Inborn errors of metabolite repair

Maria Veiga-da-Cunha1 | Emile Van Schaftingen1,2 | Guido T. Bommer1,2

1de Duve Institute, Université Catholique de Louvain (UCLouvain), Brussels, Belgium
2Walloon Excellence in Life Sciences and Biotechnology (WELBIO), UCLouvain, Brussels, Belgium

Correspondence
Maria Veiga-da-Cunha; Avenue Hippocrate 75, B-1200 Brussels, Belgium.
Email: maria.veiga@uclouvain.be

Emile Van Schaftingen; Avenue Hippocrate 75, B-1200 Brussels, Belgium.
Email: vanschaftingen@uclouvain.be

Guido T. Bommer; Avenue Hippocrate 74, B-1200 Brussels, Belgium.
Email: guido.bommer@uclouvain.be

Communicating Editor: Johan Lodewijk Karel Van Hove

Funding information
Fédération Belge contre le Cancer, Grant/Award Number: 2016-075; Fonds de la Recherche Scientifique-FRS/FNRS, Grant/Award Number: J.0104.18; H2020 European Research Council, Grant/Award Number: No 771704; Walloon Excellence in Life Sciences and Biotechnology, Grant/Award Numbers: WELBIO CR-2015A-09, WELBIO 2019-PARKINSON

Abstract
It is traditionally assumed that enzymes of intermediary metabolism are extremely specific and that this is sufficient to prevent the production of useless and/or toxic side-products. Recent work indicates that this statement is not entirely correct. In reality, enzymes are not strictly specific, they often display weak side activities on intracellular metabolites (substrate promiscuity) that resemble their physiological substrate or slowly catalyse abnormal reactions on their physiological substrate (catalytic promiscuity). They thereby produce non-classical metabolites that are not efficiently metabolised by conventional enzymes. In an increasing number of cases, metabolite repair enzymes are being discovered that serve to eliminate these non-classical metabolites and prevent their accumulation. Metabolite repair enzymes also eliminate non-classical metabolites that are formed through spontaneous (ie, not enzyme-catalysed) reactions. Importantly, genetic deficiencies in several metabolite repair enzymes lead to ‘inborn errors of metabolite repair’, such as L-2-hydroxyglutaric aciduria, D-2-hydroxyglutaric aciduria, ‘ubiquitous glucose-6-phosphatase’ (G6PC3) deficiency, the neutropenia present in Glycogen Storage Disease type Ib or defects in the enzymes that repair the hydrated forms of NADH or NADPH. Metabolite repair defects may be difficult to identify as such, because the mutated enzymes are non-classical enzymes that act on non-classical metabolites, which in some cases accumulate only inside the cells, and at rather low, yet toxic, concentrations. It is therefore likely that many additional metabolite repair enzymes remain to be discovered and that many diseases of metabolite repair still await elucidation.

KEYWORDS
1,5-anhydroglucitol-6-phosphate, D-2-hydroxyglutaric aciduria, enzyme promiscuity, G6PC3, G6PT, galactose, inborn errors of metabolism, metabolite repair, NADP(H)X, neutropenia, PGM1, SGLT2 inhibitor, UGP2

Abbreviations: D2HGDH, D-2-hydroxyglutarate dehydrogenase; G6PC3, ubiquitous glucose-6-phosphatase; G6PT, glucose-6-phosphate translocase of the endoplasmic reticulum; GALE, UDP-galactose epimerase; GALK1, galactokinase; GALT, galactose-1-phosphate uridylyltransferase; HK, hexokinase; L2HGDH, L-2-hydroxyglutarate dehydrogenase; NAXE, NAD(P)/HX dehydratase; NAXE, NAD(P)H epimerase.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2019 The Authors. Journal of Inherited Metabolic Disease published by John Wiley & Sons Ltd on behalf of SSIEM
1 | THE CLASSICAL VIEW OF METABOLISM

Work performed by the founders of intermediary metabolism led to the notion that enzymes are fantastic catalysts compared to those that are used by chemists. Enzymes are able to work under mild conditions of pH and temperature, they are often regulated and very importantly, they are remarkably specific. They act best on their physiological substrates, which are metabolites that make up the already known metabolic pathways. All this makes a lot of sense. Enzyme specificity was rightly assumed to prevent the production of substantial amounts of useless and potentially toxic side products. It allows also intermediary metabolism to be organised in metabolic pathways that involve a series of reactions, each catalysed by a ‘specific’ and dedicated enzyme. This organisation allows one to understand easily the major metabolic consequences of the enzyme defects causing inborn errors of metabolism. No doubt that this description of metabolism is largely true ... but for the fact that enzyme specificity is not absolute!1-3

2 | ENZYME IMPERFECTION

Articles describing new enzymes usually provide information on substrate specificity. In most of the cases, one compound is a much better substrate (usually 100- to 1000-fold) than the other structurally similar molecules that have been tested. For instance, the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase acts much better on glyceraldehyde-3-phosphate than on any other aldehyde that may occur in cells. Yet, in vitro, it also acts on erythrose-4-phosphate, an intermediate of the pentose phosphate pathway, at about 1/3000 of the rate of the ‘normal’ reaction.4,5 Does this side reaction also occur in vivo? Yes, it does. The reason why we do not see any accumulation of 4-phosphoerythronate in vivo is not that glyceraldehyde-3-phosphate dehydrogenase is more specific in its physiological context than in vitro, but simply that, as recently discovered,5 a ‘4-phosphoerythronate phosphatase’ eliminates this side product in the intact cell (see below).

