Autism Insights

ORIGINAL RESEARCH

Biochemical Effects of Ribose and NADH Therapy in Children with Autism

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Abstract:

Objectives: Several studies have previously indicated that children with autism often have abnormalities in methylation, glutathione redox, and mitochondrial function. A common feature of these abnormalities is that they are affected directly or indirectly by levels of ribose, reduced Nicotinamide Adenine Dinucleotide (NADH), reduced Nicotinamide Adenine Dinucleotide Phosphate (NADPH), and Adenosine-5′-triphosphate (ATP). The objective of this study was to investigate the possible biochemical effect of ribose therapy and NADH therapy on children with autism.

Design: In a pilot study, ribose was administered orally to eight children with autism for two weeks, and NADH was administered orally to another group of eight children with autism for two weeks. Children were ages 3–9 years with clinical symptoms of low energy and/or low muscle tone. Eighteen biomarkers related to methylation (including S-adenosylmethionine (SAM)), glutathione (including the reduced form, GSH, and the oxidized form GSSG), adenosine triphosphate (ATP), and folic acid and were measured at the beginning and end of the therapy.

Results: The NADH group had significant improvements in levels of ribose-5-phosphate, GSH, NADH, NADPH, and SAM. The Ribose group had significant improvements in ribose-5-phosphate, NADH, ATP, and folic acid. There was no significant change in GSSG in either group after two weeks.

Conclusions: This small study suggests that both NADH and Ribose therapy results in some improvements in biochemistry, and may be beneficial for treating children with those abnormalities. Larger studies are recommended.

Keywords: NADH, ribose, autism, mitochondria, glutathione, methylation, adenosine

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Introduction

Several studies have indicated that children with autism often have abnormalities in methylation, glutathione redox, and mitochondrial function. A common feature of these abnormalities is that they are affected directly or indirectly by levels of ribose, reduced Nicotinamide Adenine Dinucleotide (NADH), reduced Nicotinamide Adenine Dinucleotide Phosphate (NADPH), and Adenosine-5′-triphosphate (ATP). We will discuss each of these abnormalities in detail, and explain how ribose, NADH, NADPH, and ATP are involved.

Three studies by James et al have demonstrated that children with autism have impaired methylation, decreased glutathione, and increased oxidative stress.[1–3] Specifically, these studies found that children with autism, compared to controls, had decreased levels of s-adenosyl methionine (SAM), which is the primary methyl donor for methylation of DNA, RNA, proteins, phospholipids, and neurotransmitters. The formation of SAM from methionine requires adenosine-5'-triphosphate (ATP) (see Fig. 1), so decreased levels of ATP may contribute to decreased levels of SAM. ATP is produced by mitochondria, and several studies have reported impaired mitochondrial function in some children with autism. One study[4] found that children with autism, compared to controls, had significantly decreased levels of plasma ATP.

SAM is converted to s-adenosyl homocysteine (SAH) by the transfer of a methyl group, so the ratio of SAM/SAH is a measure of the body's methylation capacity. The three studies by James et al[1–3] also reported that children with autism, compared to controls, had decreased ratios of SAM/SAH. As discussed above, this could be partially due to decreased production of SAM, but another proposed mechanism is feedback inhibition of adenosine on SAH hydrolase resulting in a build up of SAH. (see Fig. 1).

Two previous studies[1,2] reported increased levels of adenosine in children with autism compared to controls. The increase in adenosine could be due to decreased conversion of adenosine to adenosine-5′-triphosphate (ATP), due to an impairment in mitochondrial function, which has been previously reported in some children with autism.[5–8] To summarize, decreased mitochondrial function would result in decreased levels of ATP and increased levels of adenosine, both of which would impair methylation.

The three studies by James et al[1–3] also reported that children with autism, compared to controls, had a decreased ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG). The reduction of GSSG to GSH involves glutathione reductase (GR)—see Figure 2. GR is a homodimer containing flavin adenine dinucleotide (FAD), which is derived from vitamin B2. First, NADPH reduces FAD to $FADH^-$ anion. The $FADH^-$ anion breaks the S-S bond of GSSG, and through a complex series of reactions forms two molecules of GSH. Thus, one mole of GSSG reacts with one mole of NADPH to form 2 moles of GSH. One study[4] found that children with autism, compared to controls, had significantly decreased levels of NADPH (and NADH), which would decrease the reduction of GSSG to GSH; that study also found that children with autism had a decreased ratio of GSH:GSSG, consistent with other studies.

