	1.2
Luteal	Low	435	0.45
Luteal	Low	447.267	1.54

Table A.2 (continued)

<b>Menstrual Cycle Phase:</b>	<b>Treatment Dose of B-12 supplement:</b>	<b>Visual Response Time (ms)</b>	<b>Dietary B-12 Intake 24 hours before test (mcg)</b>
Luteal	Low	490.733	8.49
Luteal	Low	499.867	1.12
Luteal	Low	507.267	9.28
Follicular	Low	519.133	8.97
Luteal	Low	522.4	1.81
Follicular	Low	568	2.62
Ovulation	Low	572.667	10.14
Ovulation	Low	575.533	13.25
Follicular	Low	592.533	1.11
Luteal	Low	616.667	9.9
Follicular	Moderate	566.6	10.96
Ovulation	Moderate	567.267	12.42
Follicular	Moderate	575.667	6.57
Luteal	Moderate	585.867	2.89
Follicular	Moderate	596.2	1.65
Luteal	Moderate	620.667	4.94
Luteal	Placebo	382.667	0.99
Follicular	Placebo	408	2.7

Table A. 2 (continued)

<b>Menstrual Cycle Phase:</b>	<b>Treatment Dose of B-12 supplement:</b>	<b>Visual Response Time (ms)</b>	<b>Dietary B-12 Intake 24 hours before test (mcg)</b>
Ovulation	Placebo	442.267	2.52
Luteal	Placebo	445.8	4.03
Ovulation	Placebo	482.6	3.12
Follicular	Placebo	506.733	2.82
Follicular	Placebo	522.533	2.12
Luteal	Placebo	525.267	4.67
Follicular	Placebo	542.6	17.58
Ovulation	Placebo	582.467	0.94
Ovulation	Placebo	588.333	3.62
Luteal	Placebo	594.133	0.5
Follicular	Placebo	594.333	2.7
Luteal	Placebo	596.333	3.42
Luteal	Moderate	502.067	2.44
Ovulation	Moderate	547.133	5.74
Ovulation	Moderate	409.8	1.87
Luteal	Moderate	413.733	5.62
Ovulation	Moderate	444.867	3.26
Ovulation	Moderate	459.47	0.45

Table A.2 (continued)

<b>Menstrual Cycle Phase:</b>	<b>Treatment Dose of B-12 supplement:</b>	<b>Visual Response Time (ms)</b>	<b>Dietary B-12 Intake 24 hours before test (mcg)</b>
Follicular	Moderate	484.267	1.24
Ovulation	Moderate	487.067	3.17

Table A.3 Compiled 24-Intake of B-12 vs. RDA

RT Difference (ms)	B-12 intake 24 hours prior (mcg)	Compared to RDA:	Treatment:
19.134	3.62	1.22	Placebo
86.334	3.42	1.02	Placebo
-32.334	4.67	2.27	Placebo
42.667	0.5	-1.9	Placebo
93.467	2.7	0.3	Placebo
127.533	17.58	15.18	Placebo
37.466	2.52	0.12	Placebo
30.533	2.7	0.3	Placebo
86.266	0.94	-1.46	Placebo
39.533	3.12	0.72	Placebo
63.334	2.82	0.42	Placebo
59	2.12	-0.28	Placebo
22.4	4.03	1.63	Placebo
53.066	0.99	-1.41	Placebo
39.467	2.62	0.22	Low
163.534	8.97	6.57	Low
2.2	8.49	6.09	Low
20.133	9.9	7.5	Low
112.267	13.25	10.85	Low

Table A.3 (continued)

RT Difference (ms)	B-12 intake 24 hours prior (mcg)	Compared to RDA:	Treatment:
97.466	10.14	7.74	Low
73	3.84	1.44	Low
-8.734	1.54	-0.86	Low
76.2	1.11	-1.29	Low
-0.267	1.81	-0.59	Low
62.8	9.28	6.88	Low
81.666	1.12	-1.28	Low
33.2	0.45	-1.95	Low
58.133	1.2	-1.2	Low
60.334	5.74	3.34	Moderate
180.6	2.44	0.04	Moderate
5.866	3.17	0.77	Moderate
40.6	1.65	-0.75	Moderate
67.133	4.94	2.54	Moderate
102.866	12.42	10.02	Moderate
66	5.62	3.22	Moderate
-6.334	3.26	0.86	Moderate
82.866	2.89	0.49	Moderate
37.866	1.24	-1.16	Moderate



Table A.3 (continued)

