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Cancer, deuterium, and gut microbes: A novel perspective

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ABSTRACT

Deuterium is a natural isotope of hydrogen, containing a neutron as well as a proton, which makes it twice as heavy as hydrogen. In this paper, we develop a theoretical argument that human metabolism strives to minimize the amount of deuterium in mitochondrial water, because it causes a stutter in ATPase pumps, introducing excess reactive oxygen species and reduced ATP production. Gut microbes produce hydrogen gas that is 80 % depleted in deuterium (deupleted). This gas is recycled into organic matter that supplies deupleted nutrients to the host, such as acetate, butyrate, and choline. Mitochondrial dysfunction is associated with many chronic diseases, most notably, cancer. Dehydrogenases, through proton tunneling, typically have a high deuterium kinetic isotope effect (KIE), and they supply deupleted protons to the ATPase pumps via NADH (nicotinamide adenine dinucleotide) synthesis. We propose that a tumor may arise as a consequence of mitochondrial stress in immune cells due to excess deuterium, and that the tumor microenvironment can support immune cell recovery from mitochondrial dysfunction. Cancer cells alter protein expression to support deuterium sequestration through membrane-bound vesicular ATPase, and they release deupleted nutrients, mainly lactate, into the extracellular milieu and the circulation. Deuterium depleted water (DDW) has been shown to prolong life in cancer patients. An organic high fat diet rich in B vitamins, especially niacin, riboflavin, and folate, augmented with natural prebiotics and probiotics, supports deuterium homeostasis and likely protects from cancer.

1. Introduction

The metabolic flexibility of biological organisms allows them to respond to diverse environmental factors in order to maintain homeostasis. One factor that has not received the attention it deserves is deuterium. Deuterium is a natural heavy isotope of hydrogen, the most common atom in the universe. Deuterium contains a neutron as well as a proton, making it about twice as heavy as hydrogen and changing its biophysical and biochemical properties in important ways. Deuterium is present in sea water at 156 ppm, and, although it seems small, this translates into a level in the blood, atom for atom, that is six times more than calcium, and much more than the level of various essential elements (Yaglova et al., 2023).

The budding field of deutenomics can be defined as "the study to reveal the regulatory role of naturally occurring deuterium in living organisms." Notably, in biological organisms, deuterium is fractionated differently in different biological compartments, with excess levels found in collagen in the extracellular compartments, especially the bone, and reduced levels in fatty tissues, lysosomes, and mitochondria (Boros et al., 2024a). Large amounts of deuterium depleted (deupleted) metabolic water are synthesized in the mitochondria during the production of the cell's energy currency, ATP. The ATPase pumps use proton motive force to energize the reaction, and deuterium disrupts the process, causing the release of reaction oxygen species (ROS) and damaging the pumps (Olgun, 2007).

Experimentally, it is becoming increasingly clear that reduced deuterium intake has many benefits both to neuronal health and in cancer therapy. When cultured neurons are grown in a deuterium depleted medium, their survival rate is increased under glucose deprivation conditions (Kravtsov et al., 2021). Rats pretreated with deuterium depleted water (DDW) were resistant to oxidative stress resulting from hypoxia (Kravtsov et al., 2021). A correlation was found between deuterium content of tap water and rates of depression in regions of the USA (Strekalova et al., 2015). DDW has been shown to improve long-term memory in rats (Mladin et al., 2014a) and to be beneficial in reducing anxiety (Mladin et al., 2014b). A growing number of studies, both on cancer cells grown in culture and clinically on cancer patients, have shown that DDW has therapeutic value in treating cancer (Wang

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et al., 2013; Haseli et al., 2023; Boros et al., 2021a; Somlyai et al., 2022; Somlyai et al., 2016; Somlyai et al., 2023).

In this paper, we first explain how deuterium theoretically disrupts ATPase function. Then we provide arguments supporting the idea that gut microbial metabolism supplies deupleted nutrients to support mitochondrial health of the host. We further argue that one-carbon metabolism and methylation pathways may play an important role in maintaining low deuterium in critical nutrients such as acetate. Thereafter, the metabolic policy of cancer cells is examined under the prism of the deuterium problem, and we show some parallels with yeast cells. Cancer cells appear to concentrate deuterium internally, while synthesizing deupleted nutrients that are released into the tumor microenvironment and into the circulation. We argue that this helps alleviate the deuterium overload problem, in the tumor-resident immune cells and systemically.

By greatly reducing the activity of the ATPase pumps in the mitochondria, cancer cells reduce mitochondrial ROS generation, thus maintaining survival despite deuterium overload. They derive their ATP mainly through massive processing of glucose through glycolysis and produce large amounts of lactate that is released into the tumor microenvironment. This lactate becomes a valuable deupleted nutrient that other cells, particularly the tumor-resident immune cells, can exploit.

2. Mitochondrial ATPase likely depends on deupleted protons

Mitochondrial ATP production depends upon the universal cofactor nicotinamide adenine dinucleotide (NAD). In multiple steps in the citric acid cycle in the mitochondrial matrix, NAD^+ gets reduced to NADH by gaining two electrons and one proton from various substrates. NADH then provides protons to the intermembrane space to build up a proton gradient, while providing electrons to the electron transport chain in the inner membrane. Ultimately, the protons leave the intermembrane space to return to the matrix, exiting via ATPase pumps and providing the motive force to rotate the pump and fuel the production of ATP, while reducing oxygen to metabolic water (Stein and Imai, 2012).

