Evaluation of the Biochemical Effects of Administration of Intravenous Nutrients Using Erythrocyte ATP / ADP Ratios

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Abstract

Regardless of clinical diagnosis, many acutely and chronically sick patients benefit from intravenous vitamins and minerals, which are usually administered in multiple infusions before observing obvious benefit. We hypothesized this effect was due to improved cellular energy, and attempted to find laboratory evidence via this study. Two groups of patients, chosen at random, received a single infusion of vitamins and minerals in two different dose schedules. Controls received no treatment. Study subjects were patients who presented specifically for a nutritional therapeutic approach, and although all were treated with multiple infusions, a single infusion was selected at random for this study. Thirty patients received a single infusion of a lower dose nutritional formula, sometimes known as a Myer's Cocktail (MC), and 34 had a single infusion of a higher dose nutritional I/V (NIV). Immediately prior to and after the infusion, blood was drawn and an erythrocyte ATP/ADP ratio (EADR) was determined. The results showed that in both infusion groups if the EADR was initially low, it would increase. If it were initially high, it would decrease. This effect was not observed in control subjects. Pre-test EADR boxplot analyses, derived from the results of each protocol, showed these results were statistically predictable. An analysis of variation (ANOVA) calculation indicated the differences were significant. The family error rate used was 0.05. We conclude that this regression of the EADR to the mean, as a result of either of the two infusions and not seen in control subjects, is biochemically significant.
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Introduction

We have used both infusions for several years and have observed induced clinical benefits which are often so obvious, even dramatic, that our incentive for using these treatment modalities has increased. A number of articles have been published on the use of intravenous

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Table 1. Myer's Cocktail.

Dextrose 5% in sterile water			50 - 100 cc
Magnesium Chloride 20%			2 - 5 cc
Calcium Gluconate 10%			10 cc
Pyridoxine Hydrochloride (100 mg/cc)			1 cc
Dexpanthenol (250 mg/cc)			1 cc
Vitamin C (222 mg/cc)			30 cc
Hydroxocobalamin (1000 mcg/cc)			1 cc
Thiamine Hydrochloride (100 mg/cc)			0.5 cc
Trace Mineral Mixture			1 - 2 cc
Each ml contains:	Zinc	5 mg	
	Copper	1 mg	
	Manganese	0.5 mg	
	Chromium	10 mcg	
	Selenium	60 mcg	

magnesium[1-6] and ascorbic acid[7] in a variety of clinical conditions, but little has been published in this important area of nutritional research on the use of intravenous nutrients in combination. Alan Gaby, M.D., learned of the formula known as Myers' Cocktail (MC) from the late Dr. John Myers of Baltimore. He modified the original formula by increasing the amount of vitamin C, calcium and magnesium.[8] The formula used in this study included some minerals, but was otherwise that used by Gaby. The higher-dose nutritional I.V. (NIV) contains a similar "cocktail" but in higher dosage and, because of this, is administered over a longer period. Both were given by continuous drip method.

Table 2. Nutritional I.V.

Sterile Water			500 cc
Magnesium Chloride			2 gm (10 cc)
Potassium Chloride			5 meq (2.5 cc)
Dexpanthenol			500 mg (2 cc)
Folic Acid			10 mg (1 cc)
Manganese Chloride			1 mg (0.5 cc)
Zinc Chloride			10 mg (1 cc)
Selenium			200 mcg (5 cc)
Chromium			40 mcg (10 cc)
Ascorbic Acid			20 gm (40 cc)
Adenosine 5' Monophosphate			125 mg (0.5 cc)
Procaine 2% (pm)			100 mg (5 cc)
Pyridoxine Hydrochloride			100 mg (1 cc)
Hydroxocobalamin			1000 mcg (1 cc)
Vitamin B Complex 100			2 cc
Each ml contains:	Thiamine HCl	100 mg	
	Riboflavin 5' Phosphate Sodium	2 mg	
	Pyridoxine HCl	2 mg	
	Dexpanthenol	2 mg	
	Niacinamide	100 mg	

