Evaluation of Bulk Drug Substances Used to Compound Drug Products For Patients with Autism Spectrum Disorder (ASD):
Phase II, Review of Available Evidence of Safety and Effectiveness And Evaluation of Current and Historical Use

Johns Hopkins University Center of Excellence in Regulatory Science and Innovation (JHU CERSI)

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Executive Summary

Background
Drug products compounded by entities known as “outsourcing facilities” are exempt from certain requirements of the Federal Food, Drug, and Cosmetic Act (FD&C Act), including new drug approval requirements, if the conditions set forth in section 503B of the FD&C Act are met. One of these conditions is that bulk drug substances used in compounding (a) be used to compound drug products that appear on FDA’s drug short list at the time of compounding, distribution, and dispensing; or (b) appear on a list developed by FDA of bulk drug substances for which there is a clinical need (“bulks list”).

Drug substances may be nominated for inclusion on the bulks list. The substance inositol, was nominated for use in compounding by outsourcing facilities for many conditions, including autism spectrum disorder (ASD). Other substances nominated for use in compounding (DMPS, glutathione and methylcobalamin) or currently being compounded (melatonin) have also been used for patients with ASD. In addition, during Phase I of this project JHU-CERSI provided information that oxytocin, another substance nominated for the bulks list, is used in compound drug products for patients with ASD.

To determine whether to include nominated substances on the bulk list the FDA evaluates several factors including safety, evidence of effectiveness, and the use of this substance historically and in current clinical practice. The FDA thus requested that the JHU-CERSI assess these factors for six bulk drug substances for use in patients with ASD: inositol, DMPS, glutathione, methylcobalamin, melatonin and oxytocin.

Objective
For each of the six compounds of interest (inositol, DMPS, glutathione, methylcobalamin, melatonin and oxytocin) conduct:
1. Systematic reviews of human studies of the effectiveness and harms and animal studies of the effectiveness, and,
2. Evaluation of current and historical use drawing on national, population-based, and clinical data resources, supplemented with interview of key opinion leaders in research and practice.

**Methods**

**Part 1: Review of Available Evidence of Safety and Effectiveness**
We registered 12 protocols for the systematic reviews. We completed searches for human studies (through January 2020) and animal studies (through February 2020). Screening of search results was completed by two independent reviewers using pre-defined eligibility criteria. Data extraction was completed serially (one reviewer completed and one checked) and risk of bias assessments were conducted by independent team members using study design specific tools. The body of evidence of human studies addressing critical outcomes was graded. Integration of evidence streams (animal and human) was completed, where relevant.

**Part 2: Evaluation of Current and Historical Use**
To assess current and historical use of the substances in children with ASD, we drew on 3 distinct data resources (clinical, population, and a national sample) and conducted interviews with Key Opinion Leaders (KOLs) representing active researchers and practitioners in ASD treatment.

**Use of Compounds In A Clinical Sample**
Clinical data was accessed via the Center for Autism and Related Disorders (CARD) clinic at the Kennedy Krieger Institute. Data from the clinic’s background and history form and prescription database were abstracted from the electronic medical records of children with ASD under 17 years of age that receive care at KKI-CARD. Based on these data, we assessed frequency of treatment use for each of the six compounds of interest. Results demonstrated that at KKI, <10% of parents used melatonin for their child with ASD and all prescriptions were for oral administration; <1% used Vitamin B12, all orally administered; <1% used glutathione, all administered as an injection; and chelation (“DMPS”), oxytocin, inositol, were only reported by 3 parents via the background form, none of which were prescribed at KKI.

**Use of Compounds In A Population Sample**
Population-based data was collected via collaboration with the Simons Foundation Powering Autism Research for Knowledge (SPARK) online data repository. Parents of children under 18 years of age were invited to participate in an online survey to gather information on frequency of use, route of administration, and perceived effectiveness of biomedical treatments used to treat children with ASD. Of the compounds of interest, melatonin was most frequently used, followed by Vitamin B12 substances (6.0% Methyl B12, 5.5% B Complex). Both were most frequently
administered orally and believed by parents to be “effective”. Use of the additional compounds of interest were rarer and endorsed use in <2% of responses.

**Use of Compounds in A National Sample**
Medicaid claims data from the years 2010-2014 for children with ASD was accessed and drug administration codes used to identify filled prescriptions of interest, frequency of prescription, and route of administration. National Provider Identifier (NPI) and provider ID codes in the data were also used to calculate the total number of unique providers for each drug and to identify providers associated with the highest volume of prescriptions. Of the medications, B12 was the most frequently used although the percent of users observed was still low (1.1% among Medicaid enrolled children with ASD). Injection and solution were the most common form of B12 prescription. There was also little indication of increased use among children with ASD, compared to children without ASD, in this sample. Use of the other compounds of interest was <1% among children with and without ASD.

**Key Opinion Leaders**
Finally, phone interviews with 3 key opinion leaders (KOL), composed of currently practicing physicians and researchers with expertise in ASD and Complementary and Alternative Medicine (CAM), were conducted to obtain a qualitative understanding of the patterns of use and knowledge of the compounded drug substances of interest for ASD in mainstream clinical practice. Detailed summaries comparing and contrasting the key takeaways from each KOL were provided. Overall, melatonin was seen as a safe, common treatment used by patients with ASD. B12 was known to be safe and commonly used, but not as a treatment for ASD. Oxytocin was seen as a newly emerging therapy for ASD, with insufficient evidence at the moment to support its safe or effective use for ASD. Little was known about glutathione and inositol treatments as they are rarely prescribed or recommended for ASD in clinical practice. The use of chelation to treat symptoms of ASD was widely understood to be toxic and strongly discouraged.

**Results**
For the systematic reviews, we identified and screened a total of 3,734 human studies and 6,710 animal studies. Results for the systematic reviews and the evaluation of use data are presented by substance.

**Inositol**
We were unable to draw conclusions in the systematic review based on one very small human study (high risk of bias) with limited reporting of results.

Inositol was not commonly used by children with ASD in clinical, population, or national data sources examined.
DMPS
No relevant human or animal studies were identified in the systematic reviews.

Use of DMPS for children with ASD was very rare and all KOLs were strongly opposed to the use of chelation of any kind to treat ASD.

Glutathione
We were unable to draw conclusions based on one small human study (high risk of bias) reporting only adverse events.

Glutathione was not commonly used by children with ASD in clinical, population, or national data sources examined.

Methylcobalamin
Our systematic review identified two human studies (one trial with low risk of bias, one crossover study with high risk of bias) but evidence was insufficient to draw conclusions.

Of the six compounds of interest evaluated, methylcobalamin (B12) was the second most commonly used in children with ASD (range in samples of 1% to 6%). KOLs noted that B12 would likely have no effect unless specifically taken to address a dietary deficiency.

Melatonin
Our systematic review identified 6 human studies (published in 9 articles) and two studies in adult Wistar rats. All of the human studies included children with ASD or Asperger’s syndrome and co-occurring sleep disorders. No studies reported results for ASD relevant measures or social challenges. Two studies reported improvement in repetitive behavior. The results from rodent studies were inconsistent.

Melatonin was the most commonly used substance in our datasets (60% to 97%). Melatonin was the only one of the six substances that the KOLs prescribed and considered to be safe and effective for use by children with ASD. Melatonin was used to address sleep issues most frequently and not core symptoms of ASD.

Oxytocin
We identified 18 studies (22 publications) evaluating oxytocin in human subjects and 39 studies in animals. The animal and human evidence indicate that oxytocin did not improve repetitive behaviors. In fact, most rodent studies reported an increase in repetitive behaviors as well as cognitive inflexibility with oxytocin treatment. In humans, repetitive behaviors in children and adolescents did not improve and unclear benefits were reported in adults. With regards to social communication, evidence from studies in prairie voles, zebrafish and non-human primates
indicate that oxytocin does not improve various types of social behaviors. Evidence from human studies indicate age-related differences in outcomes with two small studies reporting potential benefit in children and no clear benefits for adolescents or adults.

Oxytocin was not commonly used by children with ASD in clinical, population, or national data sources examined.

**Conclusions**

Our systematic reviews found limited to no evidence for 4 of the 6 substances (DMPS, glutathione, inositol, methylcobalamin). For melatonin, all of the studies included people with co-existing sleep disorders, and there was limited evidence on ASD-specific outcomes. Evidence from human and animal studies suggest that oxytocin does not improve repetitive behaviors. For social communication, there may be benefit of oxytocin in children but there were no clear benefits in adolescents or adults.

Most of the substances were used very rarely in our datasets and not prescribed by our KOLs. Melatonin was by far the substance most commonly reported as used for children with ASD with methylcobalamin (B12) used second most. It should be noted that in the use of each of these substances co-occurring conditions were the target, not symptoms of ASD.
Part 1: Review of Available Evidence of Safety and Effectiveness

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Summary

Objective
To conduct a systematic review of human and animal evidence on the safety and effectiveness for the following bulk substances for treating autism spectrum disorder (ASD): inositol; methylcobalamin; glutathione; 2,3-dimercapto-1-propanesulfonic acid sodium (DMPS); melatonin; and oxytocin.

Methods
Developed and registered 12 protocols. We completed searches of PubMed, Embase, Cochrane and Web of Science for human studies (through January 2020) and animal studies (through February 2020). Screening of search results was completed by two independent reviewers using pre-defined eligibility criteria. Data extraction was completed serially (one reviewer completed and one checked) and risk of bias assessments were conducted by independent team members using study design specific tools. Details, including search strategies, are provided in the full report.

Results
We identified and screened a total of 3,734 (including ClinicalTrials.gov registry entries) human studies and 6,710 animal studies. As noted in Table 1, we identified very few studies addressing DMPS (n=0), glutathione (n=1), inositol (n=1) or methylcobalamin (n=2). This summary will thus focus on results for melatonin (n=8) and oxytocin (n=57). Full narrative syntheses and, where completed, meta-analyses, PRISMA diagrams and evidence tables for all substances are provided in the full report.
### Table A. Summary of searching and screening results

<table>
<thead>
<tr>
<th></th>
<th>DMPS</th>
<th>Glutathione</th>
<th>Inositol</th>
<th>Melatonin</th>
<th>Methylcobalamin</th>
<th>Oxytocin</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Humans</strong></td>
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<td>6</td>
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<td>56</td>
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<td># data abstractions completed*</td>
<td>--</td>
<td>1</td>
<td>1</td>
<td>6 studies (9 publications)</td>
<td>2</td>
<td>17 studies (21 publications)</td>
<td>27</td>
</tr>
<tr>
<td><strong>Animals</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>131</td>
<td>3130</td>
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<td>--</td>
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<td>39</td>
<td>41</td>
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<td><strong>ClinicalTrials.gov</strong></td>
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<td></td>
</tr>
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<td>1</td>
<td>16</td>
<td>5</td>
<td>37</td>
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<td># included for data abstraction</td>
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<td>1 trial with no results posted</td>
<td>4 trials with no posted results</td>
<td>2 trials matched to included publication</td>
<td>16 trials with no results posted</td>
<td>22 trials with no results posted</td>
</tr>
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<td></td>
</tr>
</tbody>
</table>

*Note: The data abstractions completed for the *Oxytocin* column includes 17 studies (21 publications) for Animals, but there are no studies included for Data abstraction.
DMPS
No human or animal studies identified.

Glutathione
Unable to draw conclusions based on one small human study (high risk of bias) reporting only adverse events.

Inositol
Unable to draw conclusions based on one very small human study (high risk of bias) with limited reporting of results.

Methylcobalamin
We identified two human studies (one trial with low risk of bias, one cross-over study with high risk of bias) but evidence was insufficient to draw conclusions.

Melatonin
We identified 6 human studies (published in 9 articles)\textsuperscript{1-6} (see Table 2) and two studies in adult Wistar rats.\textsuperscript{7,8}

Human Studies
See Table 3.
All of the studies included children with ASD or Asperger’s syndrome and co-occurring sleep disorders.
ASD relevant measures: no study reported on selected scales
Social challenges: no studies identified
Repetitive behavior (two studies): both studies reported improved repetitive behavior
Adverse effects: little to no differences for gastrointestinal events, respiratory events, mood events neurologic symptoms, dermatologic symptoms but evidence is insufficient to draw conclusions.

Animal Studies
Rodents (two studies): inconsistent results regarding repetitive self-grooming.
Table B. Summary of the characteristics of studies that evaluated melatonin among people with autism spectrum disorder

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Study design</th>
<th>Intervention</th>
<th>Baseline N</th>
<th>Age</th>
<th>Male, %</th>
<th>Outcomes assessed</th>
<th>Overall risk of bias*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wright, 2011&lt;sup&gt;6&lt;/sup&gt;</td>
<td>Randomized crossover trial (1 month)</td>
<td>Total</td>
<td>20</td>
<td>Mean: 9 Range: 4-16 years</td>
<td>80</td>
<td>GI AE, sleep-related AE, respiratory AE, mood AE, neurological AE, dermatologic AE</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group 1, placebo (oral: once a day (qd) for 3 months)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>GI AE, sleep-related AE, respiratory AE, mood AE, neurological AE, dermatologic AE</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group 2 melatonin, (oral: 7 mg† once a day (qd) for 3 months)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>GI AE, sleep-related AE, respiratory AE, mood AE, neurological AE, dermatologic AE</td>
<td>High</td>
</tr>
<tr>
<td>Andersen, 2008&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Retrospective cohort (electronic medical record review)</td>
<td>Melatonin, (oral: 0.75-6 mg‡ once a day (qd) for 1.8 years)</td>
<td>107</td>
<td>Mean: 8</td>
<td>80</td>
<td>Neurological AE</td>
<td>Serious</td>
</tr>
<tr>
<td>Malow, 2012&lt;sup&gt;4&lt;/sup&gt;</td>
<td>Before-after/ single-arm trial</td>
<td>Melatonin, (oral: 1-9 mg once a day (qd) for 14 weeks)</td>
<td>24</td>
<td>Mean: 5.9 Range: 3-9 years</td>
<td>83</td>
<td>RBS (subscales), GI AE, neurological AE</td>
<td>Serious</td>
</tr>
<tr>
<td>Paavonen, 2003&lt;sup&gt;5&lt;/sup&gt;</td>
<td>Before-after/ single-arm trial</td>
<td>Melatonin, (oral: 3 mg once a day (qd) for 14 days)</td>
<td>15</td>
<td>Mean: 10.3</td>
<td>87</td>
<td>GI AE, sleep-related AE, neurological AE</td>
<td>Moderate</td>
</tr>
<tr>
<td>Giannotti, 2006&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Before-after/ single-arm trial</td>
<td>Melatonin, (oral: 3-6 mg once a day (qd) for 6 months)&lt;sup&gt;§&lt;/sup&gt;</td>
<td>29</td>
<td>Range: 2.6-9.6 years</td>
<td>86</td>
<td>CARS, non-specific AE</td>
<td>Moderate</td>
</tr>
<tr>
<td>Ishizaki, 1999&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Before-after/ single-arm trial</td>
<td>Total, melatonin, oral: (2-6 mg (age&gt;=20) or 0.025 mg/kg (age&lt;20, and SMID)) once a day (qd) for 2-30 months)&lt;sup&gt;ǁ&lt;/sup&gt;</td>
<td>50</td>
<td>Range: 3-28 years</td>
<td>82</td>
<td>Improvement in repetitive behavior, improvement in stereotyped behavior, sleep-related AE, mood AE, behavioral AE, non-specific AE</td>
<td>Serious</td>
</tr>
</tbody>
</table>

AE = adverse events; CARS = Childhood Autism Rating Scale; GI = gastrointestinal; NA = not applicable; RBS = Repetitive Behavior Scale; SMID = severe motor intellectual disability

* Overall risk of bias was scored differently for randomized trials than for observational studies. The ratings for randomized trials are low, medium, or high. The ratings for observational studies are low, moderate, serious, or critical.

† Initial dose was 2 mg, max dose was 10 mg, and dose range was 2 to 10 mg.

‡ Children less than six years of age were started on 0.75-1 mg of melatonin and parents were instructed to increase melatonin by 1 mg every 2 weeks (up to 3 mg), if no clinical response was seen at lower dose. Children six years and older were started on 1.5 mg and parents were instructed to increase the dose to 3 mg after 3 weeks, if no clinical response was seen at the lower dose. In all children, if no response was seen after 4 weeks, the dose was increased to 6 mg.

§ The maximum dose allowed for children under age 4 years was 4 mg and over age 4 was 6 mg. Melatonin was given for 6 months. After 1 month of discontinuation, melatonin could be re-administered.

ǁ The dose shown is planned, doses provided were 2-6 mg (age>=20); 0.01-0.1 mg/kg (age<20 and SMID), except for patients with Angelman syndrome who received 0.2 mg/kg.
Table C. Summary of the strength of evidence and conclusions for the effects of melatonin on critical outcomes among people with autism spectrum disorder

<table>
<thead>
<tr>
<th>Population</th>
<th>Outcome</th>
<th># Studies (participants)</th>
<th>Strength of evidence</th>
<th>Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with ASD</td>
<td>ADOS total score</td>
<td>No studies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients with ASD</td>
<td>SRS total score</td>
<td>No studies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients with ASD</td>
<td>Clinical Global Impressions</td>
<td>No studies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children with ASD</td>
<td>GI events</td>
<td>1 crossover trial (20)</td>
<td>Insufficient</td>
<td>We are unable to draw a conclusion.*</td>
</tr>
<tr>
<td>Adults with ASD</td>
<td>GI events</td>
<td>No studies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients with ASD</td>
<td>Sleep events</td>
<td>1 before-after trial (27)*</td>
<td>Insufficient</td>
<td>We are unable to draw a conclusion.*</td>
</tr>
</tbody>
</table>

ADOS = Autistic Diagnostic Observation schedule; ASD = autism spectrum disorder; GI = gastrointestinal; SRS = Social Responsiveness Scale
* There were concerns about study limitations and the results were imprecise. We also suspected publication bias.
† Both children and adults were included in this study.
Oxytocin

We identified 18 studies (22 publications) evaluating oxytocin in human subjects\(^9\text{-}30\) (see Table 4) and 39 studies in animals.\(^{31\text{-}69}\)

**Human Studies**

Table 5.

Children (two studies): oxytocin may have little to no effect on repetitive behaviors and restricted interests but may have a positive effect on social communication and interaction among children with ASD.

Adolescents (four studies): oxytocin may have little to no effect on repetitive behaviors and restricted interests among adolescents with ASD. The effect of oxytocin on social communication and interaction is unclear.

Adults (seven studies): effect of oxytocin on repetitive behaviors and restricted interests among adults with ASD is unclear. Oxytocin may have little to no effect on social communication and interaction.

Adverse effects: most studies in adults and adolescents; meta-analysis results for tiredness, headaches, and nasal discomfort or congestion did not suggest an increased risk of these types of adverse events among individuals that received oxytocin; little to no evidence that use of oxytocin results in increased gastrointestinal, respiratory, sleep, neurologic, urinary, reproductive, or cardiovascular events.

**Animal Studies**

Rodents (27 studies): oxytocin treatment increases repetitive behaviors and restricted interests consistently across different rodent models representing social, non-social, and ASD-relevant genetically modified strains.

Non-human primates (seven studies): little to no change in social interactions among non-human primates that received an oxytocin intervention (inconsistent results).

Prairie voles (five studies): limited evidence of increased social behaviors in prairie voles after receiving oxytocin.