ATP synthase is the smallest known rotary motor in nature. F0F1 ATPase (F-ATPase) is a molecular motor positioned in the inner membrane of the mitochondrial intermembrane space that uses proton motive force to generate the energy needed to phosphorylate ATP, while producing metabolic water from oxygen molecules. Protons moving through the ATPase pumps need to dissociate rapidly from Asp61 in F0 as an essential part of the catalytic reaction. Deuterons bind more tightly to organic molecules and dissociate more slowly, resulting in a pump stutter (Olgun, 2007; Boros et al., 2021b), Repeated stutters can be highly disruptive of the ATPase function, leading to inefficiencies in ATP production and increases in reactive oxygen species (ROS) (Olgun, 2007; Boros et al., 2021b). Proton coupled electron transfer (PCET) reactions are also disturbed by deuterium. The electron transport chain is slowed down in the presence of deuterons, and this can cause upstream accumulation and subsequent leakage of electrons, increasing free radical release (Olgun, 2007).

Hydrogen-deuterium exchange experiments on F1-ATPase showed that the γ-helix at the C-terminal tip of the molecule exhibited elevated deuteration during ATP-hydrolysis driven rotation. The “foot” of γ protrudes into the solvent, and the H-bonds are destabilized during rotational catalysis, causing the rotor tip to stall and the helix to unfold (Murcia Rios et al., 2018). Deuterium presence alters the strength of H-bonds in alpha helices (Tomita et al., 1962), and such alterations could have significant impact on the intricate workings of a powerful motor that rotates 350 times per second (Ueno et al., 2005).

Mitochondrial dysfunction is a core feature of many chronic diseases, including neurodegenerative and neurodevelopmental diseases, metabolic diseases, autoimmune diseases, and, most notably, cancer (Giulivi et al., 2023; Nicolson, 2014; Luo et al., 2020).

3. Deuterium kinetic isotope effects

In many biological reactions catalyzed by specialized enzymes such as flavoproteins, protons are transferred from one molecule to another, exploiting proton tunneling to maintain the purity of the sequestered proton. The deuterium kinetic isotope effect (KIE) is a measure of the change in the rate of a reaction when deuterium replaces hydrogen, compared to the reaction when hydrogen is present. Generally, the KIE is greater than 1.0, meaning that deuterium weakens the enzyme's kinetics. Some enzymes, such as soybean lipoxygenase, have a remarkable ability to reject deuterium, with KIEs over 100 (Navratil et al., 2018).

At least half of all enzyme-catalyzed reactions involve hydrogen transfer. Furthermore, many hydrogen transfer reactions involve some amount of quantum mechanical hydrogen tunneling. Protons, being lighter, are much more capable of tunneling than are deuterons (Henkel et al., 2014), and therefore these reactions tend to have a high deuterium KIE (Sutcliffe and Scrutton, 2002). Flavoproteins are a large class of enzymes that exploit proton tunneling mediated by the bound flavin, such as flavin adenine dinucleotide (FAD). They tend to have a KIE for deuterium that ranges from 3.5 to 10 and can be as high as 25 (Hay et al., 2009). Most dehydrogenases are flavoproteins, and therefore they are capable of depleting deuterium in the product of their reaction.

In addition to dehydrogenases, isomerases are another class of enzymes that can strip deuterium from organic molecules (Leadlay et al., 1976). When all the protons bound to carbon atoms in glucose are replaced with deuterium, the processing during glycolysis is able to shed most of them into the cytoplasmic water. As shown in Fig. 1 from a paper tracing the ultimate fate of carbon-bound deuterium atoms during metabolism of fully deuterated glucose, there are many steps during glycolysis that strip deuterium from organic intermediates and release it into the cytoplasm (Mahar et al., 2020). The enzymes involved include mannose-6-phosphate isomerase, triosephosphate isomerase, enolase, and pyruvate kinase. As a result, only a small number of the original deuterium atoms end up on the pyruvate molecule that is delivered to mitochondria for oxidative phosphorylation. This suggests that, during glycolysis, the amount of deuterium that is delivered to the mitochondria gets minimized.

4. Microbes plausibly supply deupleted nutrients to the host

Intestinal commensal bacteria can synthesize small organic molecules by using hydrogen gas as a reducing agent. These microbial metabolic products plausibly play a critical role in supplying deupleted nutrients to the host cells. Colonocytes thrive best on the four-carbon short chain fatty acid (SCFA), butyrate, whose synthesis can be traced back to carbon dioxide and hydrogen gas. The gut microbes produce three SCFAs in abundance: acetate, propionate and butyrate, which often originate from dietary fiber. Their synthesis involves a critical step of extracting hydrogen gas from breakdown products of fiber and other small molecules and then using the hydrogen gas to produce new organic molecules from carbon dioxide. Through this unusual strategy, these SCFAs can be expected to be deupleted, a feat largely achieved by the hydrogen gas. Eighteen percent of the world's population experience bloating at least once a week (Ballou et al., 2023). This is an indicator of an impaired ability to recycle gases produced by gut microbes into organic nutrients.

