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RESEARCH ARTICLE

LOW PRESSURE HYPERBARIC OXYGEN THERAPY IN AUTISM SPECTRUM DISORDERS: A PROSPECTIVE, RANDOMIZED STUDY OF 30 CHILDREN

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ABSTRACT

Background: Hyperbaric Oxygen Therapy (HBOT) is a recommended treatment for all hypoxic-ischemic pathologies. It has been demonstrated that perfusion abnormalities are present in some areas of cerebral grey matter in autistic children and there is a lack of connectivity in areas of the white matter. The cause of these anomalies is still unknown. The areas involved (temporal lobes, frontal lobes, and other areas such as the thalamus, limbic circuits, corpus callosum, and cerebellum) are connected to the symptoms and behavior of these patients. The PET and SPECT scans demonstrate how low pressure HBOT facilitates the oxygenation of hypoxic cerebral areas in autistic children. The clinical trials recorded in the literature are still insufficient for clarifying many elements.

Goals: To demonstrate that HBOT leads to significant improvements in specific abilities and behavioral symptoms tied to the functioning of the affected brain areas, and to show the safety of the HBOT treatments.

Materials and Methods: Thirty children between five and nine years old, randomized, with autism spectrum disorder (ASD) underwent HBOT at 1.5 ATA and 100% oxygen for a total of 40 sessions. The following eight psychometric scales were used for the analysis: VMI (Visual-Motor Integration), PPVT (receptive-auditory language), BRIEF (executive function), SRS (social responsiveness scale), VABS (adaptive behavior), ABC (aberrant behavior), PSI (parental distress index), A.T.E.C. (analysis of treatment outcomes). Multiple statistical tests were used, based on the requirements of each specific analysis.

Results: Improvement was found in the following areas/abilities: receptive-auditory vocabulary (p=0.016), visual-motor integration (p=0.045), visual perception (p= 0.004), motor coordination (p=0.049), "SHIFT" scale (p=0.013), subscale I/irritability (p=0.001), subscale II/lethargy (p=0.015), subscale III/stereotypic behavior (p=0.040), subscale IV/hyperactivity (p=0.000), subscale V/inappropriate speech (p=0.008) on the ABC test, communication scale (p=0.000), global scale of adaptive behavior (p=0.001), and in the outcomes of the treatment (p=0.029). A decrease was found on the social awareness subscale (p= 0.21). The analysis of the ABC subscale revealed that there were also significant improvements during the course of the treatment.

Conclusions: A significant probability of obtaining positive clinical results using HBOT in subjects affected by ASD was demonstrated. No side effects were observed in any of the children who were treated.

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INTRODUCTION

Hyperbaric Oxygen Therapy (HBOT) consists of breathing 100% oxygen or enriched mixtures at pressures higher than atmospheric pressure, in a suitable environment (hyperbaric chamber). HBOT has been used for decades as a recommended treatment for hypoxic-ischemic and infectious disorders. The pressures utilized vary from 2.2 to 2.8 ATA (1-7).

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However, some neurological illnesses such as cerebral palsy and autism spectrum disorder can be treated at lower pressures (1.3-1.75 ATA) (8, 9, 10). The optimal parameters for placing the nerve cells in the best conditions of functioning are 1.5 ATA and 100% oxygen (10, 11).

The functions of hyperbaric oxygen (12-15), even in cases of autism, in part demonstrated and in part hypothesized, are:

1. Increase in cerebral oxygenation (8, 9, 16-19);
2. Decrease in oxidative stress levels (12-14, 20-23);

3. Anti-inflamatory-Anti-edemigenous Action (1-7, 20, 24-27);
4. Immunomodulatory Action (27-34);
5. Increased production, mobilization, and differentiation of stem cells (12, 13, 35-40).