Zebrafish (one study): unable to draw conclusions about the effect of oxytocin on autism-relevant behaviors in zebrafish.
<table>
<thead>
<tr>
<th>Author, year</th>
<th>Study design</th>
<th>Intervention</th>
<th>Baseline N</th>
<th>Age</th>
<th>Male, %</th>
<th>Outcomes assessed</th>
<th>Overall risk of bias*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bernaerts, 2020</td>
<td>Randomized controlled trial</td>
<td>Group 1, placebo (intranasal: once a day for 4 weeks)</td>
<td>18</td>
<td>Mean: 24.00 years</td>
<td>100</td>
<td>SRS, RBS, GI AE, constitutional AE, respiratory AE, sleep-related AE, mood AE, behavioral AE, neurological AE, non-specific AE</td>
<td>Low</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group 2, oxytocin (intranasal: 24 IU once a day for 4 weeks)</td>
<td>22</td>
<td>Mean: 25.00 years</td>
<td>100</td>
<td>SRS, RBS, GI AE, constitutional AE, respiratory AE, sleep-related AE, mood AE, behavioral AE, neurological AE, non-specific AE</td>
<td>Low</td>
</tr>
<tr>
<td>Yamasue, 2018</td>
<td>Randomized controlled trial</td>
<td>Group 1, placebo (intranasal: twice a day (bid) for 6 weeks)</td>
<td>53</td>
<td>Mean: 26.3 Range: 18-48 years</td>
<td>100</td>
<td>CGI-I, GAF, ADOS (subscales), GI AE, respiratory AE, neurological AE, cardiovascular AE,</td>
<td>Low</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group 2, oxytocin (intranasal: 24 IU twice a day (bid) for 6 weeks)</td>
<td>53</td>
<td>Mean: 27.6 Range: 18-48 years</td>
<td>100</td>
<td>CGI-I, GAF, ADOS (subscales), GI AE, respiratory AE, neurological AE, cardiovascular AE,</td>
<td>Low</td>
</tr>
<tr>
<td>Parker, 2017</td>
<td>Randomized controlled trial</td>
<td>Group 1, placebo (intranasal: twice a day (bid) for 4 weeks)</td>
<td>18</td>
<td>Mean: 8.13 years</td>
<td>78</td>
<td>SRS, GI AE, respiratory AE, mood AE, neurological AE</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group 2, oxytocin (intranasal: 24 IU twice a day (bid) for 4 weeks)</td>
<td>14</td>
<td>Mean: 9.35 years</td>
<td>76</td>
<td>SRS, GI AE, respiratory AE, mood AE, neurological AE</td>
<td>High</td>
</tr>
<tr>
<td>Guastella, 2015</td>
<td>Randomized controlled trial</td>
<td>Group 1, placebo (intranasal: once a day (qd) for 8 weeks)</td>
<td>24</td>
<td>Mean: 14.00 years</td>
<td>100</td>
<td>CGI-I, SRS, RBS, GI AE, constitutional AE, respiratory AE, mood AE, behavioral AE</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group 2, oxytocin (intranasal: 18 or 24 IU twice a day (bid) for 8 weeks)</td>
<td>26</td>
<td>Mean: 13.85 years</td>
<td>100</td>
<td>CGI-I, SRS, RBS, GI AE, constitutional AE, respiratory AE, mood AE, behavioral AE</td>
<td>High</td>
</tr>
<tr>
<td>Dadds, 2014</td>
<td>Randomized controlled trial</td>
<td>Group 1, placebo (intranasal: once a day (qd) for 4 days)</td>
<td>19</td>
<td>Mean: 10.74 years</td>
<td>100</td>
<td>CARS</td>
<td>Unclear</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group 2, oxytocin (intranasal: 12 or 24 IU once a day (qd) for 4 days)</td>
<td>19</td>
<td>Mean: 11.79 years</td>
<td>100</td>
<td>CARS</td>
<td>Unclear</td>
</tr>
<tr>
<td>Anagnostou, 2012</td>
<td>Randomized controlled trial</td>
<td>Group 1, placebo (intranasal: twice a day (bid) for 6 weeks)</td>
<td>9</td>
<td>Mean: 32.9 years</td>
<td>78</td>
<td>CGI-I, RBS, constitutional AE, respiratory AE, mood AE, behavioral AE, neurological AE, non-specific AE</td>
<td>Low</td>
</tr>
<tr>
<td>Author, year</td>
<td>Study design</td>
<td>Intervention</td>
<td>Baseline N</td>
<td>Age</td>
<td>Male, %</td>
<td>Outcomes assessed</td>
<td>Overall risk of bias*</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------</td>
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<td>------------</td>
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<td>--------</td>
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<td>---------------------</td>
</tr>
<tr>
<td>Kosaka, 2016&lt;sup&gt;22&lt;/sup&gt;</td>
<td>Randomized controlled trial&lt;sup&gt;†&lt;/sup&gt;</td>
<td>Group 1, placebo (intranasal: once a day (qd) for 12 weeks)</td>
<td>20</td>
<td>Mean: 24.9 years</td>
<td>80</td>
<td>CGI-S, CGI-I, GI AE, respiratory AE, sleep-related AE, reproductive AE, cardiovascular AE, non-specific AE</td>
<td>Low</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group 2, low-dose oxytocin (intranasal: 16 IU once a day (qd) for 12 weeks)</td>
<td>20</td>
<td>Mean: 23.1 years</td>
<td>90</td>
<td>CGI-S, CGI-I, GI AE, respiratory AE, sleep-related AE, reproductive AE, cardiovascular AE, non-specific AE</td>
<td>Low</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group 3, high-dose oxytocin (intranasal: 32 IU once a day (qd) for 12 weeks)</td>
<td>20</td>
<td>Mean: 24.8 years</td>
<td>65</td>
<td>CGI-S, CGI-I, GI AE, respiratory AE, sleep-related AE, reproductive AE, cardiovascular AE, non-specific AE</td>
<td>Low</td>
</tr>
<tr>
<td>Auyeung, 2015&lt;sup&gt;12&lt;/sup&gt;</td>
<td>Randomized crossover trial (1 week)</td>
<td>Total</td>
<td>32</td>
<td>Mean: 36.04 Range: 18.5-56.0 years</td>
<td>100</td>
<td>Constitutional AE, respiratory AE, non-specific AE</td>
<td>Unclear</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group 1, placebo (intranasal: once a day (qd) one time)</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>Constitutional AE, respiratory AE, non-specific AE</td>
<td>Unclear</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group 2, oxytocin (placebo, intranasal) (intranasal: 24 IU once a day (qd) one-time injection)</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>Constitutional AE, respiratory AE, non-specific AE</td>
<td>Unclear</td>
</tr>
<tr>
<td>Yatawara, 2016&lt;sup&gt;30&lt;/sup&gt;</td>
<td>Randomized crossover trial (4 weeks)</td>
<td>Total</td>
<td>31</td>
<td>Mean: 6.2 Range: 3.0-8.9 years</td>
<td>87.1</td>
<td>ADOS (total score), SRS, CGI-I, behavioral AE, non-specific AE</td>
<td>Low</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group 1, placebo (intranasal: twice a day (bid) for 5 weeks)</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>ADOS (total score), SRS, CGI-I, behavioral AE, non-specific AE</td>
<td>Low</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group 2, oxytocin (intranasal: 3-24 IU per day, escalated frequency, for 5 weeks)</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>ADOS (total score), SRS, CGI-I, behavioral AE, non-specific AE</td>
<td>Low</td>
</tr>
<tr>
<td>Guastella, 2010&lt;sup&gt;16&lt;/sup&gt;</td>
<td>Randomized crossover trial (1 week)</td>
<td>Total</td>
<td>15</td>
<td>Mean: 14.88 years</td>
<td>100</td>
<td>Constitutional AE, respiratory AE, sleep-related AE, neurological AE</td>
<td>Unclear</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group 1, placebo (intranasal: once a day (qd) one time)</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>Constitutional AE, respiratory AE, sleep-related AE, neurological AE</td>
<td>Unclear</td>
</tr>
<tr>
<td>Author, year</td>
<td>Study design</td>
<td>Intervention</td>
<td>Baseline N</td>
<td>Age</td>
<td>Male, %</td>
<td>Outcomes assessed</td>
<td>Overall risk of bias*</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------</td>
<td>-------------------------------------------------------------------------------</td>
<td>------------</td>
<td>-----------</td>
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<td>--------------------------------------------------------------------------------------------------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>Hollander, 2007&lt;sup&gt;20&lt;/sup&gt;</td>
<td>Randomized crossover trial (1 week)</td>
<td>Group 2, oxytocin (intranasal: 18 or 24 IU once a day (qd) one-time injection)</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>Constitutional AE, respiratory AE, sleep-related AE, neurological AE,</td>
<td>Unclear</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group 1, placebo (intravenous: one 4-hour period, single dose)</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>RBS</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group 2, oxytocin (intravenous: 10 to 700 ml/hr, one 4-hour period, single dose)</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>RBS</td>
<td>High</td>
</tr>
<tr>
<td>Watanabe, 2015&lt;sup&gt;20&lt;/sup&gt;</td>
<td>Randomized crossover trial (1 day)</td>
<td>Group 1, placebo first (intranasal: twice a day (bid) for 6 weeks)</td>
<td>9</td>
<td>Mean: 29.3 24-43 years</td>
<td>100</td>
<td>ADOS (subscales), RBS, GI AE, constitutional AE, respiratory AE, mood AE, non-specific AE</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group 2, oxytocin first (intranasal: 24 IU twice a day (bid) for 6 weeks)</td>
<td>9</td>
<td>Mean: 35.1 24-42 years</td>
<td>100</td>
<td>ADOS (subscales), RBS, GI AE, constitutional AE, respiratory AE, mood AE, non-specific AE</td>
<td>High</td>
</tr>
<tr>
<td>Quintana, 2017&lt;sup&gt;20&lt;/sup&gt;</td>
<td>Randomized crossover trial (24 hours)</td>
<td>Total</td>
<td>17</td>
<td>Mean: 24.76 years</td>
<td>100</td>
<td>Non-specific AE</td>
<td>Low</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group 1, placebo (intranasal: once a day (qd) for 2 days)</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>Non-specific AE</td>
<td>Low</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group 2, low-dose oxytocin (intranasal: 8 IU once a day (qd) for 2 days)</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>Non-specific AE</td>
<td>Low</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group 3, high-dose oxytocin (intranasal: 24 IU once a day (qd) for 2 days)</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>Non-specific AE</td>
<td>Low</td>
</tr>
<tr>
<td>Watanabe, 2014&lt;sup&gt;20&lt;/sup&gt;</td>
<td>Randomized crossover trial (1 week)</td>
<td>Total</td>
<td>33</td>
<td>Mean: 28.5 years</td>
<td>100</td>
<td>SRS, AQ, CARS, non-specific AE</td>
<td>Low</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group 1, placebo (intranasal: once a day (qd) one time)</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>SRS, AQ, CARS, non-specific AE</td>
<td>Low</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group 2, oxytocin (intranasal: 24 IU once a day (qd), one-time injection)</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>SRS, AQ, CARS, non-specific AE</td>
<td>Low</td>
</tr>
<tr>
<td>Author, year</td>
<td>Study design</td>
<td>Intervention</td>
<td>Baseline N</td>
<td>Age</td>
<td>Male, %</td>
<td>Outcomes assessed</td>
<td>Overall risk of bias*</td>
</tr>
<tr>
<td>----------------------</td>
<td>--------------------------------------------------------</td>
<td>------------------------------------------------------------------------------</td>
<td>------------</td>
<td>----------------</td>
<td>---------</td>
<td>--------------------------------------------------------------------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td>Munesue, 2016&lt;sup&gt;23&lt;/sup&gt;</td>
<td>Randomized crossover trial (no washout period)</td>
<td>Group 1, oxytocin first, then placebo (intranasal: 8 IU twice a day (bid) for 8 weeks)</td>
<td>15</td>
<td>Mean: 22.6 years</td>
<td>100</td>
<td>CGI-I, CARS, GI AE, respiratory AE, mood AE, neurological AE</td>
<td>Low</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group 2, placebo first, then oxytocin (intranasal: 8 IU twice a day (bid) for 8 weeks)</td>
<td>14</td>
<td>Mean: 22.4 years</td>
<td>100</td>
<td>CGI-I, CARS, GI AE, respiratory AE, mood AE, neurological AE</td>
<td>Low</td>
</tr>
<tr>
<td>Anagnostou, 2014&lt;sup&gt;9&lt;/sup&gt;</td>
<td>Cohort without a comparison group</td>
<td>Total, oxytocin** (intranasal: 0.2-0.4 IU/kg twice a day (bid) for 12 weeks)</td>
<td>15</td>
<td>Mean: 13.8 years</td>
<td>73.3</td>
<td>SRS, CGI-I, RBs, GI AE, constitutional AE, respiratory AE, mood AE, non-specific AE</td>
<td>Serious</td>
</tr>
<tr>
<td>Hirosawa, 2017&lt;sup&gt;19&lt;/sup&gt;</td>
<td>Before-after/single-arm trial</td>
<td>Total, oxytocin (intranasal: 24 IU, once a day (qd); for 10 weeks)</td>
<td>10</td>
<td>Mean: 30.3 Range: 23-41 years</td>
<td>100</td>
<td>AQ, RBS</td>
<td>Serious</td>
</tr>
<tr>
<td>Kosaka, 2016&lt;sup&gt;22&lt;/sup&gt;</td>
<td>Cohort without a comparison group&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>Group 1, past placebo (intranasal: once a day (qd) for 12 weeks), now (oxytocin, intranasal: 16-32 IU once a day (qd) for 12 weeks)</td>
<td>16</td>
<td>NR</td>
<td>NR</td>
<td>GI AE, respiratory AE, sleep-related AE, reproductive AE, cardiovascular AE</td>
<td>Serious</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group 2, past low-dose oxytocin (intranasal: 16 IU once a day (qd) for 12 weeks), now oxytocin (intranasal: 16-32 IU once a day (qd) for 12 weeks)</td>
<td>16</td>
<td>NR</td>
<td>NR</td>
<td>GI AE, respiratory AE, sleep-related AE, reproductive AE, cardiovascular AE</td>
<td>Serious</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group 3, past high-dose oxytocin (intranasal: 32 IU once a day (qd) for 12 weeks), now oxytocin (intranasal: 16-32 IU once a day (qd) for 12 weeks)</td>
<td>18</td>
<td>NR</td>
<td>NR</td>
<td>GI AE, reproductive AE, sleep-related AE, reproductive AE, cardiovascular AE</td>
<td>Serious</td>
</tr>
<tr>
<td>Tachibana, 2013&lt;sup&gt;26&lt;/sup&gt;</td>
<td>Before-after/single-arm trial</td>
<td>Total, oxytocin (intranasal: 8-24 IU twice a day (bid) for 7 months)</td>
<td>8</td>
<td>Mean: 11.9 Range: 10-14 years</td>
<td>100</td>
<td>ADOS (subscales)</td>
<td>Serious</td>
</tr>
</tbody>
</table>

ADOS = Autism Diagnostic Observation Schedule; AE = adverse events; AQ = Autism Spectrum Quotient; CARS = Childhood Autism Rating Scale; CGI-I = Clinical Global Impression – Improvement Scale; CGI-S = Clinical Global Impression – Severity Scale; GAF = Global Assessment of Functioning; GI = gastrointestinal; NR = not reported; RBs = Repetitive Behavior Scale; SRS = Social Responsiveness Scale

* Overall risk of bias was scored differently for randomized trials than for observational studies. The ratings for randomized trials are low, medium, or high. The ratings for observational studies are low, moderate, serious, or critical.
†The trial had a 12-week double-blind phase, followed by a 12-week open label phase, followed by an 8-week follow-up phase. The results for the first double-blind phase only are reported here, the second phase is reported below.
‡This is the second phase of a three-phase study. The first phase was double-blinded and placebo-controlled. The second phase was open-label. The third phase was follow-up with no drug. There was no washout period between phases 1 and 2.
Table E. Summary of the strength of evidence and conclusions for the effects of oxytocin on critical outcomes among people with autism spectrum disorder

<table>
<thead>
<tr>
<th>Population</th>
<th>Outcome</th>
<th># Studies (participants)</th>
<th>Strength of evidence</th>
<th>Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children with ASD</td>
<td>ADOS total score</td>
<td>1 crossover trial (39)</td>
<td>Insufficient</td>
<td>We are unable to draw a conclusion.</td>
</tr>
<tr>
<td>Adolescents with ASD</td>
<td>ADOS total score</td>
<td>No studies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adults with ASD</td>
<td>ADOS total score</td>
<td>No studies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children with ASD</td>
<td>SRS total score</td>
<td>1 RCT (35)</td>
<td>Low</td>
<td>SRS total scores may improve</td>
</tr>
<tr>
<td>Adolescents with ASD</td>
<td>SRS total score</td>
<td>1 RCT (50)</td>
<td>Insufficient</td>
<td>We are unable to draw a conclusion.</td>
</tr>
<tr>
<td>Adults with ASD</td>
<td>SRS total score</td>
<td>2 RCT (59)</td>
<td>Low</td>
<td>Little to no effect</td>
</tr>
<tr>
<td>Children with ASD</td>
<td>Clinical Global Impressions</td>
<td>1 crossover trial (39)</td>
<td>Insufficient</td>
<td>We are unable to draw a conclusion.</td>
</tr>
<tr>
<td>Adolescents with ASD</td>
<td>Clinical Global Impressions</td>
<td>1 RCT (50)</td>
<td>Insufficient</td>
<td>We are unable to draw a conclusion.</td>
</tr>
<tr>
<td>Adults with ASD</td>
<td>Clinical Global Impressions</td>
<td>3 RCTs (185)</td>
<td>Low</td>
<td>Little to no effect</td>
</tr>
<tr>
<td>Children with ASD</td>
<td>GI events</td>
<td>1 RCT (35)</td>
<td>Insufficient</td>
<td>We are unable to draw a conclusion.</td>
</tr>
<tr>
<td>Adolescents with ASD</td>
<td>GI events</td>
<td>1 RCT (50)</td>
<td>Insufficient</td>
<td>We are unable to draw a conclusion.</td>
</tr>
<tr>
<td>Adults with ASD</td>
<td>GI events</td>
<td>4 RCTs (238)</td>
<td>Low</td>
<td>Little to no effect</td>
</tr>
<tr>
<td>Children with ASD</td>
<td>Sleep events</td>
<td>No studies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adolescents with ASD</td>
<td>Sleep events</td>
<td>1 RCT (50)</td>
<td>Insufficient</td>
<td>We are unable to draw a conclusion.</td>
</tr>
<tr>
<td>Adults with ASD</td>
<td>Sleep events</td>
<td>1 RCT (60)</td>
<td>Insufficient</td>
<td>We are unable to draw a conclusion.</td>
</tr>
</tbody>
</table>

ADOS = Autistic Diagnostic Observation schedule; ASD = autism spectrum disorder; GI = gastrointestinal; RCT = randomized controlled trial; SRS = Social Responsiveness Scale
Evidence Integration

We integrated evidence from human and animal studies for oxytocin. To do so, we first determined which outcomes most closely match between humans and animal models and then mapped the results for these outcomes onto matrixes for the two main domains of ASD: (i) repetitive and restrictive behaviors (Figure 1), and (ii) social communication (Figure 2).

The existing animal and human evidence indicate that oxytocin did not improve *repetitive behaviors*. In fact, findings from most rodent studies report an increase in repetitive behaviors as well as cognitive inflexibility with oxytocin treatment. In humans, repetitive behaviors in children and adolescents did not improve and unclear benefits have been reported in adults. With regards to *social communication*, evidence from studies in prairie voles, zebrafish and non-human primates indicate that oxytocin does not improve various types of social behaviors. Evidence from human studies indicate age-related differences in outcomes with two small studies reporting potential benefit in children, though no clear benefits were present in adolescents or adults.

Despite these negative or inconclusive findings, studies in both animal models and humans need to be interpreted with caution due to inherent study biases, as well as methodological differences in sample characteristics, as well as oxytocin administration (e.g., dose, route, and duration). Furthermore, several factors limit the application of animal studies to humans (e.g., pure animal strains do not reflect the heterogeneous clinical profiles in ASD) and these must be considered when interpreting the findings and considering future research studies in the field.
Figure A. Integration of evidence from studies of human and non-human subjects on the effects of oxytocin on repetitive behaviors and restricted interests

<table>
<thead>
<tr>
<th>Evidence from Studies in Non-Human Subjects</th>
<th>Possibly positive effect</th>
<th>No effect</th>
<th>Possibly negative effect</th>
<th>Unclear</th>
</tr>
</thead>
<tbody>
<tr>
<td>Possibly positive effect</td>
<td><img src="image1.png" alt="Children Adolescents Rodents" /></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No effect</td>
<td></td>
<td><img src="image2.png" alt="Children Adolescents Rodents" /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Possibly negative effect</td>
<td></td>
<td><img src="image3.png" alt="Children Adolescents Rodents" /></td>
<td><img src="image4.png" alt="Children Primates Zebrafish" /></td>
<td></td>
</tr>
<tr>
<td>Unclear</td>
<td></td>
<td><img src="image5.png" alt="Children Adolescents Rodents" /></td>
<td><img src="image6.png" alt="Children Primates Zebrafish" /></td>
<td><img src="image7.png" alt="Adolescents Primates Zebrafish" /></td>
</tr>
</tbody>
</table>

Figure B. Integration of evidence from studies of human and non-human subjects on the effects of oxytocin on social communication and interaction

<table>
<thead>
<tr>
<th>Evidence from Studies in Human Subjects</th>
<th>Possibly positive effect</th>
<th>No effect</th>
<th>Possibly negative effect</th>
<th>Unclear</th>
</tr>
</thead>
<tbody>
<tr>
<td>Possibly positive effect</td>
<td><img src="image8.png" alt="Children Prairie voles" /></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No effect</td>
<td><img src="image9.png" alt="Children Prairie voles" /></td>
<td></td>
<td><img src="image10.png" alt="Children Primates Zebrafish" /></td>
<td></td>
</tr>
<tr>
<td>Possibly negative effect</td>
<td><img src="image11.png" alt="Children Prairie voles" /></td>
<td><img src="image12.png" alt="Children Primates Zebrafish" /></td>
<td><img src="image13.png" alt="Adolescents Primates Zebrafish" /></td>
<td></td>
</tr>
<tr>
<td>Unclear</td>
<td><img src="image14.png" alt="Adolescents Prairie voles" /></td>
<td><img src="image15.png" alt="Adolescents Primates Zebrafish" /></td>
<td><img src="image16.png" alt="Adolescents Prairie voles" /></td>
<td></td>
</tr>
</tbody>
</table>
Conclusions

We found limited to no evidence for 4 of the 6 substances (DMPS (n=0), glutathione (n=1), inositol (n=1), methylcobalamin (n=2)). For melatonin, all of the studies included children with co-existing sleep disorders, and there was limited evidence on ASD-specific outcomes. Evidence from human and animal studies suggest that oxytocin does not improve repetitive behaviors. For social communication, there may be benefit of oxytocin in children but overall the findings are inconsistent.
References


Methods

Objectives

The objective of the systematic review is to summarize the available evidence on the safety and effectiveness for each bulk drug substance for treating autism spectrum disorder (ASD). The Food and Drug Administration (FDA) requested review of 5 bulk substances: inositol, methylcobalamin, glutathione, 2,3-dimercapto-1-propanesulfonic acid sodium (DMPS), and melatonin. Based on the findings from preliminary searching and discussions with experts (Phase I), the Johns Hopkins team and the FDA agreed to include one additional bulk substance: oxytocin.