The study of inborn errors of metabolism makes us familiar with the idea that enzyme specificity is not perfect. The name commonly given to ‘phenylketonuria’6 (rather than hyperphenylalaninemia or phenylalanine hydroxylase deficiency) illustrates this very well. The accumulation of phenylpyruvate and phenylacetate in this disease is indeed most likely the consequence of side activities of transaminases and α-keto acid dehydrogenases, stimulated by the elevated concentrations of phenylalanine. Numerous other examples exist of such promiscuous reactions taking place in the context of inborn errors of metabolism. If such reactions can be detected in vivo when the concentration of a promiscuous substrate is elevated due to a metabolic block, there is no reason to believe that they do not occur, at lower rates, under normal conditions.

The lack of absolute specificity of enzymes is the rule and not the exception.3 The description of enzymes with the lock-and-key model (though historically extremely important for the understanding of catalysis) is misleading in this respect. Proteins are flexible structures: the main polypeptide chain and the side chains of the amino acid residues may move to accommodate a molecule that is slightly different from the ideal substrate. This will of course result in less favourable kinetic properties, but the activity will still be significant.

3 | THE INBORN ERROR OF METABOLISM L-2-HYDROXYGLUTARIC ACIDURIA ILLUSTRATES THE IMPORTANCE OF METABOLITE REPAIR

The study of L-2-hydroxyglutaric aciduria underlined the importance of metabolite repair.7 L-2-hydroxyglutaric aciduria results from a deficiency in L-2-hydroxyglutarate dehydrogenase, the FAD-linked mitochondrial enzyme that catalyses the irreversible conversion of L-2-hydroxyglutarate to α-ketoglutarate.8 L-2-hydroxyglutarate has no physiological role in higher organisms. It is made as a consequence of weak side-activities of L-malate dehydrogenase9,10 and L-lactate dehydrogenase11 that lead to the reduction of α-ketoglutarate (structurally analogous to both oxaloacetate and pyruvate) to L-2-hydroxyglutarate. These side-activities are extremely low, as illustrated by the observation that α-ketoglutarate is more than one million times less good as a substrate for mammalian L-malate dehydrogenase than oxaloacetate is.10 Yet, taking into account the enzyme abundance and the concentration of the substrates in tissues, we could calculate that L-malate dehydrogenase catalyses the synthesis of several grams of L-2-hydroxyglutarate every day in a human adult.8 The same is true for lactate dehydrogenase. This considerable production of L-2-hydroxyglutarate, despite the high degree of specificity of L-malate and L-lactate dehydrogenase, implies that an additional mechanism is needed to prevent the accumulation of...
L-2-hydroxyglutarate. This is precisely the role of L-2-hydroxyglutarate dehydrogenase, a metabolite repair enzyme.7

If this metabolite repair enzyme is inactivated by mutations, L-2-hydroxyglutarate accumulates in tissues and particularly in the brain,9,12 presumably because of the presence of the Na⁺-dependent dicarboxylate transporter (NaDC3-SLC13A3), which pumps dicarboxylic acids into astrocytes.13 Since L-2-hydroxyglutarate is a structural analog of α-ketoglutarate, its accumulation in cells may inhibit several of the many enzymes that use this Krebs cycle intermediate as a substrate, as for instance histone demethylases, methylcytidine hydroxylase14,15 and lysine-α-ketoglutarate reductase,9 the first enzyme of the lysine degradation pathway.

The metabolite repair concept leads us to rephrase the statement on the importance of enzyme specificity in intermediary metabolism. ‘Enzymes of intermediary metabolism typically show a high degree of specificity. However, this specificity is not absolute and a special set of enzymes—metabolite repair enzymes—are needed to metabolize the side-products resulting from the lack of absolute specificity’ (See Figure 1).2

4 | A METABOLITE REPAIR DEFECT EXPLAINS THE NEUTROPENIA FOUND IN G6PC3 DEFICIENCY AND IN GLYCOGEN STORAGE DISEASE TYPE IB

Ubiquitous glucose-6-phosphatase (G6PC3) was discovered16,17 as a homologue of glucose-6-phosphatase (G6PC1), the enzyme that produces glucose from glucose-6-phosphate in the endoplasmic reticulum of liver and kidney cells. Unlike G6PC1, G6PC3 is ubiquitously distributed in tissues and has very low glucose-6-phosphatase activity. In contrast to G6PC1 deficiency, which causes hypoglycemia, lactic acidosis and accumulation of glycogen in liver and kidney (Glycogen Storage Disease type Ia), G6PC3 deficiency does not cause metabolic symptoms, but it is consistently associated with a severe neutropenia and, at least in humans, with heart, blood vessel and urogenital tract malformations.18,19 Both G6PC1 and G6PC3 are trans-membrane proteins that are inserted in the endoplasmic reticulum membrane with their catalytic site oriented towards the lumen of this organelle. A ubiquitously expressed glucose-6-phosphate transporter (G6PT,
encoded by the SLC37A4 gene) mediates transport of glucose-6-phosphate from the cytosol to the lumen of the endoplasmic reticulum (reviewed in Reference 20). Its deficiency results in Glycogen Storage Disease type Ib,21,22 which combines the same metabolic symptoms as G6PC1 deficiency with a severe neutropenia similar to that observed in G6PC3 deficiency.23

Neutrophils from patients with G6PT or G6PC3 deficiency were described as showing a reduced capacity to utilise glucose, decreased levels of ATP, a decreased respiratory burst, and a defect in protein glycosylation.24,25 The mechanism by which a lack of G6PT or G6PC3 leads to neutropenia and neutrophil dysfunction remained an enigma until very recently, when we discovered that G6PC3 and G6PT collaborate to destroy a glucose-6-phosphate analog called 1,5-anhydroglucitol-6-phosphate (best described as ‘1-deoxyglucose-6-phosphate’).26 1,5-anhydroglucitol-6-phosphate is made from 1,5-anhydroglucitol, a compound normally present in blood at 100 to 150 μM27 by side activities of glucose-phosphorylating enzymes,26,28 namely low Km hexokinases and ADP dependent glucokinase (ADPGK).29 1,5-anhydroglucitol-6-phosphate is a strong inhibitor of low Km hexokinases,26,30 whose intracellular accumulation may therefore block glucose utilisation. What makes neutrophils particularly sensitive to hexokinase inhibition is the fact that the cells have lost virtually all their mitochondria during the maturation process. This makes them almost totally dependent on glucose metabolism.31,32

