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Effect of High Antioxidant Cacao Consumption on Behaviors in Children with Autism Spectrum Disorder

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by

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Each person whose signature appears below certifies that this dissertation in his/her opinion is adequate, in scope and quality, as a dissertation for the degree Doctor of Philosophy in Rehabilitation Science.

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ABBREVIATIONS

ASD	Autism Spectrum Disorder
CDC	Center for Disease Control and Prevention
WHO	World Health Organization
ABA	Applied Behavioral Analysis
EIBI	Early Intensive Behavioral Intervention
SSRIs	Selective Serotonin Reuptake Inhibitor
FDA	Food & Drug Administration
ROS	Reactive Oxygen Species
EEG	Electroencephalogram
NAC	N-Acetylcysteine
CAM	Complementary and alternative medicine
ASRS	Autism Spectrum Rating Scale
TE	Trolox equivalent
ABC	Aberrant Behavior Checklist
CF	Cocoa flavanols
FRC	Flavanoid rich cocoa

ABSTRACT OF THE DISSERTATION

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by

Amy Sadek

Doctor of Philosophy, Graduate Program in Rehabilitation Science
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Dr. Lee S. Berk, Chairperson

BACKGROUND- Children with Autism Spectrum Disorder (ASD) demonstrate a physiological imbalance between free radicals, resulting from oxidative stress, and antioxidants. Oxidative stress is linked to the pathogenesis of this neurocognitive disorder. The objectives of this pilot feasibility study: 1) to examine the effect of consumption of high concentration antioxidant cacao on behavior of children as perceived by the child's teacher and 2) as perceived by the child's parent.

METHODS- This clinical trial was a prospective experimental study. Participants consumed 8 squares (or 16 grams) per day of the dark chocolate which had a concentration of 70% cacao and 30% organic cane sugar (total antioxidant concentration was 8,320 µmoles / 100 grams. The two main behavioral measures were the *Aberrant Behavior Checklist- 2nd Edition* (ABC-2) and the *Autism Spectrum Rating Scale* (ASRS) which were completed by the child's teacher at baseline and end of week four; and by the child's parent at baseline, week two, and week four.

RESULTS- Twelve teachers and children with ASD (9 males, 3 females, mean age of 10.9 ±3.9 years) participated in this study. Teachers noted significant improvements on the Autism Spectrum Rating Scales of Social/Communication (p=0.03,

η^2=0.79), Unusual Behaviors (p=0.02, η^2=0.70), and Self-Regulation (p=0.04, η^2=0.59). No significant changes were noted on any of the Aberrant Behavior Checklist-2 subscales (p>.05). For the second study, sixteen parents and children with ASD participated in the study (12 males, 4 females, aged 4 to 17 years). Parent reports showed significant improvements on the ABC-2 subscales of Irritability (p= .03, η^2 = 0.25), Social Withdrawal (p=.01, η^2 = 0.29), Stereotypic Behavior (p=.05, η^2 = 0.13), Hyperactivity/Noncompliance (p=.04, η^2 = 0.20), and Inappropriate Speech (p=.05, η^2 = 0.16). Significant improvements were noted on the ASRS scales of Social/Communication (p=.04, η^2 = 0.25), Unusual Behaviors (p=.003, η^2 = 0.20), Self-Regulation (p=0.02, η^2=0.32), and Total Scores (p<.001, η^2=0.54).

CONCLUSION- Results from this study support the therapeutic benefit of antioxidants in improving social communication, unusual behaviors, and self-regulation behaviors of children with ASD. Further robust randomized controlled trials are necessary to elaborate the validity of these finding.

CHAPTER 1

INTRODUCTION AND REVIEW OF THE LITERATURE

Autism Spectrum Disorder

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder that is characterized by deficits in social and communication skills, functional impairments across multiple settings, repetitive and restrictive behaviors, as well as hyper or hypo responsiveness to sensory stimuli in the environment [1]. According to the Center for Disease Control and Prevention (CDC), currently in the United States (U.S.), 1 in 68 children are identified as having ASD with it being four and a half times more prevalent in males than females [2]. According to the World Health Organization (2017), worldwide, one in 160 children have ASD. Although the etiology of ASD remains elusive, there are genetic, environmental, immunological, and oxidative stress influences that are related to the pathogenesis of the disorder [3,4].

Additionally, perinatal and neonatal risk factors such as, "abnormal fetal presentation, umbilical-cord complications, fetal distress, birth injury or trauma, multiple birth, maternal hemorrhage, summer birth, low birth weight, small for gestational age," among others have been identified as part of the association with ASD [5]. The heterogeneity of ASD lends itself to the need for a variety of interventions, both traditional and non-traditional, as no one technique works across the spectrum.

Traditional Therapies

Behavioral therapists, speech and language pathologists, and occupational therapists are common interventionists that work with children on the spectrum.

Behavioral therapists work with ASD children on maladaptive behaviors, language skills, daily living skills, and social functioning. This type of applied behavioral analysis (ABA), uses a Skinner approach with rote discrete trials in a one on one setting and is typically recommended at 40 hours per week for years on end [6,7]. Speech and Language Pathologists also work with children with ASD to target social communication skills, request making, speech production, alternative communication strategies, and reciprocal interaction skills (American Speech-Language-Hearing Association, 2006). Occupational therapists target sensory processing skills through regulatory strategies across environments, activities of daily living related to school and home performance, and motor skills required for activities in their educational setting [9]. In general, behavioral therapists work on maladaptive behaviors, speech therapists work on communication skills, and occupational therapists work on self-regulation skills as they relate to hypo or hyper responses to environmental stimuli.

Behavioral Therapy

Early intensive behavioral intervention (EIBI) is a common behavioral approach used in children with ASD. It is typically recommended for 40 hours per week for a minimum of two years [6]. A meta-analysis on the effectiveness of this approach revealed that children in experimental groups had better IQs and adaptive behavior outcomes compared to control groups who received a less intense approach [6]. Although ABA techniques are common and popular in addressing aberrant ASD behaviors, the evidence remains mixed as studies did not have a uniform or equally replicable methodology to show consistency of results.

Special Education and Autism

Children with ASD often receive the aforementioned services through their special education program. Furthermore, it should be noted that both special education and general education teachers educate students with ASD [10]. Evidence based practices are limited in nature for the ASD population due to the heterogeneous profiles of the disorder and often times practitioners implement a variety of strategies that have not been studied in combination [11]. Additional barriers for educators and practitioners include the child's behaviors which make conducting standardized assessments challenging to accurately develop goals and treatment plans in the school environment [12]. Overall, there is limited evidence on teachers' perceptions of ASD behaviors in the classroom setting yet these behaviors bring challenges when it comes to educational engagement from the ASD student.

Family Stress and Autism

As with any disease or disorder that has a life-long course, the stress of being a parent or caregiver of a child with ASD naturally comes with the territory. The child's behaviors, including but not limited to, anxiety, tantrums, self-injury, or outbursts may cause the family unit additional stress making daily life tasks more burdensome [13]. Parents have reported feelings of anger, doubt, and disbelief at the time of their childs' diagnosis [13]. Additionally, maladaptive behaviors had a negative effect on parent sleep and many parents reported missing work or quitting work as a result [13]. Parental stress should also be considered when weighing the consequence of aberrant behaviors of children with ASD. These ripple effects on the family unit show the colossal impact that

ASD behaviors can have and as a result require our attention to address and alleviate as much as possible.

Pharmacological Interventions

In terms of pharmacological interventions, approximately one third of the ASD population take Selective Serotonin Reuptake Inhibitor (SSRIs) drugs because their behaviors are similar to other serotonin related disorders [14]. There are only two Food & Drug Administration (FDA) approved drugs for ASD, Risperidone and Abilify [14]. Risperidone is a second-generation antipsychotic that has helped children with ASD reduce their number of tantrums, aggressive behaviors, and self-injurious behaviors [14]. Abilify is a psychotropic drug designed to treat mood irritability in children with ASD [14,15]. However, as with any drug, there are associated adverse effects including increased appetite, weight gain, dizziness, drooling, drowsiness, fatigue, vomiting, nasopharyngitis, fever, insomnia, and upper respiratory infections [14-16]. Non-pharmacological lifestyle oriented interventions may compliment traditional therapies in targeting behavior, mood, and performance issues associated with ASD without side effects of medication [17].

When the aforementioned therapies and pharmaceutical agents are considered the cost of ASD becomes colossal. A recent study reported that the cost of ASD, across one's lifetime, in the U.S. is $1.4 million, and $2.4 million, respectively, if they also have an intellectual disability [18]. However, as with any disability or disorder the cost is indefinite as there are also psychological and stress costs that cannot be quantified for the individuals or families caring for them. Lifestyle oriented approaches may, then, be

necessary not only to potentially offset costs but to improve quality of life in a long term sustainable fashion.

A Physiological Perspective: Oxidative Stress

Oxidative stress leads to an excess accumulation of free radicals in the body and has been linked to a variety of diseases including cancer, cardiovascular disease, neurological diseases, pulmonary disease, rheumatoid arthritis, nephropathy, epilepsy, allergies, inflammation, as well as ocular disease [19,20]. Although oxygen is a life sustaining necessity, the influence of oxidative stress causes oxygen to have harmful effects on the human body [21]. Oxidative stress can be simply defined as disequilibrium between reactive oxygen species (ROS), also known as free radicals, and antioxidants (Hossain, ASM Matiur, & Alam, 2014). Essentially these ROS donate oxygen to otherwise stable molecules and thus disrupt their function by oxidizing them [21]. This imbalance can damage any one of our organ systems and can alter DNA structure and compromise the integrity of cellular membranes (Hossain, ASM Matiur, & Alam, 2014). Furthermore, the human brain occupies approximately two percent of our body weight yet utilizes a quarter of our energy required at rest [23]. This is important in the pediatric population because young children are highly susceptible to oxidative stress during early development because their protective barriers against oxidative damage are not fully formed.

Though the exact mechanisms on the pathogenesis of neurocognitive disorders remains under scientific investigation, there is an identifiable link between the excess of free radicals and neuronal damage. Recently, scientists have also studied oxidative stress

as part of the pathophysiology of ASD, as well its potential link to other neuropsychiatric disorders such as schizophrenia, major depressive disorder, anxiety, panic disorder, Alzheimer's disease, Parkinson's disease, and obsessive compulsive disorders [3,23]. Due to the brain's high energy requirements, specifically neurons, which require the most energy of any brain cell are limited in their antioxidant defense making them the first cells to be negatively impacted [23]. It is clear that the deleterious effects of oxidative stress can significantly impact brain and cellular function of many organ systems.

Oxidative Stress and ASD

Although the etiology of ASD is multifactorial, research suggests that there are some neurophysiological differences between individuals with and without ASD [24,25]. For example, irregularities in neuronal connections have been associated with "cognitive, social, and sensory behavioral symptoms," (Dinstein et al., 2011, p. 1218). Furthermore, due to the limited number of studies that have examined where the brain abnormalities are and the various regions studied it is difficult to determine if the differences are localized in the brain or more systemic [25]. Of particular relevance to the current study is research on oxidative stress and ASD symptomology. Damodaran, Arumugam [3] reported a significant increase in oxidative stress markers in children with ASD compared to matched controls and the level of antioxidants excreted in urine samples were significantly lower in children with ASD compared to matched controls. Furthermore, the severity of autistic behaviors was positively correlated with oxidative stress markers, whereas antioxidant levels were negatively correlated with autistic behaviors [3,26].

Additional evidence from Ming et al. (2005), who measured urinary excretion of 8-iisoprostane and 8-hydroxy-2-deoxyguanosine in children with ASD and matched controls and found that children with ASD had increased levels of lipid peroxidation, indicating further mechanism for oxidative stress patterns. In addition, elevated blood samples of glutathione, glutathione peroxidase, methionine, oxidized glutathione, and cysteine have also been found in children with ASD [26]. Since free radicals, when in excess, can be so deleterious to mind-body health it is imperative that antioxidants be incorporated as supportive therapies for neutralizing oxidative stress.

Ming and colleagues (2005) suggests that children with ASD tend to have greater markers of oxidative stress compared to age matched controls or their non-Autistic siblings. Oxidative stress can be due to mitochondrial impairment, environmental factors, metabolic issues, and genetic predisposition [20,25]. In turn, oxidative stress can lead to a cascade of events such as inflammation, damage to cell membranes, autoimmunity, methylation damage, cell death, and neurological impairments [26]. Oxidative stress can be identified through markers of antioxidant enzymes, lipid peroxidation, and protein oxidation, all of which are higher in children with autism compared to their matched controls [3,20,25]. It is evident that this imbalance between free radicals and antioxidants creates a plethora of detrimental physiological outcomes and are contributory to the pathogenesis of ASD. In summary, these studies provide growing evidence regarding the physiological differences in children with ASD that show an imbalance in the oxidation of antioxidants and thus an excess of free radicals.

Antioxidants and Nutrition

The human body offers natural mechanisms to counter the damaging effects of free radicals that lead to oxidative stress by producing antioxidants. Antioxidants can be produced endogenously by the body or can be ingested through dietary nutrients. Endogenous antioxidants include glutathione, ceruloplasmin, transferrin, catalase, superoxide dismutase, and glutathione peroxidase [4]. Examples of dietary antioxidants include but are not limited to Vitamin E, Vitamin C, Beta-carotene, Lycopene, Selenium, Omega-3 fatty acids, Omega-6 fatty acids, and Flavonoids [19]. Polyphenols are also considered a larger category of antioxidants which are phytochemicals that are found in large quantities in fruits, vegetables, and other natural nutrients [27].

Many antioxidant rich foods and vitamins have been used in the treatment of disease [28]. For example, vitamin C and vitamin E are both rich in antioxidants and have been used for cardiovascular disease and cancer [28]. Carotenoids have also been used in diseases that are neurodegenerative, inflammatory, or cardiovascular in nature [28]. Additionally, polyphenols such as flavanols and flavonoids have free radical scavenger properties making them beneficial in combatting chronic diseases [29]. The cocoa bean has higher antioxidant properties than teas and wines as they have higher concentrations of flavonoids making it a highly valuable nutrient in health promotion [29]. Overall, antioxidants can be considered beneficial for protection against chronic diseases and are often found in functional super foods such as berries, cacao, and green tea [30].

To date, there is not an antioxidant specific validated and reliable nutrition survey that applies to children or adults. However, the Youth Adolescent Food Frequency Questionnaire is a validated measure developed out of Harvard [31]. The questionnaire

comprises nine main content areas of foods frequently consumed; eight questions on demographics, 20 questions on consumption of various beverages, 16 dairy food items, 30 questions on main dishes, six questions on types of sandwiches, eight questions on other food consumption, 17 questions on breads and grains, 41 questions on fruits and vegetable consumption, and 31 questions on snacks and desserts. The questions are ranked depending on how frequent the food items are consumed; from never to less than once per month, one to three times a week, once per week, two to four times per week, more than four times per week, and once per day. The category related to fruits and vegetables is the closest measure of children's intake of foods that would be rich with antioxidants and thus this questionnaire was employed in this study.

Cacao as an Antioxidant

In the typical U.S. diet, chocolate is the leading source of antioxidants followed by fruits and vegetables [32]. Cocoa is potent in antioxidants such as flavanols which play a role in neuroregulatory mechanisms such as anti-inflammation and vasodilatory properties [33]. Polyphenols, such as those found in cacao, are known to have the ability to remove free radicals from the body and can offer protective properties in disease such as arteriosclerosis, heart disease, and alcoholic fatty liver [28]. In addition, flavanoids have potent antioxidant content and are derived from foods such as cacao, green tea, grapes, apples, ginkgo biloba, berries, among other foods [19]. Antioxidants found in cacao include several subclasses such as polyphenols, flavonoids, and flavanols. Within flavanols, we find catechin, epicatechin, epigallocatechin, and epigallocatechin gallate all of which are

found naturally in cacao [29,34]. Consequently, it is evident that dietary antioxidants can be easily consumed and beneficial for overall health.

