Metabolism, molecular hypometabolism and inflammation: Complications of proliferative physiology include metabolic acidosis, pulmonary hypertension, T reg cell deficiency, insulin resistance and neuronal injury

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Abstract
The complexity of crosstalk between metabolism and inflammation was further defined by the discovery by James Ryan of “molecular hypometabolism (MHM),” a transcriptomic finding seen in over 80% of cases of SEID and CIRS. MHM is characterized by the suppression of ribosomal mRNA for both large and small subunits; suppression of mitoribosomal mRNA in both large and small subunits; and suppression of mRNA from nuclear-encoded mitochondrial genes including ATP synthases, electron transport chain genes (ETC), and translocases. Open-label treatment of CIRS patients with MHM, following a published protocol, ending with vasoactive intestinal polypeptide (VIP), showed clinical improvement over stereotyped baseline proteomic abnormalities. The use of transcriptomic measures showed (i) reversal of gene suppression, seen as an overshoot of mRNA number in cases compared to controls and (ii) correction by VIP of the overshoot to equal controls. Cases with MHM commonly had activation of IRS2 [MHM (+)/ IRS2 (+)] that was associated with refractory systemic symptoms, pulmonary hypertension, grey matter atrophy, and a widened anion gap. Mean age-stratified numbers of atrophic grey matter nuclei were excessive in untreated MHM (+)/IRS2 (+) compared to other cases with either (i) absence of MHM or (ii) those without IRS2 (+). Following treatment, symptoms and MHM abated; widened anion gap returned to normal; mean numbers of grey matter nuclei fell; and pulmonary hypertension improved. Non-MHM/IRS2 (+) cases had a much lower incidence of systemic complications.

Given that aerobic glycolysis is commonly found in pulmonary hypertension, we looked for the commonality of metabolic indicators for aerobic glycolysis seen in (i) pulmonary hypertension; (ii) mismatching of MHM and IRS2 (+); (iii) a symptom-dense illness; (iv) widened anion gap; (v) central nervous system injury found in patients with (vi) evidence of reduction of outer mitochondrial membrane translocases. Closure of voltage dependent anion channels (VDAC) on the outer mitochondrial membrane can lead to the activation of aerobic glycolysis. A cohort of 112 patients with transcriptomic findings and measurements for CNS volumetrics, echocardiograms and widened anion gap are presented to show the effects of proliferative physiology in clinical care. Of note, the most significant grey matter atrophy, as shown by a volumetric software program, was found with concomitant upregulation of tubulin A4A or in patients treated with azole antifungals, known microtubular dissembling drugs. The role of aerobic glycolysis and proliferative physiology as a source of chronic fatigue has not been addressed in CIRS/SEID previously.

Background
Metabolism could be defined as the group of biochemical processes needed to maintain cellular life [1]. This broad term classifies all individual metabolic processes into a single over-arching, process-interacting system. Such an all-encompassing term does not provide an understanding of the distinct bases of metabolism or their interplay with other systems, including inflammation. Specific elements of the definition of metabolism support an enhanced understanding of unique metabolic functions but lost is the cohesive consideration of all metabolic processes acting in concert to maintain life and health [1]. New data on the interaction of metabolism with inflammation, combines with transcriptomic findings in syndromes characterized by chronic inflammation are uncommon.

An indirect understanding of research foci in metabolism comes from the prevalence of search words on PubMed. Citations assessed 2/15/2020 included 7,914,000 that were devoted just to [metabolism], with over half of those citations (4,819,000) indexed to [metabolism, protein]. [Disorders of protein metabolism] brought up nearly 1,000,000 hits. [Metabolism, glucose] brought 435,000 matches, but [disorders of glucose metabolism] had only 177,000 matches. So here is a massive scientific database on metabolism and disorders of metabolism of proteins and sugars, but there is a discrepancy between general versus specific topics in metabolism. Against this dense background of the discussion of the essential elements of metabolism, we have the new transcriptomic findings of “molecular hypometabolism (MHM),” coined by Dr. James Ryan in 2016 describing the simultaneous reduction in the number of copies of ribosomal mRNA and mitoribosomal mRNA made by individuals with a chronic, multisystem, multi-symptom

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illness, characterized by chronic fatigue [2-4], of a particular type, called chronic inflammatory response syndrome (CIRS). Of note is the increased production of inflammatory compounds that accompanies suppression of production of ribosomal mRNA [4].

Nearly all CIRS cases also meet the case definition for Chronic Fatigue Syndrome (CFS) [5] and Severe Exercise-Induced Disease (SEID), though CFS and SEID have no specific biomarkers [6] CIRS has at least 15 biomarkers [7]. For these individuals, compromise of metabolism may be adaptive, providing the potential for cell survival in the face of an inflammatory or ribotoxin-mediated attack on protein synthesis at the sarcin-rin loop [8] (SRL), an evolutionarily conserved structure located between the large and small subunits of ribosomes. Found in all living organisms, and directly involved with initiation, elongation, and termination of amino acid chains, one may speculate as to the source of the paucity of papers on the incredibly important role in the metabolism of ribosomal protein production played by the SRL. (number of papers on [metabolism, SRL] =177) compared to [metabolism, ribosomes] (=44,400).

Added to decreased protein production and innate immune inflammation in MHM is the severe cellular impairment created by the simultaneous suppression of nuclear-encoded mitochondrial genes. While putative mitochondrial dysfunction in CFS and CIRS has many proponents, we must account for differential gene activity for mitochondrial genes that have migrated to the nucleus over several billion years. While nuclear-encoded mitochondrial genes number in the hundreds, only 37 genes remain in the mitochondrial genome [9]. Specifically, genes for ETC and ATP synthases were noted by Dr. Ryan to be suppressed, as were translocases. Translocases move nuclear gene-encoded products out of the cytosol, across the outer mitochondrial membrane, in conjunction with porins, small electrically charged channels (voltage-dependent anion channels, VDAC) that permit entry of ions, solutes, ADP and pyruvate against a gradient into the intermembranous space [10-13]. There, specific carriers will complete delivery across the inner mitochondrial membrane to the matrix.

Failure of entry of pyruvate into the mitochondrial matrix compartment from the cytosol has very important consequences for metabolism (see below). Survival adaptations using MHM are maintained at a cost, however, as organisms with MHM are alive but might not be vigorous in either of two main cellular energy processes, (i) mitochondrial production in patients with CIRS or SEID. They have a deficiency of translocases. For perspective, while [metabolism, mitochondria] yields 167,000 papers, nuclear-encoded mitochondrial genes, little is published on energy production in patients with CIRS or SEID. They have a deficiency of translocases. For perspective, while [metabolism, mitochondria] yields 167,000 papers, nuclear-encoded mitochondrial genes are only referenced by 1471 papers under [metabolism, nuclear-encoded mitochondrial genes] and only 4 for [metabolism, fatigue, and voltage-dependent anion channels]. Given that the estimates of the prevalence of the chronic fatigue illness in the US alone exceed 50,000,000 cases, with that number increasing yearly, it was disturbing to find the dearth of papers on [metabolism, CIRS] (=1334) and [metabolism, ribotoxins], the source of SRL injury (=72).

Fortunately, a diagnostic and treatment protocol that identifies and corrects the transcriptomic abnormalities of MHM has been in wide use [2,4], since 2010, with correction of MHM noted in over 90% of cases (manuscript in preparation). This paper will review the impact of MHM on metabolism as those pathways interact with inflammation seen in CIRS, with particular attention to aerobic glycolysis and VDAC effects on metabolic acidosis, grey matter atrophy and pulmonary hypertension in CIRS. We follow that thread with study data on 112 patients with CIRS.

**SEID, CIRS and metabolism**

The recent addition of transcriptomic testing to the approach to patients with chronic fatiguing illnesses, defined as multisystem, multi-symptom illnesses, including Severe Exercise Intolerance Disease (SEID), fibromyalgia, Post Lyme disease and chronic inflammatory response syndrome (CIRS), among others, has demonstrated not only (i) commonality of the complexity and near universality of responses of genes of inflammation and coagulation, but also (ii) the relationship of suppression of production of ribosomal mRNA and nuclear-encoded mitochondrial genes to multiple facets of glucose metabolism, especially aerobic glycolysis [14,15], resulting in neuronal injury, including cognitive impairment [16-20], and pulmonary hypertension (see PAH below) These findings in chronic illnesses parallel the findings of acute sepsis (systemic inflammatory response syndrome, SIRS), with excessive inflammation, coagulation, and immunosuppression persisting beyond the treatment of infection [21].

Each of these elements is consistent with the classic observations of Lewis Thomas that the host immune response, once initiated, can become the disease. When combined with disturbances in glycolysis and pyruvate uptake into mitochondria through VDAC, identified in part by transcriptomics, it is clear that ongoing maladaptive responses to immune and metabolic initiators underlie multiple sources of SEID (excluding CIRS) typified by the absence of objective diagnostic parameters without which there are no guides to physiology-based therapies.

For patients who survive sepsis, a chronic illness called persistent inflammation and catabolism syndrome (PICS) [21], often develops. While we have chosen the term CIRS to describe "chronic PICS" and other related illnesses related to chronic inflammation, the illness concepts are the same. The difference is that CIRS literature has data on many recognized biomarkers and a published treatment protocol [7], but PICS does not.

