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Contaminated Waters and Depleted Soils: Impact in Nutrition II Biochemical Detoxification Pathways

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Abstract

One of the main interest in reviewing the detoxification processes and ability of human body to do so, is that in not being aware how to do the detoxifying process correctly, one could be faced with all sort of metabolic diseases in the course of his/her life. More and more researchers are dedicating their lives in studying these ramping diseases as each day more xenobiotics are being developed and dumped in the planet. Many protocols can be found in many different sources of information, but the foundations behind the human detox biochemistry are not so widely broadcasted. Here are presented the details.

As our external as well as our internal environment can be polluted as it gets, revisiting the mechanisms and pathways as well as the natural ways to detoxifying can be put to our service at the right time to cope with the myriads of toxic compounds we are daily exposed to. Many natural substances that provide, enhance or help the detoxification process are addressed, as well as its main biochemical pathways. The knowledge of food and soil composition and quality are forever intertwined and it is also highlighted in this work.

The 3 detox phases are explained and the scientific review on this subject presented. The influence of life style in the gene expression for the health of the emunctories are in a simple way addressed, also how diet influences them are described.

The toxins can be accumulated all over the cells in the body, but they tend to concentrate in lipophilic tissues like the brain, fat, lymphatic system, extracellular matrix and in the endocrine receptors.

Keywords: *Detox; Biotransformation; Conjugation; Elimination; Mitochondria; Leaky Gut; Mold; Parasites-Cytochrome P450; Medicinal Plants*

Introduction

Part I of this review [1] accounted for some toxic metal ions as well as radioactive metals and some pesticides as potential xenobiotics in our daily life. Some solutions for acute toxicity were proposed. This second part of the above theme will visit the way how human body was built to detoxify itself in the presence of certain conditions involving certain nutrients. The detoxifying mechanisms (Detox) were academically classified in steps or phases to a better description of the way the body deals with the produced wastes either coming from the daily produced metabolism products and/or from the majority of foreign substances we are ubiquitously exposed.

The main interest in reviewing the human body detoxification process, is that not being aware how this cleaning steps are correctly carried out, one could be faced with metabolic diseases, even developing serious body disfunctions in the course of life. More and more

researchers are dedicating their lives in studying these ramping diseases as each day, more xenobiotics are being developed and dumped in the planet interfering with our biochemical metabolism.

As our external as well as our internal environment can be as polluted as it gets, revisiting the mechanism and pathways and their right timing, as well as the support substances for the Detox process is the best way to have a perfect gradual betterment of the whole process. Our body is capable to cope with myriads of toxic compounds we are daily exposed to if the proper biochemical conditions are met. For more details on xenobiotics, refer to references [2,3] among many others.

Lifestyle as well as eating habits can certainly play a very important role in the perfect development of the whole Detox process. Ultimately, knowledge is what will provide both professionals and laymen how to define and lead their lives the best way they find suit.

The human Detox process comprises 3 steps or phases. They are going to be addressed in this part II review as well as some food complements and plants and/or their extracts that can boost or promote these phases. These are key substances to the point that in their complete absence or in absence of some of them, one or more steps cannot be correctly accomplished, thus compromising the whole process.

The Detox phases initiate when a person stops eating solid food for several hours. The main organs that are involved in the Detox process are the liver, kidneys, lungs and the intestines. The emunctories need to be in a good health for performing their role accordingly and the presence of chemical or enzymatic support from some specific nutrients are a must. This optimum condition for all the biochemical Detox reactions can be achieved by providing the right needed molecules at the right timing. These molecules can be found in numerous foods, supplements, medicinal plants along with their extracts or essential oils, among others. Some of them are going to be discussed further in this work.

Discussion

Phase I - Biotransformation

This phase is the beginning of the detox process, when the biochemical transformation of the generally liposoluble xenobiotics into hydrophilic substances takes place. This phase is started mainly in the liver and the derived metabolites from phase I and later from phase II, are to be finally (phase III) excreted by the intestines, kidneys, skin and lungs.

Phase I is triggered when a person stops eating solid food and continue for several hours. The biotransformation is made by several hepatic enzymes capable of breaking down the xenobiotics by oxidation, reduction, hydrolysis, among others. As the expression of these specific enzymes are gene dependent, healthy organs and life style are key to the whole Detox process.

The liver, the main and larger organ in the body has between 70 - 85% of its volume composed of parenchymal hepatocytes. These hepatocytes are responsible for expression and release of large amount of proteins to the blood. Amongst them there is the superfamily of proteins involving in the synthesis and metabolism of a range of internal and external cellular constituents named Cytochrome P450 (CYP) enzymes. This CYP superfamily of enzymes are the most significant contributors to drug biotransformations [4].

The number 450 refers to the specific spectral absorbance wavelength of these proteins at 450nm. Some of them are responsible for the enzymatic action in the metabolization to facilitate the excretion of potentially harmful xenobiotics and endogenous compounds, either by S-oxidation using iron (CYP are some dozens closely related heme-containing enzymes) or o-dealkylation, epoxidation and hydroxylation [5]. This enzymatic superfamily is highly influenced by diet, exposure to pesticides, genus, environment and alcohol consumption, and medication - natural or synthetic… - briefly, by epigenetic factors [6,7].

CYP comprises many isozymes - enzymes that slightly differ by amino acid sequences - embedded primarily in the lipid bilayer of the endoplasmic reticulum of hepatocytes. The most significant of them are CYP2D6, CYP3A4, CYP2C9, CYP1A1, CYP1A2, CYP2C19 and CYP2E1. Two of the most important in dealing with metabolizing synthetic xenobiotics are CYP3A4 and CYP2D6, not only expressed by hepatic genes but also by the intestinal tissue [5,7-9].

