Nutrients in Energy and One-Carbon Metabolism: Learning from Metformin Users

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Abstract: Metabolic vulnerability is associated with age-related diseases and concomitant co-morbidities, which include obesity, diabetes, atherosclerosis and cancer. Most of the health problems we face today come from excessive intake of nutrients and drugs mimicking dietary effects and dietary restriction are the most successful manipulations targeting age-related pathways. Phenotypic heterogeneity and individual response to metabolic stressors are closely related food intake. Understanding the complexity of the relationship between dietary provision and metabolic consequences in the long term might provide clinical strategies to improve healthspan. New aspects of metformin activity provide a link to many of the overlapping factors, especially the way in which organismal bioenergetics remodel one-carbon metabolism. Metformin not only inhibits mitochondrial complex 1, modulating the metabolic response to nutrient intake, but also alters one-carbon metabolic pathways. Here, we discuss findings on the mechanism(s) of action of metformin with the potential for therapeutic interpretations.

Keywords: diabetes mellitus; energy intake; epigenetics; folic acid; food-drug interactions; food source; obesity; vitamins B

1. Introduction

Food restriction extends health and lifespan in some models [1], but what nutrients should be restricted and to what extent? Qualitative changes in the provision of dietary macronutrients induce metabolic and endocrine adaptations that are clinically relevant to the nutritional status of both, patients with low food availability and those with a persistent intake of excessive amounts of food [2]. In particular, the relationship between energy and one-carbon (1C) metabolism is extremely sensitive to food intake [3–6]. We envision that the inclusion of metformin in current strategies promoting metabolic fitness [7] is in accordance with the clinical response to dietary environment in disease states and the beneficial effects in metabolically vulnerable cells [8–11]. That is, it is important to revise what we can learn from metformin users and which are the potential implications. Metformin, as a

calorie-restriction mimetic drug affecting mitochondrial function, integrates metabolic signals and the direct effect on folate metabolism may provide therapeutic clues [12–15]. We highlight the effects of metformin on signaling pathways associated with some of the hallmarks of aging and the likely beneficial effects in the pathogenesis of comorbidities associated with metabolic diseases (Figure 1). Whether metformin can be safely used with these new indications remains to be established.
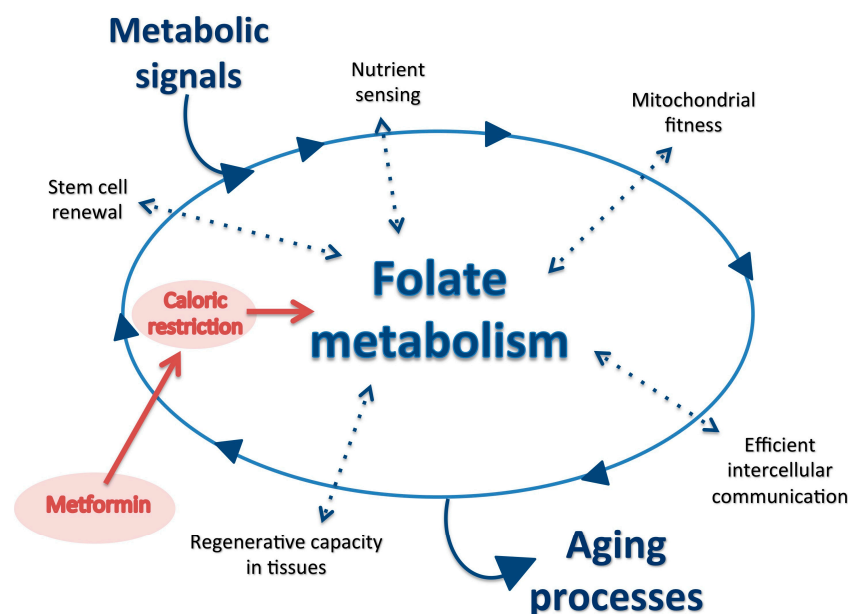

Figure 1. The effect of metformin on signaling pathways associated with nutrient excess. These effects are likely regulated by folate metabolism and represent direct effects on some of the hallmarks of tissue aging. It remains to be determined whether metformin can safely slow the development of age-related comorbidities in metabolic diseases.

