Expressive Language and the Impact of Zinc/Copper Ratio

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ABSTRACT

The interaction between genes and the environment such as mineral and trace elements are now regarded as the most probable explanation of autism (Yasuda, Yoshida, Yasuda, & Tsutsui, 2013). Weismer, Lord, and Esler (2010) identified children associated with pervasive developmental disorder not otherwise specified (PDD-NOS) and autism have exhibited substantial language delays compared to children of their chronological age. Language delays were found to profoundly impact both receptive and expressive language. The participant in this study was a three year-old female who was previously diagnosed with Autism Spectrum Disorder (ASD). For the experiment, the child was administered articulation and language standard evaluations in order to create expressive language goals. The participant's clinician created language goals and skilled speech therapy initiated. The participant was administered a 50mg zinc supplement nine weeks into therapy to measure the effects of this trace element on expressive language. The results were inconclusive in that the participant exhibited improved language abilities with and without zinc. Continuous research is warranted to gain better understanding of the effects of zinc supplementation on expressive language in children associated with ASD.

Table of Contents

LIST OF TABLES

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Chapter I

INTRODUCTION

Autism Spectrum disorders (ASD) are a group of neural developmental disorders which may be characterized by impairments in reciprocal social interaction and communication. ASD also presents with restricted repetitive behaviors, which affect 1 in 150 children (H.Yasuda, Yoshida, Y. Yasuda, & Tsutsui, 2013). Language and communication skill deficiencies are a critical dimension of the autism phenotype and are one of the most variable characteristics in individuals with ASD. Delayed language acquisition remains a hallmark to ASD for early to middle childhood. Language deficits associated with pragmatics, such as initiation and maintaining meaningful conversation, as well as differences in use of basic aspects of syntax, such as difficulties in the use of grammatical morphemes, remain key symptoms of ASD (Kim, Junker, & Lord, 2014). These deficits are particularly apparent in low-functioning children with ASD (Tek, Mesite, Fein, Naigles, 2014).

Results from research findings have contributed to the understanding that children with ASD exhibit substantial delays in language development relative to age-level, that there is considerable individual variation in language development within the autism spectrum, and that both expressive and receptive language are significantly hindered (Tek et al., 2014; Weismer, Lord, & Esler, 2010). One possible factor in the development of ASD, as well as the language deficits associated with the disorder, may be related to dietary deficiencies. Yasuda et al., (2013) reported that many infants with ASD are suffering marginal to severe zinc deficiency, suggesting a relationship of infantile zinc deficiency and autism.

Zinc (Zn) is an essential mineral for growth, skin integrity, mental activity, wound healing, and immunocompetence and is considered a trace element (Schumacher, Domingo, & Corbella, 1994). Minerals and trace elements such as zinc play an essential role in the central nervous system. A lack or excess of these minerals are known to disrupt human body functions, including the immune system (Li, Wang, BjØrklund, Zhao, & Yin, 2014). Individuals deficient in zinc experience an increased susceptibility to various pathogens. Zinc is necessary in order to excrete toxic levels of minerals and pathogens. Due to the curial role of zinc in the immune system, this trace element has zinc dependent enzymes, which is necessary for nucleic acid metabolism (Russo, Bazin, & Bigega, 2012). Low plasma zinc may lead to copper toxicity, which could lead to liver dysfunction and neurological impairment in children (Faber, Zinn, Li, & Kingston, 2009).

Children with autism are known to have a suppressed immune system. Studies have also shown children with ASD have a decreased ability to excrete toxic metals, which leads to a higher body burden (Li et al., 2014). Copper (Cu) has many important roles in the human body. Copper is used for mechanisms of cell propagation and growth, and it also supports effective immune response through the promotion of inter-leukin-2 production using lymphocytic cells. Copper is a cofactor in many metalloenzymes including cytochrome c oxidase, cooper/zinc superoxide dismutase, and lysyl oxidase. Metalloenzymes contains a metal ion, typically held by a co-ordinate-covalent bond on the amino acid side chain, or they may bond to a prosthetic group (Li et al, 2014) Copper is considered a cofactor contributor, copper affects the human bodies response to oxidative stress, oxidative phosphorylation, and collagen biosynthesis. Increased copper concentrations may contribute to oxidative damage to lipids, nucleic acids, and proteins. Significant amounts of copper have been linked to several neurological diseases including

amyotrophic lateral sclerosis, Alzheimer's disease, and Creuztzfeldt-Jacob disease (Faber et al., 2009).

Among the ASD community, the incidence rate of zinc deficiency is significantly increased compared to age-matched healthy control subjects. The occurrence of zinc deficiencies in ASD is particularly pronounced in younger children. Low levels of zinc found in the presence of copper excess contribute to an elevated cooper/zinc ratio, which was reported to correlate with the severity of symptoms associated with autism (Vela et al., 2015).