Autism Spectrum Disorders (ASD) consist of neurological development disorders that manifest before three years of age and affect relationships and communication, including a series of repetitive, obsessive, and stereotypic behaviors. Many patients also manifest some physical symptoms such as chronic diarrhea, persistent constipation, epilepsy, and changes of the sleep-wake rhythm (41-46). PET scan (Positron Emission Tomography) (47-56), SPECT scan (Single Photon Emission Computed Tomography)(9,18,19, 57-60), fMRI (Functional Magnetic Resonance Imaging) (61-65), and DTI (Diffusion Tensor Imaging) (66-85) show some areas of hypoxic grey matter and a lack of connectivity in cerebral white matter represented by decreases, excess, or aberrations in the number of synapses. The Superior Temporal Sulcus (STS) and the Superior Temporal Gyrus (STG) are hypoperfused in 76% of autistic children (19, 48-51, 55, 72). These cortical surfaces are fundamental for the perception of social stimuli and for connections with other important brain areas(12,13, 48-51, 61). The inadequate perfusion was observed even in other cortical areas located in the Frontal Lobes, parietal lobes, and the Cerebellum **(Tab. 1 e 2)** (12, 13). Anomalies in the white matter are frequent, above all in the Corpus Callosum(73-76), which connects the two cerebral hemispheres, but lack of connectivity was also found in numerous other bundles of fibers, such as the inferior and superior longitudinal fasciculus, the arcuate fasciculus, the left inferior occipitofrontal fasciculus, the uncinate fasciculus, the thalamic radiation, the internal capsule and the external capsule(79, 81, 83). Each bundle of fibers, in normal conditions, connects areas of the cerebral cortex and/or cerebral nuclei for superior executive functions. Recent neuroscience studies have demonstrated that changes in function in these areas and in these “circuits” are responsible for autistic symptoms and behaviors (47, 62-66, 68, 71). KH. Holbach identified the pressure (1.5 ATA) and the percent of oxygen (100%) that allow the nerve cells to obtain the best metabolic balance. Observing those parameters, one indeed obtains the best oxidative quotient of glucose, lesser production of lactic acid and pyruvic acid, and therefore aerobic glycolysis (10, 11).

Goals

The goals are to demonstrate that HBOT leads to significant improvement in some specific abilities and in some behavioral symptoms correlated with the functioning of the affected brain areas and to demonstrate the safety of the treatment.

MATERIALS AND METHODS

Thirty children were randomly selected: 26 boys and 4 girls, between 5 and 9 years old, with confirmed diagnoses of ASD, who had never before undergone HBOT. The sample of children was obtained observing the criteria of stratification beginning with gender and extrapolating 3:1. The criteria of exclusion were: Asperger's Syndrome, Rett Syndrome, secondary autism and known metabolic or genetic illnesses, and epilepsy.

Table 1. Brain structures in which Hypoperfusion is highlighted and main clinical correlations

BRAIN AREAS	CLINICAL CORRELATES
Thalamus	Repetitive, Self Stimulatory Behavior
Temporal Lobes	Obsessive Habits, Discomfort in Communication
Temporal Lobes and Amygdala	Difficulty in processing facial expressions
Fusiform Gyrus	Difficulty recognizing familiar faces
Broca's Area and Wernicke's Area	Decrease in the development of language and inability to process problems
Cerebellum	Cognitive and Affective Difficulties
Temporal Lobes, Frontal Lobes and Cerebellum	Decrease in I.Q.

Table 2. Main connections between STS - STG and other cerebral areas

Broca's Area (area 44)
Wernicke's Area (area 22)
Primary Auditory Cortex (area 41)
Secondary Auditory Cortex (area 42)
Primary Visual Cortex (17)
Area for eye movement (area 8)
Hippocampus-Amygdala

During the study, the participants did not undergo any pharmaceutical or behavioral treatment. The research was conducted in compliance with the norms of the 2008 Declaration of Helsinki and the families signed informed consent. Each child underwent a cycle of 40 sessions of HBOT over four weeks: two sessions daily, seven hours between sessions, from Monday to Friday. A multiplace pressurized hyperbaric chamber was used. The sessions were monitored by specialized medical and technical personnel, with the aid of audio visual devices, in compliance with regulations. The specialist in hyperbaric medicine and an attendant for each child were always present in the chamber. The sessions took place over a total period of 65 minutes. There were ten minutes of compression, 45 minutes of oxygen, and ten minutes of decompression. Oxygen at 100% was administered at a pressure of 1.5 ATA. The oxygen was breathed with the use of Sea-Long® helmets. In the compression phase, the children performed the equalization technique ear pressure by swallowing small sips of water, aided by the trained attendant.

