18 Use of Whey Protein in Children with Autism

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ABBREVIATIONS

ABC: : Aberrant Behavior Checklist
ADI-R: : Autism Diagnostic Interview, Revised
ADOS: : Autism Diagnostic Observation Schedule
ASD: : Autism spectrum disorder
BCM7: : Seven-amino acid peptide beta casomorphin-7
BV: : Biological value
CARS: : Childhood Autism Rating Scale
CBC: : Complete blood count
CBCL: : Child Behavior Checklist
CMP: : Comprehensive metabolic panel
CRWP: : Cysteine-rich whey protein
EAAT3: : Excitatory amino acid transporter-3
GF/CF: : Gluten-free/casein-free
GSH: : Glutathione (active, reduced form)
GSSG: : Glutathione (inactive, oxidized form)
MSEL: : Mullen Scales of Early Learning
NAC: : N-acetylcysteine
PLS-5: : Preschool Language Scale, 5th edition
RBS-R: : Repetitive behaviors using the Repetitive Behavior Scale, Revised
ROS: : Reactive oxygen species
RRBI: : Restricted and repetitive behaviors and interests
SAM: : S-adenosylmethionine
SAH: : S-adenosylhomocysteine
SCQ: : Social Communication Questionnaire
SIDS: : Sudden infant death syndrome
SNP: : Single nucleotide polymorphism
SRS: : Social Responsiveness Scale
tGSH: : Total glutathione (reduced + oxidized)
VABS-II: : Vineland Adaptive Behavior Scale, 2nd edition

AUTISM AND OXIDATIVE STRESS AND GLUTATHIONE

Autism is a complex neurodevelopmental disorder that affects 1 in 36 children in the United States, with four times as many males diagnosed as females (Maenner et al. 2023). The exact etiology remains undetermined. The spectrum of impairments noted in this disorder has led to the umbrella

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term "autism spectrum disorder" (ASD). This condition is associated with "deficits in social interaction and communication together with restricted and repetitive behaviors and interests" (American Psychiatric Association 2013). A myriad of behavioral assessments are used for diagnosis, with the Autism Diagnostic Observation Schedule (ADOS) (Lord et al. 1989, 2000) considered the gold standard. Despite extensive research, researchers have not identified a definite biomarker for diagnosis or treatment (Loke, Hannan, and Craig 2015). Early intervention programs and special schooling are the most effective treatments for those with this neurodevelopmental disorder, and although outcomes of early intervention vary, all children benefit (Alabdali, Al-Ayadhi, and El-Ansary 2014).

The heterogeneity of ASD can be observed in the neurologic, metabolic, and immunologic systems, etc. (Ratajczak 2011; Zimmerman et al. 2005). Therefore, it is speculated to be a multifactorial disorder involving epigenetics, genetics, and environmental factors. Oxidative stress may serve as a link between the different systems affected by this condition (Zimmerman et al. 2005). Oxidative stress occurs when there is an imbalance between reactive oxygen species (ROS) and/or reactive nitrogen species and antioxidant capacity. Glutathione, the major endogenous antioxidant, is the body's primary defense against damage from ROS and is low in those with ASD (Chauhan, Audhya, and Chauhan 2012; Geier et al. 2010; Geier et al. 2009; Ghanizadeh et al. 2012; James et al. 2004, 2006, 2008; Kern and Jones 2006; Zoroglu et al. 2004).

Most importantly, children with ASD also have decreased levels of total glutathione and the reduced or active form of glutathione (James et al. 2004; James et al. 2006). Cysteine, the rate-limiting amino acid in glutathione synthesis, is significantly decreased in ASD relative to control children, suggesting that glutathione synthesis is insufficient to maintain redox homeostasis (James et al. 2004). These significant decreases in total and free plasma glutathione and the redox ratio (active reduced: inactive oxidized glutathione) in children with ASD are of particular concern due to the importance of this system for normal cell function.

Glutathione (GSH) is a ubiquitous intracellular peptide found in all mammalian tissues, performing many diverse functions, including detoxification, antioxidant defense, immune function regulation, and modulation of cell proliferation (Meister and Anderson 1983; Vašková et al. 2023; Lu 2013; Grimble 2006; Aw 2003).

Raising systemic glutathione levels in humans is poorly achieved through the ingestion of glutathione itself (Allen and Bradley 2011). Elevating GSH levels is most efficiently achieved through the use of glutathione precursors, notably containing the limiting amino acid for glutathione synthesis, cysteine (Forman, Zhang, and Rinna 2009). The most commonly used precursor is N-acetylcysteine (NAC) (Raghu et al. 2021). A lesser-known cysteine-delivery vehicle is the use of undenatured whey protein isolate (Ross et al. 2012).