Thus, although oxidative stress is associated with a variety of neurological disorders, promising research from ASD and Down Syndrome participants suggests that the counteracting effects of antioxidants may minimize free radical damage, thereby reducing symptoms of irritability, hyperactivity and improving the physiological balance between free radicals and antioxidants [35,36]. For example, in a recent study on children and adolescents with Down Syndrome, researchers found support for antioxidant supplementation through use of vitamin E, vitamin C, E-TABS, and Energil C in reducing oxidative stress biomarkers with lasting effects even after the six-month intervention [36]. Nutrition plays an integral role on health via antioxidants. Antioxidants have great physiological and neurological benefit given their defense against toxic free radicals when normal mechanisms experience disequilibrium.

Oxidative stress biomarkers are particularly noteworthy as they have the potential to be modulated by means of non-pharmacological interventions, such as nutritional interventions. Cacao is a food rich in flavonoids and their sub group flavanols. For the purposes of this introduction, cacao and cocoa were used interchangeably. Both are associated with improved cognitive processing, alertness, and processing speed [37]. Further benefits of cocoa include increased cerebral blood flow, improved cardiovascular activity, improved insulin resistance, improved blood pressure, and improved endothelial function [37]. Antioxidants' benefits include improvement of cellular structure, neutralizing free radicals, neuroprotective, anti-inflammatory, accumulation in the hippocampus and cortex, and improvement of vascular endothelial function [27,32,34].

More specifically, cacao is a natural and potent antioxidant with benefits linked to improvements in blood pressure, lipid peroxidation, cognitive response time, gamma frequency, and cerebral blood flow [27,32,38-40]. Additionally, flavanoids' ability to cross the blood brain barrier demonstrates their neuroprotective mechanisms as well as their role in cognitive functions such as learning and memory [34,41]. The aforementioned studies summarize the inherent value in cacao as a natural superfood the mind and the body.

Scholey, French, Morris, Kennedy, Milne, Haskell [42] studied healthy adults and randomly assigned participants to one of three groups with one group consuming a beverage containing 520 milligrams (mg) of cocoa flavanols (CF), a second group consuming a beverage containing 994 mg of CF, and a matched control. Results showed that the groups who consumed 520 mg of CF and 994 mg of CF significantly improved scores on a cognitive battery test [42]. In addition, the 994 mg of CF beverage resulted in significant improvement in rapid visual information processing [42]. Field, Williams, Butler [43] also studied healthy adult subjects who consumed chocolate containing 720 mg of CF and a matched quantity of white chocolate, which served as the control group as white chocolate lacks cacao. Researchers found that the flavanol group improved in visual contrast sensitivity, reduced the time required to detect motion direction, and improved spatial memory [43]. Both of these studies noted that while the mechanisms underlying the effects are unknown they may be related to effects of CF on endothelial function and blood flow which allows improved transfer and delivery of oxygen and nutrients systemically.

Mastroiacovo, Kwik-Uribe, Grassi, Necozione, Raffaele, Pistacchio, Righetti, Bocale, Lechiara, Marini, Ferri, Desideri [39] examined elderly subjects who showed

clinical evidence of cognitive deficits and had them consume a drink everyday for eight weeks with various concentrations of flavanols. One group consumed high flavanols 993 mg, the intermediate group consumed 520 mg of flavaonol, and the low flavanol group consumed 48 mg [39]. Results revealed an overall improvement in response time and verbal fluency scores in all three groups with greatest improvement being in the highest concentration group [39]. They also found physiological benefits with improvements in insulin resistance, blood pressure, and lipid peroxidation in the high flavanol group and in the intermediate flavanol group [39]. These results are noteworthy as lipid peroxidation is a mechanism of oxidative stress that occurs when free radicals damage the lipid cellular membrane and thus destroy the integrity of the cell and its functions.

Davison, Berry, Misan, Coates, Buckley, Howe [44] aimed to determine a minimum dose effect of flavanol rich cocoa products relative to lowering blood pressure in men and women with mild untreated hypertension. Participants consumed a daily cocoa beverage containing 33, 372, 712 or 1052 mg of cocoa flavanols per day [44]. Significant reductions were noted only in the 1052 mg/day group, no reduction was seen at any other dose in this particular study [44]. In another study, Grassi, Desideri, Necozione, di Giosia, Barnabei, Allegaert, Bernaert, Ferri [45] examined cocoa flavonoids on cardiovascular functions in healthy adults. Total flavanoid concentration ranged from zero mg for the control group to 80, 200, 500 and 800mg per day on each week. Compared with controls, all cocoa-active treatments significantly and dose-dependently increased flow mediated vasodilation [45]. Results also showed improvements in arterial stiffness and blood pressure during each week of treatment [45]. A previously conducted cross sectional study on older adults demonstrated a dose response relationship with a flavonoid rich beverage

consumption at 10 grams per day for optimal cognitive benefit [46]. However, the antioxidant concentration and percentage of cacao were not noted or controlled for in this study which limits the generalizability of the dose dependent outcomes. Further research is still needed to reproduce these effects in similar samples and eventually show a consistent dose response.

A meta-analysis was conducted to evaluate the effect of flavanoid rich cocoa (FRC) on cardiovascular risk factors and to assess a dose-response relationship and the studies reviewed included samples of healthy, diabetic, overweight, and heart disease subjects [47]. Results showed that in response to FRC consumption, systolic blood pressure decreased, insulin resistance decreased, Low Density Lipoprotein cholesterol decreased, flow mediated vasodilation increased, and High Density Lipoprotein cholesterol increased [47]. A dose-response relationship was found between FRC and flow mediated vasodilation with a maximum effect observed at a flavonoid dose of 500 mg/day [47]. The above literature points to the benefits that cocoa has, particularly, the flavanols and flavonoids which are nutritional antioxidants, on a variety of physiological mechanisms.

Richelle, Tavazzi, Enslen, Offord [48] conducted a study on adult males, both smokers and non-smokers, analyzing the peak concentration of epicatechins from blood samples following consumption of dark chocolate. Findings from this study suggest that epicatechins, a subgroup of polyphenol antioxidants, peak in the blood at approximately two hours after 40 grams were consumed and at approximately three hours after 80 grams were consumed [48]. More recent research echoes these findings that epicatechins, antioxidants naturally found in cacao, peak in the blood two to three hours following consumption and resume a baseline level six to eight hours later [49,50]. In a review article

by Vauzour [40], the neurological benefits of polyphenols, including those found in cacao are identified as promoting neurogenesis and neurocognitive functions. For example, polyphenols have been noted to cross the blood brain barrier in both animal and human studies suggesting their neuroprotective features not only to support the antioxidant defense against oxidative stress but also in modulating inflammation in the brain [40]. Both, Richelle, Tavazzi, Enslen, Offord [48] and Vauzour [40], may support the benefit of maintaining a certain level of concentration in the bloodstream for physiological and neurocognitive benefits. Future studies need to examine the amount of specific dietary antioxidants' and their absorption for dosing recommendations and therapeutic purposes in human subjects.

Neurocognitive Benefits of Cacao

In addition to physiological health benefits noted above, there are also neurocognitive benefits to cacao consumption. Berk et al. (2016) found that when healthy adults consumed 70% cacao gamma wave frequency was elicited as measured by electroencephalogram (EEG) of the brain. Gamma wave frequency (γBA 31–40 Hz) is the highest brain frequency associated with long term recall, neural synchronization, learning, and heightened meditation (Berk et al., 2016). In a follow up study, they found similar results in healthy adults who consumed 70% cacao with nine cerebral cortical EEG leads to measure brain activity which again produced gamma wave frequencies (γBA 31–40 Hz) (Berk et al., 2017).

Furthermore, gamma frequency has been studied in both animal and human models and findings support that this high-level frequency is associated with short term

memory, long term memory, enhanced neuronal communication and synchronization [51]. The rapidity of gamma wave frequencies results in firing of the tight and rhythmic oscillations of the neuron thus allowing for faster interneural communication [51]. This research suggests both the neuroprotective and neuromodulatory benefits of high antioxidant cacao on brain health. Gamma frequency also plays a role in working memory, memory retrieval, and attention skills [52]. Future studies will need to identify if these changes in brain wave frequency carryover to attention and task performance on participants with neurocognitive disorders. The aforementioned studies point to the critical and valuable role that gamma wave frequencies play in higher cognitive processing skills which would be advantageous for the ASD population.

The above literature repeatedly points to both the health and physiological benefits of cacao on adults. To date, to the author's knowledge, only one study has looked at potential health benefits of cacao on children. Children with idiopathic constipation between the ages of three and six consumed four grams of cocoa husk per day and children between the ages of seven and 10 consumed eight grams per day [53]. The children who received cocoa husk supplements tended to increase the number of bowel movements compared to the placebo group [53]. Although this study was not related to ASD, nor to the concentration of cacao or flavanols, it shows limited evidence on health benefits of cacao for children.

Antioxidants, Autism, and Behavior

In the ASD population, five studies have been conducted looking at an antioxidant intervention relative to behavior change. According to the first identified

study of its kind, researchers examined use of high antioxidant vitamin C providing eight grams per 70 kilograms per day for 30 weeks and found a reduction in symptom severity [54]. This study examined antioxidants' potential benefit to children with ASD. In 2013, Ghanizadeh and Moghimi-Sarani, studied children between the ages of three and a half and 16 and the children were randomly assigned to one of two groups. One group received N-Acetylcysteine (NAC) (1200 mg/day) plus risperidone and the other received placebo plus risperidone [55]. N-Acetylcysteine is an antioxidant pro-drug and a precursor to arguably the most important antioxidant, glutathione. Results showed a significant improvement in irritability in the NAC group on the Aberrant Behavior Checklist [55].

More recently, researchers examined children between four and 12 years of age where one group received risperidone plus NAC and the control group received risperidone plus a placebo [56]. The dose of NAC ranged from 600 to 900 mg/day [56]. Results showed a significant improvement in both irritability and hyperactivity/noncompliance subscales on the Aberrant Behavior Checklist [56]. The most common noted adverse effects in the NAC plus risperidone group were constipation, increased appetite, fatigue, nervousness, and daytime drowsiness [55,56].

Lastly, a recent pilot study assessed NAC in children with ASD between ages three and 12 [35]. Subjects were randomized to the NAC or control group and were initiated at 900mg/daily for four weeks, then 900 mg twice daily for four weeks, and 900 mg three times daily for four weeks [35]. Follow-up data revealed that the NAC group showed significant improvements on the Aberrant Behavior Checklist irritability subscale compared to the control group [35]. Also, stereotypy behaviors improved on the repetitive behavior scale and social cognition improved on one of the subtests on the social

responsiveness scale [35]. These studies are key in showing some of the reproducible evidence of potential therapeutic effects and improved behavioral outcomes following antioxidant interventions in children with ASD.

Significance of the Present Study

Traditional behavioral interventions for ASD, although valuable, are missing a holistic approach as they typically do not target the physiological components to neurocognitive deficits related to oxidative stress. The antioxidant and free radical imbalance in ASD may require the combination of traditional therapies and nutritional interventions to complement each other for optimal outcomes and quality of life for children and families living with ASD. To date, to the author's knowledge, previous literature has not examined the potential association between antioxidant rich foods, such as cacao, and ASD behaviors. However, based on the literature reviewed, oxidative stress markers, such as free radicals, are elevated in children with ASD and thus can be reduced by high antioxidant consumption, specifically cacao. Thus, the purpose of the study was to provide information on the relationship between consuming high antioxidant cacao and behavior in children with ASD. Specifically, this study aimed to examine the following research questions:

1. What is the impact of consuming high antioxidant cacao on the severity of Autistic behaviors?
2. What is the parents' perspective on their child's Autistic behaviors following cacao consumption?
3. What is the teachers' perspective on their Autistic student's behaviors following

cacao consumption?

4. What is the impact on parental stress following the cacao consumption intervention?

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CHAPTER 2

ANTIOXIDANTS AND AUTISM: TEACHERS' PERCEPTIONS OF BEHAVIORAL CHANGES

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Abstract

BACKGROUND- Children with Autism Spectrum Disorder (ASD) demonstrate a physiological imbalance between free radicals, resultant from oxidative stress, and antioxidants. Oxidative stress is linked to the pathogenesis of this neurocognitive disorder. The aim of this pilot feasibility study was to examine the effect of consumption of high concentration antioxidant cacao on behavior of children with ASD.

METHODS- This was a 4-week pre-test post-test experimental pilot study of high antioxidant cacao and children with ASD. Participants consumed 8 squares (or 16 grams) per day of the dark chocolate which had a concentration of 70% cacao and 30% organic cane sugar (total antioxidant concentration was 8,320). The two main behavioral measures were the *Aberrant Behavior Checklist- 2nd Edition* and the *Autism Spectrum Rating Scale* which were completed by the child's teacher at baseline and end of week four.

RESULTS- Sixteen participants were recruited for this study. Follow up data was available on 12 participants (9 males, 3 females, mean age of 10.9 ±3.9 years). Significant improvements on the Autism Spectrum Rating Scale were noted in Social/Communication (p=0.03, η^2=0.79), Unusual Behaviors (p=0.02, η^2=0.70), and Self-Regulation (p=0.04, η^2=0.59). No significant changes were noted on any of the Aberrant Behavior Checklist-2 subscales (p>.05).

CONCLUSION- Results from this study support the potential therapeutic benefit of antioxidants in improving social communication, unusual behaviors, and self-regulation behaviors of children with ASD. Further robust randomized controlled trials are now necessary to elaborate the validity of these findings.

ClinicalTrials.gov Identifier- NCT 03195465

Key words: cacao, antioxidants, autism, social/communication, behavior, teacher, complementary therapies

Introduction

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by deficits in social and communication skills, functional impairments across multiple settings, repetitive and atypical behaviors, as well as self-regulation impairments related to sensory input from the environment (APA, 2013). According to the Center for Disease Control and Prevention (CDC), one in 68 children are identified as having ASD with it being 4.5 times more prevalent in males than females (CDC, 2016). Although the specific etiology of ASD remains elusive, there are genetic, environmental, immunological, and oxidative stress influences that are related to the pathogenesis of the disorder [3,4]. Therefore, the complexity and broad spectrum of ASD lends itself to the need for a variety of interventions and therapies for these children.

Behavioral therapists, speech and language pathologists, and occupational therapists often work in parallel in both educational and medical settings to provide therapy for children with ASD [17]. Children with ASD often receive these services through their special education program. Furthermore, it should be noted that both special education and general education teachers educate students with ASD [10]. Evidence based practices are limited in nature for the ASD population due to the heterogeneous profiles of the disorder and often times practitioners implement a variety of strategies that have not been studied in combination [11]. Additional barriers for educators and practitioners include the child's behaviors which make conducting standardized assessments challenging to accurately develop goals and treatment plans in the school environment [12]. Overall, there is limited evidence on teachers' perceptions of ASD behaviors in the classroom setting.

The Food and Drug Administration approved two pharmaceutical agents for ASD, Risperdal and Abilify. Both drugs target maladaptive behaviors and irritability [14,15]. In addition, almost one third of the ASD population are on Selective Serotonin Reuptake Inhibitor (SSRIs) [14]. However, as with any drug, there are associated adverse effects that include increased appetite, weight gain, dizziness, drooling, drowsiness, fatigue, vomiting, nasopharyngitis, fever, insomnia, and upper respiratory infections [14,16]. Thus, non-pharmacological lifestyle oriented interventions may compliment traditional therapies in targeting behavior, mood, and performance issues associated with ASD without side effects of medication [17].