These biomarkers include symptom clusters for CIRS acquired following environmental exposures to biotoxins and inflammagens in water-damaged buildings (WDB), finding at least 8 of 13 clusters present. Proteomic biomarkers compared to controls [4] include (i) increased relative risk for specific HLA haplotypes; (ii) presence of a distinctive deficit seen in visual contrast sensitivity (VCS); (iii) reduction of mean levels of melanocyte-stimulating hormone (MSH); (iv) dysregulation of ACTH to cortisol and (v) ADH to osmolality; (vi) elevated C4a, (vii) TGF beta-1, and (viii) MMP9; (ix) suppression of vascular endothelial growth factor (VEGF); (x) increased incidence of antigliadin and (xi) anticardiolipin antibodies [7]. Functional CIRS-WDB biomarkers include (a) a distinctive “fingerprint” of volumetric abnormalities [22-24] seen on NeuroQuant”; (b) reduction of VO2 max and (c) anaerobic threshold shown by pulmonary stress testing; (d) elevated pulmonary artery pressures at rest, or (e) after exercise on echocardiogram; (f) transcriptomics.

**Focus on molecular hypometabolism**

The advances of understanding the physiology associated with MHM brought to light by use of transcriptomics are highlighted by the application of differential gene activation, stratified by age, comparing cases to controls for mRNA for ribosomal genes for the small ribosome subunit and differentiating those mRNA findings from the large
ribosomal subunit. Further, transcriptomics allows us to understand mRNA findings on mitoribosomes, both large and small subunits. These findings are important when evaluating NeuroQuant, looking at the role of commensal multiple antibiotic-resistant coagulase-negative staphylococci (MARCoNS), now found to make unidentified polycyclic ether toxins (unpublished). The role of mitochondrial gene suppression in association with MARCoNS is fertile ground for continued research.

When taken as a whole, with a correction for the high and low number of copies of mRNA, combined with an adjustment for age, we have seen that transcriptomics will identify molecular hypometabolism accurately in patients with CIRS and SEID. We also see these fundamental abnormalities in less commonly represented illnesses in our data set, including multiple sclerosis and cancer, for example. Disorders of inflammation, metabolism, and loss of regulation of differential gene activation, commonly seen in CIRS, SEID, and other chronic fatiguing illnesses, hold importance by defining pathological abnormalities in metabolism that may apply to other chronic illnesses.

Changes in MHM with therapy also provide a window on the treatment of CIRS, as the use of a peer-reviewed protocol [4] shows the correction of suppression of ribosomal and nuclear-encoded mitochondrial genes, especially translocases. The completion of initial treatment is marked by an overshoot of the number of copies of targeted genes compared to controls, with a return to control levels with the use of intranasal vasoactive intestinal polypeptide (VIP). The changes in individual mRNA counts seen in each of the MHM entities, are parallel in shape when graphed, describing the “CIRS curve” [25].

Metabolism overview

Trying to simplify the known biochemical pathways of metabolism runs the risk of oversimplification. Conversely, a more detailed discussion of metabolism creates a learning curve for the new reader with the use of multiple lengthy and often unfamiliar terms for reactants in a given metabolic pathway, especially given the ubiquity of acronyms. Looking at a typical metabolic pathway, say glycolysis leading to pyruvate production and then with pyruvate entering into the Krebs cycle and ETC, we see a deluge of names of new reaction products in the pathways, with each term also identified by a new acronym that interacts with others making another new compound with its own acronym, produced by a catalyzing enzyme with its new name and its acronym. Suffice to say, metabolism can be presented to students in a way that seems to be an inordinately complicated manner when the names of compounds and definitions of acronyms are not used frequently. It remains concerning that many physicians in primary care learned these pathways in medical school only to lose recollection of pathways and enzymes over time due to lack of daily use.

Like inflammation, metabolism is present in every physiological function; metabolism can regulate energy and growth, as well as direct the fate of cells, i.e., living or dying, not being functional or creating disease [1]. As always, regulation of metabolism and metabolic flux is under control of gene activity. Differences in gene activity create differences in the regulation of metabolic activity. As we will see, metabolism can regulate the differentiation of immune cells, including the reduction of the production of T regulatory cells, under conditions of proliferation.

We will focus on glucose, pyruvate, Warburg physiology, insulin receptor substrate 2 (IRS2), proteins, and amino acids, in the discussions below. Each of the sections that follow will look at the major sections of this initial discussion in greater detail.

Metabolic complications: metabolic acidosis specifics of glycolysis and pyruvate

Once glucose enters the cell, often by the facilitated transport from a family of solute carriers, called Glut 1 (SLC2A1) and Glut 4 (SLC2A4), it enters into a cytosolic pathway called glycolysis (also called the Embden-Meyerhof pathway). This series of enzymatic steps can generate needed precursors that can be used for other metabolic pathways, small amounts of ATP and pyruvate. Glycolysis is an evolutionarily conserved process used by all living organisms. As seen with the SRL, evolutionary conservation means all the possible mutations and nucleotide polymorphisms that surely had to occur over three billion years amount to no replacement of the glycolysis genes or pathway.

Glycolysis is a ten-step pathway of intracytoplasmic conversion of a 6-carbon ring, glucose, to create two copies of a three-carbon fragment, pyruvate. As an example of the abstruse names and the plethora of acronyms, we will use the glycolysis pathway as a typical example of jargon in metabolism, highlighted in the text that follows.

There are two phases to glycolysis. The first is called the “preparatory phase,” and the second is the “payoff phase.” The preparatory phase consumes two ATP to place phosphate moieties strategically on metabolites, with the return of a total of four ATP in the payoff phase. Diversion of metabolic precursors to other pathways subtracts from the net four generated ATP [26, 27].

The conversion of glucose to the first metabolite, glucose 6-phosphate, is accomplished at the cost of one ATP to fuel an important enzyme, hexokinase. As an aside, hexokinase has recently become the focus of much attention in degenerative central nervous system diseases, especially Alzheimer’s, as Apo E2 patients rarely will have Alzheimer’s and have a rich endowment of hexokinase, but Apo E4 is associated with CNS deterioration, with reduced hexokinase [28-30]. Once the first change of glucose has been made by hexokinase, the first diversion of glucose from its ultimate goal of producing pyruvate can occur. Here, glucose 6-phosphate can be shunted to participate in the synthesis of a storage compound, glycogen, a complex carbohydrate. A second diversion occurs when glucose-6-phosphate dehydrogenase (G6PD) is activated to convert G-6-P to ribose-5-P, the first step of the pentose phosphate shunt, a significant source of metabolites, especially nucleotides, needed for added cell division. This shunt is a crucial diversion used in proliferative physiology.

If glycolysis continues, the next step is to make fructose 6-phosphate by an enzyme phosphohexose isomerase. Fructose 6-phosphate is primed with its new stereochemistry, and at a cost of one additional ATP, the enzyme phospho-fructokinase-1 will add a phosphate group making fructose 1,6-bisphosphate. We can see where this change is headed, as now the 6-carbon ring can be cleaved into two pieces. Note that fructose-6-phosphate can lead to the hexoseamine biosynthetic shunt, part of the cell’s metabolic response to stressors such as infection, ischemia or trauma, for example (see HBP below).

This cleavage is accomplished by the enzyme aldolase, known for its role in muscular diseases, to make glyceraldehyde 3-phosphate and dihydroxyacetone phosphate, with the latter then converted by triose phosphate isomerase, thus making two molecules of glyceraldehyde 3-phosphate. If the word glycerol seems to be hidden in glyceraldehyde, there can be a diversion of glyceraldehyde to make glycerol. Alternatively, dihydroxyacetone phosphate can be diverted to participate in the synthesis of lipids. This step marks the crossover from the preparatory
phase to the payoff phase. Now the two molecules of glyceraldehyde 3-phosphate can be converted by oxidation and phosphorylation using the enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH) to make two molecules of 1,3 biphosphoglycerate. GAPDH is a vitally important enzyme that serves as a rate-controlling regulator of pyruvate production that, in turn, serves as a vital component of GAIT (see below) as will be discussed. GAIT provides a mechanism for cellular feedback control to downregulate inflammatory cytokine responses and maintain metabolism. GAPDH has other important roles as well.

GAPDH will also generate reduced NADH in this pathway in the next step, which is then used to facilitate the production of two additional ATP. Step 7 uses phosphoglycerate kinase to make two copies of a single phosphate molecule, 3-phosphoglycerate. Cleaving a phosphate group from each permits conversion of ADP to make a total of two ATP for each pyruvate. Remember, two ATP were consumed in the preparatory phase; now, two sets of two ATP are returned, making a net yield of positive two molecules of ATP. 3-phosphoglycerate can be converted to an amino acid, serine, fueling the production of other amino acids needed for protein synthesis.

Next, moving one phosphate is a necessary step to manufacture of two copies of 2-phosphoglycerate by phosphoglycerate mutase. Each 2-phosphoglycerate is converted by enolase to phosphoenolpyruvate. This compound is further converted by pyruvate kinase to make pyruvate. Pyruvate, if it can traverse the outer mitochondrial membrane and reach the mitochondrial matrix, feeds into the Krebs cycle (tricarboxylic acid pathway, TCA) where it can be converted to acetyl-CoA by pyruvate dehydrogenase (PDH), leading to lipid synthesis. PDH can be blocked by pyruvate kinase (PDK1). PDK1 is induced by hypoxia-inducible factor 1 alpha (HIF-1α), a compound that also activates the conversion of pyruvate to lactate. Further, HIF-1α can increase uptake of glucose through Glut 1 and 4 and activate hexokinase, setting off glycolysis. HIF-1α is important in aerobic glycolysis (see the section on PAH below). Interest in the use of PDK1 as a possible chemotherapeutic agent is noted, as use aerobic glycolysis typifies many cancers. HIF-1α also has a significant role in suppression of the production of T regulatory (reg) cells by activating the production of T effector cells.

