

PHARMAX TECHNICAL MONOGRAPH
INTESTINAL EFFECTS ON AUTISM AND
ADHD – POTENTIAL FOR A NOVEL
PROBIOTIC APPROACH

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1. INTRODUCTION

Autistic Spectrum Disorder (ASD) is estimated to affect 1 in 166 children (*Richardson & Puri, 2002*) and has been increasing in incidence at a rate of 3.8% per year from 1966 to 1997 (*White, 2003*).

The three classical symptoms of autism are:

- ❖ Lack of verbal communication
- ❖ Lack of social awareness and response
- ❖ Desire for sameness as expressed in repetitive rituals

Symptoms of autism can occur either from birth or alternatively development can appear normal for up to 24 months before there is a sudden loss of acquired skills, which in some cases resumes slowly after a number of years. This latter type is often referred to as regressive autism (*White, 2003*). In some cases, this manifests the frequent observation of children 'growing out' of autism to some extent - or at least there being symptomatic improvement sufficient to allow a re-diagnosis to the less severe Asperger's syndrome. ASD affects three times as many boys as girls.

The prognosis for quality of life of autistics is often poor, with assisted or fully dependent day-to-day care being common. Reduced life expectancy is also apparent due to increased disease risk factors, particularly seizure-induced cardiac failure (*Kidd, 2002*).

The causes of autism are multifactorial with genetic and environmental factors playing a major role. Monozygotic twins have over 90% concordance of autism, leading to the conclusion that environmental factors play a role. Also the rate of autism amongst both monozygotic and dizygotic twins is significantly above the expected frequency. Monozygotic twins have an autism frequency 10-fold higher than would be expected from population frequency, while dizygotic twins show a greater than 5-fold increase (*Greenberg et al. 2001*)

2. THE GUT BRAIN AXIS – LINKAGE IN AUTISM

Over the past 30 years both anecdotal and published data have supported a linkage between symptoms of ASD and profound intestinal pathology in a large subset of sufferers. For instance, in a comparison between 116 ASD children and matched siblings the following types and relative rates of G.I tract symptomology were detected.

Table 1. Comparison of the prevalence of G.I. tract symptoms between autistic children and their healthy siblings (from Horvath & Perman, 2002)

		Autistic	Sibling
Number of G.I. symptoms	0	17	72
	1	10	16
	2	17	7
	3	15	0
	4	41	5
Specific symptoms	Abdominal discomfort	44	9
	Gas (2-3 events per week)	54	19
	Bloating	34	5
	Belching	24	9
	Reflux	16	5
Stool frequency	>3 per day	20	2
	2-3 per day	25	13
	1-2 per day	35	72
	3-4 per week	7	2
	1-2 per week	6	7
Stool consistency	Loose/watery	32	2
	Soft	18	21
	Normal	13	81
	Changing	23	0
	Foul smelling	49	0
	Sour smelling	8	7
	Normal smelling	43	93

Data collected from 116 ASD-diagnosed children aged between 3 and 10 years of age, compared with 43 aged matched siblings

Moreover, in a series of landmark papers, Wakefield & colleagues (2002) proposed that a unique type of gut pathology, which was associated with a sub-group of autistic children, was characterised by both ileal and colonic lymphoid node hyperplasia and merging, epithelial layer ulceration, lymphocyte infiltration of epithelium and lumen, and loss of brush border enzyme activity. This intestinal pathology was expressed in an extremely high incidence of ileitis and particularly colitis of affected children.

In addition to the overt linkage of intestinal symptoms and subgroup(s) of autistic children, D'Eufemia *et al* (1996) identified profound paracellular intestinal permeability in a group of autistic children but not in matched controls. Moreover, this permeability was not accompanied by overt symptomology, leading to the conclusion that G.I. tract abnormalities in autistic children are much more likely than previously thought.

3. GUT DERIVED EXORPHINS AND THE OPIATE THEORY OF AUTISM

A further observation of parents of autistic sufferers is that there is a pre-disposition to intolerance and adverse reactions to foods with wheat and milk products being particularly implicated. Similarly, it has been observed both anecdotally and in trials that autistic symptoms improve with the elimination of these food groups from the diet. For instance, in celiac patients with ASD, it was found that elimination of wheat not only improved the celiac condition but also the symptoms of autism (White, 2003).

