

# Effects of Metformin on Spatial and Verbal Memory in Children with ASD and Overweight Associated with Atypical Antipsychotic Use

Michael G. Aman, PhD,<sup>1</sup> Jill A. Hollway, PhD,<sup>1</sup> Jeremy Veenstra-VanderWeele, MD,<sup>2</sup> Benjamin L. Handen, PhD,<sup>3</sup> Kevin B. Sanders, MD,<sup>4</sup> James Chan, MS,<sup>5</sup> Eric Macklin, PhD,<sup>5</sup> L. Eugene Arnold, MD, MEd,<sup>1</sup> Taylor Wong, BS,<sup>1</sup> Cassandra Newsom, MD,<sup>4</sup> Rianne Hastie Adams, MSW,<sup>6</sup> Sarah Marler, MA,<sup>4</sup> Naomi Peleg, MSc,<sup>6</sup> and Evdokia A. Anagnostou, MD<sup>6</sup>

## Abstract

**Objectives:** Studies in humans and rodents suggest that metformin, a medicine typically used to treat type 2 diabetes, may have beneficial effects on memory. We sought to determine whether metformin improved spatial or verbal memory in children with autism spectrum disorder (ASD) and overweight associated with atypical antipsychotic use.

**Methods:** We studied the effects of metformin (Riomet<sup>®</sup>) concentrate on spatial and verbal memory in 51 youth with ASD, ages 6 through 17 years, who were taking atypical antipsychotic medications, had gained significant weight, and were enrolled in a trial of metformin for weight management. Phase 1 was a 16-week, randomized, double-blind, placebo-controlled, parallel-group comparison of metformin (500–850 mg given twice a day) versus placebo. During Phase 2, all participants took open-label metformin from week 17 through week 32. We assessed spatial and verbal memory using the Neuropsychological Assessment 2nd Edition (NEPSY–II) and a modified children’s verbal learning task.

**Results:** No measures differed between participants randomized to metformin versus placebo, at either 16 or 32 weeks, after adjustment for multiple comparisons. Sixteen-week change in memory for spatial location on the NEPSY–II was nominally better among participants randomized to placebo. However, patterns of treatment response across all measures revealed no systematic differences in performance, suggesting that metformin had no effect on spatial or verbal memory in these children.

**Conclusions:** Although further study is needed to support these null effects, the overall impression is that metformin does not affect memory in overweight youth with ASD who were taking atypical antipsychotic medications.

**Keywords:** metformin, autism spectrum disorder, overweight, atypical antipsychotic, memory

## Introduction

SOME EVIDENCE SUGGESTS that metformin, a medication typically used to treat type 2 diabetes (T2D), could have beneficial cognitive effects. This could be due to either neuroprotection from the negative effects of T2D itself or due to independent effects of metformin on brain function, potentially through enhanced neurogenesis.

The suspicion that metformin might be neuroprotective first arose from the observation of inflated risk of Alzheimer’s disease (AD) among patients with T2D, leading some to dub the co-

occurring deterioration as “type 3 diabetes” (Steen et al. 2005; de la Monte and Wands 2008). In a large observational cohort ( $N=127,209$ ) of older adults ( $\geq 50$  years), T2D was strongly associated with AD, but the hazard for dementia was markedly lower among adults taking metformin, sulfonylureas, or a combination of the two (Hsu, Wahlquist, Lee, and Tsai 2011). Guo et al. (2014) randomized 58 adults, 40–65 years of age with depression and T2D, to placebo ( $n=29$ ) or metformin ( $n=29$ ). After 12 weeks, the metformin group had significant improvement on the Wechsler Memory Scales-Revised and on two depression rating scales. Improvements in memory and depression were inversely correlated,

<sup>1</sup>Nisonger Center, Ohio State University, Columbus, Ohio.

<sup>2</sup>Columbia University and New York Psychiatric Institute, New York, New York.

<sup>3</sup>Western Psychiatric Institute and Clinic, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania.

<sup>4</sup>Vanderbilt University, Nashville, Tennessee.

<sup>5</sup>Biostatistics Center, Massachusetts General Hospital and Department of Medicine, Harvard Medical School, Boston, Massachusetts.

<sup>6</sup>Bloorview Research Institute, Holland Bloorview Kids Rehabilitation Hospital, University of Toronto, Toronto, Canada.

making it difficult to assess whether the primary benefit of metformin was on memory, mood, or both.

A number of *in vitro* and animal models of T2D have studied metformin's effect on markers of neurodegeneration or neuroprotection. Chen et al. (2009) found that metformin, when administered alone, significantly increased  $\beta$  amyloid ( $A\beta$ ) peptides in cultured mouse neuroblastoma cells and primary neurons, but that insulin and metformin, when administered together, reduced  $A\beta$  concentrations. Using a different approach, Gupta, Bisht, and Day (2011) exposed mouse neuroblastoma cells to exogenous insulin to produce neuronal insulin resistance, leading to classical AD neuropathological changes. However, exposure to metformin significantly reversed the insulin resistance and reduced the molecular AD-like neuropathological cell changes. Correia et al. (2008) administered 4 weeks of metformin to Goto Kakizaki rats, a substrain that develops diabetes early in life. Metformin exerted the expected antihyperglycemic effects and it was also associated with decreases in several measures of oxidative stress, suggesting the potential for neuroprotective effects.

*In vivo* studies of metformin's impact on memory function in animal models of T2D have also yielded suggestive but mixed results. Pintana et al. (2012) compared the effects of high-fat diet (HFD), which is commonly used to model T2D, on memory and exploratory behavior, as well as metabolic variables, in Wistar rats. Twelve weeks of HFD caused significant increases in body weight, plasma insulin, plasma cortisol, and homeostatic model assessment (HOMA) index. Three weeks of subsequent metformin significantly reduced all of these metabolic indices. Compared with normal-diet rats, the HFD rats took significantly longer to locate a platform in a Morris water maze test and spent less time in the target quadrant, reflecting worse learning or memory. Metformin enhanced both indices of learning, but only in the HFD rats. In contrast, Lennox et al. (2014) studied the effects of 20 days of treatment with glucagon-like peptide-1 (GLP-1) agonist and metformin in HFD mice. GLP-1 agonist alone and GLP-1 agonist + metformin improved an index of recognition memory, but metformin monotherapy had no effect.