NAC (N-acetylcysteine) is a synthetic derivative of the amino acid L-cysteine and a precursor of GSH. It has been used for several decades as a mucolytic and as an antidote to acetaminophen poisoning. Its toxicity depends on the route of administration and high dosages (Tenório et al. 2021). Arguably, it is the most widely used "antioxidant" in experimental cell and animal biology, as well as clinical studies (Pedre et al. 2021).

Cysteine-rich undenatured whey protein is a byproduct of cheese production (El-Sayed and Chase 2011), where the whey is handled at low temperatures and vigorous mechanical agitation is avoided, both of which can denature the three-dimensional configuration of the proteins (El-Sayed and Chase 2011). Loss of this native configuration negates the glutathione precursor effects of these proteins (Oka et al. 2022). The proteins predominantly acting as GSH precursors are lactoferrin, beta-lactoglobulin, alpha-lactalbumin, serum albumin, and immunoglobulin, all rich in cysteine and cystine moieties (Bounous and Gold 1991).

Unlike NAC, undenatured whey protein also delivers a rich source of protein nutrition. Containing all of the human essential amino acids, it carries the highest BV (Biological Value) of any known edible protein (Hoffman and Falvo 2004). Although a bovine milk protein, the isolate contains only negligible amounts of lactose or casein (Gangurde et al. 2011), both of which have been suspected of exacerbating autistic behavioral abnormalities (Quan et al. 2022; Kushak et al. 2011).

Immunocal® is a cysteine-rich non-denatured whey protein isolate (CRWP) that has been successfully used in several human clinical trials including cancer treatment (Tozer et al. 2008), sports performance (Lands, Grey, and Smountas 1999), lung disease (Grey et al. 2003), Parkinson's disease (Tosukhowong et al. 2016), and, notably, autistic spectrum disorder (Castejon et al. 2021). It appears in the American PDR (Physician's Desk Reference) and is described as a "glutathione precursor" (Physician's Desk Reference).

In a study examining the clinical and biochemical effects of Immunocal in Parkinson's disease patients, a statistically significant increase in both GSH and reduced/oxidized GSH levels was demonstrated after 6 months of ingesting 20 g protein/day (Tosukhowong et al. 2016). A McGill University team addressing oxidative stress in cystic fibrosis patients also used Immunocal 20 g/day vs. placebo for 3 months. After supplementation, they documented a 46.6% increase from baseline ($P < 0.05$) in lymphocytic GSH levels compared to placebo (Grey et al. 2003). Similar findings were observed in a trial looking at muscular performance in healthy young adults. Besides increases in peak power and work capacity, lymphocyte glutathione levels increased in the Immunocal-treated group (35.5%, $p = 0.02$) compared to the placebo group (Lands, Grey, and Smountas 1999). The use of whey protein as a functional food with many medical benefits continues to grow (Patel 2015).

CLINICAL STUDY EVALUATING THE EFFECTIVENESS OF CYSTEINE-RICH WHEY PROTEIN IN AUTISM

Although prior nutritional interventions addressing antioxidant capacity have successfully improved glutathione levels, the association between these changes and autistic behavior has been less compelling. Given that pharmacological alternatives targeting only specific comorbid conditions are limited and carry a significant burden of side effects, further studies utilizing complementary and alternative medicine for the treatment of ASD are justified.

A double-blind, placebo-controlled study was conducted to explore the effectiveness of a 90-day intervention with a nutritional supplement containing CRWP in treating ASD core behavioral symptoms and elevating glutathione levels in 3–5-year-old preschool children with ASD. The study also investigated whether improvements in intracellular glutathione correlated with behavioral changes. A comprehensive and age-specific behavioral assessment battery utilizing eight different tests was used to assess which core areas of ASD could be impacted by this intervention.

In brief, the effectiveness of a 90-day CRWP (Immunocal) intervention in children with ASD examined whether intracellular reduced and oxidized glutathione improvements correlated with behavioral changes. The study design was a double-blind, randomized, placebo-controlled trial in 3–5-year-old preschool children with confirmed ASD. The intervention group received daily CRWP (powder form: 0.5 g/kg for children <20 kg or a 10-g dose for those >20 kg), while the placebo group received rice protein, mimicking the protein load in the intervention group. White blood cell glutathione levels and ASD behaviors were assessed using different behavioral scales, such as the Childhood Autism Rating Scale, Preschool Language Scale, Social Communication Questionnaire, Child Behavior Checklist, and the parent-rated Vineland Adaptive Behavior Scale, 2nd edition (VABS-II) (Castejon et al. 2021).