Some researchers, particularly Reichelt *et al* (1981), believed these linkages were due to proteins in milk and wheat being broken down during the digestive process into peptides that had endorphin like structure and which could potentially act like endorphins by stimulating the opiate receptors in the brain. Excess of endorphins, as well as excessive opiate drug stimulation, produces symptoms such as low sensitivity to pain, loss of social interaction, and lowering of language communication skills - all of which are also expressed within the cardinal symptoms of autism.

Proof that these food-derived peptides have opiate properties was shown when Hemmings (1978) and Sun *et al* (1999) demonstrated that if β -caseomorphin (a milk casein-derived exorphin) and exorphin-A5 (a wheat derived-exorphin) were injected into rats, the following occurred:

- ❖ Both exorphin types crossed the blood brain barrier
- ❖ They bound to opiate receptors and induced activity in parts of the brain important in autism
- ❖ They produced symptoms in rats that were identical to autism
- ❖ These symptoms were prevented when the opiate antagonist, nalaxone was co-administered (Hemmings, 1978; Sun *et al*, 1999; Sun & Cade, 1999)

Most of these exorphins, when produced in normal healthy subjects, are broken down by brush border peptidases such as dipeptidyl peptidase IV. However, in ASD sufferers, the combination of a deficiency of these enzymes due to epithelial damage and a high frequency of intestinal permeability sets up the opportunity for these exorphins to reach very high concentrations in the bloodstream.

4. FOODS WHICH PRODUCE EXORPHINS

Foods which have been particularly implicated with exorphin production are wheat and dairy products, but other foods have also been found to exhibit exorphins, as shown in Table 2. Similarly, it is likely that other foods will also have the capability for exorphin production.

Table 2. Major food-derived exorphins (from Teshemacher, 2003)

Food derivative	Exorphin	Base protein	Opioid activity
Milk	α -caseomorphins β -caseomorphins casoxins α -lactalbumin β -lactoglobulins	α -casein β -casein K-casein α -lactalbumin β -lactoglobulin	Strong
Wheat	gluten exorphins gliadorphins	gluten α -gliadin	Strong
Rice	oryzatensins	albumin	Weak
Meat	serophin i-hemorphins	albumin haemoglobin	Weak
Spinach	rubiscolins	-	Moderate