As complex as the glycolysis pathway is with its multiple diversions, a basic tenet of metabolism is that the activation of one pathway will change the activity of competing pathways [31]. Add to that concept the idea that metabolism adapts to environmental change, which in turn creates a stimulus for differential gene activation, controlling discrete elements of metabolic pathways and inflammation. All of these changes create a cellular environment in which two consecutive measurements of gene activity and metabolism rarely are going to be similar unless the initiating stressor (exposure to ribotoxins, for example) is constant. Here is the putative role for inflammation controlling gene activation, with metabolic changes driven by gene activation that, are themselves regulated by inflammation. An exciting finding is that medications associated with salutary health effects [2,4,24] that change gene activation and inflammation will restore regulation of abnormalities of metabolism.

A prime example of gene activation, inflammation, and metabolic control comes from a review of the effect of the Randle cycle on glycolysis. As each pathway is mutually reciprocal on the other, an increase of the Randle cycle decreases glycolysis, in favor of fatty acid oxidation with its enriched source of ATP production. Increasing fatty acid oxidation, as follows a rise in IRS 2, activating Glut1 and Glut4, will suppress glucose catabolism, but at the cost of increasing oxygen consumption: burning fatty acids costs oxygen. Citrate, part of the Krebs cycle, will suppress phosphofructokinase, increasing glucose-6-phosphate, shunting the glycolysis pathway to increasing the storage of glycogen. This part of the Randle cycle creates the "second wind," as well-trained athletes know, as increased energy (9.3 calories/gram) comes from fatty acid oxidation, compared to 3.4 calories/gram from glucose oxidation.

Simply stated, direct fat burn stores glycogen. Fatty acid oxidation, in turn, generates acetyl CoA, which blocks pyruvate dehydrogenase, preventing pyruvate from being converted into acetyl CoA. This step leads to aerobic glycolysis and production of lactate, especially if pyruvate is also blocked from entering mitochondria across the VDAC by inflammation-induced reduction of translocases. The combination of the development of hypoxemia (from FAO) and aerobic glycolysis is the development of pulmonary hypertension [32]. Blocking fatty acid oxidation in isolated myocytes of the right ventricle leads to increased pyruvate burn and less oxygen consumption [32].

Meanwhile, the Krebs cycle is the site of the conversion of glutamine to glutamate, making available a free amino group crucial to glycossylation, discussed below. To find these ten metabolic steps evolutionary conserved seems surprising at first glance, and yet nature works in predictable ways. The amount of enzymatic work to make just two net ATP seems excessive, but the production of so many building blocks has two important additional benefits: (i) the manufacture of a reservoir of compounds needed for cell proliferation; and (ii) speed of ATP production. A theme in metabolism both in health and disease is the dual role of glucose in (i) energy conservation versus (ii) energy expenditure to support cell proliferation.

Glucose remains the coin of the metabolic realm as it leads to the production of pyruvate that can be further processed by an enzyme, lactate dehydrogenase (LDH), to generate lactic acid (lactate). In turn, lactate is moved by transport molecules out of the cell. Excessive amounts of lactate production can be suspected by seeing a widened anion gap in peripheral blood. However, even this seemingly simple calculation can be complicated by further metabolism of lactate in capillary beds adjacent to where it was produced, with reconversion to pyruvate, reducing the anion gap.

Pyruvate is the cellular metabolic treasure; it plays a dominant role in cell proliferation versus cellular conservation of energy primarily based on where pyruvate is further metabolized. Mitochondrial production of ATP depends on the delivery of pyruvate into the mitochondrial matrix. If delivery is compromised, cytosolic metabolism will be the default site for pyruvate metabolism. If there is too much pyruvate being made in the cytosol, or too little being consumed, look for suppression of GAPDH gene(s) to reduce pyruvate production, possibly contributing to insulin resistance. Some rapidly dividing cells will use the conversion of pyruvate to lactate as its primary source of ATP through "aerobic glycolysis," also called the Warburg Effect [33,34]. At first glance, this low yield conversion of pyruvate to make ATP, compared to what Krebs cycle and ETC pathways in the mitochondrial matrix would bring, seems dysfunctional. What benefit does a rapidly dividing cell glean from "wasting" the ability to create molecules used for energy? Cancer cells commonly use this so-called "Warburg physiology," to produce lactate, export it from the dividing cell, creating an adverse microclimate that serves as a defense to prevent T-cells from attacking growing cancer cells. Cells at the surface of the developing tumor use the reverse of cellular fermentation by reconverting lactate back to pyruvate as a source of ATP [34].
In CIRS, we worry about the Warburg Effect because it underlies the pathologic development of clinically significant metabolic acidosis, pulmonary hypertension [35]; it underlies abnormalities, including metabolism in branched-chain glycans (see below) that will lead to insulin resistance. Further, we also see multiple examples of injury to neuronal tissue associated with the Warburg Effect, both peripherally, creating peripheral neuropathy and centrally, possibly contributing to dementing illnesses, as discussed. The marker for the Warburg Effect that can harm CIRS patients, the anion gap, one that is readily calculated, but not so readily confirmed, is due to fermentation of pyruvate to make lactate. Excess lactic acid adds to or widens, the normal anion gap. To calculate the anion gap, add values of sodium (Na+) to those of potassium (K+), and from that sum, subtract the sum of CO2 and chloride (Cl-). If the number is 10-12, that is a normal anion gap. Beyond a gap of 14 or 15, however, we become concerned regarding the excessive presence of compounds that are anionic and contribute to the widened anion gap. The body uses lactic acid and bicarbonate to help regulate pH tightly; metabolic acidosis is commonly found in multiple disease states, including PICS and CIRS, among others, commonly including diabetes and renal failure.

**VDAC and pyruvate**

If pyruvate is not converted to lactate, we expect pyruvate to cross into the mitochondrial matrix to be used for fuel. Unfortunately, there is a channel, 2-3 nm in diameter [36], which creates a pore in the outer membrane of the mitochondria that is dependent upon the normal presence of translocases to function. Translocases, as the term [37] suggests, are involved in moving protein compounds from one cellular environment to another. In this case, for mitochondrial concerns, the pore will permit entry of solutes, ions, ADP and pyruvate through the outer membrane to reach the intermembranous space. Exiting the pore will be ATP that is produced by the electron transport chain in the matrix of the mitochondria and possibly reactive oxygen species (ROS). This pore, however, is closed by (i) absence of translocases, commonly seen in MHM, as discussed above but also (ii) through the activity of products made by Streptomyces and other actinomycetes [38], especially valinomycin; it is unknown if another actinomycetes product, Piericidin A, an irreversible inhibitor of Complex I, also uses the VDAC. Other factors closing the VDAC are tubulins [39], hexokinase [40], and itraconazole [41,42]. Actinomycetes metabolites can shut down flow in both directions across the pore, discharging the electrical gradient that maintains the opening [42].

Azole antifungal medications, unfortunately, are widely used by some involved in the management of illness acquired following exposure to the interior environment of WDB. This inappropriate use of azoles creates proliferative physiology; it must be recognized for its significant contribution to adverse metabolic outcomes, especially generalized metabolic acidosis, pulmonary hypertension, T reg cell deficiency, insulin resistance and neuronal injury.

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The complexity of the interaction of cytosol compartments and mitochondria, with each of its two membranes, is extraordinarily complex. Glucose metabolism products in the cytosol versus mitochondrial matrix are simply one example of compartmental complexity. The difficulty in assessing metabolic needs for each of these interactions is the difficulty measuring mitochondria activity within a living cell, understanding that there are thousands of mitochondria in a living cell, understanding that there are thousands of mitochondria functioning in each cell at a given time. Some, but not all, may be affected by other metabolic pathways. To summarize, how pyruvate is handled and where is vitally important in cell proliferation in which the Warburg Effect is effective, compared to cell conservation of energy where ETC is involved. The existence of distinct differences in the end results of pyruvate metabolism from either cytosol or mitochondrial matrix compartments underlies proliferative versus energy conservation physiology.

**Focus on the warburg effect**

Dramatic advances in medicine that are recognized by historians are relatively few. Lister and antisepsis; Jenner and cowpox vaccination to prevent smallpox; Pasteur on several fronts (including vaccination for anthrax, rabies; fermentation and Pasteurization); and Koch, regarding medical causation, come to mind. Advances in cataract care, CT scanning, and MRI technology have given the enhanced quality of life to so many. Otto Warburg gets less recognition than the thrust of this paper suggests he deserves. Perhaps if we had made broader advances in the treatment of cancers, Warburg would be better known, as his work defining aerobic glycolysis, as used by many malignant cells, was indeed seminal work [14].

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The concept that pyruvate was converted to lactate in the presence of oxygen, called aerobic glycolysis, not in the absence of oxygen (anaerobic glycolysis) was a radical idea one hundred years ago. The fact that cancer cells used aerobic glycolysis made Warburg’s observations even more dramatic. Warburg did not know enough about the role of mitochondria and oxidative phosphorylation, as Hans Krebs published his work on the eponymous Krebs’ cycle in 1937. He also did not have an explanation for the increased ammonia observed in his tissue preps, which is now recognized as a marker for active amino acid metabolism, including glutaminolysis. Warburg felt that mitochondria in cancer cells were dysfunctional, perhaps if he had known about VDAC, then he would have recognized that the dysfunction was an indicator of the closure of that pore in the outer mitochondrial membrane and that nothing was wrong with the mitochondria.