A total of 40 participants completed the 90-day treatment period (CRWP, 21; placebo, 19). At baseline, the participants' demographic and diagnostic characteristics were similar across both groups. The average age of participants in both groups was 3.9 years. Most participants were male (placebo = 83% and CRWP = 90%), and a wide variety of races were represented in the study. There were no significant differences in the medications/supplements taken by the placebo or CRWP groups.

At baseline, average scores across groups were similar for most behavioral assessments; however, some differences were noted. In the VABS-II, the placebo group exhibited less severe clinical

manifestations of ASD, and in the Child Behavior Checklist, the CRWP group had higher stress problem T-scores.

At baseline, the levels of total glutathione, oxidized glutathione, reduced glutathione, and the ratio of oxidized to reduced glutathione were measured in participants' leukocytes. There were no significant differences between groups in any of the glutathione measurements taken at baseline (Table 18.1).

BEHAVIORAL ASSESSMENTS AND GLUTATHIONE MEASUREMENTS

When comparing baseline to follow-up changes in the CRWP (n = 21) and placebo (n = 19) groups, behavioral improvements were seen in both groups; however, the CRWP group improved in more areas than the placebo group. No significant differences were observed after three months in the CARS, PLS, SCQ, or CBC behavioral scales between baseline and follow-up in the two groups (Δ vs. Δ). However, the parent-rated behavioral assessment VABS-II demonstrated significant improvements in the CRWP group compared to the placebo group in the composite score ($p = 0.03$). Further, significant changes were observed in multiple domains/sub-domains representing different aspects of adaptive behaviors associated with ASD symptoms in the intervention group.

Specifically, the socialization domain ($p = 0.04$), domestic daily living skills ($p = 0.05$), maladaptive behavior domain ($p = 0.04$), and internalizing subdomain ($p = 0.02$) all showed significant improvements with the CRWP supplementation after three months.

Higher increases in total and reduced glutathione were observed from baseline to follow-up in the CRWP group (Figure 18.1). In contrast, no significant changes in total, reduced, or oxidized glutathione levels were observed in the placebo group. After the 90-day supplementation, changes in both total ($p = 0.02$) and reduced glutathione ($p = 0.04$) in the CRWP group were significantly higher than changes in the placebo group (Figure 18.1). However, behavior improvements observed using the VABS-II were not significantly correlated with changes in glutathione levels.

SUB-ANALYSIS OF THE CRWP GROUP

Because a significant number of participants in the CRWP group showed significant improvements in adaptive behavior as measured by the VABS-II, researchers explored the common characteristics in this group of responders. Changes of >2 points or 1 SD in VABS-II composite scores were used to discriminate "responders." Out of 19 participants who completed behavioral assessments, 12 were identified as responders, compared to only 5 of the 21 who completed behavior assessments in the placebo group ($p = 0.03$). However, researchers found no difference between the two groups when comparing changes in total glutathione levels (responders $p = 0.05$, non-responders $p = 0.06$).

Further analysis of the VABS-II was used to compare the behavioral changes in the responders vs. the non-responders in the intervention group (Figure 18.2). The responders showed a much larger improvement in the given domains/sub-domains of the VABS-II, including the overall adaptive behavior composite score ($p<0.0001$ and $p = 0.0003$ compared to non-responders and placebo, respectively). In addition, researchers noted significant differences in the communication domain score, with differences in the receptive V-scale score and the expressive V-scale score, as well as in the daily living skills domain score, specifically in the personal V-scale score. Responder improvements were also noted in the socialization domain score, with significant differences in play and leisure time, coping skills, and the motor skills domain score, including the gross motor score. Moreover, the responders' group showed decreases in internalizing, externalizing, and maladaptive behavior scores (Table 18.2). It is important to highlight that no differences were found in baseline composite VABS-II scores between responders and non-responders ($p = 0.24$). These results show a favorable effect of CRWP on a range of behavior measures in those who responded to this intervention.