Hydrogen gas is one of the main gases that accumulate in the gut as a consequence of microbial metabolism. Many gut microbes possess functioning hydrogenase enzymes that can synthesize hydrogen gas from simple molecules such as glucose and formate (HCOOH). During fermentation, *Escherichia coli* (*E. coli*) strains can use a membrane-bound hydrogenase coupled with formate dehydrogenase to produce H_2 and CO_2 from formate (Sokol et al., 2019). This reaction may play an essential role in the production of deupleted SCFAs, as acetogens can subsequently produce acetate from H_2 and CO_2, where the H_2 could have been derived from formate metabolism. This sort of cycling through H_2

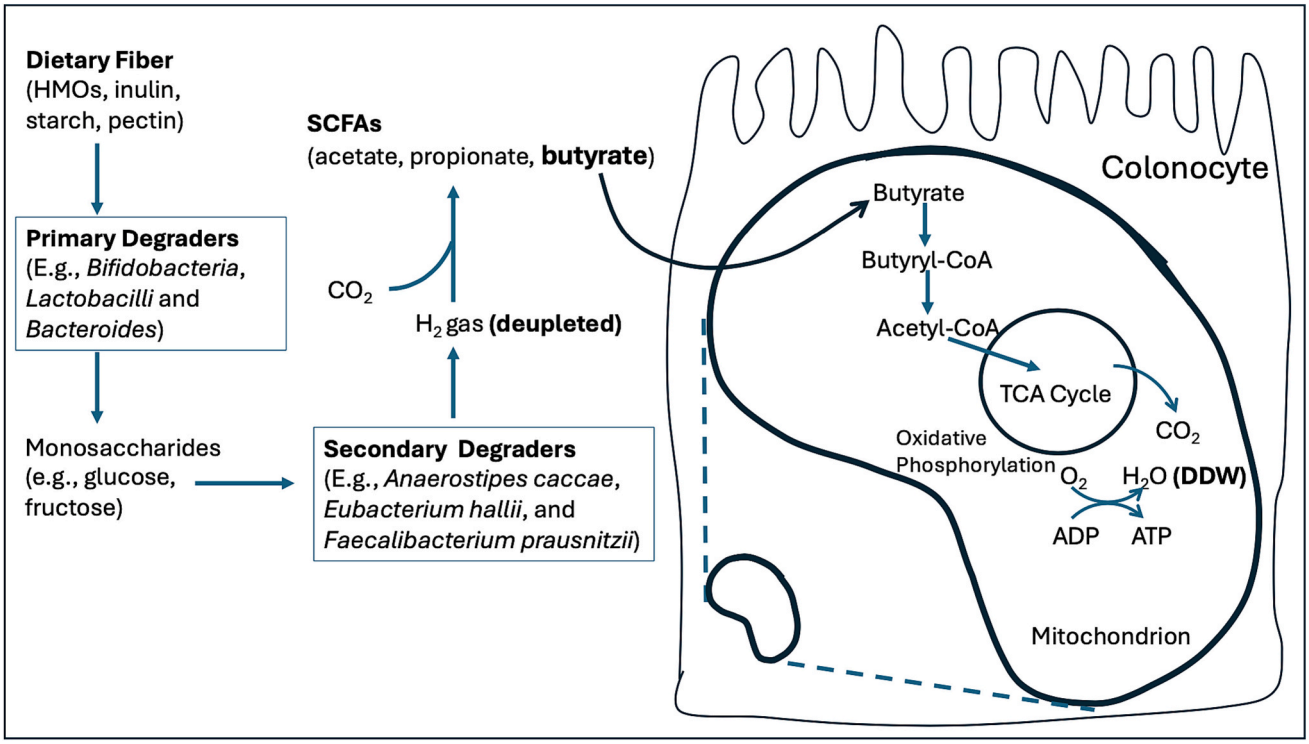

Fig. 1. Schematic of the processes by which gut microbes produce deupleted hydrogen gas and then use it to reduce carbon dioxide to acetate (acetogenic bacteria). Ultimately, further microbial enzymatic action converts acetate to butyrate. Butyrate is the preferred fuel of the colonocytes lining the colon, possibly because it is almost surely low in deuterium. HMOs: Human Milk Oligosaccharides. SCFAs: Short Chain Fatty Acids.

synthesis is a clever strategy for deuterium fractionation. Firmicutes and Bacteroides are the most prevalent hydrogen-producing bacteria in the gut, together making up over 90 % of the bacteria in the gut (Ichikawa et al., 2023). Recently, there has been a resurgent interest in the use of hydrogen gas therapeutically to treat a large list of diseases (Ohta, 2011).

It was demonstrated in 1961 that a species of *Pseudomonas* can synthesize hydrogen gas (H_2) that is 80 % depleted in deuterium, compared to normal amounts present in water. The gas is produced, along with carbon dioxide (CO_2), from formate metabolism. The enzymes that produced the hydrogen gas are similar to enzymes present in *E. coli* and other coliform bacteria that populate the human gut (Krichevsky et al., 1961). This phenomenon can be explained by the fact that many hydrogenases exhibit a very large deuterium kinetic isotope effect (KIE), as high as 43 in an acidic environment, which has been shown experimentally for the microbial class of [NiFe]-hydrogenases (Greene et al., 2015).