The fact that all cancers do not use aerobic glycolysis does not detract from the importance of Warburg’s discovery. While we know that in a given tumor, aerobic glycolysis and oxidative phosphorylation can coexist, we can speculate that slower-growing tumors use aerobic glycolysis less than faster-growing tumors. This concept can be summarized by the idea that a rapidly dividing tissue needs more cellular building blocks than energy conservation-driven cells in tissues with normal growth rates. These building blocks include nucleotides (influenced by G6PD) and lipids (influenced by TPI, triose phosphate isomerase) made during the first three steps of glycolysis.

Understanding that CIRS cases comprise the majority of those studied with transcriptomics to date, the finding of upregulation of CD14 and TLR4 are well correlated with exposure to water-damaged buildings (WDB) with elevated levels of endotoxins. Exposure to WDB with increased species abundance of actinomycetes is tentatively marked by the presence of ribosomal stress responses, including MAP kinases [45,46], with normal levels of a version of a building health index called Health Effects Roster of Type-Specific Formers of Mycotoxins and Inflamagens, version 2 [47]. Exposure to mycotoxins also can involve upregulation of MAPKs, among other genes, though greater in number than seen with exposure to actinomycetes. Exposure to mycotoxins is also marked by elevated HERTSM1-2, but simple exposure to mycotoxins is not sufficient alone to cause differential gene activation.

Transcriptomics and metabolism

The approach to metabolism in CIRS as a unique entity is complex, with multiple interacting systems of feedback regulation. While that source of complexity is daunting, understanding metabolism in CIRS is further complicated by a paucity of papers in the metabolism literature of the inflammatory gene database interacting with MHM. Novel discoveries, like MHM, form the basis for possible advances in science, but expansion of the application of new ideas can be a slow, step-by-step journey. We are further hampered by a paucity of proteomic testing results (TGF beta-1, MMP9, MSH, and VEGF) in the metabolism literature.

The use of a proprietary transcriptomic assay, GENIE, has shown us the in-depth importance of MHM as well as its multiple implications for cell physiology and metabolism. With MHM, one will first see issues regarding impairment of protein synthesis by disruption of messenger RNA (mRNA) for ribosomal genes that will impact vastly on initiation, elongation, and termination of the RNA signal at the sarciniricin loop (SRL) wedged between the large and small ribosomal subunits. The second issue has to do with energy production that is compromised by the impairment of the synthesis of mitochondrial genes found in the nucleus. Such nuclear-encoded mitochondrial RNA is produced following signals that may be disrupted by transcription factors, epigenetic factors, microRNA, as well as regulatory factors such as ribonuclear proteins. The metabolic importance of regulation of nuclear-encoded mitochondrial RNA production parallels the importance of protein interactions as well as modification of proteins seen in metabolic pathways with such modifications following glycosylation.

Similarly, when ATP synthases are also affected, mitochondrial function is impaired. There is little wrong with the mitochondria in these situations; the energy metabolism is subject to such a diverse series of dysregulations found in MHM that attempting to tease out individual aspects is difficult.

IRS2

As shown by GENIE, patients with MHM may have either up- or down-regulated responses to activation of insulin receptor substrate 2 (IRS2). IRS2 is part of a group of cytoplasmic proteins that are anabolic, preventing the development of Type 2 diabetes, in part by activating glucose transport channels Glut 1 and Glut 4, as well as activating pathways that are geared towards protein synthesis, cell proliferation, and cell survival [48]. If Glut 1 and 4 are open, but translocases are reduced, there will be an increased cytosolic load of glucose in the face of reduced pyruvate delivery to the mitochondrial matrix. Aerobic glycolysis and proliferative physiology will follow. IRS2 also helps coordinate extracellular signals via transmembrane receptors that regulate downstream pathways, including fatty acid oxidation (FAO), P13K/Akt/mTOR and MAPK [49].

IRS2 is one of the major mediators that responds to insulin and insulin-like growth factor (IGF1). If IRS2 is upregulated in the face of hypometabolism, creating a “mismatch,” in that one of the mechanisms to control cellular survival in the face of MHM is to downregulate production of pyruvate by suppressing glycolysis. If IRS2 is upregulated in MHM, activation of Akt and MAPK proceed unchecked, wasting energy and cellular building blocks both. This mismatch can be disastrous for the MHM cell metabolism as it becomes a prescription for enhanced Warburg Effect. Conversely, if MHM is absent and IRS2 is downregulated, normal cell survival and protein synthesis would again be compromised as the cell will have compromised delivery of glucose to an intact system of (i) pyruvate production by glycolysis and (ii) pyruvate delivery into the mitochondrial matrix.

When IRS2 is down-regulated, Glut1 and Glut4 transport are impaired [50]. With such impairment, a major source of glucose delivery becomes an endosome containing an insulin receptor bound to insulin and bound to glucose that is moved from the cell membrane inside the cell [51]. This endosome, functioning like an insulin bubble surrounded by a membrane, is held intact in the cytoplasm until it is acidified. If hydrogen ion is delivered inside the endosome, there will be release or recycling of insulin and insulin receptors back to the cell membrane and delivery of glucose for glycolysis [52]. In the presence of polyclassic acid ether toxins made by actinomycetes species, primarily Streptomycins, including monensin and nigericin, there will be impairment of acidification of the endosome and absent or reduced release of glucose, with little recycling of insulin and insulin receptors [53]. There is no dearth of insulin, no dearth of insulin receptors, but functionally insulin resistance is created. This mechanism of enhanced storage of insulin, insulin receptors and glucose inside the cell may be a form of “intracellular insulin resistance” [53], as opposed to the “extracellular” insulin resistance created by glycosylation of proteins in adipose tissue (see below).
Metabolic complications: pulmonary hypertension (PAH)

Pulmonary hypertension (PAH), an increase of pressure between the right ventricle and the lung in the pulmonary artery, remains underdiagnosed. The methods used to diagnose pulmonary hypertension are either a static measurement at heart catheterization or a potentially dynamic measurement of baseline echocardiogram measurements that indirectly permits the calculation of pulmonary artery pressure. Performed as an echocardiogram exam, with the potential to do a stress echocardiogram looking at the rise of pulmonary artery pressure in exercise, confirmation of elevated pulmonary artery pressure is common in CIRS [7]. Rarely is heart catheterization performed to diagnose pulmonary hypertension; measurement of elevated systolic pressure in the pulmonary artery (PASP) is regarded as an ancillary exam when concerns about left ventricular function are dominant. The difficulty of obtaining a stress echo to diagnose acquired pulmonary hypertension is multiplied by the fact that most cardiology stress test protocols are based on a search for left ventricular ischemia. Coronary artery disease and left ventricular function, especially ischemia in coronary disease and ejection fraction in heart failure, are normally of much greater clinical concern to cardiologists than evaluation for PAH.

During a baseline echocardiogram, the velocity of flow moving backward across the tricuspid valve, called the tricuspid jet, is used to calculate pulmonary artery pressure (PASP). Taking the square of the tricuspid regurgitant velocity and multiplying that number by 4, gives us a definable idea of PAH. Adding the baseline pressure found in the right atrium completes the calculated pulmonary artery pressure. For adults, any calculation of PASP, measured in four different ways during an echocardiography, was found in a 2013 study [4] to be present in nearly 50% of adults with CIRS. In the 2013 study, VIP was provided at four sprays a day (50mcg/dose). At the end of one month, if pulmonary artery pressure had not returned to normal, the dose of VIP was increased to 8 sprays a day. After two months, the dose was re-titrated to lowest dose needed for correction.

Since that study was published, there has been a surge of scholarly publications, notably, including those from Dr. John Ryan at the University of Utah Medical Center. We now know that aerobic glycolysis remains a fundamental cellular mechanism that results in PAH. In PAH, normal PASP is increased by the proliferation of endothelial tissue both lining blood vessels and in the middle of blood vessels; as well as by vascular stiffening, clotting in small arterials, and presence of an inflammatory lymphocytic infiltrate. If the condition continues undetected, possibly in concert with elevated levels of TGF beta-1, there will be endothelial to mesenchymal remodeling that stiffens small arterial vessels as well as narrows them to the point of blockage. The complications of pulmonary hypertension include right ventricular failure with a 5-year survival rate of less than 50% [54].

While PAH has been related to toxin exposure, the role of exposure to water-damaged buildings, especially actinomycetes, is evolving. We must rule out thrombotic sources of PAH, typically pulmonary emboli, when considering PAH due to proliferative physiology. Now that we know that proliferative changes resulting from aerobic glycolysis are common in CIRS, the data on the etiology of PAH will be demanded beyond interstitial lung disease, thrombosis and inflammatory. As compared to the evaluation of the Warburg effect in cancer cells, where proliferation follows increased glucose uptake, in PAH, there are greater numbers of influences, especially hypoxia and inflammation, with all leading to metabolic stress. The role of mitochondrial inputs, while possibly reduced due to closure of VDAC, still includes dysregulation of ETC, leading to ferroptosis.

The metabolic stressors of oxidative stress, inflammation, and hypoxia are a threefold stimulus to abnormalities of gene pathways involving hypoxia-induced factor 1a, but the metabolic complexity includes the pentose phosphate shunt, glycolytic programming and mitochondrial induced apoptosis [55]. These pathways include the pentose phosphate pathway, glutaminolysis, iron, hemostasis as well as fragmentation of mitochondrial itself. Also, abnormalities of fatty acid metabolism, stimulated in part by IRS2, as discussed previously, can be recognized by increased circulation of free fatty acids as well as accumulating elements that create lipotoxicity. The interaction of fatty acid oxidation (FAO) and glucose oxidation are inversely proportional, as evidenced by the function of the Randle cycle in which oxidation of fatty acids impairs glucose oxidation, and vice versa.