The follow-up- was organized:

1. Qualifying physical exam with a pediatric specialist who performed an otoscopic exam of the tympanic membranes and filled out the medical chart.
2. Meeting between the educational psychologist and the parents in the presence of the child and subsequent scheduling and planning logistics for administering the evaluation scale.

Cognitive-Behavioral and Ability Assessment Tools

The following assessment scales were chosen:

- VABS® -*Vineland Adaptive Behavior Scales*- (86).
- BRIEF® -*Behavior Rating Inventory of Executive Function*-(87).

- SRS® -*The Social Responsiveness Scale*-(88).
- ABC® -*Aberrant Behavior Checklist-Community*- (89).
- VMI® -*The Berry-Buktenica Developmental Test of Visual-Motor Integration*-(90).
- PPVT® -*Peabody Picture Vocabulary Test*- (91).
- PSI® -*Parenting Stress Index*- (92).
- ATEC® - *Autism Treatment Evaluation Checklist*-(93).

The most appropriate setting was chosen for the test in order to create an atmosphere of trust with a medical professional and to guarantee adequate conditions for the best possible cooperation of the child.

Statistical Analyses

The statistical analyses of the children took into account all of the available data without excluding any cases. Of the 30 children in the study, ten (33.33%) have not completed the VMI test and the supplementary test, and eleven (36.66%) did not meet the minimum score on the PPVT. The statistical tests that were used necessitated the inclusion of statistical analyses from this last group. The software used for the analyses was IBM SPSS Statistics 20. The selection of the most appropriate statistical test was made through a test for normality of the differences between the scores and, subsequently, the examination of the significant differences in scores on the various scales before and after treatment. The Kolmogorov–Smirnov test and the Shapiro-Wilk test with a significance threshold of 95% were used as a point of reference. The normally distributed variables underwent a t-test of differences in means (Paired Samples Test), while the non-normal variables underwent a nonparametric Wilcoxon test (Related Samples Wilcoxon Signed Rank Test). An a priori improvement of score was not hypothesized, but rather a decrease with a significance threshold of 95%. For the scales for which the scores were measured on more than two occasions, an ANOVA test for repeated measurements was used.

RESULTS

During the therapy the following behavioral improvement was observed (Tab 3):

- Between the first and the second week of treatment, on subscale I (Irritability) of the ABC scale (p=0.046);
- Between the second week and the fourth week, on subscale IV (hyperactivity)(p=0.046);
- Between the second week and the fourth week, on subscale V (inappropriate speech) (p=0.040).

At the end of therapy after completion of 40 sessions evident improvement was observed on the following scales:

1) Receptive Vocabulary (PPVT) (p=0.016).
2) Visual-Motor Integration Ability (VMI) (p= 0.045); Visual Perception (p=0.004) and Motor Coordination (p=0.049).
3) Cognitive Scale (BRIEF) (p= 0.013).
4) All of the ABC subscales: I "Irritability" (p=0.001), II "Lethargy" (p=0.015), III "Stereotypic Behavior" (p=0.040), IV "Hyperactivity" (p=0.000), V "Inappropriate Speech" (p= 0.008).
5) **VABS** on the global scale (p=0.001) and the communication scale (P= 0.000).
6) ATEC scale total score (p=0.020).
No significant improvement was recorded on the PSI and SRS scales.

A significant decrease was observed on the social awareness subscale (P= 0.21). That data is not considered to be "statistically independent aspect", due to the way that the SRS scale is structured(88).

Side Effects

Almost all of the studies of hyperbaric oxygen therapy report side effects of moderate size that can be attributed, above all, to tympanic barotrauma or, in extremely rare cases, to hyperossic crises (epileptic)(94). None of the participants in our study experienced any adverse effects, which is in line with the results of other studies conducted with low pressure (20, 95-101). The treatments were proven to be safe and tolerated-well.