D-ribose is a naturally occurring pentose monosaccharide that is a key structural component of DNA, RNA, NADH, NADPH, FADH, ATP, GTP, riboflavin, co-enzyme A and other nucleotides. NAD^+ and NADH are important co-enzymes for transport of electrons for many reactions; NAD^+ is an oxidizing agent, and NADH is a reducing agent. NADPH is an anabolic cofactor which is necessary for regeneration of glutathione, thioredoxin, and peroxiredoxins. NADPH is also required for detoxifying pathways

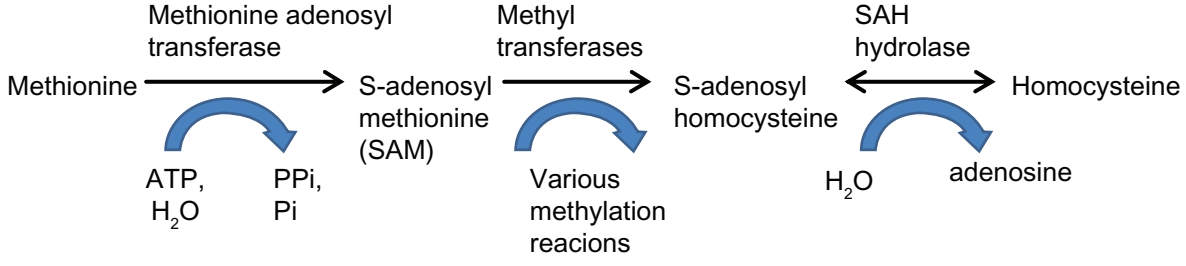

Figure 1. Conversion of methionine to SAM to SAH to homocysteine.

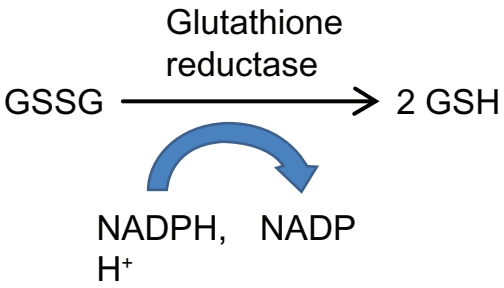

Figure 2. Reduction of GSSG to GSH (net result of a more complex process which involves FADH, see text).

such as cytochromes P450 and catalase as well as NADPH oxidase, which catalyzes the "oxidative burst" as part of the immune response. ATP is the primary energy source for many metabolic reactions in the body.

The biochemistry of ribose, NADH, NADPH, and ATP is highly inter-related. As shown in Figure 3, d-ribose is converted to ribose-5-phosphate, and then to 5-phosphoribosyl-1-pyrophosphate (PRPP). Through multiple reactions, PRPP is eventually converted to inosine monophosphate (IMP), which through two steps is converted to adenosine monophosphate (AMP). AMP is then either converted to ADP and ATP, or converted to NADH, NADPH, acetyl-coenzyme A, or flavin adenine dinucleotide ($FADH_2$). Thus, the AMP structural unit is eventually incorporated into NADH, NADPH, ATP, and other substances.

The pentose phosphate pathway (PPP) is able to produce NADPH and ribose-5-phosphate. Ribose-5-phosphate is primarily produced by the PPP, but it can also be produced from dietary ribose (see Fig. 3). NADPH is produced primarily by the pentose phosphate pathway, but it can also be produced by other pathways such as phosphorylation of NAD by NAD kinase.

NADP is required for two steps in the conversion of glucose-6-phosphate to ribose-5-phosphate via the PPP, as shown in Figure 4. Glucose-6-phosphate (6-G-P) is first converted to 6-phosphogluconolactone (6-P-GL), which is converted to 6-phosphogluconate, which is converted to ribulose-5-phosphate, which is in turn converted to ribose-5-phosphate. Thus, the formation of ribose-5-phosphate depends on adequate NADP. (We have observed that the enzyme 6-P-GL is often polymorphic in children with autism, especially those from the Mediterranean area, and should be investigated in a formal research study).