For each of the six bulk substances, we evaluated two bodies of evidence for safety and effectiveness:

1. human clinical studies
2. animal studies (using animal models of autism spectrum disorder models and/or animal behavioral phenotypes potentially relevant to ASD core symptoms)

We developed a protocol, which was modified for each bulk drug substance. The modified protocols were registered on PROSPERO (CRD42019121632, CRD42019121629, CRD42019118845, CRD42019121639, CRD42019121626, CRD42019121649, CRD42019122988, CRD42019122983, CRD42019118855, CRD42019122990, CRD42019122981, CRD42019122991).

Search Strategy

We developed a search strategy that was appropriate for each bulk drug substance. We developed separate searches for human and for animal studies.

For human studies, we searched the following databases through January 2020: PubMed®, Embase™, Cochrane Central Register of Controlled Trials (CENTRAL), and Web of Science. We developed a search strategy for PubMed using medical subject headings (MeSH®) and text words. We relied on the Safety Data Sheet (SDS) data from ChemWatch for alternate names for the bulk drug substances. We also searched ClinicalTrials.gov and the Cochrane Complementary Medicine field registry (CAM registry) in February 2020. For animal studies, we searched the following databases through February 2020: PubMed®, Embase™, Cochrane Central Register of Controlled Trials (CENTRAL), and Web of Science. Search strings are listed in Appendix A.

Study Selection

We defined the eligibility criteria for each research question a priori with input from the FDA. For human studies, we included studies in people of any age with ASD that evaluated one of the bulk drug substances of interest (DMPS, glutathione, inositol, melatonin, methylcobalamin, or oxytocin). To be included, we needed to be able to isolate the effects of the bulk drug substance. We only included studies that evaluated a non-oral formulation of oxytocin.
We included studies that assessed a core symptom of ASD or safety. We used the diagnostic criteria from the Diagnostic and Statistical Manual, 5th edition to determine core symptoms of ASD. We included original studies regardless of study design, length of followup, language of publication, and type of publication.

For animal studies, we included animal models of rats/mice, non-human primates, prairie voles, or zebrafish. Genetic mouse models for ASD include 16p11.2 deletion, SHANK3B null mutant, Engrailed2 (En2) null mutants, Pten mutants, SHANK3 heterozygotes, and Tsc1 heterozygotes. Inbred mouse models for ASD include BTBR T+Itpr3 tf/J (BTBR), BALB/cByJ (BALB), A/J, C58/J (C58), and 129S1/SvImJ. Rat models include Fmr1 and Nlgn3 knockout rats. We also included studies conducted among wild type mice and rats. We included studies that evaluated one of the bulk drug substances of interest (DMPS, glutathione, inositol, melatonin, methylcobalamin, or oxytocin). We included studies conducted in mice or rats that evaluated repetitive behaviors or restricted interests. We included studies conducted in non-human primates, prairie voles, or zebrafish that evaluated social communication or interaction. Table 1 lists the included tests for each animal model.

### Table 1. Included tests for animal models

<table>
<thead>
<tr>
<th>Animal model</th>
<th>Included tests</th>
</tr>
</thead>
</table>
| Mice or rats          | • Repetitive self-grooming  
                        | • Marble burying  
                        | • Open field holeboard exploration  
                        | • M Morris water maze reversal  
                        | • Set-shifting assay  
                        | • 5-Choice Serial Reaction Time Task  
                        | • Motor stereotypy                                                                |
| Non-human primates    | • Reciprocal social interaction  
                        | • Social communication  
                        | • Peer social networks                                                             |
| Prairie voles         | • Partner preference  
                        | • Alloparental behavior                                                            |
| Zebrafish             | • Social preference  
                        | • Shoal formation  
                        | • Inhibitory avoidance  
                        | • Zebrafish ethograms                                                             |

Two reviewers from JHU independently screened titles and abstracts and full-text articles for eligibility. To be excluded, both reviewers agreed that the study met at least one exclusion criteria. Reviewers resolved differences regarding eligibility through discussion. We used DistillerSR (Evidence Partners, 2010) to manage the screening process.

**Data Extraction**

We created and pilot tested standardized forms to abstract data on general study characteristics, participant characteristics, interventions, and outcome measures. One reviewer abstracted the data, and a second reviewer confirmed the data abstraction. We completed the data abstraction process using the Systematic Review Data Repository (SRDR). Appendix B provides the screening and data abstraction forms.
Assessment of Risk of Bias

Two reviewers from JHU independently assessed the risk of bias for each included study. For randomized controlled trials, we used the Cochrane Risk of Bias Tool. For other study designs, we used the ROBINS-I tool. JHU will select a risk of bias assessment tool that is appropriate for assessing animal studies, such as SYstematic Review Centre for Laboratory animal Experimentation (SYRCLE).

Data Synthesis and Analysis

We created evidence tables detailing all the data abstracted from the included studies. We conducted meta-analyses when there were sufficient data (i.e., at least three studies) and studies were sufficiently homogenous with respect to key variables (e.g., study design, population characteristics, study duration, treatment, and outcome definition). We calculated a pooled effect estimate of the relative risk for dichotomous outcomes, with each study weighted by the inverse variance. When there were 0 events, we calculated pooled relative risks by using the treatment-group continuity correction (inverse of the sample size of the other treatment group in cells with 0 events). We used STATA statistical software (Intercooled, version 14.2, StataCorp, College Station, TX) for all meta-analyses. We qualitatively summarized studies that were not amenable to pooling.

Grading of the Evidence

We graded strength of evidence on outcomes by using the grading scheme recommended by the Agency for Healthcare Research and Quality Methods Guide for Conducting Comparative Effectiveness Reviews. We assigned evidence grades to the most critical outcomes for each question, identified a priori. For human studies, the most critical outcomes were the total scores for the Autism Diagnostic Observation Schedule (ADOS), the Social Responsiveness Scale (SRS), and the Clinical Global Impression Scale and gastrointestinal and sleep adverse events. We assessed the strength of the available evidence by assessing the limitations to individual study quality (using individual study risk of bias), consistency, directness, precision, and reporting publication bias. We did not grade the evidence for animal studies.

We classified evidence pertaining to the research questions into four basic categories: 1) “high” grade (indicating high confidence that the evidence reflects the true effect and that further research is very unlikely to change our confidence in the estimate of the effect); 2) “moderate” grade (indicating moderate confidence that the evidence reflects the true effect and that further research may change our confidence in the estimate of the effect and may change the estimate); 3) “low” grade (indicating low confidence that the evidence reflects the true effect and that further research is likely to change our confidence in the estimate of the effect and is likely to change the estimate); and 4) “insufficient” grade (evidence is unavailable or does not permit a conclusion).

Evidence Integration

We conducted evidence integration for oxytocin; the only substance for which we identified substantial animal and human evidence. We first determined which outcomes most closely correspond between humans and animal models. We then mapped the results for these outcomes onto matrices for the two main domains of ASD: (i) repetitive and restrictive behaviors, and (ii) social communication. The matrices were based on existing frameworks and approaches for
integrating evidence from human and animal streams: the Navigation Guide and the OHAT approach from the National Toxicology Program (NTP).\textsuperscript{11, 12}
Results

2,3-Dimercapto-1-Propanesulfonic Acid Sodium (DMPS)

Search Results

We retrieved 36 records from our human subjects search, 2 records from our animal subjects search (Figure 1). After screening abstracts and full-text, we did not include any articles evaluating DMPS in human or animal subjects. Appendix C lists the studies that were excluded during full-text review with their reasons for exclusion. Our search of ClinicalTrials.gov yielded one clinical trials record; this record was excluded.

Glutathione

Search Results

We retrieved 912 records from our human subjects search, 4325 records from our animal subjects search (Figure 2). After screening abstracts and full-text, we included one study evaluating glutathione in human subjects and no studies in animal subjects. Appendix C lists the included studies and the excluded studies with their reasons for exclusion. Our search of ClinicalTrials.gov yielded 13 records. After reviewing the clinical trial records, one was identified as being relevant to the review (NCT00889538). This record refers to a completed study, but no results are posted.

Description of Human Studies

We included one study examining glutathione in human subjects with ASD, an eight-week open-label trial in which 26 children with ASD (3-13 year olds) were randomized 1:1 to either transdermal or oral lipoceutical glutathione.

Risk of Bias in Human Studies

Risk of bias in the included study was determined to be high, due to the open-label design, and incomplete data for the outcome (attrition bias). In addition the publication was unclear with respect to randomization sequence, allocation concealment, blinding of care providers and outcome assessors, and selective reporting.
Figure 1. Summary of the literature search for 2,3-dimercapto-1-propanesulfonic acid sodium

**ASD** = autism spectrum disorder; **DMPS** = 2,3-Dimercapto-1-Propanesulfonic Acid Sodium
Figure 2. Summary of the literature search for glutathione

**Human Studies**
- Records identified through database searching (N = 912)
  - Records after duplicates removed (N = 514)
    - Records screened (N = 514)
      - Records excluded (N = 511)
        - Full-text articles assessed for eligibility (N = 3)
          - Excluded (N = 2) Does not evaluate glutathione, 1 Does not include people with ASD, 0 Does not assess core ASD symptoms or safety, 0 No original data, 1
            - Studies included 1 published study (1 RCT) 0 abstracts 0 case reports

**Animal Studies**
- Records identified through database searching (N = 4325)
  - Records after duplicates removed (N = 2314)
    - Records screened (N = 2314)
      - Records excluded (N = 2314)
        - Full-text articles assessed for eligibility (N = 0)
          - Studies included 0 published studies 0 abstracts

**ClinicalTrials.gov**
- Records identified through database searching (N = 13)
  - Records screened (N = 13)
    - Records excluded (N = 12)

*ASD = autism spectrum disorder*
Efficacy from Human Studies

The included study did not report on any relevant efficacy outcomes.

Adverse Events

Adverse events were measured using the Frequency and Intensity of Side-effect Rating (FISER)/Global Rating of Side-effect Burden (GRSEB)/Patient Report of Incidence of Side-Effects (PRISE) (FISER/GRSEB/PRISE), which includes global measures on a 7-point Likert scale from 0 to 6, with one rating anchored for frequency, another for intensity, and a third for the overall burden or degree of interference in day-to-day activities. Intensity of side-effects ranges from 0 = no side-effects to 6 = intolerable side-effects.

Mood Adverse Events

There were three cases of irritability (intolerable) reported among the 26 patients followed up for 8 weeks, including 1 out of 13 (7.7%) of patients taking oral lipoceutical glutathione and 2 out of 13 (15.4%) of patients taking transdermal glutathione.13

Dermatologic Adverse Events

There were two cases of rash (intolerable) during the study, occurring in 2 out of 13 (15.4%) of patients taking transdermal glutathione.13

Non-specific Adverse Events

Among the 26 randomized participants, there were a total of 17 children across both groups reporting none to minimal side effects; 10 children reporting mild side effects; and 1 child reporting moderate side effects.13

Animal Studies

We did not find any studies evaluating glutathione in animals.

Inositol

Search Results

We retrieved 230 records from our human subjects search, 432 records from our animal subjects search (Figure 3). After screening abstracts and full-text, we included one study evaluating inositol in human subjects14 and no studies in animal subjects. Our search of ClinicalTrials.gov yielded 1 record, which was considered relevant to the review (NCT03757585). According to the latest update, this study is recruiting participants.

Description of Human Studies

We included one study examining inositol in humans, a 4-week, placebo-controlled, double-blind crossover trial in which 10 children with ASD received inositol.14
Figure 3. Summary of the literature search for inositol

**Human Studies**
- Records identified through database searching (N = 230)
- Records after duplicates removed (N = 135)
- Records screened (N = 135)
- Records excluded (N = 129)
- Full-text articles assessed for eligibility (N = 6)
- Excluded (N = 5)
  - Does not evaluate inositol, 3
  - Does not include people with ASD, 1
  - Does not assess core ASD symptoms or safety, 0
  - No original data, 1
- Studies included
  - 1 published study (1 crossover trial)
  - 0 abstracts
  - 0 case reports

**Animal Studies**
- Records identified through database searching (N = 432)
- Records after duplicates removed (N = 253)
- Records screened (N = 253)
- Records excluded (N = 250)
- Full-text articles assessed for eligibility (N = 5)
- Excluded (N = 3)
  - Does not evaluate inositol, 2
  - Not a relevant animal model, 1
  - No relevant outcomes, 0
  - No original data, 0
- Studies included
  - 0 published studies
  - 0 abstracts

**ClinicalTrials.gov**
- Records identified through database searching (N = 1)
- Records screened (N = 1)
- Records excluded (N = 0)
- Studies included
  - 1 trial report with no results posted

*ASD = autism spectrum disorder*
Risk of Bias in Human Studies

Risk of bias in the included study was determined to be high based on the non-random allocation sequence and the selective reporting of results. The study did not clearly report allocation concealment or additional bias due to outside sources. Risk of bias due to blinding of participants, care providers, or incomplete outcome data, were low.

Efficacy from Human Studies

ASD Diagnostically Relevant Measures

We did not include any studies that evaluated inositol in people with ASD and reported on ASD diagnostically relevant measures.

Clinical Global Impression Scale

No significant statistical change in the Clinical Global Impression (CGI) Scale was reported among study subjects. No numeric results for CGI score were included in the report.

The evidence for this outcome was insufficient to draw conclusions regarding efficacy, due to the fact that this study was highly limited by concerns about the randomization scheme and imprecise reporting of results, as well as suspected reporting bias (study did not report baseline nor final means and standard deviations).

Childhood Autism Rating Scale

The effects and side-effects of inositol administration were evaluated in all study subjects using the Childhood Autism Rating Scale (CARS). No statistically significant change was observed in the CARS from baseline to Week 4. At baseline, the CARS scores in the treatment arms were 42.5 (standard deviation [SD], 6.9) in the placebo group and 42.4 (SD, 5.5) in the inositol group, and at Week 4, mean CARS scores were 42.0 (SD, 6.8) in the placebo group and 41.0 (SD, 7.6) in the inositol group. A single subject responded to inositol treatment as indicated by a change in CARS score, a decrease from 40.5 at baseline to 33.0 at Week 4.

Adverse Events

Gastrointestinal Adverse Events

There were three reported gastrointestinal adverse events, all occurring during the placebo phase of the cross-over study; 1 out of 10 (10%) patients reported nausea, 1 out of 10 (10%) reported decrease of appetite, and 1 out of 10 (10%) reported diarrhea. The evidence for this outcome was insufficient to draw conclusions regarding the risk of gastrointestinal adverse events related to inositol, due to the fact that this study was highly limited by concerns about the randomization scheme and imprecise reporting of results, as well as possible undetected reporting bias.

Mood Adverse Events

There was one reported mood adverse event in the trial, also during the placebo phase; 1 out of 10 (10%) patients refused to continue to ingest the medication powder.
Melatonin

Search Results

We retrieved 1350 records from our human subjects search, 1410 records from our animal subjects search (Figure 4). After screening abstracts and full-text, we included six studies (published in nine publications) evaluating melatonin in human subjects. Our search of ClinicalTrials.gov yielded 16 records. After reviewing the clinical trial records, we considered four as relevant to the review (NCT02487082, NCT01780883, and NCT00927030). Two of the studies were recruiting participants (NCT02487082, NCT019013681). The other two studies were completed (NCT01780883, NCT00927030), but did not have results posted and we were unable to identify a corresponding publication.

Description of Human Studies

We included six studies that evaluated the efficacy and safety of melatonin in patients with ASD. One was a 3-month randomized cross-over trial with a 1-month washout period between treatments. Another was a retrospective cohort with an average of 1.8 years of followup. Four studies were before-after trials with 7 weeks to 30 months of followup. Three studies reported on funding; all three studies received support from a nonprofit organization and two studies also received funding from government agencies. None of the studies reported a registration in a clinical trials registry.

All of the studies included children with ASD or Asperger’s syndrome and co-occurring sleep disorders. The randomized crossover trial used the World Health Organization research diagnostic criteria, with further evaluation with the Autism Diagnostic Interview - Revised (ADI-R) and Autism Diagnostic Observation Scale (ADOS) when needed. The retrospective cohort study used the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR) criteria. The before-after trials used the DSM-IV criteria, the ADOS, or the Childhood Autism Rating Scale (cut-off of 29.5).

The randomized crossover trial included 20 patients (mean age 9, 80% male) and randomized them to first receive either 7 mg oral melatonin or placebo. Seventy percent of the participants were diagnosed with autism, 10% with Asperger syndrome, and 20% with atypical autism. The retrospective cohort included 107 participants (mean age 9, 80% male) who were on 0.75 to 6 mg of melatonin for an average of 1.8 years. The sample size of the before-after studies ranged from 15 to 29 participants. The mean age of the participants in three of the studies ranged from 4.6 to 10.3 years; 83% to 87% of the participants were male. The fourth before-after study included participants with other disorders and did not report population characteristics separately for the ASD subgroup.
Figure 4. Summary of literature search for melatonin

**Human Studies**
- Records identified through database searching (N = 1350)
- Records after duplicates removed (N = 935)
- Records screened (N = 935)
- Records excluded (N = 873)
- Full-text articles assessed for eligibility (N = 62)
  - Excluded (N = 49)
    - Does not evaluate melatonin, 4
    - Does not include people with ASD, 26
    - Does not assess core ASD symptoms or safety, 19
    - No original data, 9
- Studies included
  - 6 published studies in 9 citations (1 crossover trial; 1 retrospective cohort; 4 before-after trials)
  - 2 abstracts
  - 2 case reports

**Animal Studies**
- Records identified through database searching (N = 1410)
- Records after duplicates removed (N = 880)
- Records screened (N = 880)
- Records excluded (N = 850)
- Full-text articles assessed for eligibility (N = 30)
  - Excluded (N = 28)
    - Does not evaluate melatonin, 0
    - Not a relevant animal model, 25
    - No relevant outcomes, 19
    - No original data, 0
- Studies included
  - 2 published studies
  - 0 abstracts

**ClinicalTrials.gov**
- Records identified through database searching (N = 16)
- Records screened (N = 16)
- Records excluded (N = 12)

*ASD = autism spectrum disorder*
Risk of Bias in Human Studies

We considered the randomized crossover trial to have a high risk of bias because of incomplete outcome data and selective outcome reporting.\textsuperscript{20} The retrospective cohort study was considered to have a serious risk of bias because of potential confounding.\textsuperscript{15} We considered the four before-after studies to have a moderate to serious risk of bias because of concerns with confounding, the classification of interventions, deviations from intended interventions, and/or measurement of outcomes.\textsuperscript{16-19}

Efficacy from Human Studies

ASD Diagnostically Relevant Measures

Autism Diagnostic Observation Schedule, Social Responsiveness Scale, Clinical Global Impression Scale

We did not find any studies that evaluated melatonin in patients with ASD and reported on changes in the Autism Diagnostic Observation Schedule, the Social Responsiveness Scale, or the Clinical Global Impression Scale.

Other Measures

One study evaluated melatonin in children with ASD and reported on changes in the Childhood Autism Rating Scale (CARS).\textsuperscript{16} At the 2-year assessment, there were no changes in the CARS score compared with baseline scores (41 versus 40.5; $P > 0.05$).

ASD Tools Measuring Core Domain 1: Social Challenges

We did not find any studies that evaluated melatonin in patients with ASD and reported on any ASD tool measuring social challenges, such as the Autism Diagnostic Observation Schedule - social subscale, the Social Responsiveness Scale, or the Interaction Rating Scale Advanced.

ASD Tools Measuring Core Domain 2: Restricted Interests and/or Repetitive Behaviors

Autism Diagnostic Observation Schedule or Social Responsiveness Scale

We did not find any studies that evaluated melatonin in patients with ASD and reported on restricted interests and/or repetitive behaviors using the Autism Diagnostic Observation Scale or the Social Responsiveness Scale.

Repetitive Behavior Scale

Two studies evaluated melatonin in patients with ASD and reported restricted interests and/or repetitive behaviors.\textsuperscript{17, 18} One study reported on the stereotyped, self-injurious, compulsive, ritualistic, sameness, and restricted subscales of the Repetitive Behavior Scale.\textsuperscript{18} After 17 weeks, participants scored significantly better on the compulsive ($P < 0.0001$), stereotyped ($P = 0.008$), sameness ($P = 0.017$), and ritualistic ($P = 0.013$) subscales, but not the self-injurious ($P = 0.325$) and the restricted ($P = 0.13$) subscales.
In the other study, 2 out of 8 (25%) participants improved their repetitive behavior and 0 out of 1 (0%) participant improved their stereotyped behavior.\textsuperscript{17}

**Adverse Events**

**Gastrointestinal Events**

Two studies evaluated melatonin in children with ASD and reported on gastrointestinal events.\textsuperscript{19, 20} In the randomized crossover trial, there were no statistically significant differences in the frequencies of vomiting, tummy aches, and constipation (frequencies were not reported).\textsuperscript{20} Although there were more participants experiencing reduced appetite (64.7\% versus 50\%) and diarrhea (29.4\% versus 12.5\%) while on melatonin than on placebo, the differences were not statistically significant.

The before-after study reported 1 out of 15 (6.7\%) participants experiencing diarrhea while on melatonin.\textsuperscript{19}

Because of the concern for study limitations, the imprecise results, and the suspicion of reporting bias, we are unable to draw a conclusion of the effects of melatonin on gastrointestinal events in children with ASD (insufficient evidence).