Metformin has also been studied as a potential neuroprotective agent in animal models of other brain disorders. For example, in a mouse model of Huntington's disease, Ma et al. (2006) found that 2 mg/kg, but not 5 mg/kg of metformin, starting at 5 weeks of age, led to increased lifespan (27% increase) and decreased hindlimb clasp (a sign of ataxia) in male mice but not in female mice. Venna et al. (2014) employed a middle cerebral artery occlusion mouse model of ischemic stroke. They gave metformin for 3 weeks and found improved recovery of motor function that was paralleled by enhanced development of new blood vessels up to 30 days later.

These results also raise the possibility that metformin has pro-cognitive effects that are independent of protection from T2D or other brain insult. Wang et al. (2012) found that metformin promoted mouse neurogenesis *in vitro* in cultured neuronal stem cells and *in vivo* in the hippocampus. They then tested whether metformin improved memory performance in the Morris water maze, a common test of spatial learning and memory. Mice that received 38 days of metformin injections were no better at learning the initial location of a platform in the maze compared with mice that received saline injections; however, the metformin-treated mice surpassed controls in learning a *new* location when the platform was moved. This report preceded onset of a clinical trial of metformin in children with autism spectrum disorder (ASD; Anagnostou et al. 2016) and prompted us to add measures of spatial memory to the weight reduction study.

For this study, we aimed to evaluate the impact of metformin on spatial and verbal memory as an ancillary study within a random-

ized controlled trial of metformin for youth with ASD, whose overweight was associated with prescription of atypical antipsychotics. Our primary hypothesis was that individuals prescribed metformin, in comparison to those in the placebo arm, would demonstrate a beneficial effect on spatial memory. We chose a spatial memory task in an attempt to parallel the Morris water maze results (Wang et al. 2012). We included a verbal memory test as well to cover the possibility that any effect on memory may be broader than just spatial. Second, if metformin improved memory in the short term, we predicted "catch-up" in placebo participants during Phase 2, when all participants received metformin.

## Methods

### *Design and participants*

The background, methods, and primary outcomes (i.e., weight indices, side effects, and behavioral changes) of the main trial were described previously (Anagnostou et al. 2016). Participants were recruited from four academic sites participating in the Autism Speaks Autism Treatment Network (ASATN) (Bloorview Research Institute, Ohio State University, University of Pittsburgh, and Vanderbilt University Medical Center). This study was approved by the Institutional Review Boards at the four participating study sites and the ASATN clinical and data-coordinating centers. Caregivers and legal guardians signed informed consent documents and if cognitively able to do so, participating youth assented to study participation.

Intelligence Quotient (IQ) was assessed during the screen visit using the Stanford-Binet Intelligence Scale V (Roid 2003) or Mullen Scales of Early Learning AGS Edition (Mullen 1989). Most IQ assessments were done by PhD-level psychologists, and the remainder was completed by a masters-level licensed clinician or masters-level examiners who had been trained psychometrically and monitored throughout the trial by a licensed PhD-level clinical psychologist.

The trial ran in two phases. Phase 1 was a 16-week, randomized, double-blind, placebo-controlled trial testing the efficacy and safety of a liquid formulation of metformin (Riomet<sup>®</sup>) in children and adolescents with ASD. Age was balanced across the two treatments (placebo vs. metformin), precluding any confounding between age and treatment effect. Phase 2 was a 16-week, open-label extension with all participants taking metformin (total study duration, 32 weeks). Children and adolescents were eligible if they met the following criteria: (a) age was between 6 and 17 years, 4 months inclusive; (b) had a diagnosis of ASD (i.e., autistic disorder, pervasive developmental disorder not otherwise specified [PDD-NOS], or Asperger's disorder) based on the DSM-IV-TR clinical interview (American Psychiatric Association 2000) and supported by the Autism Diagnostic Observation Schedule (ADOS) (Lord et al. 2000) or ADOS-2 (Lord et al. 2012), as appropriate; (c) taking a stable dose of an atypical antipsychotic for a minimum of 1 month with no planned changes; and (d) had a documented  $\geq 7\%$  increase in body mass index (BMI) since starting the atypical antipsychotic (within past 12 months) or, if BMI  $\geq 85$ th percentile, a greater than 5% body weight increase per year since starting the medication, as documented by previous weight records. All medications other than metformin were held at constant doses.

Metformin was dispensed in a liquid formulation of 100 mg/mL, with placebo matching the appearance, smell, and taste of the metformin. For 6–9 year olds, initial dosing began with 250 mg at the evening meal and remained consistent for 1 week. During week 2, the dosage increased by another 250 mg at breakfast. At the week

2 visit, if the previous dosage was well tolerated, it was increased to 500 mg twice daily. For 10–17 year olds, the initial dosing began following a similar schedule, but at the week 4 visit, if well tolerated, the dosage was increased to 850 mg, twice daily. Study physicians could decrease the prescribed dosage in multiples of 50 mg if participants experienced adverse events (AEs). Once AEs resolved, participants could be rechallenged with higher dosages in multiples of 50 mg units.

For participants 6–9 years, the mean final dosage of both metformin and placebo was 1000 mg/day (SD=0.0) (all doses at maximum). For participants 10–17 years of age, the mean final dosage of metformin was 1622 mg/day ( $\pm$  226 mg/day), and the final dose of placebo was 1644 mg/day ( $\pm$ 284). During the open-label extension, the mean final dosage of metformin was 900 mg/day ( $\pm$  224 mg/day) for younger participants and 1578 mg/day ( $\pm$  339 mg/day) for older participants.