We have observed that some patients feel better and some feel worse immediately after any single infusion. We hypothesized, therefore, that symptomatic improvement, resulting immediately from the studied infusion, would be matched by an increase in EADR ("responder") and would decrease or remain the same in a "non-responder," a patient who did not exhibit immediate symptomatic benefit. This ratio is probably the best method of evaluating energy potential in a cell, since it represents the continuous mechanism of oxidative phosphorylation. Erythrocytes were chosen because of the ease of obtaining blood specimens, although it was understood this would not necessarily reflect similar changes in other tissues such as the brain and central nervous system. Using patients who came to our office specifically for nutritional therapy, the study attempted to ascertain whether there are post-infusion changes in EADRs immediately after completing an infusion compared to EADRs measured immediately before the infusion.

Methods

Study subjects were patients with various acute and chronic conditions who came to a Preventive Medicine office for nutritional therapy. They consisted of 64 individuals who were randomly assigned to the low dose MC or high dose NIV group. Tables 1 and 2 show the contents of the two infusions.

Thirty patients received a single infusion MC, given by drip over a half-hour period. Thirty four received a single infusion NIV. Because of the larger doses of nutrients in the NIV, this was administered over a three-hour

period. Of the 30 patients treated with MC, nine were males and 21 were females, while nine males and 25 females received NIV. For both groups the ages ranged from 10 to 78 years. Participants in this study had more than one I.V. as part of their treatment, but the pre- and post- infusion EADR was ascertained during one I.V. only. All patients were polysymptomatic. The most common symptom was fatigue, but arthralgia, recurrent "flu-like" symptoms, fibromyalgia, migraine headaches, food intolerance, recurrent sinus infections, sore throats, abdominal discomfort, heart palpitations, irritable bowel syndrome, and many varieties of autonomic and endocrine dysfunction were variably present.

The primary outcome measure was calculation of EADR before and after each infusion. The secondary outcome was to ascertain whether symptomatic correlation could be deduced in relation to the particular I/V studied, irrespective of the ultimate treatment outcome expected from repeated infusions.

Twenty healthy volunteers served as control subjects. All had blood drawn at baseline, and in 10 subjects the second specimen was taken after an interval of 30 minutes, representing the time required for a MC. In the other 10 subjects the interval was three hours, representing the time required for a NIV. No treatment was given in the interval between respective individual phlebotomies. Administration of control infusions to patients in a private practice setting is difficult to justify, as healthy volunteers know the infusion administered to them must be a placebo, since they are volunteers and have no symptoms. The intent was to show a significant change in EADR was not merely a statistical artifact.

Each patient receiving an infusion was asked to sign an informed consent for its administration as well as the blood draws before and after any infusion to be included in this study. The protocol was reviewed and passed by the Institutional Review Board of the American College for Advancement in Medicine (ACAM).

For each test, blood was drawn in a yellow-top tube and the specimen precipitated with trichloracetic acid. The specimens were stored at -20^0 C until assayed.

The reaction is based on a modification[9] of that reported by Bucher.[10] The principles are as follows: the enzyme phosphoglycerate phosphokinase (PGK) is used to catalyze the following reaction:

ATP + 3-Phosphoglycerate $\xrightarrow{PGK}$ ADP + 1,3 Diphosphoglycerate

The enzyme glyceraldhyde phosphate dehydrogenase (GAPD) is also present in the reaction mixture to catalyze the following:

1,3 Diphosphoglycerate + NADH $\xrightarrow{GAPD}$ Glyceraldehyde-3-P + NAD + P

By determining the decrease in absorbance at 340 nm which results when NADH is oxidized to NAD, a measure of the amount of ATP originally present is obtained. Results are expressed as moles ATP/g Hb. The ATP analysis was performed using kit #366-uv (Sigma Diagnostics, St Louis, Mo).

Results

The two patient groups were considered separately (see Table 3). The EADR increased or decreased variably after both MC and NIV, irrespective of the symptomatic effects expressed by the patient. Because the initial impression was that an increase in EADR would correlate with a beneficial symptomatic response, these individuals were termed "responders," whereas those whose EADRs decreased were termed "non-responders."