The proposed mechanism (Figure 2) is supported by many experimental data.26 Inactivation of G6PC3 or G6PT leads to marked accumulation of 1,5-anhydroglucitol-6-phosphate in cells incubated with physiological concentrations of 1,5-anhydroglucitol. Immortalised G6PC3-deficient mouse neutrophil progenitors accumulate 1,5-anhydroglucitol-6-phosphate and die when challenged with 1,5-anhydroglucitol, while control cells are resistant to 1,5-anhydroglucitol toxicity. The low number of neutrophils in G6PC3-deficient mice can be normalised by lowering the concentration of 1,5-anhydroglucitol in their blood with an inhibitor of the kidney sodium-dependent glucose transporter (SGLT2).33 On the contrary, administration of 1,5-anhydroglucitol to G6PC3-deficient mice further decreases their neutrophil count. Neutrophils isolated from patients with G6PC3 or G6PT deficiency, show

**FIGURE 2** Role of G6PC3 and G6PT to maintain a low level of 1,5-anhydroglucitol-6-phosphate and thereby prevent the toxic effects of this compound in neutrophils. 1,5-anhydroglucitol, which is normally present in the blood at ≈ 100 μM, is phosphorylated to 1,5-anhydroglucitol-6-phosphate by side activities of low-Km hexokinases and of ADP dependent glucokinase (ADPGK). The glucose-6-phosphate transporter G6PT and G6PC3 collaborate to hydrolyse 1,5-anhydroglucitol-6-phosphate, thereby preventing it to inhibit low Km hexokinases. This explains the lower glucose phosphorylation rates observed in neutrophils from patients with G6PC3 or G6PT transporter deficiency. G6PC3 was previously assumed to act as a glucose-6-phosphatase64 but as can be understood from this scheme, if this were the case, suppression of hydrolysis of glucose-6-phosphate should increase the flux through glycolysis and the pentose-phosphate pathway, not decrease it (from Reference 26 with the required permission)
concentrations of 1,5-anhydroglucitol-6-phosphate (≈ 3 mM) that are far above the Ki of hexokinase 3 (the main hexokinase in neutrophils)\textsuperscript{34} for this inhibitor, and close to 1000-fold higher than the concentration found in neutrophils from healthy controls.\textsuperscript{26}

Taken together, these findings indicate that G6PC3, in collaboration with G6PT, keeps the intracellular concentration of 1,5-anhydroglucitol-6-phosphate low and thereby prevents hexokinase inhibition and a reduction in the rate of glucose metabolism in neutrophils. The neutropenia observed in G6PC3 or G6PT deficiency is therefore caused by a defect of metabolite repair (see Figure 1B), which might be responsive to treatment with inhibitors of the Na\textsuperscript{+}-dependent glucose transporter of the kidney (SGLT2), currently used in the treatment of type II diabetes.

Most of the body pool of 1,5-anhydroglucitol derives from food.\textsuperscript{27} Therefore, in principle, it could be interesting to propose a 1,5-anhydroglucitol-low diet to treat the neutropenia. However, designing such a diet may be difficult, because 1,5-anhydroglucitol is present in most foods.\textsuperscript{27} Its origin is the breakdown of starch, glycogen or other glucosides to 1,5-anhydrofructose, which is then enzymatically reduced to 1,5-anhydroglucitol.\textsuperscript{35,36} The degradation of glucosides to 1,5-anhydrofructose is carried out by microbial enzymes known as alpha-1,4-glucan lyases, but it also happens as a weak side activity of some glucosidases, and this presumably explains the wide occurrence of 1,5-anhydroglucitol and the fact that there is some endogenous production of this polyl in the human body. A recent genome-wide association study of serum 1,5-anhydroglucitol concentrations allowed the identification of seven loci, four of which were close to genes encoding enzymes involved in carbohydrate digestion in the gut.\textsuperscript{37} This suggests that the gut, and probably the bacteria that it contains, play an important role in the production of 1,5-anhydroglucitol. This important point deserves further investigations.

G6PT has therefore three distinct functions: (a) to provide glucose-6-phosphate to G6PC1 in gluconeogenic tissues; (b) to provide glucose-6-phosphate to hexose-6-phosphate dehydrogenase,\textsuperscript{38} the NADPH-producing enzyme of the endoplasmic reticulum; this reaction takes place in all tissues; (c) to transport 1,5-anhydroglucitol-6-phosphate from the cytosol to the endoplasmic reticulum to allow its hydrolysis by G6PC3; the latter is a metabolite repair function that occurs in most tissues and cells, but is particularly critical for neutrophils. G6PT is therefore an example of a protein that participates both in classical metabolism and in metabolite repair.

Is the hydrolysis of 1,5-anhydroglucitol-6-phosphate the only function of G6PC3? Possibly not, since G6PC3 has a wide substrate specificity,\textsuperscript{26} suggesting that it could have more than one physiological substrate. Therefore, it is possible that the lack of hydrolysis of one of these other substrates is at the origin of the malformations frequently observed in patients with mutations in G6PC3.