TABLE 18.1
Change in Glutathione Levels from Caseline to Follow-Up in Placebo and CRWP Groups

	Placebo (Mean ± SEM)				CRWP (Mean ± SEM)					
Glutathione Levels: (nM/10^5 WBC)	Baseline (n = 24)	12 weeks (n = 16)	Δ	P-value	Baseline (n = 20)	12 weeks (n = 20)	Δ	P-value	Baseline Placebo vs. Baseline Intervention	P-value of Δ vs. Δ
tGSH	104.2 ± 15.8	88.0 ± 12.8	−16.22	0.50	113.1 ± 16.1	166.7 ± 37.2	53.6	0.17	0.77	0.02*
GSSG	10.9 ± 1.57	8.99 ± 1.36	−1.86	0.78	7.95 ± 1.34	12.88 ± 2.70	4.93	0.22	0.14	0.12
GSH	82.5 ± 13.9	66.3 ± 9.8	−16.16	0.53	97.2 ± 15.0	136.0 ± 34.4	38.8	0.47	0.13	0.04*

*$p < 0.05$.

CRWP: cysteine-rich whey protein; WBC: white blood cells; SEM: standard error of the mean; tGSH: total glutathione; GSSG: glutathione (inactive, oxidized form); GSH: glutathione (active, reduced form). Values are mean ± SEM.

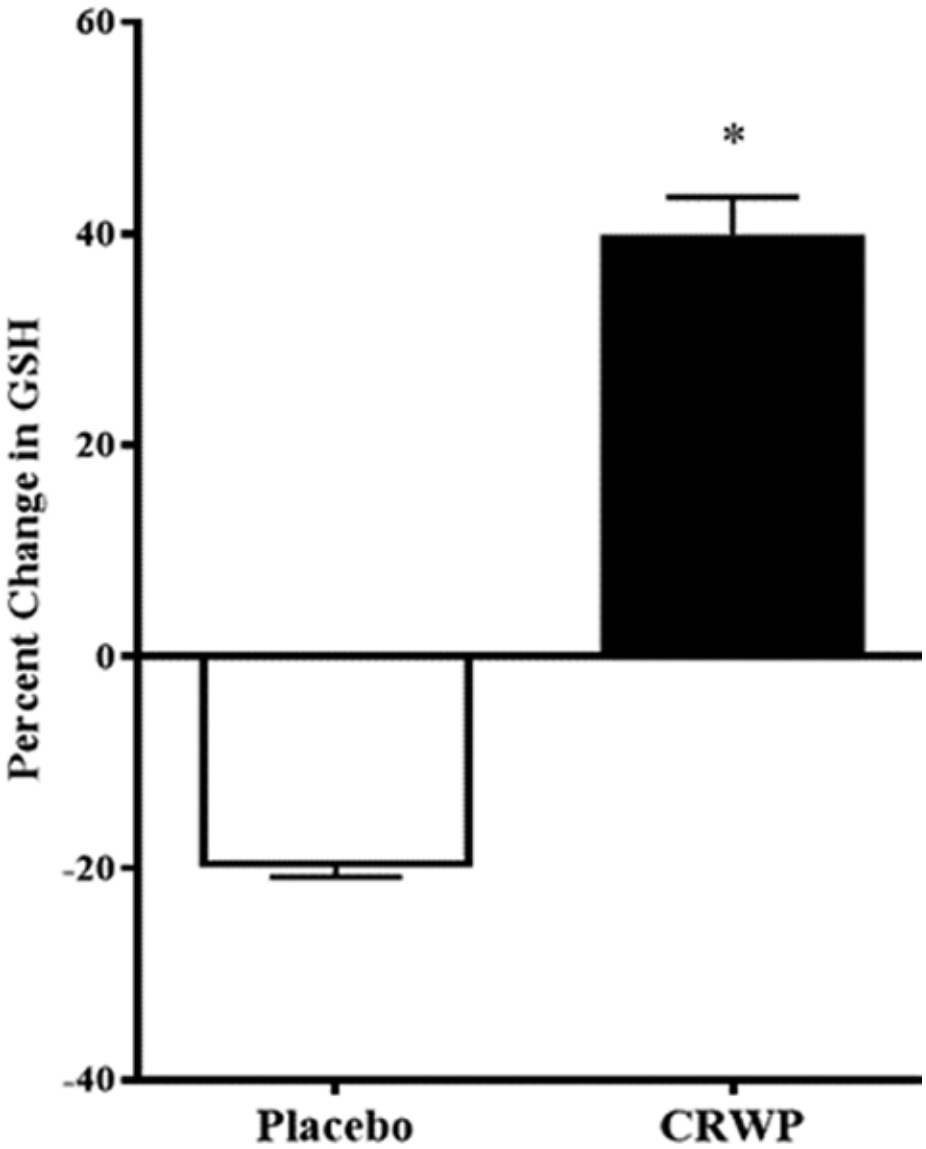

FIGURE 18.1

It is important to highlight that no serious adverse events were reported in either treatment group. Compliance, assessed by the weight of the canisters before and after treatments, showed no significant differences between the groups (90.5% used in the placebo group vs. 89.9% in the CRWP group; $p = 0.91$).