Children with ASD frequently have accompanying gastrointestinal (GI) and immunological symptoms, as well nonspecific neurological signs (Russo et al., 2012). Zinc is curial for the maintenance of the gastric mucosal integrity. Gastric mucosal integrity is essential for the blood flow and microcirculation of the gastric system (Vela, Stark, & Socha, 2015). Vela et al. (2015) performed a study on mice that experienced zinc deficiency accompanied with mucosal necrosis and ulceration as well as increased mucosal apoptosis, inflammation, edema, and structural alterations of villi. Villi are specialized for absorption in the small intestine as they consist of a thin wall, one cell thick, which enables a shorter path for diffusion. Villi have a large surface area in order to have efficient absorption of fatty acids and glycerol into the blood stream (Vela et al., 2015). Thus, when given a zinc supplementation, the mice showed beneficial effects on the mucosal integrity in many pathophysiological and inflammatory conditions of the small intestines (Vela et al., 2015).

These findings, albeit specific to mice, indicate that zinc supplementation improves GI function. Children with autism often report higher instances of GI problems, specifically the intestinal malabsorption of nutrients. In correlation to Vela et al. (2015), environmental triggers such as zinc deficiency have been linked to children with ASD. These children have been

characterized by behavioral neurodevelopmental disorders categorized by social deficits, language impairments, and repetitive behavior, which frequency occur with GI symptoms (Wasilewska & Klukowski, 2015).

Given the positive findings from these studies, the specific aim of this study was to determine if zinc supplementation could be used as a type of intervention to assist with expressive language output in children associated with ASD.

Chapter II

REVIEW OF THE LITERATURE

Pervasive Developmental Disorders

Pervasive developmental disorders are a group of neurological disorders that include autism spectrum disorder (ASD) and Asperger's syndrome (Faber et al., 2009). These disorders impair cognitive performance, reciprocal social interaction, and communication, and have unknown pathologies, but have been linked to genetic and environmental factors. Scientific breakthroughs have highlighted epigenetic alteration of gene expression by environmental influences as one possible key event in the pathogenesis of genetic diseases (Yasuda et al., 2011).

Weismer et al. (2010) observed characteristics in early language skills in a large population of toddlers with ASD. Their findings suggest that toddlers on the autism spectrum demonstrated significant delays based on age-level expectations in both comprehension and production. They found that children with developmental delay (DD), pervasive developmental disorder not otherwise specified (PDD-NOS), and autism exhibited substantial language delay compared to children of matching chronological age. Children with DD scored significantly higher on receptive and expressive language tasks related to children with ASD and PDD-NOS. Thus, children with PDD-NOS have been characterized as exhibited a significant language delay relative to typically developing children. Tek et al. (2013) conducted a longitudinal study of the trajectory as well as the variability of expressive language in young children with ASD. The authors analyzed morpho-syntactic measures, vocabulary, and sentence complexity using

samples of spontaneous speech. The results of the study concluded that high-functioning children with ASD are similar to typically developing children in language development. However, low-functioning children with ASD showed persistent language deficits over time, which the authors attributed to a global impairment in expressive language. Thus, while language deficits are typically associated with ASD, not all children with ASD will present with a language delay.

Causes/Contributing Factors to Autism

Several factors may contribute to the development of autism. A number of research studies point to genetic predisposition, environmental triggers, and dietary deficiencies, e.g., inadequate intake of mineral and/or trace elements, as playing a role in ASD. Genetic predisposition to autism is evident from family and twin studies. Family studies have shown that autism is more frequent in families with autistic proband than the general population prevalence. Genome-wide linkage mapping studies cytogenetic abnormalities in autistic individuals, and candidate gene studies support the observation that autism is a multiple gene disorder with several interacting genes of moderate effect on different chromosomes (Bespalova & Buxbaum, 2003). Bespalova and Buxbaum (2003) studied several candidates' genes and autism phenotypes. The results showed that an association with autism was reported for more than 15 genes residing at different loci of human chromosomes. The authors reported that autism is likely caused by multiple interacting genes (Bespalova & Buxbaum, 2003).

Environmental triggers have also been implicated in the development of ASD. Environmental toxins are associated with increases of oxidative stress, and can be further agitated with the combination of a suspected genetic predisposition such as ASD (Li et al., 2014). Minerals and trace elements contributing to genetic and environmental triggers play an important role in the central nervous system. The lack of, or conversely, the excess of these

minerals and trace elements, is known to cause a variety of health implications such as developmental delay, malabsorption secondary to decreased functions of intestinal dependent enzymes, and may be a contributing factor in the etiology of ASD (Li et al., 2014).

Role of Zinc

Zinc is one of the most prevalent ions in the brain and participates in the regulation of neurogenesis, neuronal migration, and differentiation, thereby shaping cognitive development and maintaining healthy brain functions (Vela et al., 2015). Zinc has a unique and extensive role in the genetic processes. Since the discovery of zinc this element has been found to be essential for nutrients of living organisms and many diverse biochemical roles for it have been identified. These roles include enzyme function, nucleic acid metabolism, cell signaling, and apoptosis (Russo, 2011).

The discovery of zinc has led scientists to believe that the element plays a crucial role in biological functions. Zinc has been identified in markers of deoxyribonucleic acid (DNA) damage, regulation of gamma-aminobutyric acid (GABA) and glutamate, and the absorption of intestinal zinc-dependent enzymes. Zinc is essential for several physiological processes, including growth and development, lipid metabolism, and brain and immune functions (Russo, 2011). Zinc also plays important roles in nucleic acid/protein synthesis, cell replication, and tissue growth and repair (Yasuda et al., 2013). The lack of zinc, commonly known as "zinc deficiency," has been associated with various pathological conditions, including impaired immunity, delayed wound healing, fetal growth delay, neural development disorders, and degenerative diseases (Yasuda et al., 2013). In regards to ASD, Yasuda et al. (2013) discovered that children with autism exhibited lower levels of zinc concentration, as measured from their

hair, when compared to normally developing children. Although only correlative, these findings suggest a relationship between zinc concentration and ASD.

