DR. SUSAN E. VECHT-LIFSHITZ
Patent Attorney
I. P. Israel Patents Ltd.

PATIENT-BASED
METABOLO-
NEUROLOGY-
TIME FOR A PARADIGM
SHIFT
Metabolo-neurology

- Analysis of the metabolome= the full complement of metabolites present in an organism to define anomolous (too much/too little) metabolites ‘ impact on nervous system of the organism.
METABOLO-NEUROLOGY- TIME FOR A PARADIGM SHIFT!

THE NEED
DESPITE DECADES OF RESEARCH AND
ENORMOUS FUNDING

• NO REAL CURES FOR ALS- MOST PATIENTS DIE
  WITHIN FIVE YEARS

• NO TRUE CURES FOR MS- MOST PATIENTS
  SUFFER AND BECOME HANDICAPPED OVER TIME

• NO CURES FOR MOST NEUROLOGICAL DISEASES-
PARKINSON’S DISEASE, MYASTHENIA GRAVIS,
ETC.

WHY?
METABOLO-NEUROLOGY- TIME FOR A PARADIGM SHIFT!

DESPITE DECADES OF RESEARCH AND ENORMOUS FUNDING
A lack of ongoing measurements of key biochemical parameters (such as serum glutamate in ALS)
  √Diabetes- ongoing serum glucose measurements
  √Hypertension- frequent blood pressure monitoring
X ALS- no monitoring of serum glutamate or other anomalous parameters
<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>VALUE IN HEALTHY INDIVIDUALS VNORM</th>
<th>VALUE IN ALS PATIENTS VALS</th>
<th>RATIO ALS:HEALTHY VALS: VNORM</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>[GLU]S</td>
<td>21.3 µM</td>
<td>40.8 µM</td>
<td>1.92</td>
<td>Plaitakis and Caroscio (61) in Plaitakis and Shashidharan*</td>
</tr>
<tr>
<td>[GLU]S</td>
<td>?</td>
<td>?</td>
<td>1.80</td>
<td>(64) in Plaitakis and Shashidharan*</td>
</tr>
<tr>
<td>[GLU]S</td>
<td>57.1 µM</td>
<td>168.3 µM</td>
<td>2.95</td>
<td>Blin et al (7) in Plaitakis and Shashidharan*</td>
</tr>
<tr>
<td>[GLU]S</td>
<td>34.1 µM</td>
<td>163.9 µM</td>
<td>4.8</td>
<td>Iwasaki et al (29) in Plaitakis and Shashidharan*</td>
</tr>
<tr>
<td>[GLU]S average</td>
<td>37.5</td>
<td>124.3</td>
<td>2.87</td>
<td></td>
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</table>
METABOLO-NEUROLOGY- TIME FOR A PARADIGM SHIFT!

ALS = AMYOTROPHIC LATERAL SCLEROSIS/ (LOU GEHRIG’S DISEASE)/MOTOR NEURONE DISEASE

- VERY HIGH SERUM GLUTAMATE
- HYPERMETABOLISM
- HIGH REACTIVE OXYGEN SPECIES/FREE RADICALS
- DNA DAMAGE- SEEN AS 8OHDG IN URINE
- HIGH SERUM PYRUVATE
- HYPOXIA

NONE OF THESE PARAMETERS ARE MONITORED ON AN ONGOING BASIS
# Metabolomic Studies - ALS