Both groups showed significant improvements from baseline to follow-up behavioral score assessments in some tests. This was expected, as all children had access to high-standard care during the study, consisting of preschool centers and therapies providing special services to this population. However, when comparing the magnitude of changes between the two groups after the 3-month trial period (Δ vs. Δ), the CRWP group showed significant behavioral improvements with medium-large effect sizes in children's adaptive behavioral skills as measured by the VABS-II. This highlights the importance of randomized, double-blind, placebo-controlled studies in revealing

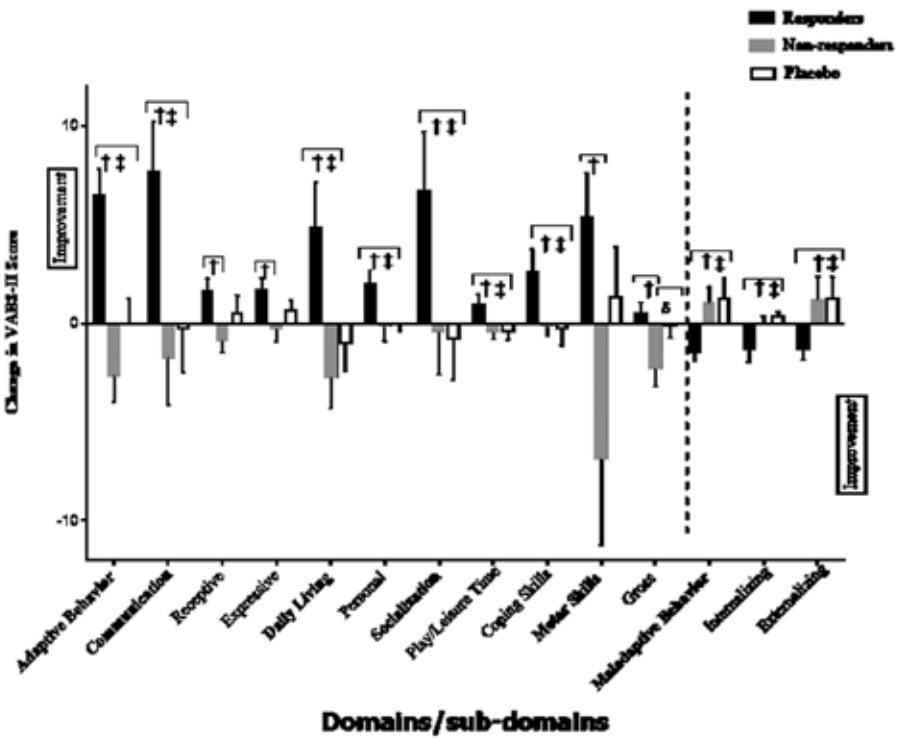

FIGURE 18.2

accurate improvements with specific interventions. It is worth noting that children in the intervention group had to improve more than the placebo group for significant outcomes to be observed, as their baseline values indicated they were more severely affected by the disorder at the start of the trial.

Several behavioral improvements in children with ASD have been associated with nutritional interventions. NAC supplementation was associated with decreased irritability using the Aberrant Behavior Checklist (ABC) and reduced repetitive behaviors using the Repetitive Behavior Scale-Revised (RBS-R) and Social Responsiveness Scale (SRS) assessments in a pilot study (Hardan et al. 2012). In contrast, Wink et al. (2016) found no behavioral improvements with a similar study design. Vitamin B6 supplementation was associated with positive changes in sleep and gastrointestinal issues in a randomized, double-blind, placebo-controlled 3-month study in 20 children with ASD using a parent-rated scale (Adams and Holloway 2004). Additionally, this study mirrored the results of another study (Frye, Melnyk, et al. 2013), which showed significant increases in many of the same VABS-II scores after supplementation with methylcobalamin plus folinic acid. Each study used a clinician, parent, or a combination of scales to assess changes in behavior. The variety of scales and study designs used to assess behavioral changes makes it difficult to compare results with most previous studies using other nutritional interventions.

Twelve out of 20 children (60%) were identified as responders to the intervention due to a greater than two-point improvement in VABS-II overall adaptive composite score. Chatham et al. (2018) showed that the minimal clinically significant difference in children with ASD ranged from 2 to 3.75 points, supporting our approach to identifying these children as responders.