Due to the heterogeneous nature of ASD severity and behaviors, which can change over the course of natural development, it is common to see this population utilizing multiple therapeutic interventions through the medical model, education system, and through alternative therapies. Common types of CAM used with ASD children include but are not limited to diet modifications, vitamin and supplement use, digestive enzymes, music therapy, among others. Complementary and alternative medicine (CAM) among children and adolescents are often used to address both behavioral and emotional issues, such as those found in children with ASD [58]. Families of children with ASD typically use CAM therapies and treatments when their child has multiple conditions [59,60]. Parents often use CAM with their ASD children to target the symptomology of mood and behavior disturbances related to hyperactivity, irritability, and attention skills [60]. Pharmacologically there are only the two aforementioned approved medications for ASD and thus any other medications prescribed are used to address behaviors related to maladaptive behaviors, mood, hyperactivity, and the like [61]. Thus, there is a need for

CAM interventions to offset potential adverse effects of pharmaceutical agents while still targeting some of the core symptoms.

Although the etiology of ASD is multifactorial, research suggests that there are some neurophysiological differences between individuals with and without ASD [24]. For example, irregularities in neuronal connections have been associated with "cognitive, social, and sensory behavioral symptoms," [24]. Of particular relevance to the current study is research on oxidative stress and ASD symptomology. Previous research indicated a significant increase in oxidative stress markers in children with ASD compared to matched controls and their non-Autistic siblings [3,20]. Furthermore, the severity of autistic behaviors was positively correlated with oxidative stress markers, whereas antioxidant levels were negatively correlated with autistic behaviors [3,26].

Oxidative stress can be due to mitochondrial impairment, environmental factors, metabolic issues, and genetic predisposition in ASD [20]. In turn, oxidative stress can also lead to a cascade of events such as inflammation, damage to cell membranes, autoimmunity, methylation damage, cell death, and neurological impairments [26]. Thus, it is evident that this lack of sufficient antioxidants creates a plethora of detrimental physiological outcomes which can contribute to the pathogenesis of ASD.

The human body offers natural intrinsic mechanisms to counter the damaging effects of free radicals that lead to oxidative stress by producing antioxidants endogenously by the body [19,62]. Oxidative stress biomarkers are particularly noteworthy as they have the potential to be modulated by means of non-pharmacological interventions, such as nutritional interventions [29]. Antioxidants' benefits include improvement of cellular structure, neutralizing free radicals, neuroprotective, anti-

inflammatory, accumulation in the hippocampus and cortex, and improvement of vascular endothelial function [27,32].

In the ASD population, several key studies have been conducted looking at antioxidant intervention relative to behavior change. According to the first identified study of its kind, researchers examined use of high antioxidant vitamin C providing eight grams per 70 kilograms per day for 30 weeks and found a reduction in symptom severity [54]. In addition, several randomized trials using N-Acetylcysteine (NAC), an antioxidant pro-drug, intervention reported significant improvements in irritability, and hyperactivity/noncompliance subscales on the Aberrant Behavior Checklist (ABC) [35,55,56].

Cacao is a natural and potent antioxidant and is a food rich in flavonoids and their sub group flavanols. For the purposes of this paper, cacao and cocoa are used interchangeably. Both flavonoids and flavanols are associated with improved cognitive processing, alertness, and processing speed [37]. Known physiological benefits of cocoa include improved cardiovascular activity, insulin resistance, blood pressure, endothelial function, lipid peroxidataion, cognitive response time, enhanced gamma frequency, and increased cerebral blood flow [27,32,37-39]. The aforementioned studies summarize the inherent value in cacao as a natural superfood for the mind and the body.

The purpose of this study was to examine changes in ASD children's behavior following 70% cacao consumption, with a high total antioxidant concentration, over a four-week period. Changes in behavior were assessed using two behavior scales: *ABC-2* and *Autism Spectrum Rating Scale (ASRS)* [63,64].

Methods

Study Design

This was a four-week pre-test post-test experimental clinical trial of children with ASD who consumed high antioxidant cacao, also known as dark chocolate. The study was conducted in Southern California, United States of America between September 2017 and March 2018. Participants were recruited through snowball sampling techniques following study approval. This study was approved by the Institutional Review Board at Loma Linda University and registered in the National Institutes of Health online database at clinicaltrials.gov (NCT 03195465). Additionally, an Investigational New Drug application was submitted, however, the application was not deemed mandatory for this study by the Food and Drug Administration.

Participants

Participants were children with a diagnosis of ASD between the ages of 4 and 18 and their classroom teachers. Sixteen children and their teachers, from 13 different school districts in Southern California, were recruited. Two teacher participants were excluded from the analysis due to missing study data, one was not able to provide post-intervention feedback because of a school break, and one was excluded because the child was home schooled thus there was no teacher rater available. For subjects' characteristics, refer to Table 1.

In order to be eligible for the study, participants had to have a clinical diagnosis of Autism Spectrum Disorder, be between the ages of four and 18, and like eating dark chocolate. A taste test was offered to the child at baseline to ensure he/she would be

willing to eat it daily for four weeks. Participants with food allergies, caffeine hypersensitivity, theobromine hypersensitivity, a history of seizures or epilepsy, a developmental age less than 24 months, diabetes, and those who were currently enrolled in another study were excluded from this study.

Procedures and Interventions

The cacao utilized in this study was analyzed for total antioxidant activity at Medallion Laboratories in Minnesota. They are considered to be a premiere and accredited nutraceutical lab that uses scientifically sound analyses on food, dietary supplements, and pharmaceutical agents. The standardized unit of analysis for total antioxidant activity is measured in µmoles trolox equivalent (TE) / 100 grams (g) [30]. The antioxidant activity of the cacao used was 52,000 µmoles TE / 100 g. The dark chocolate used in this study consisted of two ingredients, 70% cacao and 30% organic cane sugar. Participants in the study consumed eight squares of the dark chocolate, or 16 grams per day totaling 8,320 µmoles TE / 100 grams. Participants consumed four squares of the cacao twice daily with the first dose in the morning and the second dose in the afternoon. Participants consumed their first dose at approximately 8:00 a.m. and their second dose at approximately 6:00 p.m. depending on the child's school and sleep schedule. All participants were asked to be consistent at both times of day for the duration of the study and logs were kept and collected each week to check for compliance. The cacao was wrapped in foil, parchment paper, and sealed Ziploc bags to protect it from light and air so as to prevent oxidation in order to maintain the cacao's antioxidant integrity.

Measures

The primary outcome measures were the standardized assessment scales: Aberrant Behavior Checklist 2nd Edition (ABC-2) and the Autism Spectrum Rating Scale (ASRS). Both scales have been validated and are used to assess behavioral outcomes in children with ASD [63-65].

The ABC-2 scale consists of five sub-scales which include measures of Irritability, Social Withdrawal, Stereotypic Behavior, Hyperactivity/Noncompliance, and Inappropriate Speech. The ABC-2 consists of 58 questions with each question ranging from zero to three; 0 = no problem, 1 = the behavior is not a problem but slight in degree, 2 = the problem is moderately serious, and 3 = the problem is severe in degree [63,66]. The sum of each subscale was calculated and larger subscale totals indicated more severe behaviors in that category.

The ASRS consists of three subscales which include Social/Communication, Unusual Behaviors, and Self-Regulation for six to 18 year olds and Social/Communication and Unusual Behaviors for two to five year olds. The ASRS consists of 71 questions, with each question ranging from zero to four; 0 = behavior never happens, 1 = rarely happens, 2 = occasionally happens, 3 = frequently happens, and 4 = happens very frequently. In other words, the higher the score the more severe the behaviors for each subscale. Scores from each subscale were calculated using T-Scores in accordance with the standard scoring technique [64]. Teachers filled out both questionnaires (ABC-2 & ASRS) at baseline and at the end of week four.

Statistical Analyses

A sample size of 15 subjects was estimated using a medium effect size of 0.70, a power of 0.80, and a level of significance set at 0.05. Data analysis was performed using IBM SPSS Statistics Version 25.0. Data was summarized using mean ± standard deviation for quantitative variables and frequency (%) for categorical variables. The normality of the outcome variables was examined using Shapiro-Wilk test. Wilcoxon Signed Rank Test was conducted to compare changes (baseline vs. four weeks later) in ABC-2 and ASRS scores. Results were considered significant at $p \leq 0.05$.

Results

Participant characteristics are reported in Table 1. Twelve children with ASD with a mean age of 10.9 ± 3.9 participated in the study. Seventy five percent were males (n = 9) and 42% were White (n = 5). Fifty eight percent of the participants were in a special day class (n = 7) and seventy five percent were verbal (n = 9). All the participants were enrolled in an Individualized Education Program. (Table 1)

Child's medical history and type of therapy are displayed in Table 2. Seventy five percent of participants had some type of stressful event (parent divorced, changed schools, parent changed job, family moved, death in family, financial problems). Approximately 83% sleep, on average, seven or more hours per night. The majority of the participants either previously received or currently receive speech therapy, occupational therapy, and applied behavioral analysis. (Table 2)

Changes in ABC-2 and ASRS scores are reported in Table 3. There were no significant changes in the ABC-2 subscales of Irritability, Social Withdrawal, Stereotypic

Behavior, Hyperactivity/Noncompliance, and Inappropriate Speech (p >.05). A significant improvement was noted in Social/Communication (p=0.03, η^2=0.79), Unusual Behaviors (p=0.02, η^2=0.70), and Self-Regulation (p=0.04, η^2=0.59). (Table 3)

Discussion

In this pilot study, we conducted a pre-test post-test experimental clinical trial to examine the effects of high antioxidant cacao consumption on behaviors in children with ASD. Teacher perceptions were recorded as rated on the ABC-2 and ASRS at the beginning and end of the four-week study. The cacao intervention was well tolerated and no side effects were reported by any of the participants' parents over the course of the intervention. The results of this study indicated that teachers perceived a significant improvement in their ASD student's behavior in the ASRS subcategories of: 1) social/communication, 2) unusual behaviors, and 3) self-regulation. No significant changes were noted on any of the subscales on the ABC-2. This is particularly noteworthy as previous antioxidant and ASD studies used the ABC scale and found improvements in hyperactivity and irritability subscales [35,55,56]. A possible explanation for the difference between the aforementioned studies and this study may be attributed to the fact that the teacher was the informant since they are trained in objective assessments, whereas in previous studies a trained rater was the informant. Another possible explanation could be due to the different time frames used in previous studies conducted for eight weeks, 10 weeks, 12 weeks and 30 weeks compared to four weeks in the present study [35,54-56]. These investigations suggest the potential behavioral benefits of antioxidant therapies on ASD as a neurocognitive disorder [35,55,56].

The effects of antioxidants on behavior and mood in children with ASD is not well understood and the research is still in its infancy. However, based on the results from previous literature in adults, the evidence on cocoa appears promising in its neuroprotective benefits. For example, flavonoids, a subclass of flavanols found in cacao, have the ability to cross the blood brain barrier which demonstrates their neuroprotective mechanisms as well as their role in cognitive functions such as learning and memory [34]. Further, consumption of cocoa flavanols (CF) in adults significantly improved scores on a cognitive battery test and in rapid visual information processing, visual contrast sensitivity, reduced the time required to detect motion direction, and improved spatial memory [42,43]. Both of these studies noted that while the mechanisms underlying the effects are still unknown and they may be related to effects of CF on endothelial function and blood flow which allows improved transfer and delivery of oxygen and nutrients systemically. In a recent brief review, researchers concluded that consumption of CF demonstrated improvements in cognitive performance, working memory, attention, neuroprotective mechanisms, and processing speed [67]. The aforementioned studies were conducted on adult populations including cognitively at risk adults, however, no studies have been done on school-aged children with ASD [34,42,43,67].

This study had some limitations that include a lack of control group and limited sample size. Additionally, investigators did not monitor overall nutritional intake throughout the entirety of the study which means potential confounders in diet could have impacted validity. The length of the study may have not been sufficient to demonstrate more significant behavior change in the participants. Due to the pilot feasibility nature of this study, it was premature to include different intake concentrations of cacao for

participants as previous literature on adults was inconsistent and limited. All participants were receiving some form of therapy targeting behavior, communication, and regulation skills, however, it would be unethical and unrealistic to include ASD children who were not receiving any type of therapy.

Future studies are recommended so as to conduct an intervention for extended periods of time and assess changes at multiple time points. Considering analyzing biomarkers such as, urine or serum blood samples would be supportive and help validate any physiological changes in antioxidant and free radical status. Comparing different groups with different amounts of cacao, as well as running a randomized control trial will help determine if the change was in fact due to the intervention [17]. Additionally, examining behavior changes both through informant based psychometric assessments and performance based assessments (i.e. motor based tasks, attention tasks) would increase validity of this novel intervention. Complementary and alternative medicine, or therapies, are worth examining in the ASD population with consideration of the child's medical history, diet history, and efficacy of alternative therapies before their use is recommended [61]. Additionally, biologically based therapies, such as nutrition, are often used as parents tend to use CAM intervention in an effort to avoid the adverse effects of pharmaceutical agents [68]. Thus, nutrition interventions, such as this study, should continue to be analyzed as a CAM for the ASD population.

Conclusion

To the authors' knowledge, to date, this is the first pilot study linking nutritional antioxidant cacao consumption to behavior in the ASD population from a teacher's

perspective. Results from this study support the potential therapeutic benefit of antioxidants in improving behaviors of children with ASD. Additional robust randomized controlled trials are needed to further examine and validate these positive outcomes.

Ethics Statement

This study was conducted in accordance with the guidelines of the Institutional Review Board at Loma Linda University. We obtained written informed consent from all parents and teachers.

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Table 1. Participant characteristics (N = 12).

	n	%
Age (Mean ± SD)	10.9 ± 3.9	
Gender		
Female	3	25.0
Male	9	75.0
Grade		
K	1	8.3
1	1	8.3
2	1	8.3
4	1	8.3
5	2	16.7
6	3	25.0
10	2	16.7
12	1	8.3
Class setting		
SDC Mild/Moderate	6	50.0
SDC Moderate/Severe	1	8.3
General Education with RSP	2	16.6
General education full inclusion	3	25.0
IEP	12	100.0
Verbal ability		
Non verbal	1	8.3
Limited verbal	2	16.7
Verbal	9	75.0
Ethnicity		
White	5	41.7
Hispanic or Latino	3	25.0
African American	1	8.3
Mixed ethnicities	3	25.0

*Abbreviations: SDC, Special Day Class; IEP, Individualized Education Program; RSP, Resource Specialist Program

Table 2. Child's medical history and type of therapy (N=12).

	n	%
Stressful Event		
None	3	25.0
Parent divorced/separated	1	8.3
Parent changed jobs	2	16.7
Parent changed job/changed schools	1	8.3
Family moved/financial problems	1	8.3
Family moved/changed schools	2	16.7
Death in family/parent changed job/changed schools	1	8.3
Headaches	1	8.3
ADHD	4	33.3
Anxiety/Depression	2	16.7
Hours of sleep per day		
4-6 hours	1	8.3
7-8 hours	5	41.7
9 hours or more	5	41.7
Medications		
ADHD	3	25.0
ADD	1	8.3
Anxiety	2	16.7
Mood Disorder	1	8.3
Type of Therapy		
Individual psychotherapy	1	8.3
Group psychotherapy	1	8.3
Family therapy	1	8.3
Speech therapy	11	91.7
Occupational therapy	10	83.3
Applied Behavior Analysis	8	66.7
Physical therapy	3	25.0
Early intervention	8	66.7

Abbreviations: ADHD, Attention Deficit Hyperactivity Disorder; ADD, Attention Deficit Disorder

Table 3. Median (min, max) Scores for ABC-2 and ASRS over time.