### Cognitive measures

**Neuropsychological Assessment 2nd Edition Memory for Designs.** The Neuropsychological Assessment 2nd Edition (NEPSY-II) is a valid and reliable assessment of children and adolescents, for ages 3 through 16 years, on six neuropsychological domains (Korkman et al. 2007; Davis and Matthews 2010). The Memory for Designs (MD) subtest was developed to assess spatial and content memory for novel visual forms. For the current study, the starting point and level of difficulty were based on matching each child's mental age to the chronological age equivalents outlined in the NEPSY-II manual. Participants were shown a page from a stimulus book, which included between 4 and 10 geometric forms in various locations on a grid. After allowing 10 seconds of viewing the geometric forms and their locations, the examiner turned the page over and gave the design cards to the participants. The examiner then asked participants to select the matching designs from the cards and place them on a blank grid in the same location as previously shown. Four trials of the same task using different stimuli were completed.

The total number of stimulus forms correctly selected over the four trials provided a raw *Content* Score (range 0–40 for ages 3 through 4 years; range 0–60 for ages 5 through 16 years) to assess immediate recall of the *designs* shown in each trial. The total number of *stimulus locations* correctly selected over the four trials provided a raw *Spatial* Score (range 0–20 for ages 3 through 4 years; range 0–30 for ages 5 through 16 years) to assess immediate recall of locations shown in each trial. A Bonus Score was assigned if the participant responded correctly to *both* the content and the spatial elements for a given trial. The MD Total Score is the sum of the Content, Spatial, and Bonus Scores (range 0–100 for ages 3 through 4 years; range 0–150 for ages 5 through 16 years).

**NEPSY-II MD Delayed.** The MD Delayed subtest was developed to assess long-term spatial memory in children and adolescents 5 through 16 years of age. Fifteen to 25 minutes following the administration of the MD task, the child was shown an empty grid and asked to remember the final trial of the MD task. The child was asked to choose the cards and place them on the grid where he or she initially saw them.

To be administered the MD and MD Delayed subtests, participants must have had a mental age of at least 36 months. To be scored, participants must have begun the subtest at trial 1, regardless of chronological age. Because several participants had mental ages below 36 months, they were not administered the NEPSY-II test.

Standard scores were not available for some study participants whose ages exceeded 16 years (i.e., the NEPSY-II standard scores only cover 3–16 year olds). Therefore, we analyzed raw scores for all variables on this test. In all cases, higher scores reflected better performance.

We chose the NEPSY-II MD as ideal for this study for several reasons. First, it measures both recognition for two-dimensional designs and recall for spatial location, sampling both short-term and long-term spatial memory. As such, the NEPSY-II is one of very few neuropsychological batteries developed for children (Brooks et al. 2010), which samples these variables of interest. Second, it is suited for a relatively broad age range (3–16 years), making it suitable for our participants, many of whom had intellectual disability. Third, the NEPSY-II MD subtest is sufficiently brief that it could be managed by most of our study participants, many of whom had significant attentional and distractibility issues. Finally, children with autistic disorder and Asperger's disorder were among the clinical groups included during development of the NEPSY-II, indicating that such children can perform the battery.

**The Modified California Verbal Learning Test for Children.** The Modified California Verbal Learning Test for Children (MCVLT-C) (Pandina et al. 2007; Aman et al. 2008) is a modified and simplified version of the California Verbal Learning Test for Children (CVLT-C) (Delis et al. 1994). The MCVLT-C assesses young people's verbal memory ability over brief and intermediate intervals of time. Instead of the standard list of 15 nouns administered in the CVLT-C, participants in this study were administered a modified list of 10 common nouns on 5 separate learning trials. The participants were asked to recall the words in any order after each trial (measuring Immediate Free Recall). Once the Trial 5 responses were recorded, participants performed the NEPSY-II, described above, which prevented participants from rehearsing the original verbal learning list. Following this, participants were asked to recall as many of the words as possible (Long Delay, Free Recall variable). Finally, a Recognition Trial was administered to the participants, in which the 10 previously presented and 10 new words were used. Participants then had to determine whether they had heard the word before the recognition trial by indicating "yes" or "no" to the examiner.

The MCVLT-C has a provision for participants who struggle with learning the nouns in the short delay, free recall segment. If recall was  $\leq 4$  correct over the first two trials, the examiner simplified the task by using a flip chart to show drawings of the nouns as they were read aloud. Prior testing has shown that this visual aid enhances participants' ability to perform the task. Such participants were then administered all five trials with the supplementary pictures.

Three outcome variables were derived from the MCVLT-C: (a) total number of nouns recalled correctly over the five learning trials (possible score: 0–50), (b) long delay, free recall (possible score: 0–10), and (c) number of words correctly recognized + number of words correctly rejected (possible score: 0–20). Although the MCVLT-C does not assess spatial memory, we included it for three reasons. First, verbal memory is of central and undeniable importance in everyday functioning in children. Second, we knew from previous experience (Aman et al. 2009) that youth with ASD are able to perform the task. Third, our previous trial showed that the MCVLT-C was sensitive to drug intervention (Aman et al. 2008).

### Statistical analyses

A total sample size of 60 participants was planned for power to detect effects of metformin on BMI z-score. For this analysis of

cognitive effects, only 51 participants completed 1 or more usable memory tests on at least 1 session. Before data analysis, 2 authors (M.G.A.; J.A.H.) reviewed the raw data while blind to participant identity (ID) and treatment assignment. Based on within-participant variability or unacceptably low scores, we excluded data that reflected a lack of mastery or loss of mastery over the task. Depending on the variable, 7–28 sessions were excluded from the MCVLT data (mostly from the recognition trials). One to two sessions were removed from the NEPSY scores. Another participant was excluded because he was administered tasks from the wrong stratum for his mental age, but his MCVLT data were valid, still leaving 51 participants in all.