A statistically significant observation was obtained when the data were evaluated using a boxplot analysis.[11] The fundamental

Table 3. EADR and symptomatic change.

	N	Increased EADR	Decreased EADR
Myer's Cocktail			
Clinically Improved	21	11	10
No Clinical Improvement	8	7	1
Worse	1	0	1
Total	30	18	12
Nutritional I/V			
Clinically Improved	18	12	6
No Clinical Improvement	14	9	5
Worse	2	0	2
Total	34	21	13

question the statistical analysis was designed to answer was: can the EADR be statistically predicted for both groups studied and is it the same for both? The answer to this question is "yes," with the predictor being when the pre-test EADR is low, then predict the post-test EADR will increase, and when the pre-test ratio is high the post-test ratio will decrease.

The statistical analysis leading to this result is as follows**.** Both the MC and NIV groups were divided into two subgroups, defined as either "responders" or "non-responders." A pre-test EADR boxplot analysis on each of the two subgroups for each of the protocols, MC and NIV, was performed and the subgroups were compared for any significant differences between them. The particulars of this specific boxplot analysis are that the x-axis variable is the group type, either responders or non-responders**,** and the y-axis variable is the pretest ratio. The boxplot form chosen for this analysis is as follows. The bottom of the box corresponds to the twenty-fifth percentile datum point while the top of the box corresponds to the seventy-fifth percentile datum point. The line within the box represents the median and the whiskers indicate the range of the data (Figures 1 and 2).

Examination of the two graphs applicable to the treatment groups shows a remarkable consistency when the groups are defined as "responders" or "non-responders." The group characterized by a low pre-test ratio experienced an increase in the EADR after infusion. When the pre-test ratio was high, the post-test ratio was decreased. An analysis of variation (ANOVA) calculation was performed, which indicated the differences between the two groups were statistically significant. Because of this consistency we are now able to predict the EADR response to non-caloric intravenous infusions statistically.

The confidence interval calculations are a further indication that there are two distinct groups responding to the treatments and it is not the result of random error.

In order to ascertain whether this response to treatment was a statistical artifact or had biochemical significance, a similar study was performed on results obtained from the control subjects. The boxplot analyses shown in Figures 3 and 4 are for the controls.

An ANOVA analysis of "responders" and "non-responders," represented in Figures 3 and 4, and the two control groups combined, as shown in Figure 5, indicates no statistical difference between "responders" and "non-responders" for either of the two time-elapsed groups. The Tukey family error rate used was 0.05. Those differences that do exist are due solely to random error.

Table 3 indicates whether the patient felt better after the study infusion. No attempt was made to score them other than to ask the patient in each case whether they felt better. Some of the symptomatic improvement perceived by individual patients was quite striking, usually expressed as "more energy," "less fatigue" or "increased sense of well being," but the shift in post-infusion EADR could not be correlated with symptomatic improvement.

Of the total patients treated, 39 felt better and 22 did not. Only two patients felt worse, one of which was a 78-year old physician whose symptoms were related to an

inoperable meningioma. He was treated with both MC and NIV, to which he reacted adversely with vomiting for approximately 24 hours after each infusion.

Discussion

The control group were only partial controls, since none had any infusions; however, this analysis indicates the "regression to the mean" of the EADR demonstrated by the treatment groups should not be considered a statistical artifact. Rather, it should be concluded the decrease in initially high EADRs and the increase of initially low EADRs is, in fact, a biochemical effect within the cell.

This form of treatment gives nutrient assistance to the powers of self-healing of the patient. It is irrespective of the descriptive classification of the disease and makes the basic assumption that every disease process is the result of overwhelmed defense activities of the host. The assumption extends to assuming automatic responses fail because of inefficient cellular energy.

