The marker of cobalamin deficiency, plasma methylmalonic acid, may help identifying lysosomal iron trapping in patients. Its possible utility for heart failure

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ABSTRACT

Iron deficiency is known to aggravate the prognosis of patients with heart failure. Iron has functions in the mitochondrial respiratory chain. In patients with reduced mitochondrial respiration, the mitochondrial ratio between the level of nicotinamide adenine dinucleotide and its reduced form decreases. Due to the mitochondrial-lysosomal interplay, decreased mitochondrial respiration also leads to inhibition of lysosomal hydrolysis. As a result, cobalamin and iron will be trapped in lysosomes. This will, even if iron and cobalamin have been consumed and absorbed in sufficient amounts, lead to their functional deficiencies.[1] Functional iron deficiency can further impede mitochondrial respiration. Increased plasma levels of methylmalonic acid were shown to predict all-cause and cardiovascular mortality in the general population. Treatments targeting mitochondrial and lysosomal function may correct the functional deficiencies and improve prognosis in a subgroup of patients with heart failure, notably those with skeletal muscle wasting. Methylmalonic acid levels may be used for monitoring response to treatment, thereby identifying patients of the subgroup in which disease outcome may improve.

1. Introduction

Heart failure is a severe condition with substantial social and economic consequences. Its incidence of 2% in adults raises to over 8% in seniors. Its 5 years mortality rate is over 40%. Treatment aims at improving symptoms and preventing progression. A variety of diseases, including coronary artery disease, myocardial infarction, high blood pressure, atrial fibrillation, and valvular heart disease and risk factors, including alcoholism and infections, impacting the heart, can lead to heart failure. Despite the multiple pathophysiological mechanisms, targeting specific metabolic derangements is expected to offer therapeutic benefits. Studies in this context included iron, niacin (vitamin B3), and cobalamin (vitamin B12) metabolism.

Around half the patients with heart failure have iron deficiency, with or without anaemia, which has substantial adverse prognostic consequences [1]. In patients with heart failure, wasting of skeletal muscles is an independent predictor of survival [2]' and skeletal muscle myopathy is more severe in patients with iron deficiency [3]. The effects of iron deficiency on muscle energetics are associated with muscle iron content independently of anemia [3]. Iron functions in complexes of the mitochondrial respiratory chain. Its deficiency limits mitochondrial oxidative phosphorylation in cardiac and skeletal muscles [3]. Iron chelation in cultured myocytes of skeletal muscle induces loss of mitochondrial respiratory capacity [4].

Thirteen polypeptide subunits of the electron transport chain are encoded by mitochondrial DNA. Patients with coronary artery disease have increased frequencies of mitochondrial DNA lesions and common deletions [5]. These are associated with reduced mitochondrial respiration and reduced activity of electron transport chain complexes I, II, and III, that with complex IV, move electrons from the reduced form of

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[1] The plasma methylmalonic acid level will increase.

nicotinamide adenine dinucleotide (NADH) to molecular oxygen [5]. Complex I re-oxidizes NADH to NAD^+ during oxidative phosphorylation. Cells lacking complex I activity develop NAD^+ deficiency. Pathways affected include oxidative phosphorylation, the citric acid cycle, and mitochondrial one-carbon metabolism needed to produce NADPH and glutathione under nutrient stress by e.g., insulin resistance. Such cells are therefore more vulnerable to oxidative stress [6]. Indeed, circulating oxidized-LDL levels, a systemic marker of oxidative stress, predict both progression of atherosclerosis [7] and mortality in heart failure patients [8]. Of the thirteen subunits of the mammalian complex IV, the mitochondrial genome encodes subunits 1, 2, and 3, which form the catalytic core of the enzyme. *MT-COI* is the first gene in the polycistronic mitochondrial DNA, and a single missense mutation in mouse *Mt-co1* was associated with loss of cyclooxygenase activity [9]. Recently, MT-COI was linked to atherosclerosis and was found to be associated with adverse outcome in patients with coronary artery disease, linking mitochondrial oxidative stress with cardiovascular risk [10,11].

Metabolic disturbances underlying iron deficiency in heart failure patients are often unknown [1]. Clinical implications have remained limited, but recent studies suggest future perspectives. Intravenous iron repletion in heart failure patients with iron isomaltoside reduced skeletal muscle phosphocreatine recovery half-times, a proxy for improved mitochondrial oxidative function and muscle energetics [12].