Individuals who suffer from zinc deficiency often demonstrate severely suppressed immune functions and emotional disturbances. Zinc provides an essential building block for metal responsive transcription factor-1 (MTF-1). MTF-1 enhances the transcription of metallothionein (MT) genes in response to heavy metal load as well as up and down regulations in high quantity amounts of genes and enzymes related to the elimination of heavy metals. MT is a cysteine-rich, low-molecular weight, intracellular protein with high attraction to metals and is the most common intracellular protein that binds to metal. The MT system is critical in heavy metal detoxification throughout the body (Faber et al., 2009). Zinc plays a role in the production of MT, thus decreases neurotoxicity.

Copper is involved with the synthesis process of MT proteins. MT proteins have a higher attraction for copper and at times create a higher copper concentration. Copper is a catalyst for increasing physiological stress, a co-factor in neurological disease, and causes oxidative damage to lipids, nucleic acids, and proteins. Copper homeostasis needs to be controlled very closely, as copper becomes toxic in higher than normal concentrations. Uncontrolled copper absorption can become toxic to the central nervous system, especially during infancy due to incomplete liver functions. Changes in heavy metal concentrations such as zinc and copper have also been noted in neurological disorders, including Parkinson's disease, Alzheimer's disease, and Huntington's disease (Faber et al., 2009).

In recent years, efforts have been made to establish normal limits and ratios pertaining to metals in various human media. Zinc and copper have antagonist results, with admiration to each other. A nearly 1:1 ratio of zinc and copper has been associated with more effective

immune responsiveness to infectious agents. The zinc to copper ratio has been associated as a method of regulating the functional state of the MT system, which, as previously noted, is crucial in heavy metal detoxification. Low zinc/copper ratio has been linked to zinc deficiency and problems eliminating toxic heavy metals from tissues and blood through the MT system (Faber et al., 2009). In contrast, a higher copper to zinc ratio could be used as a biomarker for the diagnosis of autism (Russo et al., 2012). The study reports low zinc and high copper may control GABA receptors, ultimately changing transmitter awareness. Significant levels of copper are associated with high norepinephrine and high epinephrine levels within the body, which is further linked to perceive hyperactivity, typically correlated with symptoms of ASD (Russo et al., 2012).

There is much support for the role of GABA in the etiology of ASD. GABA is considered a primary main excitatory and inhibitory neurotransmitter (Naaijen, Bralten, & Faraone, 2015). Alterations in the levels of GABA and its receptors indicate that the GABAergic system, which is responsible for synaptic inhibition, is involved in autism (Russo et al., 2012). Zinc has been associated in GABA and glutamate regulation, specifically through the anxiolytic activity reducing GABAergic inhibition. Copper contrastively has been found to be a potent inhibitor of GABA-evoked responses. Copper toxicity might result from GABA receptor blockage. Evidence strongly suggests that copper and zinc interact with each other in GABA receptor complexes and participate in altering synaptic transmission. Due to the copper's high attraction to GABA-evoked response, zinc deficiency has been found to be associated with GABAergic impairment (Russo et al., 2012).

Early occurrence of zinc deficiency with a decline later in life, and the manifestation of some core features of ASD, such as impaired social behavior and language and communication

problems in prenatal zinc deficient mice, have recently put maternal zinc status in focus as a possible environmental factor in the etiology of ASD (Vela et al., 2015). Zinc deficiency may play a part during pregnancy and could negatively affect fetal development; this phenomenon has been observed in animal models and might be present in humans. Pregnant women that experience low zinc levels have shown correlations that affect the embryo's intestinal epithelial development symptoms, which coincides with that of children associated with ASD (Vela et al., 2015). Proper zinc levels are necessary for healthy gut development and both pre- and perinatal zinc deficiency could affect the neonate and cause downstream events that contribute to pathological processes. These processes may include but are not limited to inflammation, due to increased intestinal epithelium permeability and immune system abnormalities, including the generation of autoantibodies (Vela et al., 2015).

Children with autism have exhibited higher instances of GI problems, specifically the intestinal malabsorption of nutrients. This syndrome may be referred to as "leaky gut syndrome" (Yasuda et al., 2013). "Leaky gut syndrome" results in chronic inflammation, which influences the GI tract, due to empty spaces between cells that line the small intestine; this alteration could result in incompletely digested foods and other toxins entering the bloodstream. As a result, the immune system may respond by releasing antibodies against these substances. Inflammation of the GI can have a direct effect on the brain, causing behavioral and cognitive as well as psychiatric impairments. Appropriated together, GI abnormalities, immune system dysfunction, stress, and zinc deficiency are highly linked processes. The final result of these combined processes may be altered signaling to and within the developing brain, contributing to ASD (Vela et. al, 2015).