RT Difference (ms)	B-12 intake 24 hours prior (mcg)	Compared to RDA:	Treatment:
-5.6	6.57	4.17	Moderate
14.933	10.96	8.56	Moderate
33.2	0.45	-1.95	Moderate
25.933	1.87	-0.53	Moderate
52.467	5.66	3.26	High
103.867	3.4	1	High
-8.514	7.19	4.79	High
25.4	3.89	1.49	High
96.933	6.03	3.63	High
44	15.22	12.82	High
48.2	9.82	7.42	High
-6.6	2.7	0.3	High
38.933	5.27	2.87	High
29.533	5.29	2.89	High
69.067	3.65	1.25	High
88.8	4.49	2.09	High
33.2	4.03	1.63	High
4.066	1.11	-1.29	High

Table A.4 Progression of Tests Table in Baseline VRT Differential

Participant	Baseline	Test 1	Test 2	Test 3	Test 4
1	636.8	-42.667	-40.6	-25.4	-20.133
11	438.533	-30.533	6.6	8.734	6.334
13	522.133	0.267	-29.533	-39.533	-37.866
14	668.733	-38.933	-82.866	-76.2	-86.266
15	607.467	-19.134	-39.467	-52.467	-60.334
16	670.133	-97.466	-44	-102.866	-127.533
17	687.8	-67.133	-93.467	-96.933	-112.267
19	435.733	-25.933	-4.066	-58.133	-53.066
3	570.067	5.6	-63.334	-62.8	-69.067
4	492.933	8.514	32.334	-2.2	-5.866
6	581.533	-14.933	-81.666	-59	-88.8
7	682.667	-86.334	-103.867	-163.534	-180.6
8	468.2	-22.4	-33.2	-25.933	-43.876
9	479.733	-37.466	-48.2	-66	-73

Table A.5 Demographic Information Table

<b>Partici pant #:</b>	<b>Age</b>	<b>height (in)</b>	<b>weight kg</b>	<b>Activity Level</b>	<b>Hrs per week exercise</b>	<b>Avg Length Cycle (Days)</b>	<b>BC (Y/N)</b>	<b>B-12 Supp (Y/N)</b>	<b>MVI Supp (Y/N)</b>	<b>Vega n or Veg (Y/N)</b>
#00001	20	69	54.5	Moderately	5-6	30	N	N	N	N
#00003	20	65	56	Moderately	4-5	28	N	N	N	N
#00004	21	64	57.2	Moderately	4-5	32	N	N	N	N
#00006	21	66	62.4	Very	8-10	28-30	N	N	N	N
#00007	22	65	56.8	Very	10	32-34	N	N	N	N
#00008	20	65	54.5	Very	6-8	30	N	N	N	N
#00009	21	66	61.4	Very	5-10	32	N	N	N	N
#00011	21	64	55.4	Very	4-8	28	N	N	N	N
#00013	20	65	60.9	Moderately	5-6	28	N	N	N	N
#00014	20	62	64.5	Moderately	6-7	28-32	N	N	N	N
#00015	20	68	61.5	Very	8-10	30-32	N	N	N	N
#00016	20	63	68.2	Moderately	4-8	30-34	N	N	N	N
#00017	19	62	58.2	Moderately	6-7	28-34	N	N	N	N
#00019	19	65	52.3	Very	6-7	28	N	N	N	N

## A.2 FIGURES



Figure A.1 FitLight System

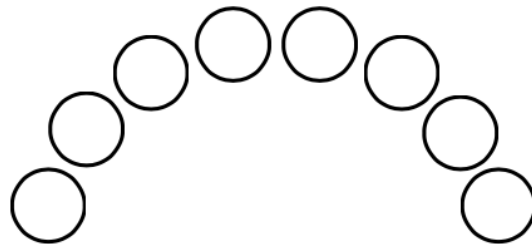


Figure A.2 FitLight System Formation

## A.3 SURVEYS

### A.3.1 Menstrual Cycle Questionnaire

**Mississippi State University**

Menstrual Cycle and General Activity Level Questionnaire

**Participant Number:** \_\_\_\_\_ **Date:** / / **Height:** \_\_\_ft \_\_\_in. **Weight:** \_\_\_\_\_lbs

1: State the last date of your menstruation.

/ /

2: How would you describe your menstrual cycle? (please check)

Regular  Irregular  Menopause

a) If answered regular, state the average length of the cycle.

\_\_\_\_\_ Days

3: Are you currently taking regular birth control?

Yes  No

a) If answered yes, please state what method:

\_\_\_\_\_

**Activity Level**

1: On average how many hours do you exercise per week?

\_\_\_\_\_ hours/week

2: How often do you take part in:

a) Moderate intensity: \_\_\_\_\_ hours/week

b) Intense activity: \_\_\_\_\_ hours/week

**Vitamin B12**

1: Are you currently consuming a Vitamin B12 supplement? **Yes / No**

If so, state the **BRAND** \_\_\_\_\_ and **FREQUENCY** \_\_\_\_\_

2: Are you currently consuming a Multivitamin supplement? **Yes / No**

If so, state the **BRAND** \_\_\_\_\_ and **FREQUENCY** \_\_\_\_\_