Scale	Baseline	Four weeks	*p* value* (η^2)
ABC-2			
Irritability	5.0 (0,31)	6.0 (0,28)	0.27 (0.18)
Social Withdrawal	13.5 (1,33)	9.0 (0,32)	0.48 (0.13)
Stereotypic Behavior	2.5 (0,15)	3.0 (0,15)	0.61 (0.05)
Hyperactivity/Noncompliance	10.5 (1,36)	8.0 (0,43)	0.20 (0.13)
Inappropriate Speech	1.0 (0,9)	0.5 (0,9)	0.59 (0.04)
ASRS			
Social/Communication	63.0 (55,82)	58.5 (47,82)	0.03 (0.79)
Unusual Behaviors	66.5 (58,85)	60.5 (53,81)	0.02 (0. 70)
Self-Regulation	61.0 (51,80)	58.0 (43,83)	0.04 (0.59)
Total of all 3 Subscales T Score	69.5 (62,79)	59.0 (52,79)	0.007 (1.4)

Abbreviations: ABC-2, Aberrant Behavior Checklist 2nd Edition; ASRS, Autism Spectrum Rating Scale, η^2 = effect size
*Wilcoxon Signed Rank Test
Effect Size = $\frac{\text{mean of difference}}{\text{SD of the difference}}$

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CHAPTER 3

A PILOT STUDY: PARENT PERCEPTIONS OF BEHAVIOR CHANGE IN THEIR ASD CHILD FOLLOWING HIGH ANTIOXIDANT CACAO CONSUMPTION

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Abstract

BACKGROUND- Children with Autism Spectrum Disorder (ASD) tend to have higher free radicals than antioxidants compared to their matched controls. The aim of this pilot study was to examine the effect of high antioxidant cacao consumption on behavior in children with ASD.

METHODS- This was a 4-week repeated measures experimental study. Sixteen participants, aged 4 to 17 years, consumed 8 squares of dark chocolate per day. The *Aberrant Behavior Checklist- 2nd Edition (ABC-2)* and the *Autism Spectrum Rating Scale (ASRS),* were completed by the parent at baseline, end of week two, and end of week four.

RESULTS- Significant improvements were noted on the ABC-2 subscales of Irritability, Social Withdrawal, Stereotypic Behavior, Hyperactivity/Noncompliance, and Inappropriate Speech ($p \leq 0.05$) and on the ASRS scales ($p \leq 0.05$).

CONCLUSION- Results indicated that antioxidant intake can improve behaviors of ASD children. More studies are needed to validate these findings.

ClinicalTrials.gov Identifier- NCT 03195465

Key words: antioxidants, cacao, autism, behavior, parents, perceptions

Introduction

Autism Spectrum Disorder

Autism Spectrum Disorder (ASD) is a life-long neurodevelopmental disorder characterized by core deficits in communication skills, maladaptive behaviors, and self-regulation impairments related to atypical responses to sensory stimuli in the environment (APA, 2013). The prevalence of ASD in the United States (U.S.) is reported at 1 in 68 children with it being four times more common in males than females (CDC, 2016). According to the World Health Organization (2017), worldwide, one in 160 children have ASD. The etiology of ASD is still unknown, however, genetic, environmental, immunological, and oxidative stress factors are linked to its pathogenesis [3,4]. Additionally, perinatal and neonatal risk factors such as, "abnormal fetal presentation, umbilical-cord complications, fetal distress, birth injury or trauma, multiple birth, maternal hemorrhage, summer birth, low birth weight, small for gestational age," among others have been identified as part of the association with ASD [5]. The heterogeneity of ASD lends itself to the need for a variety of interventions, both traditional and non-traditional, as no one therapy works across the spectrum.

Conventional Therapies

Occupational therapists, speech therapists, and behavioral therapists are the most common professionals that work with children with ASD. Occupational therapists address self-regulation as it relates to sensory dysfunction in the classroom, transitions between environments, and for task completion in home and school environments [9]. Speech and Language Pathologists work on social interaction, communication skills, and

alternative communication techniques, among other speech related impediments to enhance communication (American Speech-Language-Hearing Association, 2006). Behavioral therapists work with ASD children on aberrant behaviors, language skills, daily living skills, and social functioning. Applied behavioral analysis (ABA), employs rote discrete trials in a one on one setting and is an intensive approach that is recommended up to 40 hours per week [6,7]. All three of these intervention approaches are used in parallel to address ASD children's performance skills and behavior in both educational and home environments.

Currently, there are only two Food & Drug Administration (FDA) approved drugs for ASD, Risperidone and Abilify [14]. Risperidone is a second-generation antipsychotic prescribed for maladaptive behaviors and self-injurious behaviors whereas Abilify treats mood and irritability [14]. Adverse effects include weight gain, dizziness, drooling, fatigue, vomiting, nasopharyngitis, fever, insomnia, and upper respiratory infections [14,16]. Thus, there is both a place and need for complementary and alternative therapies in addressing the behavioral symptomology of ASD that impedes on quality of life without some of the adverse effects of pharmacological agents.

When the aforementioned therapies and pharmaceutical agents are considered, the cost of ASD becomes colossal. A recent study reported that the cost of ASD in the United States (U.S.), across one's lifetime, is $1.4 million and $2.4 million, respectively, if they also have an intellectual disability [18]. However, as with any disability or disorder the cost is indefinite as there are also psychological and stress costs that cannot be quantified for the individuals or families caring for them. Lifestyle oriented approaches may, then, be

necessary not only to potentially offset costs but to improve quality of life in a long term sustainable fashion.

Oxidative Stress and ASD

Even though ASD's etiology is multifactorial and still unknown, research suggests there may be neuron irregularity, between those with and without ASD, that is linked to the classic behavioral and social symptoms [24]. Oxidative stress, an imbalance between antioxidants and free radicals, is of significance to this study and has been linked to the pathogenesis of ASD. Damodaran, Arumugam [3] found a significant increase in oxidative stress markers in children with ASD compared to matched controls and the severity of maladaptive behaviors were positively correlated with free radicals while antioxidants were negatively correlated with autistic behaviors [3,26]. In addition, elevated blood samples of glutathione, glutathione peroxidase, methionine, oxidized glutathione, and cysteine have also been found in children with ASD [26]. These studies, although limited in number, suggest that the excess of free radicals may be linked to adverse behavioral outcomes.

Ming, Stein, Brimacombe, Johnson, Lambert, Wagner [20] suggests that children with ASD tend to have greater markers of oxidative stress compared to age matched controls or their non-Autistic siblings. Oxidative stress may be related to mitochondrial impairment, environmental factors, metabolic issues, and genetic predisposition (Ming et al., 2005). Inflammation, damage to cellular membranes, autoimmunity, methylation damage, cell death, and neurological impairments are all resultant of free radical damage [26]. Oxidative stress can be identified through markers of antioxidant enzymes, lipid

peroxidation, and protein oxidation, all of which are higher in children with autism compared to their matched controls [3,20]. In summary, the above studies support emerging evidence on the physiological differences in oxidative stress between children with ASD and those without, creating detrimental biological and behavioral outcomes.

Our bodies have an innate mechanism to endogenously produce antioxidants to counter the deleterious effects of free radicals caused by oxidative stress [19,62]. However, when these mechanisms are out of balance we may need to consider nutritional options to supplement these deficits. Antioxidants benefit us physiologically by maintaining cellular integrity, combating free radicals, and improving vascular function while also offering neuroprotection by accumulating in the hippocampus and cortex [27,32]. As a result, antioxidants may be beneficial and necessary to provide homeostasis to oxidative stress imbalance, as seen in ASD.

To date, a few important studies examined the effects of antioxidant intervention relative to behavior change in the ASD population. Dolske, Spollen, McKay, Lancashire, Tolbert [54] appear to be the first researchers to examine antioxidant therapy, in the form of Vitamin C, providing eight grams per 70 kilograms of body weight per day in 500 milligram (mg) tablets for 30 weeks and found a reduction in autistic symptom severity. Additionally, randomized controlled trials using N-Acetylcysteine (NAC), an antioxidant pro-drug, intervention found significant improvements in the irritability and hyperactivity/noncompliance subscales on the Aberrant Behavior Checklist (ABC) [35,55,56].

Cacao is a natural and potent antioxidant. For the purposes of this paper, cacao and cocoa are used interchangeably. Flavonoids and flavanols, subclasses of antioxidants found in cacao, have been correlated with improved cognitive processing, alertness, and

processing speed [37]. Cocoa's physiological and neurological benefits are vast and include improved cardiovascular activity, insulin resistance, blood pressure, lipid peroxidation, enhanced gamma frequency, and increased cerebral blood flow [27,32,37-39]. Additionally, cocoa protects neurons from apoptosis, promotes angiogenesis, and thus leads to more optimal brain functioning which enhances delivery and receipt of oxygen and nutrient supply [50]. The aforementioned studies summarize the inherent value in cacao as a natural functional food for the mind and the body which may be of significant value to neurocognitive disorders, such as ASD.

The purpose of this study was to examine changes in ASD children's behavior following dark chocolate consumption, over a four-week period. Behavioral changes were assessed by each child's parent using two behavior scales: *Aberrant Behavior Checklist 2nd Edition (ABC-2)* and *Autism Spectrum Rating Scale (ASRS)* [63,64].

Methods

Study Design

This was a four-week repeated measures experimental pilot study and clinical trial of children with ASD who consumed high antioxidant cacao, or dark chocolate. The study was conducted in Southern California, United States of America, between September 2017 and March 2018. Recruitment of participants through snowball sampling techniques was initiated following study approval. This study was approved by the Institutional Review Board at Loma Linda University and was registered in the National Institutes of Health online database at clinicaltrials.gov (NCT 03195465). An

Investigational New Drug application was submitted to the Food and Drug Administration, however, was not required for the study.

Participants

Participants were children with a diagnosis of ASD, between the ages of 4 and 17, and their parents. Seventeen children and their parents were recruited. One participant dropped leaving a total sample of sixteen with follow up data. For subjects' characteristics, refer to Table 1. In order to be eligible for the study, participants had to have a diagnosis of Autism Spectrum Disorder, be between the ages of four and 18, and like eating dark chocolate. A taste test was offered to the child at baseline to ensure he/she would be willing to eat it daily for four weeks. Participants with food allergies, caffeine hypersensitivity, theobromine hypersensitivity, a history of seizures or epilepsy, a developmental age less than 24 months, diabetes, and those who were currently enrolled in another study were excluded from this study.

Procedures and Interventions

The cacao utilized in this study was analyzed for total antioxidant activity at Medallion Laboratories in Minnesota. Medallion Labs are considered to be a premiere and accredited nutraceutical lab that use scientifically sound analyses on food, dietary supplements, and pharmaceutical agents. The standardized unit of analysis for total antioxidant activity is measured in µmoles trolox equivalent (TE) / 100 grams (g) [30]. The antioxidant activity of the cacao used was 52,000 µmoles TE / 100 g. The dark chocolate used in this study consisted of two ingredients, 70% cacao and 30% organic cane sugar.

Participants in the study consumed eight squares of the dark chocolate, or 16 grams per day totaling 8,320 µmoles TE / 100 grams. Participants consumed four squares of the cacao twice daily with the first dose in the morning and the second dose in the afternoon, or early evening. All participants were asked to be consistent at both times of day for the duration of the study and logs were kept and collected each week to assess compliance. The cacao was wrapped in parchment paper and foil, then sealed in Ziploc bags to protect it from light and air to prevent oxidation in order to maintain the cacao's antioxidant integrity.

Measures

The primary outcome measures were the Aberrant Behavior Checklist 2nd Edition (ABC-2) and the Autism Spectrum Rating Scale (ASRS). Both scales have been validated and used to assess behavioral outcomes in children with ASD [63-65].

The ABC-2 measures behaviors in populations with developmental delays and is traditionally administered on children ages five and older, however, permissions were obtained from the developer to administer to ages four and older [63]. The ABC-2 scale consists of five sub-scales including, Irritability, Social Withdrawal, Stereotypic Behavior, Hyperactivity/Noncompliance, and Inappropriate Speech. The ABC-2 has 58 questions with scores ranging from zero to three; 0 = no problem, 1 = the behavior is not a problem but slight in degree, 2 = the problem is moderately serious, and 3 = the problem is severe in degree [63,66]. Subscale sums were calculated and larger subscale totals indicated more severe behaviors in that category.

The ASRS consists of three scales which include Social/Communication, Unusual Behaviors, and Self-Regulation for six to 18 year olds and Social/Communication and Unusual Behaviors for two to five year olds. The ASRS has 71 questions, with scores ranging from zero to four; 0 = behavior never happens, 1 = rarely happens, 2 = occasionally happens, 3 = frequently happens, and 4 = happens very frequently. In other words, the higher the score the more severe the behaviors for each scale. Scores from each scale were calculated using T-Scores in accordance with the standard scoring technique [64]. Parents filled out the ABC-2 & ASRS at baseline, end of week two, and end of week four.

Statistical Analyses

A sample size of 15 subjects was estimated using a medium effect size of 0.70, a power of 0.80, and a level of significance set at 0.05. Data analysis was performed using IBM SPSS for Windows Version 25.0. Data was summarized using mean ± standard deviation for quantitative variables and frequency (%) for categorical variables. The normality of the outcome variables was examined using Shapiro-Wilk test. Friedman's test was conducted to determine overall change over time. Wilcoxon Signed Rank test was used to compare changes between different time points (baseline vs. two weeks; baseline vs. four weeks; two weeks vs. four weeks) in ABC-2 and ASRS scores. Results were considered significant at $p \leq 0.05$.

Results

Participant characteristics are reported in Table 1. Sixteen children with ASD with a mean age of 11.1 ± 3.6 participated in the study. Seventy-five percent were males (n = 12), 31% were White (n = 5), and 25% were Hispanic or Latino (n = 4). A majority of participants were enrolled in an Individualized Education Program (n =15, 93.8%) and were verbal (n = 11, 68.8%). Forty-four percent of parents worked full time (n = 7), 62% had a college degree (n = 10), 62% reported an income of $75,000 or higher (n = 9), and 81% were married (n = 13). (Table 1)

Child's medical history and type of therapy are displayed in Table 2. Twenty-five percent of participants had ADHD (n = 4) and were taking medication for attention related issues (n = 4). Approximately 56% sleep, on average, seven or more hours per night (n = 9). The majority of participants either previously received or were concurrently receiving speech therapy, occupational therapy, and applied behavioral analysis. (Table 2)

Changes in ABC-2 and ASRS scores are reported in Table 3. Friedman's test results showed there were significant improvements over time in the ABC-2 subscales of Irritability (p=.03, $\eta^2 = 0.25$), Social Withdrawal (p=.01, $\eta^2 = 0.29$), Stereotypic Behavior (p=.05, $\eta^2 = 0.13$), and Hyperactivity/Noncompliance (p=.04, $\eta^2 = 0.20$). However, no significant difference was noted for Inappropriate Speech (p=.22, $\eta^2 = 0.16$). Friedman's test also showed a significant improvement in all ASRS subscales including Social/Communication (p=.04, $\eta^2 = 0.25$), Unusual Behaviors (p=.003, $\eta^2 = 0.20$), Self-Regulation (p=0.02, η^2=0.32), and Total Scores (p<.001, η^2=0.54). (Table 3)

Post-hoc comparisons revealed that there was a significant difference in Irritability between baseline and week four (p =0.035) and between week two and week four (p=0.014). For Social Withdrawal, a significant difference was found between baseline and week two (p=0.03) and baseline and week four (p=0.023). A significant difference was found in Stereotypic Behavior between baseline and week four (p=0.05) and between week two and week four (p=0.04). Finally, there was a significant improvement in median Hyperactivity/Noncompliance scores between baseline and week four (p=0.035). No significant difference was found in median scores for Inappropriate Speech over time (p=0.22).

