Author's response to reviews

Title: Hyperbaric treatment for children with autism: a multicenter, randomized, double-blind, controlled trial

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Author's response to reviews: see over
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Original title: Hyperbaric therapy for children with autism: a multicenter, randomized, double-blind, controlled trial

Updated title: Hyperbaric treatment for children with autism: a multicenter, randomized, double-blind, controlled trial

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January 26, 2008

Dear BMC Editors and Referees:

Re: MS: 1926622929236080
Hyperbaric treatment for children with autism: a multicenter, randomized, double-blind, controlled trial

We are grateful for the constructive critique from the three referees and your editorial team. We feel the paper is significantly strengthened because of these comments. We have paid close attention to each comment and have tried to faithfully answer each issue raised and have amended the paper accordingly. For purposes of the revised paper, additions are in blue, and erased words are in red with a strike-through.

We graciously thank the editors for the opportunity to publish this article in BMC Pediatrics. We again thank the referees for their insightful and helpful comments.

Respectfully,

Dan Rossignol MD
Principal Investigator, on behalf of all authors
Referee #1

Reviewer’s report
Title: Hyperbaric therapy for children with autism: a multicenter, randomized, double-blind, controlled trial
Version: 1 Date: 8 December 2008
Reviewer: Charles J Golden
Reviewer’s report:
Major Compulsory Revisions

1. The greatest problem in the study was the lack of a long term measure to show that the effects of the treatment went beyond the positive temporary effects of increased oxygen under pressure which have been well demonstrated. Reevaluation after six months or better yet an ABBA design with alternating treatments would have strengthened the study. These limitations need to be mentioned in the study.

Since this study represents the first controlled trial of hyperbaric treatment in children with autism, it was not designed to measure the long-term effects of hyperbaric treatment (we needed to determine if it worked in a controlled fashion in the first place). As such, we added the following statement to the paper on page 28:
“Because this study was not designed to measure the long-term outcomes of hyperbaric treatment in children with autism, additional studies are needed to determine if the significant improvements observed in this study last beyond the study period. It is possible that ongoing treatments would be necessary to maintain the improvements observed, but this study was not designed to examine that possibility.”

2. The authors also need to discuss the choices of ATM and oxygen levels as well. Pat studies and many protocols have used both higher pressure and much higher levels of oxygen. Are the authors suggesting that such levels are unnecessary for long-term change? There are no absolute answers but again the issues need to be discussed in more detail.

A discussion of the different hyperbaric pressures/oxygen levels used in autism and other neurological conditions was added to the introduction on pages 5-6 as follows:
“Most typical indications for hyperbaric treatment involve the use of hyperbaric pressures above 2.0 atm. Higher atmospheric pressures are generally required to treat conditions such as carbon monoxide poisoning and to improve wound healing [8, 11]. However, improvements have been observed via treatments with 95-100% oxygen and hyperbaric pressures of 1.5-2.0 atm for some
chronic neurological conditions, including autism [7], fetal alcohol syndrome [12], cerebral palsy [10, 13], and chronic or traumatic brain injury [14-17]. Furthermore, improvements in some of these conditions, including autism [7, 9] and cerebral palsy [10], have been observed with the use of hyperbaric pressures of 1.3 atm and oxygen levels of 21-24%. In one study, significant improvements were observed in children with autism with the use of hyperbaric treatment at both 1.5 atm/100% oxygen and 1.3 atm/24% oxygen; neither hyperbaric protocol worsened markers of oxidative stress and both reduced C-reactive protein (a marker of inflammation) [7].”

And later on page 6 we added:
“Given this background, we decided to study the effects of hyperbaric treatment in children with autism using 1.3 atm and 24% oxygen compared to near-placebo hyperbaric conditions (slightly pressurized room air at 1.03 atm and 21% oxygen).”

In addition, a discussion about the feasibility of the widespread use (and long-term use) of the hyperbaric treatment parameters used in this study was added to page 28.

On page 28, we also added: “Finally, this study was not designed to determine if higher hyperbaric treatment parameters (higher atmospheric pressure and oxygen levels, which can only be provided in a clinic setting) would lead to better or more long-lasting results. Additional studies are needed to investigate that possibility.”