The majority of cellular ATP is formed in the mitochondria, from the oxidation of NADH (one molecule of NADH produces 3 molecules of ATP) and the oxidation of $FADH_2$ (one molecule of $FADH_2$ produces 2 molecules of ATP). Thus, the formation of ATP depends on both NADH and ribose (a critical building block of ATP).

Under oxidative stress, the need for ribose-5-phosphate and NADPH may be greater than that which

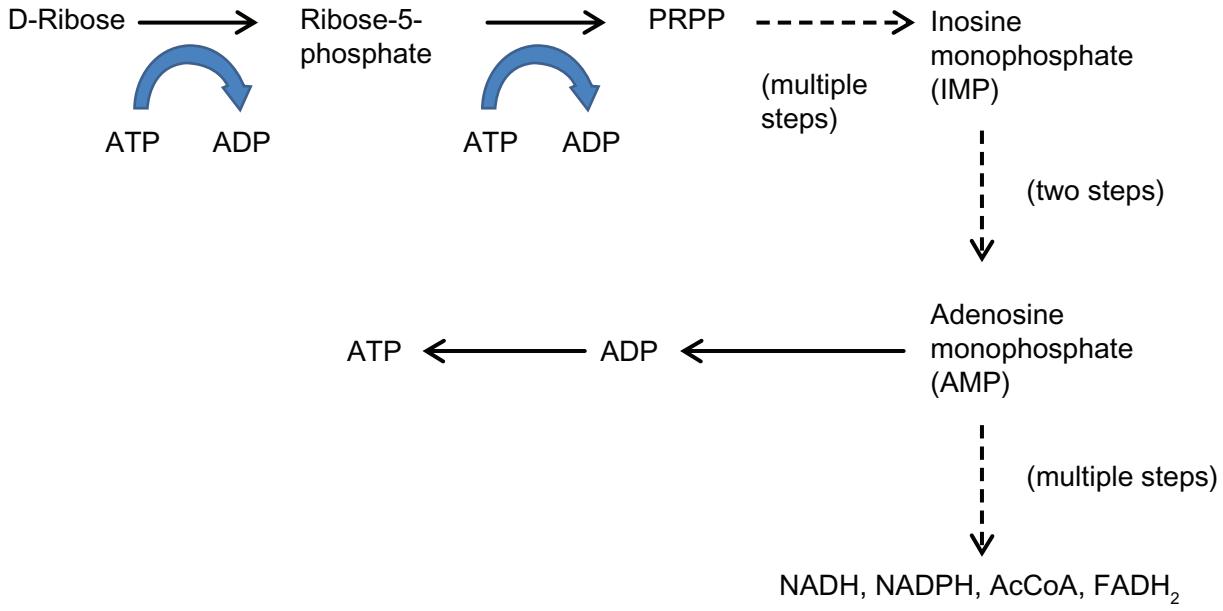

Figure 3. Conversion of Ribose to ATP, NADH, NADPH (simplified version); these reactions all occur primarily in the cytosol.

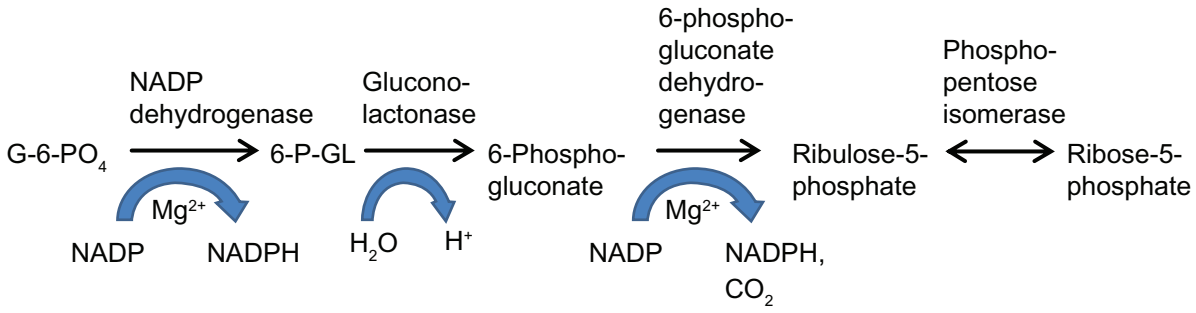

Figure 4. Conversion of glucose-6-phosphate to ribose-5-phosphate. Note that NADP is required for two of the reaction steps. These reactions are half of the Pentose Phosphate Pathway, and occur primarily in the cytosol. This reaction is the major source of NADPH in the body.