In terms of ASRS scales, a significant difference was found in Social/Communication between baseline and week two (p =0.009) and between baseline and week four (p =0.026). A significant difference was found in Unusual Behaviors between baseline and week two (p = 0.022) and baseline and week four (p =0.001). There was a significant difference in Self-Regulation between baseline and week two (p=0.018) and baseline and week four (p=0.012). Overall, there was a significant difference in median Total scores on the ASRS between baseline and week two (p=0.002), baseline and week four (p=0.002), and between week two and week four (p=0.02). (Table 3)

Discussion

In this pilot study, a repeated measures experimental clinical trial was conducted to examine the effects of high antioxidant cacao consumption on behaviors in children with ASD. Overall, participant characteristics revealed that parents were well educated, of an above average socioeconomic status, and children came from a stable home as most

were married. A previous study indicated that parents of children with ASD, who are more educated and affluent, tend to try alternative therapies and enroll their children in clinical trials more than others [70].

Based on parental report, the dark chocolate was well tolerated and no allergic reactions were reported over the course of the intervention. The results of this study indicated that parents perceived a significant improvement in their ASD child's behavior in the ASRS scales of: 1) Social/Communication, 2) Unusual Behaviors, and 3) Self-Regulation. Significant improvements were also noted on the ABC-2 subscales of 1) Irritability, 2) Social Withdrawal, 3) Stereotypic Behavior, and 4) Hyperactivity/Noncompliance. Overall, median scores decreased, indicating that participants were less irritable, socially withdrawn, and reduced stereotypic behaviors. In addition, participants improved in their social/communication skills and self-regulation, and had less unusual behaviors. The results of this study are consistent with the findings from the NAC and ASD studies that found improvements in hyperactivity and irritability ABC-2 subscales [35,55,56]. Based on these studies, there is promising benefit of antioxidant-based therapies in targeting common behavioral symptoms in ASD [35,55,56].

Although there is limited research on antioxidants, specifically cacao, and the autistic population, previous literature on adults suggests the neuroprotective benefits of cacao. In recent reviews, the benefits of the cocoa bean and its derivatives were associated with improved cerebrovascular function, permeation of the blood brain barrier via flavanols' (epicatechin and catechin), working memory, attention, processing speed, and learning [50,67]. Furthermore, ingestion of cocoa flavanols (CF) in adults significantly improved cognitive scores, visual information processing, visual contrast sensitivity, and

spatial memory [34,42,43]. The above studies acknowledge that the underlying mechanisms of cocoa on brain health are still under investigation, however, observed benefits may be related to effects on endothelial function and blood flow which promote oxygen and nutrient delivery systemically. Although the research available on cocoa's physiological and neurological benefit for adults is useful there is still a gap in the literature on how cocoa can be used in the pediatric population, specifically those with neurocognitive disorders.

Our study had some limitations. First, a lack of control group limits the generalizability of these results. Second, our assessments, although validated and reliable, were self-report measures which may include biases. However, previous research suggests the impact that ASD behaviors have on the parent and so their observations of behavior change may be most accurate as they understand the intricacies of their child's maladaptive behaviors [13]. Behaviors associated with ASD not only affect the parent child dynamic but also dually stress both the parent and child individually [13]. Additionally, parents may be strong raters because their stress has been correlated with the severity of their child's behaviors so if behaviors are more or less severe they would be directly impacted and thus able to report changes [71]. Thirdly, nutritional intake was not monitored over the course of the study which means dietary confounders could have impacted results. In an attempt to control for dietary effects, we avoided starting participants near major holiday breaks, such as Christmas and Thanksgiving, where diet may change more drastically. Fourth, the study length may not have been enough to show more significant behavior change in the participants. Previous antioxidant studies used different study lengths ranging from eight weeks, 10 weeks, 12 weeks, and up to 30 weeks compared to

four weeks in the present study [35,54-56]. The results of this study demonstrated an overall improvement in four of five ABC-2 subscales and all three ASRS scales with the most significant changes being after two weeks of intervention. Without a control group, it is difficult to rule out the potential for a placebo effect. However, some of the non-significant findings between baseline and week two strengthens the interpretation of results by suggesting that the significant effects observed after two weeks may not simply be driven by parents' belief but an actual intervention effect on behavior.

A major strength of the present study was the low attrition rate since only one participant withdrew from the study. Previous antioxidant and ASD studies did not mention total antioxidant activity of interventions used nor was amount of NAC based on anthropometric assessments of individual participants [35,54-56]. Thus, another strength of the present investigation is the analysis and report of total antioxidant activity of the cacao validating its use as an antioxidant intervention. It should be noted that because this was a pilot feasibility study, it seemed premature to include different intake concentrations of cacao for participants as previous literature on adults was inconsistent and limited. However, future studies may want to consider offering different concentrations based on body weight as nutritional needs differ across the lifespan. All participants were receiving one or more traditional therapies targeting core symptoms, however, it would be unethical and unrealistic to include ASD children who were not receiving any type of therapy.

Future studies are recommended to run the intervention for longer time periods and measure changes throughout the study to determine when the effect occurs. Analysis of biomarkers including urine or serum blood samples will be imperative to determine

any physiological changes in oxidative stress status. Comparing groups using different concentrations of cacao based on anthropometric assessments, such as weight, through randomized controlled trials will further validate if observed changes are in fact due to the antioxidant intervention. Complementary and alternative medicine (CAM) need to be researched to determine efficacious approaches in the ASD population with consideration of the child's medical history and diet history before recommendations can be made [61]. Biologically based therapies, including nutraceuticals, are commonly employed by parents of children with disabilities because CAM interventions do not have the same adverse side effects as pharmaceutical interventions [68]. Thus, nutrition interventions, more specifically antioxidant therapies, as used in this study, need to remain under investigation as a potential CAM for addressing and hopefully improving ASD behavioral symptoms.

Conclusion

To the authors' knowledge, to date, this is the first pilot study associating consumption of a nutraceutical, high antioxidant cacao, to behavioral outcomes in the ASD population from a parent's perspective. Results from this study add to the existing literature on the potential benefits of antioxidants on improving behavior in children with ASD. Further robust randomized controlled trials are needed to further validate these positive findings.

Ethics Statement

This study was approved by and conducted in accordance with the guidelines of the Institutional Review Board at Loma Linda University. We obtained written informed consent from all parents.

Acknowledgments

We gratefully acknowledge all of the families and children who generously gave us their time and effort to make this project possible and also a special thank you to the following research assistants: Vivian Noimanivone, Amy Trinh, Cindy Trinh, and Roshelle Vanta.

Table 1. Participant characteristics (N=16).

	n	%
Age (Mean ± SD)	11.1 ± 3.6	
Male	12	75.0
Grade		
K	2	12.5
1	1	6.3
2	1	6.3
4	1	6.3
5	2	12.5
6	3	18.8
7	2	12.5
9	1	6.3
10	2	12.5
12	1	6.3
Verbal ability		
Non-verbal	3	18.8
Limited verbal	2	12.5
Verbal	11	68.8
Ethnicity		
White	5	31.3
Hispanic or Latino	4	25.0
Black or African American	1	6.3
Asian	1	6.3
Native Hawaiian	1	6.3
Mixed ethnicity	4	25.0
Diagnosed by		
Pediatrician	4	25.0
Neurologist	5	31.3
Inland Regional Center	5	31.3
Psychologist	2	12.5
Employment		
Full time	7	43.8
Part time	3	18.8
Out of work and looking	1	6.3
Home maker	4	25.0
Out of work not looking	1	6.3

Table 1. Continued

	n	%
Education		
Some HS	1	6.3
Some college	2	12.5
Associate's degree	3	18.8
Bachelor's degree	6	37.5
Master's degree	4	25.0
Income		
$20,000-$34,999	2	12.5
$35,000-$49,999	2	12.5
$50,000-$74,999	2	12.5
$75,000-$149,999	4	25.0
$100,000-$149,999	5	31.3
$150,000 or more	1	6.3
Marital status		
Single	1	6.3
Married	13	81.3
Divorced	1	6.3
Separated	1	6.3
Custody[a]		
Mother	2	12.5
Shared	1	6.3

Abbreviations: SDC, Special Day Class; IEP, Individualized Education Program; RSP, Resource Specialist Program

[a]Percentages do not add to 100% because parents are married

Table 2. Child's medical history and type of therapy (N=16).

	n (%)
Headaches	2 (12.5)
ADHD	4 (25.0)
Anxiety/Depression	3 (18.8)
Sleeping Disorder	1 (6.3)
Hours of sleep per night	
4-6 hours	2 (12.5)
7-8 hours	9 (56.2)
9 hours or more	5 (31.3)
Medications	
Attention	4 (25.0)
Anxiety	3 (18.8)
Mood Disorder	2 (12.5)
Headache	1 (6.3)
Behavior	3 (18.8)
Type of Therapy	
Individual psychotherapy	1 (6.3)
Group psychotherapy	2 (12.5)
Family therapy	1 (6.3)
Speech therapy	15 (93.7)
Occupational therapy	14 (87.5)
Applied Behavior Analysis	11 (68.8)
Physical therapy	4 (25.0)
Early intervention	11 (68.8)

Abbreviations: ADHD, Attention Deficit Hyperactivity Disorder

Table 3. Median (min, max) Scores for ABC-2 and ASRS over time.

Scale	Subscale	Baseline	Week 2	Week 4	*P* value* (η^2)
ABC-2	Irritability	13.0 (0,35)	13.0 (0,31)	7.0 (0,34)	p= .03, η^2 = 0.25
	Social Withdrawal	13.0 (1,34)	9.0 (0.37)	7.5 (0,34)	p=.01, η^2 = 0.29
	Stereotypic Behavior	3.5 (0,15)	3.0 (1,14)	1.0 (0,16)	p=.05, η^2 = 0.13
	Hyperactivity/Noncompliance	15.0(4,42)	12.0 (2,39)	9.5 (0,42)	p=.04, η^2 = 0.20
	Inappropriate Speech	3.0 (0,9)	2.0 (0,9)	1.5 (0,10)	p=.22, η^2 = 0.16
ASRS	Social/Communication	70.0 (58,85)	65.5 (52,82)	68.0 (43,85)	p=.04, η^2 = 0.25
	Unusual Behaviors	69.0 (45,83)	66.0 (7,74)	61.0 (43,74)	p=.003, η^2 = 0.20
	Self-Regulation	65.0 (48,76)	64.0 (47,75)	60.0 (38,77)	p=.02, η^2 = 0.32
	Total of 3 Subscales T Score	72.0 (54,83)	68.5 (50,77)	62.0 (45,79)	p<.001, η^2 = 0.54

Abbreviations: ABC-2, Aberrant Behavior Checklist 2nd Edition; ASRS, Autism Spectrum Rating Scale, η^2 = effect size

*Friedman Test

** [d] $\eta^2 = \frac{\text{Treatment Sum of Squares}}{\text{Total Sum of Squares}}$

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CHAPTER 4

DISCUSSION

In this pilot study, we conducted a prospective experimental clinical trial to examine the effects of high antioxidant cacao consumption on behaviors in children with Autism Spectrum Disorder. Teacher perceptions were recorded as rated on the Aberrant Behavior Checklist-2 (ABC-2) and Autism Spectrum Rating Scale (ASRS) at the beginning and end of the four-week study. The cacao intervention was well tolerated and no side effects were reported by any of the participants' parents over the course of the intervention. The results of this study indicated that teachers perceived a significant improvement in their ASD student's behavior in the ASRS scales of: 1) social/communication, 2) unusual behaviors, and 3) self-regulation. No significant changes were noted on any of the subscales on the ABC-2. The results of this study indicated that parents perceived a significant improvement in their ASD child's behavior in the ASRS scales of: 1) Social/Communication, 2) Unusual Behaviors, and 3) Self-Regulation. Significant improvements were also noted on the ABC-2 subscales of 1) Irritability, 2) Social Withdrawal, 3) Stereotypic Behavior, and 4) Hyperactivity/Noncompliance. Overall, median scores decreased, indicating that participants were less irritable, socially withdrawn, and reduced stereotypic behaviors. In addition, participants improved in their social/communication skills and self-regulation, and had less unusual behaviors. The results of this study are consistent with the findings from the N-Acetylcysteine (NAC) and ASD studies that found improvements in hyperactivity and irritability ABC-2 subscales [35,55,56]. Based on these studies, there is

promising benefit of antioxidant-based therapies in targeting common behavioral symptoms in ASD [35,55,56].

Overall, participant characteristics revealed that parents were well educated, of an above average socioeconomic status, and children came from a stable home as most parents were married. A previous study indicated that parents of children with ASD, who are more educated and affluent, tend to try alternative therapies and enroll their children in clinical trials more than others [70].

Although there is limited research on antioxidants, specifically cacao, and the autistic population, previous literature on adults suggests the neuroprotective benefits of cacao. In recent reviews, the benefits of the cocoa bean and its derivatives were associated with improved cerebrovascular function, permeation of the blood brain barrier via flavanols (epicatechin and catechin), working memory, attention, processing speed, and learning [50,67]. Furthermore, ingestion of cocoa flavanols in adults significantly improved cognitive scores, visual information processing, visual contrast sensitivity, and spatial memory [34,42,43]. The above studies acknowledge that the underlying mechanisms of cocoa on brain health are still under investigation, however, observed benefits may be related to effects on endothelial function and blood flow which promote oxygen and nutrient delivery systemically. Although the research available on cocoa's physiological and neurological benefit for adults is useful, there is still a gap in the literature on how cocoa can be used in the pediatric population, specifically those with neurocognitive disorders.

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Conclusion

To the authors' knowledge, to date, this is the first pilot study associating consumption of a nutraceutical, high antioxidant cacao, to behavioral outcomes in the ASD population from both parent and teacher perspectives. Results from both studies add to the existing literature on the potential benefits of antioxidants on improving behavior in children with ASD. Further robust randomized controlled trials are needed to further validate these findings.

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APPENDIX A

PARENT INFORMED CONSENT FORM

INFORMED CONSENT (Parent)

TITLE:	**THE EFFECT OF HIGH ANTIOXIDANT CACAO ON BEHAVIORS IN CHILDREN WITH AUTISM SPECTRUM DISORDER**
SPONSOR:	**School of Allied Health Professions, Loma Linda University**
PRINCIPAL INVESTIGATOR	Lee Berk, DrPH, Associate Dean for Research Affairs
CO-INVESTIGATOR	Amy Sadek, Rehabilitation Science PhD Candidate

WHY IS THIS STUDY BEING DONE?

This study is to see if eating cacao changes Autistic behaviors in children.

Your child can be in this study if you understand and read English and you are 18 years of age or older, and your child has autism and is between 5-12 years old. Your child must like 70% cacao and not be allergic.

Children with food allergies, caffeine hypersensitivity, theobromine hypersensitivity, a history of seizures or epilepsy, a developmental age less than 24 months, or diabetics cannot be in this study.

Approximately 35 subjects will participate in this study.

This study may last up to 4 weeks with 5 visits at your child's school for approximately 30 minutes per visit. The first visit will take up to an hour for all paperwork and initial questionnaires.

HOW WILL I BE INVOLVED?

This study involves the following:

- When you come for the study, we will obtain informed consent for you and your child to be in the study.
- You will explain to your child their level of involvement in a manner they can understand to the best of their ability.
- Your child will also be offered a taste testing sample of the chocolate bar to be used in the study to see if they like it as that is a requirement to be included in the study.
- On day one, you will fill out a Food Frequency Questionnaire based on your child's typical dietary habits, an Autism Spectrum Rating Scale, an Aberrant Behavior Checklist, and the Parental Stress Index Questionnaire.
- The Food Frequency Questionnaire asks how often your child consumes foods like bread, meat, sweets, vegetables, and fruit. The Autism Spectrum Rating Scale asks questions about play skills, social interaction skills, communication skills, rigid behaviors, and understanding of humor. The Aberrant Behavior Checklist asks questions about your child's irritability, agitation, crying, social behavior, repetitive behaviors, speech, and hyperactivity. The Parental Stress Index asks questions about how you're feeling as a parent, your child's behavior, and your interaction with them.
- Your child will consume 3 squares of the 70% dark chocolate every four hours daily for four weeks during waking hours between 8:00 a.m. and 8:00 p.m.
- You will fill out the Autism Spectrum Rating Scale and the Aberrant Behavior Checklist at the end of week two and at the end of week four.
- You will fill out the Parental Stress Index Questionnaire at the end of week four.
- Your child's special education teacher will also fill out the Autism Spectrum Rating Scale and the Aberrant Behavior Checklist at the beginning of week 1, end of week 2, and end of week 4. They will also give any doses of the cacao that are during school hours.