Besides iron, also niacin plays a role in mitochondrial respiration. Niacin administration compensated NAD^+ deficiency and improved mitochondrial function in laboratory animals [6]. Recently, pharmacological doses of niacin substantially improved mitochondrial function and muscle metabolism and performance in humans with myopathy caused by genetic mitochondrial DNA deletions [13]. Importantly, niacin administration "normalized" muscle metabolism without affecting the primary gene defects.

2. Mitochondrial-lysosomal interplay and iron deficiency

Lysosome-dependent autophagy assures the recycling of components, including iron, organelles, and proteins damaged by, e.g., oxidative stress. A proper balance between mitochondrial biogenesis and autophagy helps to prevent cardiac dysfunction [14]. Mitochondrial respiratory chain deficiency inhibits lysosomal, acid pH-dependent hydrolysis [15]. Defective lysosomes lead to transcriptional repression of mitochondrial biogenesis. Impaired lysosomal acidification triggers functional iron deficiency and inflammation in vivo, associated with instability of mitochondrial DNA [16]. A vicious circle is thus operative between lysosomes and mitochondria in which defects in either of them lead to further deterioration of the other's function, to iron trapping in lysosomes, iron deficiency, inflammation, reduced mitochondrial biogenesis, and reduced mitochondrial DNA. Cellular proliferation requires adequate iron homeostasis dependent on lysosomal acidity [17]. When vacuolar-ATPase is inhibited, acidity is lost. Iron supplementation, bypassing the endo-lysosomal pathway, is sufficient to restore proliferation and cellular processes related to depleted iron. Lysosomal functions requiring internal acidity are, however, not restored under vacuolar-ATPase inhibition [16,17]. Iron deficiency may thus limit the proliferation of, e.g., immature cardiac resident stem cells, believed to protect against heart failure [18].

Heart failure patients form a heterogeneous group. Not all patients have primary dysfunction in mitochondria. In patients with coronary artery disease and in patients with longstanding iron deficiency, lesions in mitochondrial DNA are expected [5,16]. In such patients, NAD^+ deficiency can be the primary reason for iron trapping in lysosomes. Administration of niacin may improve mitochondrial respiration and therefore indirectly lysosomal hydrolysis [13].

The effects of niacin, administered for dyslipidemia, on the outcome of cardiovascular disease, were extensively studied, but results were inconclusive. This may be related to the heterogeneity of patient groups. It is therefore essential that in future trials on disease outcome, patients are selected in which niacin can interrupt the vicious circle of the mitochondrial-lysosomal interplay. The effects of niacin treatment on mitochondrial and lysosomal function should be measured. While, e.g., phosphocreatine recovery half-times can be used to quantify mitochondrial functioning, there is no method for measuring improving lysosomal function in patients. Interestingly determining methylmalonic acid (MMA), a marker of cobalamin deficiency, may allow this.

3. An unresolved question in cobalamin deficiency diagnosis

Till the 1980s cobalamin plasma levels were used for diagnosing deficiency in patients with haematological and/or neurological symptoms. However, it remained unexplained why some patients with normal cobalamin levels respond to treatment. More refined methods for diagnosis were sought. Two metabolites of which the enzymatic formation depends on cobalamin, homocysteine and methylmalonic acid (MMA), are used as biological parameters for predicting therapeutic response. Homocysteine levels depend on the functioning of the methionine cycle. The cofactor methyl-cobalamin of the enzyme methionine synthase plays a role. This enzyme catalyses the formation of methionine from homocysteine using a methyl group from 5-methyltetrahydrofolate. In mitochondria, the enzyme methylmalonyl-CoA mutase, using adenosylcobalamin as cofactor, transforms methylmalonyl-CoA into succinyl-CoA for the citric acid cycle.

Deficiencies of methyl-cobalamin and/or folate lead to elevated levels of homocysteine while only insufficiency of adenosylcobalamin will inhibit the enzyme methylmalonyl-CoA mutase, hamper the formation of succinyl-CoA and consequently favour the formation of MMA from methylmalonyl-CoA (Fig. 1). MMA is therefore considered a specific marker of cobalamin insufficiency. However, even today, it remains unclear whether the clinical response in patients with normal cobalamin and elevated MMA levels relates to correction of deficiency or results from administering pharmacological rather than physiological doses [19]. Pharmacological doses of cobalamin can bypass the physiological mechanisms that bring cobalamin to its cellular destinations.

