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Dark Chocolate (70% Cacao) Modulates Gamma Wave Frequencies in Vigorously Active Individuals

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Abstract

Background: Visualization and the demand to increase cognitive function to improve performance is an area of growing interest in athletes and physically active individuals. Gamma (25-45 Hz) waves, the fastest of the brainwave frequencies, are optimal for cognitive function.

Objective: Determine if savoring and ingestion of dark chocolate (70% cacao) can modulate gamma wave frequencies between visualization at a state of rest and a state of exercise performance in vigorously active individuals.

Participants/setting: The study recruited 10 vigorously active individuals from Loma Linda University. Participants' mean age was 23.7± 2.2 years. Vigorous activity was defined by the Centers for Disease Control and America College of Sports Medicine.

Intervention: Participant visualization of rest and exercise were assessed by EEG Power Spectral Density (PSD) during three 60-second trials: without cacao (1.4 g of 70% cacao) consumption, savoring cacao, and after fully ingesting cacao. EEG wave band activity was recorded from 9 cerebral cortical scalp locations.

Statistical analyses performed: Z-scores, using a reference baseline at visualization or rest without cacao were utilized.

Results: During visualization of rest, there was a significant increase of 541.5% in gamma wave frequency when comparing no cacao to savoring cacao ($p=<.001$). During exercise visualization, there was a significant increase of 207.5% in gamma wave frequency when comparing no cacao to savoring cacao ($p=<.001$). Z-score PSD of overall gamma wave frequencies were lowest in the absence of cacao during rest and exercise respectively (.32, .75). During visualization of rest, there was a significant decrease of 58.0% in gamma waves when comparing savoring cacao to fully ingesting cacao ($p=<.001$).

Conclusions: We suggest that EEG gamma waves are heightened during savoring and ingestion of 1.4 g of cacao, when visualization of rest and exercise take place.

Introduction

Visualization and the demand to increase cognitive function to improve performance is an area of growing interest in athletes and physically active individuals. Research shows that limiting factors to performance are more cognitive than strictly motor ability alone.[1-3] Recent research has highlighted the correlation of mental practice with the ability to synchronize visual information with physical movement.[4] Visualization is the process of purposefully generating a visual mental image and stimulating or recreating visual perception with the intent to see if there is a psychological effect.[5] Studies conducted on visualization and athletic performance have shown that there are improvements in physical performance when asked to visualize playing a sport or engaging in physical activity.[6-9] This research suggests that during visualization changes occur in neurophysiological networks. Neurophysiological networks are important to athletes to achieve peak performance, as athletes demand an array of heightened cognitive functions. Some of these functions include: increased memory recall, heightened sensory perception, ability to process large amounts of information very quickly, and a heightened state of focus.[10-13] These tasks are closely associated with gamma wave frequency.

Humans display five different types of electrical patterns or "brain waves" across the cerebral cortex. The five brain waves in order of highest frequency to lowest are: gamma (30-80 Hz), beta (12-30 Hz), alpha (8-12 Hz), theta (4-8 Hz), and delta (<4 Hz).[14] Gamma waves are the fastest of the brainwave frequencies and signify the highest state of focus.[15] In addition, they are associated with peak concentration and the brain's optimal frequency for cognitive function.[14]

There are several ways to improve cognitive function. Cacao has been shown to be one of them. Cacao has health benefits not only improving cognitive function, but decreasing cardiovascular disease risk, protecting skin against damaging sun rays, and as a powerful source of antioxidants.[16,17] Cacao, found in dark chocolate, is a major source of flavonols. Cacao flavonols, epicatechin and catechin, are potent antioxidants and anti-inflammatory components.[16-18] To exert an effect on the brain, these antioxidants need to cross the blood-brain barrier (BBB).[18] These cacao flavonols have been associated with stimulating the cascade of the expression of neuroprotective and neuromodulatory proteins that increase function and communication, improve blood flow, and promote formation of blood vessels in the brain.[19-23] Initiation and modulatory control of action from cacao flavonols on brain state remains unknown. However, current research demonstrates the ability of different cacao sensory awareness tasks to initiate gamma frequencies.[24]

Methods

Subjects:

Our research, a within subject design, recruited participants from Loma Linda University (Loma Linda, CA), and included 5 *vigorously active* male and 5 *vigorously active* female participants ages 21-65. Recruitment was conducted via flyers and word of mouth. Inclusion criteria is as follows; must be between 21-65 years of age, participants must be *vigorously active* individuals based on the "General Physical Activities defined by Level of Intensity" guidelines and must participate in an exercise routine for more than 75 minutes per week. Participants must score a 6 or higher on the Dark Chocolate Likert scale (Appendix A). Exclusion criteria includes the following; the participant cannot take caffeine, sedatives, tranquilizers, vitamins within 12 hours of the study, take ginseng supplementation, creatine or other performance enhancing steroid supplementation within 24 hours of the study, barbiturates within at least 36 hours of study, take any anti-epileptic medicines or have any allergies, colds or sinus infections day of the study. Additionally, participants were excluded if they have diabetes or high blood pressure.