3. They describe the procedure as double blind, but since the technician who gave the treatments knew what condition the individual was in, this is not a truly double blind study. The knowledge of the technician may have led to differences in the ways in which the treatments were handled.

If this criteria was used (that the technician must be blinded), then double-blind studies in HBOT would be impossible. All individuals evaluating the child (parents and physicians) were blinded to group assignment. We took great care to ensure that the hyperbaric technician would not have an influence over the study, as described in the paper. The study design that we used was similar to other studies in HBOT that were considered double-blinded, but the technician was aware of the group assignment:


The following statement was also added to page 11:

“In hyperbaric treatment studies, the study is considered double-blinded if the study participants and the evaluators of outcome measures are both blinded to group assignment (as they were in this study), even though the hyperbaric technicin is aware of the assignment [37, 38].”

4. More detailed Demographic information needs to be presented in the study sample section.
We added the following to the demographic information on page 15:
“At the onset of this study, the use of nutritional supplements, medications, and applied behavioral analysis (ABA) therapy was similar in both groups (p = ns), see Table 1. One-way analysis of variance (ANOVA) demonstrated no significant differences (p = ns) between the six centers that participated in this study for: age, initial autism severity, initial ABC total scores, final ABC total scores, initial ATEC total scores, final ATEC total scores, physician CGI scores, or parental CGI scores.”

5. More detail is necessary in the analysis section regarding which comparisons were made using the different types of statistical procedures, and the fact that a post-hoc analysis was run on some of the results, but not others.

The post-hoc analysis was expanded to include the ATEC scores and CGI scores, and is found on pages 19-20. Figure 4 (post-hoc analysis by age) and Figure 5 (post-hoc analysis by severity) were removed from the paper. The type of statistical analysis used was added to Tables 1 through 3.

The following was added to page 27 to explain the reasoning for the performing the post-hoc analysis:
“Our previous studies suggested children who were younger and those who had higher initial autism severity responded more robustly to hyperbaric treatment [7, 17]. However, these studies were small and uncontrolled, and thus we analyzed these two parameters (age and autism severity) in this study with a post-hoc analysis.”

Minor Essential Revision
6. There appeared to be a misstatement under the discussion of Physician CGI results where the article stated 6/30 of the treatment group participants had received a CGI score of 4 or 5 and then indicated a sentence later that no children in the treatment group received a score of 5

This statement was clarified on page 16:
“Conversely, 16/26 (62%) children in the control group had a “no change” or “minimally worse” score (CGI score of 4 or 5) compared to 6/30 (20%, all 6 had a score of 4) in the treatment group (p = 0.0024).”

The authors thank Dr. Golden for his thoughtful comments.
Reviewer's report
Title: Hyperbaric therapy for children with autism: a multicenter, randomized, double-blind, controlled trial
Version: 1 Date: 14 December 2008
Reviewer: David J Posey
Reviewer's report:
This is a potentially important multi-site clinical trial of hyperbaric oxygen treatment in young children with autism. The authors have done a good job at describing the methods and blinding procedures.

Major Compulsory Revisions:
1. One of the benefits of multi-site studies is that they are often more credible and less open to bias than single-site studies. To that end, some statistical analysis should be done looking at site differences in terms of baseline comparisons as well as whether site may have played a role in outcome.

We performed a statistical analysis on the 6 study sites, and there was no significant differences found, and the following was added to page 15:
“One-way analysis of variance (ANOVA) demonstrated no significant differences (p = ns) between the six centers that participated in this study for: age, initial autism severity, initial ABC total scores, final ABC total scores, initial ATEC total scores, final ATEC total scores, physician CGI scores, or parental CGI scores.”

The following was also added to page 23 in the discussion:
“The findings of this study are significantly strengthened because of the presence of a control group which previous hyperbaric treatment studies in autism lacked, and also because of the use of six separate centers which should have minimized potential bias, especially since there were no significant differences between study sites in age, initial autism severity, and initial and final scores on all of the scales used in this study.”