<table>
<thead>
<tr>
<th>Disease</th>
<th>Increase in Metabolite Concentration Relative to Healthy Controls</th>
<th>Reduction in Metabolite Concentration Relative to Healthy Controls</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALS (Lou Gehrig’s Disease)</td>
<td><strong>Glutamate</strong> <em>(P &lt; 0.001)</em>, beta-hydroxybutyrate <em>(P &lt; 0.001)</em>, acetate <em>(P &lt; 0.01)</em>, acetone <em>(P &lt; 0.05)</em>, and formate <em>(P &lt; 0.001)</em></td>
<td><strong>Glutamine</strong> <em>(P &lt; 0.02)</em>, histidine <em>(P &lt; 0.001)</em> and N-acetyl derivatives</td>
<td>Alok Kumar, Lakshmi Balab, Jayantee Kalitaa, U.K. Misra, R.L. Singh, C.L. Khetrapal and G. Metabolomic analysis of serum by (1) H NMR spectroscopy in amyotrophic lateral sclerosis <em>Clinica Chimica Acta Volume 411, Issues 7-8</em>, 2 April 2010, Pages 563-567</td>
</tr>
</tbody>
</table>
## METABOLOMIC STUDIES - PARKINSON’S DISEASE

<table>
<thead>
<tr>
<th>DISEASE</th>
<th>INCREASE IN METABOLITE CONCENTRATION RELATIVE TO HEALTHY CONTROLS</th>
<th>REDUCTION IN METABOLITE CONCENTRATION RELATIVE TO HEALTHY CONTROLS</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>PARKINSON’S DISEASE</td>
<td><strong>PYRUVATE</strong> ethymalonate, myoinositol, sorbitol and propylene glycol</td>
<td>galactitol, glycerol, methylamine, trimethylamine, ethanolamine, suberate, glutarate, <strong>malate</strong>, methylmalonate, <strong>succinate</strong>, acetate, gluconate, threonate, gluctose, ascorbate, <strong>isocitrate</strong>, and <strong>citrate</strong></td>
<td>Journal of Biomedical Science 2009, 16:63 Metabolic profiling of Parkinson's disease: evidence of biomarker from gene expression analysis and rapid neural network detection</td>
</tr>
<tr>
<td>PARKINSON’S DISEASE</td>
<td><strong>GLUTAMATE</strong></td>
<td></td>
<td>Neuroscience Letters Volume 145, Issue 2, 12 October 1992, Pages 175-177 Increased plasma concentrations of aspartate, glutamate and glycine in Parkinson's disease</td>
</tr>
</tbody>
</table>
METABOLOMIC STUDIES – MULTIPLE SCLEROSIS

<table>
<thead>
<tr>
<th>DISEASE</th>
<th>INCREASE IN METABOLITE CONCENTRATION RELATIVE TO HEALTHY CONTROLS</th>
<th>REDUCTION IN METABOLITE CONCENTRATION RELATIVE TO HEALTHY CONTROLS</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>METABOLO-NEUROLOGY-TIME FOR A PARADIGM</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
COMMON METABOLIC FEATURES OF NEUROLOGICAL DISEASES

- √ increased serum concentration of excitotoxins glutamate, aspartate and glycine
- √ increased reactive oxygen species (ROS)
- √ increased inflammatory cytokines
- √ increased DNA damage, lipid peroxidation and ROS-induced damage
- √ hypermetabolism
- √ fatigue and poor energy metabolism

a) How are these parameters connected?
b) Why aren’t they being monitored? When does ignorance become medical negligence?
c) So are neurological diseases outcomes of faulty metabolism?
COMMON METABOLIC FEATURES OF NEUROLOGICAL DISEASES

- √ increased serum concentration of excitotoxins glutamate, aspartate and glycine
- √ increased reactive oxygen species (ROS)
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- √ increased DNA damage, lipid peroxidation and ROS-induced damage
- √ hypermetabolism
- √ fatigue and poor energy metabolism

a) How are these parameters connected?

ANSWERS:

1. We have developed some disease-specific metabolic models explaining the connections for ALS and MS.
2. These include interconnected cascades of vicious cycles of faulty metabolism.
3. In order to treat these diseases and stop all these cascades simultaneously, COMBINATION THERAPIES are required.
Faulty metabolism precedes neurological deficits in ALS

EXAMPLE 1

Metabolic progression markers of neurodegeneration in the transgenic G93A-SOD1 mouse model of amyotrophic lateral sclerosis.
Department of Neurology II, Otto-von-Guericke University Magdeburg, Leipziger Str 44, Magdeburg, Germany.