All methods and procedures were approved by the Institutional Review Board of Loma Linda University prior to the start of the study. An explanation of the methods and procedures were provided to potential participants. All recruited participants signed a statement of informed consent prior to admission into the study.

Instruments:

After signing an informed consent, subjects were instructed to complete the following to assess if the participant was a *vigorously active* individual and if the participant met the inclusion criteria.

Demographic Questionnaire:

The demographic questionnaire took approximately 5-10 minutes to complete and included the following information; age, gender, current use of supplementation/medication, and type, duration and frequency of physical activity regularly performed. Participants were also asked duration of sleep the night before the study and female participants were asked when their last menstrual cycle took place. Physical activity listed on the Demographic Questionnaire was compared to physical activity guidelines listed on the "General Physical Activities Defined by Level of Intensity" to ensure that the participant fit the *vigorously active* guidelines.

General Physical Activities Defined by Level of Intensity

The General Physical Activities Defined by Level of Intensity is in accordance with the Centers for Disease Control and Prevention (CDC) and American College of Sports Medicine (ACSM) guidelines. *Vigorous activity* is defined by activities that provide greater than 6.0

metabolic equivalents or METs. MET is the ratio of the rate of energy expended during an activity to the rate of energy expended at rest.[25] For example, 1 MET is defined as the energy expenditure at rest. For the average adult, this approximates to 3.5 ml of oxygen uptake per kilogram of body weight per minute (1.2 kcal/min for a 70-kg individual).[25] The activity intensity levels portrayed in this guideline are most applicable to healthy individuals aged 20-50 years. According to the Office of Disease Prevention and Health Promotion, *vigorous activity* of 75 minutes or greater provides substantial health benefits.[26] Participants were instructed to circle all physical activities performed throughout the week on average. Participants must show the majority of their physical activities defined as *vigorous* based on the guidelines of the General Physical Activities Defined by Level of Intensity. This should take no longer than 5 minutes to complete.

Dark Chocolate Likert Scale:

Participants were provided Parliament Chocolate (70% cocoa) from Redlands, CA. The Parliament chocolate is made with cacao and cane sugar (see Figure 1). Each participant was given half a piece (1.4 grams) of dark chocolate for this experiment. Participants filled out the "Dark Chocolate Likert scale" where participants rated their degree of preference for 70% dark chocolate on a scale from 1 to 8 with 1 being "dislike extremely" and 8 being "like extremely" (Appendix A).

Figure 1: *Parliament chocolate (70% cacao) manufactured in Redlands, CA. Contains cacao and cane sugar.*

EEG:

The EEG, electroencephalogram, is a test that detects abnormalities in the brain waves or the electrical activity of the brain. During the procedure, electrodes consisting of small metal discs with thin wires were pasted onto the scalp. The electrodes detect tiny electrical charges that result from the activity in the brain cells. EEG wave band activity was recorded from 9 cerebral cortical scalp locations: F3, Fz, F4, C3, Cz, C4, P3, Pz, and P4 using the EEG B-Alert 10X System, Carlsbad, CA. Each of the brain frequencies were measured in 5 second increments. Data collection took no longer than 2 hours for the session.

Interested participants arrived at Nichol Hall, Room A 117 on the Loma Linda University campus to start the study. On day one, interested individuals filled out the informed consent document, Dark Chocolate Likert scale, and Demographic Questionnaire. This visit lasted no more than one hour.

Upon arriving for day 2 of the study, qualified participants came with dry, clean hair with zero hair products applied to hair or scalp. This visit was no longer than 2 hours. Participants were instructed to drink 1 glass of water and sleep for a minimum of 6 hours without using sleep aids the night before. The morning of the study, participants were instructed to not eat anything prior to the start of the study. The participants sat in a chair where "synapse conducting gel" was applied to their scalp along with the 9 electrodes. In a dark room, participants were seated and monitored by way of 9 electrodes through an EEG machine. The entropy monitor measures the irregularity of the processed EEG signals and displayed it as a numerical value. This study included three EEG measurements: 1) at baseline, 2) during visualization of rest for one minute, and 3) during visualization of usual vigorous exercise for one minute. Participants completed the first series of the three EEG measurements without dark chocolate. Participants were then provided a 10-minute break where they listened to "Autumn Leaves" by Tim Janis to promote relaxation. Following the break, participants were given 1.4 grams of dark chocolate (70% cacao) and asked to perform the following visualizations: 1) visualization at rest for one minute while savoring the dark chocolate in his/her mouth. 2) visualization performing usual vigorous exercise for one minute while savoring the dark chocolate in his/her mouth. This same sequence of visualizing rest and usual vigorous exercise was conducted again after participants had fully ingested the dark chocolate provided. Second by second, the nine-band width for each subject was recorded.