From our observations, many patients experience significant symptomatic improvement after MC and NIV, although it is unpredictable and most patients respond symptomatically only after repeated infusions, particularly in chronic disease. We have observed no long-term harm in any patient so treated, although symptoms may be temporarily exacerbated before any benefit is experienced, particularly with a solitary infusion, so the patient is informed of the possibility before infusions begin. This phenomenon makes it virtually impossible to draw a conclusion in regard to symptomatology for a single infusion. It is also apparent, in our experience, that some patients respond better symptomatically to the lower dose MC than the higher-dose NIV, whereas in others the reverse is true. This may be due to the patient's nutritional status at the time of the I.V. It is also clear the change in EADR does

Figure 1. Single test - Myers protocol

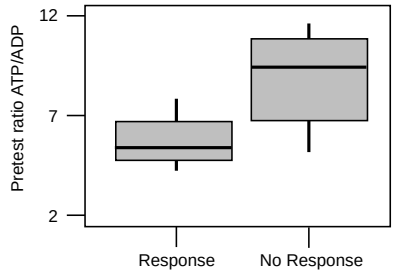


Figure 2. Single test - NIV protocol

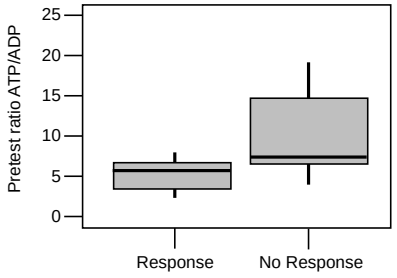


Figure 3. Control (30 minute interval)

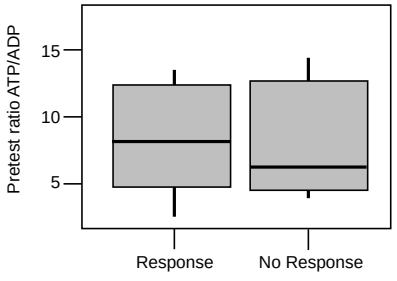


Figure 4. Control (3 hour interval)

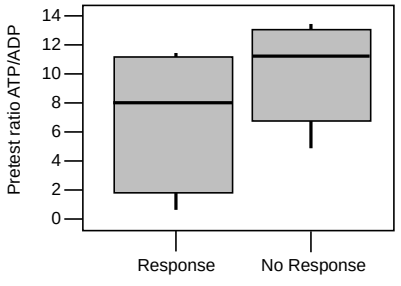
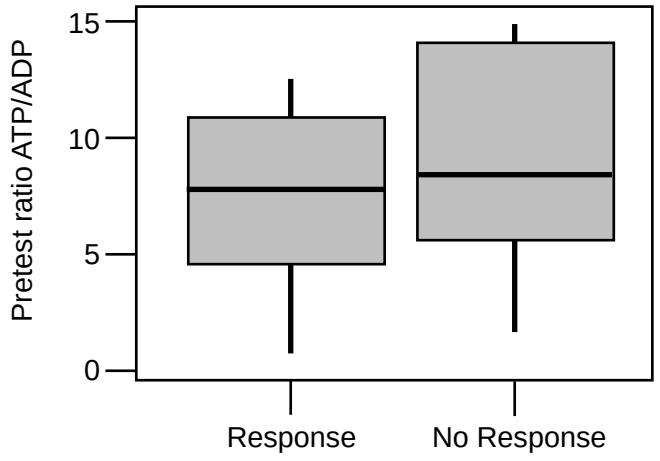


Figure 5. Control (30 minute interval + 3 hour interals combined)



not correlate with the stated, short-term symptom response of the patient after the studied infusion. The outcome EADR appears to be a function solely dependent upon whether the initial determination is high or low.

There seems to be biochemical similarity between fibromyalgia (FMS), chronic fatigue syndrome (CFS) and multiple chemical sensitivity.[12] Although regarded generally as different diagnostic clinical entities, symptoms of all three are commonly seen in one individual. Fibromyalgia is causally related to defective oxidative phosphorylation. Decreased ATP and associated low cellular oxygen tension were reported in both Chronic Fatigue Immunodysregulation Syndrome (CFIDS)[13] and FMS.[14] It has also been reported that decreased mitochondrial respiration occurs in the muscles of some individuals with a fatigue syndrome associated with accelerated aging.[15]

As suggested by Bland,[16] mitochondria may be thought of as an energy storage battery. The electrochemical gradient of electrons produced by oxidative phosphorylation in the redox cycle through half-cell reactions provides the power which drives aerobic physiological function. Thus, ATP serves as an energy conduit between the "high energy" phosphate donors and "low energy" phosphate acceptors.