Effects of metformin on cognitive outcomes were estimated from shared-baseline, random-slope, linear mixed models with fixed effects of age stratum (3–4 vs. 5–16 years)  $\times$  visit (categorical: baseline, week 16, and week 32) and stratum  $\times$  treatment  $\times$  postbaseline visit interaction, and random participant-specific intercepts and slopes with unstructured covariance. For NEPSY-II scales, the covariance structure was allowed to vary across test versions for the two age strata. Our original study (Anagnostou et al. 2016) indicated that the two drug groups differed in IQ (higher for the placebo group), and this difference approached significance for the 51 participants in this study ( $p=0.10$ ). Therefore, our linear mixed models also contained fixed effects for IQ and IQ  $\times$  visit (categorical) to control for possible chance confounding from baseline IQ on cognitive outcome measures.

The mean model was unstructured in time, whereas the covariance model assumed participant-specific linear deviations from the estimated means. The shared-baseline assumption, enforced by omitting a treatment main-effect term, reflected the true state of the population before randomization and adjusted for chance differences at baseline (Liang and Zeger 2000). Effects of treatment

assignment on 16 and 32-week change were estimated by linear contrasts of the baseline and 16 and 32-week least-square means using the observed stratum frequencies. Effect sizes (ESs) for treatment differences were calculated relative to the pooled standard deviation for 16- or 32-week change for each measure among completers. Cognitive endpoints were tested at two-tailed  $\alpha=0.05$ , without adjustment for multiple comparisons.

In response to reviewer enquiries, we also analyzed to determine whether certain subject or treatment variables influenced outcome. These variables included age, severity of ASD, type of antipsychotic taken (risperidone vs. other), presence or not of central nervous system stimulant cotherapy, and number of co-occurring medications. In general, these analyses were negative. They are available on-line as Supplementary Data (Supplementary Data are available online at [www.liebertpub.com/cap](http://www.liebertpub.com/cap)).

## Results

### Participants

Whereas 60 participants were enrolled in the full trial to assess safety and effects on weight, only 51 participants successfully completed 1 or more of the memory tests on at least 1 session. Age ranged from 7.2 to 17.4 years, with a mean of 12.6 and 12.8 years in the placebo and metformin groups, respectively. The large majority of participants were Caucasian and non-Hispanic. As shown in Table 1, most participants had autistic disorder, with sizeable subgroups diagnosed with Asperger's disorder (29%) or PDD-NOS (17%). In general, the parents/caregivers were well educated, with 59% having a college degree or higher. All participants were taking one or more psychotropic drugs in addition to atypical antipsychotics, with a mode of two psychotropic drugs in addition to the antipsychotics (see Anagnostou et al. 2016, eTable 4, Supplement 2).

TABLE 1. DEMOGRAPHIC FEATURES OF STUDY PARTICIPANTS

Variable	Level	Placebo	Metformin	p
Age (years)	Mean (SD, <i>n</i> )	12.6 (2.7, 30)	12.8 (3.02, 21)	0.879
Gender	Female, % ( <i>n</i> )	26.7 (8)	28.6 (6)	0.881
	Male, % ( <i>n</i> )	73.3 (22)	71.4 (15)	
IQ	Mean (SD, <i>n</i> )	84.1 (20.3, 30)	75.3 (23.3, 18)	0.175
Race	Asian American, % ( <i>n</i> )	6.7 (2)	4.8 (1)	1.000
	Black or African American, % ( <i>n</i> )	3.3 (1)	4.8 (1)	
	Caucasian/White, % ( <i>n</i> )	86.7 (26)	85.7 (18)	
	Other/Multiracial, % ( <i>n</i> )	3.3 (1)	4.8 (1)	
Ethnicity	Hispanic, % ( <i>n</i> )	6.9 (2)	0.0 (0)	0.503
	non-Hispanic, % ( <i>n</i> )	93.1 (27)	100 (21)	
ASD diagnosis	Autistic disorder, % ( <i>n</i> )	53.3 (16)	52.4 (11)	0.175
	PDD/NOS, % ( <i>n</i> )	10.0 (3)	28.6 (6)	
	Asperger's disorder, % ( <i>n</i> )	36.7 (11)	19.0% (4)	
Primary caregiver education level	College or less, % ( <i>n</i> )	40.0 (12)	42.9 (9)	0.133
	College graduate, % ( <i>n</i> )	46.7 (14)	23.8 (5)	
	Graduate degree, % ( <i>n</i> )	13.3 (4)	33.3 (7)	
Annual household income	Under \$50,000, % ( <i>n</i> )	41.4 (12)	28.6 (6)	0.224
	\$50,000–\$99,999, % ( <i>n</i> )	24.1 (7)	47.6 (10)	
	\$100,000 and over, % ( <i>n</i> )	34.5 (10)	23.8 (5)	
Additional psychotropic medications	1 Additional, % ( <i>n</i> )	20.0 (6)	9.5 (2)	0.256
	2 Additional, % ( <i>n</i> )	23.3 (7)	52.4 (11)	
	3 Additional, % ( <i>n</i> )	30.0 (9)	14.3 (3)	
	4 Additional, % ( <i>n</i> )	16.7 (5)	19.0 (4)	
	5 Additional, % ( <i>n</i> )	10.0 (3)	4.8% (1)	
BMI (z-score)	Mean (SD, <i>n</i> )	2.13 (0.38, 30)	1.94 (0.48, 21)	0.113

SD, standard deviation; *n*, sample size; PDD-NOS, pervasive developmental disorder not otherwise specified; ASD, autism spectrum disorder; BMI, body mass index; IQ, intelligence quotient.

The participants from both groups had BMIs two standard deviations or more above normative means. Mean final dosage of metformin at the end of Phase 2 did not differ between the randomized treatment groups by *t*-tests ( $p=0.61$  for younger,  $p>0.99$  for older participants). Detailed comparisons of the placebo and metformin groups appear in Table 1.