Statistical Analysis:

The statistical analysis that was used is z-scoring to convert the relative Cognitive State Metrics into values that can be compared among participants. Z-scoring is intended for repeated-measures, within-subjects experimental design. Raw EEG data collected was sent to B-Alert where B-Alert lab modified the raw EEG data. The raw EEG data was filtered from signals that did not include data collected from the visualization tasks including: blinking, hand movements, and outlier frequencies that was detected. From there, B-Alert sent the modified data and was exported into Excel. In Excel, utilizing the Visual Basic for Application, z-scores were computed for each participant for the three tasks: Task 1: Visualization of rest (no cacao), Visualization of exercise (no cacao); Task 2: Visualization of rest (savoring cacao), Visualization of exercise (savoring cacao); Task 3: Visualization of rest (after ingestion of cacao), Visualization of exercise (after ingestion of cacao).

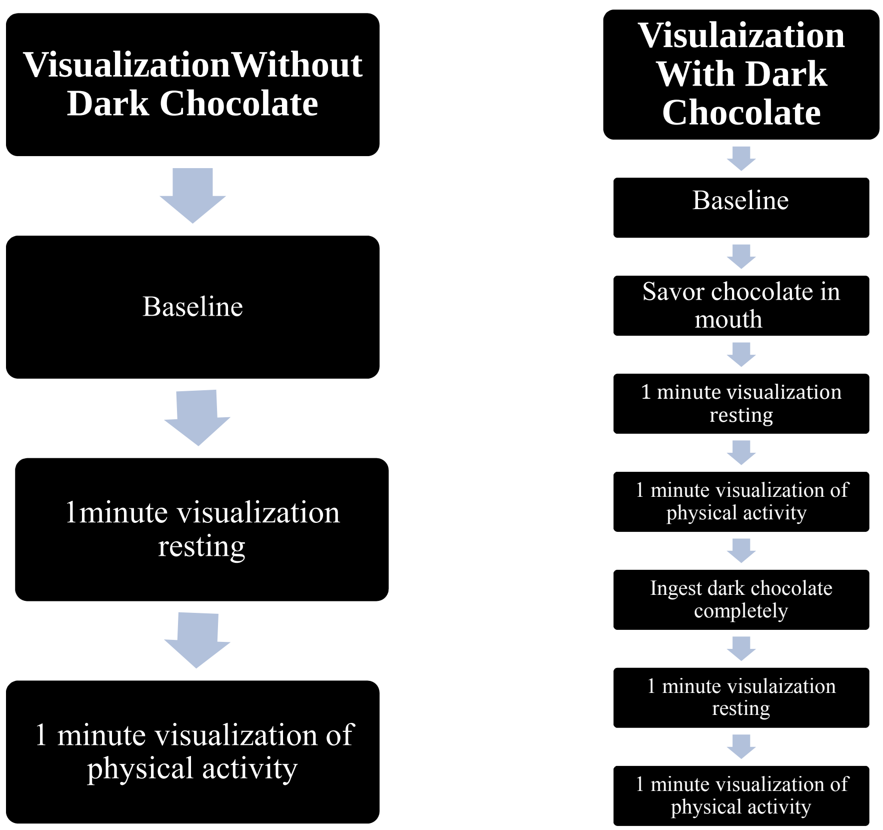

Figure 2: Sequence of data collection for one day. On the left, three trials of without dark chocolate sequence and three trials with dark chocolate occurred in this order.

Results:

Demographic information included: age, gender, sleep duration, and vigorous activity frequency. Demographics of all 10 participants are presented in Table 1. Among the 10 participants, the mean age was 23.8± 2.1. The average hours of sleep the night before the experiment was 6.7±0.6, to account for any additional independent brain modulation. In addition, all *vigorously active* individuals exercised for 6.2 ±2.2 hours each week. To determine if ingestion of dark chocolate (70% cacao) will change brain frequencies between visualization at a state of rest and a state of exercise performance via electroencephalography (EEG) in *vigorously active* individuals, percent change was computed for analysis within and between groups for each of the three tasks. The three tasks include: Task 1: Visualization of rest (no cacao), Visualization of exercise (no cacao); Task 2: Visualization of rest (savoring cacao), Visualization of exercise (savoring cacao); Task 3: Visualization of rest (after ingestion of cacao), Visualization of exercise (after ingestions of cacao).