Zinc Supplementation Related to Treatment

During prenatal stages, females with insufficient zinc levels may take a zinc amino acid complex resulting in an increased zinc measurement during pregnancy, preserving the embryotic gut and brain development. Preserving the embryotic gut will decrease the chances of ASD-GI tract associated symptoms, decrease immune system activation, and decrease stress levels. Zinc supplementation has been suggested for infantile autistic participants with mineral disorders, such as zinc deficiency (Vela et al., 2015).

Zinc supplementation has been shown to improve function in individuals with disorders other than autism. For example, Russo (2011) conducted a study in which individuals with diagnosed with anxiety were tested for zinc, copper, and anti-oxidant levels. Based on deficiencies, participants were prescribed an appropriate dose of antioxidants and supplements for a period of eight weeks. The results from the study found higher levels of copper and lower levels of zinc in participants with anxiety compared to control participants. After the period of supplementation, the Cu/Zn ratio improved as a result of an increase in zinc concentration levels. Ratings of anxiety also decreased in conjunction with the change in copper and zinc levels. Russo (2011) suggested that zinc supplementation may play a role in improving symptoms associated with anxiety

Language Deficits

While language is affected in many developmental conditions, profiles usually retain the normative balance of a receptive ability advantage over expressive skills. For many children with ASD, however, contrasting profiles are often seen, whereby raw receptive skills fail to show clear advantage over expressive skills (Hudry, Chandler, & Bedford, 2013). Thus, expressive language skills are often the focus of assessment and intervention. Language skills are often

examined in a play-based format. For example, Kim, Junker, and Lord (2014), conducted an investigation in which they studied the fidelity of measuring observations of spontaneous and expressive language (OSEL). OSEL was adapted as a 30-45 minute observational assessment, which focused on children's expressive language use in standardized, but natural context. The assessment tool uses seven tasks to evaluate the child's abilities. The *Play Figure Assembly* is an opportunity to interact within relatively easy and usually familiar play context, namely to construct different play figures through adding body parts and accessories to plastic figures. *Clarifications*, focused on eliciting requests for clarifications from a child based on the examiner's comments and questions. During *Telling a Picture Story* the child was presented with four photographs which depicted a story or plot and then asked the child to describe the story to the examiner. *Camping Trip/Picnic* elicited spontaneous language productions within a loosely structured, motivating pretend-play activity. The next task consisted of the*"Where is it?" Game;* this task intended to elicit the use of spatial prepositions. The *Retell a Story: Where Are My French Fries?* task gave the examiner a chance to observe the child's semantic and narrative skills. Finally, the *Picture Description* gave the child the opportunity to describe objects, people, and events at multiple levels. The child is expected to use different word classes such as nouns, verbs, adjectives, and verb tenses such as future, present, pass, and forms while describing the vignette. The results of the study found that the OSEL was found to be a successful and measurable tool for evaluating children's spontaneous expressive language.

Results from an assessment tool such as the OSEL are useful in developing therapy goals, as the goals may be based on spontaneous productions. Lane, Lieberman-Betz, and Gast (2015) conducted an investigation to identify effective naturalistic language interventions of promoting spontaneous expressive language. The authors found that children with ASD benefit from

procedures in which adults embed opportunities to communicate during typical activities based on the child's needs. Four behavioral interventions to promote expressive language may be used: (a) verbal model of the target behavior, (b) mand-model procedure where an adult controls opportunities to use language, (c) time delay presentation of a stimulus and a specific delay for an independent response, and (d) incidental teaching. These strategies are used to target pivotal behaviors such as motivation responding to multiple cues, self-management, and initiating interactions.

In order to maximize the impact of therapy on expressive language skills, it would be beneficial to ensure that an individual's abilities to process and produce language are functioning to his/her maximum ability. As previously noted, zinc plays a vital role in mental function (Schumacher, Domingo, & Corbella, 1994). However, individuals with autism may exhibit marginal to severe deficiency, as well as exhibiting higher levels of copper (Yasuda et al., 2013; Vela et al., 2015). This imbalance may contribute to autism and its associated symptoms (Vela et al., 2015). Prior research has documented improvement in other disorders, such as anxiety, following a period of zinc supplementation (Russo, 2011). It is possible that zinc supplementation may also improve function, specifically expressive language abilities, in individuals with autism. Hence, the purpose of this study was to investigate the use of zinc supplementation to improve homeostasis in the bodies of children associated with ASD whose zinc to copper ratio would affect expressive language output. The results from this study will be used to answer the follow question:

Will zinc supplementation improve expressive language skills in a child with ASD?

Based on this question, the following directional hypothesis was developed:

Zinc supplementation will improve expressive language skills in a child with ASD.

Chapter III

METHODS

Participants

This study was approved by the Valdosta State University Institutional Review Board (see Appendix A). One participant was recruited from the Valdosta State Speech and Hearing Clinic. The participant had been previously diagnosed with ASD and was three years of age. The participant's caregiver gave verbal consent to participate. The participant's current level of communication included single word utterances and gesturing.