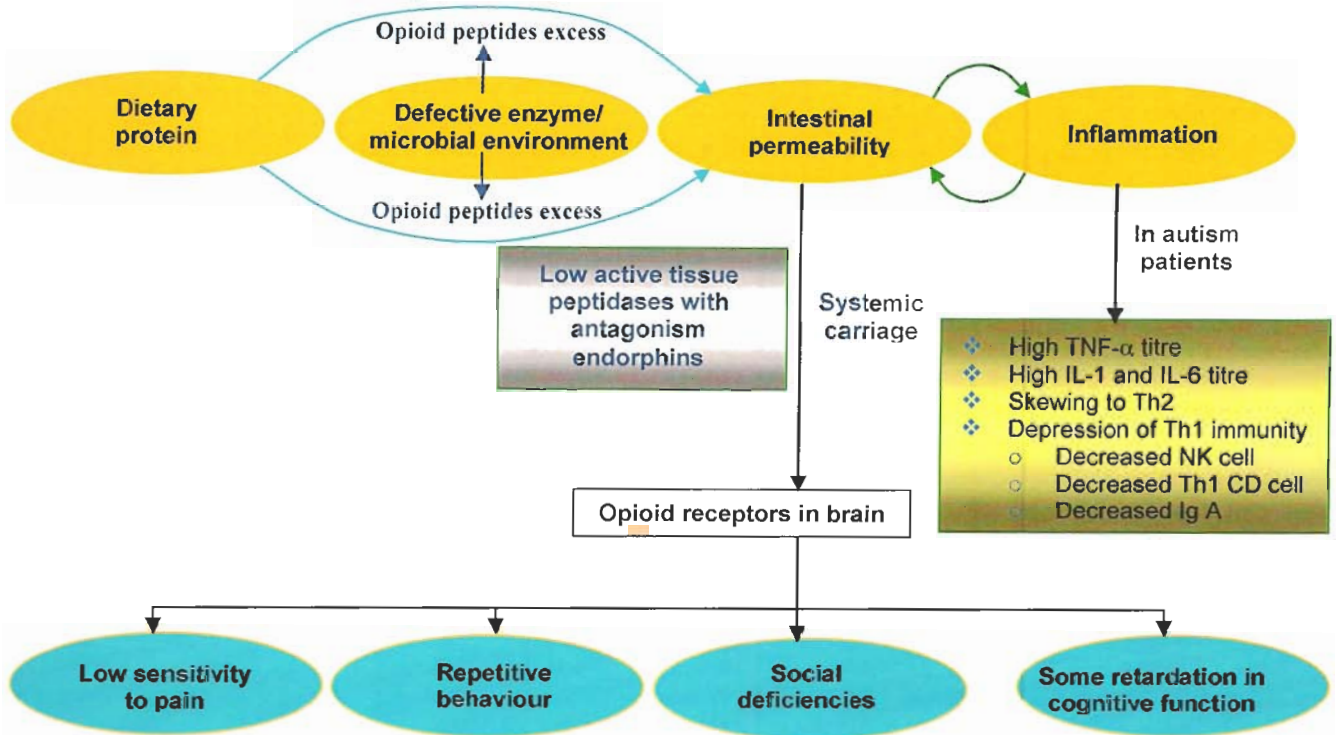
5. THE INFLAMMATORY CONNECTION TO AUTISM

In common with most diseases, autism is known to be correlated with an up-regulated inflammatory response. This was demonstrated by Jyonouchi *et al* (2001) who showed that TNF α production potential was significantly raised in autistics compared to controls.

It is unknown, but probably unlikely, that simply an up-regulated inflammatory response could trigger autism even in susceptible individuals, but it is very probable that inflammation would make ASD symptoms substantially more severe.

As can be seen, there is significant interplay with regard to exorphin excess, intestinal permeability and inflammation in the aetiology of at least a subset of ASD sufferers. This interplay is summarised in Fig. 1.

Figure 1. The gut-brain axis of pathology in autism



6. PREVENTING THE EFFECTS OF EXORPHIN EXCESS

Given the above evidence of exorphin excess in ASD, the logical preventative measure would be to exclude the offending foods from the diets of autistics. Indeed, anecdotal evidence from parents of ASD children strongly suggests benefits of this intervention. Controlled trials have however been equivocal in their results, and in part this has led to a Cochran review (Millward *et al*, 2005) of the evidence of benefits from exclusion diets in ASD. Cochran reviews independently assess the quality of the trials and, in this regard, only one trial (Knivsberg *et al*, 2002) was robust enough to be included.

This trial compared 20 ASD children with abnormal urinary pattern, assigned to matched pairs based on severity of condition and then placed on an exclusion or control diet for 12 months. The results of the trial are shown in Fig. 2.

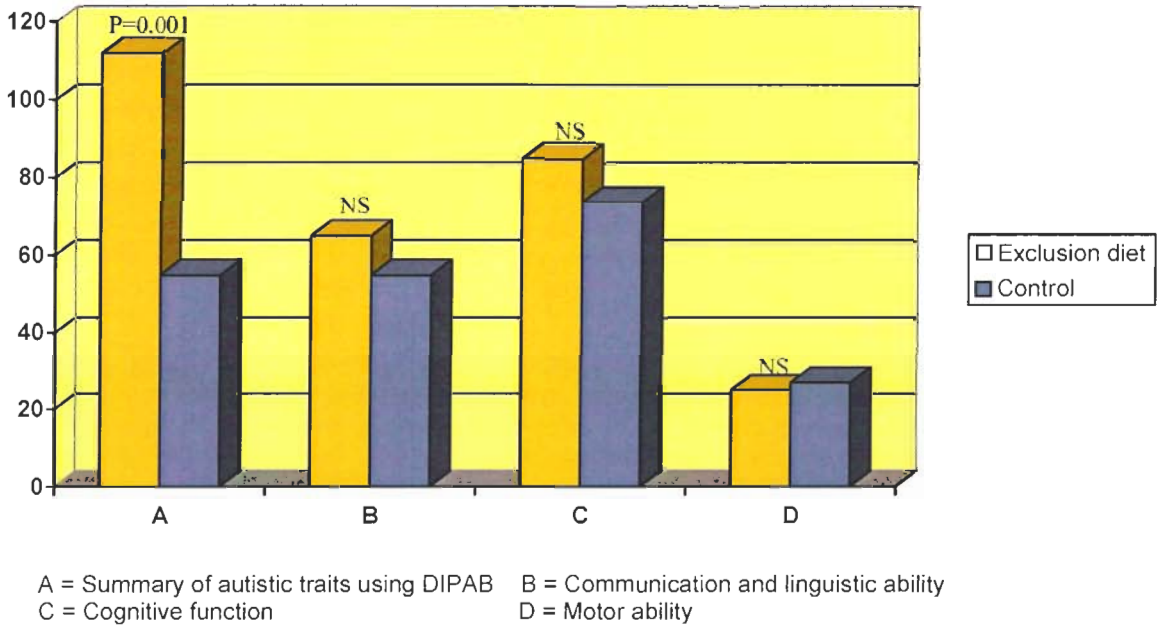
In summary, the results show that due to the small scale of the trial there was insufficient power to show differences in the individual cognitive, linguistic and motor functionality. However, in the most important category of 'Summary of Autistic Traits' using the DIPAB measure, there was a very significant improvement in the autistic symptoms of the children on the exclusion diet, leading the authors to conclude that exclusion diets are effective for appropriate subgroups of ASD.