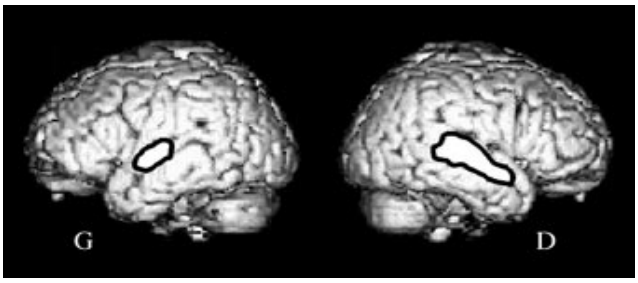


Fig 1. Bitemporal hypoperfusion in a group of 21 autistic children. Significant bitemporal hypoperfusion observed in a group of 21 autistic children compared to 10 non-autistic children (p<0.001). The temporal hypoperfusion is shown on three-dimensional rendering of images of the left and right surfaces of the lateral cortex.
From: Dysfonctionnement bitemporal dans l'autisme infantile: étude en tomographie par emission de positon. Boddaert N. et al. J Radiol 2002;83:1829-33

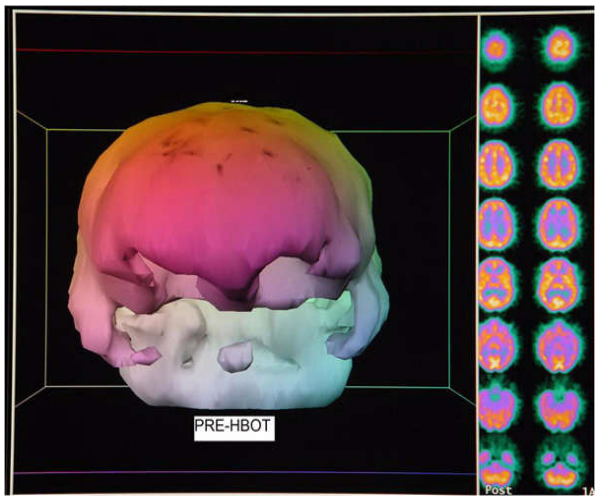


Fig 2. HBOT SPECT brain scan three-dimensional surface reconstruction and processed transverse images. Note orbital frontal and temporal lobe defects and diffuse heterogeneous pattern of blood flow. From: Low pressure hyperbaric oxygen therapy and SPECT brain imaging in the treatment of blast-induced chronic traumatic brain injury (post-concussion syndrome) and post traumatic stress disorder: a case report. Harch PG. Et al. Cases 2009 Jun 9;2:6538.

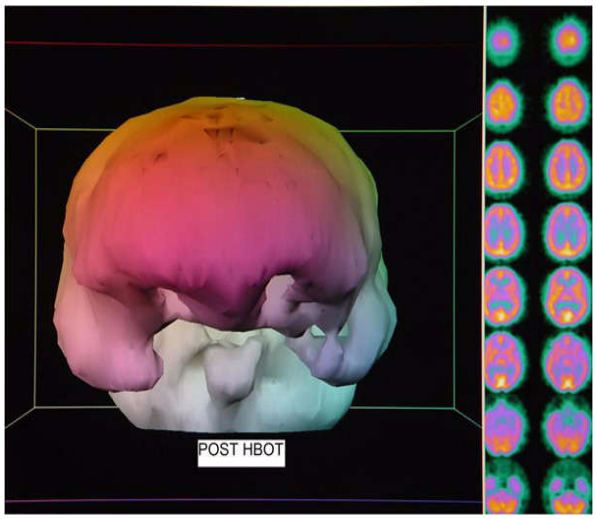


Fig 3. HBOT SPECT brain scan three-dimensional surface reconstruction and processed transverse images. Note relative improvement in brain blood flow to bilateral focal frontal and temporal defects and overall normalization of flow to a more homogeneous pattern. From: Low pressure hyperbaric oxygen therapy and SPECT brain imaging in the treatment of blast-induced chronic traumatic brain injury (post-concussion syndrome) and post traumatic stress disorder: a case report. Harch PG. Et al. Cases 2009 Jun 9;2:6538