Procedures

Initial speech and language evaluation. After the caretaker gave consent, the participant's language skills were assessed by formal and informal means. Formal assessment included administration of the *Preschool Language Scale -3* (PLS-3) (Zimmerman, Steiner, & Pond, 1992), which is an assessment tool used to assess receptive and expressive language skills in infants and young children. The PLS-3 also examines behaviors considered to be language precursors. *The Goldman-Fristoe Test of Articulation- 2* (GFTA-II) (Goldman & Fristoe, 2000) was also administered during the child's initial evaluation to assess the child's articulation ability for both spontaneous and imitative sound production by responding to picture plates and verbal cues from the examiner. The primary purpose of the GFTA-II is to provide a means of assessing an individual's articulation of consonant sounds.

Informal assessment of expressive language abilities was completed through direct and indirect tasks. The direct activity was a picture naming task during which the participant was asked to identify 10 images of verbs and 10 images depicting different emotions; the images were administered in random order. Correct and incorrect responses were recorded on a data sheet (see Appendix B). For the indirect task, the clinician tracked the number of word utterances produced during the picture naming task, structured therapy tasks, and pretend play, similar to some of the components described in the OSEL by Kim et al. (2014), during initial baseline sessions

Articulation and expressive language goals were created based on the participant's baseline testing. The articulation goal for the phoneme /p/ in isolation was created by means of sounds the child should have mastered by the age of three years. Language goals were developed based on the child's performance on the PLS-3 and informal observations made during pretend play; the goals were created to increase spontaneous speech and sequence events, as well as increase the participant's use of verbs and emotions for functional communication (Lane et al., 2015).

After the clinician collected baseline data for two therapy sessions, the participant's semester of therapy was separated into two phases. The first nine weeks of therapy sessions (Series A) was completed without zinc supplementation. The last seven weeks of therapy sessions (Series B) was completed with zinc supplementation. During the seven-week period of zinc supplementation, a week of data was not accounted for due to spring break.

Measurement of zinc and copper levels. Prior to initiation of the Series B therapy sessions, urine specimens were collected from the participant from the first urination of the day to measure zinc and copper levels. The child provided two 10mL of urine into a sample cup; the

first was tested by means of an Osumex heavy metal (Zinc) test. The urine specimen was measured in parts per million (ppm) or milligrams per liter (mg/L); for the current study, the unit of measure was ppm. Per the directions for determining zinc levels by Osumex, reagent 1 was added to the urine sample and carefully blended. Following this procedure, reagents 2 and reagent 3 were stirred into the solution. Once all three reagents were added to the urine specimen, the sample was allowed to settle for five minutes. After exactly five minutes, a fourth reagent was added to the urine and mixed into the composite. Approximately one minute after the last reagent was added, a color strip provided by the manufacturer was compared to the result in the vial in order to evaluate zinc levels.

The second 10 mL urine sample was analyzed using an Osumex heavy metal (Copper) test, measuring copper in ppmL. Per the directions for determining cooper levels, reagent 1 was added to the urine and mixed until completely dissolved. Next, a second reagent was stirred into the solution. Once the reagents were both completely dissolved, the mixture sat for five minutes. Finally, the color of the solution was again compared to the color of a strip provided within the test box.

At the completion of the Series B therapy sessions, zinc and copper levels were measured again, using the same procedure previously described. The participant was able to provide only a 5 mL urine sample rather than a 10 mL sample.

Administration of zinc supplement. Once the zinc/copper ratios had been determined, the participant was administered a 50mg zinc supplement (Nature Made) crushed into applesauce for six weeks. The supplement was taken once daily with the first meal of the day. Over-the-counter zinc supplements are commonly provided in 50mg doses. The caretaker was encouraged to monitor the participant for side effects associated with excessive zinc levels, e.g., nausea,

vomiting, respiratory difficulties, lethargy (Plum, Rink, & Haase, 2010). The caregiver checked with the primary physician before starting the participant on zinc.

Collection of data during therapy. The participant's clinician tracked expressive language goals on the provided data sheet and recorded a check if the child produced a correct response, and conversely, a cross if the child was incapable of generating the expected response. The five expressive tasks were as follows:

(a) Goal 1- imitatively produce the phoneme /p/ in isolation by following visual cues presented by the clinician with 80% accuracy or above for three consecutive sessions

(b) Goal 2 - correctly using progressive verbs presented on picture cards in sentences when prompted by the clinician with 100% accuracy for three consecutive sessions

(c) Goal 3 - correctly use emotion words presented on picture cards in sentences when prompted by the clinician with 100% accuracy for three consecutive sessions

(d) Goal 4 - correctly sequence events with 80% accuracy or above for three consecutive sessions using a simple, three-step sequence provided by the clinician on picture cards

(e) Goal 5 - spontaneously producing three to four-word sentences, (e.g., describing objects and answering questions) responding to the clinician during play-based activities and structured therapy tasks with 100% accuracy for three consecutive sessions.

During play-based activities, the clinician elicited spontaneous speech production, production of the phoneme /p/ in isolation by following visual cues, and sequencing events, e.g., getting a doll dressed, making a sandwich with pretend kitchen food, and putting rings in size formation around a stick. In structured therapy tasks, the clinician used picture-naming cards clinician to elicit correct progressive verb usage and proper emotion usage.

Analysis

Data were collected and analyzed separately from the therapy sessions for Series A and Series B. The average for each goal over the nine weeks of therapy in Series A and the six weeks of Series B was determined and compared to each other and to the baseline data. Results from Series B were also subtracted from those obtained during Series A to determine the change in function in conjunction with natural progression.