can be provided by these pathways. One possible way to address these problems is with supplementation with ribose or NADH. Supplementing with ribose bypasses the rate limiting step in this pathway. The supplied ribose is converted to ribose-5-phosphate which in turn is converted to 5-phosphoribosyl-1-pyrophosphate (PRPP) and results in accelerated nucleotide synthesis. Supplementation with ribose has been reported to increase cellular energy production in heart muscle in patients with congestive heart failure,[9] and it is now patented for that application.[10] One open-label study[11] investigated ribose supplementation (5 g T.I.D) for 41 people with Chronic Fatigue Syndrome or Fibromyalgia, and they found an average improvement in overall well-being of 30% ($P < 0.0001$). Because ribose utilizes adenosine in the production of ATP, it seems possible that ribose could not only increase ATP levels but may also decrease elevated adenosine levels and thereby normalize SAM/SAH ratios.

Similarly, NADH supplementation seems likely to increase levels of NADH and NADPH. NADH is easily converted to NAD and NADP—see Figure 5. The reducing power of NADH is what drives ATP production in oxidative phosphorylation. NADH has been reported to increase ATP production and energy levels,[12] and symptomatic improvement in patients with chronic fatigue syndrome was demonstrated in a small group of patients using NADH.[13] NADH also improves lipid peroxidation in rats,[14] and oxidative stress is a problem for children with autism as discussed above.

NADPH is also important in production of essential fatty acids, including DHA, and there are several studies reporting low levels of essential fatty acids in children with autism.[15–17]

Therefore, Ribose therapy and NADH therapy seem to be potential candidates for treating problems with methylation, glutathione, and energy production in children with autism. This paper reports on the results of a short, 2-week pilot study to determine the effect of Ribose therapy and NADH therapy on biochemistry (two weeks is too short to assess effect on behavior). The goals of this pilot study were to determine if either supplement affected methylation, glutathione level, and/or ATP level, and if the dosages were appropriate. This information will be very useful in determining whether future treatment studies should be conducted, and to provide guidance as to dosages for such studies.

Materials and Methods

This study involved sixteen patients, age 3 to 9 years, with autism spectrum disorders (ASD) diagnosed previously by a licensed physician or psychologist. The patients were clinically assessed to have low energy or

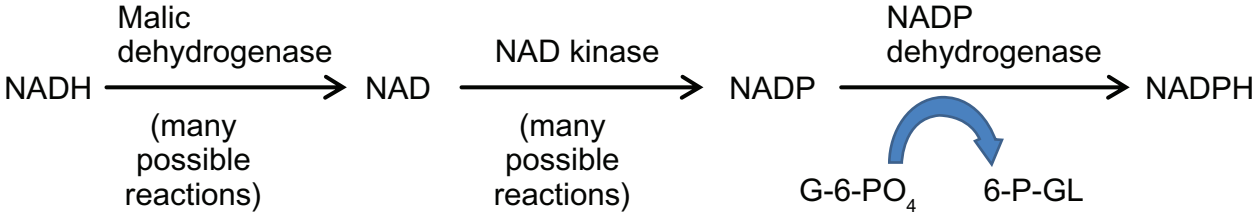

Figure 5. Conversion of NADH to NAD, NADP, and NADPH.

low muscle tone based on parental reports of symptoms and/or clinical exam (see Table 1). The participants were all current patients of Dr. Freedenfeld (lead author). The patients and their parents were informed about Ribose and NADH therapy, and were invited to consider a two week trial of one of the therapies, as a part of their overall treatment by Dr. Freedenfeld. Consenting patients were randomly assigned to receive either two weeks of NADH or ribose. No other changes in dietary or medical interventions were made during that trial. Blood tests were obtained at baseline on day one of entry into the study and again on day 14 at the end of the intervention. Each child was given either NADH (5 mg/day if under 50 pounds or 10 mg/d if over 50 pounds) as a single daily oral dose as a chewable tablet. Ribose was given as an oral drink containing 5 grams of the powder supplement once daily. These dosages are not necessarily optimal, but were chosen based on the dosages used in studies for treating other conditions.