The study also confirmed significant improvements in the glutathione levels of children with ASD using a nutritional approach known to increase glutathione biosynthesis. Children assigned to the supplement experienced a 40% increase in the reduced form of glutathione because this

TABLE 18.2
Changes in VABS-II Scores in Responders, Non-responders, and Placebo Groups

Vineland Adaptive Behavior Scale	Responders (n = 12)	Non-Responders (n = 7)	Placebo (n = 21)	P-value Responders vs. Non-Responders	P-value Responders vs. Placebo
Adaptive Behavior Composite Standard Score	6.5 ± 1.32	−2.86 ± 0.91	0.00 ± 1.25	<0.0001†	0.0003†
Communication Standard Total Score	7.67 ± 2.55	−1.71 ± 2.40	−0.29 ± 2.20	0.008†	0.01†
Receptive V-scale score	1.58 ± 0.69	−0.86 ± 0.59	0.48 ± 0.93	0.01†	0.21
Expressive V-scale score	1.67 ± 0.63	−0.29 ± 0.60	0.62 ± 0.54	0.03†	0.08
Written V-scale score	0.64 ± 0.47	0.29 ± 0.52	−0.29 ± 0.49	0.32	0.12
Daily Living Skills Standard Total Score	4.83 ± 2.31	−2.7 ± 1.6	−1.00 ± 1.39	0.008†	0.007†
Personal V-scale score	2 ± 0.71	−0.14 ± 0.77	−0.05 ± 0.36	0.03†	0.004†
Domestic V-scale score	−0.08 ± 0.57	−0.71 ± 0.60	0.05 ± 0.63	0.23	0.45
Community V-scale score	0.33 ± 0.45	−0.43 ± 0.62	−0.05 ± 0.51	0.19	0.20
Socialization Standard Total Score	6.67 ± 3.04	-0.43 ± 2.17	−0.81 ± 2.06	0.04†	0.02†
Interpersonal Relationships V-scale score	0.83 ± 0.82	0.29 ± 0.57	−0.38 ± 0.59	0.30	0.12
Play and Leisure Time V-scale score	0.92 ± 0.58	−0.43 ± 0.37	−0.38 ± 0.44	0.03†	0.04†
Coping Skills V-scale score	2.58 ± 1.18	−0.15 ± 0.51	−0.24 ± 0.89	0.03†	0.04†
Motor Skills Standard Total Score	5.33 ± 2.32	−6.86 ± 4.4	1.33 ± 2.56	0.02†	0.05
Gross V-scale score	0.5 ± 0.57	−2.29 ± 0.92	−0.14 ± 0.60δ	0.01†	0.09
Fine V-scale score	1.33 ± 0.54	0 ± 0.62	0.57 ± 0.39	0.07	0.13
Maladaptive Behavior Total V-Scale Score	−1.42 ± 0.47	1.0 ± 0.84	1.26 ± 1.05	0.01†	0.003†
Internalizing V-scale score	−1.33 ± 0.64	0 ± 0.37	0.37 ± 0.22	0.04†	0.0003†

Externalizing V-scale score	−1.33 ± 0.51	1.17 ± 1.20	1.26 ± 1.08	0.02†	0.008†

†$p < 0.05$, one-tail t-test.
δSignificant difference between placebo and non-responder subgroup where $p < 0.05$.
VABS-II, Vineland Adaptive Behavior Scale, 2nd edition. Values are mean ± SEM.

supplement provides a natural source of cysteine, the rate-limiting step in glutathione biosynthesis, leading to increased intracellular levels.

Other interventions have demonstrated comparable glutathione increases to those in our CRWP group. The combination of methylcobalamin and folinic acid induced increases of 15% in total glutathione and 20% in reduced glutathione (Frye, Melnyk, et al. 2013), while NAC treatment has demonstrated a 60% increase from baseline (Wink et al. 2016). Targeting glutathione levels is relevant because significant differences in glutathione and its related metabolites have been found in plasma (James et al. 2004, 2006), white blood cells (Ghanizadeh and Moghimi-Sarani 2013; Melnyk et al. 2012), and post-mortem brains (Chauhan, Audhya, and Chauhan 2012; Rose et al. 2012) of participants with ASD. This finding is supported by several genetic variations seen in ASD patients related to the transmethylation/transsulfuration pathway, where glutathione is one of the byproducts (James et al. 2004; James et al. 2006, 2008). The hypothesis that low glutathione levels are related to ASD symptoms has been partially validated, as several treatments targeting this deficiency have been proven to be efficacious at modifying behavioral symptoms in children with this condition (Frye, Sequeira, et al. 2013).