**Sleep**

One before-after study reported 3 out of 27 (11.1\%) participants awakening during the middle of sleep while on melatonin.\textsuperscript{17}

Because of the concern for study limitations, the imprecise results, and the suspicion of reporting bias, we are unable to draw a conclusion of the effects of melatonin on sleep events in patients with ASD (insufficient evidence).

**Constitutional Events**

Three studies evaluated melatonin in children with ASD and reported on constitutional events.\textsuperscript{17, 19, 20} In the randomized crossover trial, there were more participants experiencing daytime drowsiness (64.7\% versus 31.2\%) while on melatonin than on placebo, however, the differences were not statistically significant.\textsuperscript{20} One of the before-after studies reported 6.7\% of their 15 participants experienced extensive tiredness while on melatonin.\textsuperscript{19} The other before-after study reported 11.1\% of 27 participants experienced residual drowsiness the next morning.\textsuperscript{17}

**Respiratory Events**

One randomized crossover trial reported on respiratory events among children with ASD who received either 7mg of melatonin or placebo.\textsuperscript{20} This trial reported no statistically significant differences between melatonin and placebo in terms of sore throat, earaches, or asthma (frequencies not reported).

**Mood Events**

Two studies evaluated melatonin in children with ASD and reported on mood events.\textsuperscript{17, 20} In the randomized crossover trial, there were no statistically significant differences between melatonin and placebo in terms of low mood, anxiety, irritability, and tearfulness (frequencies not reported).\textsuperscript{20} Among the 27 participants in the before-after study, 11.1\% experienced excitement after awakening, 7.4\% experienced excitement during the day, and 3.7\% experienced excitement before going to bed.\textsuperscript{17}
Behavioral Symptoms
One before-after study reported 1 out of 27 (3.7%) participants becoming more aggressive while on melatonin.17

Neurologic Symptoms
Four studies evaluated melatonin in children with ASD and reported on neurologic events.15, 18-20 In the randomized crossover trial, there were no statistically significant differences between melatonin and placebo in terms of dizziness, headaches, confusion, and mild tremor.20 The trial also reported non-statistically significantly more people experiencing reduced alertness (41.2% versus 18.7%) while on melatonin compared with placebo. There were no reports of fits or seizures while on melatonin or placebo in the trial.

In the retrospective cohort study, there were no new-onset seizures and no increase in seizures among children with epilepsy.15 In one before-after study, 4.8% of the 21 participants reported experiencing generalized tonic-clonic seizure.18 In the other before-after study, 6.7% of the participants experienced headache and 6.7% experienced dizziness.19

Dermatologic Symptoms
In the randomized crossover trial, there were no statistically significant differences in the number of participants reporting rashes while on melatonin compared to placebo (frequencies not reported).20

Urinary/Reproductive Events
We did not include any studies evaluating melatonin in patients with ASD that reported on urinary or reproductive events.

Cardiovascular Events
We did not include any studies evaluating melatonin in patients with ASD that reported on cardiovascular events.

Nonspecific Events
In one before-after study, 4.2% of the 24 participants experienced loose stool.18 In another before-after study, none of the participants discontinued the trial due to adverse events.16 In a third before-after study, 37% of the participants reported any adverse event.17

Evidence from Animal Studies

Rodent Model Summary
Two studies with a medium risk of bias assessed the effects of melatonin in adult Wistar rats.21, 22 Rats were given either intraperitoneal melatonin or placebo. In one study, rats were given 50 g per 100 g of melatonin as a single dose.21 In the other study, rats were given 10 mg/kg of melatonin for 7 days.22

Both studies evaluated repetitive self-grooming among rats. One study reported significantly more self-grooming episodes following intraperitoneal melatonin,21 but the other study did not.22
Methylcobalamin

Search Results

We retrieved 411 records from our human subjects search, 176 records from our animal subjects search (Figure 5). After screening abstracts and full-text, we included two studies (evaluating melatonin in human subjects\textsuperscript{23,24} and no studies in animal subjects. Our search of ClinicalTrials.gov yielded 4 records. After reviewing the clinical trial records, we considered two as relevant to the review (NCT01039792, NCT00273650). Both of these records were matched to an included study.\textsuperscript{23,24}

Description of Human Studies

We included two studies evaluating methylcobalamin in human subjects.\textsuperscript{23,24} The first study was a randomized controlled trial in which 57 children with ASD were allocated 1:1 to subcutaneous methylcobalamin or placebo for 8 weeks.\textsuperscript{23} The second was a placebo-controlled cross-over study in which 30 subjects received subcutaneous methylcobalamin for 6 weeks.\textsuperscript{24}

Risk of Bias in Human Studies

The randomized controlled trial was deemed to have low risk of bias overall, with all subdomains rated “low.”\textsuperscript{23} The crossover study was deemed to have high risk of bias overall, due to a high risk of selective reporting, as well as unclear reporting of randomization sequence, allocation concealment, blinding of care providers and outcome assessors, and complete outcomes.\textsuperscript{24} Risk of bias due to blinding of participants and from outside sources was judged to be low.

Efficacy from Human Studies

ASD Diagnostically Relevant Measures

Social Responsiveness Scale

In the randomized controlled trial, efficacy of methylcobalamin was assessed using the social responsiveness scale (SRS).\textsuperscript{23} Baseline scores were 83.5 (SD, 10.6) in the placebo group and 90.0 (SD, 13.7) in the methylcobalamin group, respectively, and both treatment arms experienced mean decreases in total SRS score at Week 8 (-4.1; SD, 7.7 and -1.6; SD, 7.7, respectively). The between-arms difference in change in SRS total score from baseline to Week 8 was not statistically significant. Within the individual SRS sub-domains, only Social Motivation experienced a statistically significant difference in change in SRS score between treatment arms, -6.3 (95% confidence interval [CI], -11.9 to -0.7, p=0.02).

The evidence for this outcome was insufficient to draw conclusions regarding efficacy, due to imprecision in the reporting of the outcome in the included study (reported confidence interval was wide), as well as suspected reporting bias - as several unpublished studies of methylcobalamin in children are registered on ClinicalTrials.gov without results reported.
Figure 5. Summary of literature search for methylcobalamin

**Human Studies**
- Records identified through database searching (N = 411)
- Records after duplicates removed (N = 283)
- Records screened (N = 203)
- Records excluded (N = 267)
- Full-text articles assessed for eligibility (N = 16)
- Excluded (N = 10)
  - Does not evaluate methyl-B12, 6
  - Does not include people with ASD, 0
  - Does not assess core ASD symptoms or safety, 5
  - No original data, 1
- Studies included
  - 2 published studies (1 RCT; 1 crossover trial)
  - 3 abstracts
  - 1 case report

**Animal Studies**
- Records identified through database searching (N = 176)
- Records after duplicates removed (N = 131)
- Records screened (N = 131)
- Records excluded (N = 129)
- Full-text articles assessed for eligibility (N = 2)
- Excluded (N = 2)
  - Does not evaluate methyl-B12, 1
  - Not a relevant animal model, 2
  - No relevant outcomes, 2
  - No original data, 0
- Studies included
  - 0 published studies
  - 0 abstracts

**ClinicalTrials.gov**
- Records identified through database searching (N = 5)
- Records screened (N = 4)
- Records excluded (N = 2)
- Studies included
  - 2 trial reports that were matched to an included publication

*ASD = autism spectrum disorder; methyl-B12 = methylcobalamin*
Clinical Global Impression Scale

Both included studies assessed methylcobalamin efficacy using the Clinical Global Impression (CGI) scale. In the randomized controlled trial, change in CGI from baseline at Week 8 was 3.1 (SD, 0.8) for placebo and 2.4 (SD, 0.8) for methylcobalamin, and the between-arms difference in change in CGI was statistically significant (p<0.005). The crossover study reported that the difference between arms in change in CGI at Week 6 was not statistically significant, but identified a sub-group of 9 “responders” in which statistically significant improvements in CGI and at least 2 measures of additional behavioral measurements was observed.

The evidence for this outcome was insufficient to draw conclusions regarding efficacy, due to the fact the results of the metric were inconsistent between the two studies, reporting of the outcome was imprecise in one included study, and reporting bias is suspected - as several unpublished studies of methylcobalamin in children are registered on ClinicalTrials.gov without results reported.

Adverse Events

During each study visit of the randomized controlled trial, study staff and the study clinician asked parents if their child had experienced any adverse events. The crossover study did not report on adverse events.

Gastrointestinal Adverse Events

There were a total of three gastrointestinal adverse events reported. In the placebo arm, 1 out of 23 (4%) patients reported diarrhea and 1 out of 23 (4%) reported vomiting. In the methylcobalamin arm, 1 out of 27 (4%) patients reported stomach flu.

The evidence for this outcome was insufficient to draw conclusions regarding the risk of gastrointestinal adverse events related to methylcobalamin, due to the fact that the outcome was measured in a single study, was imprecisely reported, and was subject to suspected reporting bias.

Sleep Adverse Events

There were four sleep adverse events reported; 3 out of 23 (13%) patients in the placebo arm and 1 out of 27 (4%) patients in the methylcobalamin arm reported trouble sleeping.

The evidence for this outcome was insufficient to draw conclusions regarding the risk of sleep adverse events related to methylcobalamin, due to the fact that the outcome was measured in a single study, was imprecisely reported, and was subject to suspected reporting bias.

Constitutional Adverse Events

There were three constitutional adverse events reported; 1 out of 23 (4%) patients in the placebo group and 2 out of 27 (7%) patients in the methylcobalamin group reported fever.

Respiratory Adverse Events

There were nine respiratory adverse events reported; 2 out of 23 (9%) patients in the placebo group and 3 out of 27 (11%) patients in the methylcobalamin group reported a cold; 1 out of 23 (4%) patients in the placebo group reported croup; 1 out of 27 (4%) patients in the
methylcobalamin group reported flu; and 2 out of 27 (7%) patients in the methylcobalamin group reported nosebleed.\textsuperscript{23}

**Mood Adverse Events**

There was one mood adverse event reported; 1 out of 27 (4%) patient in the methylcobalamin group reported increased irritability.\textsuperscript{23}

**Behavioral Adverse Events**

There were 17 behavioral adverse events reported; 7 out of 23 (30%) patients in the placebo group and 2 out of 27 (7%) patients in the methylcobalamin group reported increased hyperactivity; 1 out of 23 (4%) patients in the placebo group reported increased biting; 1 out of 23 (4%) patients in the placebo group reported increased tantrums; and 1 out of 23 (4%) patients in the placebo group and 5 out of 27 (19%) patients in the methylcobalamin group reported mouthing.\textsuperscript{23}

**Neurologic Adverse Events**

There were two neurologic adverse events reported; 1 out of 23 (4%) patients in the placebo group and 1 out of 27 (4%) patients in the methylcobalamin group reported lack of focus.\textsuperscript{23}

**Dermatologic Adverse Events**

There were three dermatologic adverse events reported; 1 out of 23 (4%) patients in the placebo group reported superficial cellulitis; and 1 out of 23 (4%) patients in the placebo group and 1 out of 27 (4%) patients in the methylcobalamin group reported rash.\textsuperscript{23}

**Urinary/Reproductive Adverse Events**

There were two urinary/reproductive adverse events reported; 2 out of 23 (9%) patients in the placebo group reported wetting pants.\textsuperscript{23}

**Nonspecific Adverse Events**

There was one nonspecific adverse event reported; 1 out of 27 (4%) patient in the methylcobalamin group reported growing pains.\textsuperscript{23}

**Oxytocin**

**Search Results**

We retrieved 3191 records from our human subjects search, 4988 records from our animal subjects search (Figure 6). After screening abstracts and full-text, we included 17 studies (22 publications) evaluating oxytocin in human subjects\textsuperscript{25-45} and 39 studies in animal subjects\textsuperscript{46-84} Appendix C lists the included studies and the excluded studies with their reasons for exclusion.
Figure 6. Summary of literature search for oxytocin

**Human Studies**
- Records identified through database searching (N = 3191)
- Records after duplicates removed (N = 1770)
- Records screened (N = 1770)
- Records excluded (N = 1597)
- Full-text articles assessed for eligibility (N = 172)
  - Excluded (N = 137)
    - Does not evaluate oxytocin
    - Does not include people with ASD
    - Does not assess core ASD symptoms or safety
    - No original data
  - Studies included
    - 17 published studies in 21 citations
    - 6 RCT; 8 crossover trial; 1 before-after trial; 2 cohorts
    - 12 abstracts
    - 2 case reports

**Animal Studies**
- Records identified through database searching (N = 4988)
- Records after duplicates removed (N = 3130)
- Records screened (N = 3130)
- Records excluded (N = 2942)
- Full-text articles assessed for eligibility (N = 188)
  - Excluded (N = 147)
    - Does not evaluate oxytocin
    - Not a relevant animal model
    - No relevant outcomes
    - No original data
  - Studies included
    - 39 published studies
    - 2 abstracts

**ClinicalTrials.gov**
- Records identified through database searching (N = 37)
- Records screened (N = 37)
- Records excluded (N = 16)

*ASD = autism spectrum disorder*
Our search of ClinicalTrials.gov yielded 38 records. After reviewing the clinical trial records, 21 were identified as being relevant to the review. Eight records were for completed studies with no posted results (NCT01788072, NCT03337035, NCT01908205, NCT01914939, NCT03033784, NCT01944046, NCT01945957, NCT01308749); seven records were for ongoing studies (NCT02918864, NCT02985749, NCT01931033, NCT02493426, NCT04007224, NCT03370510, NCT03466671); and one record was for a terminated study with no results (NCT02007447). Four records were matched to publications that are included in this review (NCT01337687, NCT01256060, NCT00490802, NCT01624194). One record (NCT02940574) was a completed study which we were able to match to a publication which was not identified in our search because it was published after our search date. Therefore, the total number of included human studies is 18.

Overview of Studies

Thirteen studies examined the efficacy of oxytocin in autism spectrum disorder (ASD). Studies were conducted across a wide age group and included children under 10 years (2 studies), adolescents (4 studies), and adults (7 studies). The majority of studies were double blind randomized placebo-controlled trials (DBRCT, 10 studies), whereas a few studies (3 studies) were open label designs. Across all studies, oxytocin was administered intranasally, most often twice a day. In most studies, treatment duration was short, ranging from 4 days to 12 weeks. Some studies examined outcomes up to one year (Tachibana, 2013; Anagnostou, 2014; Kosaka, 2016; Bernaerts, 2020). Each study included a measure of social functioning as the primary outcome measure. Some studies also examined changes in restrictive and repetitive behaviors, global improvement, and global functioning.

In terms of sample characteristics, sample sizes were generally small with ages ranging from 3 to 60 years, and samples were either exclusively or predominantly male with an average IQ over 80. Most studies did not report on the presence of co-occurring medical or mental health conditions, or psychotropic medication use.

Child Studies

Two studies were conducted in children with a mean age under 10 years. Parker et al. (2017) conducted a DBRCT in 32 children including those with intellectual disability. Children received oxytocin, 24 IU twice daily, for 4 weeks. Yatawara et al. (2016) conducted a DBRCT in 31 children that included children as young as 3 years old, and those with and without intellectual disability. Children were given oxytocin, 12 IU twice daily, for 5 weeks. Results of both studies showed significantly greater improvements in social functioning in the oxytocin versus placebo group using the Social Responsiveness Scale (SRS) total score. No improvements in the Repetitive and Restricted Behavior Scale-Revised (RBS-R) in either study. Yatawara et al. (2016) also did not find any effects of oxytocin on the Autism Diagnostic Observation Schedule (ADOS) reciprocal social interaction and communication score, yet reported significantly greater improvement in the oxytocin group on the Clinical Global Impressions-Improvement Scale (CGI-I).

In conclusion, oxytocin may have little to no effect on repetitive behaviors and restricted interests, but could have a possible positive effect on social communication and interaction among children with ASD.
Adolescent Studies

Two DBRCTs found no improvements in ASD symptoms in adolescents. Guastella et al. (2015) examined 50 high functioning male adolescents who received either placebo, or either 18 IU or 24 IU of oxytocin twice daily over 12 weeks. No improvements were noted on the SRS total score, RBS-R, or the CGI-I. Dadds et al. (2013) examined 38 high functioning adolescents. The oxytocin group received either 12IU or 24IU once a day for 4 days. No improvements were noted in repetitive behaviors as measured by the Social Reciprocity Scale, or global autism symptoms as measured by the Childhood Autism Rating Scale (CARS).

Two small open label studies of adolescents, however, found improvements with oxytocin at both the short and long-term follow up time points. Anagnostou et al. (2014) examined 15 adolescents, who were predominantly high functioning, and received up to 0.4 IU/kg/day of oxytocin over 12 weeks. A near significant reduction in the SRS total score as well as improvement in the CGI-I score was present at three months, and gains on both of these measures were maintained at six months for some adolescents. Tachibana et al. (2013) examined long term outcomes in eight adolescents in which the oxytocin dose was increased in a stepwise manner up to 24IU. Significant improvements were present at six months in the social communication and interaction domains of the ADOS but not play/imagination and repetitive and restricted behavior domains.

In conclusion, oxytocin may have little to no effect on repetitive behaviors and restricted interests among adolescents with ASD. However, the effect of oxytocin on social communication and interaction is unclear.

Adult Studies

Seven studies examined the effects of oxytocin on core ASD symptoms, of which three reported positive effects. Yamasue et al. (2018) conducted the largest DBRCT in 106 adults (53 per group) over six weeks. Results showed no group differences between oxytocin (24 IU twice a day) and placebo on the ADOS reciprocity score. Oxytocin, however, resulted in a significant reduction in the ADOS repetitive behavior score compared to placebo (Cohen’s d = 0.44). No significant effects were observed for the CGI-I and Global Assessment of Functioning (GAF) scales.

Three small studies also reported improvements in ASD symptoms. In a DBRCT over six months, Kosaka et al. (2016) examined three groups (20 per group): high-dose oxytocin (32 IU), low-dose oxytocin (16 IU), and placebo. Findings showed no group differences on the CGI-I or CGI-S, however, when examining the subgroup of males only, a significant improvement on the CGI-I was found in the high-dose oxytocin compared to placebo but not low-dose oxytocin compared to placebo. In a DBRCT of 18 adult males, Watanabe et al. (2015) reported significantly greater improvements in ADOS reciprocity scores at 6 weeks with 48 IU oxytocin compared to placebo. However, no group differences were noted in any of the other outcome measures (i.e., ADOS communication and repetitive behavior scores, SRS, Autism Quotient, CARS total score, Quality of Life Scale). Last, Bernaerts et al. (2020) conducted a DBRCT in 40 high functioning adult males who received 24IU once daily in the morning. Findings showed no improvements in social communication on the SRS, but found that repetitive behaviors (as measured by the RBS-R) improved in the oxytocin group compared to the placebo group immediately after treatment and up to one year posttreatment.
Three small studies found no effect of oxytocin on ASD symptoms.\textsuperscript{26, 34, 38} In a DBRCT of 19 high functioning adults, Anagnostou et al. (2012) reported that the oxytocin (24IU twice daily) resulted in a decrease in lower order repetitive behaviors as measured by RBS-R, however, results were at a trend level when baseline behaviors were accounted for in the analyses.\textsuperscript{26} Furthermore, no improvements were noted on the CGI-I. Munesue et al. (2016) conducted a DBRCT for 8 weeks in 29 young adults, including those with severe to profound intellectual disability.\textsuperscript{38} Results showed no differences between the oxytocin and PBO groups on the CARS or CGI-I scale. Oxytocin dosing in this study was lower (8 IU twice daily) compared to other studies. Last, Hirosawa et al. (2017) conducted a small open-label single-arm study of 10 adult males with ASD. All participants were given 24 IU of oxytocin per day for 10 weeks.\textsuperscript{34} No significant changes in the Autism Quotient and RBS-R scores were observed.

In conclusion, the effect of oxytocin on repetitive behaviors and restricted interests among adults with ASD is unclear. However, oxytocin may have little to no effect on social communication and interaction.

**Adverse Events**

There were a total of 14 studies that reported adverse event data related to non-oral use of oxytocin among individuals with ASD.\textsuperscript{25, 26, 28, 31, 32, 37-40, 42-45, 85} Several types of adverse events were collected spanning several body systems including cardiovascular, urinary and reproductive, neurological, behavioral and mood, sleep, respiratory, and gastrointestinal as well as non-specific and constitutional events (Appendix D-6, Tables 9-18). A sufficient number of studies reported adverse event data on tiredness, headaches, and nasal discomfort and congestion for meta-analyses. Results from the meta-analyses are summarized immediately below followed by a paragraph that summarizes the potential impact of oxytocin use on other types of adverse events. Because tiredness was assessed by multiple studies, including 3 randomized crossover trials, we performed a meta-analysis and observed no significant differences in tiredness risk related to oxytocin use, although the trend for the point estimates showed individuals given oxytocin were less tired (Figure 7A; risk ratio [RR], 0.70; 95% CI, 0.23 to 2.08, p=0.517; I-squared, 0\%).\textsuperscript{28, 31, 43} Two randomized controlled trial studies showed similar results (Figure 7B; RR, 0.41; 95% CI, 0.10 to 1.79; p=0.238; I-squared, 48\%).\textsuperscript{32, 85}

Headache was also assessed by several studies with similar study designs which enabled meta-analysis.\textsuperscript{26, 31, 38, 39, 44, 85} Although the direction of effect was consistent across all but one study (which had a high potential for biases) the differences did not reach statistical significance (p>0.05) and had poor precision, likely due to the relatively small sample numbers (Figure 8). In addition, although the frequency of headaches across all studies appeared to increase with a longer follow up time there are serious limitations including the lack of a control group for several of the studies with the longest follow up times.\textsuperscript{25} Futures studies that include a control group are needed to more rigorously evaluate the long-term impact of oxytocin on headaches.