Collection and processing

Samples were drawn into two lavender top collection tubes and processed immediately. The tubes were inverted 3 times and spun in a standard centrifuge for 15 minutes. The plasma layer was aspirated and placed into 4 transport tubes. To each of three tubes were added 1 ml of plasma and proprietary preservatives for glutathione, NADH and folic acid. All plasma tubes were placed in the freezer and kept below 32 degrees Fahrenheit. The buffy coat was then carefully aspirated from the original lavender tubes and mixed with 1 ml saline solution and centrifuged again. The fluid was then removed from the cellular layer and placed into transport tube without preservative and frozen. The RBC layer from both original lavender tubes were placed in the refrigerator. All samples were maintained refrigerated or frozen while transported to the laboratory. All samples were sent blinded to Vitamin Diagnostics for testing.

Table 1. Participant information.

	NADH group	Ribose group
N	8	8
Males/females	6/2	8/0
Age	6.8 ± 2.4 yr	5.1 ± 2.5 yr
Diagnosis	Autism: 5 PDD-NOS: 3	Autism: 3 PDD-NOS: 5
Other conditions	Apraxia: 2 Hypotonia: 5 All had low energy and/or low muscle tone	Seizure: 1 Hypotonia: 1 All had low energy and/or low muscle tone

Laboratory methodology

Tests were done for GSH, GSSG, NADH, NADPH, SAM (RBC), SAH (RBC), 5-methy-THF, 10-formyl-THF, 5-formyl-THF, THF, Folic acid, Folinic Acid (WB), Folic Acid (RBC), Ribose-5-Phosphate, Adenosine, Inosine, Uridine and ATP. The primary markers of interest were GSH, GSSG, NADH, NADPH, SAM/SAH ratio, adenosine and ATP. Other markers were studied to help elucidate the mechanism of any observed biochemical changes and to develop a general set of markers that might be used in future studies of substances used to treat children with ASD.

Laboratory reference ranges were provided by Vitamin Diagnostics for children based on 48 children ages 3–16 yr—see Tables 2 and 3.

S-adenosylmethionine (SAM) and S-adenyosylhomocysteine (SAH) were extracted from RBC[18] and measured by LCMS.[19]

Glutathione (reduced and oxidized) was measured in plasma by a fluorescence detector.[20]

Plasma ATP was measured using a luciferin-luciferase assay.[21] NADH and NADPH in RBC were measured using spectrophotometry.[22]

Folic acid was measured in RBC using liquid chromatography tandem mass spectrometry.[23] Folic acid was measured in serum by microbiological assay.[24] Folinic acid was measured in whole blood using a microbiological assay with leuconostoc citrovorum (citrovorum factor).[25]

Adenosine, inosine, and uridine in plasma were measured by HPLC with 254 adsorption.[26]

High-pressure liquid chromatography followed by fluorometric detection was used to measure plasma levels of Ribose-5-Phosphate, THF, 5-CH3-THF, 10-formyl THF, and 5-formyl-THF.

Statistical analysis and primary hypothesis

In comparing pre and post levels, 2-sided paired t-tests were used. For individual comparisons a *P*-value of 0.05 or lower is assumed significant, and a value of 0.1 is considered marginally significant. Our primary hypothesis is that supplementation with NADH or

Table 2. NADH Treatment Group: Pre and Post levels of biomarkers, and their standard deviations. The percentage difference is listed if the *P*-value is less than 0.1.