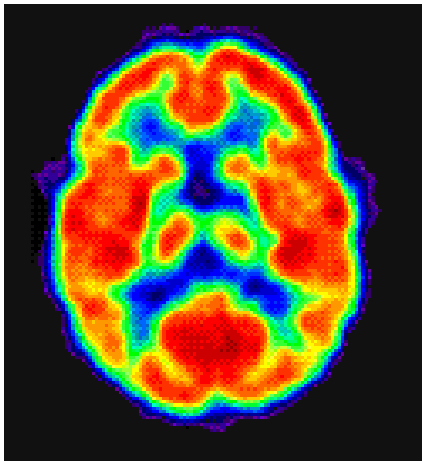
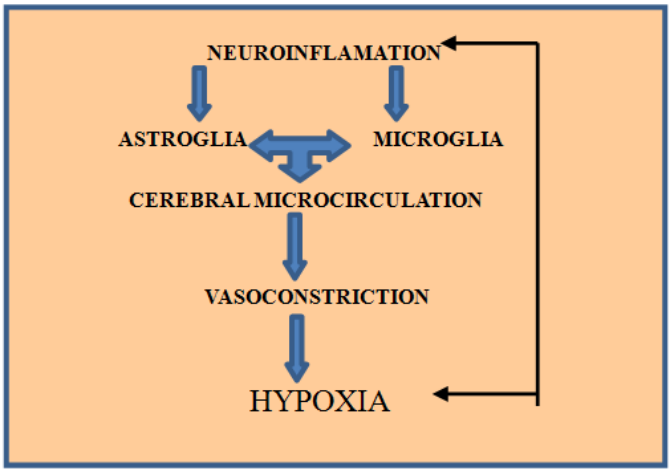


Fig 4. Typical image from a PET scan that represents the cerebral metabolic activity. The cerebral cortex and the cerebellum show elevated activity (red) while the deep structures are less active (green and blue)

Table 3. Total Results

SCALE	POST TREATMENT RESULTS expressed in p=		RESULTS DURING THE TREATMENT TEST ABC			
VABS®	Global Scale	0.001				
	Communication	0.000				
	Abilities					
BRIEF® (Area Shift)	0.013					
SRS®	0.21					
ABC®			First Week	Second Week	Third Week	Fourth Week
	Subscale I	0.001	0.046	0.046		
	Subscale II	0.015		0.046		0.046
	Subscale III	0.040				
	Subscale IV	0.000				
	Subscale V	0.008		0.040		0.040
VMI®	0.045					
Visual Perception	0.004					
Motor Coordination	0.049					
PPVT®	0.016					
PSI®	-					
ATEC®	0.020					

Table 4. Supposed mechanisms of cerebral hypoperfusion-hypoxia in the case of ASD