### Cognitive outcomes, Phase 1

The results for placebo versus metformin changes for the first 16 weeks of treatment appear in the middle panel of Table 2. Estimated treatment differences during Phase 1 ranged from  $-7.4$  to  $4.3$ . Negative estimates reflect less improvement over Phase 1 for the metformin group than for the placebo group; positive estimates indicate greater gains for metformin. Estimates for Phase 1 suggested greater improvement among placebo participants for five of nine measures (counter to the direction that we hypothesized). However, of the five measures, where placebo participants showed greater improvement, only one score showed a significant difference ( $p=0.042$ ). ESs ranged from  $0.08$  to  $0.51$ . None of the MCVT-C comparisons approached significance.

### Cognitive outcomes, for whole trial (P–M vs. M–M comparisons, across all 32 weeks)

The right-most panel of Table 2 shows the analysis for all 32 weeks of the trial. In this analysis, we asked the question of whether the group receiving placebo in Phase 1 showed “catch-up” after 16 weeks of metformin treatment. No measures differed between the randomized treatment groups at the conclusion of Phase 2, after 32 weeks of treatment, including 16 weeks of open-label metformin use in both arms ( $p=0.097$ – $0.976$ ).

We also analyzed results for the P–M (started placebo, switched to metformin) group and M–M (started metformin, maintained on metformin) (data not shown, for brevity). One of the nine measures

did show greater improvement during Phase 2 among participants in the M–M group: MD Spatial Scores ( $p=0.002$ ). For MD Spatial Scores in Phase 2, the M–M group had a  $2.79$  point improvement in scores, whereas the P–M group had a  $-0.62$  point change (deterioration) in scores. This indicates that participants performed better when medication condition was held constant for the metformin condition (i.e., M–M) in comparison to the placebo–metformin condition.

### Summary figures

We selected two figures to show the most important variables from the NEPSY-II and MCVLT-C tasks. Figure 1 shows the results for all 32 weeks for MD Total Score; Figure 2 shows the results for MCVLT-C Short Delay, Recall Score. The MD Total Score revealed nonsignificantly worse performance for the metformin condition relative to placebo over the first 16 weeks. This was followed by a slight but not significant improvement of the M–M condition over the P–M condition at week 32. Figure 2 shows nominally better performance for the placebo condition during the first 16 weeks, followed by an essentially parallel performance for P–M and M–M conditions from week 16–32 in the MCVLT-C Short Delay, Recall task. The remaining comparisons are shown in the Supplementary Data. Inspection of the figures for all nine variables suggests to us that there was no consistent difference in performance between groups. Performance was occasionally depressed for the metformin group for the first 16 weeks (e.g., for MD Spatial Score), only to surpass the P–M condition in the second phase (weeks 16–32). The remaining figures are available on line as supplemental material (Supplementary Figs. 1–7). Given a total of 18 statistical comparisons, we would expect about one “significant” finding on the basis of chance alone. Whereas, Bonferroni correction for multiple comparisons would require a  $p\leq 0.003$ , none of our comparisons met this criterion in our primary analyses.

TABLE 2. EFFECTS OF METFORMIN VS. PLACEBO (PANEL 2) AND EFFECT OF TIME AND METFORMIN (PANEL 3) ON MEMORY PERFORMANCE

Variable	n	PBO Phase 1 vs. Met Phase 1					Baseline to week 32				
		PBO-Ph1	Met Ph1	Estim.	p	E.S.	PBO-Met	Met-Met	Estim.	p	E.S.
<b>NEPSY MD</b>											
MD Content Score (0–60) <sup>a</sup>	45	2.126	2.718	0.593	0.732	0.084	3.447	6.100	2.653	0.206	0.388
MD Spatial Score (0–30) <sup>b</sup>	45	1.210	-0.769	-1.979	0.042	0.464	0.589	2.017	1.428	0.228	0.354
MD Total Score (0–150) <sup>c</sup>	45	12.727	5.370	-7.357	0.208	0.357	15.269	20.280	5.011	0.491	0.215
MDD Content Score (0–20) <sup>d</sup>	37	1.108	1.378	0.270	0.718	0.129	1.159	0.361	-0.799	0.494	0.225
MDD Spatial Scores (0–10) <sup>e</sup>	37	0.988	0.405	-0.583	0.107	0.451	0.380	0.395	0.015	0.976	0.011
MDD Total Score (0–50) <sup>f</sup>	37	6.089	2.593	-3.497	0.182	0.514	3.622	1.761	-1.861	0.592	0.185
<b>Modified California Verbal Learning Test-C</b>											
Short Delay Recall Score (0–50)	48	1.342	0.065	-1.278	0.524	0.186	4.666	3.267	-1.399	0.423	0.227
Long Delay Recall Score (0–10)	48	-0.063	0.303	0.366	0.560	0.181	0.488	1.358	0.871	0.097	0.476
Recognitions and Rejections Score (0–20)	44	0.157	4.421	4.265	0.127	0.340	4.027	4.789	0.762	0.797	0.077

In all cases, “Estim.” (estimate) refers to the estimation of differences in changes for placebo and/or metformin. Block 1 (PBO Phase 1 vs. Met Phase 1) answers the question of whether the initial placebo-controlled phase showed an effect of metformin on cognition. Block 2 (Baseline to Week 32) answers the question of whether the placebo group would show “catch-up” when treated with metformin in Phase 2. Minus (–) estimation figures indicate higher performance for the placebo group over metformin (in Phase 1) and greater improvement for the M–M group than for P–M group in Phase 2. NEPSY test difficulty levels were based on participants’ MAs.

<sup>a</sup>3–4 years MA, 0–40; 5–6 years MA, 0–48; 7–16 years MA, 0–60.

<sup>b</sup>3–4 years MA, 0–20; 5–6 years MA, 0–24; 7–16 years MA, 0–30.