Abstract
Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by a progressive loss of motor neurons. Visualizing corresponding metabolic changes in the brain of patients with ALS with proton magnetic resonance spectroscopy ((1)H-MRS) may provide surrogate markers for an early disease detection, for monitoring the progression and for evaluating a treatment response. The primary objective of our study was to evaluate whether modifications in MR metabolite levels occur before clinical disease onset, and whether these changes are directly linked to a distinct spatial progression pattern in the CNS. Therefore, age-dependent alterations in the cerebral and spinal metabolic profile in the mouse model of ALS overexpressing the mutated human G93A-superoxide dismutase 1 (G93A-SOD1) were determined by high-resolution MRS of tissue extracts at 14.1 Tesla. Both non-transgenic mice (control mice) and transgenic mice overexpressing the non-mutated human SOD1 (tg-SOD1) served as controls. In the spinal cord of G93A-SOD1 mice significantly decreased levels of N-acetyl aspartate were already detected 34 days postpartum, i.e. about 60 days before the average disease onset caused by motor neuron decline. In addition, glutamine and gamma-aminobutyric acid concentrations were significantly diminished at Day 75, which is still in the presymptomatic phase of the disease. These metabolic changes were further progressive in the course of the disease and started to involve the brainstem at Day 75. Overall, high-resolution (1)H-MRS allows a sensitive spatial and temporal metabolite profiling in the presymptomatic phase of ALS even before significant neuronal cell loss occurs.
Faulty metabolism precedes neurological deficits in ALS EXAMPLE 2

- Cell death mechanisms in the early stages of acute glutamate neurotoxicity.
- Alok Kumar, Ram Lakhan Singh and G Nagesh Babu
- Department of Neurology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, UP, India.

The present study focused on the early stages of acute glutamate (L-Glu)-induced neurotoxic mechanisms, both biochemical, e.g. intracellular reactive oxygen species (ROS) and associated parameters as well as gene expression of cell survival/death pathways, i.e. Bcl-2 and caspases. Stereotactic intracortical injections of L-Glu (1micromol/1microl) resulted in decreased size of pyramidal neurons in rat after 1h. We also observed that intracellular ROS, calcium (Ca(2+)) and peroxynitrite (ONOO(-)) production were significantly elevated, whereas, mitochondrial transmembrane potential (DeltaPs) and total glutathione were significantly decreased by L-Glu bolus. The Bcl-2/Bax ratio in the L-Glu-injected rats was found to be significantly lower than the controls. Moreover, acute L-Glu significantly induced mRNA expression of nNOS, iNOS, caspase-3 and caspase-9. It may be concluded from the present study that acute L-Glu administration, at an early stage, increases intracellular ROS accumulation, Ca(2+) levels and peroxynitrite production and decreases glutathione pool. Furthermore, it appears that decreased mitochondrial Bcl-2/Bax ratio might have upregulated the mRNA expression of caspase-3 and caspase-9 which launch cell death cascade. Regarding the chronology of the events, we presume that acute L-Glu increases ROS and decreases DeltaPs and glutathione rapidly and it is more likely that these events precede gene expression changes, ultimately resulting in neuronal damage/death. DOI: 10.1016/j.neures.2009.11.009
The neuroexcitotoxic amino acids glutamate and aspartate are altered in the spinal cord and brain in amyotrophic lateral sclerosis

Dr. Andreas Plaitakis, MD *, Evangelos Constantakakis, MD, Jeffrey Smith, MD Mount Sinai School of Medicine, New York, NY*Correspondence to Andreas Plaitakis, Mount Sinai School of Medicine, One Gustave L Levy Place, New York, NY 10029

