UNIVERSITY OF THE INCARNATE WORD

June 20 2018

PI: Dr. Jeffrey Rabin

Protocol title: The Impact of Chocolate on Visual Performance: Psychophysics and Electrophsiology

Jeffrey :

Your request for revisions to Expedited protocol 17-06-002 was approved. The following revisions to your protocol have been approved:

- Research procedures: Revisions to research procedures as indicated in document titled "1706002_Rabin_IRB_Protocol_original with 2018 tracked changes"
- Addition to the investigative team: Abebe, Fortuna; Cha, Christopher; Nguyen, Minh; Renteria, Liana; Wastani, Arzoo.
- Number of approved subjects: From 30 to 40 subjects within the age range of 18 to elderly adults with no upper limit and no exclusion based gender or ethnicity. Exclusions will include diabetic patients as well as any disease condition in which patients have dietary needs which preclude ingestion of high sugar content chocolate bars or any allergies to the bar components.
- Consent form: Revised consent form to reflect changes to protocol and investigators.
- Recruitment materials: Revisions to describe the total time required.
- Duration of study: Re-approval for one year. The new expiration date is **6/20/19.**

Please keep in mind these additional IRB requirements:

- A request for continuing review must be completed for projects extending past one year, and closure of completed studies must be reported. Use either the **IRB Continuing Review Request** or **IRB Closure** form.
- Changes in protocol procedures must be approved by the IRB prior to implementation except when necessary to eliminate apparent immediate hazards to the subjects. Use the **IRB Amendment Request** form.
- Any unanticipated problems involving risks to subjects or others must be reported immediately.

Approved protocols are filed by their number. Please refer to this number when communicating about this protocol.

Approval may be suspended or terminated if there is evidence of a) noncompliance with federal regulations or university policy or b) any aberration from the current, approved protocol. Congratulations and best wishes for successful completion of your research. If you need any assistance, please contact the UIW IRB representative for your college/school or the Office of Research Development.

Sincerely,

Ana Hagendorf, PhD, CPRA

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APPROVED AMMENDMENT

Protocol Title: The Impact of Chocolate on Visual Performance: Psychophysics and Electrophysiology

Section 1. Purpose: Our purpose is to determine if acute consumption of dark chocolate compared to white chocolate impacts:

1. Visual perception of high and low contrast black/white and colored letters at low and high luminance.
2. Objective visual electro-diagnostic measures of retinal, optic nerve and visual cortical function.
3. Sensitivity within the visual field and size of the visual field.
4. Effects of blue light on retinal and cortical electro-diagnostic measures of flicker sensitivity.

Section 2. Background and Significance: Consumption of dark chocolate has been associated with improved blood flow, cardiovascular function, slowing of degenerative aging processes, as well as enhanced mood and cognitive performance. (1-5) Dark chocolate is rich in cocoa flavanols which have both antioxidant effects to prevent and impede degenerative disease and as well as more immediate effects on local and cerebral blood flow. One study demonstrated an improvement in contrast sensitivity (CS) which is the visual ability to detect low contrast targets. (6) Hence dark chocolate may enhance vision during critical task performance in military and law enforcement settings as well as every-day tasks such as driving. Whereas it is assumed that visual enhancements from dark chocolate derive from increased cerebral and/or retinal blood flow, (6) direct measurements are lacking. Our prior research (7) demonstrated that hands free phone communication (verbal distraction) can delay reaction time and impair CS. We initiated a randomized clinical trial last year to explore potential effects of dark chocolate on:

1. Visual acuity (VA) and CS and reaction time with and without verbal distraction.
2. Objective electrophysiological measures of retinal, optic nerve and cortical function.

Thus far we have run 30 subjects through this protocol and successfully presented and published some of our results. (8) We observed small but significant effects on VA and CS but may have reached a ceiling effect on improvement from dark chocolate since we were using healthy young adults who achieved maximum performance on some tests. For example, many visual acuities (VA) were about 20/10 which is as good as the visual system permits since VA is limited by the spacing of the retinal cones which corresponds to the detail of a 20/10 letter. In addition, our comparison was between high flavanol dark chocolate and low flavanol milk chocolate and it is conceivable that in some cases the milk chocolate produced a positive effect as well as the dark chocolate decreasing the actual size of the effect of dark chocolate. Therefore, we plan to extend this study with an improved design including a baseline measure on each test to compare chocolate intervention to these baselines. In addition, we will use a white chocolate bar as the interventional control which has no flavanol content. (9) The battery of tests will be limited to those which offer enhanced sensitivity to avoid ceiling effects. In the process of doing this, we also will be establishing normative and repeatability values for FDA approved newly acquired tests and will be developing and evaluating and expedient way to measure the visual field using a standard white board and a test of blue light on flicker responsivity. Our purpose for testing monocularly in the amended protocol is to enhance our range for identifying differences, since we see less well with one eye vs. two, and we may wish to develop predictive models combining results from different tests which would be more valid if the same eye from each subject was used rather than some tests monocular while others binocular. Eventually, we hope to sufficiently refine our test battery and extend our subject pool to individuals with ocular, systemic and neurologic disease with the exception of those with diabetes, hypoglycemia or any condition in which sugar or any constituent of the chocolate bars may place the subject at risk. However, studies to be completed in Summer 2018 will be limited to visually normal healthy adults to establish normative values on the new tests and identify those most sensitive for potential application in patients with ocular, systemic and/or neurologic disease.