CIRS includes features in which excessive use of aerobic glycolysis is routinely noted. The first is in pulmonary hypertension, possibly due to toxic exposure; the second is due to deficient production of T-reg cells in a rapidly expanding pool of immune lymphocytes (see below). In pulmonary hypertension HIF-1α dependent upregulation of pyruvate dehydrogenase kinase (PDK1 and PDK2) blocks pyruvate dehydrogenase. This blockade prevents the conversion of pyruvate to acetyl-CoA in the mitochondria, which then leads to failure of the mitochondrial electron transport chain production of ATP as well. The findings in pulmonary hypertension may well involve pathways focusing on glutaminolysis, including the hexokinase and hexosaminidase pathways [56-58].

Another central question in pulmonary hypertension and, to a lesser extent, T-regulatory cell depletion is the role of hypometabolism. In hypometabolism, if IRS2 genes are suppressed, there will be reduced stimulus for glycolysis, reduced Glut-1 and Glut-4 activity, as well as reduced activation of the Akt pathways; reduced fatty acid oxidation pathways and reduced MAP kinase pathways. When these factors are combined with activation of platelet beta-tubulin genes, either TUBB1 or TUBA4A, and especially if combined with upregulation on multiple coagulant genes, we are looking at a microvascular flood of glucose shunted toward proliferative physiology; reduction of Krebs cycle, reduction of NADH production; reduction of oxidative phosphorylation; and reduction of hexokinase and hexosaminidase pathways. As opposed to the classic Warburg Effect, which will have activation of glutaminolysis to try to activate the Krebs cycle, molecular hypometabolism suppresses ribosomal and mitochondrial functions and includes both enhanced and reduced coagulant gene activity. The variability between Warburg Effect findings and current findings in pulmonary hypertension in CIRS patients is that we now know that treatment of the inflammatory response will reanimate the metabolic pathways and return the patient to normal with reduction of pulmonary artery hypertension. With the suppression of HIF-1α pathways, combined with suppression of Akt pathways and suppression of MAP kinase pathways, we have a prescription for the three elements needed to stimulate the production of T-regulatory cells [58]. In the face of stimulation of constant bombardment of innate immune receptors with inflammasgers in CIRS, there is an upregulation of metabolic pathways that is accompanied by widened anion gap, usually due to increased capillary levels of lactic acid that must be corrected in order for pulmonary hypertension itself to be corrected.

A common path of physiologic features includes endothelial dysfunction, excessive proliferation, impaired apoptosis of vascular
cells, and mitochondrial fragmentation. The proliferation/apoptosis imbalance relates in part to activation of transcription factors, hypoxia-inducible factor 1-α and nuclear factor of activated T-cells (NFAT), and apoptosis. Perivasculary inflammatory disruption of adventitial connective tissue and a glycolytic metabolic shift in vascular cells and right ventricular myocytes also occurs in pulmonary hypertension. These are also seen in target tissue in the right ventricle and skeletal muscle, reflecting the systemic nature, not of just glycolysis but the entire metabolic shift driven by nuclear transcription factors. Pulmonary arterial hypertension is an obstructive pulmonary vasculopathy, characterized by excessive proliferation, apoptosis-resistant inflammation [59].

Fortunately, the treatment protocol discussed earlier with each of the 12 steps effectively corrects hypometabolism, stops the Warburg Effect, permits correction of pulmonary hypertension, permits restoration of T-cell populations, blocks metabolic acidosis, and begins the process of neuronal healing. All these benefits have been identified from the use of a measurement of molecular hypometabolism in gene activation, by application of transcriptomic testing.

**Metabolic complications: GAIT is one of many biologic controls on inflammation**

The gamma interferon activated inhibitor of translation complex (GAIT) consists of four elements (glutamyl-prolyl-tRNA synthase); NS1-associated protein 1 (NSAP1); ribosomal protein L13a and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) [60]. Perhaps no other feedback system exemplifies the tightly regulated interactions between inflammation and metabolism than GAIT. This grouping can bind to translation regions of given inflammation genes, including VEGF, to inhibit their translation. While there are differences in the inflammatory response between mice and humans, the well-described regulatory GAIT system in mice is similar to the less well described human GAIT system [61].

GAIT is an extraordinary complex negative feedback regulation system that helps slow down the over-translation of inflammatory genes. Moreover, it acts as an additional mechanism to slow down glycolysis by removing GAPDH from the pathway of conversion of glucose to pyruvate. By blocking pyruvate production, the cell can avoid producing lactic acid when there are problems with molecular hypometabolism affecting mitochondrial pathways, including the Krebs cycle and ETC. Specifically, in the absence of normal production of mitochondrial membrane translocases, it teleologically serves the cell well to “hibernate,” reducing inflammation and reducing the creation of pyruvate until the hypometabolism has been resolved.

The GAIT proteins are metabolically expensive to produce and operate. Gamma interferon serves to activate kinases in multiple discrete steps by which a phosphate group is placed on specific amino acids (usually serine or threonine) to change protein structure and function. By linking interferon, produced as a result of inflammation, to a mechanism to control glycolysis, together with a mechanism to control the initiation of protein synthesis, the cell obtains the ability to control the potential for multiple adverse effects caused by inflammation [61].

It is instructive to note that levels of plasma VEGF in CIRS cases fall into three groups: suppressed, over-expressed, and no different from controls [4]. Each result is approximately 33% in prevalence for VEGF in CIRS cases. VEGF itself is induced following interferon-gamma treatment within 8 hours but by 24 hours has returned to basal levels. We see this phenomenon in the repetitive exposure protocol called SAIIE (sequential activation of innate immune elements) in which on day one of re-exposure, VEGF levels rise rapidly only to fall by day 3 to nadir. The mechanism here is translational silencing by the GAIT system of VEGF.

There are GAIT effects on other genes of uncertain influence on the progression of CIRS, including ZIKP, Cdks as well as ceruloplasmin. DIPK and ZIPK are felt to be similar to housekeeping genes in that they mediate phosphorylation of the protein L13a, therefore functioning as GAIT internal regulatory elements [62].

**Myeloid cells:** In CIRS, we see the diverse metabolic effects seen in myeloid cells during innate immune responses [63]. First studied in a Toll 4, CD 14 system looking at the programming of myeloid cells following endotoxin exposure, thought initially to be replicated in other toxin exposures, is now showing the remarkable diversity of “rewiring” that takes place after a diversity of exposures [63]. This emerging field, called immunometabolism, is now felt to be a problem in innate immune responses specifically increasing the availability of small molecules able to manipulate a diverse array of metabolic roots.

Cellular metabolic pathways are felt to serve three major functions: (i) generation of energy; (ii) production of building blocks necessary for cellular maintenance; and (iii) proliferation and modulation of cellular signaling. Immune cells in forced stimulation are not activated to either proliferate or increase uptake of nutrients. Following antigen presentation to myeloid cells, there is a need for the rapid expansion of myeloid cell population such that building blocks, including nucleotides and lipids, are generated by repurposing metabolic pathways. As the immune cell population is selectively expanded, there will be new signaling molecules produced to organize the inflammatory response [63].

As expected, with the rapid expansion of cell lines, there will be a tendency to use aerobic glycolysis. In the presence of pyruvate dehydrogenase kinase, there will be suppression of the Krebs cycle, reducing the production of acetyl-CoA (and therefore, lipids from citrate). Much of the evidence involved with the metabolism of myeloid cell activation comes from stimulation of toll receptor 4 (TLR4) by bacterial endotoxin, lipopolysaccharide (LPS).

As discussed previously in this chapter on aerobic glycolysis, this is the function of the rapidly expanding cell lines. New data suggests that in addition to increasing the rate of glycolysis, there are additional metabolic signatures in myeloid cells separate from the Warburg Effect. The diversity of metabolic responses in myeloid cells is remarkably conserved. One of the mechanisms to bring about the long-term reduction of innate immune memory involves epigenetic changes involving methylation and demethylation, or acetylation of histones or deacetylation of histones. These epigenetic findings can impact on Krebs cycle physiology as well as differential gene activation [63].

The presence of chronic inflammatory environmental conditions can alter the microenvironment but also alter transcriptomic responses. Both elements can contribute to the migration of macrophages into tissue as well as T-regulatory cells. While perhaps better studied in tumor cells, lactate can initiate pro-inflammatory effects as well as prompting tumor angiogenesis. Thus, metabolic changes in immune cells accumulating in blood vessel walls, as in margination, or in joint capsules promoting inflammation as the disease progress, possibly including atherosclerosis [64].

Of interest are the associated findings with the pentose-phosphate shunt in which accumulation of succinate, brought about the
disruption of the Krebs cycle, leads to stabilization of HIF-1α, which as a transcription factor will induce production of an inflammatory cytokine, interleukin-1β (IL-1β). If the mitochondrial mechanisms, including translocases, are not adversely impacted as seen in hypometabolism, LPS stimulation will cause remodeling of the electron transport chain itself, promoting generation of mitochondrial ROS which serves a role to assist in killing microbes [65].