5 | DEFICIENCY IN TWO SUPER-CONSERVED REPAIR ENZYMES LEADS TO A NEW METABOLIC DISEASE

Coenzymes and cofactors have the appropriate reactivity to facilitate a whole diversity of reactions. However, because of their high reactivity, these molecules are more likely to be damaged than other more stable molecules. This is true for the pyridine nucleotides, NAD and NADP, which under their reduced form (NADH and NADPH), are rather easily converted to hydrated forms (called NADHX and NADPHX), that is, to forms that have undergone a water addition reaction. This reaction may for example be caused by a side activity of glyceraldehyde-3-phosphate dehydrogenase.\textsuperscript{39,40} But it may also be spontaneous, and this is particularly true for NADPH, which is converted to NADPHX at a rate of 10\% per hour at 37°C.\textsuperscript{41} The enzyme that reverses this damage by removing a water molecule from NAD(P)HX in an ATP-dependent manner was first described in 1956.\textsuperscript{42} Yet, it was only recently that its sequence was identified.\textsuperscript{41} This raised the awareness that NADPHX dehydratase is an extremely conserved protein, which is present in virtually all eukaryotes, prokaryotes, and archaea. It also revealed the existence of an epimerase needed to change the orientation of the hydroxyl group resulting from the water addition reaction.\textsuperscript{41} As the dehydratase is specific for the S-epimer of NAD(P)HX, the epimerase is required for the repair of the R-epimer, which is equally formed when NADH or NADPH are hydrated. The epimerase is also an extremely conserved protein. This high conservation is not surprising: NAD and NADP are common to all living organisms and the problem of their hydration may have been particularly critical for the early forms of life, which appeared in surroundings with a high temperature. Indeed, increasing the temperature strongly enhances the rate at which spontaneous reactions take place. NADHX and NADPHX are known to inhibit several enzymes that use NAD or NADP as cofactors.\textsuperscript{43} This, together with the loss of functional nicotinamide nucleotides are certainly very good reasons to have a dedicated repair system that costs only one high energy bond per repaired NAD or NADP molecule (see Figure 1B).

Inactivating mutations in the epimerase\textsuperscript{44,45} and in the dehydratase\textsuperscript{46} have recently been shown to cause a new form of leukoencephalopathy, which is characterised by the fact that fever episodes lead to a dramatic deterioration of the clinical state of the patients. In this disease, fever may not only result in a favoured unfolding of
mutated enzymes, but also in enhancing the rate of formation of NAD(P)HX. The details of the pathophysiological mechanisms are still unknown, but both enzymes deficiencies lead to the accumulation of NADHX in cells and to perturbations in mitochondrial respiration.\textsuperscript{47}

6 | D-2-HYDROXYGLUTARIC ACIDURIA TYPE I, ANOTHER DEFECT OF METABOLITE REPAIR

Two enzymes produce D-2-hydroxyglutarate from \(\alpha\)-ketoglutarate in normal mammalian cells (Figure 3). (a) 3-Phosphoglycerate dehydrogenase, the first enzyme in the serine synthesis pathway shows a side activity on \(\alpha\)-ketoglutarate, a structural analog of the normal product of the 3-phosphoglycerate dehydrogenase reaction, and reduces it to D-2-hydroxyglutarate\textsuperscript{48}; (b) hydroxyacid:oxoacid transhydrogenase (HOT) an unusual oxidoreductase that uses \(\alpha\)-ketoglutarate as an electron acceptor, converting it to D-2-hydroxyglutarate. The physiological role of HOT is to oxidise 4-hydroxybutyrate to succinate semialdehyde.\textsuperscript{49} This reaction is required because part of the succinate semialdehyde that is produced from GABA by GABA transaminase is transiently reduced by non-specific aldehyde reductases to 4-hydroxybutyrate, a metabolic dead end. Both sources of D-2-hydroxyglutarate are clearly the result of enzyme side activities, rather than mainstream metabolism.

D-2-hydroxyglutarate dehydrogenase is the repair enzyme that metabolises D-2-hydroxyglutarate. It has common features with L-2-hydroxyglutarate dehydrogenase, in that it is an FAD-linked mitochondrial enzyme that irreversibly converts its substrate to \(\alpha\)-ketoglutarate.\textsuperscript{50} Its deficiency leads D-2-hydroxyglutaric aciduria type I, which because of the origin of its substrate, is also a

\[\text{Veiga-da-Cunha et al.} \quad 19\]

**FIGURE 3** Production of D-2-hydroxyglutarate and its metabolism by the FAD-dependent D-2-hydroxyglutarate dehydrogenase. The mitochondrial enzyme D-2 hydroxyglutarate dehydrogenase catalyses the irreversible oxidation of D-2-hydroxyglutarate and its inactivation by mutations causes D-2-hydroxyglutaric aciduria type I. D-2-hydroxyglutarate can be produced from \(\alpha\)-ketoglutarate by four different enzymes. Hydroxyacid-oxoacid transhydrogenase (HOT) oxidises 4-hydroxybutyrate using alpha-ketoglutarate as an electron acceptor. 3-P-glycerate dehydrogenase, an enzyme involved in the pathway of serine synthesis (not shown), has a side activity on \(\alpha\)-ketoglutarate due to the structural similarity of the latter with 3-phosphohydroxypropionate, the normal product of this enzyme. Mutated forms of IDH1 and IDH2, as described in various cancers, particularly in glioblastomas,\textsuperscript{51,52} and in D-2-hydroxyglutaric aciduria type II (mutations in IDH2) very efficiently catalyse the reduction of \(\alpha\)-ketoglutarate to D-2-hydroxyglutarate; in this condition, the metabolic capacity of D-2-hydroxyglutarate dehydrogenase is exceeded and D-2-hydroxyglutarate accumulates
metabolite repair defect. D-2-hydroxylglutarate dehydrogenase has a very high affinity for D-2-hydroxylglutarate (Km < 10 μM), but only a very low metabolic capacity, which is sufficient to cope with normal production of D-2-hydroxylglutarate, but can be overwhelmed when production increases.