There are at least three broad classes of microbes that inhabit the gut that can use hydrogen gas as a reducing agent, methanogens, sulfate-reducing bacteria (SRB), and acetogens (Smith et al., 2019; Lajoie et al., 1988). Methanogens produce methane from H_2 and CO_2, and acetogens produce acetate from H_2 and CO_2, according to the following equation:

$$4H_2 + 2CO_2 = CH_3COOH + 2H_2O; \Delta G0 = -95\ \text{kJ/mol}. \quad (1)$$

Studies on several different strains of acetogenic bacteria obtained from human feces found that all of them could grow when provided with only hydrogen gas and carbon dioxide, and acetate was the sole metabolite produced, via reductive acetogenesis (Bernalier et al., 1996a; Bernalier et al., 1996b). The acetate that is produced by acetogenic bacteria is further processed by other gut microbes into butyrate, an essential nutrient for the epithelial cells lining the colon (colonocytes) (Singh et al., 2023). Fig. 1 schematizes the complex biological pathways by which gut microbes convert fiber to butyrate.

The production of deupleted nutrients by gut microbes necessitates microbial internal deuterium enrichment, but microbes, unlike human cells, are surprisingly able to tolerate extremely high levels of deuterium in their environment (Xie and Zubarev, 2014).

5. Are methyl groups involved in methylation pathways deupleted?

It is possible that the primary reason why methylation pathways are so critical in metabolism is that methyl groups are a storage form of deupleted protons. The cytosine residues in cytosine-guanine (CG) pairs in DNA are commonly methylated, and the methylation pattern regulates gene expression. Many types of cancer are associated with DNA hypomethylation (Mahmoud and Ali, 2019). The methyl groups attached to guanine can be traced back to S-adenosylmethionine, which has been called the "universal methyl donor" for all the methylation pathways via one-carbon metabolism (Ducker and Rabinowitz, 2017).

A case can be made that the methyl group that is attached to the sulfur atom in methionine is significantly deupleted. Human methionine synthase transfers the methyl group from methyltetrahydrofolate to homocysteine to produce methionine (Mascarenhas et al., 2022). Methyltetrahydrofolate in turn is produced in two steps from tetrahydrofolate. First, formaldehyde (derived from methane gas) reacts with tetrahydrofolate, either spontaneously or enzymatically via microbial enzymes, to produce methylenetetrahydrofolate (Ducker and Rabinowitz, 2017; Crowther et al., 2008; He et al., 2020). The enzyme that reduces methylenetetrahydrofolate to methyltetrahydrofolate in the second step is methylenetetrahydrofolate reductase, a flavoprotein which has a deuterium KIE of 2.9 (Schmidt et al., 2000). Thus, the two protons in the methylene unit have a short path back to H_2 gas, and all three protons in the methyl group can be expected to be deupleted, although this needs to be verified experimentally.

6. The many benefits of butyrate to human health

About 300 to 600 mmol. of SCFAs are produced on average every day in the human intestine, and most of them are absorbed by the colonocytes (Recharla et al., 2023). Members of the Firmicutes genus, particularly *Faecalibacterium prausnitzii*, are the most common butyrate-producing bacteria in the gut (Verstraeten et al., 2024; Duncan et al., 2004). These microbes combine two acetate molecules to synthesize butyrate, and, if the acetate is deupleted, then the butyrate will be, too, although, to our knowledge, there is no published literature on the amount of deuterium in butyrate. Many studies have shown that inflammatory bowel disease (IBD) is linked to low butyrate levels (Recharla et al., 2023; Parada Venegas et al., 2019).

Butyrate has become a promising therapeutic treatment for cancer (Sun et al., 2024; Son and Cho, 2023). Butyrate suppresses cancer cell growth, migration, and invasion by inhibiting histone deacetylase (HDAC) activity (Davie, 2003; Chambers et al., 2018). HDACs remove acetyl groups from histones, which generally alters expression of proteins involved in cancer (Glozak and Seto, 2007). HDAC inhibitor drugs have recently shown promise as anti-cancer agents, as they induce growth arrest and apoptosis in several different cancer cell lines (Di Gennaro et al., 2004). Butyrate causes apoptosis of cancer cells through multiple mechanisms. It induces death-associated protein kinase (DAPK) expression in human gastric cancer cells, leading to apoptosis (Shin et al., 2012). This effect is likely mediated by its action as an HDAC inhibitor. Butyrate also activates the mitochondrial apoptotic pathway by increasing the ratio of pro-apoptotic Bax to anti-apoptotic Bcl-2, which leads to cytochrome *c* release, caspase 3 activation and apoptosis (Chen et al., 2019).

A primary beneficial effect of gut microbes on host metabolism is the production of SCFAs. SCFAs are beneficial in maintaining gut barrier function, glucose homeostasis, immunomodulation, appetite regulation and obesity (Chambers et al., 2018). Butyrate can be passed into the blood stream by colonocytes, and it can cross the blood-brain barrier. It plays an important role in the communication channels of the gut-brain axis (Silva et al., 2020).

7. Choline, cancer, and cardiovascular disease

Choline ((2-hydroxyethyl)trimethylammonium) is an essential nutrient for humans, acting both as an important structural part of phospholipids and as a methyl donor in one-carbon chemistry. It is a precursor to multiple membrane lipids including phosphatidylcholine, sphingomyelin and plasmalogens. The choline in these membrane lipids is used by cholinergic neurons to synthesize acetylcholine, an important neurotransmitter (Blusztajn et al., 1987). It is important to note that S-adenosylmethionine is used as the methyl donor to supply three methyl groups to membrane phosphatidylethanolamine to produce phosphatidylcholine, via the enzyme phosphatidylethanolamine *N*-methyltransferase (PEMT) (Vance, 2013). This implies that the three methyl groups, all attached to the nitrogen atom of choline, would be deupleted.