Similarly, another element of this Krebs cycle, citrate, can be exported from mitochondria to the cytoplasm following LPS stimulation, which in turn will stimulate the expansion of endoplasmic reticulum as well as Golgi bodies, that in turn support cytokine release from dendritic cells [63]. What we are seeing is use of metabolic networks to regulate inflammatory processes throughout the cell, including nuclear transcription and mitochondrial function.

As discussed, IRS2 activation will stimulate the activation of mTOR, which is an important stimulator of the metabolic response of the cell, affecting nutrient availability and energy demands, together with PI3K/ Akt. This combination drives aerobic glycolysis to meet the demands of actively dividing cells. The regulatory counter-balance is the activation of AMPK, which enhances regulation of oxidative phosphorylation, promoting both ATP production and inflammatory properties [66].

**Metabolic complications: insulin resistance hexosamine biosynthetic pathway (HBP)**

The layers of regulatory interaction of the transcriptome, inflammation, and glucose metabolism are well-exemplified by an important metabolic pathway for glucose (but only affecting 3-6% of intracellular glucose), the hexosamine biosynthetic pathway (HBP). This pathway provides another “side street” for glucose entering glycolysis. Remember that when glucose is converted into glucose-6-phosphate, that compound can be used (i) to make glycogen or (ii) enter into the pentose phosphate shunt; or (iii) be converted into fructose-6-phosphate. If (iii) applies glycolysis can continue, or fructose-6-phosphate can enter the HBP, especially if GAPDH is suppressed. This pathway is intimately involved with the response to stressors, particularly inflammation, ischemia, trauma, or oxidative stress from ROS. The HBP is called the survival pathway [67] by keeping serum glucose higher than normal. Recently, the HBP is identified as the survival pathway that leads to insulin resistance, as will be discussed. In addition to providing extra nucleotides needed for cell proliferation, HBP provides the needed building block, UDP-GlcNAc, required for the production of glycans (O-linked-N-acetyl glucosamines, O-GlcNAc) that in turn regulate countless proteins and transcription factors [68,69].

Looking more loosely, in the necessary presence of glutamine, we see the regulatory control which impacts on HBP begins at the second step in glycolysis where fructose-6-phosphate is changed by glutamine-fructose-phosphate amidotransferase (GFAT), especially in the concomitant presence of hypoxia, by adding glutamine to make glucosamine-6-phosphate. An acetyl CoA is added to make glucosamine-N-acetyl-6-phosphate, followed by rearrangement of the location of the phosphate. Then the nucleotide uridine phosphate is added from nucleotide pools to make uridine diphosphate N-acetyl-glucosamine (UDP-GlcNAc).

This product can now be added by posttranslational protein modification to membrane-bound or secreted proteins on serine or threonine moieties on hundreds of nuclear and cytoplasmic proteins via a modular process called O-glycosylation involving the enzyme O-GlcNac transferase (OGT). OGT is evolutionarily conserved and is the only enzyme that does the actual glycosylation. Acute cellular stress increases UDP-GlcNAc; increasing levels results in increased cell survival [67], and then feeds back to suppress GFAT [68]. While O-glycosylation is involved with many feedback control systems, the survival pathway also stimulates O-glycosylation to make defensins, non-specific acute phase neutrophilic peptides used in host defence against bacteria and viruses.

Opposing UDP-GlcNAc and OGT is N-acetylglucosaminidase (O-GlcNacase; OGA), also evolutionarily conserved, which is the only enzyme that removes the glucosamine from previously O-glycosylated proteins, acting primarily in the cytosol. The flux through the HBP that excessively favours OGT over OGA is implicated in the development of insulin resistance. Work on adipocytes shows impairment of insulin-stimulated glucose uptake, caused in part by (i) increasing glucose toxicity; (ii) decreasing GLUT 4; (iii) increasing glycosylation of IRS-1 and Akt [70]; and (iv) is blocked by inhibition of GFAT [68].

A known biological antagonist of OGA is O-(2-acetamido-2-deoxy-D-glucopyranosylidene) amino-N-phenyl carbamate, (PUGNAc) [67, 69, 70]. PUGNAc blocks the removal of glycans placed by OGT by inhibiting OGA. Glucose utilization was markedly impaired in the presence of PUGNAc; phosphorylation of IRS and Akt was impaired, and insulin resistance was markedly increased. Theoretically, downregulating gene activation for PUGNAc could have salutary health effects in insulin resistance and via OGA activation, decreasing inflammation.

Understanding that the sources of insulin resistance are multifactorial; and returning to the role of capillary hyperperfusion in CIRS, we can speculate to what extent prevention of hypoxia, which activates HBP, by (i) reducing metabolic acidosis, by (ii) preventing proliferative physiology; by (iii) enhancing VDAC, thereby (iv) enhancing energy conservation from mitochondrial matrix function would provide salutary benefit in protection from decreased glucose uptake and protection from O-glycan induced insulin resistance.

**Inside insulin resistance**

Using a T3-L1 cell line that expresses the GLUT4 transporter, a well-characterized model of the study of insulin resistance, Vosseller and Wells show the effect of excessive glycosylation by OTG by reduction of OTA or blockade of OTA by PUGNAc created a reduced activation of downstream effectors of insulin receptors [71]. By identifying the glycosylation status of individual members of the insulin signaling cascade, including insulin receptor substrate and β-catenin, insulin resistance was recognized. The study shows that metabolism incorporates post-translational regulatory modification of proteins, accentuated by the flux of glucose through the hexosamine biosynthetic pathway.

The role of glycosylation extends beyond insulin resistance. Glycosylation is catalyzed by only one enzyme, OGT. Removal of O-GlcNAc from proteins is catalyzed only by one enzyme, OGA. The balance of OGT versus OGA can be altered by the activity of PUGNAc, which preferentially blocks OGA [69]. Abnormalities of proteins, including transcription factors, are linked by glycosylation which can influence (i) transcription activity; (ii) DNA binding; (iii) localization; (iv) for stability in interaction with cofactors. The role of regulation of glycosylation affecting transcription factors shows excellent potential for new approaches for the treatment of diabetes and obesity.
Despite the promise for new insights, awareness of O-glycosylation is not new. Indeed, Kamemura, in 2003 discussed the balance of glycosylation versus phosphorylation of nucleocytoplasmic proteins as having a role in the metabolic control of signaling and transcription [72]. The role of OGT back then was identified as necessary for stem cell viability, making GlcNAc essential for life in multicellular creatures. Curiously, the gene encoding OGA maps to a locus important to late-onset Alzheimer's disease. This link of the blockade of glycosylation to neuronal injury underscores the importance of the combination of inflammation, increasing csgylation elements bound to beta-amyloid with metabolism. The interaction of glycosylation and phosphorylation is of great importance for regulatory proteins, variously including estrogen receptors, Sp1, endothelial nitric oxide synthase and B-catenin. This paper emphasizes the interaction of protein modifications in metabolism to which we add the additional role of interactions with known confounders, inflammatory responses.

Consistent with the research done in the early 2000s, Park, in 2005 [70], extends the cell culture basis of insulin resistance to glycosylation and modification of IRS2 and Akt2 by PUGNAc. PUGNAc has no effect on GLUT4; it has an increased effect on glycosylation of insulin signaling intermediates, reduces the insulin-stimulated phosphorylation on IRS2 and Akt, subsequently leading to insulin resistance in adipocytes.

By 2010, additional studies of insulin resistance, focusing on the main tissues involved with glucose clearance, i.e., adipose tissue and striated muscle, added to understanding the important role of the hexosamine biosynthesis pathway [68]. Once again, we see modifications of protein by glycosylation being a modulator of insulin sensitivity in fat cells. Here we see the interaction of adipose tissue for disposal of glucose, interacting with leptin as a central element in energy homeostasis. The conclusion is that adipose tissue factors are key regulators in maintaining glucose homeostasis, shown in animal studies. Metabolism focusing on glucose extends to fat cell hormones and adipocytokines. The recurrent theme is the hexosamine biosynthesis pathway in glycosylation controls the endocrine function of adipocytes mediating insulin resistance.

A tantalizing approach to the treatment of insulin resistance has to do with blockade of the crucial enzyme GFAT. The use of azaserine or 6-diaz-o-oxonolouric acid inhibited glucose-glycolate resistance in fat cells. On the other hand, the treatment of cells with glucosamine showed a reduction of insulin-mediated glucose uptake. This effect is not blocked by azaserine.

Additional work from Wells and Teo published in 2014, underscores that while glycosylation is involved in the regulation of adipocyte cytokine secretion upon induction of insulin resistance in human fat cells, the investigators identified 190 proteins that are secreted, as well as 20 proteins that are upregulated and 6 that are downregulated in insulin resistance [73]. Glycosylation can be more than just oxygen mediated; nitrogen and sulfur both can contribute to the creation of glycans. Here 91 glycosylation sites were derived by 51 secreted proteins as well as 155, and 29 N-O glycosids, respectively, were also identified. Quantification of N-Glycan structures shows how genuinely complex insulin resistance is. None of these studies done in cell culture or animals controlled for molecular hypometabolism, which further compiles the issue of precisely what contributes to insulin resistance.

The HBP generates aminosugars; and provides building blocks for glycosyl side chains, proteins, and lipids. All these effects begin with the regulation of GFAT together with OGT, which catalyses a reversible, posttranslational protein modification in O-linkage to specific serine/threonine residues [74]. In this sense, HBP acts as a cellular nutrient sensor and plays a role in the development of insulin resistance, not to mention the vascular complications of diabetes. Crucial in this last function, complications of diabetes are the induction of TGF beta-1 and plasminogen activator inhibitor-1 in vascular smooth muscle cells, renal cells and aortic endothelial cells. In this era of implantable insulin pumps, speculation about titrating inhibitors and activators of GFAT has been discussed, but there are no published studies to confirm a "magic bullet" for the treatment of diabetes and obesity.

Metabolic complications: T regulatory cells and proliferative physiology

The fourth element of CIRS in which proliferative physiology plays a crucial role has to do with the balance of production of T-regulatory cells versus T-effector cells. Years ago, Quest Baltimore set up a T-regulatory cell assay for my office use. Acquired T-regs were CD4+CD25++. Thymus-derived T-regs were CD4+CD25++ CD127- io. The CD25 indicates a binding site for IL2; the CD127 is a binding site for IL7. Having these two types of T reg cells enumerated by flow cytometry provided a rapid determination of whether CIRS patients had deficiencies of T-regulatory cells or not. It also became clear that treatment with VIP corrected the deficiency of T reg.

T-reg cells will interact with retinoic acid orphan receptors in tissue to suppress tissue-based inflammation and reduce autoimmunity. If ROR is not present in normal amounts, then the T-reg cells were plasticized and made into T-effector cells. These cells made more TGF beta-1, setting up a positive feedback loop leading to a deficiency of T reg. For years, the feedback loop idea provided a working hypothesis to explain why low levels of T-reg were linked to putative deficiencies of ROR, but there were no assays to demonstrate ROR without obtaining tissue by biopsy. In retrospect, the unconfirmed idea assigning causation by ROR had little support. What is supported now is that impairment of normal production of T-reg cells in favor of T-effector cells accrues when lymphocytes are being stimulated to proliferate in response to a metabolic or inflammatory stressor. Indeed, this mechanism is one that has been identified for years. Unfortunately, the link between metabolism and inflammation; and T reg suppression has received reduced attention in current literature. What we find in T-regulatory cells in that repression of Akt/mTOR, hypoxia-inducible factor-1 alpha (HIF-1α), and aerobic glycolysis are important for suppression of efficient generation of T-reg cells. Those same pathways suppress T-reg cell development and mature T-reg cells as well [75].

The link between IRS2 and Akt/mTOR, glycogen, HIF-1 alpha and aerobic glycolysis, as discussed in metabolic acidosis and pulmonary hypertension, follows the same pathways in lymphocytes. The net result is that there is a varied effect of the same molecular basis in metabolism that affects human health.

This basic mechanism is added to by the role of Toll-like receptor signaling that will promote T-reg cell differentiation increases Akt/mTOR, HIF-1 alpha and aerobic glycolysis. If we add in an additional confounding factor, transgene expression. For this article, I have added this activation of Akt/mTOR so that the reduction of glycolysis that follows can increase oxidative metabolism and increase T-reg cells.

Metabolic complications: Brain atrophy

Given the commonality of aerobic glycolysis in proliferative physiology seen in the systemic illness of those with chronic fatigue, a search was extended to the literature looking for evidence for
proliferative physiology in the brain. The observations of (i) grey matter nuclear atrophy (ii) atrophy of cortical grey; and (iii) superior lateral ventricle enlargement as seen on NeuroQuant are consistent with what we would expect to see if there were adverse effects of proliferative physiology in the brain. Finding such evidence would complete the unifying thread that encircles CIRS as a metabolic disorder causing the end-organ injury seen in chronic fatigue-symptoms.

Work from the lab of Yellen, including Diaz-Garcia, has provided evidence of the extrapolation of systemic proliferative physiology to the brain [76]. Neuronal stimulation itself triggers neuronal glycolysis, not surprising since 20% of whole-body energy is required for normal brain function. During brain stimulation, aerobic glycolysis will preferentially be used by neurons as opposed to oxidative phosphorylation and ETC activation in mitochondria. Whether proliferative physiology is confined to neurons or generalizes to other brain cells, is not clear. The authors used hippocampal slices of mice to show that neuronal metabolic response to stimulation does not depend on astrocytic contribution, nor do they require neuronal uptake of lactate.

This paper from Yellen’s lab looks for aerobic glycolysis in transient response of the brain to activation [77]. The possible cellular mechanisms for metabolic resupply of energy and neurons were also addressed. Unfortunately, without consideration of voltage-dependent anion channels, we are left with an open question regarding the continuation of proliferative physiology following stimulation.

An additional question regarding neuronal consumption of oxygen and glucose has to do with the plasticity of activity given a different set of activity patterns. The speculation was that different gene expression programs would be involved. The authors note the crucial role of MAPK/ERK but again do not discuss MAPK activation following IRS2 activation [78]. Based on MAPK activation of other tissues, one would expect to find IRS2 activation as a precursor. Here again, changes in gene activation will be subject to translocase function and VDAC opening.

Again, from Yellen’s lab [79], metabolism will increase during stimulation, but not all energy metabolism is equally affected. Given an increase in local cerebral blood flow with increased glucose uptake, not matched by a similar increase in oxygen consumption, suggests that glycolysis becomes a dominant metabolic pathway producing energy. This would correspond to a temporary increase in lactate production well measured at the bedside except with magnetic resonance spectroscopy. This paper provides evidence that stimulated neurons become exporters of lactate, suggesting proliferative physiology.

If proliferative physiology is present as the review above suggests, can we extrapolate to adverse effects of proliferative physiology with neurodegeneration, particularly Alzheimer’s disease? Here a variety of central nervous system injuries, including the presence of beta-amyloid, reactive astroglisis (which can disrupt normal glycolysis), weakens the relationship of astrocytes to neurons in the purported astrocyte shuttle of lactate to neurons; impairs normal brain homeostasis; impairs clearance of beta-amyloid; promotes cytokine release and inexorably leads to neurodegeneration [80].

Microglial cell metabolic physiology similarly is at risk for proliferative physiology. Microglial cells routinely will use glucose, fatty acid oxidation, and glutamine, with glucose transporters expressed, to supply sufficient glucose intake [81]. Microglial-fueled metabolism may be associated with glial reactivity with a fuel switch contributing to an underlying cause of hypothalamic dysregulation associated with obesity. No comment on proliferative physiology is made.

Supportive cells, including astrocytes and microglial cells aside, neurons also have cellular metabolic demands at nerve terminals. Neurotransmission requires ATP to support energetic demanding steps, including maintenance of ionic gradients or sodium/potassium pumps, as well as reversing changes in intracellular calcium that arise from opening voltage-gated calcium channels [82]. Energy demands in the brain are met by glycolysis and oxidative phosphorylation, with glycolysis dominating the early stages of recovery of synaptic function; and with dysregulation of glycolysis contributing to neurodegeneration.

Finally, the role of glycogen availability comes into play during the intense activity of the nervous system when energy demand exceeds supply. Glycogen in astrocytes is converted to lactate, some of which is transported to neurons, thereby providing protection against hypoglycemia and preservation of neuronal function [83]. If the conversion of lactate to pyruvate is impaired during cerebral metabolic stress, the protective effect of lactate will be adversely impacted.

Taken together, we have supportive data on the adverse effects of proliferative physiology in the brain involving neurons, astrocytes, glial cells, and synapses. Given the role of molecular hypometabolism in proliferative physiology, the next steps in brain research on metabolic abnormalities found in aerobic glycolysis in SEID AND CIRS are supported.

**The study**

Using a retrospective waiver for transcriptomic studies from Copernicus Group IRB, Cary, NC, clinical data were collated from chart review of 112 consecutive de-identified patients who were having transcriptomics testing done, including MHM testing; but also usually having anion gap, pulmonary artery pressures, and NeuroQuant testing. Not all cases with transcriptomics had an ancillary study. These results were compared to published control groups from a single medical clinic.

**Methods**

The molecular methods used for transcriptomic testing are presented with permission.

**RNA collection, extraction, and labelling**: Venous blood was drawn from the arms of subjects into PAXGene RNA blood collection tubes (http://www.preanalytix.com/product-catalog/blood/rna/products/paxgene-blood-rna-tube/), incubated for four hours at room temperature, then frozen at −80 °C until RNA extractions were performed. Total RNA was extracted with the Qiagen PAXGene Blood miRNA System kit according to the manufacturer’s protocol. The total RNA was analyzed using an Agilent 2100 bioanalyzer (Agilent Technologies, USA) for RNA integrity, quantified using a NanoDrop ND-2000 (Wilmington, DE). Only samples with Agilent RIN scores ≥ 8 were used for sequencing.

**Sequencing**: Sequencing libraries were made from 1ug of total RNA starting material with the Kapa Biosystems stranded mRNA-Seq kit (www.kapabiosystems.com), according to manufacturer’s instructions for the Illumina platform. The amplified library fragments were purified and checked for quality and concentration using an Advanced Analytical Fragment AnalyzerTM to check the size and quality of the individual libraries and a Qubit for concentration. Equimolar amounts of individual libraries were pooled into groups of eight with a final round of quantification using an Agilent 2100 Bioanalyzer with a High Sensitivity DNA chip (Agilent Technologies, USA). The pooled samples of eight were sequenced on an Illumina Nextseq 500 DNA sequencer, using Version 2, high output, 75 bp single-end sequencing reagent kit
Shoemaker RC (2020) Metabolism, molecular hypometabolism and inflammation: Complications of proliferative physiology include metabolic acidosis, pulmonary hypertension, T reg cell deficiency, insulin resistance and neuronal injury.

The raw data files were streamed to the BaseSpace data warehouse (basespace.illumina.com) and de-multiplexed by sample into fastq files.

**Sequencing analysis:** Fastq sequencing data were imported into CLC Biomedical Genomics Workbench (BGW) analysis software version 4.0 and mapped to gene regions of the human genome using the USC hg37 build. Samples were divided into patient and control classes and subjected to Empirical analysis of DGE, as available in EdgeR Bioconductor package (Robinson et al., 2010). Empirical analysis of DGE was run using all raw data, as suggested in the user guide, but also after using different quality filters on the data. Data filtering consisted of removing hemoglobin alpha and beta reads and scaling remaining entities into reads per million. Scaled data were then quantile normalized and filtered at 4 different, increasing expression levels, using genes that were present in either class at > 0.3, 0.5, 0.75, and 1 reads/million. Significantly expressed genes were selected for gene ontology (GO) and molecular pathway analysis to identify the possible enrichment of genes with specific biological themes using Elsevier’s Pathway Studio and the Database for Annotation, Visualization, and Integrated Discovery, v6.8 (DAVID).

**Nanostring:** To validate RNA Seq data, 24 total RNA samples (12 controls and 12 patients) were analyzed using the NanoString platform for direct, multiplexed analysis of mRNA (www.nanostring.com). This platform uses to capture and detection probes on purified RNA, with no enzymatic manipulation of the sample. One hundred ng of total RNA was used as input material against a panel of 185 genes. Samples were run at the NanoString proof of principal lab in Seattle, WA, according to the manufacturer’s recommendation.

**Results**

We identified four groups in our total cohort of 112 Stage 1 patients.

In Group 1, molecular hypometabolism was present with IRS2 upregulation in this cohort of 62 patients. The combination of both metabolic hypometabolism and IRS2 upregulation yield results suggestive of a mechanism for enhanced grey matter nuclear atrophy in our cases (Table 1). A mean number of atrophic nuclei seen on NeuroQuant (NQ) was 4.1. The mean IRS2 was 1.78, with 16.1% of patients exhibiting an increased size of a superior lateral ventricle, an untoward finding consistent with either (i) obstruction of the flow of cerebral spinal fluid contributing to hydrocephalus (NPH) or (ii) loss of substance of cortical grey. No patients showed an increased Evan’s Index, ruling out NPH. Pulmonary hypertension (> 30 mm Hg) was identified in 80% of cases with widened anion gap seen in 85% of cases.

In group 2, 26 patients had MHM present but IRS2 negative. Mean atrophic grey matter nuclei were 3.0, which suggests that the enhanced glucose delivery for glycolysis seen in IRS2 positive has an increased incidence of atrophy of grey matter nuclei compared to those also with MHM but with IRS2 negative. Mean IRS2 in cohort 2 was -0.97. Superior lateral ventricle enlargement was less common; 7.6% of patients were found to have this abnormality. PAH was seen in 8%, with a widened anion gap seen in 20%.

Group 3 was comprised of 16 patients, MHM negative and IRS2 negative. Mean atrophic nuclei was 2.8, with a mean IRS2 value of -1.67. Only one patient had superior lateral ventricle enlargement (6.1%). PAH was seen in no patients, while anion gap was widened in 33%. Group 4 only had 8 cases with MHM negative and IRS2 positive. Atrophic nuclei were 1.16, the lowest of all measured groups, with a mean IRS2 of 1.14. No superior lateral ventricle enlargement was noted. Not found were any cases of PAH; or widened anion gap.

**Discussion**

As we have discussed in detail, the MHM patients will have suppression of translocases and/or closed VDAC, as shown by elevated tubulins, use of itraconazole or reactive exposure to actinomyces. An open VDAC is required to deliver pyruvate across the outer mitochondrial membrane. If pyruvate cannot get into the mitochondria, there will be no additional ATP production. If enhanced glycolysis due to IRS2 positivity is present, there will be an additional influx of glucose into the cell for cytosolic aerobic glycolysis conversion to lactic acid. Lactic acid is then exported outside of the cell, contributing to metabolic acidosis.

The combination of both metabolic hypometabolism and IRS2+ yield results suggestive of a proliferative mechanism for enhanced grey matter nuclear atrophy in our cases. Taken together, however, what we see is that in the absence of translocases due to molecular hypometabolism, there is an effect of an increase in delivery of glucose, flooding the cytosol without any transport of breakdown products of glycolysis into mitochondria. This excessive amount of pyruvate, when converted to lactic acid, will contribute to systemic metabolic acidosis together with neuronal injury both to grey matter nuclei as well as to cortical grey in addition to fomenting underlying pulmonary artery hypertension.

Note that PAH was defined by finding elevated levels of tricuspid regurgitation on baseline echocardiogram with a value for tricuspid regurgitation exceeding 2.5 meters per second and assuming right atrial pressure was 5mm Hg. As noted previously, the measurement of TR was not required for the performance of GENIE and not all cases had echocardiogram testing done. These results will need to be validated in a larger trial.

Interestingly, measurement of anion gap was not required for performance of transcriptomic testing but was frequently submitted with additional clinical materials by physicians. It is only when group 4 is reviewed that we see that absence of MHM and the presence of IRS2 positivity result in essentially an ablation of metabolic complications related to the interaction of inflammatory genes, ribosomal genes, nuclear coded mitochondrial genes, and metabolism. If we do not have

<p>| Table 1. Combination of both metabolic hypometabolism and IRS2 upregulation |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th></th>
<th>MHM+IRS2+</th>
<th>MHM+IRS2-</th>
<th>MHM-IRS2-</th>
<th>MHM-IRS2+</th>
<th>CONTROLS</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>62</td>
<td>26</td>
<td>16</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td>A Nuclei/6</td>
<td>4.1</td>
<td>3.0</td>
<td>2.8</td>
<td>1.2</td>
<td>1.4</td>
</tr>
<tr>
<td>SLV increase</td>
<td>16.1</td>
<td>7.6</td>
<td>6.1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mean IRS2</td>
<td>1.78</td>
<td>-0.97</td>
<td>-1.67</td>
<td>1.14</td>
<td>1.33</td>
</tr>
<tr>
<td>Anion gap &gt; 12</td>
<td>85</td>
<td>20</td>
<td>33*</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>PASP increase</td>
<td>80</td>
<td>8</td>
<td>0*</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

excessive glucose inputs due to IRS2 negativity, but MHM is present, the metabolic complications of metabolic acidosis and pulmonary hypertension are less severe.

**Conclusion**

The layers of complexity of cellular use of glucose include diversion of metabolites from glycolysis; interaction with inflammatory mediators; aerobic glycolysis; and biosynthetic pathways used to make needed nucleotides, lipids, amino acids, and intermediates. The metabolic stress response elements are influenced by differential gene activation/suppression. Both metabolic and inflammatory systems interact to maintain cell viability, energy conservation, and cell proliferation. When systems go awry, for example, when there is an imbalance of glycosylation genes OGT and OGA that persists beyond recovery from stressors such as trauma, ischemia, or infection, an acute response can be converted into a chronic disease, such as insulin resistance.

MHM is such a process. When chronic fatigue, as part of a multisystem, multi-symptom illness, becomes entrenched, possibly after exposure to ribotoxins, especially those made by actinomycetes, we now know to look for adaptive, survival-based abnormalities in ribosomal genes, translocases, and ATP synthases, among others. Compared to control data sets, we see a group of patients here with a correlation of MHM+/IRS2 (+) with worsening end-organ injury in untreated, Stage 1 CIRS patients. There is a widened anion gap, consistent with metabolic acidosis. There is an increased percentage of cases in this group with atrophic grey matter nuclei or enlarged superior lateral ventricle enlargement, as shown by NeuroQuant. PAH is far more common in this group compared to MHM+/(-) IRS2; MHM(-)/(-) IRS2; and MHM(-)/IRS2 (+).

When evaluated by published metabolic literature, these data are consistent with the adverse effects of proliferative physiology, which has its roots in aerobic glycolysis. In turn, aerobic glycolysis requires compartmentalization of glucose metabolism. A closed VDAC hastens the process. Patients found by transcriptomic testing showing MHM+/IRS2 (+) may well benefit from additional testing by NeuroQuant, a comprehensive metabolic panel to determine anion gap, and echocardiogram. If available, flow cytometry for CD4+CD25+CD127-/-0 is recommended.

While proliferative physiology, a systemic process, is required for acute host defenses, persistent proliferative states are manifestly not healthy when the host has MHM. The host response once again becomes the enemy. As long as standard CIRS treatment protocols are followed carefully and in order, without omissions, salutary benefits, including repair of CNS injury, can reasonably be expected.

Weaknesses of this study include missing data, as not all transcriptomic patients had echocardiograms, NeuroQuant and CMP performed. As such, a prospective study will be necessary. Further, the absence of T reg assays leave a hole in analysis, as the proliferative model would predict marked T reg deficiency in Group 1, with steadily increasing numbers through Group 4.

This study, with its focus on proliferative physiology, holds great promise for expanded use of less commonly used protocols and less commonly used medications, especially including VIP.

**Thanks**

To Debbie Waidner for excellent technical assistance, to David Lark and Jacki Meinhardt who kindly reviewed the manuscript and made excellent suggestions that have strengthened the paper. James Ryan PhD, CSO, ProgeneDx, supplied the methods for transcriptomic testing.

**Potential conflict of interest**

Dr. Shoemaker holds interest in ProgeneDx, a company that sells GENIE tests.

**References**


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Complications of proliferative physiology include metabolic acidosis, pulmonary hypertension, T reg cell deficiency, insulin resistance and neuronal injury.