4. Methylmalonic acid and heart failure

Increased levels of MMA in patients with normal cobalamin levels have been found in conditions (including iron deficiency and old age) and diseases (including heart failure, renal insufficiency, and diabetes) associated with oxidative stress, in particular, if several comorbidities are present in the same patient [20]. Values for MMA were more often increased than those of homocysteine. This is understandable since oxidative stress may accelerate the conversion of homocysteine into glutathione [21]. In heart failure patients, increased plasma MMA levels are associated with the presence of comorbidities, not with cobalamin levels [22]. Of patients with cobalamin levels >350 pg/ml, considered sufficient, one third had elevated MMA values (>32 ng/ml). Plasma MMA levels in patients with heart failure were significantly increased as compared to those in healthy controls. Moreover, plasma MMA values were higher in acutely decompensated heart failure than in newly diagnosed disease. In both cases the differences appeared to be independent of renal function [22]. In patients without comorbidities, MMA levels were negatively associated with cobalamin [22].

5. Both cobalamin and iron are trapped in dysfunctional lysosomes

As discussed before, impaired lysosomal acidification triggers functional iron deficiency due to lysosomal iron trapping [16]. Cobalamin's mechanism of action involves its passage through lysosomes. The subsequent release in mitochondria, like for iron, also depends on lysosomal acidification. In cultured cells, both increasing lysosomal pH with the lysosomotropic drug chloroquine or inhibiting lysosomal proteolytic enzymes with leupeptin trapped cobalamin in lysosomes and lowered
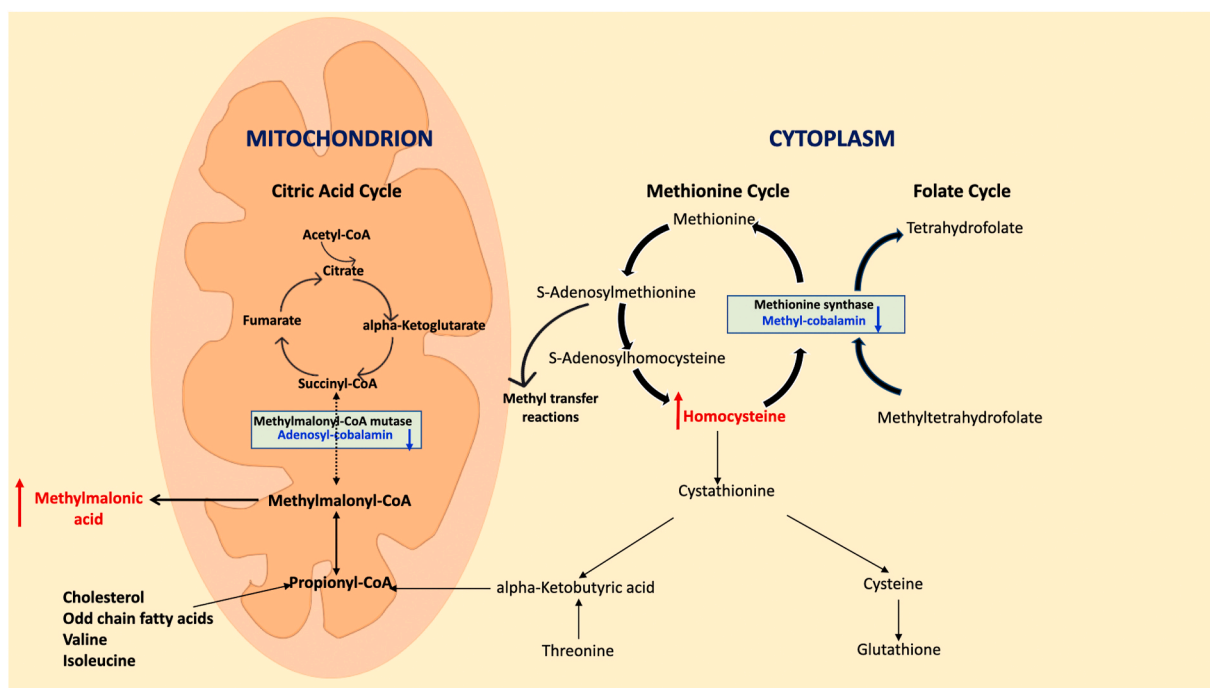
Fig. 1. Schematic representation of the effects of cobalamin deficiency in cytoplasm and mitochondria.

levels in cytosol and mitochondria. Cobalamin dependent methylmalonyl-coenzyme A mutase was inhibited [23].

As a consequence of the above, patients with increased MMA levels due to lysosomal malfunction will suffer from both functional cobalamin and iron deficiencies (see Graphical Abstract). Pharmacological doses of cobalamin and iron can attenuate these deficiencies and lower MMA levels but cannot restore the underlying lysosomal dysfunction. In the absence of neurological symptoms, one has time to verify whether the elevated MMA levels were caused by lysosomal trapping of cobalamin.