Many of the studies administered oxytocin intranasally and, thus, collected adverse events specifically related to the nasal passage including congestion and general discomfort. Again, no significant differences (p>0.05) were found between individuals that received oxytocin and those that received a placebo (Figure 9) and the overall frequency of nasal discomfort reported across both control and intervention groups was less than 16\% (Appendix D-6, Table 11). These results were consistent across randomized controlled\textsuperscript{32, 37, 39, 44} and crossover trials.\textsuperscript{28, 38, 43} For congestion, the meta-analysis point estimate across four randomized control trials showed
increased congestion among participants that received oxytocin compared to those that received placebo (Figure 10), however, these findings did not reach statistical significance (RR, 1.99; 95% CI, 0.70 to 5.61, p>0.05; I-squared, 18%). Results were inconsistent across controlled and crossover study designs. Two out of the four randomized controlled trials have biases due to lack of assessor blinding and/or incomplete outcome data [Parker, 2017; Guastella, 2015].

In sum, meta-analysis results for tiredness, headaches, and nasal discomfort or congestion did not suggest an increased risk of these types of adverse events among individuals that received oxytocin. However, this conclusion is based on a relatively small number of studies that examined adverse events, some of which had serious potential biases, and thus, it should be interpreted with caution.

Meta-analyses were not possible for several other adverse event categories due to a lack of multiple studies with a similar design and adverse event data collection. For these studies we assessed the body of evidence for trends related to oxytocin treatment. As shown in Appendix D-6 Table 9, there was consistent evidence across nine studies, representing three different study designs including randomized controlled trials, observational cohort, and randomized crossover, that the prevalence of gastrointestinal symptoms did not increase among individuals treated with oxytocin; the prevalence of GI symptoms ranged from 0% to 33% which is similar to reported increased prevalence of co-occurring GI symptoms among individuals with ASD. Of note, the study reporting the highest GI symptom prevalence had a high risk of potential biases [Anagnostou, 2014].

For the respiratory system, about half of the randomized trial studies showed a slight increase in the prevalence of cough, nose bleed, olfactory hypersensitivity, nose irritations and itching, and sore throat related to receiving oxytocin but these findings are based on a small number of individuals and they were not consistent across studies (Appendix D-6, Table 11). One study reported a high frequency of upper respiratory infections (46.7%) but it did not include a placebo control group and reported events up to 24 weeks post treatment [Anagnostou, 2014].

Prevalence of nasopharyngitis and rhinitis, also considered to be a result of upper respiratory infections, were substantially lower in all other randomized study designs and importantly showed few differences between the placebo and oxytocin treatment groups (Appendix D-6, Table 11). Thus, the reported high frequency of respiratory infections are likely a result of biases in the study design. The results from two studies suggest drowsiness, i.e., somnolence, may be slightly elevated among individuals receiving oxytocin, particularly at high doses (Appendix D-6, Table 12). As shown in Appendix D-6, Table 13, there was little to no evidence to support adverse mood changes related to oxytocin treatment with the exception of irritability but only 2 studies evaluated this specific event and one of those lacked a control group and was subject to serious biases [Anagnostou, 2014]. In addition, one randomized controlled study observed an increase in the frequency of crying and more emotionalness, up to 4 weeks post treatment, among individuals that received oxytocin. Although only 2 studies reported data for hyperactivity/increased energy, both studies showed a slight increase in the prevalence among individuals that received an oxytocin intervention compared to a placebo control group (Appendix D-6, Table 14). Evidence also did not support increased neurologic events including seizures or headaches related to oxytocin use (Appendix D-6, Table 15). There were no adverse urinary or reproductive events reported for any participant although these results were reported by a single study and for a very small number of individuals (Appendix D-6, Table 16). Very few total adverse cardiovascular events were reported and
most frequencies were similar or higher in the placebo group compared to the oxytocin treatment group (Appendix D-6, Table 17). Finally, there were no non-specific adverse events that were consistently reported by participants. The total number of events was similar between placebo and oxytocin groups in randomized controlled trials and somewhat elevated, 41.9% compared to 22.6%, in a randomized crossover trial study (Appendix D-6, Table 18). Of note, there did appear to be an increase in either thirst, day/night urination, or constipation in one study [Yatawara, 2016]. Based on the data presented, we conclude that there is little to no evidence showing treatment with oxytocin results in increased gastrointestinal, respiratory, sleep, neurologic, urinary, reproductive, or cardiovascular events. While there were some higher frequencies observed for drowsiness, hyperactivity, and thirst, day/night urination, or constipation, the evidence to date is not sufficient to definitively conclude oxytocin treatment results in an increase of these side effects. Additional rigorous studies with larger samples sizes are needed.

One important limitation to these findings is that nearly all of the studies examined side effects in adolescents and/or adults. Only 2 studies collected side effects in young children and they reported an increased frequency of nasal, headache, behavioral, and non-specific side effects related to oxytocin use. Because ASD has an early childhood onset studies to evaluate potential side effects of oxytocin administration specifically in children are needed.

Figure 7. Results from meta-analyses of randomized crossover trials (A) and randomized controlled trials (B) to assess impact of oxytocin use on tiredness

<table>
<thead>
<tr>
<th>Study design and Length of followup</th>
<th>Author, year</th>
<th>n/N in oxytocin</th>
<th>n/N in placebo</th>
<th>RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crossover</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guastella 2010 day of test</td>
<td></td>
<td>1/16</td>
<td>4/15</td>
<td>0.23 (0.03, 1.87)</td>
</tr>
<tr>
<td>Watanabe 2015 12 weeks</td>
<td></td>
<td>1/18</td>
<td>1/18</td>
<td>1.00 (0.07, 14.79)</td>
</tr>
<tr>
<td>Auyeung 2015 2 weeks</td>
<td></td>
<td>3/32</td>
<td>2/32</td>
<td>1.50 (0.27, 8.38)</td>
</tr>
<tr>
<td>Subgroup (I-squared = 0.0%)</td>
<td></td>
<td></td>
<td></td>
<td>0.70 (0.23, 2.08)</td>
</tr>
<tr>
<td>RCT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guastella 2015 8 weeks</td>
<td></td>
<td>1/26</td>
<td>5/24</td>
<td>0.18 (0.02, 1.47)</td>
</tr>
<tr>
<td>Bernaerts 2020 4 weeks</td>
<td></td>
<td>1/22</td>
<td>0/18</td>
<td>2.82 (0.11, 73.61)</td>
</tr>
<tr>
<td>Subgroup (I-squared = 47.6%)</td>
<td></td>
<td></td>
<td></td>
<td>0.41 (0.10, 1.79)</td>
</tr>
</tbody>
</table>

NOTE: Weights are from Mantel-Haenszel model; continuity correction applied to studies with zero cells
Figure 8. Results from meta-analyses of randomized crossover trials (A) and randomized controlled trials (B) to assess impact of oxytocin use on headaches

<table>
<thead>
<tr>
<th>Study design and Author, year</th>
<th>Length of followup</th>
<th>n/N in oxytocin</th>
<th>n/N in placebo</th>
<th>RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Crossover</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guastella 2010</td>
<td>Day of test</td>
<td>0/16</td>
<td>1/15</td>
<td>0.33 (0.01, 7.19)</td>
</tr>
<tr>
<td>Munesue 2016</td>
<td>8 weeks</td>
<td>3/15</td>
<td>1/14</td>
<td>2.80 (0.33, 23.36)</td>
</tr>
<tr>
<td>Subgroup (I-squared = 20.5%)</td>
<td></td>
<td></td>
<td></td>
<td>1.32 (0.28, 6.27)</td>
</tr>
<tr>
<td>(B) RCT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parker 2017</td>
<td>4 weeks</td>
<td>1/14</td>
<td>0/18</td>
<td>3.29 (0.16, 66.46)</td>
</tr>
<tr>
<td>Yamasue 2018</td>
<td>6 weeks</td>
<td>4/53</td>
<td>0/53</td>
<td>9.00 (0.50, 163.12)</td>
</tr>
<tr>
<td>Anagnostou 2012</td>
<td>6 weeks</td>
<td>1/10</td>
<td>0/9</td>
<td>2.90 (0.12, 67.36)</td>
</tr>
<tr>
<td>Bernaerts 2020</td>
<td>4 weeks</td>
<td>1/22</td>
<td>1/18</td>
<td>0.82 (0.05, 12.19)</td>
</tr>
<tr>
<td>Subgroup (I-squared = 0.0%)</td>
<td></td>
<td></td>
<td></td>
<td>3.27 (0.84, 12.70)</td>
</tr>
</tbody>
</table>

NOTE: Weights are from Mantel-Haenszel model; continuity correction applied to studies with zero cells

Figure 9. Results from meta-analyses of randomized crossover trials (A) and randomized controlled trials (B) to assess impact of oxytocin use on nasal discomfort

<table>
<thead>
<tr>
<th>Study design and Author, year</th>
<th>Length of followup</th>
<th>Nasal discomfort</th>
<th>n/N in oxytocin</th>
<th>n/N in placebo</th>
<th>RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Crossover</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Munesue 2016</td>
<td>8 weeks</td>
<td>Congestion</td>
<td>0/15</td>
<td>2/14</td>
<td>0.19 (0.01, 3.58)</td>
</tr>
<tr>
<td>Auyeung 2015</td>
<td>2 weeks</td>
<td>Runny nose</td>
<td>3/32</td>
<td>5/32</td>
<td>0.60 (0.16, 2.30)</td>
</tr>
<tr>
<td>Watanabe 2015</td>
<td>12 weeks</td>
<td>Nose irritation</td>
<td>2/18</td>
<td>2/18</td>
<td>1.00 (0.16, 6.35)</td>
</tr>
<tr>
<td>Subgroup (I-squared = 0.0%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.57 (0.21, 1.55)</td>
</tr>
<tr>
<td>(B) RCT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parker 2017</td>
<td>4 weeks</td>
<td>Congestion</td>
<td>3/14</td>
<td>0/18</td>
<td>7.86 (0.51, 121.24)</td>
</tr>
<tr>
<td>Guastella 2015</td>
<td>8 weeks</td>
<td>Blocked nose</td>
<td>1/26</td>
<td>0/24</td>
<td>2.92 (0.12, 71.65)</td>
</tr>
<tr>
<td>Yamasue 2018</td>
<td>6 weeks</td>
<td>Rhinitis</td>
<td>0/53</td>
<td>1/53</td>
<td>0.33 (0.01, 8.00)</td>
</tr>
<tr>
<td>Anagnostou 2015</td>
<td>6 weeks</td>
<td>Congestion</td>
<td>2/10</td>
<td>0/9</td>
<td>4.80 (0.24, 94.76)</td>
</tr>
<tr>
<td>Kosaka 2016</td>
<td>12 weeks</td>
<td>Itchy nose</td>
<td>0/40</td>
<td>1/20</td>
<td>0.25 (0.01, 4.51)</td>
</tr>
<tr>
<td>Subgroup (I-squared = 11.0%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.82 (0.63, 5.25)</td>
</tr>
</tbody>
</table>

NOTE: Weights are from Mantel-Haenszel model; continuity correction applied to studies with zero cells
Evidence from Animal Studies

Rodent Model Summary

In rats and mice, we analyzed changes in restricted and repetitive behaviors (RRB), a core component of autism spectrum disorder (ASD), related to oxytocin treatment. Oxytocin was administered across several life stages, ranging from infancy through adulthood, and in several ways including intranasally, intracerebroventricularly, intraperitoneally, intracranially, and subcutaneously. The impact of oxytocin administration on RRB were captured using a number of established and well-validated mouse behavioral tasks including time spent grooming, repetitive self-grooming, marble burying, motorstereotypy, rewarded T-maze reversal, and 5 choice serial reaction time. The inbred mouse models used to assess RRB included those genetically modified to contain human ASD risk variants (Ctnnap2), less social strains (BTBR, C58), wild type or more sociable background strains (C57BL/6N), and genetically modified strains (e.g. Oxtr −/−, Grin1). An outbred ICR CD-1 mouse strain was also examined in one study (Appendix D-7, Table 1). Assessing the RRBs in mouse strains with varying levels of sociability can provide important insights, via contrasts between the results across social strains, into oxytocin effects on RRB in the context of sociability traits – both of which are core components of ASD deficits in humans. These tasks and models have been reported to have good face validity for human ASD. Similarly, as shown in Appendix D-7 Table 1, rat models included wild type, genetically modified inbred (Shank3; Shank3B), and outbred (Wistar; mongrel albino) strains.

We evaluated whether oxytocin impacts ASD-relevant repetitive behaviors by examining the results of studies that measured grooming behaviors, marble burying, and motorstereotypies.
Eight studies examined the frequency or total amount of time spent on self-grooming behaviors after oxytocin treatment.\(^{47-49, 59, 60, 62, 65, 69}\) Five of those studies assessed acute short-term responses to oxytocin treatment and consistently showed substantial and significant increases in the amount of time spent grooming among mice that received oxytocin compared to those that received a placebo including in a mouse genetic model of ASD and wild type rats (Appendix D-7, Table 3).\(^{49, 59, 60, 62, 69}\) Two of the three studies that did not observe a significant change in RRB with oxytocin treatment \(^{47-49}\) measured long-term repetitive grooming outcomes, one week or more after acute oxytocin administration,\(^{47, 49}\) in a non-social ASD-relevant mouse strain (BTBR).\(^{47}\) Eleven studies examined acute repetitive self-grooming scores, primarily in wild type rats, after administration of oxytocin via several routes of delivery. Nearly all of them showed significant increases in grooming scores among rodents that received oxytocin compared to those that received placebo (Appendix D-7, Table 4).\(^{46, 51-56, 58, 61, 64, 68}\) These effects were consistent across routes of oxytocin administration and across males and females. This suggests oxytocin administration increases repetitive grooming behaviors. Two out of five studies that reported on other measures of grooming in wild type mice or rats reported significant differences in repetitive self-grooming behaviors related to treatment with oxytocin (Appendix D-7, Table 5).\(^{63, 71}\) Tests for motorstereotypies, i.e., repetitive movements, in outbred Wistar rats showed decreased motorstereotypies 1 hour after oxytocin administration compared to rats treated with saline (Appendix D-7, Table 9).\(^{60}\) Finally, two out of three studies that assessed repetitive behaviors in mice using marble burying based tests reported fewer repetitive behaviors in mice that received oxytocin relative to those that did not (Appendix D-7, Table 6).\(^{67, 70, 72}\) The effects of oxytocin were observed in both social and non-social mouse strains,\(^{67, 72}\)

The rewarded T-maze reversal\(^{66}\) and 5 choice serial reaction time tasks\(^{57}\) were used by one study, in a genetically modified inbred mouse strain (Oxtr \(-/-\)), to assess cognitive inflexibility and restricted interests. Intracerebroventricular administration of oxytocin improved cognitive flexibility but only in the subset of mice that were deficient in oxytocin due to a genetic mutation.\(^{66}\) Mice without a genetic deficit in oxytocin showed decreased cognitive flexibility in response to oxytocin treatment (Appendix D-7, Table 7). A separate test for restricted interests showed increased interest in new stimuli in mice that lacked a functional oxytocin receptor gene and received oxytocin relative to those that received saline and those with a functional oxytocin gene suggesting administration of oxytocin can rescue the behavioral deficits associated with loss of the oxytocin receptor (Appendix D-7, Table 8).\(^{57}\) In conclusion, the body of evidence in rodents suggests that oxytocin treatment actually increases repetitive behaviors and restricted interests consistently across different rodent models representing social, non-social, and ASD-relevant genetically modified strains.

Several important points must be taken into account when considering the relevance of these results to humans. First, even if oxytocin reduced RRBs which is not supported by the evidence to date, some of the oxytocin delivery modes (e.g., intracranial and intracerebroventricular) are not possible to administer in living humans without serious adverse risks. Second, findings from genetically modified inbred rodent strains may not be applicable to a large number of individuals with ASD because the specific genetic mutations examined in these rodent models occur in a relatively small proportion of total ASD cases (each variant observed in ~1-2% of all ASD cases, at most). Third, most of the rodent model data comes from inbred strains which do not reflect complex genetic heterogeneity, like that observed in humans, which is critical for ASD given common genetic variants play a large role in ASD risk. Fourth, most of the rodent studies examined acute short-term changes in RRB and the few that examined longer-term RRB showed
no change in behaviors with oxytocin treatment. A better understanding of how the oxytocin dosing schedule relates to short and long-term effects of the treatment on ASD core features is also critical to understand. Finally, the vast majority of the rodent model studies did not provide critical information regarding the age of the mice under investigation. Given the early childhood onset of ASD core symptoms these studies provide little information about the effectiveness of oxytocin at different developmental windows or age groups, and in particular there is a paucity of data during childhood periods when most treatment would likely begin.

Non-Human Primates
A total of seven non-human primate studies (published in six publications), mostly crossover in design, assessed the effects of oxytocin on juvenile and adult social behaviors. Oxytocin was administered intranasally as either a single dose or repeatedly for up to 6 months. Most studies tested acute responses to oxytocin, i.e., within 45 minutes of administration of the compound. Qualitative results for the number of instances and total amount of time spent in close contact with or huddling with other non-human primates of the same type were inconsistent with studies reporting increases [Smith, 2010; Cavanaugh, 2015; Arias del Razo, 2020], no change [Proctor, 2016; Cavanaugh, 2018; Cavanaugh, 2015; Smith, 2010], and decreases [Arias del Razo, 2020] after the animals received oxytocin (Appendix D-7, Table 12). The inconsistencies do not appear to be completely explained by differences in non-human primate type, time of testing, rigor of study design, sex, age, or test setting (e.g., familial versus non-familial interactions). Food sharing [Smith, 2010; Cavanaugh, 2018] and parenting behaviors [Taylor, 2019] consistently showed no changes in response to intranasal oxytocin administration (Appendix D-7, Table 12), although this evidence is not strong given the small number of studies that assessed these behaviors. Finally, female titi monkeys that received oxytocin showed an increase in the amount of time spent with parents compared to those who received the placebo but this effect was opposite in males who preferred to spend more time with unfamiliar animals when treated with oxytocin [Arias del Razo, 2020] parental preference in titi monkeys]. There is no strong evidence to support improved social interactions among non-human primates that received an oxytocin intervention.

Prairie Voles
A total of five controlled trial studies assessed the effects of oxytocin on social behaviors in prairie voles. Oxytocin was administered via several routes including intranasally and subcutaneously, intraperitoneal, and intracerebroventricular injections and often at a range of doses within each route of administration. Qualitative assessment of social behaviors across multiple studies consistently showed social contact and approach was not increased for females that received oxytocin and this result did not change by dose or route of administration (Appendix D-7, Table 15). There was conflicting evidence for males with one study reporting increased social contact and approach and two others reporting no significant change. It isn’t clear whether this discrepancy was due to differences in the routes of administration, timing of the social contact testing, or is related to study biases. The overall time prairie voles spent in a cage with another vole did not differ, qualitatively, between those that received oxytocin and those that did not. However, two studies reported females who received oxytocin spent significantly more time with a familiar partner than a stranger (Appendix D-7, Table 15; Bales, 2013; Cushing, 2000). Finally, no changes in social experiences at different life stages, assessed via alloparenting and juvenile affiliation tests, were observed in relation to oxytocin administration...
(Appendix D-7, Table 15). In sum, there is limited evidence supporting increased social behaviors in prairie voles after receiving oxytocin. However, this conclusion should be interpreted with caution because all of these studies had substantial risk of bias limiting the rigor of the conclusion.

**Zebrasfish**

Zebrasfish exhibit social traits through schooling behaviors. Thus, assessing these behaviors in zebrasfish in response to oxytocin may provide insights into the impact of oxytocin on social traits, a core feature of ASD. One paper met our systematic review criteria and reported changes in social interactions and aggressiveness in zebrasfish with and without oxytocin treatment. For a subset of fish, the authors also induced deficits in the N-methyl-D-aspartate (NMDA) glutamate receptor, which plays a critical role in synaptic plasticity and cognition, using the MK-801 compound. As shown in Appendix D-7 Table 18, fish with deficits in synaptic plasticity showed increased social interactions and aggressive behaviors in response to oxytocin treatment. Similar increases in social interactions in response to oxytocin were also observed among fish without MK-801 induced deficits in synaptic plasticity, however, these changes were not significant. Decreases in aggressiveness in response to oxytocin were reported in synaptically sufficient zebrasfish but significance levels were not reported making it difficult to interpret the robustness of the observations. Given the paucity of data in zebrasfish and lack of statistical rigor in the one relevant study, there is too little evidence to make any conclusions about the effect of oxytocin on autism-relevant behaviors in this animal model system.

**Evidence Integration**

We were able to complete an integration of evidence from human and animal studies for oxytocin. To do so, we first determined which outcomes most closely match between humans and animal models. We then mapped the results for these outcomes onto matrices for the two main domains of ASD: (i) repetitive and restrictive behaviors (Figure 11), and (ii) social communication (Figure 12).

In summary, there appears to be moderate correspondence between animal and human studies regarding the effects of oxytocin on social communication and repetitive behaviors associated with ASD. Both animal and human studies have used standardized behavioral paradigms and clinical measures, respectively, to assess outcomes. With regards to social communication, evidence from studies in prairie voles, zebrasfish and non-human primates indicate that oxytocin does not improve various types of social behaviors. Data from human studies indicate age-related differences in outcomes with two small studies reporting potential benefit in children, though no clear benefits were present in adolescents or adults.