DISCUSSION

In children affected by ASD the images provided by the PET scan (Positron Emission Tomography), SPECT scan (Single Photon Emission Computed Tomography), fMRI (Functional Magnetic Resonance Imaging), and DTI (Diffusion Tensor Imaging) show anomalies both in the grey matter and the white matter. Seventy six percent of the autistic children compared to the control group and even children with mental delays of other types, as reported by Zilbovicius, Boddaert *et al*. (Fig. 1), show perfusion abnormalities in the Superior Temporal Sulcus (STS) and the Superior Temporal Gyrus (STG) (48-51). These anomalies therefore constitute a marker for autism because they are only present in that situation. A lack of "connectivity" has been observed in the white matter consisting of a lack and/or overabundance of synaptic connections and/or abnormal connections in some bundles of fibers. The frequency with which these anomalies are manifested in the white matter is not known and in total (white matter and grey matter) not even the cause of these changes has been identified. According to the most accepted theories, the perfusion abnormalities are correlated with the "neuroinflammation" found in the autopsies of autistic subjects. Neuroinflammation influences the cerebral microcirculation, through the activation of the astroglia and the microglia, and causes through vasoconstriction, hypoxic events which, in a sort of vicious cycle, in turn promote the inflammatory cerebral processes (27, 102-104) (Tab.4). The anomalies in white matter could, instead, result from the altered neural synaptogenesis migration in the first years of life, which would build an abnormal neural structure (12, 13, 48-51, 105). As previously stated, STS and GTS are not only extremely important areas for perception of social stimuli, they are also connected to the verbal areas (Broca and Wernicke), the primary auditory area, the primary visual area, the area for eye movement, and the hippocampus-amygdala. These last structures are involved in the regulation of emotional-instinctive functions and in memory. The above-mentioned areas are the polymodal association portion of the cerebral cortex in which information, from various sources, is integrated and processed. The insufficient perfusion and lack of "connectivity" in those association areas could explain the inadequate, insufficient coordination of activity and therefore some of the manifestations of clinical autism. In some subjects it is also possible that anomalies in the white matter predominate over those of the grey matter. This heterogeneity could explain the differences in the quality and intensity of responses to the hyperbaric treatment, considering that supplying the cells with oxygen and returning them to good metabolic conditions is without doubt a more rapid process than stimulating the production and mobilization of stem cells to substitute or change the connectivity of the damaged nerve fibers. We report a summary of the clinical trials currently published. The literature is somewhat controversial (106-109), at present the double-blind studies are limited to those of Rossignol *et al*. (2009) and of Granpeesheh *et al*. (2010). Rossignol *et al* (2007) described an open-label study, in which 12 children between three and sixteen years old underwent 40 session of hyperbaric oxygen therapy at 1.5 ATA and 100% oxygen for 45 minutes. A control group of six children was treated at 1.3 ATA and 24% oxygen. The authors demonstrate the decrease in the CRP (C-reactive protein) that is an indicator of inflammation (in this case attributed to neuroinflammation and to the entero-imflammation) and no variation of the indicators of oxidative stress. The evaluation scales (ABC-C, SRS, ATEC), administered before and after the treatment, show significant improvement in the areas of sociability and communication, of the state of awareness and eye contact (20). Levy *et al*. (2008), in a review of the complementary and alternative treatments for autism, Levy *et al*. discuss Rossignol's open-label study and do not take a position, limiting themselves to pointing out the necessity of randomized and controlled trials (108).

Yldiz *et al*. (2008), in a letter to the editor, criticize the methodology used in the Rossignol study. They conclude that, given the modest difference between the subjects treated at 1.5 ATA and those treated at 1.3 ATA, hyperbaric oxygen is not superior to breathing normobaric oxygen at a higher percentage (110). Chungpaibulpatana *et al*. (2008), in a nonrandomized, open-label study examined seven children after HBOT treatments at 1.3 ATA and 100% oxygen. After ten sessions, 75% showed a significant level of improvement in five areas (sociability, coordination, visual fine motor skills, language, gross motor coordination skills, state of awareness) (95). Lerman *et al*. (2008), treated three children with 40 HBOT sessions at 1.3 ATA and 88% oxygen in a randomized trial. No evident benefits were proven (96). It seems opportune to discuss in greater detail the results obtained in a multicentric, double-blind, randomized, controlled study by Rossignol *et al*. (2009). In this study, 62 autistic children between two and seven years of age underwent treatment with hyperbaric oxygen. The first group, composed of 33 children, underwent 40 session of HBOT at 1.3 ATA and 24% oxygen. This group was compared to a control group of 29 children who received the same number of sessions at 1.03 ATA and 21% oxygen, that is pressurized air. All of the children were evaluated on three scales (CGI, ABC, and ATEC) before and after the treatment. Eighty percent of the group treated with HBOT showed significant improvement in the areas of receptive language, social interaction, state of awareness, and eye contact, as opposed to 38% of the control group (97). Granpeesheh *et al*. (2010), treated 18 autistic children in a randomized, double-blind trial. The children underwent 80

session at 1.3 ATA and 24% oxygen. They showed that treatment at that pressure and percentage of oxygen did not produce statistically significant results. The results were derived from an analyses of evaluation scales (ABC, ADOS, BRIEF, CGI, PSI, SRS, VABS, RSB, PPVT, and VMI) administered before, during, and after the treatment (98). Analogously, Jepson *et al*. (2010), treated 16 autistic children with HBOT at 1.3 ATA and 24% oxygen for 40 sessions and did not find significant improvements (99). Conflicting results were observed by Bent *et al*. (2011), in a non controlled study of ten autistic children who underwent 80 session of HBOT. The authors observed two areas of improvement on the CGI-I scale, but no change in levels of cytokines. The study found that the improvements on the CGI-I scale might not be attributable to the HBOT and that in any case the levels of cytokines, found to be unchanging, were not correlated with the degree of the autism (100).