WHAT ARE THE REASONABLY FORESEEABLE RISKS OR DISCOMFORTS I MIGHT HAVE?

This study poses no greater risk to you than what you routinely encounter in day-to-day life. Participating in this study will involve the following risks: possible breach of confidentiality and feeling uncomfortable answering questions about parental stress.

All records and research materials that identify you and your child will be held confidential. Any published document resulting from this study will not disclose your identity without your permission. Information identifying you will only be available to the study personnel.

All data will be secured in a locked cabinet in a locked office.

The use of your Protected Health Information is explained in the separate authorization form.

WILL THERE BE ANY BENEFIT TO ME OR OTHERS?

Although you may not personally benefit from this study, your participation may help clinicians and teachers when working with autistic children.

WHAT ARE MY RIGHTS AS A SUBJECT?

Your participation in this study is entirely voluntary. You may refuse to participate or withdraw once the study has started. Your decision whether or not to participate or terminate at any time will not affect your future standing with the researchers, the schools, or the Inland Empire Autism Assessment Center of Excellence. You do not give up any legal rights by participating in this study.

If, at any time, you feel uncomfortable with the questions on the surveys, you may refuse to answer questions.

WHAT COSTS ARE INVOLVED?

There is no cost to you for participating in this study.

WILL I BE PAID TO PARTICIPATE IN THIS STUDY?

You will be paid a $50 gift card for completing this study in full.

WHO DO I CALL IF I HAVE QUESTIONS?

You may call 909-558-4647 or e-mail patientrelations@llu.edu for information and assistance with complaints or concerns about your rights in this study.

SUBJECT'S STATEMENT OF CONSENT

- I have read the contents of the consent form and have listened to the verbal explanation given
  by the investigator.
- My questions concerning this study have been answered to my satisfaction.
- This study has been explained to my child at a level she/he can comprehend and I give

permission for my child to participate in the study.

- Signing this consent document does not waive my rights nor does it release the investigators,
  institution or sponsors from their responsibilities.
- I may call Dr. Lee Berk during routine office hours at (909) 651-5828 (ext: 15828) if I have
  additional questions.
- I hereby give voluntary consent to participate in this study.

I understand I will be given a copy of this consent form after signing it.

Signature of Parent/Guardian	Printed Name of Parent/Guardian
Date	Name of Child

Authority to act for subject:_____

INVESTIGATOR'S STATEMENT

I have reviewed the contents of this consent form with the person signing above. I have explained potential risks and benefits of the study.

Signature of Investigator	Printed Name of Investigator
Date	

APPENDIX B

SPECIAL EDUCATION TEACHER INFORMED CONSENT

INFORMED CONSENT (Special Education Teacher)

TITLE:	THE EFFECT OF HIGH ANTIOXIDANT CACAO ON BEHAVIORS IN CHILDREN WITH AUTISM SPECTRUM DISORDER
SPONSOR:	School of Allied Health Professions
PRINCIPAL INVESTIGATOR:	Lee Berk, DrPH, Associate Dean for Research Affairs
CO-INVESTIGATOR:	Amy Sadek, Rehabilitation Science PhD Candidate

WHY IS THIS STUDY BEING DONE?

The purpose of the study is to determine if consuming high antioxidant cacao changes the severity of Autistic behaviors.

You are invited to be in this study because you are an English-literate special education teacher, 18 years of age or older. We enrolled a child you are teaching in our study and need your feedback.

Approximately 35 subjects will participate in this study.

This study may last up to 4 weeks with 5 visits at your school site for approximately 30 minutes per visit.

HOW WILL I BE INVOLVED?

Participation in this study involves the following:

- Following your school districts' agreement to participate, we will obtain informed consent from you.
- On day one, you will fill out an Autism Spectrum Rating Scale and an Aberrant Behavior Checklist for the child.

- Your student who is autistic will consume 3 squares of the 70% dark chocolate every four hours daily for four weeks during waking hours between 8:00 a.m. and 8:00 p.m.
- You will provide the doses that occur during school hours such as 8:00 a.m. and 12:00 p.m.
- You will fill out the Autism Spectrum Rating Scale and the Aberrant Behavior Checklist at the end of week two and at the end of week four.

WHAT ARE THE REASONABLY FORESEEABLE RISKS OR DISCOMFORTS I MIGHT HAVE?

This study poses no greater risk to you than what you routinely encounter in day-to-day life. Participating in this study will involve the following risks: possible breach of confidentiality and feeling uncomfortable answering questions.

All records and research materials that identify you and the child will be held confidential. Any published document resulting from this study will not disclose your identity without your permission. Information identifying you will only be available to the study personnel.

All data will be secured in a locked cabinet in a locked office.

WILL THERE BE ANY BENEFIT TO ME OR OTHERS?

Although you may not personally benefit from this study, your participation may help clinicians and teachers when working with children with a diagnosis of Autism Spectrum Disorder. Results of the study can provide insights to practitioners and teachers while working with children with Autism Spectrum Disorder.

WHAT ARE MY RIGHTS AS A SUBJECT?

Your participation in this study is entirely voluntary. You may refuse to participate or withdraw once the study has started. Your decision whether or not to participate or terminate at any time will not affect your future standing with the researchers, the schools, or the Inland Empire Autism Assessment Center of Excellence. You do not give up any legal rights by participating in this study.

If, at any time, you feel uncomfortable with the questions on the surveys, you may refuse to answer questions.

WHAT COSTS ARE INVOLVED?

There is no cost to you for participating in this study.

WILL I BE PAID TO PARTICIPATE IN THIS STUDY?

No.

WHO DO I CALL IF I HAVE QUESTIONS?

You may call 909-558-4647 or e-mail patientrelations@llu.edu for information and assistance with complaints or concerns about your rights in this study.

SUBJECT'S STATEMENT OF CONSENT

- I have read the contents of the consent form and have listened to the verbal explanation given by the investigator.
- My questions concerning this study have been answered to my satisfaction.
- Signing this consent document does not waive my rights nor does it release the investigators, institution or sponsors from their responsibilities.
- I may call Dr. Lee Berk during routine office hours at (909) 651-5828 (ext: 15828) if I have additional questions.
- I hereby give voluntary consent to participate in this study.

I understand I will be given a copy of this consent form after signing it.

Signature of Subject	Printed Name of Subject
Date	

INVESTIGATOR'S STATEMENT

I have reviewed the contents of this consent form with the person signing above. I have explained potential risks and benefits of the study.

Signature of Investigator	Printed Name of Investigator
Date	

APPENDIX C

AUTHORIZATION FOR USE OF PROTECTED HEALTH INFORMATION

INSTITUTIONAL REVIEW BOARD
Authorization for Use of Protected Health Information (PHI)

Per 45 CFR §164.508(b)
RESEARCH PROTECTION PROGRAMS
LOMA LINDA UNIVERSITY | Office of the Vice President of Research Affairs
24887 Taylor Street, Suite 202 Loma Linda, CA 92350
(909) 558-4531 (voice) / (909) 558-0131 (fax)/e-mail: irb@llu.edu

TITLE OF STUDY:	The Effect of High Antioxidant Cacao on Behaviors in Children with Autism Spectrum Disorder
PRINCIPAL INVESTIGATOR:	Lee Berk, DrPH
Others who will use, collect, or share PHI:	All Authorized Personnel

Use of the terms "I," "you" and "your" addresses, where appropriate, the study patient, the parent or legal representative if the study patient is a minor, any unborn fetus(es) and child(ren) once born. The study named above may be performed only by using personal information relating to your health. National and international data protection regulations give you the right to control the use of your medical information. Therefore, by signing this form, you specifically authorize your medical information to be used or shared as described below.

The following personal information, considered "Protected Health Information" (PHI) is needed to conduct this study and may include, but is not limited to: name, address, telephone number, date of birth, medical records and charts, height, weight, school of attendance, current therapy services the child receives such as but not limited to occupational therapy, physical therapy, and speech therapy. Medical history of food allergies, caffeine hypersensitivity, theobromine hypersensitivity, seizures or epilepsy, developmental age < 24 months, and diabetes will also be obtained.

The individual(s) listed above will use or share this PHI in the course of this study with the Institutional Review Board (IRB) of Loma Linda University,

the sponsor of the study School of Allied Health Professions and its affiliates, government agencies such as the Food and Drug Administration (FDA), other research sites involved in this study, health care providers who provide services to you in connection with this study, central labs, central review centers and central reviewers.

The main reason for sharing this information is to be able to conduct the study as described earlier in the consent form. In addition, it is shared to ensure that the study meets legal, institutional, and accreditation standards. Information may also be shared to report adverse events or situations that may help prevent placing other individuals at risk.

All reasonable efforts will be used to protect the confidentiality of your PHI, which may be shared with others to support this study, to carry out their responsibilities, to conduct public health reporting and to comply with the law as applicable. Those who receive the PHI may share with others if they are required by law, and they may share it with others who may not be required to follow national and international "protected health information" (PHI) regulations such as the federal privacy rule.

Subject to any legal limitations, you have the right to access any protected health information created during this study. You may request this information from the Principal Investigator named above but it will only become available after the study analyses are complete.

- The authorization expires upon the conclusion of this research study.

You may change your mind about this authorization at any time. If this happens, you must withdraw your permission in writing. Beginning on the date you withdraw your permission, no new personal health information will be used for this study. However, study personnel may continue to use the health information that was provided before you withdrew your permission. If you sign this form and enter the study, but later change your mind and withdraw your permission, you will be removed from the study at that time. To withdraw your permission, please contact the Principal Investigator, Dr. Lee Berk, or study personnel at 909-651-5828 (ext 15828).

You may refuse to sign this authorization. Refusing to sign will not affect the present or future care you receive at this institution and will not cause any penalty or loss of benefits to which you are entitled. However, if you do not sign this authorization form, you will not be able to take part in the study for which you are being considered. You will receive a copy of this signed and dated authorization prior to your participation in this study.

I agree that my personal health information may be used for the study purposes described in this form.

Signature of Patient *or* Patient's Legal Representative	**Date**
Printed Name of Legal Representative (if any)	Representative's Authority to Act for Patient
Signature of Investigator Obtaining Authorization	**Date**

APPENDIX D

CALIFORNIA EXPERIMENTAL SUBJECT'S BILL OF RIGHTS

You have been asked to participate as a subject in an experimental clinical procedure. Before you decide whether you want to participate in the experimental procedure, you have a right to:

1. Be informed of the nature and purpose of the experiment.

2. Be given an explanation of the procedures to be followed in the medical experiment, and any drug or device to be utilized.

3. Be given a description of any attendant discomforts and risks reasonably to be expected from the experiment.

4. Be given an explanation of any benefits to the subject reasonably to be expected from the experiment, if applicable.

5. Be given a disclosure of any appropriate alternative procedures, drugs or devices that might be advantageous to the subject, and their relative risks and benefits.

6. Be informed of the avenues of medical treatment, if any available to the subject after the experiment if complications should arise.

7. Be given an opportunity to ask any questions concerning the experiment or the procedure involved.

8. Be instructed that consent to participate in the medical experiment may be withdrawn at any time and the subject may discontinue participation in the medical experiment without prejudice.

9. Be given a copy of any signed and dated written consent form used in relation to the experiment.

10. Be given the opportunity to decide to consent or not to consent to a medical experiment without the intervention of any element of force, fraud, deceit, duress, coercion or undue influence on the subject's decision.

I have carefully read the information contained above in the "California Experimental Subject's Bill of Rights" and I understand fully my rights as a potential subject in a medical experiment involving people as subjects.

_____ _____

Date Patient

APPENDIX E

PARTICIPANT'S DEMOGRAPHIC FORM

1. Name of Child _____
2. Name of Parent (Informant) _____
3. Date of Birth _____
4. Current Grade _____
5. Gender:
   - □ Male
   - □ Female
6. How was your child diagnosed with Autism Spectrum Disorder?
   - □ Pediatrician
   - □ Neurologist
   - □ School Psychologist
   - □ Inland Empire Autism Assessment Center of Excellence
   - □ Other (please specify):_____
7. Ethnicity: **(Please check only one answer)**
   - □ White
   - □ Hispanic or Latino
   - □ Black or African American
   - □ Native American or American Indian
   - □ Asian
   - □ Native Hawaiian or Other Pacific Islander
   - □ Other (please specify): _____
8. Current Employment: **(Please check only one answer)**
   - □ Full-time
   - □ Part-time
   - □ Self-employed
   - □ Out of work and looking for work
   - □ Out of work but not currently looking for work (unemployed)
   - □ Homemaker
   - □ Military
   - □ Retired
   - □ Unable to work
   - □ Other (please specify): _____

9. Highest Level of Education: **(Please check only one answer)**
   - ☐ No schooling completed
   - ☐ Primary school to 8th grade
   - ☐ Some high school, no diploma
   - ☐ High school graduate, diploma or the equivalent (GED)
   - ☐ Some college credit, no degree
   - ☐ Trade/technical/vocational training
   - ☐ Associate Degree
   - ☐ Bachelor's Degree
   - ☐ Master's Degree
   - ☐ Doctorate Degree

10. What is your current marital status? **(Please check only one answer)**
   - ☐ Single
   - ☐ Married
   - ☐ Divorced
   - ☐ Separated
   - ☐ Widowed

11. If divorced, who has custody of the child?
   - ☐ Mother
   - ☐ Father
   - ☐ Shared
   - ☐ Other (please specify): _____

12. What is your annual household income? **(Please check only one answer)**
   - ☐ Less than $20,000
   - ☐ $20,000 - $34,999
   - ☐ $35,000 - $49,999
   - ☐ $50,000 - $74,999
   - ☐ $75,000 - $99,999
   - ☐ $100,000 - $149,999
   - ☐ $150,000 or more

13. Have any of the following stress events occurred in your life? **(Check all that apply)**
   - ☐ Parents divorced or separated
   - ☐ Family illness
   - ☐ Death in the family
   - ☐ Parent changed job
   - ☐ Change schools
   - ☐ Family moved
   - ☐ Family financial problems
   - ☐ Other (please specify): _____

14. Child's Medical History: **(Check all that apply)**
    - ☐ Seizures or Epilepsy
    - ☐ Diabetes
    - ☐ Food allergies
    - ☐ Headaches
    - ☐ Migraine headaches
    - ☐ Attention Deficit Hyperactivity Disorder (ADHD)
    - ☐ Anxiety or Depression
    - ☐ Autism Spectrum Disorder
    - ☐ Eating Disorder
    - ☐ Sleeping Disorder
    - ☐ Other: (please specify): _____

15. Average hours per night your child sleeps on weekdays: **(Please check only one answer)**
    - ☐ 0-3 hours
    - ☐ 4-6 hours
    - ☐ 7-8 hours
    - ☐ 9 hours or more

16. Average hours per night your child sleeps on weekends: **(Please check only one answer)**
    - ☐ 0-3 hours
    - ☐ 4-6 hours
    - ☐ 7-8 hours
    - ☐ 9 hours or more

17. Has your child ever had any of the following forms of treatment? **(Check all that apply)**
    - ☐ Individual psychotherapy
        - ➢ If so, for how long: _____
    - ☐ Group psychotherapy
        - ➢ If so, for how long: _____
    - ☐ Family therapy with child
        - ➢ If so, for how long: _____
    - ☐ Speech therapy
        - ➢ If so, for how long: _____
    - ☐ Occupational therapy
        - ➢ If so, for how long: _____
    - ☐ Applied Behavioral Analysis (ABA)
        - ➢ If so, for how long: _____
    - ☐ Physical therapy
        - ➢ If so, for how long: _____
    - ☐ Early Intervention (Inland Regional Center, Early Head Start)
        - ➢ If so, for how long: _____

18. Does your child have an Individualized Education Program?
    - No
    - Yes

19. What classroom setting is your child currently in?
    - Special education: SDC mild/moderate
    - Special education: SDC moderate/severe
    - Special education: SDC severe/profound
    - Mostly special education with some mainstreaming
    - General education with RSP support
    - General education (full inclusion)
    - Has a "one on one" aid in special education or general education
    - Other (please specify):_____

20. Where did you get a copy of the flyer to participate? **(Check all that apply)**
    - Inland Empire Autism Assessment Center of Excellence
    - WonderLAB
    - Loma Linda University Outpatient Pediatric Rehabilitation Center
    - Loma Linda University Interdisciplinary Outreach Clinic
    - Drayson Center (Loma Linda)
    - SenseAbilities
    - Precious Hearts Academy
    - High Desert Speech and Language Center Inc.
    - Inland Regional Center
    - Pediatric Therapy Associates (Chino)
    - Anaheim Hills Pediatric Therapy Inc.
    - Five Oaks Speech Clinic
    - Horizon Therapy Services
    - Nichols Speech Therapy
    - Up & Movin Pediatric Therapy
    - Redlands Community Hospital
    - Autism Society Inland Empire (Facebook or newsletter)
    - Clinical Trials Website
    - Facebook
    - Email
    - Other (please specify): _____

APPENDIX F

ABERRANT BEHAVIOR CHECKLIST 2ND EDITION QUESTIONNAIRE

Aberrant Behavior Checklist–Community

200

If in School, Type of Class? (Check **all** applicable)

- ☐ Regular class
- ☐ Mainstream with "pull-outs"
- ☐ Intellectual disability
- ☐ ASD: Autism spectrum disorder
- ☐ Emotional disability
- ☐ Physical disability
- ☐ Other _____

Where Did You Observe the Client?