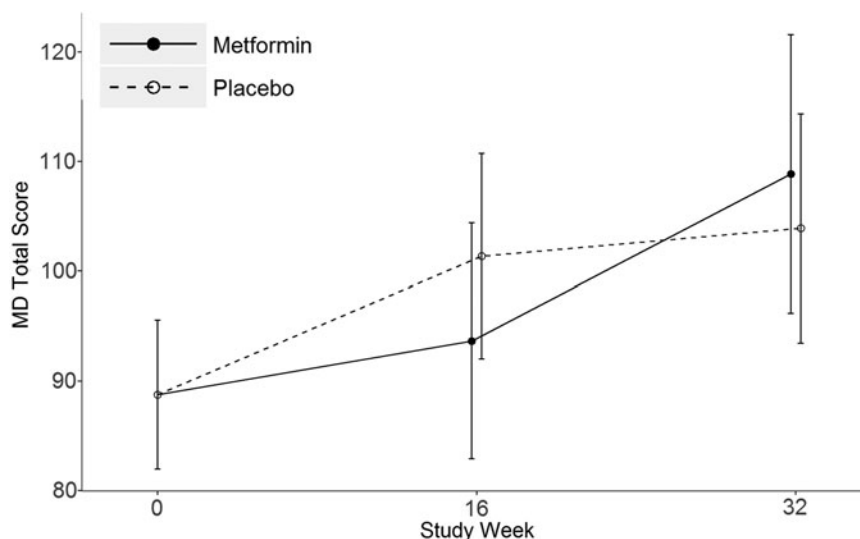
<sup>c</sup>3–4 years MA, 0–100; 5–6 years MA, 0–120; 7–16 years MA, 0–150.

<sup>d</sup>3–4 years MA, N/A; 5–6 years MA, 0–16; 7–16 years MA, 0–20.

<sup>e</sup>3–4 years MA, N/A; 5–6 years MA, 0–8; 7–16 years MA, 0–10.

<sup>f</sup>3–4 years MA, N/A; 5–6 years MA, 0–40; 7–16 years MA, 0–50.

PBO, placebo; Met, metformin; MD, Memory for Designs; MDD, Memory for Designs Delayed; MAs, mental ages; E.S., effect size.



**FIG. 1.** Memory for designs total score. Weeks 0–16 compared placebo and metformin treatment. During weeks 17–32, open-label metformin was given to all participants. Possible scores ranged from 0 to 150, with higher scores reflecting better performance.

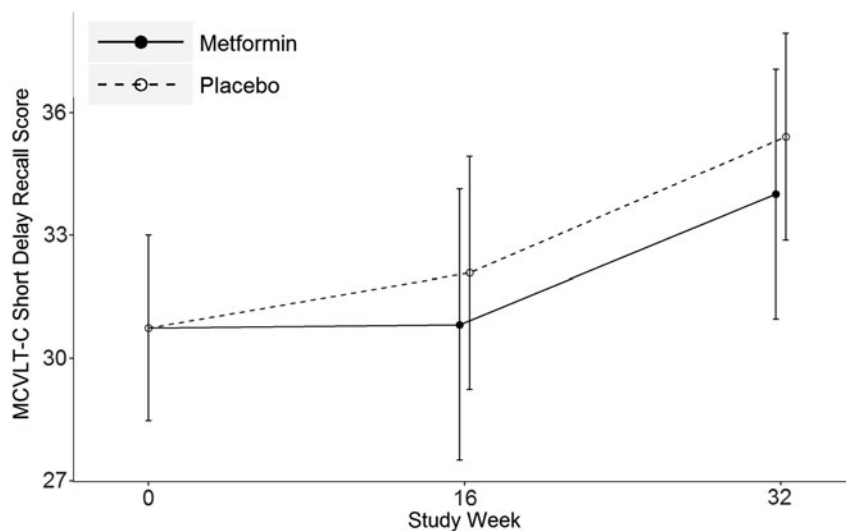
## Discussion

Despite human and rodent data suggesting that metformin treatment could potentially enhance memory (especially spatial memory), we saw little evidence of improvement or worsening. Visual inspection of memory performance on all nine variables of interest from the NEPSY-II and MVLTC revealed no consistent pattern of treatment effect. ASD is a neurodevelopmental disorder often accompanied by intellectual disability and other learning difficulties; therefore it is important that we also saw little interference with memory variables either.

As all participants were being treated with atypical antipsychotic medications, it is probable that severe irritability and/or disruptive behavior were ongoing concerns for these youth. It is important to recognize that such children can be exceptionally difficult to assess for cognitive effects of pharmacological interventions. Comorbidities, such as attention-deficit/hyperactivity disorder,

are often present in addition to the disruptive behaviors that led to prescribing of the atypical antipsychotic medication. Indeed, most participants were taking *two additional psychotropic medications* other than the atypical antipsychotic and metformin. Troost et al. (2006) compared effects of risperidone and placebo in children with predominantly PDD-NOS treated for irritability and found that only about 50% of the sample could perform two attentional tasks. Likewise, Aman et al. (2008) reported that only 35% of 101 youth with autistic disorder could comply with *any* test procedures when assessed on a cognitive battery that incorporated five tasks.

It is worth noting that researchers in Toronto have preliminary data suggesting that female rodents, but not males, were able to recover spatial working memory in an injury model when treated with metformin (Rebecca Ruddy, unpublished observations, University of Toronto, March, 2017). Thus, given that our data were derived from only 14 female participants (27%) out of 51, they might not be capable of detecting any such sex-specific effect, if one exists in human



**FIG. 2.** Short delay recall score on the MCVLT-C task. Weeks 0–16 compared placebo and metformin treatment. During weeks 17–32, open-label metformin was given to all participants. Possible scores ranged from 0 to 50, with higher scores reflecting better performance. MCVLT-C, Modified California Verbal Learning Test for Children.

beings. Therefore, one should not conclude from our data that metformin has no cognitive effects. Instead, there appears to be no discernable pattern, thus far, in children with ASD and irritable behavior. Additional studies of this type, both with children having ASD (ideally with enhanced female representation) and with totally different clinical populations, are certainly warranted.