While it is clear that exclusion of exorphin-producing foods from the diet is effective in reducing ASD symptoms, the improvement was limited rather than total, in that the children still expressed autism. It would be optimistic perhaps to expect complete resolution from simple dietary exclusion but nevertheless, reasons for limited success could include the fact that exorphin excess over a long period of time, especially in the critical childhood period, may be partially irreversible. Alternatively, foods other than wheat and milk products are known to produce exorphins (see above) and these will still be present in the diet.

Pharmax has therefore taken a different approach and has developed a probiotic with specific capabilities to degrade these exorphin peptides, as well as having the known intestinal benefits associated with probiotic organisms.

The advantage of this approach is that the probiotic organisms are able to colonize the intestinal epithelium and would hence be 'in situ' to degrade exorphin peptides. Moreover, as these peptides are very similar in structure, the likelihood is that degradation of exorphins from all foods would be achievable.

Figure 2. Single, blind, randomized control trial of effect of exclusion diet on ASD (from Knivsberg et al, 2002; Millward et al, 2004)



7. PHARMAX HLC MINDLINX

Pharmax scientists, in collaboration with Drs Gunnar Bronsted and Gregor Lundfell in Norway, have screened hundreds of isolates of probiotic bacteria for their ability to degrade exorphins from wheat and milk. Several isolates showed some capability to degrade these two peptides but, in intestinally-simulated conditions that would be necessary during the digestive process, only two expressed extensive elimination of these peptides over a period of two hours. The two isolates were identified as strains of *Lactobacillus crispatus* (CLT221) and *Lactobacillus rhamnosus* (CLT341) respectively. Their ability to degrade both β -caseomorphin (from milk) and exorphin-A5 (from wheat) is shown in Fig. 3 and Fig. 4 respectively.

Figure 3. Breakdown of milk β -caseomorphin-7 by selected HLC lactic acid bacteria