Section 3. Location, Facility and Equipment to Be Used: All measurements will be conducted in the University of the Incarnate Word Rosenberg School of Optometry (UIWRSO) in air-conditioned laboratory and clinical rooms. The specific tests to be used are described in section 7 but all are FDA approved clinical tests administered in standard clinical exams and to patients referred to our Visual Neurophysiology Service (VNS) for special testing.

Testing requires no eye drops, dilation, or contact with the eyes. All subject data will be de-identified by assigning each subject a number and storing performance data in a password-protected Excel spreadsheet. CS and electro-physiological reports of performance will be printed as hard copy and/or PDF with subjects identified only by their subject numbers and stored in the Principal Investigator's locked office in a locked filing cabinet.

Section 4. Subjects and Informed Consent: A total of 40 subjects will be recruited from UIW and RSO students, staff, faculty and patients. The age range will be 18 to 65 years and we will attempt to include comparable numbers of male and female participants. Inclusion criteria include visual acuity of at least 20/20 in each eye based on a comprehensive eye exam within 1 year ago with no evidence of ocular, systemic or neurologic disease or ocular trauma. The smallest difference in VA we consider significant is 0.04 log MAR and the highest standard deviation is 0.06. Hence the targeted effect size is mean difference/SD = 0.04/0.06 = 0.67. The minimum number of subjects to achieve this VA difference at the 5% level of significance with power of 80% is: (1/effect size)2 x 16 = (0.06/0.04) 2 x 16 = 36 subjects. (8,9). Hence 40 subjects will be recruited to account for subject attrition during the course of the study. Prior to obtaining written informed consent, each subject will be briefed on the nature of the study by one or more members of the research team. Subjects will be informed that they will be evaluated with standard vision tests 30 minutes after consuming a chocolate bar to assess its effects on performance. Recent evidence indicates that dark chocolate can afford positive effects on cognition in as little time as 30 minutes (10). The dark chocolate and white chocolate bars to be used (Figure 1 2018) are commercially available from Trader Joe's and have comparable ingredients and nutrients except for the higher percentage (72%) of cacao dark chocolate in the experimental bar while the control bar contains only white chocolate which does not include flavanols or antioxidants due to removal of cocoa and associated polyphenol flavonoids. (11) As stated in detail in the informed consent document, subjects will be asked if they have allergies to any of the ingredients contained in the chocolate bars illustrated in Figure 1 2018, and will be excluded from participating if they answer affirmatively. As noted above, any subjects with ocular, systemic or neurologic disease, including diabetes and hypoglycemia, will not be allowed to participate in the study.

Section 5. Subject Compensation: Each subject will be given a $25 gift card after completion of the entire study.

Section 6. Duration: One year.

Section 7. Research Design: A double-blinded crossover design will be used to assess possible effects of dark and white chocolate bars on visual performance and visual electro-diagnostic responses. Subjects will be tested in an initial 1-hour baseline session followed by two 90-minute interventional sessions in which each subject will consume either the dark or white chocolate bar 30 minutes prior to testing with order of testing randomized across subjects. Testing sessions following chocolate consumption will be separated by at least 72 hours as in our prior study based on rapid metabolism of chocolate within 8 hours. Subjects will be asked to refrain from consuming coffee or caffeinated drinks on the day of testing (including baseline) and to consume the bar without milk or milk products which can lessen beneficial effects of dark chocolate. In the chocolate bar interventions each In order ensure that subjects are unaware of the type of chocolate to be consumed, an eye patch (like a "pirate's patch) will be placed over each eye with a sterile cotton eye pad between the patch and the eyes. This will be done after the subject is seated comfortably and has water available. The subjects will then be asked to consume the chocolate bar, taking her/his time and an investigator will be nearby should the subject need any assistance. Music will be played during this time to ensure a relaxed environment. The subject will be offered water during consumption of the bar which will take place in the research room. Insofar as many of the subjects will be in-house student volunteers, she/he will then remain in UIWRSO to study, work-study, finish clinical records, etc. for about 15 minutes followed by application of ERG and VEP recording electrodes prior to testing at 30 minutes after chocolate bar consumption. Testing to be administered during baseline and chocolate interventional sessions include:

- Monocular VA, small letter CS (20/25 and 20/50 letter sizes), and large letter CS (20/670; Pelli-Robson, Precision Vision, Inc. for all VA and CS letter tests) with each subject's preferred eye. VA and small letter CS will initially be measured with a dark green filter placed before the rear illuminated chart which simulates the decreased luminance (brightness) and color of a night vision goggle display. This will be followed by measurement at the normal luminance levels. Two measures will be recorded for each test and the average of the two used for data computation unless one measure is much less than the other (>0.04 log

MAR for VA, >0.10 log CS for CS) suggesting an attentional or error in scoring. In these case the higher of the two measures will be used. Time for testing: 5 minutes.