6. Identifying patients with improved lysosomal function upon treatment

Existing methods for measuring circulating, functional, and stored iron do not specifically identify lysosomal iron trapping. Patients with elevated MMA levels, despite adequate cobalamin levels, might suffer from lysosomal dysfunction. Lysosomal dysfunction is expected to lead to trapping of both iron and cobalamin in these patients. After identification of this patient subgroup, trapping of iron and cobalamin needs confirmation. Strong evidence for lysosomal dysfunction can be obtained by methods releasing both the trapped iron and cobalamin. In case lysosomal dysfunction results from reduced mitochondrial oxidative phosphorylation and thus NAD^+ deficiency, increasing mitochondrial respiration may improve lysosomal function. This will render both iron and cobalamin bioavailable, reduce phosphocreatine recovery half-times and normalize MMA levels, without administration of iron and cobalamin. While this combined approach is useful for obtaining proof of principle, the measurement of phosphocreatine recovery half-times is cumbersome and not suitable for routine clinical practice. On the other hand, measuring MMA is feasible in the clinical situation. Response of MMA to treatment for NAD^+ deficiency, without cobalamin administration, in patients with a clinical history including iron deficiency, adequate cobalamin levels, inflammation, muscle wasting, and oxidative stress is highly suggestive for improved mitochondrial-lysosomal interaction.

7. Treatment may already be available

In patients with adult-onset mitochondrial myopathy NAD^+ deficiency was demonstrated in both muscles and blood [13]. Pharmacological doses of niacin (750–1000 mg/day) were administered for 10 months. Muscle NAD^+ levels reached control values, and blood levels increased 8-fold [13]. Niacin improved muscle strength and metabolism, mitochondrial biogenesis, and respiratory chain activity, despite the continued presence of the primary mitochondrial gene defects [13].

The effect of niacin on lysosomal disorders remains to be demonstrated. However, as a NAD^+ precursor it is expected to improve lysosomal function in view of the following molecular mechanisms: mitochondrial dysfunction leads to a reduction in the NAD^+/NADH ratio; - NAD^+ precursors have shown beneficial effects in several mouse models characterized by reduced NAD^+/NADH ratios [6]. This includes a marked improvement of the respiratory chain defect and exercise intolerance in a mouse model of mitochondrial disease [24]; - NAD^+ dependent activation of Sirtuin1 is mechanistically involved in this improvement of the phenotype [24]; - Sirtuin1 is regulating the activity of vacuolar-ATPase, needed for lysosomal acidification [25,26]; - NAD^+ is needed for correcting lysosomal acidification lost by mitochondrial translation dysfunction [27], - loss of lysosomal acidification was shown to trap iron and cobalamin [16,23]; – also in patients with NAD^+ deficiency mitochondrial function improves substantially after niacin administration [13].

For these combined reasons clinical trials of long duration with niacin may be warranted in a subgroup of heart failure patients in which evidence for mitochondrial and lysosomal dysfunction can be obtained after evaluation of the effect on MMA (and phosphocreatine recovery half-times) after shorter term niacin treatment.

8. Conclusion

Patients, including those with heart failure, with high MMA-despite normal cobalamin levels, especially if they also have iron deficiency, muscle wasting, inflammation, and increased levels of oxidized-LDL, may suffer from mitochondrial and lysosomal malfunction. Upon treatment for, e.g., NAD^+ deficiency, both mitochondrial and lysosomal

function may improve, and the trapped cobalamin and iron may be released. It is advisable that long-term disease outcome after treatment is measured in groups of patients who respond to therapy. Treatments leading to both improved phosphocreatine recovery half-times and normalization of MMA levels, without iron and cobalamin administration, are candidates for improving lysosomal function.

This conclusion implies that lowering elevated plasma levels of MMA upon treatment with NAD^+ precursors, e.g., niacin, in patients with normal cobalamin levels may lead to a better prognosis. While this remains to be proven, our hypothesis describes a biological mechanism that can explain the recent finding that plasma levels of MMA predict all-cause and cardiovascular mortality in the general population [28]. We agree with the authors of the latter paper that the biological mechanisms involved in the association of plasma MMA levels and cardiovascular disease warrant further study. The present hypothesis may accelerate such studies and, if confirmed, application in clinical practice would be relatively straightforward.

Funding

Authors' contributions

AV conceived the molecular and clinical ideas for the review, drafted and revised the manuscript. JZ provided updates on the literature and discussed the manuscript. PH critically discussed and revised the manuscript. YC critically discussed and revised the manuscript. All authors read and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no competing interest.

Acknowledgement

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