Chapter IV

RESULTS

The purpose of this research was to determine whether zinc supplementation would increase expressive language output in child with ASD. Two periods of therapy were conducted; the first for nine weeks and the second for six weeks. The first period, deemed Series A, was completed without zinc supplementation. The second period, Series B, was completed while the participant took a daily over-the-counter 50mg zinc tablet.

Summary of Expressive Language Function without Zinc Supplementation

To control for natural expected progression of the participant over the course of therapy, an exercise was undertaken to plot the expected results against actual outcomes using historical data gathered from nine prior therapy seasons once baseline goals had been established for the semester. Tables 1 through 5 shows the change in function from baseline and in therapy without zinc supplementation (Series A) and therapy with zinc supplementation (Series B) for each goal.

As noted in Table 1, the participant had a total increase of 250% improvement on Goal 1 (imitative production of /p/ in isolation) from baseline through Series A and 31.41% improvement for Series B. The improvement was noted during both therapy periods, with the greatest amount of change occurring during the Series A sessions. As noted in Table 2, for Goal 2 (correct use of progressive verbs shown on picture cards), the participant demonstrated no change in both Series A and Series B sessions. Similar results were found for Goal 3, noted in Table 3 below (correct use emotion words presented on picture cards); the participant had 0%

increase in Series A. She did, however, exhibit a 14% decrease in Series B. The results for Goal 4 (correctly sequence events), shown in Table 4 showed a total increase of 300% from baseline during Series A but a 20% decrease in Series B. Similar to Goal 1, the greatest change occurred during Series A sessions. Lastly, for Goal 5 (production of 3-4 word sentences by responding to clinician prompting) and noted in Table 5, the participant exhibited a total increase of 90.91% from baseline during Series A and a 36% increase in Series B.

Heavy Metal Test Results

Before daily supplementation began, the participant's zinc levels appeared to be at 0 ppm using the Osumex heavy metals zinc test. The participants' copper levels using the Osumex heavy metals copper test showed a level of 10 ppm. These results indicated the participant zinc to copper ratio 0ppm:10ppm, significantly lower levels of zinc in their body when compared to copper. Recommended normal limits a person should have a zinc to copper ratio equivalent of 1:1.

After the six weeks of zinc supplementation, the participant's zinc levels stood at 0.5 ppm, while copper levels were at 0.0 ppm; these results indicated that the participants copper levels were significantly lower than zinc. Recommended normal limits indicate that an individual should have a zinc to copper ratio equivalent of 1:1.

Session	Clinical Data	% Change
Baseline	0%	
no zinc 1	0%	
no zinc 2	10%	
no zinc 3	16%	60.00%
no zinc 4	33%	106.25%
no zinc 5	20%	-39.39%
no zinc 6	35%	75.00%
no zinc 7	35%	0%
no zinc 8	20%	-42.86%
no zinc 9	30%	50.00%
Maximum change from measurable data point (session 7 vs session 2)		**250%**
with zinc 1	46%	53.33%
with zinc 2	20%	-56.52%
with zinc 3	30%	50.00%
with zinc 4	30%	0%
with zinc 5	46%	53.33%
with zinc 6	37%	-19.57%
Maximum change with zinc supplementation (no zinc 7 vs with zinc 1)		**31.43%**

Table 1. Clinical data and percent change during the nine sessions of therapy without zinc supplementation and six sessions of therapy with zinc supplementation for Goal 1 (imitatively produce the phoneme /p/ in isolation when provided with visual cues).

Session	Clinical Data	% Change
Baseline	100%	
no zinc 1	100%	0%
no zinc 2	100%	0%
no zinc 3	90%	-10.00%
no zinc 4	100%	11.11%
no zinc 5	100%	0%
no zinc 6	100%	0%
no zinc 7	100%	0%
no zinc 8	100%	0%
no zinc 9	100%	0%
Maximum change from baseline (session 9)		**0%**
with zinc 1	90%	-10.00%
with zinc 2	95%	5.55%
with zinc 3	90%	-5.26%
with zinc 4	95%	5.55%
with zinc 5	100%	5.26%
with zinc 6	100%	0%
Maximum change with zinc supplementation (no zinc 9 vs with zinc 6)		**0%**

Table 2. Clinical data and percent change during the nine sessions of therapy without zinc supplementation and six sessions of therapy with zinc supplementation for Goal 2 (correctly using progressive verbs presented on picture cards).

Session	Clinical Data	% Change
Baseline	100%	
no zinc 1	100%	0%
no zinc 2	100%	0%
no zinc 3	100%	0%
no zinc 4	100%	0%
no zinc 5	100%	0%
no zinc 6	100%	0%
no zinc 7	100%	0%
no zinc 8	100%	0%
no zinc 9	100%	0%
Maximum change from baseline (session 9)		**0%**
with zinc 1	100%	0%
with zinc 2	100%	0%
with zinc 3	80%	-20.00%
with zinc 4	100%	25.00%
with zinc 5	93%	-7.00%
with zinc 6	86%	-7.53%
Maximum change with zinc supplementation (no zinc 9 vs with zinc 6)		**-14.00%**

Table 3. Clinical data and percent change during the nine sessions of therapy without zinc supplementation and six sessions of therapy with zinc supplementation for Goal 3 (correctly using emotion words presented on picture cards).