Finally, we note that the cognitive benefit reported from previous studies in our Introduction was in the context of brain dysfunction from T2D, high blood sugar, high insulin, or trauma. It is possible that metformin offers cognitive benefit, but only in circumstances of metabolic brain stress. Alternatively, it is also possible that it may only work through the mechanism of normalizing blood sugar and insulin receptor sensitivity.

### Limitations

Limitations of this study include the small sample, with 51 youth able to provide any data. Additionally, a substantial portion of the MCFLT-C data was excluded because of lack of mastery over the task. All participants were receiving an atypical antipsychotic and at least one additional psychotropic medication, and 84% were receiving at least two other psychotropic medications. Hence, the possibility of other drugs interacting with metformin cannot be discounted. Finally, based on limited data relating to cognitive effects with metformin, we only assessed these youth for drug effects on memory functions; it is possible that metformin may affect other cognitive domains, such as aspects of attention or executive functioning.

### Conclusion

In 51 youth with ASD participating in a trial of metformin for weight reduction, we observed no clear-cut effects of treatment on spatial or verbal memory. However, evaluating cognitive functioning in children with ASD and irritable behavior presented numerous challenges. The matter deserves more study.

### Clinical Significance

Despite some evidence to the contrary from studies of humans and animals, our data offer little reason to believe that metformin treatment affects memory performance in children and adolescents with ASD.

### Acknowledgments

Funding/Support: This project was funded by the HRSA of the U.S. Department of Health and Human Services (HHS) under cooperative agreement grant no. UA3 MC11054—Autism Intervention Research Network on Physical Health. Ranbaxy Laboratories Ltd. donated both metformin and placebo for the purposes of this study. This work was conducted through the Autism Speaks Autism Treatment Network serving as the Autism Intervention Research Network on Physical Health.

### Disclaimer

This information or content and conclusions are those of the authors and should not be construed as the official position or policy of, nor should any endorsements be inferred by HRSA, HHS, or the U.S. government.

### Disclosures

Dr. M.G.A. has received consultation fees and research contracts, served on advisory boards, and provided investigator training for

AMO Pharma Ltd., Bristol-Myers-Squibb, CogState, Ltd., Confluence Pharmaceutica, Coronado Biosciences, Forest Research, Roche, Janssen Pharmaceuticals—Johnson and Johnson, Lumos Pharma, MedAvante, Inc., Novartis, Ovid Therapeutics, ProPhase LLC, and Supernus Pharmaceuticals. Dr. J.A.H. has received funding from Roche, Supernus, Forest Research Institute, Sunovion, Young Living Essential Oils, and Autism Speaks. Dr. J.V.-V.W. has consulted for or served on the advisory boards of Roche, Novartis, SynapDx; received research funding from Roche, Novartis, Seaside Therapeutics, Forest, and SynapDx; and has received other funding from the Sackler Foundation, New York Collaborates for Autism, Autism Speaks, Landreth Family Discovery Grant, Vanderbilt University, Columbia University, National Institute of Mental Health, National Institute for Child Health and Human Development, Autism Speaks, HRSA, Agency for Health Research and Quality. Dr. B.L.H. has received funding from Lilly, Curemark, and Roche, National Institute of Mental Health, National Institute of Aging, and Autism Speaks. Dr. K.B.S. has received research funding from Roche, Forest, Curemark, Sunovion, and Stemina; and other funding from Autism Speaks, Health Resources and Services Administration (HRSA), and National Institute for Child Health and Human Development. Dr. E.M. is a Data Safety Monitoring Board member of Acorda Therapeutics and Shire Human Genetic Therapies and has received grant support from Autism Speaks, Adolph Coors Foundation, ALS Association, ALS Therapy Development Initiative, ALS Therapy Alliance, Biotie Therapies, Michael J. Fox Foundation, Muscular Dystrophy Association, HRSA, and National Institutes of Health. Dr. L.E.A. has received research funding from Curemark, Forest, Lilly, Neuropharm, Novartis, Noven, Shire, Supernus, Roche, and YoungLiving (as well as NIH and Autism Speaks); has consulted with Gowlings, Neuropharm, Organon, Pfizer, Sigma Tau, Shire, Tris Pharma, and Waypoint; been on advisory boards for Arbor, Ironshore, Novartis, Noven, Otsuka, Pfizer, Roche, Seaside Therapeutics, Sigma Tau, Shire; and received travel support from Noven. Dr. E.A. has received consultation fees and served on advisory boards for Roche; industry funding from SynapDx and Sanofi-Aventis; royalties from APPI, Springer International Publishing; and other funding from Canadian Institutes of Health Research, Ontario Brain Institute, Department of Defense, Autism Speaks, National Centers of Excellence, National Institute of Health and Physician Services Incorporated. Mr. J.C., Ms. T.W., Dr. C.N., Ms. R.H.A., Ms. S.M., and Ms. N.P. have no competing financial interests.

### References

- Aman MG, Hollway JA, McDougale CJ, Scahill L, Tierney E, McCracken J, Arnold LE, Vitiello B, Ritz L, Gavaletz A, Cronin P, Swiezy NB, Wheeler C, Koenig K, Ghuman J, Posey DJ: Cognitive effects of risperidone in children with autism and irritable behavior. *J Child Adolesc Psychopharmacol* 18:227–236, 2008.
- American Psychiatric Association: Diagnostic and Statistical Manual of Mental Health Disorders (4th ed TR). Washington DC: American Psychiatric Association, 2000.
- Anagnostou E, Aman MG, Handen BJ, Sanders KB, Shui A, Hollway JA, Brian, Arnold LE, Capano L, Hellings JA, Butter E, Mankad D, Tumuluru R, Kettel J, Newsom CR, Hadjiyannakis S, Peleg N, Odrobina D, McAuliffe-Bellin S, Zakrofsky P, Marler S, Wagner A, Wong T, Macklin EA, Veenstra-VanderWeele J: Metformin for treatment of overweight induced by antipsychotic medication in young people with autism spectrum disorder. A randomized clinical trial. *J Am Med Assoc Psychiatry* 73:928–937, 2016.