- ☐ Family home
- ☐ School
- ☐ Day program
- ☐ Apartment
- ☐ Group home
- ☐ Workplace
- ☐ Other _____

Conditions: Is the client's functioning limited in some way due to the following impairments? (Please circle appropriate numbers)

	No	Mild	Moderate	Severe
a) Hearing impairment?	0	1	2	3
b) Visual impairment [requiring *more* than glasses]?	0	1	2	3
c) Speech and language impairment?	0	1	2	3
d) Physical disability?	0	1	2	3
e) Seizures or epilepsy?	0	1	2	3
f) Autism spectrum disorder (ASD)?	0	1	2	3
g) Intellectual disability?	0	1	2	3
h) Chronic sleep problems?	0	1	2	3
i) Gastrointestinal (GI) issues?	0	1	2	3

j) Medical conditions/Other (please describe): _____

_____

Current medications (Please list any medication and frequency of dosing):_____

_____

_____

_____

_____

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SLOSSON EDUCATIONAL PUBLICATIONS, INC.
P.O. Box 280, East Aurora, New York 14052

INSTRUCTIONS

The ABC-Community rating scale is designed to be used with clients living in the community. Please note that the term *client* is used throughout to refer to the person being rated. This may be a child of school age, an adolescent, or an adult.

Please rate this client's behavior for the last four weeks. For each item, decide whether the behavior is a problem and circle the appropriate number:

0 = not at all a problem
1 = the behavior is a problem but slight in degree
2 = the problem is moderately serious
3 = the problem is severe in degree

When judging this client's behavior, please keep the following points in mind:

(a) Take ***relative frequency*** into account or each behavior specified. That is, *consider this person with respect to others of similar age and sex, in general.* For example, if the client averages more temper outbursts than most other people you know of similar age and sex or most others in his/her class, it is probably moderately serious (2) or severe (3) even if these occur only once or twice a week. Other behaviors, such as noncompliance, would probably have to occur more frequently to merit a high rating.

(b) If you have access to this information, consider the experiences of other caregivers with this client. If the client has problems with others but not with you, try to take the whole picture into account.

(c) Try to consider whether a given behavior interferes with his/her *development, functioning or relationships.* For example, body rocking or social withdrawal may not disrupt other children or adults, but it almost certainly hinders individual development or functioning.

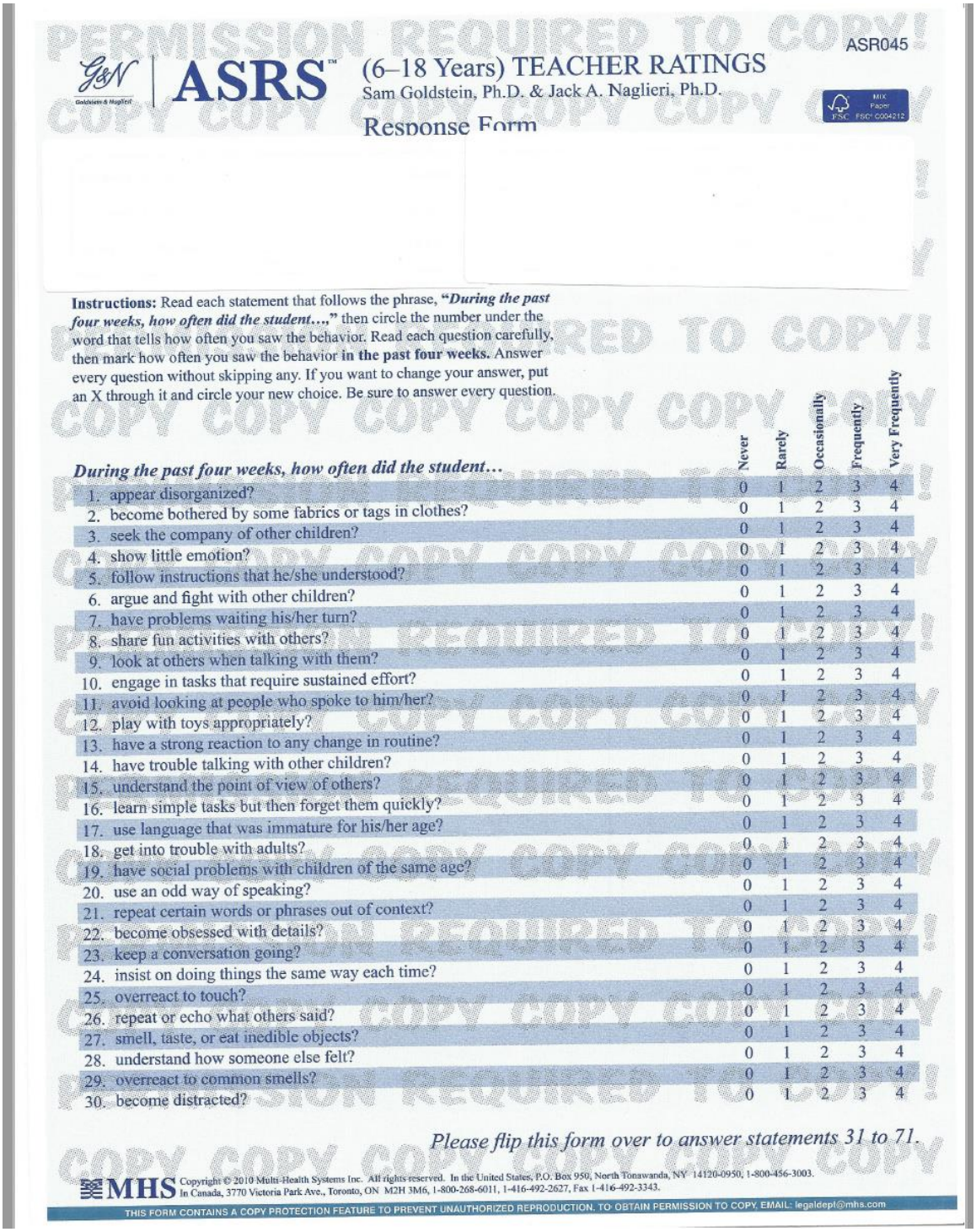
Do not spend too much time on each item - your first reaction is usually the right one.

1.	Excessively active at home, school, work, or elsewhere	0	1	2	3
2.	Injures self on purpose	0	1	2	3
3.	Listless, sluggish, inactive	0	1	2	3
4.	Aggressive to other children or adults (verbally or physically)	0	1	2	3
5.	Seeks isolation from others	0	1	2	3
6.	Meaningless, recurring body movements	0	1	2	3
7.	Boisterous (inappropriately noisy and rough)	0	1	2	3
8.	Screams inappropriately	0	1	2	3
9.	Talks excessively	0	1	2	3
10.	Temper tantrums/outbursts	0	1	2	3
11.	Stereotyped behavior; abnormal, repetitive movements	0	1	2	3
12.	Preoccupied; stares into space	0	1	2	3
13.	Impulsive (acts without thinking)	0	1	2	3
14.	Irritable and whiny	0	1	2	3
15.	Restless, unable to sit still	0	1	2	3
16.	Withdrawn; prefers solitary activities	0	1	2	3
17.	Odd, bizarre in behavior	0	1	2	3
15.	Disobedient; difficult to control	0	1	2	3
19.	Yells at inappropriate times	0	1	2	3
20.	Fixed facial expression; lacks emotional responsiveness	0	1	2	3

21.	Disturbs others	0	1	2	3
22.	Repetitive speech	0	1	2	3
23.	Does nothing but sit and watch others	0	1	2	3
24.	Uncooperative	0	1	2	3
25.	Depressed mood	0	1	2	3
26.	Resists any form of physical contact	0	1	2	3
27.	Moves or rolls head back and forth repetitively	0	1	2	3
28.	Does not pay attention to instructions	0	1	2	3
29.	Demands must be met immediately	0	1	2	3
30.	Isolates himself/herself from other children or adults	0	1	2	3
31.	Disrupts group activities	0	1	2	3
32.	Sits or stands in one position for a long time	0	1	2	3
33.	Talks to self loudly	0	1	2	3
34.	Cries over minor annoyances and hurts	0	1	2	3
35.	Repetitive hand, body, or head movements	0	1	2	3
36.	Mood changes quickly	0	1	2	3
37.	Unresponsive to structured activities (does not react)	0	1	2	3
38.	Does not stay in seat (e.g., during lesson or learning periods, meals, etc.)	0	1	2	3
39.	Will not sit still for any length of time	0	1	2	3
40.	Is difficult to reach, contact, or get through to	0	1	2	3
41.	Cries and screams inappropriately	0	1	2	3
42.	Prefers to be alone	0	1	2	3
43.	Does not try to communicate by words or gestures	0	1	2	3
44.	Easily distractible	0	1	2	3
45.	Waves or shakes the extremities repeatedly	0	1	2	3
46.	Repeats a word or phrase over and over	0	1	2	3
47.	Stamps feet or bangs objects or slams doors	0	1	2	3
48.	Constantly runs or jumps around the room	0	1	2	3
49.	Rocks body back and forth repeatedly	0	1	2	3
50.	Deliberately hurts himself/herself	0	1	2	3
51.	Pays no attention when spoken to	0	1	2	3
52.	Does physical violence to self	0	1	2	3
53.	Inactive, never moves spontaneously	0	1	2	3
54.	Tends to be excessively active	0	1	2	3
55.	Responds negatively to affection	0	1	2	3
56.	Deliberately ignores directions	0	1	2	3
57.	Has temper outbursts or tantrums when he/she does not get own way	0	1	2	3
58.	Shows few social reactions to others	0	1	2	3

APPENDIX G

AUTISM SPECTRUM RATING SCALE



ASR045

G&N Goldstein & Naglieri

ASRS™ (6–18 Years) TEACHER RATINGS

Sam Goldstein, Ph.D. & Jack A. Naglieri, Ph.D.

Response Form

Instructions: Read each statement that follows the phrase, ***"During the past four weeks, how often did the student…,"*** then circle the number under the word that tells how often you saw the behavior. Read each question carefully, then mark how often you saw the behavior **in the past four weeks.** Answer every question without skipping any. If you want to change your answer, put an X through it and circle your new choice. Be sure to answer every question.

During the past four weeks, how often did the student…	Never	Rarely	Occasionally	Frequently	Very Frequently
1. appear disorganized?	0	1	2	3	4
2. become bothered by some fabrics or tags in clothes?	0	1	2	3	4
3. seek the company of other children?	0	1	2	3	4
4. show little emotion?	0	1	2	3	4
5. follow instructions that he/she understood?	0	1	2	3	4
6. argue and fight with other children?	0	1	2	3	4
7. have problems waiting his/her turn?	0	1	2	3	4
8. share fun activities with others?	0	1	2	3	4
9. look at others when talking with them?	0	1	2	3	4
10. engage in tasks that require sustained effort?	0	1	2	3	4
11. avoid looking at people who spoke to him/her?	0	1	2	3	4
12. play with toys appropriately?	0	1	2	3	4
13. have a strong reaction to any change in routine?	0	1	2	3	4
14. have trouble talking with other children?	0	1	2	3	4
15. understand the point of view of others?	0	1	2	3	4
16. learn simple tasks but then forget them quickly?	0	1	2	3	4
17. use language that was immature for his/her age?	0	1	2	3	4
18. get into trouble with adults?	0	1	2	3	4
19. have social problems with children of the same age?	0	1	2	3	4
20. use an odd way of speaking?	0	1	2	3	4
21. repeat certain words or phrases out of context?	0	1	2	3	4
22. become obsessed with details?	0	1	2	3	4
23. keep a conversation going?	0	1	2	3	4
24. insist on doing things the same way each time?	0	1	2	3	4
25. overreact to touch?	0	1	2	3	4
26. repeat or echo what others said?	0	1	2	3	4
27. smell, taste, or eat inedible objects?	0	1	2	3	4
28. understand how someone else felt?	0	1	2	3	4
29. overreact to common smells?	0	1	2	3	4
30. become distracted?	0	1	2	3	4

Please flip this form over to answer statements 31 to 71.

ASRS™ (6–18 Years) TEACHER RATINGS

Sam Goldstein, Ph.D. & Jack A. Naglieri, Ph.D.