"Mitochondrial resuscitation" has been partially achieved through administration of nutrients in a patient with known mitochondrial DNA deletion.[17] Chiarantini et al[18] studied ATP/ADP ratios in hexokinase-deficient fibroblasts and concluded the ratio was better than the absolute values of either component in evaluating the energy status of the cell. At a normal rate of utilization the total ATP pool in heart muscle turns over six times per minute. The control and integration of mitochondrial high-energy production depends upon ATP production being exactly balanced to the high rates of utilization required by contraction.[19] With the addition of phospho-creatine(PCr) the "resting" heart turns over the total high energy phosphate pool (ATP + PCr) between two and four times per minute. Production of ATP must be under very tight control with little margin for error. The "shifting sand" which represents the relationship between ATP and ADP at any one time does not indicate the rate of hydrolysis of ATP versus its reconstitution from ADP. Although maintenance of a sufficiently high ratio is essential to overcome the thermodynamic burden of uphill processes, it is not clear to what degree enzymes which control this ratio also control cell physiology.[20]

From their data using mitochondrial fractions from rat heart and liver, Jacobus et al concluded respiratory control in tissues is a function of the rate of transport of ADP into the mitochondrial matrix, mediated by the kinetic properties of adenine nucleotide translocase.[21] From the perspective of cell high-energy phosphate metabolism, the direct regulation of ATP production by ADP availability makes sense. In order to couple the critical balance between energy supply (metabolism) and energy demand (work) tightly, tissue respiration would best be controlled by a signal indicative of the

magnitude of cell labor. Changes in the availability of ADP directly reflect the steady state magnitude of energy demand. Slater et al[22] proposed mitochondrial respiratory control was a function of the ATP/ADP ratio, independent of tissue phosphate content.

The amount of ATP in a cell is usually enough to supply its free energy needs for several minutes. It is constantly being hydrolyzed and regenerated. For example, the turnover of ATP in vertebrate skeletal muscle can increase more than a hundredfold during high-intensity exercise, while the actual total muscle content is unchanged.[23] This requires ATP hydrolysis and synthesis rates to be exactly balanced, despite their marked reaction fluctuations. It would not be unreasonable to find that an intermediate ATP/ADP ratio represented optimum cellular efficiency.

Korge and Cambell[24] concluded ATP consumption is well balanced with ATP generation, even in fatigued muscles. This balance is achieved by down-regulation of ATP consumption, although the maximum rate of local ATP regeneration relative to that of ATP hydrolysis *in vivo* is not known, mainly because *in vitro* determinations underestimate this value.

Conclusions

There are two major questions we have attempted to address. The first is why primary essential nutrients administered in very large doses result in clinical improvement in many sick people. The second question is whether measuring cellular energy metabolism through the determination of EADRs might correlate with observed symptomatic status. Our hypothesis was EADRs would increase with symptomatic improvement and decrease or remain the same without it.

Our experience suggests significant changes in relationships between ATP and ADP, even after single infusions, but our hypothesis proved incorrect. The fact that our controls did not exhibit this statistically-significant change reinforces this. It is not apparent why an initially low EADR becomes high or a high EADR becomes low after either of the infusions studied. It is clear, however, that there is a balance between the rate of ATP synthesis versus its utilization, and this phenomenon of regression to the mean appears to be biochemically significant.

With so many variables the energy state of the cell must be in a continuous state of flux. It is possible there is an ideal cellular ATP/ADP ratio which is intermediate between "high" and "low." Therefore, the fall in EADR which we have termed a "non responder," versus a "responder" when the ratio increases, appears to be an incorrect interpretation of the chemistry. There seems to be insufficient scientific information about these shifting relationships to be able to accurately interpret the statistically-significant changes observed in EADR in both groups of treated patients versus untreated controls.

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