Colorectal cancer is the third most commonly diagnosed cancer type worldwide, and it is second only to lung cancer in mortality (Sung et al., 2021). A combined deficiency in folate, choline and methionine causes DNA hypomethylation, hepatic steatosis, cirrhosis, and ultimately liver cancer in rodents, even when they are not exposed to carcinogenic agents (Davis and Uthus, 2004). A study investigating DNA methylation levels in colon cancer biopsies revealed that the tumor cells had significantly reduced DNA methylation compared to the adjacent healthy cells, which was associated with reduced expression of the folate receptor (Szigeti et al., 2022). Higher intake of dietary choline was associated with a reduced risk of colorectal cancer (Lu et al., 2015). The authors suggested that protection might be due to choline's role as a methyl donor. Insufficient methyl donor capacity might disrupt DNA methylation and impair DNA repair mechanisms (Davis and Uthus, 2004).

Choline is almost surely a valuable deupleted nutrient to support mitochondrial health. It is informative to trace the pathways of choline metabolism in the gut, which critically involve the gut microbes. In the gut, choline is converted to betaine (trimethylglycine) which then provides methyl groups to convert homocysteine back to methionine, via the enzyme betaine-homocysteine methyltransferase (BHMT). Choline can be metabolized by the gut microbes into trimethylamine (TMA), which is then passed to the liver where it is oxidized to trimethylamine oxide (TMAO). Betaine and carnitine can also be converted to TMAO. Elevated serum levels of TMAO are associated with cardiovascular disease (Zhen et al., 2023).

There are several strains of methanogenic archaea that inhabit the human gut, and these strains can metabolize tri-, di-, and monomethylamine (all derivatives of choline) to methane, using hydrogen gas as a reducing agent (Dridi et al., 2012; Chhibber-Goel et al., 2016). A unique group of methanogenic archaea that populate the human gut, in the order Methanomassiliicoccales, are even restricted to utilizing only methyl compounds such as TMA as substrates to synthesize methane using H_2 as a reducing agent (Brugre et al., 2014; de la Cuesta-Zuluaga et al., 2021). This pathway, which involves oxidizing methyl groups to CO_2 and H_2, and then recombining these products to synthesize methane, may play an essential role in further fractionating deuterium out of organic molecules.

The methane that is synthesized is likely to be extremely low in deuterium, since the methyl groups it was derived from most likely came from a previous cycle of deuterium scrubbing.

Probiotics containing these archaea species may be a way to reduce the levels of TMAO in the blood and hence to treat cardiovascular disease (Janeiro et al., 2018). Indeed, gut colonization with *Methanobrevibacter smithii* in ApoE−/− mice led to a significant reduction in plasma TMAO levels, as well as attenuation of the atherosclerosis burden (Ramezani et al., 2018).

Acetylcholinesterase is the enzyme that converts acetylcholine to acetate and choline, freeing up choline for future metabolism. Organophosphate pesticides are well known acetyl-cholinesterase inhibitors. A study revealed that cancer patients had significantly reduced acetylcholinesterase activity compared to controls (53.4 vs. 93.8 (*p*-value 0.001)) (Ahmed et al., 2024). It is possible that the reduced potential for converting choline into valuable deupleted nutrients via gut microbial activities explains this link.

8. Yeast overgrowth and serum ethanol

A study on human non-small cell lung cancer cells showed that lactate is the preferred fuel used by tumor cells in the citric acid cycle. The cells release lactate derived from glucose through glycolysis into the circulation and then retrieve it later for further processing in the mitochondria (Faubert et al., 2017). It is likely that the primary purpose of these extra steps is to further reduce the likelihood that the proton that ultimately becomes the hydrogen atom in NADH, produced in the mitochondria, is a deuteron.

This same principle probably applies for yeast cells populating the gut, except that the glucose is converted to ethanol rather than lactate. A rare condition aptly named “auto- brewery syndrome” happens when a person experiences a massive overgrowth of yeast in the gut, which are producing large quantities of ethanol from glucose via fermentation and releasing it into the gut, from which it is ultimately absorbed into the bloodstream (Zewude et al., 2024). The person experiences symptoms of severe inebriation, despite not having consumed any alcohol. Two genera of yeast are most noted for causing this condition, namely *Saccharomyces* spp. and *Candida* spp. (Din et al., 2020).

Ethanol and lactate have long been viewed as waste products of glycolysis, but this view is rapidly changing. Tracer studies conducted in 2020 on ^{13}C-labeled lactate have shown that this nutrient is taken up by every tissue in the body, even by tumor cells (Rabinowitz and Enerbäc, 2020). A study on *Saccharomyces cerevisiae* showed that they practice a

near complete uncoupling of glycolysis from oxidative phosphorylation. These authors wrote: "Specifically, we show that fermenting budding yeast simultaneously release and uptake ethanol, much as many mammalian cells simultaneously produce and consume circulating lactate." (Xiao et al., 2022) Two dehydrogenase steps take ethanol to acetaldehyde and then acetyl-CoA, which directly feeds into the citric acid cycle. Both steps convert NAD^+ to NADH. Under oxidative stress conditions, an even larger portion of the supply of protons for NADH production in the mitochondria is contributed by externally supplied ethanol, compared to pyruvate derived from glucose metabolism (Xiao et al., 2022). Fig. 2 illustrates the common but surprising metabolic practice of yeast cells.