Session	Clinical Data	% Change
Baseline	25%	
no zinc 1	not addressed	n/a
no zinc 2	not addressed	n/a
no zinc 3	60%	140.00%
no zinc 4	100%	66.67%
no zinc 5	50%	-50.00%
no zinc 6	100%	100%
no zinc 7	20%	-80.00%
no zinc 8	80%	300.00%
no zinc 9	100%	25.00%
Maximum change from baseline (session 9)		**300.00%**
with zinc 1	100%	20.00%
with zinc 2	100%	0%
with zinc 3	80%	-20.00%
with zinc 4	100%	25.00%
with zinc 5	100%	0%
with zinc 6	80%	-20.00%
Maximum change with zinc supplementation (no zinc 9 vs with zinc 6)		**-20.00%**

Table 4. Clinical data and percent change during the nine sessions of therapy without zinc supplementation and six sessions of therapy with zinc supplementation for Goal 4 (correctly sequence events using a simple, three-step sequence provided on picture cards).

Session	Clinical Data	% Change
Baseline	11%	
no zinc 1	10%	-9.09%
no zinc 2	15%	50.00%
no zinc 3	13%	-13.33%
no zinc 4	20%	53.85%
no zinc 5	17%	-15.00%
no zinc 6	20%	-17.65%
no zinc 7	21%	5.00%
no zinc 8	18%	-14.29%
no zinc 9	25%	38.89%
Maximum change from baseline (session 7)		**90.91%**
with zinc 1	21%	16.00%
with zinc 2	19%	-9.52%
with zinc 3	24%	26.32%
with zinc 4	30%	25.00%
with zinc 5	34%	13.33%
with zinc 6	24%	-29.41%
Maximum change with zinc supplementation (no zinc 7 vs with zinc 5)		**36%**

Table 5. Clinical data and percent change during the nine sessions of therapy without zinc supplementation and six sessions of therapy with zinc supplementation for Goal 5 (spontaneously produce three- to four-word utterances during play-based and structured therapy tasks).

Chapter V

DISCUSSION

The aim of the investigation was to examine the effect of zinc supplementation on expressive language function in a child with ASD. Specifically, it was hypothesized that zinc supplementation would increase expressive language abilities. The results of the study partially support the hypothesis of this study. The participant did demonstrate an improvement in two of the five goals (imitative production of /p/ in isolation and spontaneous production of 3-4 word utterances) during the period of zinc supplementation; however, a larger increase of growth was shown in Series A treatment without zinc supplementation. It should be noted, however, that the participant also exhibited a slight decrease in two other goals (using emotion words correctly and sequencing events) during the period of zinc supplementation.

For both Goal 1 (imitative production of /p/ in isolation) and Goal 5 (spontaneous production of 3-4 word sentences), a considerable increase in performance was observed during Series A, with continued improvement, albeit on a much smaller scale, during Series B. Given that the increase observed during Series B falls within the range of change observed during Series A for both goals, these findings do not suggest that zinc supplementation had a marked effect on the participant's skills in these areas. In addition, the substantial gains made by the participant during Series A indicate that she experienced significant growth in these tasks during the initial phase of therapy. As the semester progressed, subsequent changes may have been minimal as she acquired these skills and/or reached a plateau in her learning abilities at this time.

Similarly, for Goal 4 (sequencing events), the participant demonstrated significant improvement in function during Series A, suggesting significant learning and growth during the initial phase of therapy. She did exhibit a slight decline in function during Series B; however, this decrease was smaller than that observed during Series A, suggesting stabilization of this skill as she progressed.

For Goal 2 (progressive verb use) and Goal 3 (use of emotional words), no change in overall function occurred during Series A. She maintained his performance level for Goal 2 but did demonstrate a slight decrease in proficiency for Goal 3. It is noteworthy, however, that the decline in performance during Series B for Goal 3 was still at a level high enough to meet the criteria established by her clinician for successful achievement of this skill. There are several possible reasons as to why she exhibited a decrease in performance during Series B for these goals. One possibility is that the zinc supplementation had a negative impact on her abilities in these areas, given that the declines were observed after zinc supplementation was initiated. It is unclear why certain skills would be enhanced with zinc supplementation, e.g., phoneme imitation and spontaneous speech production, while others would be disrupted. Alternatively, the decrease in function for these two skills may be spurious in nature and/or related to extraneous factors, e.g., fatigue, participant engagement in therapy, etc. Her scores still succeeded the threshold of mastery for those skills. Further investigation is warranted to determine if zinc supplementation affects language skills differentially.

In sum, the effects of zinc supplementation on expressive language did not show conclusive evidence of an improvement on the effects of articulation, sequencing, progressive verbs, and emotion identification, and speech production Due to zinc supplementation being introduced halfway through the semester, it is inconclusive if the results would have been

different if zinc had been used at the beginning of the semester then towards the end of the study. The participant's ability to improve upon a task may show a higher percentage of learning at the start of a new concept then towards the middle of learning. As the participant gains mastery, large improvements may not be readily seen. Whether the small declines in performance noted for two of the goals reflect natural variability in function that may occur over time or are a result of zinc supplementation are not clear.