- Brooks BL, Sherman EM, Strauss E: Test review: NEPSY-II: A developmental neuropsychological assessment, second edition. *Child Neuropsychol* 16:80–101, 2010.
- Chen Y, Zhou K, Wang R, Liu Y, Kwak, YD, Ma T, Thompson RC, Zhao Y, Smith L, Gasparini L, Luo Z, Xu H, Liao Z: Antidiabetic drug metformin (GlucophageR) increases biogenesis of Alzheimer's amyloid peptides via up-regulating BACE1 transcription. *Proc Natl Acad Sci* 106:3907–3912, 2009.
- Correia S, Carvalho C, Santos, MS, Proenca T, Nunes E, Duarte AI, Monteiro P, Seica R, Oliveira CR, Moreira PI: Metformin protects the brain against the oxidative imbalance promoted by type 2 diabetes. *Med Chem* 4:358–364, 2008.
- Davis JL, Matthews RN: NEPSY-II review. *J Psychoeduc Assess* 28: 175–182, 2010.
- de la Monte SM, Wands JR: Alzheimer's disease is type 3 diabetes—Evidence reviewed. *J Diabetes Sci Technol* 2:1101–1113, 2008.
- Delis DC, Kramer JH, Kaplan E, Ober BA: California Verbal Learning Test—Children's Version. San Antonio, TX, Psychological Corporation, 1994.
- Guo M, Mi J, Jiang QM, Xu JM, Tang YY, Tian G, Wang B: Metformin may produce antidepressant effects through improvement of cognitive function among depressed patients with diabetes mellitus. *Clin Exp Pharmacol Physiol* 41:650–656, 2014.
- Gupta A, Bisht B, Dey CS: Peripheral insulin-sensitizer drug metformin ameliorates neuronal insulin resistance and Alzheimer's-like changes. *Neuropharmacology* 60:910–920, 2011.
- Hsu CC, Wahlqvist ML, Lee MS, Tsai HN: Incidence of dementia is increased in type 2 diabetes and reduced by the use of sulfonylureas and metformin. *J Alzheimers Dis* 24:485–493, 2011.
- Korkman M, Kirk U, Kemp S: NEPSY—Second Edition (NEPSY-II). San Antonio, TX, Harcourt Assessment, 2007.
- Liang KY, Zeger S: Longitudinal data analysis of continuous and discrete responses for pre–post designs. *Sankhyā Indian J Stat* 62: 134–148, 2000.
- Lennox R, Porter DW, Flatt PR, Holscher C, Irwin N, Gault VA: Comparison of the independent and combined effects of sub-chronic therapy with metformin and a stable GLP-1 receptor agonist on cognitive function, hippocampal synaptic plasticity and metabolic control in high-fat fed mice. *Neuropharmacology* 86: 22–30, 2014.
- Lord C, Risi S, Lambrecht L, Cook EH, Leventhal BL, DiLavore PC, Pickle A, Rutter M: The Autism Diagnostic Observation Schedule—Generic: A standard measure of social and communication deficits associated with the spectrum of autism. *J Autism Dev Disord* 30: 205–223, 2000.
- Lord C, Rutter M, DiLavore PC, Risi S, Gotham K, Bishop S: Autism Diagnostic Observation Schedule (2nd ed.). Torrance, CA, Western Psychological Services, 2012.
- Ma TC, Buescher JL, Oatis B, Funk JA, Nash AJ, Carrier RL, Hoyt KR: Metformin therapy in a transgenic mouse model of Huntington's disease. *Neurosci Lett* 411:98–103, 2006.
- Mullen EJ: Mullen Scales of Early Learning. Bloomington, MN, Pearson Assessments, 1989.
- Pandina GJ, Bilder R, Harvey PD, Keefe RS, Aman MG, Gharabawi G: Risperidone and cognitive function in children with disruptive behavior disorders. *Biol Psychiatry* 62:226–234, 2007.
- Pintana H, Apajai N, Prachayasakul W, Chattipakorn N, Chattipakorn SC: Effects of metformin on learning and memory behaviors and brain mitochondrial functions in high fat diet induced insulin resistant rats. *Life Sci* 91:409–414, 2012.
- Roid GH: Stanford-Binet Intelligence Scales (5th ed.). Itasca, IL, Riverside Publishing, 2003.
- Steen E, Terry BM, J Rivera E, Cannon JL, Neely TR, Tavares R, Xu XJ, Wands JR, de la Monte SM: Impaired insulin and insulin-like growth factor expression and signaling mechanisms in Alzheimer's disease—is this type 3 diabetes? *J Alzheimers Dis* 7:63–80, 2005.
- Troost PW, Althaus M, Lahuis BE, Buitelaar JK, Minderaa RB, Hoekstra PJ: Neuropsychological effects of risperidone in children with pervasive developmental disorders: A blinded discontinuation study. *J Child Adolesc Psychopharmacol* 16:561–573, 2006.
- Venna VR, Li J, Hammond MD, Mancini NS, McCullough LD: Chronic metformin treatment improves post-stroke angiogenesis and recovery after experimental stroke. *Eur J Neurosci* 39:2129–2138, 2014.
- Wang J, Gallagher D, DeVito LM, Cancino GI, Tsui D, He L, Keller GM, Frankland PW, Kaplan DR, Miller FD: Metformin activates an atypical PKC-CBP pathway to promote neurogenesis and enhance spatial memory formation. *Cell Stem Cell* 11:23–35, 2012.

Address correspondence to:  
 Michael G. Aman, PhD  
 Nisonger Center  
 Ohio State University  
 McCampbell Hall  
 1581 Dodd Drive  
 Columbus, OH 43210

E-mail: aman.1@osu.edu