9. Yeast and cancer cells have much in common

There are striking parallels between the metabolic policies of yeast cells and cancer cells.

It seems that both have a role to play in restoring deuterium homeostasis to the organism, or perhaps more specifically to the immune cells that infiltrate their microenvironment. *Candida albicans* is an opportunistic pathogenic fungus, and it tends to infect those with impaired immune function, including cancer patients (Yu and Liu, 2022). Ironically, *Candida* overgrowth is also a risk factor for cancer (Wang et al., 2023). As was discussed previously, *Candida* overgrowth leads to excess accumulation of ethanol in the blood. Ethanol is metabolized into acetate in two steps involving ethanol dehydrogenase (producing acetaldehyde) and acetaldehyde dehydrogenase (Aldh), producing acetate. Because these dehydrogenases are flavoproteins, both steps produce deupleted NADH.

The primary enzyme in the liver for metabolizing acetaldehyde is Aldh2, localized to the mitochondria. Genetic mutations causing deficiencies in Aldh2 increase the risk to hepatic cell carcinoma (HCC) following alcohol-related liver fibrosis. The description of this process by Seo et al. is precise and revealing: "Mechanistic studies revealed that after chronic alcohol exposure, Aldh2-deficient hepatocytes produce a large amount of harmful oxidized mitochondrial DNA via extracellular vesicles, which can be delivered into neighboring HCC cells and subsequently activate multiple oncogenic pathways, promoting HCC." (Seo et al., 2019).

Chronic ethanol consumption increases cancer risk, not only in the liver but also in the digestive tract and breast tissues (Seitz and Stickel, 2010). When acetaldehyde levels are very high, particularly when Aldh is defective, acetaldehyde becomes toxic. People with genetic defects in Aldh are especially vulnerable to cancer from chronic ethanol exposure, because acetaldehyde lingers long enough to cause DNA damage (Seitz and Stickel, 2010). It may be for this reason that frequent episodes of *Candida* overgrowth are a risk factor for cancer (Wang et al., 2023). Several *Candida* species, including *Saccharomyces cerevisiae* and *Cyberlindnera jadinii*, are transcriptionally active in gastrointestinal tumors and predictive of worse tumor outcomes (Dohlman et al., 2022). Oncogenesis might alleviate the burden on the *Candida* fungi to repair the deuterium overload problem, a Faustian bargain. Chemotherapy can increase the risk of systemic Candidiasis in cancer patients, and this may simply be because the cancer cells can no longer adequately support deuterium detoxification for the host, so *Candida* species rise to the occasion (Teoh and Pavelka, 2016).

Yeast cells can survive in both an aerobic and an anaerobic environment. They are quite capable of surviving long term on glycolysis alone, and apparently, they can alter their metabolism strategically towards aerobic glycolysis, in the presence of high deuterium exposure, shutting down ATP production by the mitochondria. Similarly, cancer cells switch to glycolysis as an energy source and release deupleted nutrients such as lactate and formate (Meiser et al., 2018), likely triggered by elevated deuterium levels in their growth medium. Interestingly, reactivating oxidative phosphorylation can be a useful chemotherapy strategy, because it induces mitochondrial-stress-dependent apoptosis in cancer cells. The anticancer agent dichloroacetate (DCA) activates the TCA cycle in the mitochondria, which restores oxidative phosphorylation, increasing oxidative stress leading to apoptosis (Bhat et al., 2015).

10. Explaining metabolic reprogramming in cancer

Several of the abnormal aspects of cancer cell metabolism can be linked to deuterium homeostasis. Cancer cells adopt an unusual metabolic policy, exploiting aerobic glycolysis as the main source of ATP and invoking anabolic pathways in the mitochondria to provide resources to support proliferation and migration (Park et al., 2020). They upregulate