In several cancer cells, and particularly glioblastoma, mutations of isocitrate dehydrogenase 1 or 2 (IDH1 and IDH2) make that these enzymes become NADPH-dependent α-ketoglutarate reductases and no longer display their normal isocitrate dehydrogenase activity.51,52 Hereditarily transmitted mutations of IDH2 with a similar effect are found in patients with D-2-hydroxylglutaric aciduria type 2.53 Mutated IDH1 or IDH2 become so efficient at producing D-2-hydroxylglutarate that the low metabolic capacity of D-2-hydroxylglutarate dehydrogenase is no longer adequate to prevent D-2-hydroxylglutarate accumulation.

It is outside the scope of this short review to discuss the role of D-2-hydroxylglutarate in oncogenesis. Intriguingly, patients with D-2-hydroxylglutaric aciduria type 1 show widely variable clinical spectra, and the same is true for the type II form.54 This is possibly the result of D-2-hydroxylglutarate affecting the pattern of gene expression by inhibiting α-ketoglutarate dependent enzymes such as proline hydroxylase, histone demethylases, and methylcytosine hydroxylase.14,15

7 | HOW MANY METABOLITE REPAIR ENZYMES ARE THERE?

The rapid pace of discovery of new metabolite repair enzymes in recent years2,55 indicates that many metabolite repair enzymes still remain to be discovered. Glycolysis is the metabolic pathway for which the inventory of metabolite repair enzymes is the most complete.55 Eleven different repair reactions have been identified. In addition to those described above and encoded by the genes L2HGDH, G6PC3, NAXD, and NAXE, we should add PGP (encoding phosphoglycolate phosphatase), which destroys three toxic glycolytic side products: 4-phosphoerythronate, L-2-hydroxyglutarate, and 2-phosphoglycolate5; glyoxalase I and II, which destroy methylglyoxal, a toxic compound formed from triose-phosphates spontaneously or by a side activity of triose-phosphate isomerase56; and finally two enzymes that serve to repair glycation products resulting from the spontaneous reaction of glucose or glucose-6-phosphate with amines.57,58 Thus, the number of known repair reactions just matches the number of classical enzymatic reactions involved in the conversion of glucose to lactate. If this ratio of about one repair enzyme per conventional enzyme applies to the whole metabolic map, we are probably still missing hundreds of metabolite repair enzymes, and possibly the explanation for many inborn errors of metabolism.

8 | WHEN IS A METABOLITE REPAIR ENZYME CRITICAL?

Two main reasons justify the existence of a metabolite repair enzyme. The main one is that the repair enzyme removes a toxic metabolite. This accounts for the importance of eliminating NAD(P)HX, D-2-hydroxylglutarate, L-2-hydroxylglutarate and 1,5-anhydroglucitol-6-phosphate (see Figure 1B) and 4-phosphoerythronate.

Another reason may be to recover potentially useful metabolites. Accordingly, Nit1 which is a highly conserved amidase found in animals, yeast, plants, and many bacteria, hydrolyzes deaminated glutathione, a damaged form of glutathione resulting from the side activities of various transaminases.59 Nit1 thereby allows the cells to recycle the useful molecules of which deaminated glutathione is made of, that is, α-ketoglutarate, cysteine and glycine. Interestingly, absence of this repair enzyme in mice, yeast, eukaryotic cell lines and Arabidopsis thaliana has no detrimental consequences, despite the striking accumulation (and urinary loss) of deaminated glutathione.59,60 Deaminated glutathione is likely innocuous. Yet, because of the important urinary loss of deaminated glutathione induced by its accumulation, the deficiency of Nit1 may increase the need for sulfur amino acids in the diet and therefore be problematic when the diet is deficient in these.

Therefore, it is likely that diseases of metabolite repair occur mostly when the abnormal metabolite that needs to be destroyed is toxic, because, in most of the cases, it acts as an inhibitor of one or several enzymes of intermediary metabolism. In these cases when the abnormal metabolite is an extremely potent inhibitor (4-phosphoerythronate5; NADHX46), low levels of accumulation are enough to cause dramatic perturbations. Identifying the toxic compound may be very difficult in the absence of ad hoc hypotheses, particularly if this compound is an unknown metabolite. Yet, identifying the exact function of a metabolite repair enzyme in the context of human inborn errors of metabolism may be very useful as it may lead to new therapeutic strategies, as appears to be the case for 1,5-anhydroglucitol-6-phosphate.26

Many diseases of metabolite repair likely remain to be elucidated. This may be the case of diseases for which the mutated gene is already known, but for which the function of the encoded product is unknown. If the encoded protein is ‘enzyme-like’ or even simply if it is conserved in very distant organisms like bacteria (metabolic enzymes are the most conserved proteins), the possibility that it is a
yet unknown enzyme of metabolite repair needs to be taken into consideration, and the adequate strategy followed to understand its function.

9 | EPILOGUE: WHEN ENZYME PROMISCUITY IS USEFUL

As often observed in bacteria, enzyme promiscuity may be advantageous in the context of enzyme defects, also in humans. A nice example is phosphoglucomutase 1 (PGM1) deficiency, which causes problems in the synthesis of glycans because of defective production of glucose-1-phosphate and therefore of UDP-glucose (by UDP-glucose-pyrophosphorylase—UGP2) and UDP-galactose (by UDP-galactose epimerase—GALE) (Figure 4A). Remarkably, the glycosylation defect in this disease is efficiently treated by galactose supplements, which in fibroblasts and presumably also in vivo increase the UDP-glucose and UDP-galactose pools. The mechanism of this effect is more complex than it would appear at first sight.