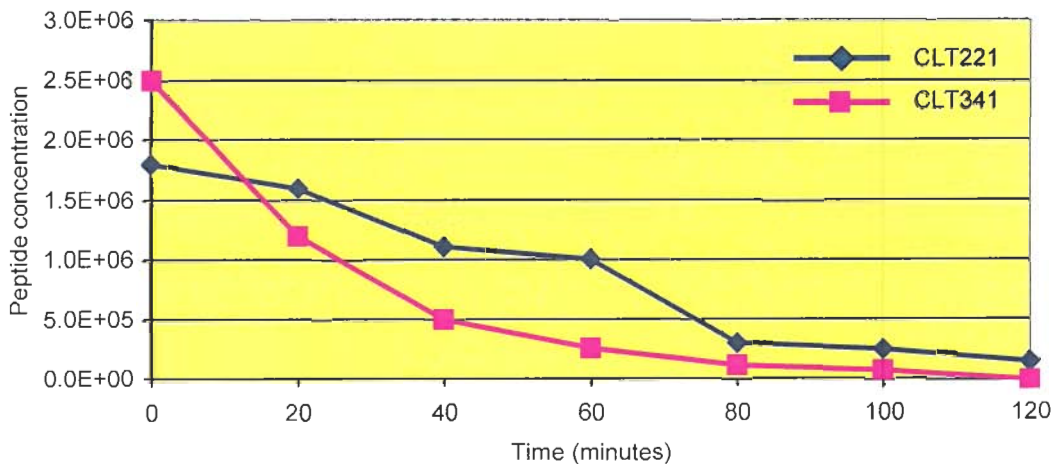
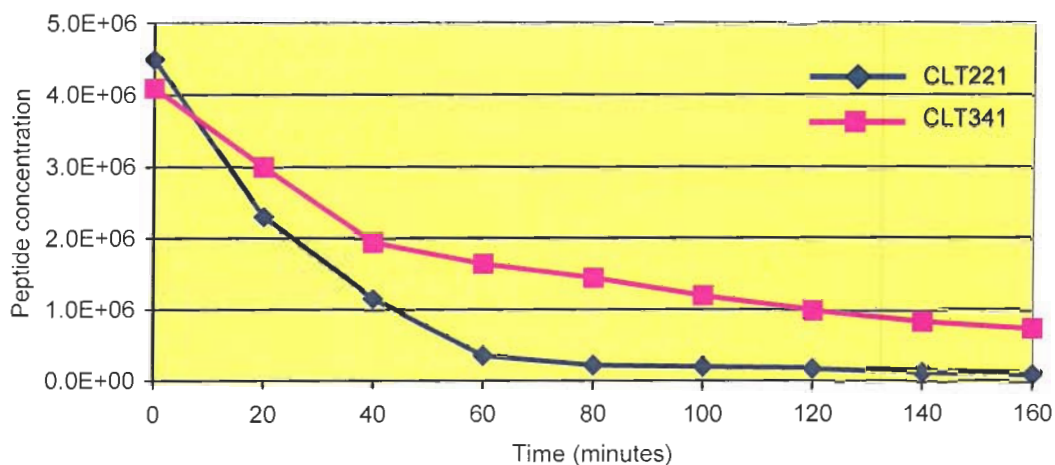


Figure 4. Breakdown of wheat exorphin-A5 by selected HLC lactic acid bacteria



These two new probiotic strains were then effectively **added** to the existing HLC High Potency product to produce HLC MindLinx. As such HLC MindLinx combines the clinically proven benefits of the HLC Lab4 consortium alongside the unique peptide-degrading attributes of the additional strains - resulting in the first probiotic developed specifically for autism. The combination of selection and clinical characteristics of HLC MindLinx are summarised in Table 3.

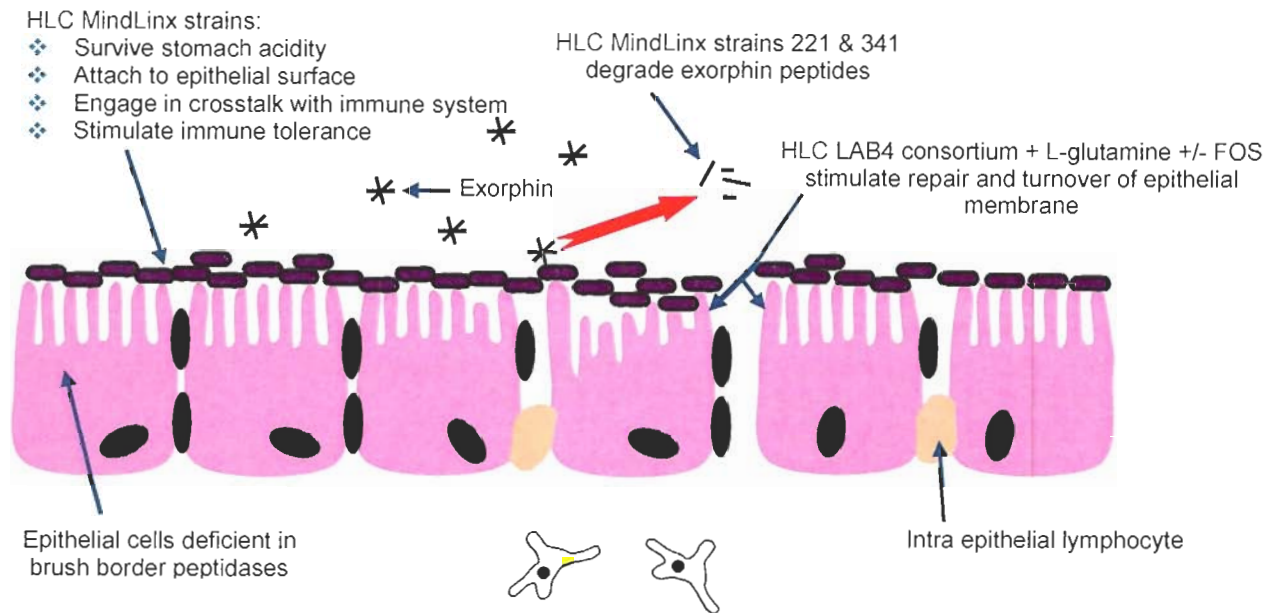
Table 3. Selection criteria and clinical characteristics of a probiotic specifically developed for autistic spectrum disorder (ASD)