Percent change within each group for the 3 tasks are shown in Table 2. When visualizing rest, there was a significant increase of 541.5% in gamma wave frequency when comparing no cacao to savoring cacao ($p=<.001$) and a 169.3% increase when comparing no cacao to after ingesting cacao ($p=.04$). Similar trends were seen when visualizing exercise. There was a significant increase of 207.5% in gamma wave frequency when comparing no cacao to savoring cacao ($p=<.001$) and a 45.7% increase when comparing no cacao to after ingesting cacao ($p=.42$). Results displayed z-score PSD of overall gamma wave frequencies were lowest in the absence of cacao during rest and exercise (.317; .752) respectively. Results indicated that gamma wave frequencies were more predominant with cacao than without cacao.

Additionally, percent change between groups for the 3 tasks are shown in Table 2. When visualizing rest there was a significant decrease in gamma waves of 58.0% when comparing savoring cacao to after fully ingesting cacao ($p=<.001$). Similarly, when visualizing exercise there was a significant decrease in gamma waves of 52.6% when comparing savoring cacao to after fully ingesting cacao ($p=.01$). Results further showed that z-score PSD of overall gamma wave frequencies were higher when comparing savoring to ingesting during visualization of rest (2.031; .852) and visualization of exercise (2.311; 1.095).

Table 1. Mean (SD) of Demographic Characteristics by Study Group (n=10)

	Study Group (n=10)
Gender	
Male	5
Female	5
Age, y	23.8 (±2.1)
Sleep, hr	6.7 (±0.6)
Vigorous Activity Frequency, hr	6.2 (±2.2)

Abbreviations: SD= standard deviation

Table 2. Overall Z score of PSD Gamma by Task with percent change of no cacao vs. cacao

VISUALIZATON TASK	Z-Scored PSD Overall Gamma	
A) Rest (no cacao)	0.317	
B) Rest (savor cacao)	2.031	
C) Rest (ingest cacao)	0.852	
D) Exercise (no cacao)	0.752	
E) Exercise (savor cacao)	2.311	
F) Exercise (ingest cacao)	1.095	
	% Change	**p-value**
A-B	541.5	< .001
A-C	169.3	.04
B-C	-58.0	<.001
D-E	207.5	<.001
D-F	45.7	.42
E-F	-52.6	.01

PSD-Power Spectral Density

Discussion/Conclusion:

The current study observed whether savoring and ingesting 1.4 grams of dark chocolate (70% cacao) could modulate gamma wave frequency and amplification between visualization at a state of rest and a state of exercise performance via electroencephalography (EEG) in vigorously active individuals. Our results showed that gamma wave frequencies were substantially more prevalent in the presence of cacao (savoring and after fully ingesting) than without cacao, when subjects were both visualizing rest and exercise respectively. This provides evidence that cacao sensory and ingestion enhances gamma modulation. Berk et. al found that different cacao sensory awareness tasks initiated gamma frequencies.[27] Further studies have led us to believe that gamma waves are heightened in the presence of cacao. Nehlig et. al tested improved cognitive performance through a series of subtraction tasks with consumption of drinks containing various milligrams of cacao flavonols. They found that higher amounts (994 mg) of cacao containing beverages significantly accelerated rapid visual information processing. [20]

Gamma waves are associated with processing speed. Through gamma waves, the brain is able to process substantial amounts of information at a rapid speed, remember it, and retrieve that memory later. Therefore, as shown in our study, the presence of cacao increases gamma frequency, which is involved in processing speed. Crichton et al. found that habitual cacao consumption was significantly associated with better neuropsychological tests including spatial memory and organization, working memory, scanning and tracking and abstract reasoning.[28] High gamma activity is linked to increase memory recall, sensory perception, and focus. Similarly, based off the result of our study enhancing gamma frequency and amplification through cacao, we suggest that in agreement with Crichton[28] that cacao is associated with better neuropsychological testing due to gamma state associations.

