

The Remarkable Effects of “ASEA redox Supplement” In A Child with Duchenne Muscular Dystrophy – A Case Report

Abstract

This research aims at discovering dietary supplements which may show comparable or even stronger beneficial effects (with less or none adverse effects) than corticosteroids in children with Duchenne Muscular Dystrophy (DMD). This paper presents a case report on the effects of an ionized “saline water” called “ASEA redox Supplement®” (ARS) oral solution in a ~2-year-old boy with DMD from Bucharest, Romania. In vitro studies showed that ARS is a very potent selective NRF2 activator, thus a very potent (indirect) antioxidant: the studies conducted in vivo also support this main pharmacological mechanism of ARS, with no toxicity up to high doses, in contrast with the much more toxic corticosteroids. From the first months of ARS treatment all the rhabdomyolysis markers (with very high initial serum levels) dropped significantly, with no found toxicity. The main conclusions of this paper are: (1) ARS has remarkable antioxidant and immunomodulatory effects and should be studied on larger groups of children with DMD under the age of 4 years old (but also on other age groups of children and even young adults), as an alternative to early corticosteroids; (2) Given its immunomodulatory effect (NRF2 selective activation and NF-κB inhibition), ARS deserves future cohort studies on its potential to replace corticosteroids and other non-steroidal immunosuppressants (at least partially) in many types of pulmonary/renal/hepatic/ articular/skin autoimmune autoimmune and even malignant diseases of both children and adults; (3) Given its very strong antioxidant effects (by highly selective NRF2 potent activation), ARS deserves future cohort studies on acute/chronic diseases that imply high levels of tissular oxidative stress, especially some acute/chronic cardiovascular and respiratory diseases like acute myocardial infarction with acute/chronic heart failure, stroke, Chronic Obstructive Pulmonary Disease (COPD), asthma etc. of both children and adults (so that ARS may help millions and even billions worldwide).

Keywords: ASEA redox supplement (ARS) oral solution, 3-year-old boy, Duchenne muscular dystrophy (DMD), NRF2 selective activation, corticosteroids

Main abbreviations: DMD: Duchenne Muscular Dystrophy; ARS: ASEA redox Supplement®; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; NRF2: Nuclear factor erythroid 2-related factor 2; COPD: Chronic Obstructive Pulmonary Disease

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Case Report

Andrei-Lucian Drăgoi*


Independent MD pediatrician specialist, Romania

*Address for Correspondence

-Lucian Drăgoi, Independent MD pediatrician specialist (from Bucharest), Romania

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Main Text

An introduction to DMD

Duchenne Muscular Dystrophy (DMD) [1,2] is the most common type of muscular dystrophy and has an incidence of ~1/3600 born male infants. DMD is a severe X-linked recessive muscular dystrophy caused by a mutation (inherited from a person's parent in ~2/3 of DMD cases and non-inherited de novo mutation in ~1/3 of DMD cases) in the gene encoding dystrophin (dys) (located on the short [p] arm of X chromosome, at locus 21 [Xp21]), which dys is a cytoplasmic protein and an essential component of a protein complex (with many subunits) that connects the myocyte cytoskeleton to the surrounding basal lamina of the extracellular matrix through the muscular cell membrane (sarcolemma). Normal skeletal myocytes contain small amounts of dys but its total/partial absence or abnormal length leads to excess calcium cations penetrating the sarcolemma and causing excess water to enter into all mitochondria which then burst, causing intracellular oxidative stress, sarcolemma permanent damage and myocytes/cardiomyocytes necrosis. Progressive rhabdomyolysis causes muscular fibers to be progressively replaced by adipose and connective tissue (pseudohypertrophic muscular dystrophy or muscular pseudohypertrophy). Muscle weakness associated with progressive muscle atrophy (with secondary fatigability, frequent falls and progressive difficulty in walking and getting up from lying or sitting position) usually begins around the age of 4 years and worsens rapidly in boys with DMD, so that most of them become unable to walk by the age of 12 years. In advanced stages, DMD patients may have respiratory disorders (due to respiratory muscles damage), swallowing difficulties (with high risk of aspiration pneumonia) etc. Due to rhabdomyolysis (including myocardium cytolysis), DMD patients have extremely high Creatine Kinase (CK) and possibly (very) high CK-MB isomer (CK-MB) serum levels: the serum levels of Aspartate Transaminase (AST) and Alanine Transaminase (ALT) are also very increased. Consequently,

Myoglobin (MG) (produced by rhabdomyolysis) also attains high concentrations in serum and urine. *Electromyography* (EMG) distinguishes the weakness caused by destruction of muscle tissue. *Echocardiography* may show *dilatative cardiomyopathy* secondary to *myocardial fibrosis* (which can occasionally lead to *congestive heart failure* and/or *cardiac arrhythmias*). *DNA testing* demonstrating mutation(s) in one or more of the 79 exons of *dys-gene* can often make the diagnosis at birth or confirm the diagnosis in most suspected cases. DMD doesn't have a curative treatment, but only a pathophysiological and symptomatic treatment which may delay the onset of symptoms and increase the quality of life. *Several types of medications* were proved to be relatively useful in the treatment of DMD and its complications, such as: *steroids* such as *prednisolone* and *deflazacort* (which were demonstrated to slow muscle degeneration and to produce short-term improvements in muscle strength and function up to 2 years, including walking period prolongation according to some reports); β 2-agonists such as *salbutamol* (which were demonstrated to increase muscle strength, but don't modify disease progression), *anticonvulsants* (for possible seizures control), *ataluren* (which is indicated for DMD patients that can walk and are more than 5 years old; ataluren probably makes ribosomes less sensitive to premature stop codons, especially for the 'UGA' stop codon, by promoting insertion of certain near-cognate transfer RNA at the site of nonsense codons, with no apparent effects on downstream mRNA transcription, processing, stability nor on the resultant protein), *sildenafil* (which was also demonstrated to improve the muscular blood flow in DMD boys) etc. There are also several new *genetic treatment* approaches to DMD patients. The *exon-skipping gene therapy* (ESGT) with *antisense oligonucleotides* (*oligos/AONs* like *eteplirsen* or *drisapersen*) triggers skipping of an exon (adjacent to the exon affected by mutation) so that to restore the reading frame and production of a (still-truncated but) more functional version of *dys*. For ESGT to be efficient on medium and long term, AONs must be periodically redelivered into muscles. *Stem cell replacement therapy* (SCRT) was also proposed. SCRT is a therapy using *pericytes* (a type of multipotent stem cells which have the ability to be delivered systemically and uptaken by crossing the vascular barrier, then to fuse and form myotubes): pericytes are injected arterially, crossing through arterial walls into muscles, where they can differentiate into potentially functional myocytes. *CRISPR/Cas9-mediated genome editing* (not currently feasible in humans, but potentially feasible in the future) is the most ambitious hope in the treatment of DMD: this is a technique which can precisely remove a targeted mutation of *dys-gene*, by allowing the DNA repair mechanisms of myocytes to replace that mutant *dys-gene* with a normal *dys-gene*. Despite all these efforts in finding new and more efficient treatments, the average life expectancy of DMD patients is ~ 26 years (with a maximum between 30-50 years in rare cases who benefit from excellent care). Most DMD patients become *wheelchair-dependent* early in life and the gradual development of *cardiac hypertrophy* and/or *restrictive respiratory insufficiency* typically results in *premature death* between ages of 20-30 years. Important note. Among the differential diagnosis of DMD are other genetic/non-genetic muscular dystrophies

(MDs), from which the more rare (1.5-6/100 000 male births) (X-linked recessive) *Becker muscular dystrophy* (BMD) is similar to DMD (regarding etiology and pathogenesis which include less severe mutations of the same *dys-gene* [thus making BMD a dystrophinopathy too]) but with less affected ("milder") phenotypes.

An introduction to ARS, NF-kB and NRF2

ARS is produced by an international direct selling and multi-level marketing company called "ASEA, LLC" ^[1] founded in 2007 and headquartered in Salt Lake City, Utah, USA. The present ARS is based on a technology initially created, developed and patented ^[2] by a former company called "Medical Discoveries Inc." and was previously called "MDI-P". ARS is a clear, colorless liquid generated by electrolysis of a highly purified sterile saline. ARS is distributed in ~1 liter plastic bottles/vials. ARS has a saline concentration of ~0.27g NaCl /100ml (0.27%) and additionally contains (in a total concentration of ~1%) highly reactive (but stabilized) chlorine and oxygen species (see ARS patent previously referenced as a footnote):

The Oxidant (OX) Species from ARS mainly include hydrogen peroxide (H_2O_2), superoxide anions species (O_2^- and HO_2^-), hypochlorous acid (HOCl), hydronium cation (H_3O^+), hypochlorite radical (OCl^*), singlet oxygen $^1[O_2]$, (partially soluble) oxygen biatomic molecules (in triplet ground state) and oxygen triatomic molecules (O_3) (ozone);

The Reductive (RED) Species from ARS mainly include: hypochlorite anions (ClO^-) (also paired as sodium hypochlorite Na^+ClO^- [NaOCl]), chlorine anions (Cl^-), chlorine biatomic molecules (Cl_2), (partially soluble) hydrogen biatomic molecules (H_2) and hydrogen anions (H^-). In contrast with ARS, MDI-P had a relatively high concentration (~25-50%) of those reactive (mainly OX) species and was initially tested for its microbicidal properties: it was found to be a very fast-acting, broad-spectrum microbicidal solution effective against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Legionella pneumophila* and *Candida albicans* [3]. ARS was launched by ASEA LLC in 2009 as dietary supplement certified for oral consuming safety by the Department of Agriculture and Food, State of Utah, USA [3]. ARS is currently produced in a production facility which is FDA registered, NSF certified and GMP compliant. In 2015, ASEA partnered with BioAgilytx Labs ^[4] (specialized in biomarker testing) which periodically validates (as also monitored by FDA) the existence of reactive oxygen species in the ARS solution ^[5]. After ARS being officially registered in the European Union (EU) (with number "NUT 1936" ^[6]), the first European country in which ARS was also registered and launched as a dietary supplement was Italy (a founding member of EU), under the name "ASEA advancing life" (with index number 54229 in the official list of dietary supplements approved in Italy ^[7]).

A secondary introduction to the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) NF-kB is a ubiquitous DNA transcription factor that "governs" the Phase I cellular stress response, implying cytokine production and cell

survival when exposed to free radicals, heavy metals, ultraviolet irradiation, oxidized LDL, bacterial or viral antigens etc. NF- κ B essentially regulates the immune response to infection: incorrect NF- κ B regulation has been linked to cancer, inflammatory and autoimmune diseases, septic shock, viral infection and improper immune development.

A secondary introduction to the (erythroid-derived 2)-like 2 nuclear factor (NRF2) NRF2 is a ubiquitous DNA transcription factor (usually activated after NF- κ B activation) which “governs” the Phase II cellular stress response, so that NRF2 activation can induce an over-expression of more than 150 genes (which are all involved in the Phase II cellular stress response), many of these genes encoding antioxidant proteins/enzymes (like superoxide dismutase [SOD], glutathione peroxidases [GPx], catalases etc) and tissular lipases (which supply higher amounts of fatty acids as “fuels” for cell repairing) that protect cells against oxidative damage triggered by injury and inflammation.

The demonstrated biological activity of ARS ARS was tested *in vitro* and *in vivo* on animals and humans too. *In vitro* studies on various human cells showed that ARS is a very potent (but transient) selective NRF2 activator (by increasing 5 to 8 times the cytoplasmatic concentrations of SOD and GPx, even at low ARS *in vitro* concentrations), avoiding the activation of NF- κ B (thus having interesting favorable immunomodulating properties) [4]: the studies conducted *in vivo* (on both animals

and humans) also support this main pharmacological mechanism of ARS, with no toxicity (as previously presented) up to high doses (calculated per kilogram of body mass) and a satisfactory bioavailability (which assures the strong effect demonstrated *in vivo*); all studies on ARS until present are briefly presented in one official brochure published by ASEA LLC on its official website [8]. ARS oral consumption was already demonstrated *in vivo* to over-express by 20-30% five protein-coding genes (Potassium Channel Tetramerization Domain Containing 12 gene [KCTD12], Early Growth Response 1 gene [EGR1], Pyridine Nucleotide-Disulphide Oxidoreductase Domain 1 [PYROXD1], Interleukin 1 Receptor Associated Kinase 3 [IRAK3], C-C Motif Chemokine Receptor 10 [CCR10]), all implicated in various genetic signaling pathways, including regulatory network pathways (the inflammation pathway reduction [the NRF2 pathway], the innate immune system function pathway, vascular integrity signaling pathways, digestive enzymes signaling pathways and hormone modulation pathways) [9]. As NRF2 is ubiquitously expressed with the highest concentrations in the cytoplasm of cells from the vital organs (in descending order of NRF2 cytoplasmic concentrations: kidneys, muscles, lungs, heart, liver and brain), it is expected that ARS to mainly protect human vital organs (with the additional argument that these vital organs have the largest blood supply, which is directly proportional with the concentration that ARS may reach in these vital organs). ARS was also shown to induce apoptosis in cultures of dysfunctional, stressed or damaged cells [4]. Additionally, ARS activates human tissular lipases (most

¹For more information and verifications, the page dedicated to the (redox) science behind ARS can be accessed at this URL: aseaglobal.com/science/ (see all the sections from the “Science” menu tab)

²One of the main ASEA patents (patent no. 8367120) can be accessed at this URL: www.patentgenius.com/patent/8367120.html

³The ARS certificate of safety for oral consuming can be accessed at this URL (saved by A. L. Drăgoi in 2016 in his personal database and stored on his personal domain dragooi.com, as this certificate isn't publicly accessible in the present on ASEA LLC company official site): asea.dragooi.com/ASEA_certificate_of_safety_for_human_consumption.jpg. The other documentation demonstrating ARS oral consumption safety can be also accessed on ASEA LLC official website or on www.dragooi.com at URLs:

(1) asea.dragooi.com/Asea_Safety_Studies_from_2002_to_2006_Collection_brochure.pdf (a list of endotoxicity and cytotoxicity studies abstracts, a list which is not accessible from ASEA LLC official website in the present); (2) mediafilelibrary.myasealive.com/src/media/xmlfile/ASEA%20REDOX%20Safety%20&%20Classification%20Summary.pdf (alternative source URL: asea.dragooi.com/Asea_Safety_Classification_Summary_brochure.pdf)

⁴For more information and verifications, the official website of BioAgilytix Labs can be accessed at this URL: www.bioagilytix.com

⁵ARS redox certification by BioAgilytix Labs has a dedicated page on ASEA LLC official website that can be accessed at this URL: aseaglobal.com/science/bioagilytix-redoxcertified/

⁶For more information and verifications, the official list of all the dietary supplements approved in EU can be accessed at this URL (for ARS entry, see page 842 of the linked pdf list): asea.dragooi.com/ASEA_Registration_In_UE_NUT1936_see_pag_842.pdf

⁷For more information and verifications, the official list of all the dietary supplements approved in Italy can be accessed at this URL (for ARS entry, see page 149 of the linked pdf list): asea.dragooi.com/ASEA_Registration_In_Italy_No54229_see_pag_149_.pdf

probably via NRF2 pathway) and significantly increases the fatty acids serum levels that are further internalized by skeletal muscles and myocardium and used as “fuels” by myocytes and cardiomyocytes, partially sparing the glycogen reserves of myocytes/cardiomyocytes and so raising the resistance of skeletal muscles to effort and possibly the resistance and contractility myocardium: see prof. D.C. Nieman’s first metabolomics study on ARS ^[10]. Based on this first metabolomics study on ARS (and its encouraging results) conducted by prof. D.C. Nieman, the University of North Carolina (Chapel Hill) also started a second trial on ARS called “*Effect of ASEA on Energy Expenditure and Fat Oxidation in Humans*” ^[11] with results summarized in ARS “all main studies” brochure at page 2, in a rubric entitled “*Influence of ASEA redox supplement ingestion on oxidative stress*” ^[12]. Given its antidoping certification ^[13], ARS is also widely used in the present by various athletes around the world ^[14]. ARS can be thus considered a potentially valuable nutrigenomic treatment resource which deserves extended studies on progressively larger cohorts. There are many known natural molecules (especially flavonoids) and plant extracts that were demonstrated to be natural NRF2 activators in vitro and/or in vivo: sulforaphane, resveratrol, quercetin, curcumin, Ginkgo biloba plant extract, ginseng plant extract, catechins etc. There are also some synthetic NRF2 activators like: dimethyl fumarate, monomethyl fumarate, metformin etc. However, all these molecules have demonstrated tissular toxicity (especially liver toxicity) at high doses, in high contrast with ARS which was demonstrated to have an excellent safety profile (as previously presented in this paper). Caloric restriction (which was demonstrated to prolong life span in humans and animals) was also demonstrated to also imply a significant NRF2 activation effect (due to induced oxidative stress in caloric restricted cells): physical exercise has a similar activation effect on NRF2 by (“naturally”) producing a large palette of reactive oxygen species (ROS) which ROS (in some specific

amounts) were proved to be essential to muscle fibers survival and development, by activating the endogenous antioxidant enzymes via NRF2 pathway (inversely explaining the high health risks of sedentary).

The main scope of this case report study is to emphasize the multiple advantages that ARS has over corticosteroids and the potential of ARS (if extensively studied in the future) to fully replace corticosteroids in children with DMD, given the remarkable (and very promising) biological effects of ARS in this Romanian boy with DMD and also given the absence of ARS adverse reactions until present.

Other authors have also focused on the importance of NRF2 pathway activation in the treatment of DMD (5, 6, 7, 8, 9).

The Detailed Description of this DMD Case Report

The first consult of the DMD boy in my pediatric office (from January 11th 2018, at 2 years and 8 months of age). The DMD diagnosed boy first came to my pediatric office for consult on January 11th 2018 when he was aged approximately 2 years and 8 months. Anamnesis (including familial history). When he was ~1-year-old (in the summer of year 2016), the boy had a high fever episode and he was suspected for high risk bacterial infection, so that he was hospitalized in “Victor Gomoiu” Children Hospital from Bucharest where he received a short cure (7 days) with antibiotics for urinary tract infection (UTI) with urine culture positive for *Escherichia coli* (with a slight enlargement of the left kidney, but no other abnormalities on the abdominal ultrasound): with that hospitalization, the boy was discovered to have high serum levels of AST,

⁸This brochure can be accessed using these URLs:

- (1) mediafilelibrary.myasealive.com/src/media/xmfl/file/ASEA%20REDOX%20Scientific%20Validation%20Summary.pdf
- (2) asea.dragoii.com/ASEA_All_studies_Until_Present_Summary_Brochure.pdf

⁹The main results of this genetic study on ARS are briefly presented in a brochure that can be accessed at these URLs:

- (1) aseascience.com/asea-science/initial-gene-study-showed-asea-redox-affected-important-signaling-pathway-genes/
- (2) mediafilelibrary.myasealive.com/src/media/xmfl/file/ASEA%20REDOX%20Gene%20Study%20Summary.pdf
- (3) asea.dragoii.com/ASEA_First_Gene_Study_Summary_brochure.pdf

¹⁰The results of this study are contained in a pdf brochure (Microsoft PowerPoint Presentation) can be freely accessed using this URL (recovered and available on dragoii.com domain): asea.dragoii.com/ASEA_Prof_Nieman_Metabolomics_study_results.pdf

¹¹The details of this trial study can be accessed at this URL: clinicaltrials.gov/ct2/show/record/NCT01884727

¹²This ARS “all main studies” pdf brochure can be accessed at these URLs:

- (1) mediafilelibrary.myasealive.com/src/media/xmfl/file/ASEA%20REDOX%20Scientific%20Validation%20Summary.pdf (official URL)

the boy was redirected to the children neurology ward of “Alexandru Obregia” Hospital from Bucharest, for further investigations and diagnosis: the DNA testing (ready on September 12th 2016) of both the child and the mother showed the same mutation in the 52nd exon of dys-gene (a duplication of its 7547th nucleotide) which very probable implies a premature interruption of dys-gene reading from exon 53 to its last exon 79. Based on this genetic result, the boy was then diagnosed by the neurologist with “oligosymptomatic progressive muscular dystrophy” and was recommended physical therapy and periodic control (at every 6 months) in the children neurology ward (but with no reassessment of the muscle damage biological markers at that time). The mother also gave information about her brother (the maternal uncle of her boy) who “couldn’t walk and was immobilized to bed from ~7 years of age until his death at ~18 years of age”, which is very suggestive for a DMD phenotype, thus for positive history of familial DMD (but the boy’s mother couldn’t show any medical documents of her brother and his diagnosis). As expected, the dys-mutation-carrier mother has no biological or clinical signs of muscle damage (including no biological markers of heart muscle damage and normal electrocardiography (ECG)). The parents were told by the neurologist to wait until the boy will be 4-year-old, for him to start a cure with prednisone P.O, to delay the progress of the disease: this (quite long) prednisone P.O, temporization (decided by the neurologist) and the symptoms/signs of the boy at that time (impaired extension of the right limb when walking and running plus calves enlargement) worried parents and these were the main reason which determined the parents to ask a second medical opinion from me, as an MD pediatrician specialist.

Physical examination. The main clinical signs found were: impaired extension of the right limb (when walking), calves pseudo hypertrophy (with 23/23 cm maximum circumference of both calves), slight tonus deficit of the axial/spinal muscles, extreme anxiety at the physical exam, hyperkinetic child, moderate language delay (he only used ~20 correctly pronounced words at that age and only used pairs of words, but rarely sentences with verbs). The rest of the physical (including neurological) examination results were normal: cranial nerves tests in normal limits, normal breath rate and normal pulmonary sounds, normal heart rate (with no heart murmurs), normal abdomen (without clinically detectable hepato/splenomegaly), but with increased consistency stools

(with defective discomfort), normal diuresis and urination (with no kidney pain/sensibility), normal genital apparatus. Body mass: 14 kg (Age: 2y8m) (in the normal range for sex and age). Body height: 91 cm (Age: 2y8m) (in the normal range for sex and age). Based on diagnosis, anamnesis and physical examination (previously presented), I have requested some basic imaging and laboratory exams (see next).

Medical imaging exams of the DMD boy (in chronological order): see next. Heart ultrasound (January 28th 2018): echographically normal (with the reserve that the child was very anxious and hyperkinetic during this examination). Abdominal ultrasound (*April 10th 2018; *parents delayed this exam because of objective reasons): minimal hepatomegaly; all the other examined organs were

echographically normal (with the reserve that the child was very anxious and hyperkinetic during this examination).

Laboratory exams (rhabdomyolysis and inflammatory markers serum levels) of the DMD boy (in chronological order: blood probes taken on January 16th 2018 and January 22nd 2018): see next. Gamma-glutamyl-transferase (GGT) (GGT was periodically used as a liver toxicity marker): 10 U/ml (within the normal range [wnr]); AST serum level: 473 U/L (~10 times the normal superior limit [nsl] [~10 x nsl]; of muscle origin, given the GGT wnr); ALT serum level: 558 U/L (~17 x nsl; of muscle origin, given GGT wnr); CK: 34 453 U/L (~200 x nsl; of muscle origin, given the GGT wnr); CK-MB: 1241 U/L (~52 x nsl; myocardial origin). MG (22.01.2018): 2006 ng/mL (~28 x nsl; of muscle [including myocardial] origin) (the MG serum level was chosen, because the child didn’t want to cooperate for determining the urinary concentration of MG by urine sampling). C-reactive protein (CRP) serum level (22.01.2018): 0.61 mg/L (wnr). Erythrocyte sedimentation rate (ESR) (22.01.2018): 9 mm/h (wnr).

Treatment (from ~ January 22nd 2018) Given all the DMD patient information previously given (plus the argumentative notes on giving ARS orally to a child under 12 years of age), I have decided to give the following recommendations (including medical treatment): ARS solution, P.O. 30+30+0 ml/day (~4 ml/body_kg/day) (started from ~22.01.2018); L- carnitine oral solution (in concentration 1g/10 ml, 10 ml vials), P.O. 0+½+0 vials/day (500 mg/day ~ 36 mg/body_kg/day) (after lunch, with fruit juice) (also started from ~22.01.2018).

(2) asea.dragoi.com/ASEA_All_studies_Until_Present_Summary_Brochure.pdf (alternative URL)

¹³ The antidoping certificate of ARS can be accessed at this URL (as recovered on author’s dragoi.com personal site): asea.dragoi.com/ASEA_antidoping_certificate.pdf

¹⁴ The site of ASEA LLC dedicated to the most known athletes around the world consuming ARS can be accessed at this URL: aseaathletes.co

Important argumentative note on L-carnitine prescription L-carnitine acts as a transporter of long-chain fatty acids into the mitochondria (where to be oxidized for energy production): given the anticipated high serum levels of fatty acids produced by ARS (as previously mentioned by citing the metabolomics study conducted by prof. D. C. Nieman on ARS), I have used L-carnitine as an adjuvant for ARS. I have also included omega-3 fatty acids plus multivitamins oral supplement as syrup (conc. ~8 mg DHA/ml, 1.8 mg EPA/ml; also containing vitamins A, D, C, E, B1 (thiamine), B2 (riboflavin), B6, B12 (cobalamin), niacin [vitamin B3], pantothenic acid [vitamin B5], biotin [vitamin B7]) (started from 22.01.2018) 1.5+1.5+1.5 ml/day (4.5 ml/day in total).

Important argumentative note on omega-3 plus vitamins prescription. I have chosen this DHA-EPA-multivitamin mix especially for the boy's language delay: I have chosen however a very small dose (4.5ml/day) given the age and the possibility that the multivitamin (antioxidant) mix may inhibit ARS effect (by partially/totally neutralizing the free radicals from the ARS solution already absorbed in the blood). I have also (re)prescribed physical therapy (reinforcing the same recommendation given by the neurologist, as previously mentioned): however, the parents weren't compliant to this recommendation, with their argument that the boy "is already very active and full of energy". I have also prescribed a psychological consult: the parents weren't compliant to this recommendation either, because of some prejudices on this kind of consult, as they consider their boy psychologically "normal".

Important note (interpretation) Given the small age of the boy, the small number of signs/symptoms up to present, the fact that there is no "cut-off" exon number for a dys-gene mutation to exactly predict when an affected boy will develop a DMD phenotype and when he will develop a milder BMD phenotype, the diagnosis of DMD isn't 100% sure yet: however, the severe form of MD of his maternal uncle (with loss of walking from 7 years of age until his death at 18 years of age) indicates this boy's duplication of the 7547th nucleotide (from 52nd exon of dys-gene, which is probably shared with his maternal uncle and surely inherited from his maternal grandmother [also mother of his maternal uncle]) will more probably generate a DMD phenotype: additionally, there is a study in which a targeted disruption of exon-52 in the mouse dys-gene had induced muscle degeneration similar to that observed in DMD [10]. The extremely (initial) high CK and CK- MB serum levels also indicate/suggest a DMD phenotype found in a clinically oligosymptomatic initial stage. That is why we have decided to start an aggressive therapy with ARS at least one year before the age of 4 years (when he was temporized to begin a corticosteroid treatment or other treatment like ataluren or even experimental treatments with AONs or stem cell [pericytes] replacement therapy).

All the pediatric consults (including this previously described initial pediatric consult given to this boy with DMD), imaging and labs were condensed in the next

(Table 1: <https://biomedress.com/pdf/CJBRT-19-04-018-01.pdf>); the significant decrease of all the rhabdomyolysis markers (in the period in which this boy with DMD received ARS P.O.) was also quantized in the next figures.

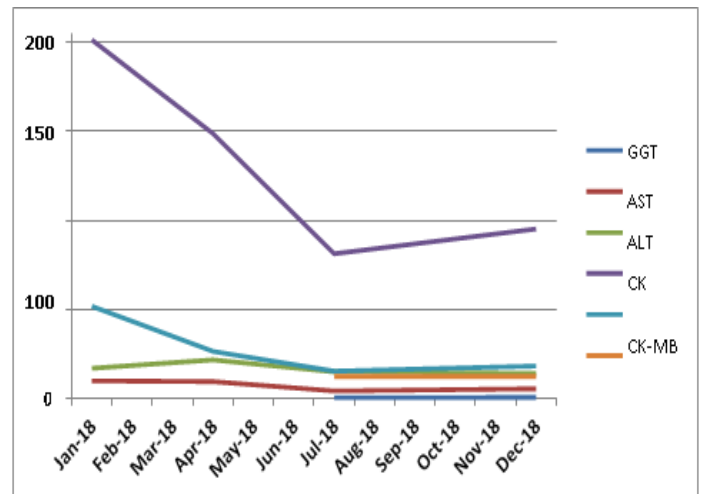


Figure 1a: The significant global decrease of all the rhabdomyolysis markers serum levels (expressed adimensionally, as multiples of their normal superior limits), in the period in which this boy with DMD received ARS P.O

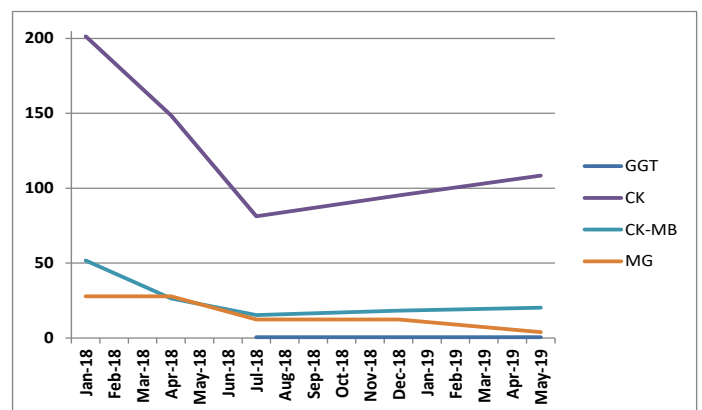


Figure 1b: The significant global decrease of some rhabdomyolysis markers (CK, CK-MB and MG) serum levels plus GGT serum level showing no liver toxicity (expressed adimensionally, as multiples of their normal superior limits), in the period in which this boy with DMD received ARS P.O.

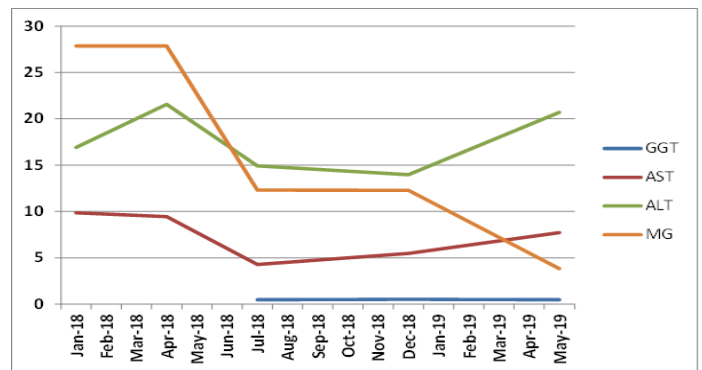


Figure 1c: The significant global decrease of some rhabdomyolysis markers (AST, ALT and MG) serum levels plus GGT serum level showing no liver toxicity (expressed adimensionally, as multiples of their normal superior limits), in the period in which this boy with DMD received ARS P.O.

Results and Interpretations

- a. At my request, the parents send some movies (filmed with a phone in mp4 format) with their boy climbing stairs, walking and running; short selected sequences from these movies (so that the face of the child to not be visible frontally) were uploaded on author's personal site (www.dragoi.com) and are available at the following URLs:
- i. www.dragoi.com/ARSinDMD_HighStairStepsClimb_6sec.mp4 (the boy climbing stairs with high steps – 6 seconds movie selection)
 - ii. www.dragoi.com/ARSinDMD_LowStairStepsClimb_9sec.mp4 (the boy climbing stairs with low steps – 9 seconds movie selection)
 - iii. www.dragoi.com/ARSinDMD_HorizontalWalk_4sec.mp4 (the boy walking on a horizontal plane - 4 seconds movie selection)
 - iv. www.dragoi.com/ARSinDMD_HorizontalRunning_4sec.mp4 (the boy running on a horizontal plane - 4 seconds movie selection)
- b. The ARS-based treatment in the last ~1.5 years, from 22.01.2018 up to 29.05.2019 (with ARS P.O. 60 ml/day [~4 ml/body_kg/day] in the first ~3 months, then 90 ml/day [~6-6.5 ml/body_kg/day] from 18.04.2018 until 17.12.2018, then 100 ml/day [~7 ml/body_kg/day] from 18.12.2018 until 29.05.2019) was associated with a slight ALT serum level total increase of ~22% (despite a significant decrease of ALT serum levels from ~17 nsl on January 2018 to 14 nsl on December 2018), an AST serum level total decrease of ~22% (with normal GGT serum levels in all this ~1.5 years interval, thus no detectable liver toxicity of ARS), a marked CK serum level total decrease of ~46%, a very significant CK-MB serum level decrease of ~61% and a quite spectacular MG serum level total decrease of ~86% (with CK, CK-MB and MG being actually the main target of my ARS recommendation and which may be explained by the fact that ARS has stronger NRF2 activation effect on the myocardium, where the expression of NRF2 is larger than in skeletal muscles, an additional indirect subtle potential “proof” that ARS acts via NRF2 pathway). This results suggest that ARS may have very potent muscular (including myocardial) protective effects, significantly limiting the muscular damage in DMD patients, with the potential of even stronger effects in (milder) BMD phenotypes: this comes in the “same pack” with no liver toxicity, no adverse effect on growth and development of the child and no other adverse effects in other clinical spheres until the present. The slight increase of AST, ALT, CK and CK-MB in the last year (from July 2018 until present, as seen in the previous graphics, but only a small

- increase when compared to the significant drop of these rhabdomyolysis markers in the first ~6 months of ARS) has many possible explanations: (i) it is plausible that ARS dosage of 4-7 ml/body-kg/day to have progressively generated a phenomenon of ARS-resistance in myocytes (which is possible, as in the case of many other pharmacological agents): however, the spectacular drop of MG serum levels (which continued to decrease despite the slight increase of all the other markers in the last ~1 year) appears to contradict this hypothesis (because MG is the most specific muscular marker, with the highest eloquence in this set of markers); (ii) At doses of 4-7 ml/body-kg/day, it's also possible that ARS may sustain the myocytes (including cardiomyocytes) and keep them functional, but at their “limits” of survival, so that myocytes may continue to lose CK, CK-MB, AST and ALT through their cellular membranes, but without apoptosis and thus keeping MG in the intracellular medium; (iii) There's also the possibility that ARS may accelerate the clearance of red blood cells (RBCs) (and their rate of renewal implicitly) so that AST and ALT may (at least) partially be produced by this higher rate of RBCs replacement; (iv) it is also possible that ARS to have produced a greater number of young myocytes (including cardiomyocytes) (by some ARS-specific mechanisms, that are detailed in the next paragraphs), which myocytes may still lose CK, CK-MB, AST and ALT (through their cellular membranes) but maintain their viability so that not to lose MG too; (iv) the muscular mass of this DMD boy has surely increased in the last 1.5 years and the present ARS doses may not be adequate anymore (for this increased muscular mass) so that larger doses up to 10 ml/body-kg/day (and even higher) may be needed in the future (doses which may however exceed the financial possibilities of the family and this may also be a problem in the future, especially if ataluren won't show any clinical and paraclinical benefits); (v) the slight increase of CK, CK-MB, AST and ALT in the last ~1 year also superposed to the temporary removal of L-carnitine and omega-3 plus multivitamin supplements (because we had the suspicion that A, D and E vitamins may have generated that hyperechogenicity of the liver and its moderately increased dimensions as shown by the abdominal ultrasound from December 2018): this may indicate that omega-3, multivitamins and L-carnitine may yet have a synergic effect with ARS and may help ARS in decreasing the global rhabdomyolysis rate of this boy affected by DMD; however, the normalization of the abdominal ultrasound from June 2019 indicates that L-carnitine and/or omega-3 plus multivitamins may have exposed the liver to a (obviously reversible) pharmacological stress, which suggests that L-carnitine and omega-3 plus multivitamins may be reintroduced (given their possible beneficial synergic effects with ARS), but in shorter cures of 1-2 months (given their possible hepatotoxicity when given in cures of 3 to 6 months);
- c. The significant decrease of both CK and CK-MB serum levels may be explained by a (significant) decrease of the

oxidative stress in DMD myocytes, a decrease produced by ARS via NRF2 pathway (and more pronounced in the myocardium). Similarly to steroids, it is not excluded that ARS may also induce additional over expression of UTRN gene and utrophin synthesis subsequently [11]. ARS may act on UTRN gene via NRF2 pathway or, more probably (and also by NRF2-pathway), by upregulating sarcospan (SSPN) (a 25-kDa transmembrane protein located in the dystrophin-associated protein complex of skeletal muscle cells; SSPN depends on dys for its proper transmembrane localization). SSPN up regulates the levels of Utrophin-glycoprotein complex (UGC) to compensate dys loss in the neuromuscular junction of DMD patients [12,13]: utrophin expression is extremely increased in DMD patients (and also female carriers) as a compensatory mechanism, both in dys- lacking muscle fibers and in rare (revertant) fibers that express dys, as a small proportion of muscle fibers of DMD patients continue to show strong dys staining (and these "revertant fibers" are thought to arise by a mechanism that restores the reading frame [14,15]. Normally, SSPN upregulates both UGC and Dystrophin-associated Glycoprotein Complex (DGC), which DGC has the very important role to form a critical link between the (intracellular) cytoskeleton and the extracellular matrix. SSPN regulates the amount of utrophin produced by the UGC to restore laminin binding due to dys absence or inefficiency (of too-short dys isoforms from DMD phenotypes): if laminin binding is not restored by SSPN, the cell membrane diminishes its surface and its adherence to the extracellular matrix. In dystrophic mdx mice (mice with various artificially induced point mutations in their dys-gene [producing early artificial STOP codons in the dys-gene, which will produce various small non-functional dys variants], used as an experimental model to study DMD), SSPN increases levels of utrophin (by inducing UTRN gene over expression) and restores the levels of laminin binding, reducing the symptoms of DMD. SSPN is also an essential regulator of Akt/PKB signaling pathway (a signal transduction pathway with protein kinase B [Akt] and phosphatidylinositol 3-kinase [PI3K] as key-components, a pathway that promotes survival and growth in response to extracellular signals): this signaling pathway will be hindered and muscle regeneration will not occur in the absence of SSPN.

- d. There is also a small probability for ARS to modify the sensibility of ribosomal protein synthesis (translation) to stop codons, so that ARS may also induce other DMD isoforms (based on the skipping of reading stop codons) like ataluren is hypothesized to act.
- e. It is not excluded that ARS may also (directly or indirectly) partially restore the reading frame of dys- gene and so to increase the percent of "revertant fibers" in DMD patients: only an immunohistological study on a DMD child (or adult) treated with ARS could confirm or infirm such a hypothesis.

Discussions

- a. This specific DMD case (treated with ARS P.O.) may

inspire new possible future studies based on ARS. Given its clinical and biological effects in this DMD child case and its "prototype" selective NRF2 activator features, ARS and all the other known NRF2 activators may be tested in DMD and BMD patients (in future blinded [b] randomized controlled trials [bRCTs]). RCTs on NRF2 activators versus steroids in DMD/BMD cases (started before OR after 4 years of age) may also be conducted. DMD patients treated with ARS can be verified for UTRN gene over expression and utrophin high cellular levels. MD patients treated with ARS can also be verified for intracellular existence of DMD isoforms other the DMD pathological isoform expected in the specific exon-52 mutation (or other dys-exons mutations) of each DMD patient in part. Immunohistological studies can also demonstrate various muscular effects of ARS, to check if ARS actually increases the percent of "revertant fibers" in DMD patients.

- b. Only 4-5% of DMD patients have exon-52 dys mutations [16], so that ARS may have a great potential to be tested in all types of mutations on all dys-gene exons, including in the patients with milder BMD phenotype. ARS may be an important potential tool to test for and combat cellular oxidative stress in vital organs (where NRF2 reaches its highest concentrations) and muscles (including the heart muscle, of course) in a large spectrum of acute and chronic adult and child diseases: myocardial infarction, stroke, chronic liver disease, chronic kidney disease, cancers (including protective effects for healthy cells in various [highly toxic] chemotherapy regimens) etc.
- c. Given the estimated DMD prevalence worldwide (4.78/100 000 males), the estimated BMD prevalence worldwide (1.53/100 000 males) [17] and the present human population on Earth ($\sim 7.7 \cdot 10^9$ persons, from which $\sim 50\% \sim 3.85 \cdot 10^9$ males, according to April 2019 real-time statistics¹⁵), we predict $\sim 242\ 935$ cases worldwide of DMD and BMD together. Extrapolating the estimated average frequency of DMD (1/3600 male births) and the estimated minimum frequency of BMD (1.5/100 000 male births) to the global human birth rate on Earth of 386 000 births/day (from which $\sim 50\%$ are male births, according to the last estimation from 2015¹⁶), we estimate that ~ 57 new cases (of DMD and BMD together) appear every day on Earth (corresponding to ~ 20625 new "DMD+BMD" cases per year). ARS may thus have significant potential in helping in all these old and new cases. Given its extrapolated strong antioxidant effect in other child/adult high-oxidative stress acute and chronic diseases (as previously discussed), ARS may help millions and even billions worldwide.
- d. Corticosteroids were proven to ameliorate both the motor and intellectual functions in children with DMD, but with modest decrease of the rhabdomyolysis markers (and even increasing the CK serum level in some studies, without a clear explanation yet) and with a very large palette of side effects on children with DMD (treated with corticosteroids) [18-20]: in contrast, we have

demonstrated (at least in this presented case) that ARS P.O. at doses 4-6.5 ml/body kg/day has the same ameliorative effects (as corticoids have) plus significant reductions of rhabdomyolysis markers plus no demonstrated side effect after ~1 year of treatment with ARS.

- e. This boy has the right to receive ataluren starting from the age of 5 (because the Romanian law doesn't allow yet the initiation of ataluren earlier than this age, but supports the expenses in DMD boys with non-sense mutations after this age): *we predict that ARS may act synergically with ataluren either by producing even more revertant muscular fibers or by significantly diminishing the oxidative stress in myocytes* (by producing longer/heavier dys isoforms together with protecting myocytes from oxidative stress and intracellular irreversible damage implicitly). ARS generally increases the capacity of animal cells to defend from various toxic agents (including pharmacological agents), that is why *ARS has also the potential to decrease the rate of adverse effects of ataluren (especially nausea and vomiting)*.

Conclusions

- a. ARS has remarkable antioxidant and immunomodulatory effects and should be studied on larger groups of children with DMD under the age of 4 years old (but also on other age groups of children and even young adults), as an alternative to early corticosteroids.
- b. Given its immunomodulatory effect (NRF2 selective activation and NF-kB inhibition), ARS deserves future cohort studies on its potential to replace corticosteroids and other non-steroidal immunosuppressants (at least partially) in many types of pulmonary/renal/hepatic/ articular/skin autoimmune autoimmune and even malignant diseases of both children and adults;
- c. Given its very strong antioxidant effects (by highly selective NRF2 potent activation), ARS deserves future cohort studies on acute/chronic diseases that imply high levels of tissular oxidative stress, especially some acute/chronic cardiovascular and respiratory diseases like acute myocardial infarction with acute/chronic heart failure, stroke, Chronic Obstructive Pulmonary Disease (COPD), asthma etc. of both children and adults (so that ARS may help millions and even billions worldwide).

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- b. **Author contributions:** The conceptualization, data curation, formal analysis, investigation, methodology, project administration, software (used for keeping the evidence of all patients, including this boy), supervision, validation, visualization, writing (the original draft plus review & editing) were all done by dr. Andrei-Lucian Drăgoi, the single author of this article. Funding acquisition and resources were mainly supported by the parents of this boy and secondarily supported by RNHIS; we have also obtained the oral consent of both parents to publish this medical case in both English and Romanian, with the only condition to not mention the names of the boy, parents or other relatives;
- c. **Competing interests:** the author of this paper was invited a couple of times to present ARS and his clinical experience with ARS, but with no financial remuneration and no competing interests.
- d. **Data and materials availability:** we have initially published this case report as a simple preprint (DOI: 10.13140/rg.2.2.21420.36486) entitled "(ASEA in DMD - version 1.1 - 1.08.2018 - 13 pages) The remarkable clinical and biological effects of ASEA ionized water /"redox supplement" (co-administered with L-carnitine and omega-3 fatty acids plus multivitamins dietary supplements) in a ~3-year-old boy with Duchenne muscular dystrophy (DMD) from Romania - a case report" on: Research Gate platform (see URL: www.researchgate.net/publication/325371161), Academia.edu platform (see URL: www.academia.edu/36909338), Vixra platform (see URL: <http://rxiv.org/abs/1806.0354>) and GSJournal platform (see URL: <http://gsjournal.net/Science-Journals/Research%20Papers/View/7338>); we have also created a simple webpage about ARS (asea.dragoii.com), in the purpose of extensively informing the parents of children-patients on ARS, when we have recommended ARS to specific patients; this preprint was also taken over by other platforms like: Data city (see URL: <https://search.datacite.org/works/10.13140/rg.2.2.21420.36486>). This present article is an updated and revised version of that initial preprint: a great part of that preprint was rewritten for this paper. All data is available in the main text of this present article.

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¹⁵For real-time statistics on world’s human population see URL: www.worldometers.info/world-population

¹⁶See the website “Our World in Data” page at URL: <https://ourworldindata.org/fertility-rate#empirical-view> (subchapter “Births and the birth rate - Births Globally”)

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