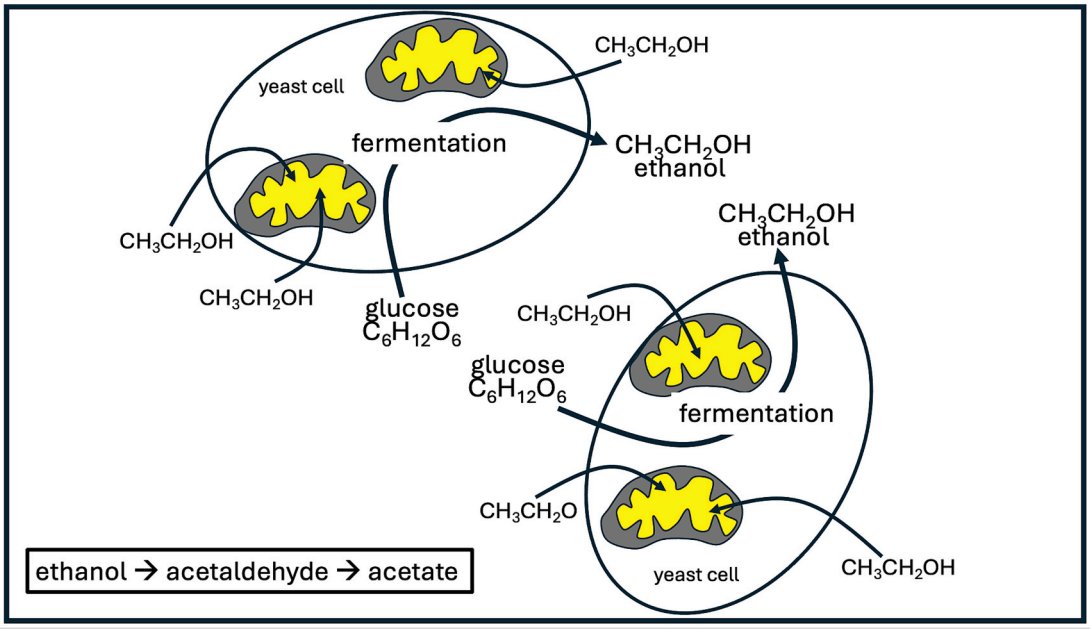

Fig. 2. During fermentation, yeast cells convert glucose to ethanol in the cytoplasm and release the ethanol into the extracellular space. During oxidative phosphorylation, they take up external ethanol from a collective pool and use it to fuel the citric acid cycle.

vacuolar ATPase (V-ATPase) to pump predominantly ^{1}H protons out of the cell, reversing the membrane polarity, acidifying the external space, and increasing the deuterium content in the cancer cell cytoplasm (Stransky et al., 2016; Tejeda-Muñoz et al., 2022; Kotyk et al., 1990).

Cancer cells commonly overproduce lactate and then release it into the circulation, allowing the recipient cell to skip glycolysis and efficiently produce ATP directly from the tumor-provided lactate molecule, a process known as the Warburg effect. Meanwhile, the cancer cell repurposes its own mitochondria towards anabolic pathways, thus minimizing exposure to ROS generated by oxidative phosphorylation (Potter et al., 2016). Lactate has powerful signaling capabilities, especially towards tumor-resident immune cells. Lactate, combined with the low pH tumor microenvironment, promotes tumor survival and growth by suppressing $CD8^{+}$ T cell activation and inducing an M2 phenotype in macrophages that facilitate immune escape (Caslin et al., 2021). Lactate accumulation in the tissue microenvironment is a common feature of both inflammatory disease and cancer (Li et al., 2022). The Warburg effect reflects cancer cell plasticity: cancer cells produce ATP primarily through glycolysis even in the presence of adequate oxygen for oxidative phosphorylation (Warburg et al., 1927). This is similar to the strategy that yeast cells use in the presence of excess deuterium. Aerobic glycolysis produces lactate under a variety of stressful conditions, such as trauma, infection, myocardial infarction, and heart failure (Li et al., 2022).

Lactate is a very useful fuel for the mitochondria, because it can easily be converted to pyruvate via lactate dehydrogenase. Pyruvate produced via glycolysis can be transported into the mitochondria and used directly to generate large amounts of ATP via the citric acid cycle. However, most of the time this is not what happens. Instead, pyruvate is converted to lactate in the cytoplasm, and then the lactate is transported across the mitochondrial membrane and converted back to pyruvate (Brooks et al., 1999). While this appears to be a superfluous step, it can actually be viewed as a very clever strategy for assuring that the hydrogen atoms in the mitochondrial NADH are deupleted. Cytoplasmic lactate dehydrogenase has a high deuterium KIE (~3–4) (Grimshaw and Cleland, 1980), so it provides lactate with a deupleted proton, which is then delivered to NAD^{+} in the mitochondria, to finally produce a further deupleted NADH molecule within the mitochondria, again, most likely, since the enzyme is a flavoprotein, with a high deuterium KIE. The proton in NADH is delivered directly to the intermembrane space of the mitochondria by the enzyme NADH dehydrogenase. The extra steps of passing a proton to lactate and then from lactate to NAD^{+} provide a simple mechanism for further shedding of deuterium. Fig. 3 depicts the lactate shuttle.

Very frequently, the metabolism of glucose to produce ATP is carried out in two steps in two different cells, with the first cell producing lactate from glucose via glycolysis in the cytoplasm, and the second one metabolizing lactate to CO_2 and water in the mitochondria. A paper published in 2022 brings awareness to the fact that lactate is a very common metabolic intermediate between glycolysis in one cell and the citric acid cycle in another distant cell. The authors wrote: "Indeed, it seems that most carbohydrate oxidation in mammals, rather than occurring by a tissue taking up glucose and fully oxidizing it to carbon dioxide, instead involves carbon flowing through circulating lactate as a metabolic intermediate." (Xiao et al., 2022). The release of lactate and acidification by tumor cells accompanies metastasis, angiogenesis and poor outcomes of cancer progression (Pérez-Tomás and Pérez-Guillén, 2020).

11. Discussion

Theoretically, deuterium can damage mitochondrial ATPase pumps, leading to inefficient ATP synthesis and excessive production of ROS (Olgun, 2007). Biological organisms seem to have evolved to have clever strategies to reduce deuterium levels in the mitochondria, and the gut microbiome plays an essential role in supplying deupleted nutrients for the host, including the short chain fatty acids acetate, propionate and butyrate, and the methyl groups that participate in methylation pathways. The microbes collectively achieve this feat by synthesizing significantly deupleted H_2 gas and using it as a reducing agent to synthesize deupleted small organic molecules (Krichevsky et al., 1961; Smith et al., 2019).