	Survival through stomach	Rstant to bile salts	Attach to epithelial cells	Modulate immune system	Opiate peptide degradation	Industrial scale-up/stability	Proven in human DBPC trials
Existing HLC strains							
L. acidophilus CUL60	Y	Y	Y	Y	N	Y	Y
L. acidophilus CUL21	Y	Y	Y	Y	N	Y	Y
B. bifidum CUL20	Y	Y	Y	Y	N	Y	Y
B. lactis CUL34	Y	Y	Y	Y	N	Y	Y
New opiate-degrading strains							
L. crispatus CUL221	Y	Y	Y	?	Y	Y	N
L. rhamnosus CUL341	Y	Y	Y	?	Y	Y	N

Additionally, HLC MindLinx capsules and powder contain L-glutamine to help repair intestinal permeability. The powder form of HLC MindLinx also contains the prebiotic FOS (fructooligosaccharide).

A schematic illustrating how the combined benefits of HLC MindLinX would operate *in vivo* is shown in Fig. 5.

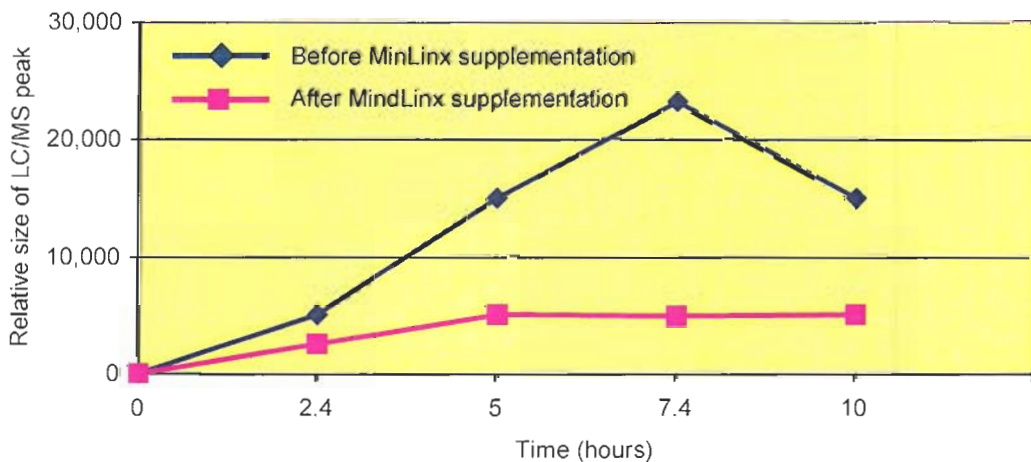
Figure 5. Synergistic action of components of HLC MindLinX



8. THE EFFECTS OF HLC MINDLINX ON NORMAL AND AUTISTIC HUMAN SUBJECTS

To assess the effect of HLC MindLinX on exorphin level in normal human subjects, six volunteers were assessed for urinary exorphin level before supplementation, and after 21 days of supplementation of the probiotic. The results are shown in Fig. 6, which demonstrates profound ability of the HLC MindLinX product to reduce urinary levels of exorphin.