The existing animal and human data indicate that oxytocin did not improve repetitive behaviors. In fact, findings from most rodent studies report an increase in repetitive behaviors as well as cognitive inflexibility with oxytocin treatment. In humans, repetitive behaviors in children and adolescents did not improve and unclear benefits have been reported in adults.

Despite these negative or inconclusive findings, studies in both animal models and humans need to be interpreted with caution due to inherent study biases, as well as methodological differences in sample characteristics, as well as oxytocin administration (e.g., dose, route, and duration). Furthermore, several factors limit the application of animal studies to humans (e.g., pure animal strains do not reflect the heterogeneous clinical profiles in ASD) and these must be considered when interpreting the findings and considering future research studies in the field.
Figure 11. Integration of evidence from studies of human and non-human subjects on the effects of oxytocin on repetitive behaviors and restricted interests

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Figure 12. Integration of evidence from studies of human and non-human subjects on the effects of oxytocin on social communication and interaction

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References


41. Tachibana M, Kagitani-Shimono K, Mohri I, et al. Long-term administration of intranasal oxytocin is a safe and promising therapy for early adolescent boys with autism spectrum


Abbreviations

ADI-R = Autism Diagnostic Interview – Revised
ADOS = Autism Diagnostic Observation Schedule
ASD = autism spectrum disorder
CARS = Childhood Autism Rating Scale
CENTRAL = Cochrane Central Register of Controlled Trials
CGI = Clinical Global Impression Scale
CI = confidence interval
DMPS = 2,3-dimercapto-1-propanesulfonic acid sodium
DSM = Diagnostic and Statistical Manual of Mental Disorders
FDA = Food and Drug Administration
FISER = Frequency and Intensity of Side-effect Rating
GRSEB = Global Rating of Side-Effect Burden
JHU = Johns Hopkins University
MeSH = medical subject headings
NTP = National Toxicology Program
PRISE = Patient Report of Incidence of Side-Effects
RBS-R = Repetitive and Restricted Behavior Scale - Revised
RR = risk ratio
SD = standard deviation
SRDR = Systematic Review Data Repository
SRS = Social Responsiveness Scale
SYRCLE = Systematic Review Centre for Laboratory Animal Experimentation
Part 2: Evaluation of Current and Historical Use

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Summary

In order to assess the patterns and correlates of use for the six bulk drug substances up for evaluation, qualitative and quantitate data from various sample types including clinical, population, national, and Key Opinion Leaders were collected. This sample and method diversity allowed us to collect and compare data gleaned from different kinds of stakeholders, e.g. parents of children with ASD and medical professionals, to strengthen the conclusions and fill any gaps of information from individual groups.

Overall, the relative patterns of use frequency for each of the bulk drug substances were consistent across studies. Opinions from different stakeholder groups provided insights that could possibly explain these observed patterns.

B12

Of the six compounds of interest evaluated, B12 was the second most commonly used to use ASD; however, the rates of use were considerably low. This ranged from 1% in the KKI clinical prescription database to 6% in the SPARK population survey data. Based on reports of high multivitamin and B-complex vitamin use, it is likely that additional children receive B12 supplements incidentally, even though dedicated B12 use for ASD was uncommon. On average, most SPARK respondents reported that B12 was of little benefit or presented no change. This comports with KOL opinions that B12 would likely have no effect, positive or negative, on individuals with ASD unless specifically taken to address a dietary deficiency.

Melatonin

Melatonin was overwhelmingly the most common of the compounds of interest to be used to treat ASD. This ranged from 60% in the SPARK survey data to 97% in the KKI prescription database. Only 0.15% of children with ASD were observed to use melatonin in the Medicaid data, however this is likely because a significant majority of melatonin is acquired over-the-counter, as observed in the SPARK data. Melatonin was rated as being very helpful by SPARK respondents and was the only one of the six drug substances that the KOLs prescribed and considered to be a safe and effective for use by patients with ASD to address sleep issues.

Oxytocin

Oxytocin was not commonly used by children with ASD. Reported use ranged from 0% in the KKI prescription database to just 0.2% in the SPARK survey data. Response rates were too low to ascertain information about most common providers of oxytocin or its perceived effectiveness. Based on KOL reports, the scientific evaluations of oxytocin as a treatment for ASD are still the early stages so there is not yet sufficient data to speak to its effectiveness or the best route of administration.

Inositol

Inositol was not commonly used by children with ASD. Reported use ranged from 0% in the Medicaid claims data to 3.3% in the KKI prescription database. Based on the 12 SPARK respondents that reported use, inositol was most commonly acquired over-the-counter or online and was perceived on average as having little benefit or presenting no change. The KOLs knew
little about the use of inositol for ASD and all reported that they do not prescribe or recommend it because there is insufficient evidence.

**Glutathione**

Glutathione was not commonly used by children with ASD. Reported use ranged from 0.05% in the Medicaid claims data to 1.6% in the SPARK survey data. Based on the 19 SPARK respondents that reported use, glutathione was most commonly acquired online and was perceived on average as causing no change. The KOLs knew little about the use of glutathione for ASD and all reported that they do not prescribe or recommend it because there is insufficient evidence.

**DMPS Chelation**

Use of DMPS chelation to treat children with ASD was very rare. Reported use ranged from 0% in both the SPARK survey data and KKI prescription database to >0.1% in both the Medicaid claims data and KKI medical records. The few captured instances of use were broadly categorized as “chelation” in general and may not have necessarily referred to DMPS chelation. While there was no data to speak to parent perception of DMPS chelation effectiveness, all KOLs were strongly opposed to the use of chelation of any kind to treat ASD.
Evaluation of Current and Historical Use

Objective

The objective of this project was to evaluate evidence regarding historical and current use of 6 bulk drug substances that may be compounded for treatment of autism spectrum disorder (ASD). The six substances evaluated were melatonin, B12 (methylcobalamin), oxytocin, inositol, glutathione, and 2,3-Dimercapto-1-propanesulfonic acid (DMPS) chelation. We will refer to these 6 substances generally as compounds of interest throughout this report.

Project Goals

This report summarizes findings regarding historical and current use of the compounds of interest to inform policy addressing treatments for patients with ASD. To achieve these goals we drew on 3 distinct data resources (clinical, population, and a national sample), and conducted interviews with Key Opinion Leaders (KOLs) to evaluate current and historical use of melatonin, B12, oxytocin, inositol, glutathione, and DMPS acid chelation for ASD treatment.

Background

History and Nosology of ASD

The earliest published account of autism was detailed in 1943 by Dr. Leo Kanner at the Johns Hopkins University Henry Phipps Psychiatric Clinic. In Autism Disturbances of Affective Conduct, Dr. Kanner described 11 children who were markedly similar in terms of their lack of social reciprocity, the hallmark characteristic of autism. Just one year later across the globe another physician, Dr. Hans Asperger of Austria, described 4 boys with similar social and stereotypic deficits as those identified by Dr. Kanner.

Since the inaugural reports by Drs. Kanner and Asperger detailing children with “autistic disturbances of affective contact” and “autistic psychopathology”, respectively, the nosology of autism has changed considerably. Autism was first formally classified as “infantile autism” in the 3rd edition of the American Psychiatric Association’s Diagnostic and Statistical Manual (DSM). The five criteria used to define autism in DSM-III included early onset (criteria a), social deficits (criteria b), language perturbations (criteria c), and stereotypic behaviors (criteria d and e). In DSM-IV, the narrowly defined infantile autism was replaced with a broader set of conditions termed Pervasive Developmental Disorders (PDD). These syndromes retained similarities in the core deficits of autism (language, social relatedness, and stereotypies) and childhood onset, but were unique in terms of clinical presentation and course. The DSM-IV diagnoses under PDD included Autistic Disorder, Rett syndrome, Childhood Disintegrative Disorder, Asperger’s Disorder, and PDD-NOS. Except for Rett syndrome, which was dropped from DSM-V after the molecular etiology was identified, all of these diagnoses were removed from DSM-V due to concerns about lack of validity and temporal stability.

Today, autism is classified and colloquially spoken of as Autism Spectrum Disorder (ASD). Even 70 years later, criteria for ASD in DSM-V echoes what was originally described by Kanner. To receive a diagnosis of ASD, a child must display an early onset of deficits in “social-emotional reciprocity” and present with at least two of the following symptoms: 1) stereotyped use of objects, 2) inflexibility or insistence on sameness, 3) highly fixated interests, and 4)
sensory abnormalities\textsuperscript{27}. The fifth edition of the DSM also subsumed previous diagnostic criteria (e.g., speech language difficulties) and even specific diagnoses (e.g., Asperger’s disorder) under a special section on specifiers where clinicians can utilize these terms to characterize the child’s developmental needs\textsuperscript{27}. This shifting nosological landscape is just one explanation for the exponential increase in ASD prevalence estimates over the last 50 years.

**Epidemiology of ASD**

In 1967, Dr. Victor Lotter conducted the first epidemiologic study of autism. Data for his study were from a screening of over 78,000 children, 8-10 years of age, living in Middlesex, United Kingdom\textsuperscript{4}. From this sample Dr. Lotter identified 32 children with autism, resulting in a prevalence of 4.5 per 10,000 children\textsuperscript{4}. The first US epidemiologic study in Madison, WI - conducted by Dr. Darold Treffert in 1970 - produced a very similar prevalence estimate of 3 per 10,000 children\textsuperscript{29}.

Since the inception of DSM-IV in the mid-90s, the US and developed countries across the world have witnessed a precipitous rise in ASD prevalence estimates. Studies published in the UK and US between 2001 and 2003 reported prevalence estimates at 30-60 per 10,000 children, representing a 10-20 fold increase compared to their earlier counterparts\textsuperscript{2,5,31}. Similar increases have been reported in developed countries, including those in the Nordic regions, the South Pacific, Asia (including Japan and South Korea), and Europe\textsuperscript{5}.

In response to the public concern regarding the “autism epidemic” in the US, the CDC established the Autism and Developmental Disabilities Monitoring (ADDM) to track national trends across the US\textsuperscript{31}. The first report, in 2002, from the CDC’s ADDM found 1 in 150 children met criteria for ASD\textsuperscript{31}. Prevalence estimates increased to 1 in 110 children\textsuperscript{31} in 2009 and 1 in 68 in 2014, with the latter representing a 123% increase since 2002\textsuperscript{1}. The estimate of 1 in 68 held in 2016 firm as the CDC reported no change in autism prevalence estimates for the first time since beginning ASD surveillance\textsuperscript{32}. However, in 2018, the CDC reported 1 in 59 children had ASD, representing a 15% increase since 2014.

**Natural History and Diagnosis**

Until the last ten years, children with ASD were often detected and diagnosed when they entered the school system\textsuperscript{36,38}. However, a landslide of early detection studies has now armed clinicians with the tools to reliably detect autism as early as 18 months of age. Detection of ASD is often contingent on the developmental profile of the child, such that those with greater speech-language delays, more pronounced delays in joint attention, and loss of developed skills (also called developmental regression) may have an earlier detection of their ASD. Unfortunately, socio-economic factors also play a role, as Mandell and colleagues (2002) found blacks were diagnosed 3 years later than whites\textsuperscript{41}. Underreporting and lack of detection of ASD symptoms have also been historically found among Hispanics\textsuperscript{42}. Delayed diagnosis among both of these ethnic minorities is thought to be due to poor access to healthcare, lack of education on early development, and cultural differences in terms of help seeking and trust of the medical system\textsuperscript{42}.

At present, no biomarker or biological test is available for autism. This relegates diagnosis to the evaluation of a behavioral phenotype. Two gold standard measures exist for diagnostic purposes. The first is the Autism Diagnostic Observation schedule (ADOS)\textsuperscript{45}, a standardized, semi-structured play-based assessment. There are 5 different modules of the ADOS, ranging from the ADOS-G for toddlers to ADOS-4 for late adolescents and adults with fluent language abilities. The ADOS is often employed by a clinician, such as speech-language pathologist or
psychologist, and boasts very strong psychometric properties. The second gold standard measure is the Autism Diagnostic Interview-Revised (ADI-R). The ADI-R is a structured parent report instrument conducted by a trained professional. The interview often takes 1-2 hours and covers the child’s entire history of development. Established algorithms and cutoff scores for both the ADI-R and ADOS exist and are well-validated. It should be noted that neither the ADOS nor ADI can produce a diagnosis; they are meant to inform clinical decision, which can only be designated by a physician or psychologist.

Burden of Disease and Comorbidities

As reflected in the term autism spectrum, disease course, symptom severity, and outcomes of children with ASD is highly variable. The strongest predictors of outcomes often relates to the child’s developmental profile, including cognitive and language abilities. Despite this variability, ASD is associated with a large burden of disease. In 2010, ASD was listed as the 1st and 4th leading cause of psychiatrically-related disability worldwide among children under 5 and 5-14 years, respectively.

Even in the first report of ASD, Dr. Kanner observed a number of physical problems, such as gastrointestinal symptoms, among his patients with ASD. Since that report, a large and ever-expanding body of work has shown children with ASD suffer from high rates of neurological, physiological, and psychiatric problems. These rates are so high that Atladottir (2012) found an increased probability of hospital contact for 15 of the 16 ICD-9 categories of disease for children with ASD, compared to those without ASD, using a total population sample in Denmark.

Children with ASD suffer from a host of physiological and neurological problems. Roughly half of this population has an intellectual disability and about 10% of the disorder is attributable to genetic (e.g., Fragile X, Rett’s Syndrome), metabolic, or neurological conditions. The three most pressing and prevalent physical conditions in ASD include: 1) seizure disorders, which occur in roughly a third of the population and has been linked to early mortality among this group; 2) gastrointestinal problems, which seems to occur in at least half of the population; and, 3) sleep disorders, which range from 40%-80% of the population. Metabolic syndromes, hormonal dysregulation, eczema and skin allergies, and headaches are often associated with ASD as well.

Numerous studies document that individuals with ASD have extremely high rates of psychiatric disorders, including both internalizing and externalizing disorders. Methodological differences between studies and the fact that no standardized measure of psychiatric symptoms has been validated in ASD make it difficult to pinpoint exact prevalence rates. Nevertheless, rates as low as 63% and as high as 96%, for a single disorder, have been reported. In a clinic-referred sample, Joshi and colleagues (2010) found over 90% of youth with ASD had 3 or more psychiatric disorders.

Treatments in ASD

Only two treatments have been approved by the Food and Drug Administration for use in ASD. This includes use of risperidone and aripiprazole to treat tantrums or aggression among early school-aged children with ASD. Serious side-effects are known to accompany these medications, however. This includes, but is not limited to, weight gain, gynecomastia, drowsiness, and involuntary movements such as tardive dyskinesia. Combining parent training interventions with these medications have also shown to modestly increase treatment effects. The Cochrane group has reviewed the literature on numerous treatments for co-occurring
psychiatric symptoms among this population\textsuperscript{71}. Outside of risperidone and apipripazole, the Cochrane group has not supported the efficacy or effectiveness of any other pharmacologic or non-pharmacologic treatment of psychiatric symptoms in ASD\textsuperscript{71}.

**Use Of Compounds In A Clinical Sample**

**Executive Summary**

For this study, data was collected through collaboration with the Center for Autism and Related Disorders (CARD) clinic at Kennedy Krieger Institute (KKI). Data was abstracted from the electronic medical records of children with ASD under 17 years of age that receive care at KKI-CARD. Based on these responses, we assessed frequency of treatment use for each of the six compounds of interest.

**Background**

KKI-CARD is one of the oldest, largest, and well-known ASD specialty clinics in the United States. Founded 24 years ago by Dr. Rebecca Landa, CARD provides evaluation and ongoing treatment for children and adolescents with ASD. CARD provides a host of services, including, but not limited to, interdisciplinary diagnostic evaluation for youth ages 0-18 years, speech language pathology, physical and occupational therapy, psychiatric evaluation and treatment, social work services, and care coordination.

CARD serves a range of populations, both within Baltimore and beyond. Roughly 80% of referrals to CARD come from the state of Maryland and slightly more than half are between 0-6 years of age (58%) and Caucasian (51%; 27% African American, 5% Hispanic, 17% Other/Multiracial). Most children are covered under private insurance (60%; 31% receive Medical Assistance; 9% Other types of insurance).

**Objective**

To examine the use of compounds of interest at KKI-CARD.

**Methods**

**Inclusion Criteria**

To be included in this analysis, the child must be under 17 years of age, have a KKI-confirmed Autism Spectrum Disorder (ASD) diagnosis, and the parent must agree to join the clinical research registry. The age limit, of 17 years, was put in place to avoid any legal complications involving the need for receiving consent from the adult with ASD themselves since the registry consent is only provided by the parent. The registry sample was exclusively used since it provides prospective consent, from parents, for researchers at CARD to use the child’s medical records for investigation. All parents have the option to join the IRB-approved registry at two points in time, during their intake (the point of contact with the institution) and when they fill out their background and history forms (3 months before the appointment). Registry consent is provided online. On average, 80% of parents agree to join the registry, and 70% complete their background and history form. The final inclusion criterion, which required the child to have an ASD diagnosis, was put in place to ensure sample validity. All diagnoses
were provided by a licensed physician or doctoral level clinical psychologist based on ASD diagnostic criteria set forth by the *Diagnostic and Statistical Manual, Fourth or Fifth Edition*. For more details on the registry, see Kalb and colleagues (2019). Figure 1 shows the sample derivation.

**Figure 1: Sample Derivation**

![Sample Derivation Diagram]

**Sample**

A total of 1,788 youth met these criteria. Children in the KKI-CARD Registry were, on average, 7 years of age (Mean = 7.35, SD = 4.10), 80% were from Maryland (12% Virginia, 8% other states), 29% were receiving Medical Assistance, and most (82%) were male. Slightly more than half were Caucasian (55%), with the remaining portion being African American (22%), Asian (8%), and other race (15%).

**KKI Background and History Form**

The first set of data comes from the KKI-CARD’s background and history form. This form covers a range of clinical topics, from developmental milestones to family history, which providers use to inform the child’s evaluation and/or treatment. From this form, three items are of interest. Question 89 asks parents “Does the child receive any of the following treatments (check all that apply)”. The list provided includes three relevant items: Chelation, Glutathione, and Other (where parents can write in any other related treatments). The Chelation and Glutathione items are dichotomous (yes/no), while the “Other” provides an open text box for parents to list any additional treatments. Question 61 provides parents an open text box to list “any treatments they are currently taking”. Question 63 is similar in format, asking caregivers to “provide any vitamins and supplements and their reason for taking them”. Parents are able to list up to 7 medications (Q61) and vitamins/supplements (Q63). Unfortunately, neither route of administration nor prescriber is known, since it is not asked in the questionnaire.

Since only chelation and glutathione are directly asked in the form, a string search was conducted for Q89, Q61, and Q63. A total 6 different terms [melatonin, glutathione, Vitamin B12, chelation (“DMPS”), oxytocin, inositol] were search across all open text boxes. This occurred using the regular expression (regexm) command in STATA 15.0. This command searched all strings for any string that included the first four letters (to account for misspelling) of the compound of interest. If a string was identified, it was then manually reviewed, and appropriately coded, by Dr. Kalb.

**KKI Prescription Database**

The second dataset came from the KKI’s prescription database. This database originates as a combination of parent and clinician report. More specifically, at the appointment, a caregiver can request a medication from their medical provider or a physician can initiate a prescription. A nurse or intake specialist then enters that information into the prescription database, which is signed off by medical (physician) provider. Prescriptions can be new or refills and prescriptions can be generated from any KKI site, not just CARD, if a child is seen at another location.
The KKI prescription database was retrieved from the information systems department. That department used the drug alias list (N=132) to string search the prescription database using Python. Results of the search, including the name of the compound and route of administration, were then returned to Dr. Kalb.

Analysis

Descriptive statistics were employed to understand the proportion of children using the compound of interest.

Results

KKI Background and History Form

Less than 10% (N=166) of parents reported their youth used one or more of these compounds across all 3 relevant items. In fact, only six youth co-used two of these compounds; none used three or more simultaneously. Overall use of these compounds was rare. Results from the analysis are shown in Table 1.

KKI Prescription Database

A total of 240 youth (13%) from the KKI registry matched in the prescription database. Of those in the prescription database, most only had one prescription (N=111, 46%), 51 had two prescriptions (21%), 29 had 3 (12%), and the remaining had 4+ (N=49, 21%).

From the list of compounds, a total of 603 prescriptions were provided between 2013 and 2019. Of those prescriptions, almost all were for Melatonin, while a few were provided for Vitamin B12 and glutathione. Only two of these medications (glutathione) were non-oral (injection). No prescriptions for oxytocin, chelation, or inositol were found.

Discussion

Results from this analysis demonstrate melatonin was the most frequently used compound. Per parents, use was still infrequent (<10%). If parents received a prescription of melatonin at KKI, it was always orally administered. The second most common compound was Vitamin B12, although use was rare (<1%) per parents and prescriptions (<3% of all prescriptions); all
prescriptions for Vitamin B12 were oral. Glutathione was also rarely (<1%) reported by parents and prescribed at KKI (n=2); although when prescribed at KKI, it was administered as an injection. Chelation (“DMPS”), oxytocin, inositol, were only reported by 3 parents via the background form, none of which were prescribed at KKI.
Use of Compounds in a Population Sample

Executive Summary

For this cross-sectional study, data was collected through collaboration with the Simons Foundation Powering Autism Research through Knowledge (SPARK) initiative, an online registry of self-referred parents/caregivers of individuals with autism. Parents of children under 18 years of age were invited to participate in an online survey capturing basic demographic information and answer questions about specific treatments and interventions used to address ASD symptoms in their children. Based on these responses, we assessed frequency of treatment use, motivating symptoms for treatment use, and perceived effectiveness by the respondent.