Significant limitations of this study include the small sample size, narrow age sample, and short treatment intervention. Future studies, including a broader age group and using specific behavioral assessments and scales, are warranted. It would also be pertinent to include a cohort of neurotypical children in a similar study to compare any changes in glutathione levels and behaviors in that population with those of children with ASD.

INHIBITION OF CYSTEINE TRANSPORT BY CASEIN AND GLUTEN-DERIVED OPIOID PEPTIDES: IMPLICATIONS IN ASD

Cow milk-derived whey proteins are well known for their high content of the sulfur amino acid cysteine, but the absorption and systemic availability of this important nutrient are restricted by an opioid peptide released upon digestion of the milk curd protein beta casein. Specifically, the seven-amino-acid peptide beta casomorphin-7 (BCM7; Tyr-Pro-Phe-Pro-Gly-Pro-Ile) inhibits cellular cysteine uptake mediated by excitatory amino acid transporter-3 (EAAT3), also designated as EAAC1 in mice (Trivedi et al. 2014). While other members of the EAAT family transport glutamate and aspartate, EAAT3 preferentially transports cysteine and is expressed by gastrointestinal (GI) epithelial cells, especially in the distal ileum, as well as by neurons and immune cells. As a result, BCM7 can exert significant effects on antioxidant status, brain function, and inflammatory conditions.

Trivedi et al. (2014) demonstrated dose-dependent BCM7-mediated inhibition of cysteine uptake in GI-derived Caco-2 cells and SH-SY5Y neuronal cells, which paralleled inhibition by morphine and was blocked by a mu-opioid receptor antagonist. Inhibition of cysteine uptake was associated with decreased glutathione (GSH) and GSH/GSSG, as well as a lower ratio of S-adenosylmethionine (SAM) to S-adenosylhomocysteine (SAH), indicative of increased oxidative stress and impaired methylation, respectively. These actions were further accompanied by significant changes in the expression of redox-related genes as well as substantial effects on DNA methylation status, including gene pathways related to gastrointestinal disease and inflammation (Trivedi et al. 2015). Taken

together, these actions of milk-derived BCM7 indicate an important potential influence on cellular metabolism and genomic regulation, with special importance for early infant development when milk is the sole or primary source of nutrition.

The discovery of BCM7-mediated inhibition of cysteine uptake can be traced back to the groundbreaking studies carried out in Norway by researchers who observed behavioral improvements in ASD subjects upon administration of a gluten/casein-free (GF/CF) diet accompanied by a decrease in urinary excretion of peptides (Knivsberg et al. 1990). Cessation of the GF/CF diet resulted in worsening behaviors and resumption of excessive urinary peptides. Reichelt and Knivsberg (2003) later reported that BCM7 and a similar opioid peptide released upon digestion of gluten, gliadinomorphin-7 (YPQPQPF; GM7), were among the elevated urinary peptides found in the ASD subjects. Since BCM7 and GM7 were food-derived, this prescient observation suggested that an ASD-associated "leaky gut" allowed systemic exposure to these opioid peptides (Reichelt and Knivsberg 2003). However, it should be noted that Dettmer et al. (2007) failed to identify BCM7 or GM7 in the urine of autistic subjects.

The amount of BCM7 released from bovine beta casein depends on the nature of the amino acid immediately following its sequence. A single nucleotide polymorphism (SNP) specifies either a histidine or a proline residue, denoted as A1 beta casein or A2 beta casein, respectively. Since the proline side chain is cyclic, it impairs protein cleavage at this locus, resulting in very little BCM7 being released from A2 beta casein compared to A1 beta casein. Consistent with this relationship, consumption of milk from homozygous A2/A2 cows results in an approximately twofold higher increase in plasma GSH compared to mixed A1/A2 (Deth et al. 2016), illustrating a crucial role for BCM7 in regulating GI absorption of whey protein-derived cysteine, whose availability is rate-limiting for GSH synthesis. Notably, the action of milk-derived BCM7 can also restrict cysteine absorption from other proteins consumed in the same meal.