In reviewing deuterium homeostasis, we have come to believe that the best way to both prevent and treat cancer is to maintain a wholesome diet of organic whole foods that are rich in micronutrients. Ultra-processed foods, which are deficient in micronutrients and highly contaminated with toxic additives, have become a greater component of the diet in modern times, and their overconsumption is associated with many adverse health outcomes (Elizabeth et al., 2020). It is essential to consume adequate amounts of the B vitamins, niacin, riboflavin and

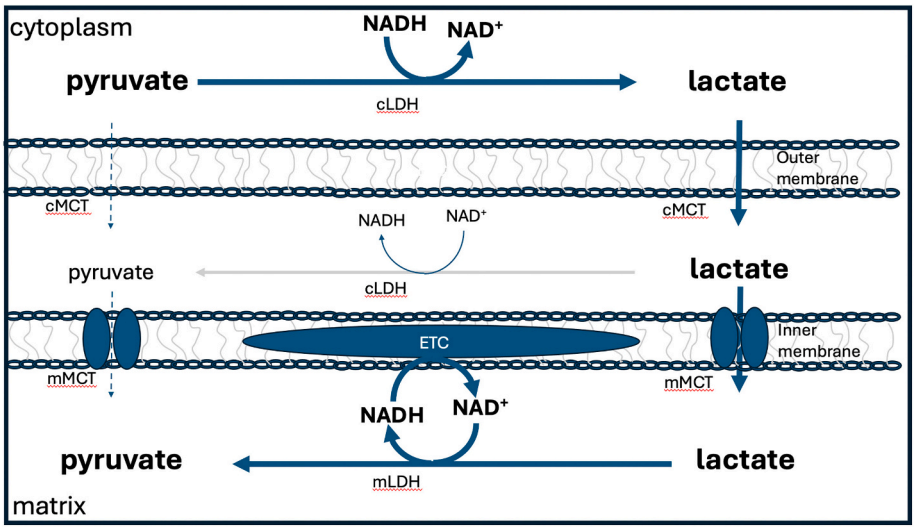

Fig. 3. Illustration of the lactate shuttle. Most of the pyruvate produced in the cytoplasm gets metabolized to lactate in the cytoplasm, and then the lactate is taken up into the mitochondrial matrix, utilizing mitochondrial MCT. Mitochondrial LDH is primarily responsible for converting lactate back to pyruvate, which can then enter the citric acid cycle for metabolism to CO_2 and H_2O. This assures that the proton attached to NADH in the matrix is derived from matrix metabolic water, i.e., is low in deuterium. mMCT = mitochondrial monocarboxylate transporter. ETC = electron transport chain. mLDH = mitochondrial lactate dehydrogenase. cLDH = cytoplasmic lactate dehydrogenase. Figure adapted from Fig. 5 in Brooks et al. (1999).

folate to assure abundant supplies of NAD, FAD, and methyltetrahydrofolate.

Fatty acids are low in deuterium compared to other foods, likely because acetyl-CoA (derived from acetate) is the primary building block of fatty acids. A paper published in 2024 argued, based on a case study, that a diet high in fatty acids is particularly appropriate in training for extreme exercise under hypoxic conditions, i.e., as preparation for climbing Mount Everest, primarily because they provide deupleted protons to the mitochondrial ATPase pumps (Boros et al., 2024b). Butter is an excellent source of butyrate, and it is low in deuterium. Animal-based fats and coconut oil are other low-deuterium nutrients. A high fiber diet can supply prebiotics to support butyrate synthesis by the gut microbiota. Choline (abundant in animal-based foods) is a good source of methyl groups, as well as betaine and the amino acids glycine, alanine and methionine. Eating certified organic foods will minimize exposure to toxic agrichemicals that could be disrupting the gut microbiome (Glibowski, 2020; Chiu et al., 2020). Fermented foods are a great source of acetate and other SCFAs, and they also support renewal of the gut microbiome (Shimizu et al., 2019).

12. Conclusion

Deutenomics is a new research field in biology that is gaining momentum in recent times, and it is giving us new insight into metabolic pathologies. Deuterium is a pervasive natural element that presents special challenges to biological organisms. Theoretical studies suggest that the mitochondrial ATPase pumps are very sensitive to deuterium, which accumulates in the motor and causes stutters, leading to an increase in reactive oxygen species and inefficiencies in ATP production. The gut microbiome likely plays a significant role in supplying deupleted nutrients to the host, via hydrogen recycling. An organic diet, rich in low-deuterium nutrients, prebiotics, probiotics, and B vitamins is a good strategy for reducing cancer risk. The importance of methylation pathways may be primarily due to the low deuterium content in the methyl groups, although this needs to be verified experimentally. Oncogenesis may be driven by deuterium overload in mitochondria, and the unusual metabolic policies of cancer cells can be explained as a strategy of their cancer pathogenesis to hoard deuterium and supply deupleted nutrients, mainly lactate, to the resident immune cells as well as to distant organs. Lactate and a low pH in the tumor microenvironment signal to the immune cells to suppress immune activation and promote tumor survival. Much more research is needed to further clarify the roles that deuterium plays in health and disease.

CRediT authorship contribution statement

Stephanie Seneff: Writing – original draft, Conceptualization. **Anthony M. Kyriakopoulos:** Writing – review & editing, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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