Given that the positive results that appeared in the first publications were not subsequently reconfirmed (111) Ghanizadeh (2012), through meta-analysis of the studies on the use of HBOT in autistic children, concluded that additional in-depth studies would be advisable. In contrast, in the very recent meta-analysis of all of the studies that have been published on the subject, Rossignol *et al*. (2012), highlight the differences between the two main studies examined and reconfirm the efficacy of HBOT in the treatment of autism (112). Despite the fact that the data provided by Sampanthavivat *et al*. (2012) is difficult to interpret, it seemed to us that it would be useful and correct to mention it. The authors compared 60 autistic children, divided into two groups. The first group underwent 20 sessions of hyperbaric oxygen therapy at 1.5 ATA and 100% oxygen. This group was compared with the second group that underwent 20 pressurized air sessions. The two groups were examined with the ATEC and CGI evaluation scales. Although both groups demonstrated statistically significant behavioral improvements, no differences were found between the two groups (101). The explanation could be found in previous hypotheses published by Rossignol *et al*. (2006-7-8) according to whom the positive results could be attributed not only to the oxygen, but also to the pressure used (12,13,14). The current official position of the UHMS (Undersea Hyperbaric Medical Society Position Paper, 2009), examining the scarce literature on the subject, was stated by Bennett *et al*. They do not recommend the routine use of HBOT in treatment for autism and suggest further in- depth studies (113).

The discrepancies emerging from different studies gave rise to the need for a study that would follow the parameters identified by Holbach (1.5 ATA and 100% oxygen) to allow for the proper functioning of the damaged nerve cells. The improvements discovered at the end of the 40 HBOT sessions are significant in the visuomotor area, but also independently in visual perception and in motor coordination. The neurological research indeed identifies separate cerebral pathways for "what" (visual perception) and for "where" (visuomotor). The cerebral areas dedicated to these abilities are precisely localized in both the motor-sensory association areas (right hemisphere and motor cortex opposing to the dominant hand), in the cerebellum and in the subcortical nuclei. The lack of development of neural connections in the white matter creates visual motor and integrative difficulties (114-122). Important results were also obtained from the values of receptive-auditory vocabulary, abilities tied to the functions of Wernike's Area and the Arcuate Fasciculus (123, 124). Improvements were measured in the "shift" area of the executive functions and also in all of the subscales of the test of abnormal behavior. The executive functions depend on the prefrontal cortex. Deficits there are manifested as behavioral symptoms. We therefore attribute those results to the improved oxygenation of the prefrontal cortex (125-134). The Vineland scale indicated improvements in adaptive function, in particular the communication scale. The A.T.E.C. scale showed consistent improvements in both specific and total examined areas:

Speech/Language/Communication, Sociability, Sensory/ Cognitive Awareness and Health/Physical/Behavior. In particular the sleep-wake rhythm, consistency of the feces, eye contact, and bladder and bowel control were positively improved. That tool was indispensable for evaluating the areas not considered by the other tests, for example the physical symptoms and the state of health of the patient. The test also allowed us to measure the parents' state of satisfaction with the treatment. The data regarding significant decrease on the "social awareness" subscale is difficult to interpret. By definition, social awareness consists of the ability to understand social signals through empathy (sensory aspects of social reciprocal behavior). Our first point is that the SRS subscales cannot be considered to be "statistically independent aspects", but must be considered in entirety. They can be considered individually only for the purpose of determining personalized therapeutic interventions. Our second point derives from the observation that, while the communicative abilities are much improved (as shown on the Vineland scale), the same improvement was not shown for behavioral abilities. Rather, the patients were not placed in a condition to learn other people's behavioral models, or to acquire their own autonomy to improve their sphere of social awareness (we emphasize that participants in the study did not undergo any therapy during the treatments).