Figure 6. Effect of 21 days supplementation of HLC MindLinX on urinary excretion of opioid peptide exorphin B5 in six normal adult subjects



9. CASE HISTORY OF USE OF HLC MINDLINX IN AUTISM

HLC MindLinX has been tested on one child formerly diagnosed with autism, and now re-diagnosed as Asperger's Syndrome. This child was a classic case of regressive autism manifest at approximately 18 months of age and expressing

severe intestinal pathology from age 5 onwards. Elimination of wheat and dairy products considerably reduced intestinal symptoms and possibly autistic symptomology.

In this case, the supplementation of HLC MindLinx, alongside the re-introduction of small amounts of wheat and dairy into the diet, resulted in the urinary exorphin level being normal. Removal of HLC MindLinx resulted in a sharp increase in the urinary exorphin level (results of this work will be published in full in 2006).

10. AN INTEGRATED NUTRITIONAL APPROACH TO AUTISM AND ADHD

The review above has concentrated on the link between the intestine and the brain in the expression of autistic behaviour, with special reference to the exorphin production and a novel probiotic approach to achieving improvement of symptomology. However, other nutritional approaches in combination with the probiotic approach show considerable merit in terms of scientific support and also anecdotal feedback of benefits. It is not possible to provide the full background to these in this review but the suggested protocol below takes account of this integrated approach.

11. SUGGESTED PROTOCOL FOR NUTRITIONAL SUPPLEMENTATION IN AUTISM AND ADHD

1) FATTY ACID SUPPLEMENTATION

Both DHA and EPA are important, and providing the ratio of DHA:EPA is anything between 1:3 and 2:3, then the total amount of the two combined fatty acids is more important than the exact ratio.

Dosages should be varied dependent upon severity of condition:

Children 1-4 years 600 to 1,800mg DHA + EPA per day

Children 4-12 years 1,000 to 3,500mg DHA + EPA per day

The Pharmax products **Finest Pure Fish Oil**, **Berry Frutol** or original **Frutol** are all suitable for supplementing the above protocols.

2) INTESTINAL SUPPORT

PROBIOTIC:

HLC MindLinx 1 capsule or 1g powder (¼ teaspoon) take twice daily with meals.

This product would need to be taken indefinitely to ensure continued benefits

INTESTINAL PERMEABILITY:

Glutamine and arginine 2 to 5g per day for minimum of 60 days.

The Pharmax product **L-Glutamine Powder (with arginine)** is recommended.

3) ANTIOXIDANT

Oxidative stress, like inflammation, is strongly associated with ASD. Therefore, adequate antioxidant provision is a vital component of any ASD and ADHD protocol.

The Pharmax product **Endogenous Antioxidant & Cofactors** is particularly recommended.

4) VITAMIN AND MINERALS

Sufficient levels of the B-vitamin group, along with magnesium, zinc and iron, have been found by different studies to be important in ASD and ADHD.

Pharmax has found that the concentrated liquid supplements **B Complex CWS** and **Mag:Cal CWS (with boron & B6)** are particularly useful for children, where compliance in taking capsules and tablets is poor.

12. Summary

- ❖ **ASD is a complex constellation of disorders of varied but uncertain aetiology**
- ❖ **The incidence of ASD, as measured by most parameters, is increasing sharply**
- ❖ **A sub-group of ASD presents with intestinal pathology that has unique characteristics and which is variable in severity**
- ❖ **The intestinal pathology results in both the excessive production of dietary exorphins and their translocation to the brain, where they cross the blood brain barrier and stimulate opiate receptors**
- ❖ **Exorphin excess exposed to the brain has been found to produce autism symptoms in animals**
- ❖ **Diets which exclude the major exorphin-producing foods have been found to significantly reduce autistic symptoms. However, other foods also produce exorphins, so another mechanism of decreasing exorphin level is desirable**

- ❖ Probiotics have been found anecdotally to improve symptoms of autistics, particularly those with intestinal pathology. This is due to intestinal mucosal repair, reduction of inflammation and re-establishing immune tolerance and re-balancing the flora
- ❖ Pharmax has discovered new strains of probiotic that specifically degrade dietary exorphins
- ❖ These have been added to the original HLC Lab4 strains to produce a probiotic with unique characteristics for specific use in autism and ADHD
- ❖ Initial data show that this probiotic is effective in reducing dietary exorphin levels on both normal and autistic subjects
- ❖ A comprehensive nutritional approach to autism and ADHD is advised in order to achieve maximised improvements.

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