Metformin is currently used exclusively in the treatment of type 2 diabetes mellitus (T2DM), but there is potential for additional indications in obesity, inflammatory disorders, cardiovascular diseases and cancer. For instance, in obese patients without diabetes, the weight loss effects of metformin are not inferior to those of a recently approved drug for obesity [16] and the antenatal administration of metformin during pregnancy reduces maternal weight gain without effects on neonatal outcomes [17–19]. Moreover, the Diabetes Prevention Program has found beneficial effects in diabetes prevention and a durable weight loss attributable to metformin [20–22]. Cardiovascular benefits are also likely, and, when compared to sulfonylurea or insulin therapy, metformin monotherapy is associated with a higher reduction in cardiovascular events [23,24]. In addition, the anti-inflammatory actions of metformin can be separated from its metabolic effects, and there is ongoing research assessing the role of metformin in the prevention and recurrence of breast cancer [25,26].

All these effects are crucial in preventing disease and promoting health. We discuss here the metabolic alterations connecting the metformin response and the function of essential nutrients, which emphasize that careful attention to diet might shape clinical strategies [27]. Our arguments are framed in the relationship between energy and one-carbon metabolism, reinforced by the mechanisms of action proposed for metformin.

2. One-Carbon Metabolism: Inputs and Outputs

Folate coenzymes play a crucial role in health and disease and are present in virtually all organisms and cell types. Moreover, although controversial, some dietary arguments support the addition of folic acid or related compounds to common foods [28–30]. In humans, dietary folic acid is reduced

to 7,8-dihydrofolate (DHF) and then to 5,6,7,8-tetrahydrofolate (THF) by dihydrofolate reductase (DHFR), initiating the folate cycle. Biochemical reactions converge here, using the ability of THF to carry 1C units in different oxidation states. Folate coenzymes contain poly-γ-glutamate tails attached to a p-aminobenzoic acid moiety by the activity of folylpolyglutamate synthetase (FPGS), an essential enzyme that regulates the distribution of different folate forms in cellular compartments and specific actions in cell proliferation pathways [31,32]. Serine, glycine and methionine are readily provided in the diet. Serine is oxidized in the mitochondria and transferred to THF by serine hydroxyl-methyl-transferase (SHMT), resulting in glycine and 5,10-methylene-THF (CH2-THF). Glycine may be incorporated directly into purine nucleotide bases or glutathione (GSH) and CH2-THF can be converted to 5-methyl-THF in a reaction catalyzed by methylenetetrahydrofolate reductase (MTHFR) or 10-formyl-THF according to cellular needs. Of note, serine and glycine may be synthesized de novo through glycolysis, aldol cleavage (from threonine, in some cells) or reactions involving demethylation (from choline, betaine, dimethylglycine and sarcosine) [31,32].

The folate cycle, closely linked to the methionine cycle, regulates the availability of methyl groups (CH3) through the sequential conversion of methionine to S-adenosylmethionine (SAM), S-adenosylhomocysteine (SAH) and homocysteine. Conversely, in the presence of 5-methyl-THF (5-mTHF) and methionine synthase, which requires vitamin B12 (cobalamin), methionine can be regenerated. Transmethylation reactions and intermediate metabolites are crucial to the synthesis of high-energy molecules, structural macromolecules, thymidine, and purines and to the maintenance of the cellular redox state and the transsulfuration pathway [33,34]. Dietary vitamins also communicate bioenergetics and 1C metabolism. For instance, the conversion of homocysteine to cysteine (transsulfuration pathway) and the synthesis of glutathione require vitamin B6 (pyridoxine) and vitamin B12 is essential (via the mitochondrial enzyme methylmalonyl-CoA mutase) to form succinyl-CoA, a key substrate of the citric acid cycle (Figure 2).
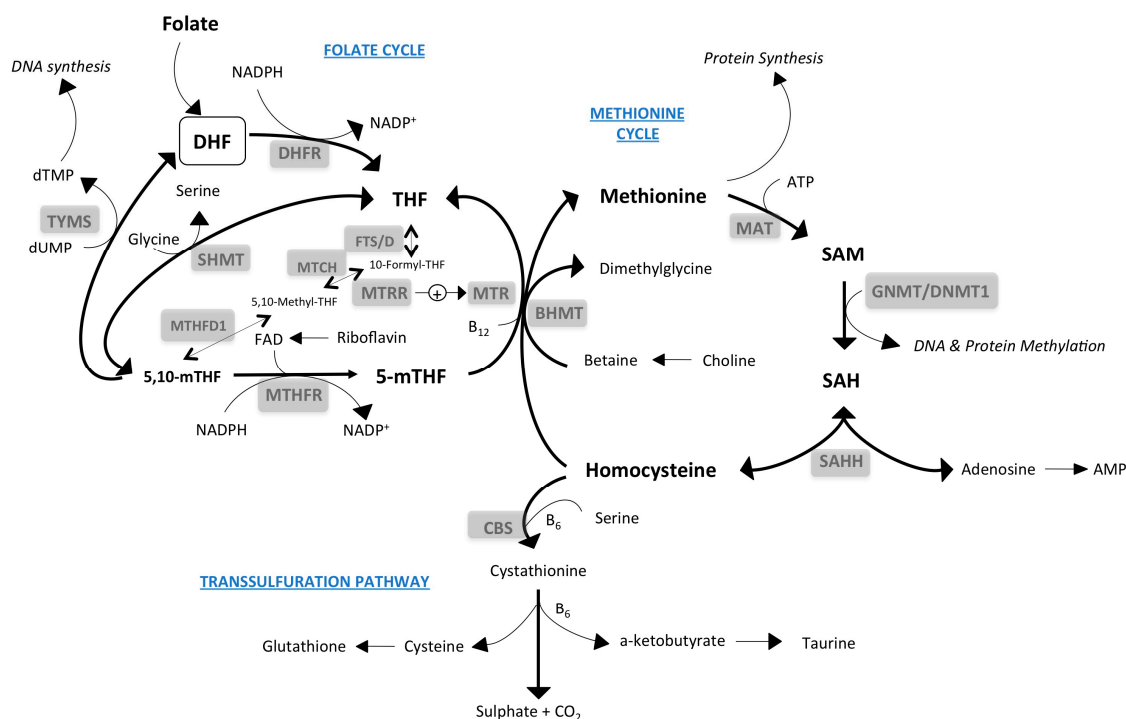
Figure 2. Metabolic pathways indicating the close dietary dependence in the folate cycle, methionine cycle and transsulfuration pathways. The role of B vitamins is paramount in regulating the expected complexities in relevant enzymes and circulating levels of metabolites. BHMT, betaine-homocysteine *S*-methyltransferase; CBS, cystathionine beta-synthase; DHFR, dihydrofolate reductase; DNMT, DNA methyltransferase; FAD, flavin adenine dinucleotide; GNMT, glycine *N*-methyltransferase; MAT, aminomethyltransferase; MTHFR, methylenetetrahydrofolate reductase; MTR, methyltransferase; SAH, S-adenosylhomocysteine; SAHH, S-adenosylhomocysteine hydrolase; SAM, S-adenosylmethionine; SHMT, serine hydroxymethyltransferase; THF, tetrahydrofolate; TYMS, thymidylate synthase; UDP, uridine diphosphate.

The cellular functions that depend on these micronutrients illustrate how important careful dietary intake is to avoid deficiencies and how difficult it is to establish clinically based data to be used in both the management of disease states and drug treatments. It may appear paradoxical, but diseases associated with excessive food intake may present deleterious effects that could result from excess in micronutrients [30]. The challenge is to achieve an adequate nutrient balance in the different groups within the general population. In this context, several complexities at the organismal level may be illustrative. For instance, the hepatic effects of diets deficient in choline and methionine are practically indistinguishable from those observed with methionine rich, high-fat, high-energy diets. In contrast, the balanced provision of methionine increases healthy lifespan in experimental models [35–37]. These nutritional observations exemplify the close dependence among regulatory pathways in energy and one-carbon metabolism and the critical importance of the equilibrium between inputs and outputs (Figures 2 and 3).
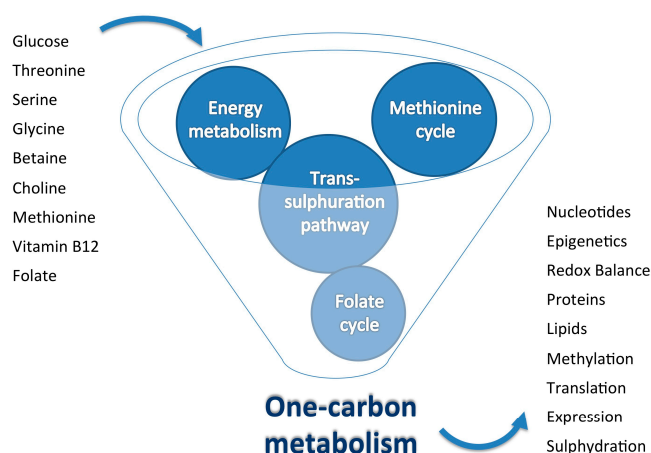
Figure 3. Schematic representation summarizing the importance of appropriate equilibrium in inputs and outputs of one-carbon metabolism. Excessive intake of a given nutrient influences the availability of other nutrients and can perturb metabolism with deleterious consequences. Understanding the events linking metabolism and diet-dependent pathways may provide crucial insight into their role in health and disease.

3. The Lack of Redundancy between Cytoplasmatic and Mitochondrial One-Carbon Metabolism Affects Tissue-Specific Responses to Nutrients

Deficient or excessive intake of nutrients causes changes in the mitochondrial electron transport chain that model the organismal response to the environment. In human cells, the specific forms of folate cofactors and the extent of polyglutamylation differ in cytosolic and mitochondrial pools. Curiously, 1C metabolism is compartmentalized, and mechanisms controlling the flux of components through these compartments are strictly regulated ([38] and references therein). Whether mitochondrial dysfunction is a cause or a consequence of disease remains debatable, but it appears that mitochondria receive monoglutamate THF, which is polyglutamylated, charged with 1C units, and later released to the cytosol [39,40], ensuring the correct function of cellular 1C metabolism.

Energy and one-carbon metabolism jointly integrate the cellular nutrient status. Reactions of glycine and serine are particularly important connecting nutrition, 1C metabolism and the effects of biguanides, as demonstrated in experimental models of glycine auxotrophy. Data in these models indicate that the cytoplasmic serine hydroxymethyltransferase (SHMT) isozyme is not essential, but glycine biosynthesis requires a different mitochondrial isozyme, which is not reversed by external addition of nutrients [41]. Moreover, in the absence of cytoplasmic SHMT, serine donates 1C units and formate flows normally to the cytoplasmic THF pool through the activity of mitochondrial methylene tetrahydrofolate dehydrogenase (MTHFD), a trifunctional folate-dependent enzyme with activity as a CH2-THF dehydrogenase, CH+-THF cyclohydrolase and 10-formyl-THF synthetase. Mice without these mitochondrial isozymes (MTHFD2/MTHFD2L) are not viable [42–44]. In addition, glycine is broken down by the exclusively mitochondrial glycine cleavage system (GCS) and in this reaction direction, the electrons are delivered to complex I of the mitochondrial respiratory chain [45]. Metformin affects mitochondrial biology at this point, but information on compensatory pathways is limited [8,31].

Understanding the transport processes between compartments and how these differences in cytoplasm and mitochondria control metabolic processes might provide a rationale for treating metabolic abnormalities. The strictly mitochondrial GCS activities support a model of multiple carrier-facilitated diffusion of metabolites between cytosol and mitochondria [30]. Because there is no methionine adenosyltransferase (MAT) activity in mitochondria, putative carriers are also necessary for the constant transport of SAM from the cytosol to the mitochondria [46]. The relationship between compartments might also explain the metabolism of choline, which is obtained primarily

as phosphatidylcholine in the diet. In the liver, choline is a major source of methyl groups through conversion to betaine (*N,N,N*-trimethylglycine). The cytosolic betaine hydroxymethyltransferase (BHMT) generates methionine and *N,N*-dimethylglycine (DMG), but DMG absolutely requires the activity of mitochondrial dehydrogenase (DMGDH) to act as a 1C donor in mitochondria. Similarly, sarcosine (*N*-methylglycine) can be a 1C donor in the cytoplasm, but in the mitochondria, it requires sarcosine dehydrogenase (SDH) [47].

Collectively, these multiple regulatory steps indicate essential mechanisms for preventing disease. Experiments with genetically engineered mice, examining each step, are of limited value to understand human disease [48], but in humans there are associations between genetic variants and several diseases [49–54]. Similarly, available data suggest that mitochondrial 1C metabolism is critical for metabolic adaptations and cell survival in cancer and T cell-mediated (immune) pathologies [55–57]. T cell activation does not increase the expression of proteins related to glycolysis, pentose phosphate pathway or oxidative phosphorylation. In contrast, T cell activation produces new and specialized mitochondria characterized by the massive induction of enzymes involved in folate-mediated 1C metabolism [55], indicating that the mitochondrial and cytosolic pathways for generating and processing 1C units are not redundant. That is, cytosolic 1C metabolism is insufficient to support T cell proliferation, cancer cell immortality and excessive metabolic impact when mitochondrial 1C metabolism is impaired [31,58–62] (Figure 4).

Figure 4. Mitochondrial and cytosolic pathways for generating and processing 1C units are separated but not redundant and depend on extracellular provision. Metabolically active cells may survive deficiencies in cytosolic isozymes but require the correct function of mitochondrial isozymes (marked in red). GCS, glycine cleavage system.

Mechanisms affecting energy and 1C metabolism are likely linked to cellular biosynthesis, redox maintenance and epigenetic status. Accurate information on the activities of the aforementioned enzymes in complex diseases might provide clues regarding how nutritional status or dietary and pharmacologic manipulations affect physiological regulation with evident therapeutic opportunities.

4. The Importance of Drugs and Diets That Modulate Mitochondrial Activity

Mitochondrial function, epigenetic signals and nutrient-sensing pathways are likely combined to promote health at both the cellular and the organismal levels [63,64]. Drugs and dietary regimens that directly modulate mitochondrial activity are promising [65].

Mitochondria are not only providers of energy but also of signaling units, and the existence of mitochondrial-derived peptides (MDPs) suggest the new concept of the existence of mitochondrial hormones and metabolic regulators [66–68]. Similar to metformin [12,69], targets of these MDPs include one-carbon metabolism, thiosulfate sulfurtransferase and AMP-activated protein kinase (AMPK), critical links between nutrients and health. Nutrient sensing is important for the distribution of energy, and changes in food intake alter metabolic strategies [70,71]. Notably, metformin activates AMPK, inducing changes in bioactive metabolites connected to transcriptional regulators through as yet undefined mechanisms [72,73] but probably including an interplay among food intake, metabolism and mTOR signaling in clinically relevant settings [74,75].

In particular, metabolic aspects in cancer are now a renewed source of potential therapeutic targets. Cells depleted of mitochondrial DNA identify metabolic vulnerabilities and illustrate that 1C metabolism, serine biosynthesis and transsulfuration are sensitive to mitochondrial dysfunction and stress [76]. Similarly, cancer cells without the ability to catalyze the conversion of isocitrate to α-ketoglutarate reveal that perturbing mitochondrial metabolism with metformin may help to kill cancer cells [77]. The challenge is to learn how to use cell- and tissue-specific variations in the mitochondrial management of 1C donors and how to interpret the clinical response to manipulations in nutrient availability. Several lines of evidence indicate that crosstalk between epigenetic signals and cellular metabolism may be a clinically useful field of research because it represents a mechanism to convert dietary-induced metabolic changes into stable patterns of altered gene expression [78–81].

5. Is Gene Expression Reprogrammed in Response to Metabolic and Dietary Stimuli Affecting One-Carbon Metabolism?

Whether epigenetic mechanisms are causally coupled to changes in metabolic phenotypes is a question posed by epidemiological studies examining extreme nutritional changes during fetal development [82–85]. It is not surprising, however, to find contradictory data because exposure windows and conditions are not controlled in documented "experiments" on long-term famine [82–84]. These studies do not reveal the contribution of specific nutrients, but studies in other cohorts associate defects in energy and one-carbon metabolism in pregnant women with future risk in offspring ([85] and references therein). Taken together, these data indicate that epigenetic modulation of metabolic pathways may promote adverse phenotypes in later life [86,87].

In the current context of excessive food intake, it is urgent to understand the potential implications of modifying epigenetic marks by nutrition and whether diet-induced changes in metabolic or phenotypic traits may affect future generations. Nutritional, pharmacological and metabolic signals induce epigenetic drift (i.e., metabolic states correlated with chromatin states), and several epigenetic marks influence the expression of neighboring genes through generations. It is apparently the feedback or combination among different epigenetic mechanisms that dynamically configures the chromatin landscape throughout life [88,89] but DNA methylation alone has strong mechanistic support to explain modifications by dietary changes. DNA methylation and certain metabolites generated by mitochondrial respiration interact with transcription factors, suggesting metabolo-epigenetic links between energy and one-carbon metabolism [90–92]. It is plausible that cellular transcriptional machinery and chromatin-associated proteins integrate inputs derived from food as part of the response of living organisms to continuous fluctuations in the availability of energy substrates.

Alterations in genes that encode enzymes affecting chromatin regulation, such as kinases, acetyltransferases, demethylases and methyltransferases, are common in dietary-related diseases [93]. These enzymes use cellular metabolites as sources of phosphate, acetyl or methyl groups, and interpret the metabolic state of a specific cell. For instance, the levels of SAM, a methyl donor, SAH and threonine

alter methylation status through pathways involving acetyl-coA metabolism, and specific dietary restrictions cause transcriptional and metabolic responses [94,95]. We recently provided an example of how metformin-driven reduction of acetyl-CoA is sufficient to correct histone H3 acetylation, indicating that metformin regulates mitonuclear communication and the cellular epigenetic landscape. These data may result in knowledge that can be applied to metabolo-epigenetic strategies for prevention or therapy [96]. Future research will ascertain how dietary manipulations and synthetic epigenetic modifiers contribute to DNA methylation and how plastic the genome is to dietary changes. It is also important to establish what magnitude of metabolic stimuli is required to produce appreciable changes and whether epigenetic changes can be reversed.

6. The Ability of Metformin to Target One-Carbon Metabolism: Perspectives in Clinical Practice Outcomes

The dietary inputs associated with the folate and methionine cycles support cellular integrity and health, but there is some risk in manipulating bioactive elements in the absence of well-proven associations between consumption and disease. This probably explains why nutritional manipulation is not incorporated into clinical practice against cancer, but we should not forget that the action of B vitamins led to the discovery of folate antagonists as a major class of cancer chemotherapy agents [97] and that restriction in some nutrients negatively affect tumor formation in mice [98].

The multi-faceted activity of metformin and its likely interconnected mechanisms of action are relevant to potential applications in diseases characterized by mitochondrial dysfunction, gene deregulation and failure in metabolic homeostasis [99–103]. The global metabolic impact of metformin and the generated energetic stress suggest a strong association with the overall management of food intake. Metformin stimulates glucose uptake by muscle, inhibits hepatic gluconeogenesis and stimulates AMPK through effects in NADH ubiquinone oxidoreductase, the first component of the electron transport chain. Other putative actions with dietary associations include AMPK-independent signaling through glucagon-dependent cyclic AMP and the inhibition of mitochondrial glycerophosphate dehydrogenase [103–105]. These findings on metformin action suggest an approach for treating metabolic diseases not restricted to the field of diabetes.

It could be argued that drugs reducing insulin levels may reduce cancer risk, and drugs that increase circulating insulin may increase cancer risk, but the effects of metformin in cancer cells seem specific, preventing the boost in glycolytic intermediates, decreasing citric acid cycle intermediates, and depleting the cellular glutathione content [106]. Moreover, metformin impairs one-carbon metabolism in a manner similar to drugs that target folate-related enzymes and have long been used to treat inflammatory diseases and cancer [8,12,106–109]. Efforts to clinically address this issue remain incomplete and difficult to interpret because heterogeneity in metformin response is considerable and only partially explained by genetic and nutritional factors. Metformin pharmacodynamics and pharmacokinetics have been reviewed recently ([110] and references therein). Current formulations have a bioavailability of ~50% (approximately 40% is absorbed in the duodenum and proximal jejunum, and ~10% in the ileum and colon). Unabsorbed drug is eliminated in the feces, and metformin circulates in the plasma unbound and without transformation until cleared by the kidneys. Metformin is a hydrophilic molecule requiring specific mechanisms of transport, primarily organic cation transporters (OCT). Briefly, plasma membrane monoamine transporter (PMAT) and OCT3 contribute to gastrointestinal uptake, and OCT1 may be responsible for transport into the interstitial fluid of the intestine. OCT1, OCT3 and multidrug and toxin extrusion proteins (MATE) are expressed on the basolateral membrane of hepatocytes. In the kidney, metformin is taken up into renal epithelial cells by OCT2 and excreted into the urine via MATE1 and MATE2. OCT1 and PMAT may contribute to metformin reabsorption. Genetic variants of these and other transporters may be important to explain the therapeutic action of metformin, the high inter-individual variability in gastrointestinal tolerance and drug–drug interactions [111–114]. It would be useful to ascertain whether these genetic variants could have predictive value in patients before they take the drug [115], but genetics alone

frequently fail to explain phenotypes. In this context, we foresee that considering metformin for non-diabetic indications is an example of the value of applying metabolomics to useful clinical research. The next task is connecting energy and one-carbon metabolism to physiology, describing heterogeneous phenotypes and defining the role of nutrition in the response to drug treatment.

7. The One-Carbon Cycle in Metformin Users and Potential Adverse Effects

We believe that metformin is best described as an "antimetabolite" drug, and, as such, several deleterious effects might be expected among long-term metformin users. Trials to ascertain these effects are scarce, and data are frequently contradictory, but people at risk of deficiency in one-carbon-related metabolites include vegans, vegetarians, pregnant women, breastfeeding women, the elderly and patients with anemia or poor renal function [116].

There is no evidence of risk in humans from taking metformin during pregnancy, but this issue requires attention [17]. Folate deficiency in metformin users is rare, but a decrease in serum and red blood cell (RBC) folate concentration is common [117]. It is difficult to ascertain the effect of folate fortification in some foods due to discrepancies in results [118] and the lack of data regarding folate bioavailability in metformin users [34,119]. Favoring the consumption of foods that are endogenously high in folates is probably reminiscent of decades using folates with cytotoxic agents, but this empirical practice requires caution. For instance, lessons from a prospective study in rural India indicate that normal/high folate concentrations associated with deficiencies in vitamin B12, attributable to a lacto-vegetarian diet combined with folic acid supplementation, may be associated with a higher risk of insulin resistance in the offspring [120]. This is relevant because metformin might facilitate the mechanism known as "methyl folate trap", which acts on the regulation of the methionine/folate cycle and cysteine oxidation [121]. According to this concept, although only demonstrated in pernicious anemia, the cell would mistakenly interpret vitamin B12 deficiency as a lack of methionine, and will divert the remaining folate away from DNA biosynthesis towards the methylation of homocysteine to methionine, building up 5 methyl THF that the cell will not be able to use [121].

The relationship between vitamin B12 deficiency and metformin treatment has been studied since the beginning of the 1970s with some clinical confusion likely related to the extreme heterogeneity among studies [122–124]. Among metformin users, a slight reduction in serum vitamin B12 concentrations is common, but some studies have reported contradictory results indicating that metformin has no effect or even might improve vitamin B12 metabolism [125–127]. No mechanism has been proposed and the issue requires further research but there is probably no clinical relevance in these observations as discussed below. Moreover, the possible benefits of vitamin B12 supplements in metformin users have not been assayed and the notion of causality is complicated because diabetes and obesity are also associated with vitamin B12 reductions [128].

Metformin does not significantly increase blood lactate levels, but we are probably depriving some patients from potential benefits based on the putative risk of lactic acidosis [129]. After 70 years of real-world clinical experience, the incidence of lactic acidosis is mostly based in anecdotal reports. However, caution is important in individuals with reduced metformin clearance (i.e., poor renal function), reduced lactate clearance (i.e., impaired hepatic metabolism), and/or increased lactate production (i.e., sepsis or reduced tissue perfusion). Advanced age may also increase risk because of age-related decline in renal function and increased risk for acute renal failure (i.e., dehydration), drug–drug interactions and other medical conditions. Moreover, elderly patients with type 2 diabetes are independently at greater risk for hyperlactatemia and have a reduced threshold for the development of lactic acidosis in response to a secondary event [130].

The most frequent adverse effects are gastrointestinal in nature: diarrhea, nausea, and to a lesser extent, vomiting, flatulence or heartburn. Of note, in randomized controlled trials, similar effects are found in the placebo group, indicating potential bias [131]. Those patients without preexisting gastrointestinal conditions are apparently free of these effects, but it is not uncommon that some patients decline using metformin [112,113]. Metformin response and tolerance are intrinsically

associated with the gut and the intake of nutrients [132]. Metformin could increase serum glucagon-like peptide 1 concentration by increasing its secretion from L cells, distributed throughout the intestine, and/or by reducing its breakdown by dipeptidyl peptidase-4 in the intestinal mucosa and portal system [133]. Curiously, serotonin and histamine release from the intestine are associated with similar effects (nausea, vomiting, increased gut motility and diarrhea). It is possible that metformin inhibits diamine oxidase, which is highly expressed in enterocytes and responsible for the metabolism of these gut peptides [134]. Metformin might also disrupt the enterohepatic circulation of bile salts, predominantly through reduced ileal absorption and osmotic effects facilitating diarrhea [135]. Finally, the gut microbiome is considered an environmental factor contributing to the development of metabolic diseases and a possible target of metformin. A reduction in butyrate-producing bacteria and an increase in opportunistic pathogens are common in type 2 diabetes and could potentially influence gastrointestinal tolerance [136–138].

8. Measuring the Impact of Folate-Related Deficiencies in the Clinical Setting: Potential for Targeted Metabolomics

Results from immunoassays in clinical laboratories are difficult to interpret and limited by the availability of reagents and automated biochemistry platforms. Methodological constraints, confounding factors, and poor inter-laboratory reproducibility are common pitfalls [139–141]. Consequently, the picture obtained when exploring folate metabolism is partial at best. For instance, total circulating B12 in serum is unreliable in some clinical conditions and practically useless as a method to detect true, functional B12 deficiency [142]. Most (80%) of B12 is bound in serum to haptocorrin, and a variable proportion (5%–20%) is bound to transcobalamin II and ready for tissue uptake (holotranscobalamin). Measuring in the same batch serum holotranscobalamin, homocysteine and/or methylmalonic acid can mitigate limitations [125,143]. Some pitfalls were made evident in a large cohort of participants enrolled in the REasons for Geographic and Racial Differences in Stroke (REGARDS) study [144]. The proportion of participants with low serum B12 concentration was exactly the same (2%; not clinically evident) in participants without diabetes and in long-term metformin users with diabetes. However, serum B12 concentrations were lower in metformin users than in those who did not use metformin. Curiously, metformin users were less likely to have taken multivitamins (6–25 μg of vitamin B12 per dose), and multivitamin users had a significantly higher serum B12 concentration compared to those who did not take multivitamins [144]. A longitudinal study to assess the impact of metformin is warranted, but it appears that laboratory biomarkers do not add significantly to clinical decisions and that dietary advice might contribute to better management of metformin users [145].

Similarly, measurement of serum folate with standard immunoassays is accompanied by possible errors in interpretation. For instance, obesity appears to be associated with decreased serum (measuring recent folate intake) but also with increased RBC folate (measuring a long term intake) concentrations compared with lean subjects. The association is plausible but the presence of obesity-associated metabolic disturbances hampers further interpretations [146]. It was recently clarified that folate measurements in serum and in RBC display similar performance in assessing folate status [147]. The use of both measures generates higher and unnecessary costs, but the RBC folate assay is less likely to provide falsely normal levels attributable to dietary behavior or recent supplements [147]. The observation that metformin is associated with a slight, but sometimes significant, raise in homocysteine and/or decrease in folate needs careful consideration, but there is no sufficient evidence to recommend folic acid supplements to metformin users [148,149].

It remains unknown how nutritional manipulations may affect the complex relationships among metabolites involved in the 1C cycle, but studies in women with seasonal variations in nutrient intake highlight the need to measure all metabolites and cofactors [150,151]. Analytical platforms should provide measurements of different folate species, especially 5-methyltetrahydrofolate as the active 1C donor [150,151]. Specifically, it is important to confirm data suggesting that levels of maternal

one-carbon metabolites at conception influence DNA methylation in the early embryo and that offspring methylation correlates with the paternal somatic methylation pattern [152]. This effort implies better tools for measuring intermediate metabolites either side of the involved reactions [153,154]. Targeted metabolomics may help in pursuing a better interpretation. In this context, ultra-high pressure liquid chromatography coupled to an electrospray ionization source and a triple-quadrupole mass spectrometer is an affordable choice to quantitatively examine the methionine/folate bi-cyclic 1C metabolome [155]. This method has been used to explore the activation of methylogenesis in some models. This essential function of 1C metabolism provides a labile pool of methyl groups required for successfully establishing and maintaining the DNA methylation imprint [155]. A similar approach has been used to explore energy metabolism with a simple and rapid method based on gas chromatography coupled to quadrupole time-of-flight mass spectrometry and an electron impact source [156]. The accurate and simultaneous measurement of selected metabolites facilitates the understanding of metabolic responses to changing environmental factors and has the potential for searching quantitative biomarkers of disease and signals indicating the ability of drugs to restore cellular homeostasis [157]. In addition, this analytical approach may serve to assess the expected toxicity in potential applications for metformin employed in oncology at doses notably higher than those used chronically in the management of diabetes [8].

9. Conclusions

Metformin users may provide data on the effect of nutrients in health and disease. There is growing evidence demonstrating the multiple protective effects of metformin against obesity-associated diseases, a major challenge to global public health. Some findings support the idea that metformin mediates the mitochondrial response to excessive food intake and the effect of different micronutrients. In particular, the mechanism of action of metformin involves effects on both energy and one-carbon metabolism and suggests novel strategies that involve the combination of lifestyle modification with pharmacotherapy. The concept is more important in individuals whose risk factors are not reduced by dietary interventions and dietary regimens in metformin users may provide valuable information. We envision that several analytical approaches in the field of metabolomics can provide diagnostic indicators on multiple metabolic aspects and may ascertain the effects of nutrient intake. Accordingly, it is especially relevant to assess the role of significant nutrients, such as serine, glycine, methionine, folic acid or other B vitamins, affecting one-carbon metabolism. Efforts to repurpose metformin, the first choice as an oral treatment of type 2 diabetes, as an antimetabolite drug, reinforce the interest in understanding food and drug interactions and the expected toxic effects caused by a change in dose range.

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