Galactose is phosphorylated to galactose-1-phosphate (GALK1), which is converted to UDP-galactose by transferase (GALT). Importantly, this transuridylylation reaction does not increase the (UDP-glucose + UDP-galactose) pool (circled in green in Figure 4), even if large amounts of galactose-1-phosphate are present, because one mole of UDP-glucose is consumed for each mole of UDP-galactose that is formed in the transuridylylation reaction. There is therefore no net formation of UDP-hexose (Figure 4A).

The simplest way of explaining the increase in the UDP-hexose pool under these conditions is to assume that galactose-1-phosphate can be converted to UDP-galactose by a reaction that uses another uridylyl donor than UDP-glucose. Such a reaction does indeed exist: specificity studies have shown that UDP-glucose pyrophosphorylase (UGP2) has a weak (a few percentage of the classical reaction) but significant side activity, where UTP and galactose-1-phosphate are converted to UDP-galactose and inorganic pyrophosphate (UTP + galactose-1-phosphate → UDP-galactose + PPi) (Figure 4B). This reaction is certainly facilitated by the high concentration of galactose-1-phosphate resulting from the galactose supplements, and by the low concentration of glucose-1-phosphate (one of the substrates of UGP2) due to the PGM1 deficiency. The UDP-galactose that is formed in this way can be converted to UDP-glucose by the epimerase (GALE) and, via the GALT reaction, replenish the glucose-1-phosphate pool. Glucose-1-phosphate is in turn converted to a second molecule of UDP-glucose, initiating a virtuous cycle that progressively expands the UDP-hexose pool and will cease only when galactose-1-phosphate is exhausted (Figure 4).

To conclude, this a contrario example does not detract from the importance of metabolite repair. It just highlights the complexity of metabolism and how far we need to go in the details of pathophysiological mechanisms to provide the best help to the patients. There is little doubt that the number of diseases that will be explained by a default in metabolite repair mechanisms will greatly expand in the coming years.

ACKNOWLEDGMENTS

The work performed in the authors’ laboratory is supported by the Fonds de la Recherche Scientifique-FRS/FNRS...
GB is Maria Veiga-da-Cunha ORCID described in the article. All authors have contributed to the reporting of the work AUTHOR CONTRIBUTIONS

CONFLICT OF INTEREST Maria Veiga-da-Cunha, Emile Van Schaftingen and Guido Bommer declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS All authors have contributed to the reporting of the work described in the article.

ORCID

Maria Veiga-da-Cunha https://orcid.org/0000-0002-2968-7374

REFERENCES

1. Khersonsky O, Tawfik DS. Enzyme promiscuity: a mechanistic and evolutionary perspective. Annu Rev Biochem. 2010;79: 471-505.
2. Linster CL, Van Schaftingen E, Hanson AD. Metabolite damage and its repair or pre-emption. Nat Chem Biol. 2013;9: 72-80.
3. Peracchi A. The limits of enzyme specificity and the evolution of metabolism. Trends Biochem Sci. 2018;43:984-996.
4. Ishii Y, Hashimoto T, Minakami S, Yoshikawa H. The formation of erythronic acid 4-phosphate from erythrose 4-phosphate by glyceraldehyde-3-phosphate dehydrogenase. J Biochem. 1964;56:111-112.
5. Collard F, Baldin F, Gerin I, et al. A conserved phosphatase destroys toxic glycolytic side products in mammals and yeast. Nat Chem Biol. 2016;12:601-607.
6. Scrivener CR. The PAH gene, phenylketonuria, and a paradigm shift. Hum Mutat. 2007;28:831-845.
7. Van Schaftingen E, Rzem R, Veiga-da-Cunha M. 1-2-Hydroxyglutaric aciduria, a disorder of metabolite repair. J Inherit Metab Dis. 2009;32:135-142.
8. Rzem R, Veiga-da-Cunha M, Noel G, et al. A gene encoding a putative FAD-dependent L-2-hydroxyglutarate dehydrogenase is mutated in L-2-hydroxyglutaric aciduria. Proc Natl Acad Sci U S A. 2004;101:16849-16854.
9. Rzem R, Achouri Y, Marbaix E, et al. A mouse model of L-2-hydroxyglutaric aciduria, a disorder of metabolite repair. PLoS One. 2015;10:e0119540.
10. Rzem R, Vincent MF, Van Schaftingen E, Veiga-da-Cunha M. L-2-hydroxyglutaric aciduria, a defect of metabolite repair. J Inherit Metab Dis. 2007;30:681-689.
11. Intlekofer AM, Dematteo RG, Venneti S, et al. Hypoxia induces production of L-2-Hydroxyglutarate. Cell Metab. 2015;22: 304-311.
12. Angileri E, Bertolino N, Salsano E, et al. In-vivo brain H1-MR-spectroscopy identification and quantification of 2-hydroxyglutarate in L-2-Hydroxyglutaric aciduria. Brain Res. 2016;1648:506-511.
13. Brauburger K, Burckhardt G, Burckhardt BC. The sodium-dependent di- and tricarboxylate transporter, NaCT, is not responsible for the uptake of D-, L-2-hydroxyglutarate and 3-hydroxyglutarate into neurons. J Inherit Metab Dis. 2011;34:477-482.
14. Chowdhury R, Yeoh KK, Tian YM, et al. The oncometabolite 2-hydroxyglutarate inhibits histone lysine demethylases. EMBO Rep. 2011;12:463-469.
15. Xu W, Yang H, Liu Y, et al. Oncometabolite 2-hydroxyglutarate is a competitive inhibitor of alpha-ketoglutarate-dependent dioxygenases. Cancer Cell. 2011;19:17-30.
16. Martin CC, Oeser JK, Svitek CA, Hunter SI, Hutton JC, O’Brien RM. Identification and characterization of a human cDNA and gene encoding a ubiquitously expressed glucose-6-phosphatase catalytic subunit-related protein. J Mol Endocrinol. 2002;29:205-222.
17. Shieh JJ, Pan CJ, Mansfield BC, Chou JY. A glucose-6-phosphate hydrolase, widely expressed outside the liver, can explain age-dependent resolution of hypoglycemia in glycogen storage disease type Ia. J Biol Chem. 2003;278:47098-47103.
18. Boztug K, Appaswamy G, Ashikov A, et al. A syndrome with congenital neutropenia and mutations in G6PC3. N Engl J Med. 2009;360:32-43.
19. Cheung YY, Kim SY, Yiu WH, et al. Impaired neutrophil activity and increased susceptibility to bacterial infection in mice lacking glucose-6-phosphatase-beta. J Clin Invest. 2007;117:784-793.
20. van Schaftingen E, Gerin I. The glucose-6-phosphatase system. Biochem J. 2002;362:513-532.
21. Gerin I, Veiga-da-Cunha M, Achouri Y, Collet JF, Van Schaftingen E. Sequence of a putative glucose 6-phosphate translocase, mutated in glycogen storage disease type Ib. FEBS Lett. 1997;419:235-238.
22. Veiga-da-Cunha M, Gerin I, Chen YT, et al. A gene on chromosome some 11q23 coding for a putative glucose-6-phosphate translocase is mutated in glycogen-storage disease types I b and I c. Am J Hum Genet. 1998;63:976-983.
23. Chou JY, Jun HS, Mansfield BC. Type I glycogen storage diseases: disorders of the glucose-6-phosphatase/glucose-6-phosphate transporter complexes. J Inherit Metab Dis. 2015;38:511-519.
24. Bashan N, Potashnik R, Peist A, Peleg N, Moran A, Moses SW. Deficient glucose phosphorylation as a possible common denominator and its relation to abnormal leucocyte function, in glycogen storage disease 1b patients. Eur J Pediatr. 1993;152 (Suppl 1):S44-S48.
25. Hayee B, Antonopoulos A, Murphy EJ, et al. G6PC3 mutations are associated with a major defect of glycosylation: a novel mechanism for neutrophil dysfunction. Glycobiology. 2011;21: 914-924.
26. Veiga-da-Cunha M, Chevalier N, Stephene X, et al. Failure to eliminate a phosphorylated glucose analog leads to neutropenia in patients with G6PT and G6PC3 deficiency. Proc Natl Acad Sci U S A. 2019;116:1241-1250.
27. Yamanouchi T, Tachibana Y, Akanuma H, et al. Origin and disposal of 1,5-anhydroglucitol, a major polyol in the human body. *Am J Physiol*. 1992;263:E268-E273.
28. Sols A, Crane RK. Substrate specificity of brain hexokinase. *J Biol Chem*. 1954;210:581-595.
29. Richter JP, Goroncy AK, Ronimus RS, Sutherland-Smith AJ. The structural and functional characterization of mammalian ADP-dependent Glucokinase. *J Biol Chem*. 2016;291:3694-3704.
30. Crane RK, Sols A. The non-competitive inhibition of brain hexokinase by glucose-6-phosphate and related compounds. *J Biol Chem*. 1954;210:597-606.
31. Borregaard N, Herlin T. Energy metabolism of human neutrophils during phagocytosis. *J Clin Invest*. 1982;70:550-557.
32. Maianski NA, Geissler J, Srinivasula SM, Alnemri ES, Roos D, Kuijpers TW. Functional characterization of mitochondria in neutrophils: a role restricted to apoptosis. *Cell Death Differ*. 2004;11:143-153.
33. Fortuna D, McCloskey LJ, Stickle DF. Model analysis of effect of canagliflozin (Invokana), a sodium-glucose cotransporter 2 inhibitor, to alter plasma 1,5-anhydroglucitol. *Clin Chim Acta*. 2016;452:138-141.