The potential functional consequences of the inhibition of cysteine uptake by BCM7 and GM7 are broad and incompletely appreciated, reflecting the regulatory roles of redox status and methylation, including their influence on inflammation and immunity. Lower cysteine and GSH levels promote oxidative stress, and the folate and vitamin B12-dependent enzyme methionine synthase is highly sensitive to oxidative stress, which may account for BCM7/GM7 effects on DNA methylation and gene expression (Trivedi et al. 2014). Low GSH and oxidative stress increase the activity of the NLRP3 inflammasome (Bourgonje et al. 2021), while recent evidence indicates that cysteine-dependent persulfidation is crucial for terminating its cytokine-producing activity (Takeda et al. 2023). Thus, restriction of cysteine absorption could increase the risk of inflammation, not only in the GI tract but throughout the body.

Autoimmunity is associated with a Th2 pattern of CD4+ T-cell cytokine production, and autoimmune disorders have been linked to gluten and casein consumption and/or reversal by a GF/CF diet (Bangarusamy et al. 2021; Vojdani et al. 2003). Interleukin-1 (IL-4) is a key Th2 cytokine whose transcription is regulated by methylation of its promoter region, in an age-dependent manner reciprocal to that of interferon-gamma (IFN-γ), with IL-4/IFN-γ being normally higher in early development (Xia et al. 2016). Consumption of A1 milk was associated with higher IL-4 levels and inflammation in GI tissues (Ul Haq et al. 2014), while gluten-dependent celiac disease was associated with elevated IFN-γ in children (Björck et al. 2015), but elevated IL-4 in adults (Manavalan et al. 2010). Together, these findings indicate abnormal epigenetic regulation of cytokines, which might be related to redox and methylation consequences of BCM7/GM7.

THE EFFECT OF OTHER PROTEINS: CASEIN AND GLUTEN

This chapter has discussed the relationship between cysteine availability and its correlation with multiple physiological processes influencing cellular metabolism and genomic regulation, especially during early infant development.

Numerous studies have confirmed the benefits of a GF/CF diet, not only in autism but also in a variety of other conditions, as well as its widespread adoption as a health-promoting lifestyle choice (Poslt Königová et al. 2023; Whiteley et al. 2012). The link to cysteine transport arose because cysteine and GSH levels are markedly lower in ASD subjects (James et al. 2006), leading to the hypothesis that the GF/CF benefit might somehow improve cysteine availability. Thus, the health-promoting benefits of a GF/CF diet can, in large part, be attributed to an increased availability of cysteine due to avoidance of foods that release opioid peptides during their digestion and act to inhibit cysteine absorption. Moreover, low cysteine levels in ASD individuals may make them more sensitive to the inhibitory effects of BCM7 and GM7. Furthermore, since diet-derived urinary peptides decreased with a GF/CF diet, the ASD-associated "leaky gut" can tentatively be ascribed to a damaging effect of decreased cysteine on the barrier function of GI epithelial cells, most likely associated with inflammation.

Further research is needed to prove that GF/CF diets are effective in ASD and to determine if such dietary restrictions are more effective in those showing low cysteine and GSH levels.

MINI DICTIONARY OF TERMS

- **Aberrant Behavior Checklist:** A behavioral instrument that evaluates five domains: irritability, social withdrawal, stereotyped behavior, hyperactivity, and inappropriate speech. This scale is filled out by a caregiver.
- **Biological value:** Measures the proportion of absorbed protein from a given food and how much is incorporated into other proteins in the body.
- **Maladaptive behavior domain (Vineland):** Includes a child's problem behaviors in two forms: internalizing and externalizing behaviors. Internalizing behaviors are negative and focused inward, such as fearfulness, social withdrawal, and concentration problems. Externalizing behaviors are negative towards the environment, such as bullying, physical aggression, and disobeying rules.
- **Redox-related genes:** Genes that can regulate the expression of antioxidants under oxidative stress conditions.
- **Vineland Adaptive Behavior Scale:** A standardized test used to diagnose intellectual and developmental disabilities, autism, and developmental delays.

SUMMARY POINTS

- Lower antioxidant capacity, specifically lower GSH levels, is a common finding in ASD.
- Oxidative stress can be a major factor in many manifestations of ASD, such as abnormal methylation, inflammation, and immunity.
- GSH levels rely on cysteine availability, and dietary manipulations aimed at improving antioxidant capacity are centered on cysteine levels.
- A cysteine-rich whey protein supplement showed an effective increase in intracellular levels of GSH as well as some improvements in autistic behavior.
- Opioid peptide released upon digestion of the milk curd protein beta casein can decrease cysteine availability, providing a rationale for gluten- and casein-free diets in ASD.

The scientific evidence discussed in this chapter has been reported by Castejon et al. (2021).

ChatGPT was used to generate the abstract of this chapter.

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