The baseline testing indicated that the participant's zinc/copper ratio was out of normal limits by having a 0ppm zinc level and having a 0.05ppm sensitivity to copper. Testing completed at the end of the semester, after six weeks of zinc supplementation, showed that the participant's zinc levels were raised to .5ppm, and copper levels were decreased to 0ppm. Thus, the participant's zinc levels showed a minor increase and copper levels decreased.

Limitations and Recommendations

An investigation that incorporates toddlers associated with ASD and consent to take a daily supplement is a complicated topic. This research only included one participant; increasing the size of the sample would add to the current information and increase the generalizability of these results. Another limitation that was not accounted for was the child's ability to provide a urine sample because he was not potty-trained. Although the participant's caregiver gave consent for the study to proceed, there was a delay prior to starting the zinc supplementation due to the toilet training. As a result, the participant was only able to take the zinc supplementation for six weeks. This contrasts with prior studies using zinc supplementation, which have utilized an eight week protocol. Subsequent studies should ensure that participants would be able to complete all testing requirements. Lastly, an extraneous variable was administration of the supplementation.

Since the investigator could not perform the regimen in person, one must assume that the child received the supplementation at the appropriate time and on a daily basis.

Conclusions

Overall, the results do not clearly indicate that zinc supplementation in children with ASD could increase expressive language for length of utterances and expressing events. However, as noted above, unanticipated factors limited the duration of zinc supplementation and thus the potential impact on expressive language skills. These results did show a change in the zinc/copper ratio, which has been associated with severity of autism (Vela et al., 2015). This research highlights the need to investigate the levels of heavy metals present within children with ASD with subsequent dietary changes as needed, and the possible effects on language abilities.

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Appendix A:

Institutional Review Board Approval

Institutional Review Board (IRB)

for the Protection of Human Research Participants

PROTOCOL NUMBER: IRB-03521-2017

RESPONSIBLE RESEARCHER: Ms. Gabrielle Strauss

SUPERVISING FACULTY: Dr. Mary Gorham-Rowan

PROJECT TITLE: *Impact of Zinc Supplementation on Language Function in Children.*

LEVEL OF RISK: ☒ Minimal ☐ More than Minimal

TYPE OF REVIEW: ☒ Expedited Under Categories **3 & 7** ☐ Convened (Full Board)

CONSENT REQUIREMENTS:

☐ Adult Participants – Written informed consent with documentation (signature)

☐ Adult Participants – Written informed consent with waiver of documentation (signature)

☐ Adult Participants – Verbal informed consent

☐ Adult Participants – Waiver of informed consent

☒ Minor Participants – Written parent/guardian permission with documentation (signature)

☐ Minor Participants – Written parent/guardian permission with waiver of documentation (signature)

☐ Minor Participants – Verbal parent/guardian permission

☐ Minor Participants – Waiver of parent/guardian permission

☐ Minor Participants – Written assent with documentation (signature)

☐ Minor Participants – Written assent with waiver of documentation (signature)

☐ Minor Participants – Verbal assent

☐ Minor Participants – Waiver of assent

☐ Waiver of some elements of consent/permission/assent

APPROVAL: This research protocol is **approved** as presented. Please use the stamped consent document(s) as your copy master(s). Once you duplicate the consent form(s), you may begin participant recruitment. **Please see Attachment 1 for additional important information for researchers.**

COMMENTS:

EXPEDITED PROTOCOL APPROVAL REPORT

Attachment 1

ADDITIONAL INFORMATION FOR RESEARCHERS:

If your protocol received expedited approval, it was reviewed by a two-member team, or, in extraordinary circumstances, the Chair or the Vice-Chair of the IRB. Although the expediters may approve protocols, they are required by federal regulation to report expedited approvals at the next IRB meeting. At that time, other IRB members may express any concerns and may occasionally request minor modifications to the protocol. In rare instances, the IRB may request that research activities involving participants be halted until such modifications are implemented. Should this situation arise, you will receive an explanatory communiqué from the IRB.

Protocol approvals are generally valid for one year. In rare instances, when a protocol is determined to place participants at more than minimal risk, the IRB may shorten the approval period so that protocols are reviewed more frequently, allowing the IRB to reassess the potential risks and benefits to participants. The expiration date of your protocol approval is noted on the approval form. You will be contacted no less than one month before this expiration date and will be asked to either submit a final report if the research is concluded or to apply for a continuation of approval. It is your responsibility to submit a continuation request in sufficient time for IRB review before the expiration date. If you do not secure a protocol approval extension prior to the expiration date, you must stop all activities involving participants (including interaction, intervention, data collection, and data analysis) until approval is reinstated.

Please be reminded that you are required to seek approval of the IRB before amending or altering the scope of the project or the research protocol or implementing changes in the approved consent process/forms. You are also required to report to the IRB, through the Office of Sponsored Programs & Research Administration, any unanticipated problems or adverse events that become apparent during the course or as a result of the research and the actions you have taken.

Please refer to the IRB website (http://www.valdosta.edu/ospra/HumanResearchParticipants.shtml) for additional information about Valdosta State University's human protection program and your responsibilities as a researcher

Appendix B:

Data Sheet

Name: _____ Date Range: _____

DATE	GOAL	1	2	3	4	5	6	7	8	9	10	%

NOTES: