Suggested pathology of systemic exertion intolerance disease: Impairment of the E₃ subunit or crossover of swinging arms of the E₂ subunit of the pyruvate dehydrogenase complex decreases regeneration of cofactor dihydrolipoic acid of the E₂ subunit

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ABSTRACT

Systemic Exertion Intolerance Disease (SEID) or myalgic encephalomyelitis (ME) or chronic fatigue syndrome (CFS) has an unknown aetiology, with no known treatment and a prevalence of approximately 22 million individuals (2%) in Western countries. Although strongly suspected, the role of lactate in pathology is unknown, nor has the nature of the two most central symptoms of the condition – post exertional malaise and fatigue. The proposed mechanism of action of pyruvate dehydrogenase complex (PDC) plays a central role in maintaining energy production with cofactors alpha-lipoic acid (LA) and its counterpart dihydrolipoic acid (DHLA), its regeneration suggested as the new rate limiting factor. Decreased DHLA regeneration due to impairment of the E₃ subunit or crossover of the swinging arms of the E₂ subunit of PDC have been suggested as a cause of ME/CFS/SEID resulting in instantaneous fluctuations in lactate levels and instantaneous offset of the DHLA/LA ratio and defining the condition as an LA deficiency with chronic instantaneous hyperlactataemia with explicit stratification of symptoms. While instantaneous hyperlactataemia has been suggested to account for the PEM, the fatigue was explained by the downregulated throughput of pyruvate and consequently lower production of ATP with the residual enzymatic efficacy of the E₃ subunit or crossover of the E₂ as a proposed explanation of the fatigue severity. Functional diagnostics and visualization of instantaneous elevations of lactate and DHLA has been suggested. Novel treatment strategies have been implicated to compensate for chronic PDC impairment and hyperlactataemia. This hypothesis potentially influences the current understanding and treatment methods for any type of hyperlactataemia, fatigue, ME/CFS/SEID, and conditions associated with PDC impairment.

Background

Myalgic encephalomyelitis (ME) or chronic fatigue syndrome (CFS) has an unknown aetiology, with no known treatment and a prevalence of approximately 22 million individuals (2%) in Western countries. To emphasize its somatic nature, it was renamed systemic exertion intolerance disease (SEID) in 2015 [1].

CFS is recently hypothesized to be a cell danger response, type 3 (CDR3) disorder, suggesting an impairment of autonomous oxidative phosphorylation in anti-inflammatory M2 type mitochondria [2]. Abnormalities previously reported in 20 metabolic pathways, with reductions in 80% of metabolites, suggest a hypometabolic syndrome [3]. Among 144 metabolites examined previously, lactate and pyruvate ranked among the 5 most relevant biomarkers of CFS [4]. Increased blood lactate levels along with a concomitant reduction in glucose levels in CFS patients may inhibit lactate gluconeogenesis (Cori cycle); however, further concomitant reductions in urine pyruvate and alanine might suggest either inhibition of alanine gluconeogenesis (glucose-alanine cycle) or glycolysis with consequently reduced availability of acetyl-CoA [5]. Simultaneously, an impairment of pyruvate dehydrogenase was evidenced by increased mRNA expression of regulatory kinases, citrulline-4 and PRARδ, and excessive lactate production by myoblasts in the presence of patients’ serum [6]. In contrast, elevated brain levels of lactate in CFS patients rather than in those with generalized anxiety disorder [7] and healthy volunteers, but not major depressive disorder (MDD) [8] and fibromyalgia [9], indicate that lactate is not a specific biomarker, as revealed through proton magnetic resonance spectroscopy. However, ventricular brain lactate in pooled samples of CFS-MDD-healthy volunteers revealed a positive correlation with fatigue across multiple health conditions, with a weak positive
correlation with CFS symptoms [7–9].

Although strongly suspected, the role of lactate in CFS pathology is unknown, nor has the nature of the two most central symptoms of the condition – post exertional malaise (PEM) and fatigue.

Considering the increasing evidence regarding dysfunctional energy production, we suggest a novel functional model of the pyruvate dehydrogenase complex (PDC). Although the mechanisms underlying ATP production are well-known, three features remain unexplained. It is still unknown (a) why, two pyruvate molecules are simultaneously required to maintain the stoichiometric balance of ATP gain through glycolysis and oxidative phosphorylation. Simultaneous utilisation of two pyruvate molecules does not contribute to understanding the underlying, especially since it does not (b) explain the origin of a driving force within the PDC, or (c) explain the purpose of the swinging arms of the E3 subunit. Unfortunately, the current model of PDC function does not identify the final acceptor from the NAD⁺/FADH₂ pair and is thus neither complete nor dynamically functional.

We believe that the novel functional model of PDC will unveil the cause of ME/CFS/SEID, fatigue, PDC impairments, and any type of hyperlactataemia, and will be exploited in the development of a new treatment strategies and diagnostics.

The hypothesis

Novelty of the approach

The proposed mechanism indicates a central role of the PDC in maintaining energy production with LA and its counterpart, DHLA, its regeneration being a rate limiting factor.

Considering the current understanding of the structural organisation of the PDC [10], we suggest that entire process of pyruvate oxidation to acetyl-CoA occurs in a triplex structure comprising E₁ and E₃ subunits compactly associated with the E₂ subunit, which altogether comprise one functional PDC unit, which we further refer to as “the PDC electrochemical circuit” or “circuit”. Functionally, one circuit comprises two paths, ‘proton’ and ‘carbon’ flow, which are tightly coordinated by the swinging arms mechanism for crossover exchange between DH LA and LA, which serves as circuit breaker and is necessary to initiate a new cycle, thus being a central mechanism for circuit function.

DH LA regeneration results in proton transfer to the final acceptor NAD⁺*, which is suggested to be produced for this requirement in the third path, termed the ‘pyruvate/NAD⁺ shuttle’. This shuttle is mandatory for functioning of the circuit; hence, it is provided with a final acceptor, i.e. generation of the electrochemical driving force. The shuttle is not a part of the PDC. The PDC may have several circuit triplexes; hence, it comprises multiple copies of subunits [10] organized in a pentagonal dodecahedral configuration. The suggested disturbances in the functioning of a new model have been considered a potential cause of ME/CFS/SEID and hyperlactataemia. The model does not include the regulative elements of the PDC for conceptual clarity.

Normal functioning of the PDC circuit

Glycolysis yields two pyruvate and two NADH₂ molecules from one glucose molecule, and pyruvate has to be transported into the mitochondrial matrix [11] prior to its oxidation by the PDC (Fig. 1, step 1). However, it is still unclear why the simultaneous mitochondrial entry of two pyruvate molecules is required to maintain the stoichiometric balance of ATP gain through glycolysis and oxidative phosphorylation. The lactate shuttle hypothesis [12] suggests that (a) lactate is produced under both anaerobic and aerobic conditions, and (b) cells use lactate as an energy source. This hypothesis has been controversial until recent evidence suggested that (a) respiration is inhibited when transmembrane pyruvate transport is inhibited [13] and conversely increased in the presence of substrates and co-factors of lactate dehydrogenase (LDH) [13], and (b) LDH colocalises with both respiratory chain proteins and more precisely with Bcl-2 on the outer mitochondrial membrane (OMM), altogether indicating that LDH is associated with muscle mitochondria thus suggesting the presence of lactate in the mitochondrial matrix [13].

We hypothesize that lactate has a unique function: it serves as an intermediate for NAD⁺ synthesis in the pyruvate/NAD⁺ shuttle. The hypothesized functional mechanism of the PDC circuit is initiated with lactate and NAD⁺ synthesis by LDH from pyruvate translocated across the OMM (step 2). Thus, the pyruvate:lactate ratio in healthy individuals tends towards 1, and LDH activity is required to maintain that ratio through regulation. We postulate that half of the pyruvate molecules within the pool are not reduced to lactate, but rather are oxidised to acetyl-CoA [11], while the other half generates a driving force operating the circuit (NAD⁺). During pyruvate oxidation, which we termed the ‘carbon path’ (steps 3–5), cofactor LA of E₃ accepts two protons and is reduced to DHLA. To repeat the ‘carbon flow’ with a new pyruvate molecule, reduced DHLA must be regenerated. Hence, co-factor FAD⁺ of E₃ accepts protons from DHLA and reduces it to FADH₂ (step 6). DH LA is thus regenerated and the swinging arms are required to switch their positions (step 10) to introduce LA into the ‘carbon flow’ and a new DH LA molecule to the ‘proton flow’. Within the proton flow, NAD⁺ accepts protons from FADH₂, the latter being oxidised to FAD⁺, which readily accepts protons from the next crossover of DH LA (step 7). Upon serving as a proton acceptor, NAD⁺ is converted to NADH + H⁺ (step 7), which again readily reacts with a new translocated pyruvate (step 2) to subsequently yield lactate and NAD⁺.

In situ clearance. Lactate is toxic; therefore, a mechanism for its immediate clearance has been suggested to occur in the mitochondrial matrix and is postulated to be primary to the well-known Cori and glucose-alanine cycles occurring systemically. In situ clearance harnesses the unique diphorase activity of membrane-bound LDH. Diphorase catalyses the reversible conversion of pyruvate to lactate. Hence, the shuttle is a turnover path comprising the by-product lactate and substrate pyruvate, with the production of NADH + H⁺ (step 8) for its further utilization in step 2 for the formation of proton/electron acceptor – NAD⁺. Lactate is converted to pyruvate simultaneously with the reduction of NAD⁺ to NADH + H⁺; therefore, our model, as opposed to the current model, is complete, accounting for both redox pairs displaying coordinated activity. Theoretically, pyruvate formed in step 8 may follow the carbon path (step 9) or the shuttle (steps 2 and 8). Newly formed NADH + H⁺ reacts with one of two translocated pyruvate molecules (step 2). Thus, PDC functions uninterruptedly if the required amounts of substrates are supplied and if all other regulatory elements and cofactors are available.

It is logical to predict that the action of diphorase-LDH and LDH are not synchronised; consequently, a moment of time is observed when the pyruvate:lactate ratio is not ideally equal to 1. Lactate levels are elevated immediately before the action of diphorase-LDH and vice versa. Therefore, instantaneous fluctuations of lactate (IFL) are potentially detected in any living cell. If this hypothesis holds true, unveiling this unique two-tact mitochondrial mechanism would proceed in a manner similar to a cardiogram visualizing the four-tact heart beating mechanism.

Both halves of the circuit are synchronized by the crossover action of the swinging arms of the PDC. Considering this viewpoint, the rate of DH LA regeneration is limited and depends on enzymatic efficacy of the E₃ subunit. Furthermore, we suggest the functional synchronization between diphorase-LDH activity (step 8) and the crossover mechanism (step 10) essential for the functioning of the circuit. In healthy mitochondria, either the products of step 4/6 or PDC regulative elements or the final intermediate of the TCA cycle, oxaloacetate, or translocation of two pyruvate molecules may be a signal for step 8. If true, disorder resulting from dysfunction of either the crossover mechanism or E₃ impairment may result in hyperlactataemia even under aerobic conditions. If this hypothesis holds true, both healthy and affected
individuals would present IFL: while lactate levels in healthy individuals are within the normal range owing to timely functioning of the shuttle, high-amplitude IFL is expected to occur in affected individuals.

The alternative suggested herein for systemic lactate clearance by the Cori and glucose-alanine cycles may be supported by the reduction in lactate levels in hepatectomized dogs [14] treated with dichloroacetate (DCA) and temporary hyperlactataemia in post-hepatectomy patients [15].

Either E3 impairment or the crossover of swinging arms of the E2 subunit is the cause of ME/CFS/SEID

The aetiology of E3 impairment is unknown. Gene mutations, radiation, conjugation with exogenous molecules with PDC (pollutants, adverse nutritionals, and molecules of bacterial, viral, and prion origin), and structural or functional defects might impair DHLA regeneration (Fig. 2), resulting in an LA deficit, thus deranging normal energy production and instantaneously increasing DHLA levels. This impairs diphorase activity of LDH owing to disturbed synchronization of the DHAA/LA crossover, consequently increasing the amplitude of IFL (hyperlactataemia). Impairment of the crossover of the swinging arms of the E2 subunit may also cause the abovementioned scenario. Furthermore, the aetiology of this impairment is unknown. Thus, independent of a site, the condition may be redefined as an LA deficiency with instantaneous hyperlactataemia. This new definition explains two central symptoms from the list of the Canadian criteria [16], fatigue and PEM. We suggest that fatigue results from the downregulated throughput of pyruvate within the carbon flow path and lower acetyl-CoA (i.e. ATP) output owing to synchronization with the rate of the impaired proton flow, while its severity is the reverse of the residual enzymatic efficacy of the E3 subunit. Further, the present data expand the current understanding of PEM by including the fact that malaise and exacerbation occur not only after physical work, but also after any metabolic function at any level of organization in the body. Because any metabolic function in the body at any level of organization demands energy, we suggest instantaneous hyperlactataemia as an explanation of PEM occurring during and after the performance of energy-demanding activities of daily life or maintenance of allostasis within the body.

Further, we believe that all symptoms from the list of the Canadian criteria [16] might be explained by the present hypothesis and either result from dihydrolipooyl dehydrogenase (DHDL, E3) or dihydrolipooyl transacetylase (DLAT, E2) crossover impairment owing to (a) altered levels of carbon matter (elevated lactate levels and lowered levels of signalling molecules); (b) disturbances in electrochemical electron transport; (c) allostatic offsets of PDC co-factors, including NAD (niacin), FAD (riboflavin), and TPP (thiamine) in addition to LA/DHLA; (d) downregulation of PDC or any other yet unknown consequence. In addition, normal functioning of muscle tissues is generally believed to be affected by a reduced ATP pool, thus not being associated with the initial symptoms including but not limited to frequent urination, tachycardia, sore throat, hypertension, blurry vision, and abdominal and muscular pain. These symptoms occur in one of three types of muscle tissue: skeletal, heart, and smooth. Thus, adequate ATP levels are essential for normal dissociation of myosin from actin within microfilaments; consequently, the generally low ATP levels are believed to increase the duration of their association, which is suspected to underlie the decreased elasticity of muscle tissues within the urinary bladder, gut, uterus, blood vessels, heart, and eyes. Therefore, we suggest that instantaneously decreased elasticity of muscle tissues or instantaneous spasms of various duration accounts for painful muscle contractions and causes abdominal, ophthalmic, and fibromyalgic pain, sore throat,
blurred vision, and contractions leading to decreased elasticity of tissues in the urinary bladder; therefore, frequent urination results from decreased bladder volume, increased numbers of heart contractions (tachycardia) and blood vessels, essentially causing hypertension, altogether suggesting stratification of symptoms.

If this assumption holds true, successful life-long compensation of the DLD or crossover of DLAT impairment (Fig. 2) will progressively improve physiological functioning at all levels, thus eliminating all symptoms as long as energy used for allostatics and performing activities of daily life does not exceed the available energy pool at any particular time. Overall, this implies that appropriate treatment may result in hormesis, wherein patients might achieve a higher stamina than that before entering the disease state, if the condition is inherited. Clinical evidence regarding a successful treatment protocol based on the hypothesis presented herein will be published elsewhere.

Novel life-long therapeutically compensation of the impairment

PDC function does not generally require an excessive exogenous supply of LA because of its regeneration within the complex. Therefore, LA is not of high nutritional significance, and only a modest dietary intake of LA is needed. Although no clinical trials have been conducted, a short-term effect of LA followed by worsening [17], in the same manner as from the exogenous overload of ascorbic acid, tocopherols, and GSH [18], has been reported in some cases. Although the above-mentioned molecules are reportedly regenerated by the DHLA/LA pair [19], in the present context, the inverse is suggested – they serve as its alternative regenerators. Independent of the endogenous supply or alternative regeneration, hyperlactataemia still occurs; hence, shuttle work will not be restored.

The therapeutic effect of nutritional oxalates on hyperlactataemia resulting from different aetiologies has been unveiled, which is currently pending a patent, probably owing to the favourable reversible enzymatic activity of LDH (Fig. 2 step 8). Oxamate and oxalate inhibit LDH in different directions, in competition with pyruvate and lactate, respectively [20], and this activity is observed only with bound enzymes. Moreover, a precursor of oxalate, DCA, has reduced therapeutic effects in ME/CFS/SEID patients [21]. Altogether, these findings strengthen our assumption. Because enzymatic conversion of lactate to pyruvate does not affect DHLA regeneration, this therapy should be combined with a compensatory exogenous supply of LA (step 11). If this holds true, a combination of continuous supply of LA and enzymatic conversion of lactate would increase stamina, decrease symptom burden, fatigue, and IFL amplitude and decrease blood lactate levels in ME/CFS/SEID patients. Other therapies involving blockade of LDH would most probably result in extreme fatigue instead of reversing of its activity, e.g. after chemotherapeutic treatment of some cancer types. Based on the present model, this may result from the satisfactory DHLA regeneration, which in turn decelerates pyruvate oxidation.

Evaluation of the hypothesis

To our knowledge, this is the first postulation of the present hypothesis to explain the completely different roles of two identical pyruvate molecules simultaneously translocated into mitochondrial matrix. Moreover, it ascribes a central role of the PDC in maintaining energy production to LA and its counterpart, DHLA, with its regeneration as a novel rate limiting factor. Successful regeneration of DHLA to LA is considered to be limited by either one of two impairments: functional alteration of DLD (subunit E3) or defective crossover of the swinging arms of DLAT (subunit E2) of PDC. Conventional isotope techniques may help map these pathways. The tests explained...
levels. Detection of ‘up’ and ‘down’ peaks of lactate reflect the activity difference between two consecutive measurements of capillary lactate might reflect the dysfunctional in situ clearance of lactate. IFL is the difference between two consecutive measurements of capillary lactate levels. Detection of ‘up’ and ‘down’ peaks of lactate reflect the activity of the pyruvate/NAD⁺ shuttle in a two-tact mechanism of in situ clearance of lactate. The hypothesis may be successfully assessed through functional analysis based on consecutive measurements of lactate levels within short intervals (5 min) before, during, and after the applied stimuli (test 1). Stimuli should not generate anaerobic conditions within the cell and not be of muscular work by nature. Any physiological or cognitive functions, such as reading or digestion of a light meal could be examples of appropriate stimuli. No studies have attempted to determine lactate levels in ME/CFS/SEID patients in response to stimuli under aerobic conditions; however, elevated lactate levels [22] and impaired proton homeostasis [23] after physical exertion, e.g. under anaerobic conditions, have been reported.

As postulated, either impaired E₃ or crossover skews the DHLA/LA ratio towards DHLA; this ratio trends towards 1 in healthy individuals. We suggest that DHLA in addition to regenerative the proton flow path may have alternate systemic, extramitochondrial functions similar to the Cori cycle for lactate gluconeogenesis. Therefore, only short-term, instantaneous, elevations of DHLA might be expected, similar to lactate. Measurements should be performed in the same manner as that in test 1, eventually reflecting only the consequence without identifying the cause (test 2). At the present time, the limit of DHLA and LA detection in healthy volunteers is higher than the physiological range [24] and therefore development of a new methodology is highly required.

Upon overloading with the precursor of E₃ co-factor FAD⁺, riboflavin, further differentiation of the site of impairment was suggested. We postulate that impaired enzymatic activity of E₃ decelerates the carbon flow path even if crossover (step 10) is functional. Furthermore, we postulate that impaired E₃ uses less cofactor; thus, excessive amounts of its precursor, riboflavin, would not be tolerated by individuals with ME/CFS/SEID. Moreover, we postulate that daily intake of riboflavin favours reactions upstream of E₃, in steps 2 and 7, resulting in an increased IFL amplitude. Therefore, by performing test 1 but with chemical stimulation by riboflavin instead of functional stimuli, in the same manner as glucose is used to diagnose diabetes, higher-amplitude IFL will be induced only in affected individuals (test 3). In addition, the degree of E₃ impairment can be measured through analysis of enzymatic efficacy (test 4). Thus, both the cause and the degree of impairment can be identified.

To exclude the inherited nature of DLD or swinging arms of DLAT impairment, the test for any alterations within corresponding encoding genes may be performed (test 5 and 6). To our knowledge, no clinical cases associated with impairment of crossover of DLAT have been reported yet, while clinical manifestation of DLD deficiency was previously described with a wide range of severity: from exercise-induced weakness with severe acidosis at baseline levels of lactate [25] to death from severe intractable metabolic acidosis [26]. A homozygous missense mutation p.1480M, in the interface domain, was responsible for the mildest form [25], and p.D479V and p.R482G mutations result in milder, exclusively myopathic forms [25]. Evaluation of 13 Ashkenazi Jew patients revealed the severity of the condition [26] through two coincident mutations: the more widespread G229C mutation in the NAD⁺-binding domain and the rare insertion mutation 105insA (Y35X). Thus, patients with disease onset in early childhood or in later life were homozygous for the G229C, while those demonstrating disease onset at birth and with more severe grade of disability harboured both mutations. Novel cases [27] have shown that mutations p.140Lfs*4 and p.G461E also caused progressive exertional fatigue with mitochondrial proliferation, which was reversible upon riboflavin supplementation. In such cases, riboflavin was suggested to structurally stabilise DLD, thus exerting a chaperon-like effect. In contrast, DLD impairment in individuals harbouring the G229C and p.1480M mutations could not be successfully treated because of intractable metabolic acidosis.

Can E₃ or E₂ crossover impairment cause hyperlactataemia of any type?
Hyperlactataemia is a potentially lethal condition characterized by elevated blood lactate levels beyond the physiological range of 0.5–2.0 mmol/L. Type A hyperlactataemia has been considered a result of tissue hypoxia, while type B combines all other cases with normal oxygenation of the tissues. Since elevated lactate levels have been suggested to be strongly correlated with patient outcomes and survival [28], but do not exhibit required biomarker specificity [29], we postulate that both types have the same underlying cause – an impaired immediate in situ clearance of lactate. The reason underlying this impairment may be a factor other than E₃ or crossover of E₂ impairment; however, numerous pathways including glycolysis (cytoplasm), pyruvate oxidation (PDC circuit), lactate oxidation by OMM-bound LDH (pyruvate/NAD⁺ shuttle), oxidation of acetyl-CoA (TCA), oxidative phosphorylation (electron transport chain), or signalling and regulation may be involved. Therefore, identification of IFL in a healthy population, with an IFL amplitude ensuring the maintenance of normal lactate levels, may indicate the hypothesized novel mechanism for in situ lactate clearance in general. Because both lactate levels and exposure to lactate during a given time depend on the IFL amplitude, the treatment strategies for any type of hyperlactataemia should exploit diphosphoryl activity of OMM-bound LDH alone or in combination with an exogenous supply of compounds in the deficit, resulting from a particular impairment. For example, combinatorial therapy may be advantageous when patients are successfully administered endogenous compounds that they are deficient in, but still die because of acidosis, similar to those with mitochondrial encephalomyopathy [30].

Consequences of the hypothesis and discussion

We believe that the present hypothesis, if successfully confirmed, may further the current understanding of the biochemistry of energy production and in turn influence further generation and understanding of knowledge within a broad range of disciplines, including but not restricted to medicine, biology, biotechnology, and sports. Novel insights into the functional organization of the PDC, the cause and treatment of ME/CFS/SEID, LA deficiency and hyperlactataemia of any aetiology might help develop new treatment strategies. Moreover, the diagnostic tests discussed herein may inspire the development of new diagnostic tools for ME/CFS/SEID and hyperlactataemia and to confirm the present hypothesis.

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Declaration of Competing Interest

Both authors, Øyvind and Victoria Bohne have no conflict of interest to declare. We are owners of the family business Bohne Askøy AS, having applied for patents to treat and diagnose ME/SEID/CFS and hyperlactataemia. One of the authors is affected by ME/CFS/SEID and they both are genuinely interested to present their working hypothesis.

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Appendix A. Supplementary data

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References