The worsening that was observed could therefore be explained by their improved ability for communication and the contemporaneous lack of appropriate behavioral models which would lead to the frustration of feeling inadequate on a social level. But that hypothesis requires confirmation through specific studies. It is of use to cite an isolated case, equally difficult to interpret, of a boy included in our study who, at the end of treatment, showed an increase in difficult to manage behavior, accompanied by aggressive reactions, as reported by the family. A possible explanation could lie in the fact that some children affected by ASD have high baseline levels of oxidative stress or a deficit in the "scavengers" due to which they are not able to eliminate the excess of oxygen (free radicals) administered through the treatment (20, 135-143). We repeat that in the period in which the patients underwent the hyperbaric treatment, they did not undergo any other treatment. We therefore deduce that the changes that were observed can be attributed the low pressure HBOT, recognized in the international literature cited:

1. Increase in cerebral oxygenation (Fig 2 e 3).

2. Decrease in levels of oxidative stress.
3. Antiinflamatory-oedematigenous actions

These actions occur at high pressure and at low pressure (4-6,12-14). The other actions proposed (immunomodulatory action and stem cells changes) were demonstrated at high pressure (≥ 1.8 ATA), but, at the moment, there is not sufficient evidence to verify them also at low pressure or at percentages of oxygen less than 100%, as has also been reported by other authors (12,13, 111).

We would like to make a few additional observations:

1) In normal conditions the cerebral cortex and the cerebellum are more oxygenated and are therefore more metabolically active than the deeper structures, as showed in the PET images (Fig. 4). That situation could explain the motive for which the autistic tied to certain areas of the cortex (STS and GTS) responded favorably and more quickly than symptomatology tied to deeper structures (for example the limbic system and the amygdala) Therefore the levels of sociability, receptive vocabulary, communication, etc, respond in a more evident manner than the emotional or instinctive levels, because they stimulate areas that begin with a less serious base metabolic situation than the other areas.

2) Since almost all of the current studies on the effect of HBOT on stem cells were conducted at pressures of ≥ 1.8 ATA, we could hypothesis protocols at pressures higher than 1.5 ATA to increase the production, mobilization and differentiation of the stem cells to their maximum, in those situations where prevalent anomalies in the white matter have been demonstrated (it is necessary to conduct neuroimaging on all of the subjects)(144).

All of the elements mentioned above lead us to conclude that there are sub-populations of patients affected by ASD with evident and different characteristics, which are difficult to identify. This hypothesis could also explain that percentage of "non responders" identified in the literature (20, 95, 97, 98-101) and the differences in the intensity of responses to the hyperbaric treatment, varying from subject to subject.

Conclusion

The epidemiological and social characteristics of autism spectrum disorder inspire researchers to search for ever more effective treatments. In this randomized study, we administered 40 sessions of HBOT at 1.5 ATA and 100% oxygen to 30 children affected by ASD, with the aim of demonstrating that this treatment leads to significant improvement in some specific abilities and in some behavioral symptoms correlated with the function of the affected cerebral areas. Within the limits of the small sample treated, improvement in visuomotor abilities emerged, of receptive language, of the cognitive "shift" ability, as well as the adaptive functioning and the abnormal behavior described above: irritability, social withdrawal, stereotypic behaviors, hyperactivity, and inappropriate speech. This resulted in improvements even the patients' level of attention, participation and presence (145). The treatment was well-tolerated. All of the participants demonstrated an excellent adaptability during the treatment and no averse physical effects or side effects were observed. On the basis of neuroscience studies which correlate the dysfunction of some cerebral areas with clinical autism, the mechanisms of action of the hyperbaric oxygen, and the results obtained in this study, it is possible to confirm the efficacy of 'HBOT in the treatment of ASD. Considering the importance of the subject, it is necessary for confirmation and more in-depth research through further studies with the end of identifying, for example, different procedures, data from monitoring over time, related to a new possibilities and data that will allow future clinical progress.

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