One possible explanation of cacao achieving gamma state involves the flavonols in cacao. These flavonols are considered to affect cognitive function by influencing the signaling pathways that are involved in normal memory processing and learning, [29,30] including those involved in long term potentiation and synaptic plasticity.[31] This leads to enhanced neuronal connection and communication with a greater capacity for memory acquisition, storage and retrieval. A proposed mechanism is (-) epicatechin interacts with cellular signaling pathways, primarily with mitogen-activated protein (MAPK), extracellular-signal-regulated (ERK) and phosphoinositide 3-kinase (PI3-kinase/Akt) signaling cascades. These cascades trigger gene expression and protein synthesis for maintaining long-term potentiation (LTP) and establishing long-term memories. [29,32] (-) Epicatechin, induces both extracellular-signal-regulated (ERK1/2) and CREB activation in cortical neurons and subsequently increases CREB regulated gene expression, a transcription factor which binds to the promoter regions of many genes associated with memory and synaptic plasticity. The exact mechanism of action has yet to be clarified.

Results further showed that gamma wave frequency and amplification was higher during visualization of exercise than during visualization of rest (Figure 2). It is well recognized that motor imagery improves motor learning efficiency.[4,33] These studies have revealed that visualization produces the same brain modulation as actions. Visualization thus influences motor control, attention, perception, planning and memory. Further, Berk et al. found that through visualization of exercise performance, gamma wave frequencies were the predominant wave band.[34] Through research we suggest that mental visualization can affect the configuration of brain neural functional networks for performing skillful behaviors.

We, as humans, display 5 brain waves. These waves correlate to a different state of consciousness. For athletes, flow state is a hot topic. Flow state is colloquially known as being *in the zone*.[35] In other words, this state is defined as being completely immersed in what one is doing. Flow state is the alpha-theta brain wave that borders between the conscious and subconscious.[36] Interestingly, recent research has shown that the Alpha-Theta zone is the only brain wave zone in which Gamma waves can occur. [37-40] Gamma and Theta are paired brain waves, thus Gamma can only happen when someone is in Theta. Through research, we believe that enhancing Gamma waves can improve peak performance in *vigorously active individuals*, helping them become *in the zone*.

All thoughts influence neurochemical changes, both temporary and long lasting. In addition, there are physiological, metabolic and neuronal factors that determine the complexity of brain wave frequency. Science has only begun to scratch the surface. With that, there were limitations to this study. It takes 30 minutes to metabolize cacao flavonols. There was only 5 minutes between savoring and ingestion of the cacao in the first trial. Future research should consider waiting approximately 30 minutes post cacao ingestion to confirm similar effects seen in our study. Berk et al. found that stimulation with taste and smell elicited gamma wave activity. [1] This would suggest another mechanism of action besides the flavonols crossing the blood brain barrier.

As part of inclusion criteria, each participant had to score a 6 or higher on the Likert scale ("like moderately" to "like extremely"). Gamma brain waves are as pleasurable as they are powerful. The neurochemical dopamine, flows freely when Gamma waves prevail. Berk et al found that the pleasure caused from laughter enhanced gamma frequencies when compared to feelings of distress.[41] In accord, Small et al. demonstrated changes in brain activity when participants were eating chocolate out of pleasure vs aversion.[42] This leads us to believe that there is a neurochemical mechanism that stimulates Gamma frequency beyond the nutritive effects of cacao.

<u>Conclusion:</u>

Besides physical training, a high demand is placed on visuospatial and other cognitive skills. Improving cognitive skills helps athletes make better and faster decisions, absorb information and maintain high speed during performance. We suggest that gamma waves are heightened with the presence of 1.4g of cacao during visualization of rest and exercise similarly. Our study demonstrates the potential increase in cognitive function for *vigorously active* individuals to achieve a level of peak performance. Further research is needed to expand these positive findings.

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Appendix A: Dark Chocolate Likert Scale

Please rate your degree of preference for 70% dark chocolate by marking one of the following:

Dislike Extremely (1)	Dislike Moderately (2)	Dislike Slightly (3)	Neither Like nor Dislike (4)	Like Slightly (5)	Like Moderately (6)	Like Very Much (7)	Like Extremely (8)

Appendix B: Demographic Questionnaire

1) Full name: ____
2) How old are you? ___________
3) What is your gender?
    a. Male
    b. Female
        a. When was your last menstrual cycle? ____________________________
4) Do you take any supplements or medications?
    a. No
    b. Yes (Please write **ALL current** supplements **and** medications)
    ____
    ____
    ____
5) How much sleep did you get last night? _________________
6) **Physical Activity Questionnaire**
    Using this questionnaire, please write all of the following physical activities that you typically engage in

Type of Activity (walking, jogging, etc)	Duration (minutes/hours per day)	How often (daily, once a week, twice a week, etc)