	Units	Laboratory pediatric reference range (ages 4–16 years)	Autism average before treatment	Autism average after treatment	Percent difference	*P*-value (pre vs. post, paired t-test)
GSSG (plasma)	µmol/L	0.16–0.50	0.48 ± 0.14	0.45 ± 0.12		n.s.
GSH (plasma)	µmol/L	3.8–5.5	3.15 ± 0.34	3.63 ± 0.34	15%	0.004
NADH (RBC)	nmol/ml	16.8–30.6	15.7 ± 2.3	19.2 ± 2.5	22%	0.02
NADPH (RBC)	nmol/ml	24.5–49.6	21.0 ± 3.1	26.3 ± 4.2	25%	0.02
Ribose-5-PO4 (plasma)	µmol/L	0.8–4.9	2.61 ± 2.1	7.13 ± 2.0	173%	0.004
ATP (plasma)	nmol/L	17–21	13.0 ± 4.8	14.6 ± 7.4		n.s.
SAM (RBC)	µmol/dl	221–256	209 ± 10	223 ± 14	6%	0.01
SAH (RBC)	µmol/dl	38.0–49.0	45.4 ± 9.7	44.6 ± 7.4		n.s.
SAM/SAH			0.22 ± 0.04	0.20 ± 0.03		n.s.
5-CH3-THF (plasma)	nmol/L	8.4–72.6	13.7 ± 3.9	14.1 ± 3.4		n.s.
10-formyl-THF (plasma)	nmol/L	1.5–8.2	2.40 ± 0.87	2.48 ± 0.94		n.s.
5-formyl-THF (plasma)	nmol/L	1.2–11.7	2.39 ± 0.93	2.59 ± 1.11		n.s.
THF (plasma)	nmol/L	0.6–6.8	1.12 ± 0.43	1.51 ± 0.92		n.s.
Folic acid (serum)	nmol/L	8.9–24.5	14.9 ± 4.1	16.0 ± 3.1		n.s.
Folinic acid (WB)	nmol/L	9.0–35.5	12.6 ± 3.7	12.4 ± 4.1		n.s.
Folic acid (RBC)	nmol/L	400–1500	406 ± 18	409 ± 19		n.s.
Adenosine (plasma)	10^{-8} mol/l	16.8–21.4	22.1 ± 4.2	19.7 ± 2.3	–11%	0.08
Inosine(plasma)	10^{-6} mol/l	3.0–5.0	3.78 ± 1.6	3.48 ± 1.1		n.s.
Uridine (plasma)	10^{-6} mol/l	4.2–7.4	10.6 ± 2.6	11.0 ± 1.7		n.s.

Table 3. Ribose Treatment Group: Pre and Post levels of biomarkers, and their standard deviations. The percentage difference is listed if the *P*-value is less than 0.1.

	Units	Laboratory pediatric reference range (ages 4–16 years)	Autism average before treatment	Autism average after treatment	Percent difference	*P*-value (pre vs. post, paired t-test)
GSSG (plasma)	μmol/L	0.16–0.50	0.48 ± 0.14	0.47 ± 0.11		n.s.
GSH (plasma)	μmol/L	3.8–5.5	3.08 ± 0.16	3.31 ± 0.38	8%	0.10
NADH (RBC)	nmol/ml	16.8–30.6	16.0 ± 2.4	19.0 ± 1.4	18%	0.04
NADPH (RBC)	nmol/ml	24.5–49.6	20.9 ± 2.3	25.9 ± 4.8	24%	0.07
Ribose-5-PO4 (plasma)	μmol/L	0.8–4.9	1.65 ± 1.6	5.33 ± 2.7	222%	0.004
ATP (plasma)	nmol/L	17–21	14.4 ± 3.3	19.3 ± 3.4	34%	0.009
SAM (RBC)	μmol/dl	221–256	209. ± 9.5	218 ± 13	4%	0.08
SAH (RBC)	μmol/dl	38.0–49.0	45.9 ± 10	46.0 ± 9.7		n.s.
SAM/SAH			0.22 ± 0.05	0.21 ± 0.05		n.s.
5-CH3-THF (plasma)	nmol/L	8.4–72.6	12.0 ± 4.6	12.8 ± 3.7		n.s.
10-formyl-THF (plasma)	nmol/L	1.5–8.2	2.64 ± 2.2	2.13 ± 1.1		n.s.
5-formyl-THF (plasma)	nmol/L	1.2–11.7	1.79 ± 1.0	1.68 ± 0.79		n.s.
THF (plasma)	nmol/L	0.6–6.8	1.22 ± 0.36	1.37 ± 0.44		n.s.
Folic acid (serum)	nmol/L	8.9–24.5	12.1 ± 3.3	13.3 ± 2.9	10%	0.009
Folinic acid (WB)	nmol/L	9.0–35.5	11.4 ± 5.1	11.0 ± 3.9		n.s.
Folic acid (RBC)	nmol/L	400–1500	380 ± 37	383 ± 36		n.s.
Adenosine (plasma)	10^{-8} mol/l	16.8–21.4	22.2 ± 6.0	19.9 ± 3.5		n.s.
Inosine (plasma)	10^{-6} mol/l	3.0–5.0	3.14 ± 0.85	3.56 ± 1.1		n.s.
Uridine (plasma)	10^{-6} mol/l	4.2–7.4	15.2 ± 3.5	14.4 ± 3.7		n.s.