Background

This online survey provides an up to date assessment of ASD treatment use in a community ascertainment sample, where deep phenotypic data are available in a very large sample size. Understanding which treatments are used by individuals with ASD, how they are accessed, and their perceived effectiveness has implications for clinical practice and policy. Additionally, understanding perceived indicators for treatment use (ASD core symptoms vs. comorbid behavior) has implications for developing policy guidelines regarding compounding for currently regulated and unregulated substances.

Objectives

To examine the use of the 6 compounds of interest among a national sample of SPARK-enrolled children with autism.

Methods

Recruitment was conducted through SPARK and their online autism research registry, which has 20,000 individuals with Autism Spectrum Disorders (ASD) and their families participating as of April 2017. Recorded primary family contacts were emailed regarding the opportunity to participate in the study. Inclusion criteria were: 1.) Having a child with ASD between age 2 years and 17 years and 10 months of age; 2. A Social Communication Questionnaire (SCQ) Scale score greater or equal to 12 to increase sensitivity of a reported ASD diagnosis; and 3.) Consent for data sharing with investigators for future research studies. Adopted children were allowed to participate and if a family had more than 1 child with ASD enrolled in SPARK, a single child from that family was invited to participate at random.

Up to three email invitations were sent over a 4-week period. Recipients who have not responded after 6 weeks will be considered to have refused participation. Those who accepted the invitation were prompted to complete an online consent after which they may complete and submit the Biomedical Treatments for ASD Questionnaire (see Appendix 1). Data collection began on 12/11/19 and closed out on 2/17/20. Of 1,487 total respondents, 1,171 completed the entire survey. Detailed information on response is presented in Appendix 2.

Analysis

We used descriptive statistics to examine frequencies of use for each of the 6 compounds of interest.
Results

We received a total of 1,487 responses to the survey. The survey was most often completed by the biologic mother of the child with ASD (Table 4). Participants were most often non-Hispanic white and children with ASD were more likely to be male.

<table>
<thead>
<tr>
<th>Table 4- Respondent Data and Demographics of Children with ASD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Respondent Data</strong></td>
</tr>
<tr>
<td>Total Respondents</td>
</tr>
<tr>
<td>Respondent Relationship to child</td>
</tr>
<tr>
<td>Biological Mother</td>
</tr>
<tr>
<td>Biological Father</td>
</tr>
<tr>
<td>Other Primary Caregiver</td>
</tr>
<tr>
<td>Missing</td>
</tr>
<tr>
<td>Highest level of education completed by mother</td>
</tr>
<tr>
<td>Graduate/professional degree (MD, PhD, MA/MS, MBA, etc.)</td>
</tr>
<tr>
<td>Baccalaureate degree (four-year degree)</td>
</tr>
<tr>
<td>Associate degree (two-year degree)</td>
</tr>
<tr>
<td>Completed some college</td>
</tr>
<tr>
<td>Trade or vocational school (after high school)</td>
</tr>
<tr>
<td>GED diploma</td>
</tr>
<tr>
<td>Some high school</td>
</tr>
<tr>
<td>High school graduate</td>
</tr>
<tr>
<td>Did not attend high school</td>
</tr>
<tr>
<td>Missing</td>
</tr>
</tbody>
</table>

| Demographics of Children with ASD                            |                  |
| Sex (%)                                                      |                  |
| Male                                                         | 1180 (79.4)      |
| Female                                                       | 270 (18.2)       |
| Missing                                                      | 37 (2.5)         |
| Mean Age in Years (SD)                                       | 9.19 (4.27)      |
| Race (%)                                                     |                  |
| *respondents select all that apply                           |                  |
| Asian                                                        | 76 (5.1)         |
| Black or African-American                                    | 139 (9.3)        |
| Native Hawaiian or Pacific Islander                          | 11 (0.7)         |
| White                                                        | 1247 (83.9)      |
| Other                                                        | 120 (8.1)        |
| Ethnicity                                                    |                  |
| Hispanic                                                     | 229 (15.4)       |
| Non-Hispanic                                                 | 1215 (81.7)      |

We found that 41.8% of children had been treated with any Complimentary or Alternative Treatment (Figure 2). Of the compounds of interest, melatonin was most frequently used, followed by Vitamin B12 substances (6.0% Methyl B12, 5.5% B Complex). Use of the additional compounds of interest were more rare and endorsed use in <2% of responses.
Participants reported that melatonin was most often administered orally (96.0%), followed by sub-lingual and dermal administrations (6.2% and 4.0%, respectively) (Figure 3). Melatonin was most often used to address sleep problems as reported in the survey (Figure 3).

Among those respondents that endorsed ever using melatonin to treat their child with ASD, nearly half reported the treatment to be very helpful (Figure 4). As presented in Appendix 3, Figure 5, respondents reported that a medical professional most often suggested the use of melatonin for their child, and it was most often purchased over the counter (Figure 6).
While more rare, Methyl B12 and B Complex Vitamins were also endorsed in 6.0% and 5.5% of participants. For Methyl B12, the 58.9% of use was oral, though 32.8% was administered via injection. Notably, respondents reported that Methyl B12 was administered to address difficulties with language and communication. However, 92.5% of respondents reported use of B Complex vitamins with primarily oral administration. In contrast, respondents endorsed a range of behavioral and psychiatric symptoms as motivation for use (Figure 5).

Figure 4- Perceived Effectiveness of Melatonin In Addressing Symptoms / Conditions Endorsed

Figure 5- Frequencies of Vitamin B Routes of Administration, and Symptoms / Conditions Present for Use
For both Methyl B12 and B Complex vitamins, respondents reported that use was of little benefit or presented no change. Both substances were most often suggested by a medical professional. While B Complex vitamins were most often purchased over the counter, Methyl B12 use came from multiple sources including over the counter purchase, prescription, and online orders with nearly equal frequency.

Figure 6- Perceived Effectiveness of Vitamin B In Addressing Symptoms / Conditions Endorsed

Detailed information on other substances of interest examined is reported when non-missing in Appendix 5.

Discussion

While many families in SPARK have used CAM therapies, those most commonly used were melatonin and B12. Notably, melatonin use was reported for treatment of sleep difficulties and not core symptoms of ASD. Treatment for sleep was thought to be effective. For B12, treatment was often used for core ASD symptoms. Vitamin B supplements, in contrast, were reported to be used to treat symptoms related to many psychopathologies. The effectiveness of these Vitamin B therapies was more often judged to be slightly helpful or result in no change. Limitations of this work include that this study relies solely on self-report and is subject to recall bias. In a large sample of this nature, it would not be possible to validate these reports and likely such data would not be available. Despite these limitations, this project provides valuable information regarding current treatment practices in a large sample of individuals with ASD.
Use of Compounds in a National sample

Executive Summary

The extensive and detailed administrative records of Medicaid beneficiaries are a rich source of information about the nationwide patterns of health care usage in the United States. For this phase of the project, Medicaid claims data for the years 2010-2014 was analyzed to assess the historical patterns and frequency of use for the 6 compounds of interest. A total of 369,691 children with ASD, aged 2-17 years, were included in this analysis. Drug codes used to identify filled prescriptions and provider administered infusions of the compounds of interest. Information about frequency, routes of administration, location, and discipline of providers who prescribed these drugs was also captured.

Background

Medicaid, Autism, & Claims Data

Medicaid is the primary source of health insurance for one in four children in the United States. While most Medicaid-enrolled children qualify based on their family’s low-income status, specific eligibility criteria vary across states. Some states additionally allow coverage for children with disabilities that impair functioning regardless of income and others still provide waivers to include children with specific diagnoses or conditions that are not traditionally covered by Medicaid. As a result, many children with ASD in the United States qualify for and are insured by Medicaid coverage under several different forms of eligibility.

Medicaid Claims Data

All states report detailed budgets and expenditures for Medicaid to the Centers for Medicare and Medicaid Services (CMS). In order to support research and policy analysis initiatives for Medicaid and other low-income populations, the CMS has made these data publicly available. The primary data sources for Medicaid statistical data are the Medicaid Statistical Information System (MSIS), the Medicaid Analytic eXtract (MAX) files, and the CMS-64 reports. MSIS is the basic source of state-submitted eligibility and claims data on the Medicaid population, their characteristics, utilization, and payments. The MAX data are a set of person-level data files derived from MSIS data on Medicaid eligibility, service utilization and payments. The data are available for all states and the District of Columbia beginning with calendar year 1999. For the present study, MAX data were analyzed to characterize the patterns and prevalence of use of melatonin, B12, oxytocin, inositol, glutathione, and DMPS chelation among Medicaid-enrolled children with ASD.

Objectives

To examine the frequency, route of administration and providers of the compounds of interest, among Medicaid-enrolled children with ASD, between 2010-2014.
Methods

Inclusion Criteria

Data were abstracted from the MAX General Information files for calendar years 2010-2014. Of the individuals included in these files, only those with a unique patient id (variable name `bene_id`) and complete information about sex, date of service and date of birth were eligible for inclusion. Since the focus of the study was children, those aged less than 2 and older than 18 were excluded. The lower limit of age 2 was employed since community-based diagnoses less than two are rare and may be unreliable. Individuals with fewer than 15 days of eligibility in at least one month were excluded, as were those with an eligibility gap greater than 3 months.

ASD Case Definition

Cases of ASD were identified when a child had at least two encounters in an outpatient setting with an ICD-9-CM code of 299.x between 2010 and 2014. This approach provides a measure of reliability for diagnosis and is similar to other ASD Medicaid claims-based studies. We did not apply a minimum Medicaid eligibility requirement prior to the first ASD diagnosis for inclusion as this is a study of prevalent cases.73

Measures

We assessed frequency, providers, and patterns of use of melatonin, B12, oxytocin, inositol, glutathione, and DMPS chelation within this sample of children with and without ASD who received care through Medicaid. We examined both compounding ingredient (unfinished) and pharmaceutical company-manufactured product (finished) versions of each drug substance. We abstracted data on drug utilization using National Drug Codes (NDC) for filled prescriptions and Healthcare Common Procedure Coding System (HCPCS) and Current Procedural Technology (CPT) codes for documented infusions of the substance in a medical setting.

Prescription Codes:

In the CMS prescription database, drugs are identified using NDC. In order to query this database, we searched the list of finished and unfinished drug products in the FDA NDC directory (12/11/2018 version) for the codes of all substances containing the 6 drugs of interest. We searched within the nonproprietary name and substance name columns (variable names `product.xlsx`, `package.xlsx`, `unfinished_product.xlsx`, and `unfinished_package.xlsx`) then manually reviewed the results to confirm that only drugs of interest were included. NDC codes for pure substances as well as products composed of a combination of one of the drugs of interest and other drug substances were included. In total, we identified 458 relevant “finished” NDC codes and 117 relevant “unfinished” NDC codes (See Appendix 7).

Administered Infusion Codes:

In the MAX data, medical procedures and services are denoted with HCPCS and CPT codes. These codes are used by medical providers to make billing claims for services rendered. A list of pertinent HCPCS/CPT codes for only infusions of the drugs of interest was compiled by searching within the 2014 HCPCS Alpha-Numeric Index.
Linkage of Drug Codes to ASD Cases:

Using the identified HCPCS/CPT and NDC codes, we linked the dates for the administered infusions (HCPCS/CPT) and prescription fills (NDC) for each of the 6 drugs to the child subjects with ASD in the Medicaid billing data cohort file. Only infusions or prescriptions filled on or after the first ASD encounter contributed (See Appendix 8 for NDC drugs prescribed to children with ASD). The cumulative frequencies of provider administered infusions and prescription fills for finished and unfinished drug products were recorded, as well as the frequencies of different formulations and routes of administration used (See Appendix 9 for ASD Pediatric National and State Frequencies for Each Rx).

National Provider Identifiers

In the Medicaid data, codes for prescription fills and infusions are also associated with a National Provider Identifier (NPI), hospital ID or other state assigned numerical code that identifies the prescribing and/or administering provider. We linked all filled prescriptions (NDC) and administered infusions (HCPCS/CPT) of the compounds of interest to their associated NPI and provider ID codes and reported the frequencies of both for each provider. Identification codes of providers that billed filled at least 11 prescriptions and/or administered infusions of compounds to children with ASD are listed in Appendix 10. Only NPI’s and provider IDs associated with at least 11 billed filled prescriptions of compounds of interest to children with ASD were included in the report.

A separate record was made of just NPI’s and the combined frequencies of the administered infusions and filled prescriptions for each which was used to identify providers associated with the highest volume of prescriptions. A list of all NPIs shown to have billed for at least 11 compounds of interest to children with ASD is included in Appendix 11. The names and locations of the largest providers were found by using an online NPI number directory search.

Analysis

Descriptive statistical analyses were performed.

Results

A total of 37,022,310 children aged 2-17 years were enrolled in Medicaid from 2010-2014 and met the inclusion criteria. Of these, 369,691 had at least two encounters for ASD. The majority of the children with ASD were male (79.4%) and white (49.8%) with “Blind/Disabled, Cash” being the most common Medicaid eligibility (44.5%). The median age of first ASD encounter was 9 years with a median number of ASD encounters of 16. There was a median of 0.7 years between the first Medicaid eligibility and first ASD diagnosis and 2.4 years between first ASD encounter and the end of eligibility or December 31, 2014. The median time between Medicaid eligibility and first ASD diagnosis was 0.7 years. See Table 5 for details. (See Appendix 6 for demographic means and frequencies nationally and by state).
<table>
<thead>
<tr>
<th></th>
<th>ASD</th>
<th>Non-ASD</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>369,691</td>
<td>36,652,619</td>
</tr>
<tr>
<td><strong>Sex, %</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>79.4</td>
<td>49.7</td>
</tr>
<tr>
<td>Female</td>
<td>20.6</td>
<td>50.3</td>
</tr>
<tr>
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<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>Race/Ethnicity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White, Not of Hispanic Origin</td>
<td>49.8</td>
<td>36.6</td>
</tr>
<tr>
<td>Black, Not of Hispanic Origin</td>
<td>13.6</td>
<td>21.4</td>
</tr>
<tr>
<td>Hispanic</td>
<td>9.2</td>
<td>23.2</td>
</tr>
<tr>
<td>Hispanic or Latino and one or more races</td>
<td>3.7</td>
<td>4.6</td>
</tr>
<tr>
<td>Asian or Pacific Islander</td>
<td>1.8</td>
<td>2.8</td>
</tr>
<tr>
<td>More than one race (Hispanic or Latino not indicated)</td>
<td>1.5</td>
<td>0.7</td>
</tr>
<tr>
<td>American Indian or Alaskan Native</td>
<td>0.9</td>
<td>1.3</td>
</tr>
<tr>
<td>Native Hawaiian or Other Pacific Islander</td>
<td>0.5</td>
<td>0.9</td>
</tr>
<tr>
<td>Unknown</td>
<td>19.1</td>
<td>8.5</td>
</tr>
<tr>
<td>Missing</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>*<em>Medicaid Eligibility Criteria</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blind/ Disabled, Cash</td>
<td>44.5</td>
<td>4.0</td>
</tr>
<tr>
<td>Child, Poverty (Includes Medicaid Expansion CHIP Children)</td>
<td>20.9</td>
<td>49.7</td>
</tr>
<tr>
<td>Child (NOT child of unemployed adult, not foster care child), Eligible under Section 1931 of the ACA</td>
<td>8.4</td>
<td>29.7</td>
</tr>
<tr>
<td>Other Blind/Disabled</td>
<td>7.7</td>
<td>0.4</td>
</tr>
<tr>
<td>Foster Care Child</td>
<td>6.5</td>
<td>3.0</td>
</tr>
<tr>
<td>Blind/ Disabled, Poverty</td>
<td>4.3</td>
<td>0.2</td>
</tr>
</tbody>
</table>
Using the cumulative frequency data reported, we calculated the percentages of children with and without ASD that had administered infusions and/or prescription fills for finished and unfinished products (Table 6). B12 had the greatest prescription and infusion frequencies (1.1%).

**Table 6- Percent of Children Aged 2-17 With and Without ASD Enrolled in Medicaid with Prescription Fills (identified by NDC code) and Administered Infusions (identified by HCPCS/CPT code) of Drugs of Interest, 2010-2014**
<table>
<thead>
<tr>
<th></th>
<th>Prescription or Infusion (%)</th>
<th>Prescription NDC code (%)</th>
<th>Prescription Finished Product (%)</th>
<th>Prescription Unfinished Product (%)</th>
<th>Infusion HCPCS/CPT code (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ASD</td>
<td>Non-ASD</td>
<td>ASD</td>
<td>Non-ASD</td>
<td>ASD</td>
</tr>
<tr>
<td><strong>B12</strong></td>
<td>1.1</td>
<td>1.2</td>
<td>1.1</td>
<td>1.2</td>
<td>1.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Melatonin</strong></td>
<td>0.15</td>
<td>0.01</td>
<td>0.15</td>
<td>0.01</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Oxytocin</strong></td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>&lt;0.01</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>38</td>
<td>N=104</td>
<td></td>
</tr>
<tr>
<td><strong>Glutathione</strong></td>
<td>0.05</td>
<td>0.01</td>
<td>0.05</td>
<td>&lt;0.01</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>106</td>
<td>N=33</td>
<td></td>
</tr>
<tr>
<td><strong>Inositol</strong></td>
<td>0</td>
<td>0.01</td>
<td>0</td>
<td>0.01</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>DMPS</strong></td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>N=1</td>
<td>N=1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>N=77</td>
<td></td>
</tr>
</tbody>
</table>

N only included when greater than 11; **N=369,691 ASD, N= 36,652,619 non-ASD.

For filled prescriptions, we also calculated the percentages of children with and without ASD that used different formulations and routes of administration for each drug (Table 7). Injection and solution were the most common form of B12 prescriptions (0.05%, 0.05%).

The list of NPI and provider ID codes linked with their associated frequencies of filled prescriptions (NDC) and administered infusions (HCPCS/CPT) were used to calculate the total number of unique providers for each drug (Tables 8 & 9). The frequencies for each provider were used to calculate the median and numerical range of filled prescriptions and infusions that originated from each individual provider. Finally, the total number of providers responsible for ≥50% of both filled prescriptions and infusions was calculated.
Table 7 - Percent of Children With and Without ASD Enrolled in Medicaid with Filled Prescription (NDC) for Drugs of Interest by Formulation and Route of Administration, 2010-2014

<table>
<thead>
<tr>
<th></th>
<th>B12 (%)</th>
<th>Melatonin (%)</th>
<th>Oxytocin (%)</th>
<th>Glutathione (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ASD</td>
<td>Non-ASD</td>
<td>ASD</td>
<td>Non-ASD</td>
</tr>
<tr>
<td>Inhalation</td>
<td>0</td>
<td>0</td>
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<tr>
<td></td>
<td>N=49</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Injection</td>
<td>0.05</td>
<td>0.01</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>N=184</td>
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<td></td>
<td></td>
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<tr>
<td>Powder</td>
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<td>&lt;0.01</td>
<td>0.01</td>
</tr>
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<td></td>
<td>N=116</td>
<td>N=2</td>
<td>N=33</td>
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<td>0</td>
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<td></td>
<td>N=182</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Crystal</td>
<td>0.04</td>
<td>&lt;0.01</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>N=162</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intramuscular</td>
<td>0.04</td>
<td>0.01</td>
<td>0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>N=155</td>
<td></td>
<td></td>
<td>N=4</td>
</tr>
<tr>
<td>Intravenous</td>
<td>0.02</td>
<td>&lt;0.01</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>N=81</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nasal</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>N=18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>N=79</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral</td>
<td>1</td>
<td>1.2</td>
<td>0.14</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>N=3,561</td>
<td>N=522</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 8- Physicians Who Wrote Drug Prescriptions (Minimum 11) Filled by Children with ASD Enrolled in Medicaid, 2010-2014

<table>
<thead>
<tr>
<th>Physicians</th>
<th>Total Number of Unique Prescribing Physicians</th>
<th>Median Number of Rx Filled by Each Physician (Range)</th>
<th>Total Number of Physicians Responsible for ≥ 50% of Prescriptions</th>
</tr>
</thead>
<tbody>
<tr>
<td>B12</td>
<td>388</td>
<td>16 (11 – 524)</td>
<td>89</td>
</tr>
<tr>
<td>Melatonin</td>
<td>127</td>
<td>21 (11-229)</td>
<td>30</td>
</tr>
<tr>
<td>Oxytocin</td>
<td>1</td>
<td>27*</td>
<td>1</td>
</tr>
<tr>
<td>Glutathione</td>
<td>7</td>
<td>24 (12-227)</td>
<td>1</td>
</tr>
</tbody>
</table>

*no range, only 1 provider

Table 9- Providers Who Administered Drugs of Interest (Minimum 11) to Children with ASD Enrolled in Medicaid, 2010-2014

<table>
<thead>
<tr>
<th>Providers</th>
<th>Total Number of Unique Administering Providers</th>
<th>Median Number of Infusions Administered by Each Provider (Range)</th>
<th>Total Number of Providers Responsible for ≥ 50% of Drug Administrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>B12</td>
<td>419</td>
<td>18 (11 - 1096)</td>
<td>89</td>
</tr>
<tr>
<td>Melatonin</td>
<td>160</td>
<td>20.5 (11-87)</td>
<td>52</td>
</tr>
<tr>
<td>Oxytocin</td>
<td>2</td>
<td>25.5 (25-26)</td>
<td>1</td>
</tr>
<tr>
<td>Glutathione</td>
<td>5</td>
<td>23 (11-718)</td>
<td>1</td>
</tr>
</tbody>
</table>

National Provider Identifier (NPI)

From the list of frequencies of total administered infusions and filled prescriptions for each NPI, we identify the providers associated with the highest volume of prescriptions (Figures 13-16). Lee Silsby Compounding Pharmacy in Cleveland Heights, OH (NPI 1780763276) was the
highest volume provider of any medication with 704 prescriptions for glutathione and 1126 prescriptions of Vitamin B12 (unknown route of administration). Ron’s Pharmacy Services in San Diego, CA (NPI 1851593560) was the highest volume facility to provide IM infusions of Vitamin B12 with 32 filled prescriptions. The top two highest volume providers of IV Vitamin B12 were Children’s Hospital Medical Center (Home Health IV Therapy Pharmacy) in Cincinnati, OH (NPI 1629116645) with 136 infusions and BIORX LLC in Cincinnati, OH (NPI 1851337778) with 111 filled prescriptions.