2. The statistical analyses that were chosen do not appear to be straightforward and seem to exaggerate the effect. For example, table 3 presents statistical tests of the difference between baseline and endpoint for the two treatments. However, wouldn't it be more straightforward to compare the endpoint scores between each treatment and adjust for any significant differences at baseline? It wasn't clear why this was chosen as the primary way to analyze the data? Was it because the sample size was likely inadequate for a more straightforward analysis? If so, it raises the question as to whether the results should be viewed
as preliminary rather than definitive.

Table 3 and 4 were revised to add the p-values for the comparison between the two groups. This information was found in the text, but was added to the tables to avoid this confusion.

3. It was stated that subjects could be taking nutritional supplements, medications, and treatments. The percentage of getting these other treatments would be important to know to better understand the sample at baseline.

Table 1 was updated to include a comparison of the number of children taking nutritional supplements, medications, and therapies. This was also updated in the Study Sample section on page 15: “At the onset of this study, the use of nutritional supplements, medications, and applied behavioral analysis (ABA) therapy was similar in both groups (p = ns), see Table 1.” There was no significant difference between the two groups.

4. The power calculation section was not adequate. The authors say that they have 80% power, but don't say what the effect size they have power to detect. Please clarify.

The effect size was added to the power calculation on pages 13-14.

5. Examining Table 2, the problem of having multiple comparisons becomes somewhat apparent. Should some statistical correction for multiple measures be made? Also, the parental ratings were not always congruent with physician ratings (e.g., social interaction, activity level). This should be discussed.

Table 2 was eliminated to avoid this apparent problem. A statistical analysis was also performed on the parental ratings compared to the physician ratings on pages 17-18:
“A significant correlation existed between the physician and parental CGI scales for the treatment group (r = 0.60, p = 0.0005).”
For the control group: “and therefore the correlation between the physician and parental CGI scales was not significant (r = 0.27, p = 0.1819).”

The following was also added on pages 22-23:
“In the hyperbaric treatment group, parental CGI scores significantly correlated with physician CGI scores (r = 0.60, p = 0.0005) which strengthens the CGI results in this group. In the control group, the parents were significantly more likely to rate their child as improved on the CGI scale compared to the physicians (p = 0.0245) and therefore the parental and physician CGI scales did not significantly correlate (r = 0.27, p = 0.1819). This finding further suggests that the blinding
procedure was adequate in this study and also demonstrates evidence of a participation effect in the control group.”

6. Their previous data finding younger age and greater severity being associated with response was refuted by this trial. Please discuss. In addition, since these analyses were post-hoc and unexpected, I'd view them as preliminary. Thus, delete them from the abstract. I'd also consider dropping the figures devoted to them and simply putting the data in the text. It seems like "cherry picking" to pick the best data for presentation.

We added the following discussion on pages 27-28 concerning younger age and greater severity:

“Our previous studies suggested children who were younger and those who had higher initial autism severity responded more robustly to hyperbaric treatment [7, 9]. However, these studies were small and uncontrolled, and thus we analyzed these two parameters (age and autism severity) in this study with a post-hoc analysis.”

“Moreover, children who had lower initial autism severity also had the most improvements with hyperbaric treatment in this study. The reason for this finding is not known, but may be due to greater levels of oxidative stress and other metabolic problems recently described in children with higher autism severity compared to those with lower severity [84].”

Figure 4 (post-hoc analysis by age) and Figure 5 (post-hoc analysis by severity) were removed from the paper.

The post-hoc analysis listing p-values was removed from the abstract. However, if it is acceptable to Dr. Posey, and since we found similar age and autism severity effects on both the ABC and the ATEC (additional analysis was done on the ATEC), we would like to place the following statement in the abstract:

“Post-hoc analysis indicated that children over age 5 and children with lower initial autism severity had the most robust improvements.”

We feel that leaving this comment in the abstract is reasonable and may help other researchers and parents who are deciding about whether or not to investigate hyperbaric treatments.

Discretionary Revisions:
1. Some discussion about the feasibility of the widespread use of the treatment and whether ongoing treatment sessions are needed would be informative.