Ribose will result in improvements in methylation, glutathione, ATP, ribose, NADH, and NADPH.

Results

Table 2 indicates the levels of biomarkers before and after treatment with NADH, and Table 3 shows the results before and after treatment with ribose. Figures 6 and 7 summarizes the effects of NADH therapy and Ribose therapy on the primary biomarkers of interest.

SAM/SAH (RBC): In both pretreatment groups SAM was below the laboratory reference range in 7/8 children. After treatment, SAM rose into the normal range in 5/7 treated with NADH but only in 1/7 children treated with ribose. SAH did not change in either group and SAM/SAH ratio did not show any significant change.

GSH-GSSG: GSSG was elevated at baseline in 5/8 in the NADH group and in 4/8 in the ribose group, compared to the laboratory reference range for children. After treatment, there was an increase in GSH in the NADH group (P = 0.004) and an increase in the ribose group (P = 0.1), but the average level of GSH still remained below the laboratory reference range for both groups. GSSG remained elevated, suggesting that oxidative stress remained a problem.

NADH/NADPH: Both NADH and NADPH were low in most of the children in both pretreatment groups compared to the laboratory reference range. After treatment, both groups had improvements in both NADH and NADPH, with the average values of both metabolites increasing into the normal reference range.

Ribose-5-phosphate: Both groups had initial average levels within the laboratory reference range. After treatment, there were significant increases in both groups (P = 0.004 for both groups), with final average levels ending somewhat above the laboratory reference range.

ATP: ATP was low at baseline in 6/8 children in the NADH group and in 5/8 in the ribose group. The level increased in 6 of 8 children in the ribose group (P = 0.009), but there was no significant change in the NADH group. It is interesting that one child in the NADH group had an unexplained significant drop in ATP after treatment and if this outlier was dropped from analysis then the ATP increase would have been statistically significant.

Secondary biomarkers

Adenosine: Adenosine was initially somewhat elevated in both groups. After treatment, the average level of both groups decreased into the normal reference range, but the change was only marginally significant in the NADH group (P = 0.08), and not significant in the Ribose group (P = 0.15).

Uridine: Uridine was initially elevated in 15 of 16 children at baseline. It did not change significantly after treatment.

Inosine: Inosine levels were initially below the laboratory reference range in 7 children, and elevated in 3 children. Inosine levels did not change during treatment.

THF: There were no significant changes in either treatment group in levels of THF, 5-CH3-THF, 10-formyl THF, or 5-formyl-THF. THF levels were all initially within the laboratory reference range. For 5-CH3-THF, 3/16 children started with levels below the reference range. For 10-formyl THF, 4/16 children started with levels slightly below the reference range. For 5-formyl-THF, 2/16 children had levels slightly below the reference range.

Folic Acid: There were low levels of serum folic acid (1/16), folinic acid (WB) (4/16), and RBC folic acid (7/16). The NADH did not have any significant changes in folic or folinic acid. The Ribose group had a small increase in serum folic acid (+10%, P = 0.009), but no other changes.

Behavioral Results

Table 4 summarizes the parent reports of any changes observed during the two weeks of treatment. There

Table 4. Parent reports of treatment effects after two weeks.

NADH group	Ribose group
Better energy: 1 No changes: 7 No reports of adverse effects	Increased energy and more social: 1 Slight increase in energy for first few days: 1 Better bladder control and less need for sensory input: 1 No change: 5 No reports of adverse effects

were no reports of adverse effects. There were no consistent behavioral or functional changes in either group although one child in each group reported increased energy and one child in the ribose group reported improved bladder control and less need for sensory input. The study was short and it may be that longer treatment would result in other changes. However, the primary purpose of this study was to capture biochemical changes.

Discussion

The low baseline levels of GSH, NADH, NADPH, SAM and ATP confirm previous observations[1–3] of impaired detoxification capacity, impaired reductase activity, impaired methylation and impaired mitochondrial energy production in the autism population.