Response Form

During the past four weeks, how often did the student…	Never	Rarely	Occasionally	Frequently	Very Frequently
31. play with others?	0	1	2	3	4
32. notice social cues?	0	1	2	3	4
33. respond when spoken to by adults?	0	1	2	3	4
34. avoid looking at an adult when there was a problem?	0	1	2	3	4
35. have problems paying attention when doing homework or chores?	0	1	2	3	4
36. make careless mistakes in school work?	0	1	2	3	4
37. talk too much about things that adults don't care about?	0	1	2	3	4
38. resist being touched or held?	0	1	2	3	4
39. care about what other people think or feel?	0	1	2	3	4
40. focus too much on details?	0	1	2	3	4
41. not understand why others don't like him/her?	0	1	2	3	4
42. share his/her enjoyment with others?	0	1	2	3	4
43. show an interest in the ideas of others?	0	1	2	3	4
44. leave homework or chores unfinished?	0	1	2	3	4
45. understand age-appropriate humor or jokes?	0	1	2	3	4
46. flap his/her hands when excited?	0	1	2	3	4
47. listen when spoken to?	0	1	2	3	4
48. focus on one subject for too much time?	0	1	2	3	4
49. need things to happen just as expected?	0	1	2	3	4
50. talk too much about things that other children don't care about?	0	1	2	3	4
51. insist on certain routines?	0	1	2	3	4
52. have problems paying attention to fun tasks?	0	1	2	3	4
53. become fascinated with parts of objects?	0	1	2	3	4
54. line up objects in a row?	0	1	2	3	4
55. smile appropriately?	0	1	2	3	4
56. start conversations with others?	0	1	2	3	4
57. fail to complete tasks?	0	1	2	3	4
58. ask questions that were off-topic?	0	1	2	3	4
59. have trouble talking with adults?	0	1	2	3	4
60. interrupt or intrude on others?	0	1	2	3	4
61. look at others when interacting with them?	0	1	2	3	4
62. overreact to loud noises?	0	1	2	3	4
63. become upset if routines were changed?	0	1	2	3	4
64. choose to play alone?	0	1	2	3	4
65. insist on keeping certain objects with him/her at all times?	0	1	2	3	4
66. have social problems with adults?	0	1	2	3	4
67. twirl, spin, or bang objects?	0	1	2	3	4
68. reverse pronouns (e.g., you for me)?	0	1	2	3	4
69. show good peer interactions?	0	1	2	3	4
70. respond when spoken to by other children?	0	1	2	3	4
71. appear fidgety when asked to sit still?	0	1	2	3	4

APPENDIX H

PARENTAL STRESS INDEX SHORT FORM

PSI-4 SHORT FORM

Answer Sheet

Name _____ Gender _____ Date of birth ____/____/____

Ethnic group _____ Marital status _____ Today's date ____/____/____

Child's name _____ Child's gender _____ Child's date of birth ____/____/____

SA = Strongly Agree **A = Agree** **NS = Not Sure** **D = Disagree** **SD = Strongly Disagree**

1.	I often have the feeling that I cannot handle things very well.	SA	A	NS	D	SD
2.	I find myself giving up more of my life to meet my children's needs than I ever expected.	SA	A	NS	D	SD
3.	I feel trapped by my responsibilities as a parent.	SA	A	NS	D	SD
4.	Since having this child, I have been unable to do new and different things.	SA	A	NS	D	SD
5.	Since having a child, I feel that I am almost never able to do things that I like to do.	SA	A	NS	D	SD
6.	I am unhappy with the last purchase of clothing I made for myself.	SA	A	NS	D	SD
7.	There are quite a few things that bother me about my life.	SA	A	NS	D	SD
8.	Having a child has caused more problems than I expected in my relationship with my spouse/parenting partner.	SA	A	NS	D	SD
9.	I feel alone and without friends.	SA	A	NS	D	SD
10.	When I go to a party, I usually expect not to enjoy myself.	SA	A	NS	D	SD
11.	I am not as interested in people as I used to be.	SA	A	NS	D	SD
12.	I don't enjoy things as I used to.	SA	A	NS	D	SD
13.	My child rarely does things for me that make me feel good.	SA	A	NS	D	SD
14.	When I do things for my child, I get the feeling that my efforts are not appreciated very much.	SA	A	NS	D	SD
15.	My child smiles at me much less than I expected.	SA	A	NS	D	SD
16.	Sometimes I feel my child doesn't like me and doesn't want to be close to me.	SA	A	NS	D	SD
17.	My child is very emotional and gets upset easily.	SA	A	NS	D	SD
18.	My child doesn't seem to learn as quickly as most children.	SA	A	NS	D	SD
19.	My child doesn't seem to smile as much as most children.	SA	A	NS	D	SD
20.	My child is not able to do as much as I expected.	SA	A	NS	D	SD
21.	It takes a long time and it is very hard for my child to get used to new things.	SA	A	NS	D	SD
22.	I feel that I am: (Choose a response from the choices below.)	1	2	3	4	5
23.	I expected to have closer and warmer feelings for my child than I do, and this bothers me.	SA	A	NS	D	SD
24.	Sometimes my child does things that bother me just to be mean.	SA	A	NS	D	SD

1. a very good parent.
2. a better-than-average parent.
3. an average parent.
4. a person who has some trouble being a parent.
5. not very good at being a parent.

2

SA = Strongly Agree A = Agree NS = Not Sure D = Disagree SD = Strongly Disagree

25. My child seems to cry or fuss more often than most children. ..... SA A NS D SD
26. My child generally wakes up in a bad mood. ..... SA A NS D SD
27. I feel that my child is very moody and easily upset. ..... SA A NS D SD
28. Compared to the average child, my child has a great deal of difficulty in getting used to changes in schedules or changes around the house. ..... SA A NS D SD
29. My child reacts very strongly when something happens that my child doesn't like. .. SA A NS D SD
30. When playing, my child doesn't often giggle or laugh. ..... SA A NS D SD
31. My child's sleeping or eating schedule was much harder to establish than I expected. SA A NS D SD
32. I have found that getting my child to do something or stop doing something is: (Choose a response from the choices below.) 1 2 3 4 5

    1. much harder than I expected.
    2. somewhat harder than I expected.
    3. about as hard as I expected.
    4. somewhat easier than I expected.
    5. much easier than I expected.

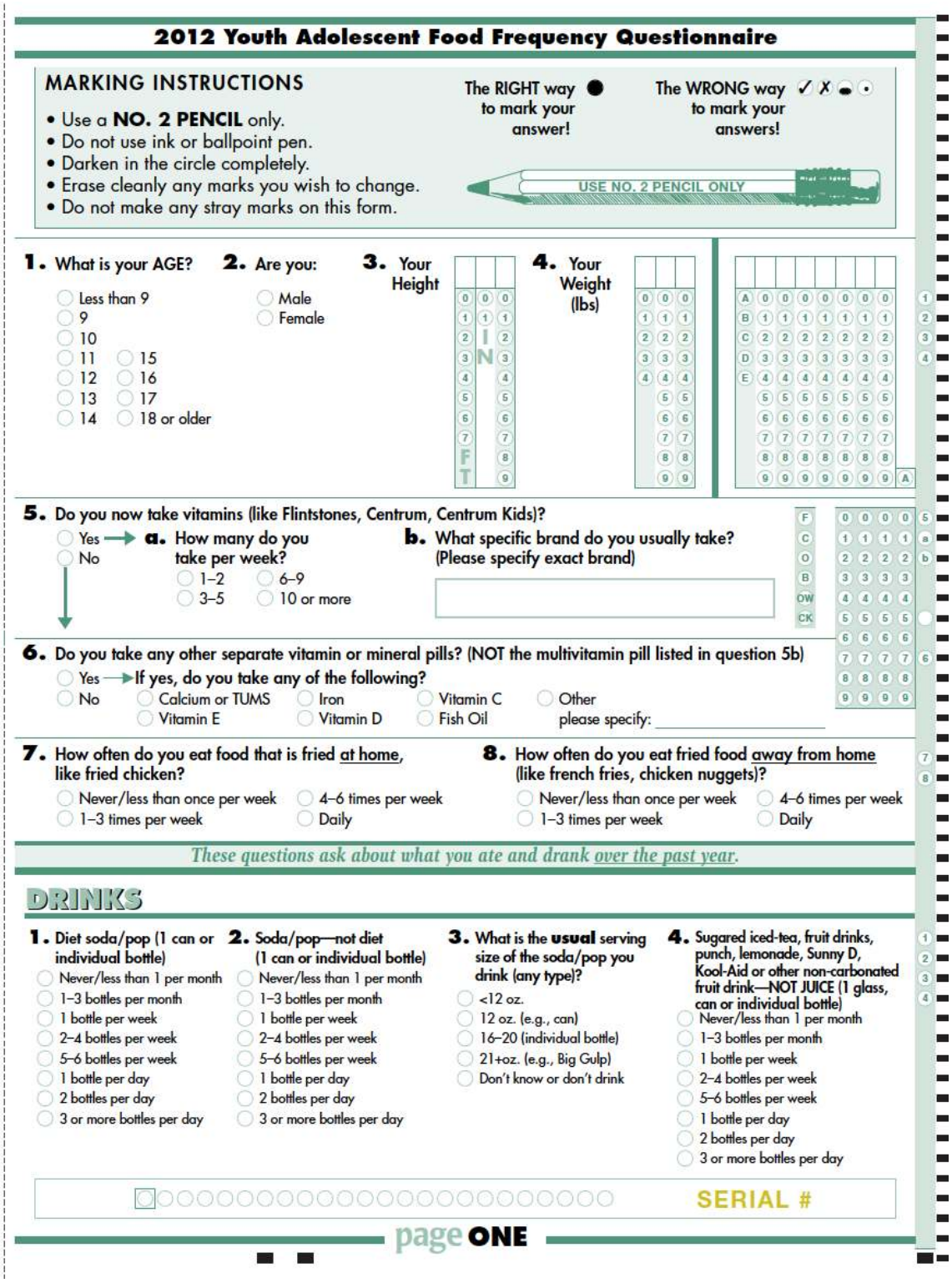
33. Think carefully and count the number of things which your child does that bothers you. For example, dawdles, refuses to listen, overactive, cries, interrupts, fights, whines, etc. (Choose a response from the choices below.) 1 2 3 4 5

    1. 1-3
    2. 4-5
    3. 6-7
    4. 8-9
    5. 10+

34. There are some things my child does that really bother me a lot. ..... SA A NS D SD
35. My child's behavior is more of a problem than I expected. ..... SA A NS D SD
36. My child makes more demands on me than most children. ..... SA A NS D SD

APPENDIX I

YOUTH AND ADOLESCENT FOOD FREQUENCY QUESTIONNAIRE



2012 Youth Adolescent Food Frequency Questionnaire

MARKING INSTRUCTIONS

- Use a **NO. 2 PENCIL** only.
- Do not use ink or ballpoint pen.
- Darken in the circle completely.
- Erase cleanly any marks you wish to change.
- Do not make any stray marks on this form.

The RIGHT way ● to mark your answer!

The WRONG way ✓ ✗ ⬬ ⊙ to mark your answers!

USE NO. 2 PENCIL ONLY

1. What is your AGE?
- Less than 9
- 9
- 10
- 11
- 12
- 13
- 14
- 15
- 16
- 17
- 18 or older

2. Are you:
- Male
- Female

3. Your Height (IN / FT)

4. Your Weight (lbs)

5. Do you now take vitamins (like Flintstones, Centrum, Centrum Kids)?
- Yes → **a.** How many do you take per week?
  - 1–2
  - 3–5
  - 6–9
  - 10 or more
- No

b. What specific brand do you usually take? (Please specify exact brand)

6. Do you take any other separate vitamin or mineral pills? (NOT the multivitamin pill listed in question 5b)
- Yes → If yes, do you take any of the following?
- No

Calcium or TUMS · Iron · Vitamin C · Other
Vitamin E · Vitamin D · Fish Oil
please specify: _____

7. How often do you eat food that is fried <u>at home</u>, like fried chicken?
- Never/less than once per week
- 1–3 times per week
- 4–6 times per week
- Daily

8. How often do you eat fried food <u>away from home</u> (like french fries, chicken nuggets)?
- Never/less than once per week
- 1–3 times per week
- 4–6 times per week
- Daily

These questions ask about what you ate and drank <u>over the past year</u>.

DRINKS

1. Diet soda/pop (1 can or individual bottle)
- Never/less than 1 per month
- 1–3 bottles per month
- 1 bottle per week
- 2–4 bottles per week
- 5–6 bottles per week
- 1 bottle per day
- 2 bottles per day
- 3 or more bottles per day

2. Soda/pop—not diet (1 can or individual bottle)
- Never/less than 1 per month
- 1–3 bottles per month
- 1 bottle per week
- 2–4 bottles per week
- 5–6 bottles per week
- 1 bottle per day
- 2 bottles per day
- 3 or more bottles per day

3. What is the **usual** serving size of the soda/pop you drink (any type)?
- <12 oz.
- 12 oz. (e.g., can)
- 16–20 (individual bottle)
- 21+oz. (e.g., Big Gulp)
- Don't know or don't drink

4. Sugared iced-tea, fruit drinks, punch, lemonade, Sunny D, Kool-Aid or other non-carbonated fruit drink—NOT JUICE (1 glass, can or individual bottle)
- Never/less than 1 per month
- 1–3 bottles per month
- 1 bottle per week
- 2–4 bottles per week
- 5–6 bottles per week
- 1 bottle per day
- 2 bottles per day
- 3 or more bottles per day

SERIAL #

page ONE

APPENDIX J

DATA COLLECTION FORM

Research Data Compliance Log

Week 1

Day	Time	Number of Squares Eaten	Comments (missed doses, other comments)
Monday Morning			
Monday Afternoon			
Tuesday Morning			
Tuesday Afternoon			
Wednesday Morning			
Wednesday Afternoon			
Thursday Morning			
Thursday Afternoon			
Friday Morning			
Friday Afternoon			
Saturday Morning			
Saturday Afternoon			
Sunday Morning			
Sunday Afternoon			

APPENDIX K

LETTER OF RECRUITMENT FOR FLYER DISTRIBUTION

Date:

Letter of Agreement to Conduct Research

To Whom It May Concern,

I, _____, _____, of
(name) (title)

_____, agree to participate in a research study by
(organization/institution)

allowing the research recruitment to take place at (Name of site) per research projects' protocol. This doctoral graduate student who is a licensed Occupational Therapist is conducting a research study through Loma Linda University, School of Allied Health Professions. The study is entitled "The Effect of High Antioxidant Cacao on Behaviors in Children with Autism Spectrum Disorder."

_____ _____
Signature Date

_____
Institution

APPENDIX L

ALLERGIC REACTION FACT SHEET

Potential allergic reactions to eating chocolate*:

"Chocolate may cause acne, allergic skin reactions, bloating, colic in infants, constipation, decreased bone density, dental caries, eczema, gas, headaches, improved insulin sensitivity, increased cholesterol levels, increased insulin levels, irregular heart rhythms, increased oxalate levels in urine, irritable bowel syndrome, irritability, jitteriness, kidney damage and disorders, migraines, nausea, neck pain, nervousness, shakiness, sleep disturbances, stomach rumbling, stomach upset, swelling under the skin, unpleasant taste, upset stomach, vomiting, weight gain."

In the event that your child/student experiences any of these side effects:

1. Stop chocolate consumption immediately.
2. Consult with the school nurse if allergic reaction happens at school.
3. Consult your child's health care provider immediately if they experience side effects at home.
4. Call 911.
5. Report incident to Dr. Lee Berk at (909) 651-5828 (ext: 15828) and Amy Sadek at 951-212-2104.

Reference

*Mayo Clinic. (2013, November 1). *Drug and Supplements: Chocolate.* Retrieved from http://www.mayoclinic.org/drugs-supplements/chocolate/safety/hrb-20058898

APPENDIX M

FLYER FOR RECRUITING PARTICIPANTS

Volunteers needed for Autism Study

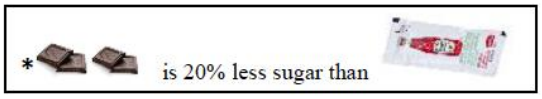
We are looking for volunteers for a graduate student research study titled "The Effect of High Antioxidant Cacao on Behaviors in Children with Autism Spectrum Disorder."

Who can participate?

- Children between 5 and 18 years of age with a Diagnosis of Autism Spectrum Disorder.
- Child must like and be willing to eat 70% cacao (also referred to as dark chocolate).
- Children with any food allergies, including chocolate, are not eligible to participate.

About the Study:

- Child will be asked to eat 4 small pieces of the 70% cacao twice daily for 4 weeks.
  - Note: 4 small pieces of the cacao equals approximately ½ a teaspoon of organic cane sugar. Four pieces of the chocolate has 20% less sugar than a packet of Heinz ketchup*. (According to the American Heart Association Children are allowed 6 teaspoons of added sugar per day).



- Parent and special education teachers will fill out questionnaires at weeks 1, 2, & 4.

Compensation for Participation:

- Participants who complete the study in full will receive a $50.00 gift card (and Special Education teachers will receive a $15.00 gift card upon completing the study in full).

For further information please contact: Doctoral Student Investigator – Amy Sadek, MOT, OTR/L, at (951) 212-2104 or email at asadek@llu.edu.

Principal Investigator: Lee Berk, DrPH, Associate Dean of Research Affairs, School of Allied Health Professions, Loma Linda University.

Note: This study has no guaranteed or even likely evidence of changing your child's behavior.
Sponsored by School of Allied Health Professions, Loma Linda University