**Figure 13- NPI's and Associated Frequencies of All Glutathione Prescriptions**

**Figure 14- NPI's and Associated Frequencies of Intramuscular (IM) Vitamin B12 Prescriptions**
Discussion

This analysis leveraged a large national dataset to characterize the historical use of our 6 drugs of interest by Medicaid enrolled children with ASD. Use of these medications was rare in this sample. Of the medications, B12 was the most frequently used although the percent of users observed was still low. There was also little indication of increased use among children with ASD, compared to children without ASD, in this sample.

Because Medicaid enrollment and reporting standards are mandated by state and federal government, the sample selection for Medicaid eligible children with ASD was comprehensive and systematic. That said, eligibility criteria and reporting practices vary significantly between states, so the national frequencies and averages likely do not represent all children with ASD in all states equally. Furthermore, not all states had data available for all years 2010 through 2014. The list of which states were available for what years can be found [here](#).
Another limitation is that drug utilization paid for out of pocket, by private insurance, or other non-Medicaid means is not captured by CMS data. The elevated median age of first encounter for ASD (9 years) also suggests that eligible children with ASD diagnoses experience some delay when enrolling in Medicaid. Any drug use prior to enrollment would likewise not be captured by the CMS data. While this missingness may contribute to the low drug use frequencies among the prevalent ASD cases, the rates of drug use are similarly low in the other branches of this project which are not limited by these criteria.

Finally, it is important to note that the reason for being prescribed or administered each drug cannot be assumed from the data. As such, we cannot know if the drugs were specifically used as a treatment for the children’s ASD. Self-report data from the SPARK survey will assist in filling these gaps in the current analysis.
Executive Summary

Healthcare providers and researchers with expertise in ASD, Complementary and Alternative Medicine (CAM) and/or compounded medications were recruited and interviewed to develop a more nuanced understanding of the practices and beliefs associated with the use of nontraditional biomedical treatments for individuals with ASD. In this study we gathered information about 1) the professional background of our participants and their self-reported level of knowledge in ASD, CAM, and/or compounding 2) their opinions on CAM and compounding utilization in the general public and individuals with ASD, 3) their familiarity with specific drug substances in these categories, and 4) their perceptions on the safety and effectiveness of these substances. Included in the specific treatments were the compounds of interest—inositol, B12, glutathione, DMPS chelation, oxytocin, and melatonin.

Background

While there is no known “cure” for ASD, there is a wide variety of treatments and therapy options used to address the core and co-occurring symptoms of children with ASD. Since these therapies are not all evidence-based, and vary in their degree of safety and effectiveness, it is important to understand: 1) which CAM and/or compounded substances different healthcare providers recommend and prescribe to their patients; 2) how much providers and experts in the field know about these treatments and others used by patients; and 3) how these different professionals feel about the safety, effectiveness and regulations of such treatments. The Key Opinion Leaders (KOL) in this study were all physicians with research experience who worked directly with patients with ASD.

Objectives

To obtain qualitative data from KOLs on the prescribing practices, patterns of use, safety, and effectiveness of compounded drug substances and CAM treatments used to treat ASD.

Subjects

Individuals considered for comment were identified by study staff as being knowledgeable in the field of ASD, CAM and/or compounding within the United States. These individuals were found by reviewing existing literature on CAM use for ASD and by reaching out to faculty at the Center for Autism and Related Disorders (CARD) at the Kennedy Krieger Institute (KKI) (See Appendix 12 for template of invitation emails sent to KOLs). Participating individuals were healthcare professionals with published research in relevant fields and/or practitioners actively treating children with ASD. Compounding pharmacies were contacted for comment as well, but none responded. KOL participant profiles are summarized in Table 10.
**Table 10 - Key Opinion Leader Profiles**

<table>
<thead>
<tr>
<th>Areas of Experience</th>
<th>KOL 1</th>
<th>KOL 2</th>
<th>KOL 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Profession and Leadership Roles</strong></td>
<td>MD, developmental and behavioral pediatrician</td>
<td>MBBS, pediatrician, neurodevelopmental disabilities specialist</td>
<td>MD, developmental and behavioral pediatrician</td>
</tr>
<tr>
<td>Division Chief of Developmental and Behavioral Pediatrics</td>
<td>Assistant Medical Director of Center for Autism and Related Disorders</td>
<td>Director of Medical Informatics and the institution’s Interactive Autism Network (IAN)</td>
<td></td>
</tr>
<tr>
<td>Professor of Pediatrics</td>
<td>Assistant Professor in Neurology and Neurodevelopmental Medicine</td>
<td>Associate Professor of Pediatrics</td>
<td></td>
</tr>
<tr>
<td><strong>Research</strong></td>
<td>Lead admin. for Leadership Education in Neurodevelopmental and related Disabilities (LEND) program</td>
<td><strong>Primary research</strong>: metabolic and mitochondrial disorders associated with ASD, regression in ASD and early identification and treatment of autism and communication disorders</td>
<td>Previous director of Interactive Autism Network (IAN), online registry for children with ASD</td>
</tr>
<tr>
<td></td>
<td><strong>Primary research</strong>: ASD, diet and nutrition, and medication management of ASD including CAM</td>
<td></td>
<td><strong>Primary research</strong>: early identification, evaluation, and treatment of children and adolescents with developmental disabilities, including ASD, learning, and attention disorders</td>
</tr>
<tr>
<td><strong>ASD</strong></td>
<td>Extensive clinical experience, primary research focus</td>
<td>Extensive clinical experience, primary research focus, close family connection (adult son with ASD)</td>
<td>Extensive clinical experience, primary research focus</td>
</tr>
<tr>
<td><strong>CAM</strong></td>
<td>Extensive clinical experience, primary research focus (esp. CAM for ASD)</td>
<td>Extensive clinical experience, refers to evidence-based literature and NIH materials for updated info</td>
<td>Extensive clinical experience, generally familiar with scientific literature, relies on colleagues, experts, and scientific reviews</td>
</tr>
<tr>
<td><strong>Compounding</strong></td>
<td>Has patients that use compounded substances but does not prescribe them, keeps up with</td>
<td>Has prescribed compounded preparations and worked with a metabolic geneticist that formulated, refers to evidence-</td>
<td>Little to no direct experience with patients that use compounded substances, does</td>
</tr>
</tbody>
</table>
Methods

For this study, JHU research staff conducted in-depth phone interviews of experts in ASD, CAM, and compounding (Appendix 13). The interview was developed and reviewed by an interdisciplinary team of child psychiatrists, epidemiologists, autism researchers, and pharmacology experts at JHU. All three participants were pediatricians with published research in relevant fields (see Table 9). Invitations to participate in the study were sent via email using publicly available contact information provided online by the individuals. Those who agreed to participate were sent an overview of the interview questions in advance. The phone interviews were conducted by a single JHU research staff member and were recorded for later review. These audio recordings were reviewed and summarized in order to provide a deeper characterization of unique individual perspectives as well as overarching themes that emerge across respondents. Key quotes were extracted from the recorded interviews to illustrate points described in the summary, however no audio clips, names, or other explicit identifiers that could link participants to responses were included in the report.

Results

Impressions on CAM for ASD

KOLs 1, 2 and 3 were all aware of the CAM practices that their patients use and reported that a high percentage of their ASD patients ask about and/or use some sort of CAM. All discuss CAM with their patients to provide guidance on safety and effectiveness; however, none prescribe or initiate CAM treatments themselves unless it is for a conventional medical indication (e.g. all have recommended melatonin because they consider it to be a conventional, evidence-based treatment for sleep). Overall, attitudes towards CAMs were ambivalent since it is a vast category that encompasses a broad array of biological and nonbiological treatments and interventions – all three providers acknowledged that some are potentially useful, some ineffective, and others harmful.

Impressions on Compounding for ASD

Opinions on compounding for ASD were more varied. KOLs 1, 2 and 3 were all familiar and generally comfortable with the practice of compounding for patients with ASD who need conventional medications reformulated for different administration routes. However, all three had very different opinions on the broader practice of compounding supplements, novel formulations and more for ASD (see Safety and Effectiveness section below). KOL 1 does not prescribe compounded substances for patients and only suggests it as a treatment option when it is medically or behaviorally indicated. They acknowledged the value of “legitimate” compounding practices for ASD but expressed skepticism and concern about drugs compounded into forms outside of their intended use in order to treat ASD.

KOL 2 on the other hand has had professional experience contacting compounding pharmacies to have conventional medications like Clonidine reformulated from solid tablets to
liquids for patients with feeding issues. KOL 2 has also worked closely with a physician that developed an original compounded formulation for mitochondrial dysfunction in ASD that is now produced as a supplement by a pharmaceutical company. KOL 2 did not express the same concerns about custom compounded preparations. KOL 3 did not have additional experience or knowledge about the broader practice of compounding for ASD.

**CAM and Compounding in Clinical Practice**

- **KOL 1**
  - Includes standard questions about CAM use in their medical history review and regularly monitors and follows-up on use at every patient visit. Does not explicitly ask about compounding or discuss it unless patient brings it up.
  - Occasionally recommends CAM substances such as melatonin, diets, and multivitamins when there is a conventional medical indication.
  - Believes the most common CAM for ASD are prayer, gluten free/casein free diets, Omega-3 fatty acids, occupational therapy, sensory interventions, melatonin (though considered this “evidence-based” and not CAM), and cannabidiol (CBD).
  - Believes the most common compounded medication for ASD is multivitamins.
  - Believes the most common route of administration is oral ie. syrup to be put in beverage or medication put in food.

- **KOL 2**
  - Typically asks parents about CAM, supplements, and compounded drug use during medical history check when asking them to list all mainstream and alternative treatments they use, though not part of medical history template as with KOL 1. Considers potential adverse interactions between treatments and asks about the CAM provider and treatment follow-up to ensure safety.
  - Believes the most common CAM for ASD are special diets, B12 shots or medication, multivitamins, compounded preparations for ADHD (“calm”), compounded preparations for anxiety, omega-3 fatty acid, fish oils, carnitine supplements for mitochondrial disorder, patches, “and more”.
  - Believes the most common compounded medication for ASD are multivitamins, probiotics, mitochondrial mixture, CBD, however, is less sure about these.
  - Believes the most common route of administration is liquid (syrup); added that transdermal is newer but also popular.

- **KOL 3**
  - Discusses thoughts and impressions of CAM treatments with patients who inquire about them during clinic visits but does not explicitly ask patients to list CAM or compounding use during routine medical history check.
  - Believes the most common CAM for ASD are commercial products, dietary additives, CBD, and melatonin. Added that these trends have changed over time.
• Is not familiar with compounding for ASD but stated that liquids are generally among the most preferred routes of administration for ASD.

Summary of Participant Characteristics and Motivation for Use
Below we summarize key features of individuals with ASD to highlight symptoms they seek to address, and motivation for use. Overall, patients seek to address core ASD symptoms as well as associated comorbidities. CAM and compounding may have specific socio-demographic correlates, though are only suggested here via subjective data.

Characteristics of Patients Using CAM and/or Compounding for ASD
• Symptoms Associated with CAM Use
  o Core ASD symptoms i.e. restricted, repetitive behaviors or social/communication issues, particularly those with severe impairment (KOL 1, 2, 3)
  o Gastrointestinal issues, feeding issues, and/or food sensitivity/selectivity (KOL 1)
  o Sensory issues (KOL 1)
  o ADHD symptoms (KOL 1, 2)
  o Down Syndrome (KOL 1)
  o Seizures (KOL 2)
  o Sleep issues (KOL 2, 3)
• Symptoms Associated with Compounding Use
  o Compounding specifically is more common in patients with food sensitivities. (KOL 1, 2)

Correlates of CAM and Compounding Use
• CAM is thought to be used by:
  o Patients whose parents use CAM (KOL 1)
  o Parents with more disposable income to spend on CAM (KOL 1)
  o Uneducated parents and/or patients who pursue online leads for CAM (KOL 1), though KOL 3 did not believe education levels made a difference.
  o Parents who do not believe in genetic etiologies for ASD (KOL 1)
  o Parents of younger children who were newly diagnosed with ASD (KOL 2, 3)
  o Older patients in transition periods of life, such as teenagers, who seek additional help with behavior problems (KOL 2)
  o More widely used in white populations than African American, doesn’t think education level makes much difference, gender of child doesn’t make much different, only difference appears to be racial (KOL???)
• Compounding is thought to be used by:
  o People with food intolerances (KOL 1 and 2)
  o More people currently than in the past (e.g. the 90’s and early 00’s) because the practice is better known to the public now and because a range of readymade, compounded preparations are more easily available in stored or online. (KOL 2)
Motivation for CAM Use and Compounding

• CAM provides a feeling of autonomy over treatment and outcomes (KOL 1)
• CAM allows patients to try to fill gaps left by conventional medical treatment and/or try to find a cure (KOL 1, 2 3). “There is a belief system that within functional medicine that there are nutritional and other biologic reasons for the symptoms of autism that are not being addressed by conventional medicine and [by] using integrative and other novel approaches you’re addressing the underlying basic reasons for the symptoms of autism and other behavioral challenges that you see” (KOL 1)
• They believe CAM are harmless (KOL 1) and may help them avoid common side effects of conventional ASD medications (KOL 2)
• They hear about CAM use online or from magazines, friends, family or others with children on spectrum (KOL 3)
• Concern about traditional medicine having offending substance (KOL 1)
• Food selectivity or feeding issues can make conventional medical preparations difficult for patients with ASD to ingest (KOL 1, 2).
• Families read about compounded substances for ASD online, in magazines or by word of mouth and are convinced to try new treatments (KOL 3)

Comments on Compounds of Interest

Inositol

KOL 1 reported that inositol was used as an alternative treatment to address symptoms of ADHD, most commonly by an oral administration. They were not in favor of its use for ASD due to the lack of credible research to support it and inability to rate its safety and/or effectiveness. KOLs 2 and 3 reported having no knowledge about inositol use for ASD.

B12

KOL 1 and 2 have known patients that use B12 supplements, though neither prescribe it to treat ASD. KOL 1 believes it is less commonly used for ASD now than in the past. The most common routes of administration are thought to be oral (KOL 1, 2), subcutaneous (KOL 1), and injection (KOL 2). Both would consider recommending B12 in standard doses to patients who demonstrate low levels due to vegetarian or vegan diet (KOL 1) or other feeding challenges or restrictions (KOL 2), however neither would recommend it for use to treat ASD. KOL 2 did not believe it would have any substantial impact, positive or negative, on children with regular diets and noted that since B12 is a water-soluble vitamin, any excess consumed would be harmlessly excreted. KOL 1 and 3 could not speak to the safety or effectiveness for ASD.

Glutathione

KOL 1 confirmed that glutathione is taken among patients with ASD, most commonly by oral administration; however, would not recommend use for ASD because there is not sufficient data to assess safety and effectiveness. KOLs 2 and 3 know that some patients with ASD take glutathione but do not recommend it themselves and have no further knowledge about its use, safety, or effectiveness.
DMPS Chelation

Though no comments were made about DMPS chelation specifically, KOLs 1, 2, and 3 were aware that chelation in general is used as an alternative treatment in the broader ASD community and they strongly advised against it. KOL 1 reported having lost patients for counseling against the harms of chelation therapy for ASD. All three have prescribed chelation for patients but only when there was a conventional medical indication for it such as lead poisoning. For its indicated purpose, all it is a safe and effective treatment in both oral and IV forms. To treat ASD, however, all three agree that it is unsafe and that there is no indication. KOL 2 expressed skepticism about the validity of lab results provided to patients by third party providers of ASD chelation therapies.

Oxytocin

KOLs 1, 2, and 3 do not have patients that use oxytocin but believe it is widely used elsewhere to treat patients with ASD. They were all aware of the nascent research into its effectiveness for treating ASD. Oral (KOL 1) and intranasal (KOL 1, 2) are the most common routes of administration with intranasal supposedly being most effective (KOL 2). All KOLs consider oxytocin an interesting, potentially promising treatment given the ongoing research and would be in favor of its use to treat ASD in the future if the literature supports it; however, at present they do not feel there is enough evidence or indication to do so safely in a clinical context. KOL 1 added that some studies have demonstrated short term effectiveness, but the version of oxytocin available online is not effective and certain propriety preparations provide doses that are outside the recommended range.

Melatonin

All three commonly recommend the use of melatonin to address sleep issues, specifically sleep onset, for their patients with ASD. KOL 2 noted that about a third of ASD patients report sleep issues. It was considered by all providers to be a conventional, evidence-based treatment which is available OTC so no need for prescription. They reported that oral routes of administration are most common, including tablets and liquid solutions. Safe in correct dosage and proper formulation with “modest” effectiveness (KOL 3).

Safety And Effectiveness

Overall, KOLs 1, 2 and 3 expressed that CAM treatments vary widely in their safety and effectiveness and that it’s important for patients to receive proper guidance and information to make wise decisions. KOL 1 emphasized the importance of “shared decision making” with patients to help them make safe and wise decisions about their own health. “The only way we can enhance safety is with education, and it has to be accessible education.” (KOL 1). KOL 1 and 3 expressed concerns about misinformation online and advocated for organizations that provide evidence-based resources the ASD community.

Regarding compounding for ASD, KOL 1 acknowledged the potential benefits of compounding for conventional medical indications but was concerned about the practice of compounding medications into forms outside of their intended use. Specifically, they expressed concerns over potentially toxic products and doses as well as harms caused by lax safety and inspection standards in certain compounding facilities.
KOL 2 was generally more comfortable with compounding for ASD, so long as it is with a reputable compounding facility. Since compounding “has always been a part of pharmacy” KOL 2 did not express significant concerns apart from making sure the parents are given all info they need to take compounded drugs in a safe/effective way. That said, both KOL 1 and 2 were particularly concerned with injectable compounded products that are intended to be administered by the parent or patient and worried about the harm this could cause if improperly injected.

KOL 3 broadly expressed concerns about the safety and potential harms of alternative treatments including compounding. They worried that some families use CAM as a replacement, rather than supplement to, conventional medicine and that certain CAM providers may mislead and take advantage of families for financial gain.

Discussion

Though all three KOLs were clinician researchers that treated patients with ASD, their familiarity and expertise with CAM and compounding treatments varied widely. All three KOLs were clear to emphasize that, regardless of effectiveness, most CAM and compounding practices used by their patients fell outside the scope of evidence-based conventional treatments that they prescribe in their respective practices. We note that while the interviewed KOLs are clinician researchers with varying levels of expertise in this area, we were not able to elicit response from individuals which routinely engage in compounding practices.

By recruiting KOLs with different backgrounds and levels of expertise we were able to present a range of opinions and perspectives that likely guide the use of such treatments in the general public. These detailed, first-hand accounts from KOLs complement existing quantitative survey data on the prevalence and correlates of biomedical treatment use for ASD. Understanding the subjective impressions of CAM and compounding use for ASD by medical professionals in the field is important when considering the implications of federal policy on clinical practice. Additionally, understanding perceived indicators for treatment use has implications for developing policy guidelines regarding compounding for currently regulated and unregulated substances.
Supplementary Documents

APPENDIX 1: File name A1_SPARK Questionnaire.pdf
*during data collection, minor changes were made to the phrasing of demographic and “time of use” variables to suit SPARK’s online survey template requirements

APPENDIX 2: Table 3- SPARK Questionnaire Response Rate

APPENDIX 3: Figure 5- Suggested Use of Melatonin in SPARK

APPENDIX 4: Figure 6- How Melatonin Was Obtained in SPARK

APPENDIX 5: Figures 7-12- Summary Data For Additional Compounds of Interest in SPARK

APPENDIX 6: File names A6_ASD Pediatric National and State Frequencies.csv and A6_ASD Pediatric National and State Means.csv

APPENDIX 7: File name A7_Finished and Unfinished NDC Autism Drugs.csv

APPENDIX 8: File name A8_NDC Drugs Prescribed to Children with ASD.csv

APPENDIX 9: File name A9_ASD Pediatric National and State Frequencies for Each RX.csv

APPENDIX 10: File name A10 Physicians Who Billed for 11 or More Filled Rx of Drugs of Interest.csv and A10_PRVDR ID That Billed for 11 or More Admin Infusions of Drugs of Interest.csv

APPENDIX 11: File name A11_NPIs That Billed 11 or More Drugs of Interest.csv

APPENDIX 12: File name A12_KOL Invitation Email.pdf

APPENDIX 13: File name A13_KOL Interview Script.pdf
References