A discussion about the feasibility of the widespread use (and long-term use) of the hyperbaric treatment parameters used in this study was added to page 28:
“Because this study was not designed to measure the long-term outcomes of hyperbaric treatment in children with autism, additional studies are needed to determine if the significant improvements observed in this study last beyond the study period. It is possible that ongoing treatments would be necessary to maintain the improvements observed, but this study was not designed to examine that possibility. Our clinical observations in children with autism suggest that additional hyperbaric treatments beyond 40 total sessions can lead to additional improvements; however, further studies are needed to formally validate these observations. Recently, several companies have started producing and marketing portable hyperbaric chambers that are approved by the U.S. Food and Drug Administration (FDA) for home use and are able to supply the hyperbaric treatment parameters used in this study. Therefore, the widespread and long-term use of this potential treatment is feasible and not necessarily costly (on a per treatment basis). Finally, this study was not designed to determine if higher hyperbaric treatment parameters (higher atmospheric pressure and oxygen levels, which can only be provided in a clinic setting) would lead to better or more long-lasting results. Additional studies are needed to investigate that possibility.”

2. Adding some discussion about the potential risks of hyperbaric oxygen treatment would be important.

The following was added to page 6 to discuss the potential risks of hyperbaric treatment: “Hyperbaric treatment for children is generally regarded as safe, even at pressures of 2.0 atm and 100% oxygen for two hours per day [36]. In descending order, the most common side effects observed during hyperbaric treatment are barotrauma (2% incidence), sinus squeeze, serous otitis, claustrophobia, reversible myopia, and new onset seizure (which occurs in 1–3 per 10,000 treatments) [8]. In children with autism, the use of hyperbaric treatment using pressures up to 1.5 atm and 100% oxygen has been shown to be safe and well-tolerated [7, 9].”

The authors thank Dr. Posey for his thoughtful comments.
Major Compulsory Revisions

Although the methodology in this study has been generally excellent, the authors have not indicated how the additional inspired oxygen fraction of 24% in the treatment group was obtained. It is essential to include this detail before the manuscript can be accepted.

This detail has been added on page 8:

“Oxygen flowing at 10 liters per minute from an oxygen concentrator was mixed with room air and pumped into the chamber following a protocol previously described [7]. This resulted in a final chamber oxygen concentration of approximately 24% as measured by an oxygen monitor.”

Page 23 It is stated that ‘it is unknown how hyperbaric therapy improves cerebral hypoperfusion. This is not correct and should be omitted. There are many studies showing that increasing the inspired oxygen fraction induces cerebral vasoconstriction and reduces even global cerebral oedema in head injury monitored in real time.

The statement “It is unknown how hyperbaric therapy improves cerebral hypoperfusion” has been omitted.

The paper does not include enough basic science. For example; increasing the plasma oxygen tension inevitably increases the gradient for oxygen transfer into tissue. Inflammation is associated with blood-brain barrier disturbance which contributes to microcirculatory oedema. There should be a brief discussion of the latest research on the role of hypoxia in the up regulation of HIF 1 alpha as hypoxia occurs when there is hypoperfusion.

A discussion on the role of inflammation, hypoxia, cerebral edema, blood-brain barrier disturbances, and HIF-1α was added on pages 24-26:

“These findings could be consistent with a cerebral vasculitis [24]. Elevated urinary levels of 8-isoprostane-F2α have also been reported in some children with autism [57]. In some studies, this
isoprostane elevation has been shown to cause in vivo vasoconstriction and increase the aggregation of platelets [58].”

and:

“Cerebral hypoperfusion is associated with hypoxia [24] and several studies in children with ASD have reported evidence of cerebral hypoxia, as measured by a reduction in brain Bcl-2 and an increase in brain p53 [60-63]. Elevated p53 is induced by hypoxia [64] and a decrease in Bcl-2 is associated with increased apoptosis provoked by hypoxia [65]. Hypoxia leads to higher brain concentrations of hypoxia-inducible factor 1α (HIF-1α) [66]. An increase in HIF-1α causes an increase in inflammation, including redness and swelling of tissues, and the attraction of lymphocytes [66]. HIF-1α is essential for inflammation mediated by myeloid cells [67]. In fact, in one study, rats that were null for HIF-1α demonstrated almost complete inhibition of the inflammatory response [68]. HIF-1α is responsible for angiogenesis that is secondary to hypoxia [68, 69] and also induces Vascular Endothelial Growth Factor (VEGF), which increases the permeability of blood vessels [66] and causes tissue edema. Evidence of cerebral edema in 19 children with autism compared to 20 typically-developing children was suggested by one recent T2-magnetic resonance imaging (MRI) study [70]. This edema can lead to increased interstitial space between cells [71] and cause an increase in the distance that oxygen must diffuse from blood vessels to reach brain cells and can thus lead to cellular hypoxia [72]. Inflammation is also associated with blood-brain barrier disturbances which can further increase cerebral edema [24]. Chronic inflammation is commonly associated with the infiltration of polymorphonuclear neutrophils and other immune cells, along with the cytokines that are released by these cells. This causes an increase in local oxygen usage due to the elevated oxygen requirements created by these newly infiltrated cells. Yet, at the same time, inflammation causes reduced oxygen extraction by normal cells [73]. For instance, in one study, elevated markers of inflammation (including IL-6, tumor necrosis factor receptors 1 and 2, and high-sensitivity C-reactive protein) were significantly correlated with decreased maximum oxygen uptake at peak exercise (VO2max) in patients with known or suspected coronary artery disease [74]. Therefore, inflammation prevents maximal uptake of oxygen by cells. Inflammation also increases oxidative stress and can cause neutrophils to become more adherent and attach to vessel walls [75]. This infiltration and increased adherence of inflammatory cells can contribute to brain injury by decreasing microvascular blood flow, causing thrombosis, and increasing the production of free radicals [76]. Hyperbaric treatment can overcome the effects of cerebral hypoperfusion and hypoxia by: increasing the plasma oxygen tension which transfers more oxygen into tissue, including the brain [77, 78], decreasing cerebral edema [79], inhibiting the expression of HIF-1α and its target genes [80], and by causing angiogenesis over time [18].”

Minor Revisions
Page 6 The DSM-IV criteria are not specified.
The actual criteria are not usually listed in studies, but a reference was added for the DMS-IV criteria.

Discretionary Revisions
Page 24 In view of the highly positive findings of this study and the fact that no other trial has demonstrated such benefits under strictly controlled conditions to open the conclusions with negative comments demeans the study. Many other inventions used for ASD children are equally time consuming and hyperbaric treatment need not be expensive.

The negative comments were removed from the conclusion.

The reviewer has a preference for the word treatment rather than 'therapy'. In view of the proven changes that relate to increased inspired fractions of oxygen it is suggested that treatment would be preferable.

The word “therapy” has been replaced with “treatment” throughout the paper.

It would be helpful to spell out the abbreviations in the title of each section of the 'Outcome Measures' as some readers will not be familiar with them and will need to refer to the list of abbreviations used.

The abbreviations were spelled out in the titles for each section on pages 16-18.

In the discussion a placebo response as high as 30-37% is referred to from other studies of ASD. It would be helpful to know over what time period this was found to allow comparisions to be made with the findings reported here.

The following was added to page 21:
“For example, one prospective study comparing a single dose of IV secretin to a placebo found that 30% of the children receiving the placebo had a significant improvement immediately after the infusion [45]. Another prospective study comparing daily treatment with amantadine to a placebo over a 4-week period found a mean placebo response rate of 37% [46].”

A brief comment on the participation effect in relation to the parental v professional scores would be worth including. Also a comment about the very remarkable demonstration of the overall accuracy of parental assessments.
An analysis of the correlation between the physician and parental CGI scores was performed and added to pages 17-18:
“A significant correlation existed between the physician and parental CGI scales for the treatment group (r = 0.60, p = 0.0005).”
For the control group: “and therefore the correlation between the physician and parental CGI scales was not significant (r = 0.27, p = 0.1819).”

Furthermore, a discussion of the participation effect and the parental assessments was added to pages 22-23:
“In the hyperbaric treatment group, parental CGI scores significantly correlated with physician CGI scores (r = 0.60, p = 0.0005) which strengthens the CGI results in this group. In the control group, the parents were significantly more likely to rate their child as improved on the CGI scale compared to the physicians (p = 0.0245) and therefore the parental and physician CGI scales did not significantly correlate (r = 0.27, p = 0.1819). This finding further suggests that the blinding procedure was adequate in this study and also demonstrates evidence of a participation effect in the control group.”

The authors thank Dr. James for his thoughtful comments.