ABSTRACT

Because recent studies showed a systemic defect in glutamate metabolism in amyotrophic lateral sclerosis (ALS), we measured the levels of free amino acids in frontal and cerebellar cortex and two areas of spinal cord obtained at autopsy from 22 patients who died of this disease. Glutamate levels were significantly decreased (by 21 to 40% of control values) in all areas investigated; cervical and lumbar spinal cord showed the greatest change. Aspartate levels were also significantly reduced (by 32 to 35%) in the spinal cord only. A positive correlation was shown between the changes of glutamate and aspartate as well as a significant alteration in the glutamate to glutamine ratio in the spinal cord of patients with ALS. Although we cannot exclude the possibility that these abnormalities may partly result from neuronal cell loss, the data suggest the presence of a generalized defect that may affect the neurotransmitter and metabolic pool of glutamate. The defect may be expressed more severely in the spinal cord than in other central nervous system areas. These results, taken together with the previously shown systemic abnormality, raise the possibility that distribution of glutamate between the intracellular and extracellular pool may be altered in ALS and may mediate the neurodegeneration.
Plasma glutamate and glycine levels in patients with amyotrophic lateral sclerosis: The effect of riluzole treatment

Clinical Neurology and Neurosurgery, Volume 110, Issue 3, Pages 222-226


Abstract

Objectives
Defective glutamate (glu) metabolism and excitotoxicity have been implicated in the pathogenesis of amyotrophic lateral sclerosis (ALS). Moreover, glycine (gly) has been shown to potentiate excitatory transmission. The “antiglutamatergic” agent riluzole has been shown to prolong survival in ALS. The aim of the study was to investigate a possible effect of riluzole on plasma glu and gly levels, correlating with clinical response to treatment.

Patients and methods
Plasma concentrations of glu and gly were measured in 20 healthy volunteers and 22 ALS patients before treatment and after 6 months on riluzole.

Results
At baseline, increased plasma glu correlated with spinal onset and male gender whereas gly levels did not differ between patients and controls. No significant change was observed for both amino acids post-treatment, despite a lower rate of disease progression.

Conclusion
These results suggest that riluzole may affect disease progression without a significant impact on plasma glu and gly levels, possibly indicating different mechanisms of drug action.
COMMON METABOLIC FEATURES OF NEUROLOGICAL DISEASES

- √ increased serum concentration of excitotoxins glutamate, aspartate and glycine
- √ increased reactive oxygen species (ROS)
- √ increased inflammatory cytokines
- √ increased DNA damage, lipid peroxidation and ROS-induced damage
- √ hypermetabolism
- √ fatigue and poor energy metabolism

b) Why aren’t they being monitored? When does ignorance become medical negligence?

The proof of the pudding is in the eating. No ALS patient has been cured since the days of Lou Gehrig. Why?

- Wrong classification of these diseases?
- Neurologists’ lack of knowledge of metabolism/biochemistry related to these diseases?
- A tendency to measure outcomes (nerve conduction, MRI changes, muscle strength) rather than metabolic causes?
- A monopoly/cartel of treatment granted only to neurologists?
c) So are neurological diseases (at least, in part) outcomes of faulty metabolism?  
YES

d) Are these disease potentially, at least partially reversible, if the underlying metabolism is corrected?  
YES
What steps are required to achieve cures of these diseases?

1) Definition of “what’s wrong” at a metabolic level.
2) Definition of “how” and “why”- Qualitative understanding of underlying disease mechanism- interconnection between anomalous parameters.
What additional steps are required to achieve cures of these diseases?

4) Development of good *in vitro* models for testing potential cure candidates.

5) **Finding funding** for running *in vitro/* *in vivo* /clinical trials.

6) **Finding partners** to make this happen including:
   * Venture partners
   * Business partners
   * Industrial partners
   * Researchers
What steps are required to achieve a cure for ALS?

1. Definition of “what’s wrong” at a metabolic level.

We have spent five years reviewing scientific journals and patent literature to define the “what’s wrong”.

2. Definition of “how” and “why”- Qualitative understanding of underlying disease mechanism- interconnection between anomalous parameters.

We have developed a metabolic model (unpublished but part of a patent application)


We have calculated ball-park figures for reactive oxygen species and have defined other metabolites from the literature data.
What steps are required to achieve a cure for ALS?

4. **Good *in vitro* models for testing potential cure candidates.**
   In progress: search for high throughput models for predicting efficacy in ALS patients, current mouse models are too slow, too expensive and not good predictors of outcomes in humans.

5. **Funding for running *in vitro/in vivo* /clinical trials.**
   We are currently looking for funding.

6. **Experienced Partners to make this happen including:**
   We are currently looking for
   * Venture partners
   * Industrial Partners
   * Business partners
   * Researchers
Increased reactive oxygen species (ROS)- free radicals **HOW MANY IS TOO MANY?**

http://www.youtube.com/watch?v=YGn21Ck4t28

**Never Say Never Again** is a 1983 James Bond film

- **M** (Edward Fox): Too many free radicals. That's your problem.
- **James Bond** (Sean Connery): "Free radicals," Sir?
- **M**: Yes. They're toxins that destroy the body and the brain, caused by eating too much red meat and white bread and too many dry martinis!
- **James Bond**: Then I shall cut out the white bread, Sir.
- **M**: Oh, you'll do more than THAT, 007. From now on you will suffer a strict regimen of diet and exercise; we shall PURGE those toxins from you!
Increased reactive oxygen species (ROS)- free radicals

HOW MANY IS TOO MANY?

Is a regimen of diet and exercise sufficient to purge the free radicals? To address this, we must quantify:

HOW MANY ARE FORMED IN A HEALTHY CONTROL?

HOW MANY ARE FORMED IN SPORTSPERSON?

HOW MANY ARE FORMED IN NEUROLOGY PATIENT?
Increased reactive oxygen species (ROS)- free radicals

HOW MANY IS TOO MANY?

A) CALCULATE OXYGEN INHALED PER DAY
B) MULTIPLY BY PERCENT CONVERTED INTO ROS
C) OBTAIN QUANTITY OF ROS IN HEALTHY PERSON
D) FOR SPORTSPERSON, MULTIPLY BY A FACTOR
E) FOR NEUROLOGY PATIENT MULTIPLY BY SECOND FACTOR
F) ASSUME QUANTITY NOT CLEARED BY NORMAL METABOLISM
G) OBTAIN RESIDUAL ROS
HOW MANY FREE RADICALS ARE FORMED PER DAY?

1) The percent of oxygen obtained from air inhalation is \( \frac{\text{inhaled-exhaled}}{\text{total}} \times 100\% = \frac{(21-17)}{100} \times 100\% = 4\% \)

2) An average male breathes 12-20 times/min with an average tidal volume at rest of 0.5 liter.
   
   \[ V_{\text{male}} = 16 \times 0.5 \text{L} = 8 \text{L/min} = 8 \times 60 \times 24 \text{ L/day} \]
   \[ V_{\text{male/day}} = 11520 \text{ L air/day} \]

3) TOTAL OXYGEN = 0.04 \times 11520 \text{ L/DAY}
   
   = 460.8 \text{ L/DAY}
   = \frac{460.8}{22.4} \text{ MOLES/DAY}
   = 20.5 \text{ MOL/DAY}
   = 20.5 \times 32 \text{ g/DAY}
   = \frac{656 \text{ g O}_2}{\text{DAY}}
METABOLO-NEUROLOGY—TIME FOR A PARADIGM SHIFT!

HOW MANY FREE RADICALS ARE FORMED PER DAY?

=656 g O₂/DAY


Superoxide = (0.02-0.05) x 656 g/day

1) Superoxide produced/day = 13.1-32.8 g/day (HEALTHY inactive PERSON)

2) According to Halliwell et al, a sportsperson may produce up to ten to 15 times as many ROSes as an inactive person, due to increased oxygen uptake and anaerobic metabolism.

SUPEROXIDE SPORTSPERSON
= (13.1-32.8) X (10-15)
= 131-492 g superoxide/day
QUANTIFICATION OF METABOLIC CHANGES

Increased reactive oxygen species (ROS)- HOW MANY FORMED IN A HEALTHY CONTROL/ SPORTSPERSON/NEUROLOGY PATIENT?

Assume that a healthy person clears most of the free radicals, this is clearly not the case for neurology patients, as demonstrated by the damage and increased 8OHDG in serum and urine. So, let us assume that an ALS patient with less/less active superoxide dismutase clears 60-80% superoxide, then there will remain 20-40% which do damage.

ALS superoxide =  (13.2-492) x (0.2-0.4)=2.64-196.8 g residual superoxide/day
or in mols = (2.64-196.8)/32 =0.0825-6.15 M/day superoxide
Clinical trials ALS- CO Q 10  MW 863.34
= 0.002 M-0.003 M CO Q 10
Study NCT00243932

http://clinicaltrials.gov/ct2/show/results/NCT00243932?sect=X6015#outcome1

<table>
<thead>
<tr>
<th></th>
<th>2700mg CoQ10</th>
<th>Placebo</th>
<th>1,800 mg CoQ10</th>
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<tbody>
<tr>
<td>= 0.002 M-0.003 M/day CO Q 10 TO SCAVENGE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0825-6.15 M/day superoxide??????</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of Participants</td>
<td>73</td>
<td>75</td>
<td>35</td>
</tr>
<tr>
<td>Decline in the ALS Functional Rating Scale-revised (ALSFRSr)</td>
<td>8.80 ± 7.34</td>
<td>9.44 ± 8.82</td>
<td>10.9 ± 9.3</td>
</tr>
</tbody>
</table>
Conclusions about clinical trials

= 0.002 M - 0.003M CO Q 10/day TO SCAVENGE 0.0825-6.15 M/day superoxide??????

Dosage in NCT00243932 clinical trial is 2-4 orders of magnitude too little (30-3000 X too little)

Other trials
28 subjects receive oral lipoic acid 1200mg daily (MW 206.33) = 0.0058 M

DOSAGE OF ONE ACTIVE AGENT IS TOO LITTLE QUANTITATIVELY TO BE EFFECTIVE
CONCLUSIONS

1) It’s time to re-think these diseases
2) It’s time to quantify the magnitude of the problem in these diseases
3) It’s time to understand these diseases
4) It’s time to develop combination therapies to cure these diseases
Thank you
Dr. Susan E. Lifshitz, Patent Attorney

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METABOLO-NEUROLOGY-
TIME FOR A PARADIGM SHIFT!
### Forbidden Foods, Drinks, and Activities

<table>
<thead>
<tr>
<th>Forbidden Subject</th>
<th>Reason</th>
</tr>
</thead>
<tbody>
<tr>
<td>All sushi, raw, marinated, smoked fish, frozen fish of certain species, certain fresh fish and other marine organisms</td>
<td>May contain significant concentrations of thiaminase</td>
</tr>
<tr>
<td>Aspartame and artificial sweeteners</td>
<td>Converts to/contains aspartate (neurotoxin)</td>
</tr>
<tr>
<td>Monosodium glutamate</td>
<td>Increases the concentration of the neurotoxin, glutamate.</td>
</tr>
<tr>
<td>High glycemic index foods and beverages. Large quantities of carbohydrates - high glycemic load.</td>
<td>ALS patients have some glucose intolerance/diabetic-like metabolic activities. Glucose drips should be replaced, if possible, with other carbon sources of lower glycemic index (amino acids (low glutamate and aspartate) and mannitol)</td>
</tr>
<tr>
<td>Carbonated and/or sweet or diet drinks</td>
<td>Glycemic load, aspartame neurotoxicity, inhibitory effects of carbonic acid in carbonated drinks on oxidative phosphorylation.</td>
</tr>
<tr>
<td>Smoking</td>
<td>Obvious deleterious effects</td>
</tr>
<tr>
<td>Large quantities of alcohol</td>
<td>Exacerbates thiamine deficiency (Wernicke's encephalopathy)</td>
</tr>
<tr>
<td>Heavy exercise</td>
<td>Increases anaerobic respiration</td>
</tr>
</tbody>
</table>