- Diopsys® multi-focal electro-retinograms (mfERGs) recorded with skin electrodes from the lower lids. The stimulus is an LCD display with 61-hexagons which flash on and off allowing the clinician to record responses from specific retinal sites which derive from retinal cone and bipolar cells. Prior to testing the lower lids of each eye and forehead will be cleaned with a non-abrasive skin cleaner followed by application of adhesive recording electrodes below at the lower lid of each eye with adhesive disposable ground electrode affixed to the forehead. Both response amplitudes and latencies (time required to elicit response) will be recorded. mfERGs will be recorded twice from the subject's preferred eye. Time for testing: 10 minutes.
- There is evidence that the increased amount of blue light emitted from contemporary computer, tablet and cell phone displays can contribute to eye fatigue, which is associated with a decreased ability to see high rates of flickering light (8). But no one knows why the ability to see flicker decreases after blue light and whether it occurs in the retina or the brain. To investigate this, we will simultaneously record brain (visual-evoked potentials, VEPs) and eye-waves (pattern electroretinograms, PERGs) from the subject's preferred eye in response to flickering checkerboard and striped patterns (Konan Medical) before and after she/he views blue and normal colored displays. After cleaning the back of the head 1 cm above the inion, the top of the head and forehead with non-abrasive Konan EEG skin cleanser, adhesive skin electrodes will be placed at the cleansed sites with electrode paste to record the VEPs. After cleaning the lower lid of the subject's preferred eye with a non-abrasive lid wipe a similar adhesive skin electrode (without paste) will be placed under the preferred eye to record the PERGs. This will be conducted once followed by 10 minutes of video game play or viewing videos (their preference) on iPad Pro adjusted to introduce a strong blue color to the game or video, followed again by the VEPs and PERGs. This procedure then will be repeated but the game or video will be colorized to be warm (yellower, much less blue light) to allow comparison of lighting effects on flickering VEPs and PERGs. Different orders (blue vs. warm color) and different videos or games will be used in separate sessions. 25 minutes.
- Konan Medical Cone Contrast Test-HD, a computer-based test which yields threshold red, green and blue cone CS, will be used to assess color vision at near from the patient's preferred eye with measures repeated twice at baseline and for each chocolate condition with orders counter-balanced across subjects and conditions. Time for testing: 5 minutes.
- Color vision and black-white CS will be measured at distance (4m for color, 6m for black white-CS) using a computer-based system which records thresholds and response times (Innova Systems, Inc.). Testing will be recorded at baseline and at each chocolate intervention. Order of testing will be counter-balanced across subjects and chocolate condition. Time for testing: 5 minutes.
- Tangent screen horizontal field and blind spot size determination will be measured using a large white board at 1m and 2m with the subject viewing with her/his preferred eye. The stimulus will be a square black target (3mm at 1m, 6 mm at 2m) on a white wand. The criterion will be disappearance of the black square as the target is moved away from fixation. The horizontal size of the visual field and blind spot will be recorded for each subject twice at each location at baseline and during each chocolate interventional session with order counter-balanced across subjects and chocolate condition. Time for testing: 5 minutes.
- Humphrey frequency doubling perimetry (FDT) will be recorded from the patient's preferred eye using the 20 deg. threshold field. Only one measure will be taken per session since normative data are included in this system. The Mean deviation (overall sensitivity) as well as Pattern Standard Deviation (focal sensitivity loss) will be recorded as well as decibel (sensitivity) levels from 19 discrete points in the visual field. Time for testing: 5 minutes.

The total time for testing in session one is 1-hour and 1.5-hours for each chocolate interventional session (2 and 3).

Section 8. Risk Analysis: This a minimal risk study with no greater risk than that associated with a standard eye exam and special testing conducted with FDA approved tests in our VNS Referral Service. No subjects with restrictive dietary needs, diabetes or hypoglycemia and/or with stated allergies to any of the chocolate bar ingredients will be included

Section 9. Confidentiality: All subject data, including CS values, reaction times, and electrophysiological amplitudes and latencies, will be de-identified by assigning each subject a number and storing these performance data in a password-protected Excel spreadsheet accessible only to the Principal and Co-Investigators. Test reports will be printed as hard copy and/or PDF with subjects identified only by their subject numbers and stored in the Principal Investigator's locked office in a secured filing cabinet.

Section 10. Literature Cited:

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