

Observational Study  
of Parents Supplementing Children  
with Duchenne Muscular Dystrophy  
with Protandim :  
Results Show Positive Benefits

*Important Disclaimer: The author of this document is a parent of a child with DMD and this document has not been peer reviewed.*

## Abstract

Summary: Duchenne Muscular Dystrophy (DMD) is a fatal disease that is caused by an absence of the Dystrophin protein. The lack of the Dystrophin protein leads to a continuous high-rate of muscle break down, regeneration and fibrosis resulting in a mean-on-set of loss of ambulation at 10 years of age and a mean on-set of death of less than 20 years of age.

Summary of the Study: A parental observational study of boys with DMD supplementing with Protandim was carried out over a 3 month period by parents of 16 boys whose ages ranged from 4-14 years of age.

Parents agreed not report any outcomes to each other for the duration of the study, so that any significant patterns reported across group in the study would act as a form of blind evidence.

Summary of Results: 8 parents have reported observable improvements in psychological factors, such as speech and mood, between 2 days and 2 weeks. 11 parents reported improvements in strength and energy levels between 2-6 weeks. 9 parents reported improvements in softness of calf muscles between 2-8 weeks. 5 Parents reported improvements in concentration levels and ability to follow instructions, 6 teachers and physiotherapists reported improvements in the boy's concentration or energy at school. 2 boys saw no benefit.

Conclusion: The distinct, closely matching temporal patterns of improvement that have been blindly reported by parents show that Protandim is conferring a benefit to boys with DMD, and therefore supplementing with Protandim warrants further investigation.

### Important Disclaimer

*The author of this document is a parent of a child with DMD and this document has not been peer reviewed. This document is written in the 3<sup>rd</sup> person as the author.*

**This document is not a recommendation to take Protandim. It is strongly advised that you contact your clinician before taking any supplement or medicine.**

**No participant taking part in this observational study, including the author of this document has any relationship, financial or otherwise with Lifeline Therapeutics, the makers of Protandim, or anybody or organization associated with them.**

**No participant taking part in this observational study, including the author of this document, is representing any organization, company or charity.**

**This observational study was initiated, run and reported by the author of this document and not by any organization, company or charity.**

## **Introduction**

### ***History***

In April 2006 the author was contacted by a parent of child with Duchenne Muscular Dystrophy saying they thought they were seeing an improvement in their son's strength, energy and a small decrease in calf muscle inflammation after taking a new food supplement called Protandim for 2 months.

After hearing this anecdotal report the author checked into the scientific rationale behind the supplement [5] and discovered that there was a scientific basis behind their observations [1,2,3,8].

The author then made checks into the safety of the supplement and contacted the Chief Scientific Officer (Prof. McCord) at the maker of Protandim (Lifeline Therapeutics) to ask about the product including dosages and safety information for the author son's age and weight. Prof. McCord then contacted their pharmacist who confirmed safety and dosage information (see below).

After 4 weeks of giving the food supplement to the author's son, the author and other family members started to see improvements in their son's energy and stamina. The benefit that was being seen could be guessed at the order of about 20%.

Following the author's observations another parent was contacted who followed the same process and also saw an improvement in their son's energy and stamina after about 4 weeks and a small decrease in the inflammation in their son's calf muscles. In addition this parent also saw an improvement in their son's language within a few days of taking the supplement.

The author decided that the observed benefits were important and that the goal would be to convince a clinician to run a double-blind random trial. It was expected that clinicians would not be able to justify a clinical trial based on the anecdotal evidence from 3 parents so better anecdotal evidence would be necessary. After contacting clinicians cost, placebo effects, anecdotal evidence and developmental reasons were (justly) cited as reasons not to run a trial and it proved necessary to adopt an alternative route.

The basis of the plan was to gather evidence in an ethical manner from a number of parents then collate this anecdotal evidence. A number of known parents were contacted and told the anecdotal evidence and were strongly advised to contact their clinician and the makers of Protandim. If the parents then decided to supplement their child, the parents were asked if they would observe their child for the purpose of capturing anecdotal data. Parents were asked not discuss their observations with other parents on the study, in order to ensure the cross-sectional data was blind so that clinicians could justify a double blind trial.

## ***Purpose***

The main purpose of the observational study was to gather anecdotal evidence from parents giving their children Protandim to determine whether it would be beneficial or not to proceed with a double-blind clinical trial.

An additional (sub) goal for the observational study was to try to determine possible end-points for a clinical trial by asking parents to observe specific outcomes. The three parents who had observed a benefit of Protandim had seen improvements across (1) energy and stamina, (2) inflammation and language and (3) cognition, so it was decided to ask parents to observe these.

Another important (sub) goal was to conduct the study in a highly ethical manner so that clinicians would be able to use the data to justify the clinical trial.

## ***Rationale***

### **Role of the Dystrophin Protein**

The primary location of the dystrophin gene is in human skeletal and cardiac muscles. The dystrophin protein also plays a role in the brain, though its function is not clear.

The pathology Duchenne Muscular Dystrophy is commonly believed to start with an excessive of free-calcium,  $Ca^{2+}$  [“calcium”] in skeletal muscle fibers, caused by an excessive influx (or a reduced efflux) of calcium [32, 39, 40, 58, 60]. However, some researchers have not found higher calcium levels in (resting) muscle fibers [59]. The relationship between the missing protein and the influx or efflux of calcium into muscle fibers is complex and still controversial.

Currently, there are several alternative models for the function of the dystrophin protein inside the long fibrous muscle cells [22,26]. The three main functional models can be characterized as the “return spring” functional model, the “shock-absorber” functional model, and the “signaling transducer” functional model.

In the “return spring” [8, 23, 41] model the dystrophin protein provides a retraction-force inside normal muscle fibers that pulls the calcium channels closed and in its absence the calcium channels stay open longer, leaking calcium into the muscle cell. This then triggers a pathological cascade causing calcium overload and lipid peroxidation in the muscle membrane which in turn causes muscle weakness [31] and further calcium influx and pathology. The two channels believed to be involved are the stretch activated calcium channel (SOC) and store operated calcium channel (SAC).

\* Note that recently a published paper has found further evidence that the TRCP1 protein involved in the stretch operated channels and the store operated channels is linked to the Dystrophin associated protein complex [65].

In the “shock-absorber” model [25,27] the dystrophin protein provides structurally-enhancing-force inside normal muscle fibers and in its absence the muscle tears causing the influx of calcium which triggers a pathological cascade eventually resulting in muscle loss and fibrosis.

In the “signaling transducer” model [24] the dystrophin protein sends signals inside muscle fibers when they are extended or contracted. The lack of these signals cause an abnormal influx and/or efflux of calcium resulting in the same pathological process, or causing inhibition of muscle growth or repair. An experiment that tried to eliminate  $Ca^{2+}$  influx through the stretch activated channels [43] showed that NF-Kappa B was activated by mechanical stretch alone. The same research found that in lung tissue stretch-induced activation of NF-kappaB involves activation of stretch-activated channels and the production of free radicals [48].

Whilst these models differ in the role of the dystrophin protein it is worth making several important points. First, that the shock absorber model is the older and best supported model in the patient literature but is not as well supported in the recent scientific literature. Second, the different models of the Dystrophin protein are not mutually exclusive. Third, the three models share similar pathological pathways involving excess calcium. Fourth, the pathological pathways involve a number of oxidative stress processes. Fifth, the functional role of Dystrophin has not been agreed upon..

Finally, an additional developmental dimension to these dystrophic models is also important. It has recently been shown [61] that in dystrophic mice calcium handling in fully developed isolated muscle fibers (myofibers) was near normal whilst calcium levels were 3 times greater in muscle fibers during their very early development (myotubes) and 22 times greater after 8 days.

Despite the lack of specific agreement on the role of the Dystrophin protein [26] all three functional models agree that oxidative stress caused by calcium plays a significant role in the pathology of the disease.

## **Pathological Pathways**

Once inside the skeletal muscle the free calcium ( $Ca^{2+}$ ) triggers a number of pathological pathways which lead to the production of Reactive Oxygen Species (ROS) such as superoxide, hydrogen peroxide, and hydroxyl radicals. There is evidence to support the hypothesis that ROS induces protein damage in human dystrophin-deficient muscle as protein carbonyl levels (an indicator of ROS) have been raised by 211% compared to normal muscle [55]. It has also been found that there are significant differences between the degree of oxidation of muscle proteins between Duchenne and Becker patients [57].

Mitochondria are the major endogenous source of ROS [34] in muscles. The calcium influx is absorbed by mitochondria inside the muscle fiber [33, 38] where they act to regulate the levels calcium inside the muscle [38]. In normal muscle the production of ROS by mitochondria can be removed by the bodies own enzyme scavenging. However, when there is an excess of calcium the mitochondria produce too much ROS for the bodies own enzyme scavenging mechanisms to remove [35]. In addition to the over production of ROS calcium overload of the mitochondrial calcium cause the mitochondrial cell death [37] further reducing calcium handling in the cell.

As well as the over production of ROS by the mitochondria, it is also thought that three other pathways (1) calpain, (2) phospholipase A2 and (3) “inflammatory” could increase the production of ROS due to excess calcium in the muscle fibers [1,32]. The calpain pathway is up regulated by abnormally high levels of calcium which causes internal cell remodeling and cell death [44,45]. The phospholipase A2 pathway damages the membrane integrity and increases the production of ROS [32, 46, 47]. The “inflammatory” pathway that is mediated by kappa-b [66, 67, 68] increases leukocytes (neutrophils and macrophages) which also produce more ROS [1,49].

The increased ROS inside the muscle cell directly damages the fats in cell membrane by a process called lipid peroxidation. Reducing lipid peroxidation in MDX mice has been shown to reduce muscle degeneration [6]. This damage to cell membranes further increases calcium influx, which may further increase the influx of calcium [50]. Later muscle fibrosis leads to reduced oxygen in the muscle tissue which further increases lipid peroxidation [50]. In addition increased ROS has been shown to reduce nitric oxide synthase (NOS) [63] reducing the Nitric Oxide and increasing the development of fibrosis.

## ***SOD, CAT and GPx***

The human body produces three enzymes whose purpose is to break down Radical Oxygen Species (ROS) with the body to less damaging substances, these are:

- Superoxide Dismutase (SOD)
- Catalase (CAT)
- Glutathione Peroxidase (GPx)

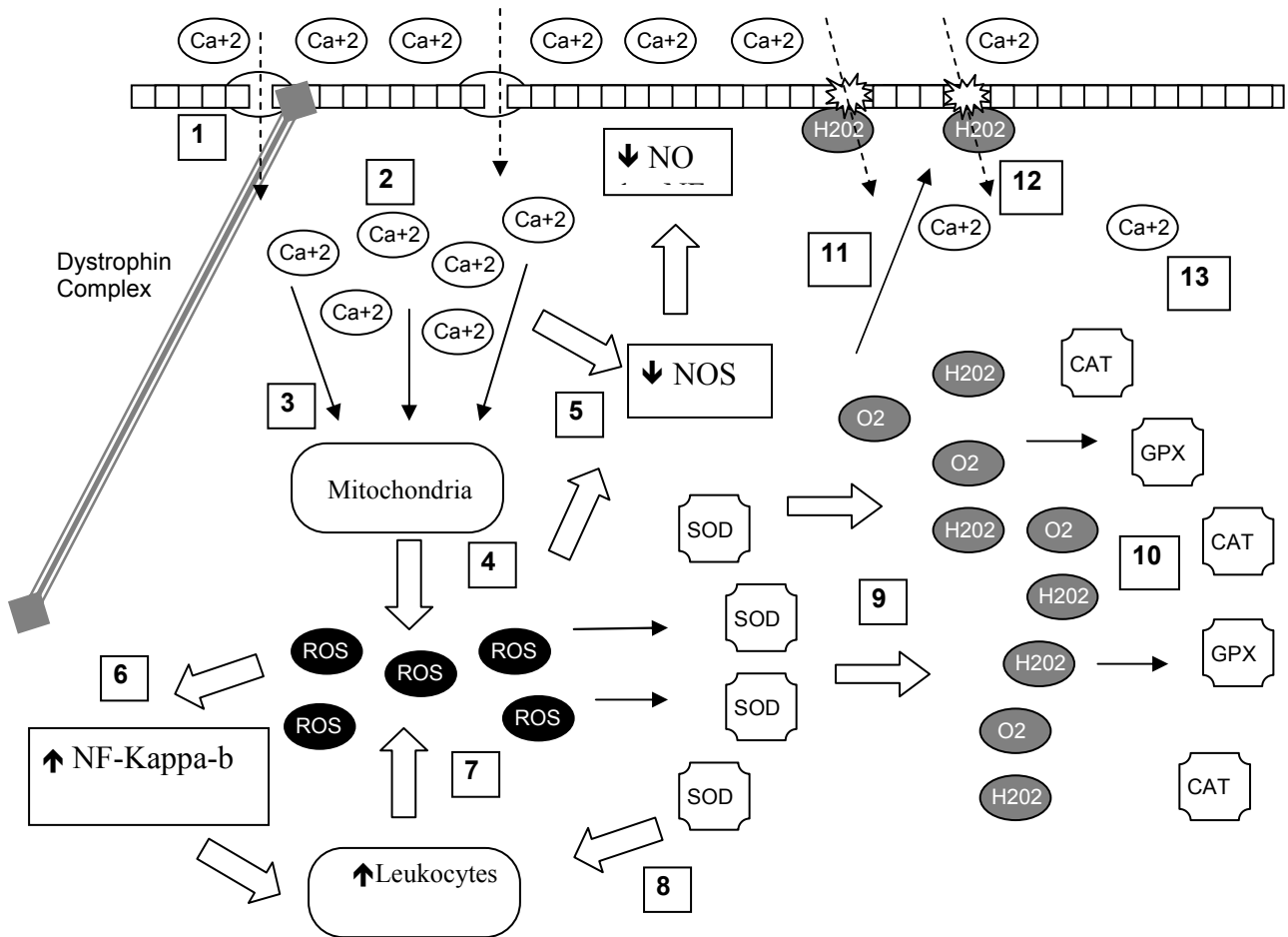
Superoxide dismutase (SOD) is an enzyme that breaks down the common ROS dismutated superoxide into oxygen and hydrogen peroxide (HP). Catalase (CAT) is an enzyme that breaks down hydrogen peroxide to water and oxygen. Glutathione Peroxidase (GPx) is an enzyme family that breaks down hydrogen peroxide to water and oxygen. Together these enzymes can break down ROS down to the less harmful substances of hydrogen, oxygen and water.

According to the complex pathological model presented SOD, CAT and GPx intervene in the pathological pathways to do the following:

1. They reduce damaging free radicals by converting them to water and oxygen.
2. They help increase Nitric Oxide, which reduces fibrosis.
3. They suppress Leukocytes activity which causes inflammation.

The pathological pathways that have been described (see diagram below) contain several feedback loops whereby an increase in ROS and HP eventually causes a further increase in ROS and HP. By dampening down the ROS and HP this, in theory at least will lead to further drops in ROS and HP.

Diagram showing a partial model of how free radical damage may occur in dystrophic muscle.



- |   |  |
|---|--|
| <ol style="list-style-type: none"> <li>2. Stretch Activated Channels are not shut by the dystrophin complex.</li> <li>3. There is an excess influx of free calcium (ca<sup>2+</sup>) into the muscle cell.</li> <li>4. The mitochondria absorb the excess ca<sup>2+</sup>.</li> <li>5. The mitochondria become overloaded and produce radical oxygen species (ROS) as O<sub>2</sub><sup>-</sup></li> <li>6. The ROS and ca<sup>2+</sup> interacts with the nitric oxide synthase (NOS) reducing the Nitric Oxide (NO) production and increasing fibrosis.</li> <li>7. ROS increases NK_Kappa-b which increases leukocyte activity,</li> </ol> | <ol style="list-style-type: none"> <li>7. Additional leukocytes (e.g. neutrophils) caused by kappa-B inflammation produce more ROS.</li> <li>8. The superoxide dismutase (SOD) suppresses Leukocytes activity</li> <li>9. The SOD enzyme converts the ROS to Hydrogen Peroxide (H<sub>2</sub>O<sub>2</sub>) and oxygen (O<sub>2</sub>).</li> <li>10. The catalase (CAT) and Glutathione peroxidase (GPX) reduce the H<sub>2</sub>O<sub>2</sub> to hydrogen and water.</li> <li>11. H<sub>2</sub>O<sub>2</sub> causes lipid peroxidation and tears in the muscle membrane.</li> <li>12. Membrane tears let in more Ca<sup>2+</sup> and due to decreased NO favor more fibrosis.</li> <li>13. The pathological cycle of fibrosis and tearing increases the intensity.</li> </ol> |
|---|--|

## Protandim

Protandim [5] is a supplement, introduced in 2005\*, that is aimed reducing the effect of free-radical oxidation by inducing SOD, CAT and HPx. Protandim has been shown to significantly reduce lipid peroxidation products after 30 days by 40%, measured by thiobarbituric acid-reacting substances (TBARS). Protandim has also been shown to increase erythrocyte SOD by 30% and CAT by 54% after 120 days.

Protandim [5] has been shown to linearly increase the erythrocyte SOD and CAT activity up to 120 days, this is because new erythrocytes (red blood cell) take 120 days to be fully replaced in the body. This means that the study would expect to last at least 120 days in order to show the benefits from the known increases SOD and CAT.

No toxicity, unwanted pharmacological effects of Protandim were found during the previous Protandim human study. There are also extensive human safety records for the supplements used in Protandim.

\*It is worth noting that a supplement that increased *both* SOD and CAT (and HPx) was wanted by the author before Protandim was introduced. The reason for wanting to increase *both* SOD and CAT was that increasing SOD alone may increase hydrogen peroxide (a lipid damaging free radical) and without an increase in CAT or HPx then there could be a possible increase in hydrogen peroxide damage. In other words maintaining the ROS/CAT+HPx balance was an important initial consideration.

## Protandim Ingredients

Protandim consists of the following 5 used herbal plant extracts:

Ingredient	Capsule (675mg)	Content
Bacopa Monniera (Brahmi)	150 mg	45% bacosides
Silybum marianum (Milk Thistle)	225mg	70–80% silymarin
Withania somnifera (Ashwagandha)	150mg	
Green Tea (low caffeine)	75mg	98% polyphenols and 45% epigallocatechin-3-gallate
Turmeric	75 mg	95% curcumin

\*Taken from Protandim website:

Each ingredient in Protandim may possibly have other beneficial *direct* effects on the pathology of DMD as well the effect caused by an increase in SOD, CAT and HPx.

Bacopa has been shown to increase SOD, CAT and GPx in the brain after 14 and 21 days of administration [69]. Bacopa has also shown [70] “A significant reduction in the serum markers of heart and kidney damage and the extent of lipid peroxidation with a

concomitant increase in the enzymatic (SOD and CAT) and non-enzymatic antioxidants (reduced glutathione) were observed”.

In addition [71] “Bacopa extract promotes the antioxidant status, reduces the rate of lipid peroxidation and the markers of tumor progression in the fibrosarcoma bearing rats.”. This is particularly interesting because “Fibrosarcoma (fibroblastic sarcoma) is a malignant tumor derived from fibrous connective tissue and characterized by immature proliferating fibroblasts or undifferentiated anaplastic spindle cells.”, and  $Ca^{2+}$  influx has been found in immature fibroblasts [61].

Curcumin has an ambiguous beneficial status in DMD. On one hand it has been shown not to prevent the activation of NF-Kappa-B in DMD [72] and in hyperglycemia [74] but it has been shown to improve sarcolemmic integrity and muscle strength [73].

Green tea has already been shown to [75,76] “protecte [DMD] muscle against the first massive wave of necrosis and stimulated muscle adaptation toward a stronger and more resistant phenotype.”

As well as inducing SOD and CAT. There is some evidence that milk thistle may directly effect the calcium handling [78], possibly through mitochondrial respiration [77, 79, 80] and reduced NF-Kappa-B [81,82] and that this may also achieve a beneficial outcome for DMD muscle.

As well as increasing SOD and CAT [85, 86] ashwagandha has also been shown to inhibit NF-Kappa-B [83] reduce lymphocyte proliferation [84] and increase NO production [88].

The previous benefits shown by green tea and curcumin, the calcium handling and NF-kappa reducing properties of milk thistle, the NF-kappa reducing and NO inducing properties of ashwagandha and the improvement in cognitive function by Bacopa show that there is a possibility that other positive factors benefit dystrophic muscle.

## **Study**

### ***Method***

Because anecdotal evidence reported in sporadic form is difficult for clinicians and researchers to assess it was decided to ask a limited number of parents if they wanted to take part in a parental study of Protandim. An international group of parents who were known were contacted and asked if they wanted to take part in this study.

All participants taking part in this observational study were supplied with a Questionnaire that detailed the background behind this observational study and it strongly recommended that they talk to their clinician about their involvement in the study, Also that they should report any negative effects immediately to their clinician and they were free to withdraw from the study at any time.

The purpose of the study was to try to determine whether the anecdotal benefit observed by the first 3 parents was due to a placebo effect or not. Because the 3 parents who had taken Protandim already had seen a slight temporal pattern in the benefits it was decided that if all the parents taking the supplement agreed not to talk to each other about the study results in their child, then if this pattern emerged strongly across the parents then it would confirm that the benefits were not due to a placebo effect.

### ***Source***

Protandim was sourced from Lifeline Therapeutics. If parents chose to supplement with Protandim they were required to source the supplement themselves. Some parents sourced Protandim directly from the website. However the makers of Protandim offered to supply Protandim for the duration of the study, which made it easier for parents in Europe to source the supplement.

### ***Dose***

The dosage was determined by a pharmacist who was contacted by Lifeline Therapeutics to be half a tablet a day for participants under 70lbs and a whole tablet a day for those over 70lbs.

## ***Ethics***

As stated earlier it was very important to get the ethical and legal aspects of the study correct so that clinicians would feel comfortable using the data from the study to justify a clinical trial.

The priority concern was that there were no short or long term adverse effects from taking Protandim. Three parents had already seen improvement, with no side effects. No side effects from the human trial of Protandim had been reported. The supplements in Protandim have a huge track record of safety, and have no known toxicity or significant side effects in the dosages to be used in the study. The pharmacist at the makers of Protandim checked for doses in children and was available (via Lifeline Therapeutics) for questions.

When parents were asked if they wanted to take part in the study they were told 3 parents have anecdotally seen an improvement and told about how to source the supplement. Parents were recommended to talk to their clinician and the makers of Protandim about the supplement before taking it. 3 of the 20 parents contacted declined to participate and were not contacted after that. No parents said they felt under any pressure to take the supplement and all parents said they were pleased to have been given the opportunity to have been informed about the supplement.

All parents were strongly recommended to contact their clinician before taking Protandim – the majority of parents informed me after the study that they spoke to their clinicians did before taking the supplement.

Parents were strongly recommended to report any side effects immediately to their clinician and to stop taking the supplement. They were also asked to let the author or the makers of Protandim know immediately about any side effects so that this information could be disseminated to the other participants in the study. Reporting side effects in this way meant an enhanced degree of mutual support across the group.

Food supplements are not required to be registered for clinical trials. Checks were made with the FDA and COREC that the ingredients in Protandim have been classified as a food supplement and did not require an ethical review. The Port Authority in the UK was checked to ensure that import licensing was valid – they required that an EU valid curcuma import license was obtained from the manufacturers to ensure that the no Sudan Red dye was present in the product. This license was obtained by the makers of Protandim.

## ***Adverse Reactions***

No adverse reactions were reported.

A small warning: 4 patients reported that after 3-4 weeks their child was less constipated (not diarrhea) and found that the time between feeling an initial urge to go to the toilet and going to the toilet was reduced. The children had adjusted themselves within a few days of the problem appearing.

From my personal experience I believe that this is due to a change in colonic motility. The reason for this is that I could just see when my child was on the toilet they were making a lot less effort than before – and looked somewhat surprised when they found their large intestine was doing more of the work for them.

After doing the study I found that improvements in colonic motility had been observed in MDX mice [18].

## **Questionnaire**

Participants were asked to complete a questionnaire.

The questionnaire was designed to allow parents to assess their child on a functional level. In other words it was designed to allow parents to make judgments based on areas of their child's activities that were easily observed and they were non-overlapping.

Parents were simply asked to report if the child's function was better, the same or worse.

Assessments were asked for every 2 weeks because it was felt that the time period between assessments was small enough that observations would be remembered and large enough that daily changes would not cloud the parent's judgments.

No objective assessments, involving measurements, were asked for as it was felt to have been too much effort for the parents and didn't fit with the purpose of the study and was considered too subjective. For example parents were not asked to say whether the improvement was "big" or "small".

Table : showing questionnaire to be completed by participants or their parents.

**Periodic Assessment**

Note: Change is reported as, observed change over the previous 2 weeks. To record your observations you can either edit this file directly or print it out and tick the boxes.

	<b>Falling, Walking Running</b>	<b>Climbing Climbing Stairs</b>	<b>Hand &amp; Arm Strength</b>	<b>Energy, Tiredness Stamina</b>	<b>Talking, Learning Reading</b>	<b>Tendons &amp; Muscle &amp; Inflammation</b>
<b>Week 2</b>  / /2006	<input type="radio"/> Better <input type="radio"/> Same <input type="radio"/> Worse	<input type="radio"/> Better <input type="radio"/> Same <input type="radio"/> Worse	<input type="radio"/> Better <input type="radio"/> Same <input type="radio"/> Worse	<input type="radio"/> Better <input type="radio"/> Same <input type="radio"/> Worse	<input type="radio"/> Better <input type="radio"/> Same <input type="radio"/> Worse	<input type="radio"/> Better <input type="radio"/> Same <input type="radio"/> Worse
<b>Week 4</b>  / /2006	<input type="radio"/> Better <input type="radio"/> Same <input type="radio"/> Worse	<input type="radio"/> Better <input type="radio"/> Same <input type="radio"/> Worse	<input type="radio"/> Better <input type="radio"/> Same <input type="radio"/> Worse	<input type="radio"/> Better <input type="radio"/> Same <input type="radio"/> Worse	<input type="radio"/> Better <input type="radio"/> Same <input type="radio"/> Worse	<input type="radio"/> Better <input type="radio"/> Same <input type="radio"/> Worse
<b>Week 6</b>  / /2006	<input type="radio"/> Better <input type="radio"/> Same <input type="radio"/> Worse	<input type="radio"/> Better <input type="radio"/> Same <input type="radio"/> Worse	<input type="radio"/> Better <input type="radio"/> Same <input type="radio"/> Worse	<input type="radio"/> Better <input type="radio"/> Same <input type="radio"/> Worse	<input type="radio"/> Better <input type="radio"/> Same <input type="radio"/> Worse	<input type="radio"/> Better <input type="radio"/> Same <input type="radio"/> Worse
<b>Week 8</b>  / /2006	<input type="radio"/> Better <input type="radio"/> Same <input type="radio"/> Worse	<input type="radio"/> Better <input type="radio"/> Same <input type="radio"/> Worse	<input type="radio"/> Better <input type="radio"/> Same <input type="radio"/> Worse	<input type="radio"/> Better <input type="radio"/> Same <input type="radio"/> Worse	<input type="radio"/> Better <input type="radio"/> Same <input type="radio"/> Worse	<input type="radio"/> Better <input type="radio"/> Same <input type="radio"/> Worse
<b>Week 10</b>  / /2006	<input type="radio"/> Better <input type="radio"/> Same <input type="radio"/> Worse	<input type="radio"/> Better <input type="radio"/> Same <input type="radio"/> Worse	<input type="radio"/> Better <input type="radio"/> Same <input type="radio"/> Worse	<input type="radio"/> Better <input type="radio"/> Same <input type="radio"/> Worse	<input type="radio"/> Better <input type="radio"/> Same <input type="radio"/> Worse	<input type="radio"/> Better <input type="radio"/> Same <input type="radio"/> Worse
<b>Week 12</b>  / /2006	<input type="radio"/> Better <input type="radio"/> Same <input type="radio"/> Worse	<input type="radio"/> Better <input type="radio"/> Same <input type="radio"/> Worse	<input type="radio"/> Better <input type="radio"/> Same <input type="radio"/> Worse	<input type="radio"/> Better <input type="radio"/> Same <input type="radio"/> Worse	<input type="radio"/> Better <input type="radio"/> Same <input type="radio"/> Worse	<input type="radio"/> Better <input type="radio"/> Same <input type="radio"/> Worse

## ***Participants***

Participants were recruited through personal contacts. Participant's ages varied between 4 and 14 years of age.

The observational study included a mix of ambulatory and non-ambulatory participants and steroid and non-steroid participants.

Because of the international nature of the internet participants were recruited from 5 countries, including the UK, US and France.

Three parents who said they were starting the study did not start the study.

Table: listing the participant's id, age and steroid and ambulatory status of the participants.

<b>Participant</b>	<b>Age</b>	<b>Steroids</b>	<b>Ambulatory</b>	<b>Response</b>
A	5	N	Y	Completed
B	5	Y	Y	Completed
C	5	N	Y	Completed
D	6	Y	Y	Completed
E	4	N	Y	Completed
F	13	Y	N	Completed
G	14	Y	N	Completed
H	8	Y	Y	Completed
I	9		Y	Completed
J	8	Y	Y	Completed
K				Didn't start
L	?	N	Y	Completed
M	?	?	?	Completed -
N		Y	Y	Didn't start - didn't like taste
O				Didn't start
P	6	Y	Y	Completed
Q	7	Y	Y	Completed
R	8	N	Y	Completed
S	6	Y	Y	Completed

## **Results**

### ***Negative Results***

There was no worsening during the study for any participant, over any period on any endpoint.

No negative results or comments have been reported

Two parents reported that they had not seen any improvements.

### ***Positive Results***

Positive Results were reported in 3 areas:

- Cognitive
- Muscle Function (grouping ambulation, strength and energy)
- Muscle Inflammation

Of the 16 who have completed the study only 1 parent has decided not to continue with Protandim after not seeing any benefits.

## ***Cognitive Function***

The most surprising result of the study was that all parents who saw an improvement in their child's language abilities saw it within the first 2 weeks of starting the study. Parents who reported problems with their child's speech were the ones that saw an improvement.

Overall 11 parents saw an improvement in their son's cognitive function; this included a combination of speech, mood and concentration.

Many emails were received after a few weeks of taking Protandim with parents saying either my "son has become much more talkative", or in some cases "my son won't shut – up". Parents were unaware of what each other was saying (most parents were unknown to each other) the similarity between the parents reports indicates similar improvements in the boys language.

No adverse reactions were found in sleeplessness or irritability, indicating that the green tea in Protandim was not acting as a stimulant. Several parents reported an improvement in mood reinforcing that the green tea was not acting as a stimulant.

Participants who did not report an improvement in chattiness and sociability were followed up after the study to see if they noticed any change in this area. Several of the participants who did not report a change in their son's language said that their son was very chatty in relation to their peers before the study.

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Table showing parents observations of changes in language and cognitive functions and the onset time of those changes. The comment section includes comments made by parents in either the diary document or private emails.

<b>Participant</b>	<b>Onset Time</b>	<b>Comment from Parent</b>
A		Post study response in question about chattiness reported “He was very chatty anyway”.
B		Post study response in question about chattiness reported no change. Previously “he’ll just chat non stop at times, interrupt people while they are trying to speak”
C	0-8 week	“There has been positive change in his speech. Talking more and giving more information.”
D	0-2 week	“He has become a lot more talkative. He talks all the time.”
E	0-2 week	“The biggest change we have seen is his speech, almost an over night transformation. He is clearer, constructs sentences better and seems to understand other people better.”
F	0-2 week	“He seems a lot happier and can’t stop him talking.”
G	0-2 week	“We have noticed an increase in M's "chattiness". He is normally an extremely quiet boy and he has been initiating conversations with people other than close family members and close friends. This is wonderful as he is extremely clever but is often let down by his lack of contributions in class.”
H	0-2 week	“A cognitive benefit at school – with more concentration.”
I		No change
J		No change
L		No change
P		No change
Q		No change
R	0-2 week	“Talking a lot more,”
S	2-3 week	Child had “extreme” behavior problems before taking Protandim. “All of a sudden I have noticed he is starting to articulate much better and talk more fluently. He is suddenly starting to write and draw all the time. He has never been into writing and drawing much but he is suddenly a little Picasso now.”

### ***Muscle Function***

Parents we asked to observe their child's muscle function in three different areas.

- Falling, Walking Running
- Climbing, Climbing, Stairs
- Hand & Arm Strength
- Energy, Tiredness Stamina

Interestingly, changes in physical functions did not appear until the 4<sup>th</sup> week of taking Protandim, unlike the cognitive changes that almost all appeared in the 1<sup>st</sup> week of taking Protandim. Again, parent's reports were being made independently of each other.

Table showing parents observations of changes in muscle function. The comment section includes comments made by parents in either the diary document or private emails.

<b>Participant</b>	<b>Onset Time</b>	<b>Comment from Parent</b>
A	4-5 week	Improvement in stamina and walking. He is less tired after school and will run around right up to bed time.
B	4-5 week	Increase in strength and stamina
C	4-5 week 10-11 week	Better falling, walking, running Better climbing stairs
D	4-5 week	He seems to be getting upstairs better, walking further
E	4-5 week	Improvement in falling, walking, and running
F	4-5 week	He has much more energy his upper body strength has improved.
G		We have not noticed any improvement in muscle function in him at all.
H	6-7 week	Walks better, he doesn't fall (except when he has done too much), more dynamic on the stairs. I think that it gives him mostly energy.
I		No change
J	8-9 week	Improvement In falling
L		No change
P		No change
Q	6-11 weeks	Better Walking, Falling Running , Energy and Stamina
R	4-5 weeks	Improvement in energy – he wants to walk further.
S	2-3 weeks	Improvement in strength - walking up the stairs carrying toys and not holding onto anything

### ***Inflammation in Calf Muscles and Tendons***

Parents were asked to report on calf inflammation as a measure. Many parents monitor their son's calf muscles and tendons quite closely because they perform stretching exercises on their sons either in the morning, evening or both.

Again parent's reports were being made independently of each other.

Table showing parents observations of changes in muscle inflammation. The comment section includes comments made by parents in either the diary document or private emails.

<b>Participant</b>	<b>Onset Time</b>	<b>Comment from Parent</b>
A	4-5 week	The upper side of his thigh muscle is softer than they were before.
B	2-3 week	Calf muscles were a lot softer
C	6-11 weeks	Back of legs softer + more movement in left foot/ankle.
D	4-5 week	His calf muscles have changed, not quite so all round hard but he still feels "lumpy" and they are still noticeably bigger than what you would expect to be normal.
E	4-5 week	I think they feel softer and don't look as swollen
F	4-5 week	His calf muscles appear softer
G		No change
H	6-7 week	The physician find his muscles and tendons less stiff than before the study
I		No change
J		No change
L		No change
P		No change
Q	6-10	Better inflammation
R	2-5 weeks	Reduction in inflammation from swollen to soft like mine (mother)
S		Have not noticed his legs getting softer

***Other Observations***

This section includes some other observations made by parents. It has been included because clinicians designing a clinical trial might want to use these observations as additional end-points.

This table also records the two parents who stopped taking Protandim, and both saw a decline in their son's strength and language.

Table showing parents observations of other changes, not reported in the questionnaire. The comment section includes comments made by parents in either the diary document or private emails.

<b>Participant</b>	<b>Onset Time</b>	<b>Comment From Parent</b>
A	2-3 weeks	Less constipated (colonic mobility)
B		
C	2-3 weeks	More regularized bowel movements
D	2-3 weeks	Less constipated (colonic mobility). After stopping Protandim for a week noticed a definite deterioration.
E		
F		Non ambulatory. Less constipated. Less moody whilst off steroids. Rash on face whilst off steroids has gone. The participant said he did not want to come off Protandim as he felt better.
G		
H		Less moody.
I		None
J		
L		None
P		None
Q		
R		Temper is less, partly due to frustration with language. A "definite increase in moodiness and inattentiveness" when stopped taking Protandim for 5 days.  After stopping Protandim for 2 weeks there was an increase in moodiness, decline in language function and calf muscles became "lumpy" again.
S		Calf muscles have become softer.

***3rd Party Observations***

Several parents sent emails during and after the study with comments they had had from teachers and physiotherapists and clinicians who had been working closely with their sons.

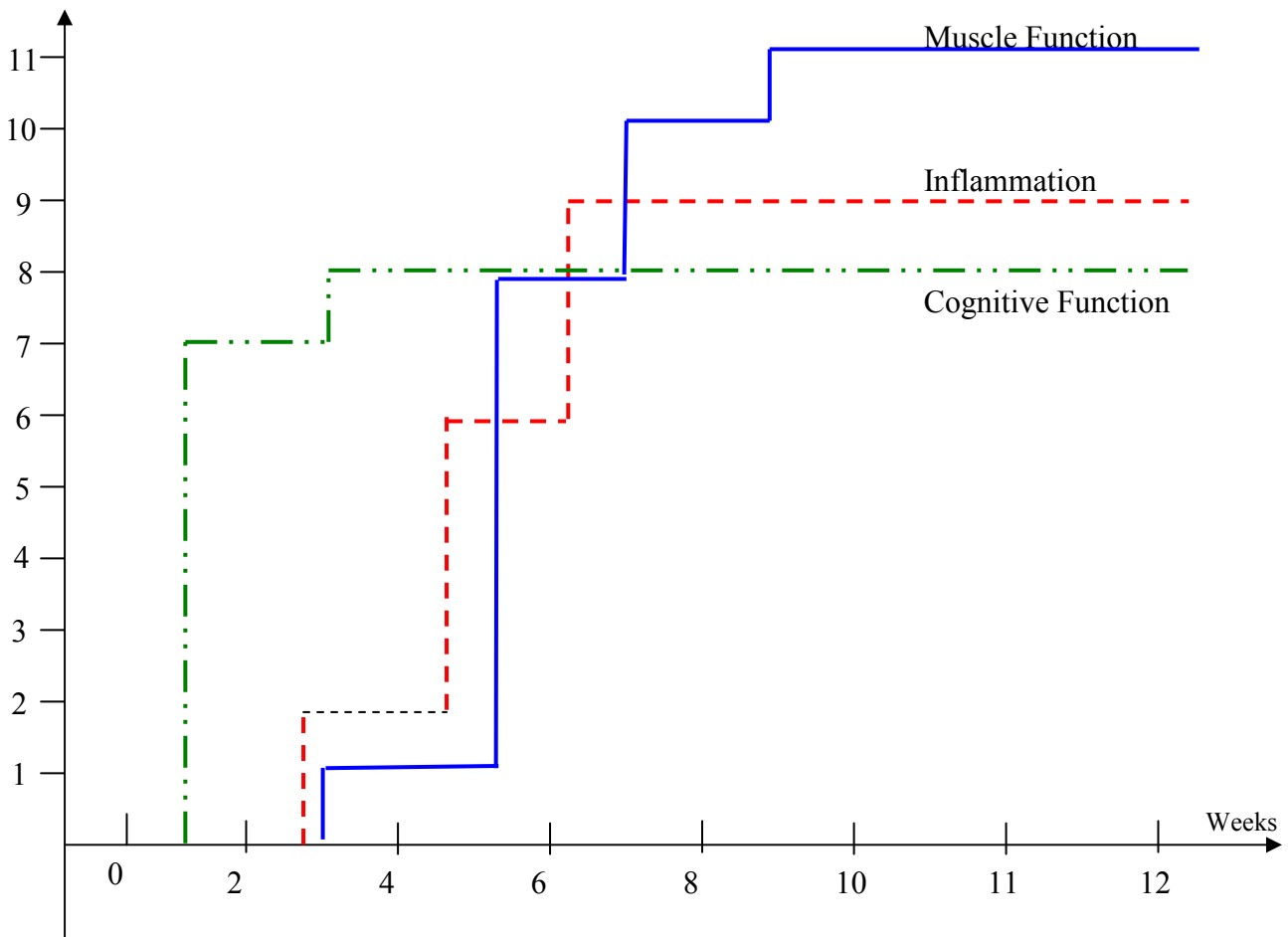
<b>Participant</b>	<b>Onset Time</b>	<b>Comment From Parent</b>
A	12-13 week	Classroom helper commented he had a lot more energy and stamina than he did before the study.
B		
C	3-4 week	Teacher noticed better concentration and more interest in learning.
D		
E	8-9 week	<p>When he went back to school a couple of weeks ago, his teacher from last year had spent some time with him in reception, she could not get over the difference in him and the head teacher has also commented.</p> <p>The physio also commented on the change in his speech since the last time she saw him.</p> <p>Pediatrician wrote “Since starting Protandim he has made some really quite remarkable progress. He is walking longer distances and he can now pull to stand using a plinth or chair. He is now able to crawl on his hands and knees and he can go up steps holding onto a rail on both sides. He has made great progress in speech and language and his mother is very pleased with the overall progress he is making in school now.”</p>
F		
G	8-9 week	His teacher told me that she found he had more concentration in comparison with last June
H	6-7 week	The physician found his muscles and tendons less stiff than before the study
I		None
J		No noticeable change
L		None
N		None
P		None
Q		Not heard back from
R		
S	4-5 weeks	School noted good interactions, polite, not appearing as anxious, able to take turns and work through a frustration with minimal assistance, they have not noticed him putting his hand to/in his mouth at all this week

## Summary of results

A pattern emerged across the parent's reports (see diagram below) showing that there was a clear improvement in cognitive functions in the first week of then study, then an improvement in muscle function and inflammation between 2-6 weeks.

Diagram: showing the number of parents reporting the onset of benefits against the time in weeks.

Number of Parents Reporting onset of benefit



## Discussion

The main result of the study shows that there is a clear difference between the observed onset of cognitive improvement and observed onset of physical improvement by parents in the study. The cognitive benefits appeared in the first week and physical and inflammatory benefits appeared in about the 4<sup>th</sup> week.

### ***Was there a Placebo Effect?***

The parent's reports were independent of each other, yet overall they observed the same patterns of cognitive and physical improvement. The consistent reports of improvement in cognition and language within the first week and physical improvements in the 2-6 week give very good cause to believe that the observations were not due to a placebo effect.

Additionally, the many of the verbal observations made by parents about language improvements were very similar – such as “he'll just chat non stop at times”, “he talks all the time”, “can't stop him talking”, “an increase in chattiness”.

Additionally the improvement in inflammation and physical improvements took place at about the same time and over a slightly longer time frame. The improvements in cognition and inflammation came as a surprise (to the author), as this was not what they were expecting - the questionnaire end-points indicate that improvement in physical functions were what was mainly expected given the observations of the first three parents.

It is worth adding that since undertaking this study parents on the DMD PTC-124 trial have also reported (surprise) cognitive benefits and cognitive end-points have now been added to that trial.

Blind observations have also been made by third parties, such as teachers, physios and clinicians who have also reported an improvement in cognitive and physical functions,

In the study there were two non-ambulatory patients. One of whom saw an improvement in upper body strength and concentration and mood and other saw an improvement in language and social confidence.

Benefits were seen by participants both on and off steroids. Steroids have not been reported to have a significant improvement in cognitive function, rather an increase in moodiness. Interestingly several parents whose sons were on steroids reported an improvement in their sons moodiness during the study. The data tends to imply that Protandim is both acting on a different part of the pathology to steroids and helping to relieve some of the effects of steroids.

Two parents who stopped Protandim, one for one week, one for 2 weeks both reported a decline in function.

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