The primary hypothesis, namely that NADH therapy and Ribose therapy would results in improvements in methylation, glutathione, ATP, NADH, NADPH, and ribose, was found to be generally true for both therapies. NADH therapy and Ribose therapy had similar effects on most of the primary biomarkers of interest (see Figs. 6 and 7). The NADH group had more increase in GSH presumably since NADPH is a co-factor for converting GSSG to GSH. The Ribose group also had a significant increase in plasma ATP, whereas the NADH group had only a small increase in ATP (not significant). Ribose is a building block for ATP, so it makes sense that Ribose therapy would be more beneficial. Since another study[12] found that NADH increased ATP in cardiomyocytes, it may be that a larger study is needed to reach significance.

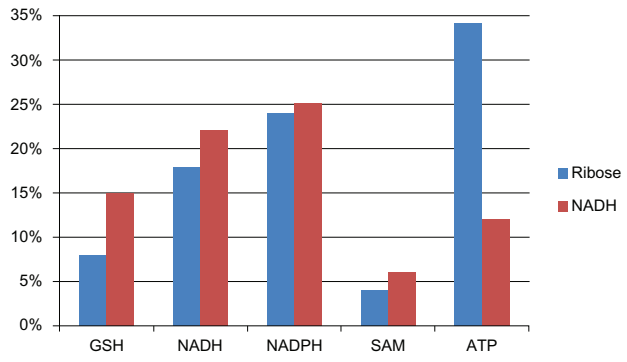

Figure 6. Effects of Ribose therapy and NADH therapy on primary biomarkers (% change). There was also a large increase in levels of ribose in both groups (not shown because the increase was so large it would be off the scale).

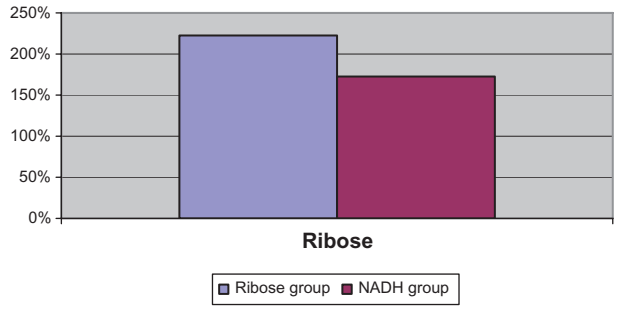

Figure 7. Effect of Ribose therapy and NADH therapy on level of Ribose (% change).

It is likely that varying the dosage and treatment duration of either Ribose or NADH would affect the results—this study did not investigate variations in dosing, and that should be considered in future studies. Since there were no side-effects at the current dosage, it appears safe to consider higher dosages, and higher dosages may have more benefit.

There was no significant improvement in GSSG in either group. The conversion of oxidized GSSG to reduced GSH requires glutathione reductase (GR) and NADPH. Supplementation with either NADH or Ribose significantly increased levels of NADPH, and resulted in some increase in GSH (NADH group: +15%, $P = 0.004$; Ribose group: +8%, $P = 0.10$), so it is surprising that this did not result in a reduction of GSSG.

We hypothesize that longer treatment may be needed to improve GSSG levels, as two weeks is a relatively short time. We also hypothesize that much longer treatment (8–12 months) may result in improvement in levels of essential fatty acids, since NADPH is a co-factor in their synthesis—it was not measured in this study because we believed that two weeks was insufficient to detect any change.

For both therapies, it appears that there were little changes in symptoms after two weeks. A larger and longer study with standardized assessments would be needed to determine if there is an effect on symptoms.

Limitations

1. This was a small pilot study involving relatively few participants, and was not designed or powered to show behavioral changes in the study population. Rather, it was intended to gather preliminary

biochemical data so that a formal study could be planned.
2. The use of laboratory reference ranges for children is reasonable, but it would be better to simultaneously measure levels in healthy neurotypical children. Also, the laboratory reference range is for a broader age range (3–16 years) than for the study participants (3–9 years).
3. The diagnosis of ASD in the participants was based on previous clinical reports, and was not independently verified in this study.
4. The dosage was not necessarily optimal, and since no adverse effects were reported, it may be useful to consider higher dosages to determine if they will have more benefit.

Conclusion

Both Ribose and NADH therapy seem to be well-tolerated, and both appear to result in several improvements in biochemistry after only two weeks of therapy. The promising results of this small study suggest that both therapies are worthy of further study in larger and longer trials with formal assessment of possible effects on symptoms, and investigation of dosage effects and treatment duration.

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Disclosure

This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors and peer reviewers of this paper report no conflicts of interest. The authors confirm that they have permission to reproduce any copyrighted material.

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