34. Rijksen G, Staal GE, Beks PJ, Streefkerk M, Akkerman JW. NAD(P)HX dehydratase producing 1,5-anhydro-D-fructose from starch and glyco-amylase acting on 1,5-anhydro-D-fructose. *Biochim Biophys Acta*. 1982;719:431-437.
35. Yu S, Bojesen K, Svensson B, Marcussen J. Alpha-1,4-glucan lyases producing 1,5-anhydro-D-fructose from starch and glyco-amylase linked to glucose metabolism. *Biochim Biophys Acta*. 1999;1433:1-15.
36. Sükuma M, Kametani S, Akanuma H. Purification and some properties of a hepatic NADPH-dependent reductase that specifically acts on 1,5-anhydro-D-fructose. *J Biochem*. 1998;123:189-193.
37. Li M, Maruthur NM, Loomis SJ, et al. Genome-wide association study of 1,5-anhydroglucitol identifies novel genetic loci linked to glucose metabolism. *Sci Rep*. 2017;7:2812.
38. Senesi S, Csala M, Marcolongo P, et al. Hexose-6-phosphate dehydrogenase in the endoplasmic reticulum. *Biolo Chem*. 2010;391:1-8.
39. Rafter GW, Chaykin S, Krebs EG. The action of glyceraldehyde-3-phosphate dehydrogenase on reduced diposphopyridine nucleotide. *J Biol Chem*. 1954;208:799-811.
40. Oppenheimer NJ, Kaplan NO. Glyceraldehyde-3-phosphate dehydrogenase catalyzed hydration of the 5-6 double bond of reduced beta-nicotinamide adenine dinucleotide (betaNADH). Formation of beta-6-hydroxy-1,4,5,6-tetrahydronicotinamide adenine dinucleotide. *Biochemistry*. 1974;13:4685-4694.
41. Marbaix AY, Noel G, Detroux AM, Vertommen D, Rider MH, Veiga-da-Cunha M. The structural and functional characterization of soluble and particulate enzymes. *Biochim Biophys Acta*. 1982;719:431-437.
42. Meinhart JO, Chaykin S, Krebs EG. Enzymatic conversion of a reduced diposphopyridine nucleotide derivative to reduced diposphopyridine nucleotide. *J Biochem*. 1956;220:821-829.
43. Yoshida A, Dave V. Inhibition of NADP-dependent dehydrogenases by modified products of NADPH. *Arch Biochem Biophys*. 1975;169:298-303.
44. Kremer LS, Danhauser K, Herebian D, et al. NAXE mutations disrupt the cellular NAD(P)HX repair system and cause a lethal Neurometabolic disorder of early childhood. *Am J Hum Genet*. 2016;99:894-902.
45. Spiegel R, Shaag A, Shalev S, Elpeleg O. Homozygous mutation in the APOA1BP is associated with a lethal infantile leukoencephalopathy. *Neurogenetics*. 2016;17:187-190.
46. Van Bergen NJ, Guo Y, Rankin J, et al. NAD(P)HX dehydratase (NAXD) deficiency: a novel neurodegenerative disorder exacerbated by febrile illnesses. *Brain*. 2019;142:50-58.
47. Becker-Ketten J, Paczia N, Conrotte JF, et al. NAD(P)HX repair deficiency causes central metabolic perturbations in yeast and human cells. *FEBS J*. 2018;285:3376-3401.
48. Fan J, Teng X, Liu L, et al. Human phosphoglycerate dehydrogenase produces the oncometabolite D-2-hydroxyglutarate. *ACS Chem Biol*. 2015;10:510-516.
49. Struys EA, Verhoeven NM, Jansen EE, et al. Metabolism of gamma-hydroxybutyrate to d-2-hydroxyglutarate in mammals: further evidence for d-2-hydroxyglutarate transhydrogenase. *Metabolism*. 2006;55:353-358.
50. Achouri Y, Noel G, Vertommen D, Rider MH, Veiga-Da-Cunha M, Van Schaftingen E. Identification of a dehydrogenase acting on D-2-hydroxyglutarate. *Biochem J*. 2004;381:35-42.
51. Dang L, White DW, Gross S, et al. Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature*. 2009;462:739-744.
52. Ward PS, Patel J, Wise DR, et al. The common feature of leukemia-associated IDH1 and IDH2 mutations is a neomorphic enzyme activity converting alpha-ketoglutarate to 2-hydroxyglutarate. *Cancer Cell*. 2010;17:225-234.
53. Kranendijk M, Struys EA, Gibson KM, et al. Evidence for genetic heterogeneity in D-2-hydroxyglutaric aciduria. *Hum Mutat*. 2010;31:279-283.
54. Kranendijk M, Struys EA, Salomons GS, Van der Knaap MS, Jakobs C. Progress in understanding 2-hydroxyglutaric acidurias. *J Inherit Metab Dis*. 2012;35:571-587.
55. Bommer GT, Van Schaftingen E, Veiga-da-Cunha M. Metabolite repair enzymes control metabolic damage in glycolysis. *Trends Biochem Sci*. 2019.
56. Richard JP. Mechanism for the formation of methylglyoxal from triosephosphates. *Biochem Soc Trans*. 1993;21:549-553.
57. Delpierre G, Rider MH, Collard F, et al. Identification, cloning, and heterologous expression of a mammalian fructosamine-3-kinase. *Diabetes*. 2000;49:1627-1634.
58. Veiga-da-Cunha M, Jacquemin P, Delpierre G, et al. Increased protein glycation in fructosamine 3-kinase-deficient mice. *Biochem J*. 2006;399:257-264.
59. Paracchi A, Veiga-da-Cunha M, Kuhara T, et al. Nt1 is a metabolite repair enzyme that hydrolyzes deaminated glutathione in Arabidopsis. *Proc Natl Acad Sci U S A*. 2017;114:E3233-E3242.
60. Niehaus TD, Patterson JA, Alexander DC, et al. The metabolite repair enzyme Nt1 is a dual-targeted amidase that disposes of damaged glutathione in Arabidopsis. *Biochem J*. 2019;476:683-697.
61. Tegtmeyer LC, Rust S, van Scherpenzeel M, et al. Multiple phenotypes in phosphoglucomutase 1 deficiency. *N Engl J Med*. 2014;370:533-542.
62. Radenkovic S, Bird MJ, Emmerzaal TL, et al. The metabolic map into the pathomechanism and treatment of PGM1-CDG. *Am J Hum Genet*. 2019;104:835-846.
63. Turnquist RL, Gillett TA, Hansen RG. Uridine diphosphate glucose pyrophosphorylase. Crystallization and properties of...
the enzyme from rabbit liver and species comparisons. *J Biol Chem.* 1974;249:7695-7700.

64. Jun HS, Lee YM, Cheung YY, et al. Lack of glucose recycling between endoplasmic reticulum and cytoplasm underlies cellular dysfunction in glucose-6-phosphatase-beta-deficient neutrophils in a congenital neutropenia syndrome. *Blood.* 2010;116:2783-2792.

**How to cite this article:** Veiga-da-Cunha M, Van Schaftingen E, Bommer GT. Inborn errors of metabolite repair. *J Inherit Metab Dis.* 2020;43:14–24. [https://doi.org/10.1002/jimd.12187](https://doi.org/10.1002/jimd.12187)