The hepatocytes also are receptors for pathogenic and inflammatory signals and respond by secreting innate immunity proteins (Refer to table 1 - Biosynthesis of secreted innate immunity proteins by hepatocytes - in reference [10]). They also produce, among others, several chemokines (Some are CXCL1 and MCP-1) to attract immune cells in response to eventually liver damage and bacterial infection. The liver is also a unique organ to have tolerogenic effect on the immune system. That is to say that the liver can induce immune tolerance where there is pathological or undesirable activation of the normal immune response. Hepatocytes, in that matter, participate in the modulation of the adaptative immune system, along with many other nonparenchymal cells in the liver [10].

As hepatocytes compose almost the entirety of the liver organ and have a vital role in innate immunity, life style can prolong or abbreviate the health of this organ.

Some enzymes needed for xenobiotic biotransformations, in phase I, adapted from reference [6] are in table 1 according to their main classes.

Dehydrogenases	Reductases	Oxidases	Mono-Oxygenases	Hydrolases
Alcohol Dehydrogenase	Ketoreductase	Aldehyde Oxidade	Cytochromes P450	Esterases
Aldehyde Dehydrogenase	Nitroreductase	Monoamine Oxidase	Flavin-Containing Mono-Oxygenase	Amidases
Dihydrodiol Dehydrogenase	Azoreductase			Epoxide Hydrolase
Xanthine Dehydrogenase	N-Oxide Reductase			
	Sulphoxide Reductase			

Table 1: *Enzymes needed in the biotransformation in the DETOX process.*

In the panoply of needed enzymes (Table 1), one can see that not only the CYP enzymes are required in Phase I.

Some of the cofactors in Phase I are flavonoids, phospholipids, branched-chain aminoacids, glutathione, folic acid, and vitamins B2, B3, B6 and B12. Through oxidation, reduction, hydrolysis, hydration or dehalogenation, the fat-soluble toxins are converted into more water soluble compounds. This is when free radicals are formed. Antioxidants are needed in the process, at the right time, to prevent tissue damage. The antioxidants will help provide less toxic and more water soluble intermediary metabolites in this step before phase II starts.

During the period of this phase, after 24h of stopping solid food ingestion, mainly antioxidant green juices must be provided to the person at regular intervals for the next 12h. These green juices should be conceived in order to provide the body with the most possible needed nutrients for the best possible accomplishment of this Detox phase I process. Further on in this review one can find some of these nutrients.

Phase II - Conjugation

After around 24 to 36 hours of having stopped the consumption of solid food, Phase II commences and will go on for around more 12h. Metabolites previously generated in Phase I are processed in this second step through conjugation with other molecules forming conjugated metabolites that are more water soluble and easily excreted. This step absolutely needs inducers which are critical for the

ability of the biochemical of the human body to continue with the second Detox phase and to take completely advantage of the potential of these inducer compounds, including the chemoprotective effect of many of them [11]. The metabolites generated in Phase I are in general, toxic and must go through phase II when then the metabolites will be transformed into less harmful products.

Michael reaction acceptors (olefins and acetylenes) conjugate to electron-withdrawing groups constituting a major class of Phase II enzyme inducers. The active α, β-unsaturated groups make Michael acceptors susceptible to react with nucleophilic groups. Under physiological conditions, potential nucleophiles that can undergo Michael addition include thiols (cysteine, glutathione) and reactive amines (aminoacids, albumin and other proteins), which have a ubiquitous presence as small molecules [12].

Michael addition reaction mechanisms, simply put, play a very important role in the detoxification process. These are conjugate-addition reactions involving doubly stabilized enols with, for instance, α-β unsaturated carbonyl compounds. In the literature, the terms Michael donor and Michael acceptor are used for the nucleophile and the electrophile, respectively. In the biochemical process of detoxification, the strength of the bases (donors) [11] will determine the stereochemistry of the obtained products: Generally, strong bases (electron donors) tend to give 1,2-carbonyl addition reactions, whereas weak bases give 1,4-conjugate addition [13].

The liver is responsible for the expression of a wide variety of xenobiotic biotransforming enzymes, whose main feature is their ability to catalyse either Phase I - the oxidation, reduction and hydrolysis, and/or Phase II - conjugation of functional groups on xenobiotic molecules. Nevertheless, some chemicals can become more toxic metabolites after these conversions. So knowledge is power.

The enzymes involved in conjugation reactions in phase II can be summarized as according to [6]: Uridine 5”-diphosphate-glucuronosyltransferases (UDP-glucuronosyltransferases); Alcohol sulphotransferase; Amine O-sulphotransferase; Phenol sulphotransferase; glutathione-S-transferase; Phenol O-methyltransferase; Catechol O-methyltransferase; Amine N-methyltransferase; Histamine N-methyltransferase; Thiol S-methyltransferase; Glycine acyltransferase; Glutamate acyltransferase; Arylamine N-acetyltransferase; Cysteine N-acetyltransferase; Cysteine conjugate β-lyases; Thioltransferase; Rhodanese.

These enzymes are mainly made in the liver and kidneys. They play a major role in protecting against the oxidant stress caused by a myriad of pollutants. Taking as first example, the UDP-glucuronosyltransferases genes are regulated by a) several liver-enriched transcription factors such as Hepatocyte nuclear factor (Hnf)-1 (an homeotic gene (homeotic genes are a group of genes that regulate pattern formation, mainly expressed in liver and kidney) and Hepatocyte nuclear factor 4α (HNF4α, NR2A1 - which is a highly conserved member of the nuclear receptor superfamily, expressed at high levels in the liver and kidney and to a small extent, in the small intestine, colon, and pancreatic β-cells), and by b) ligand-activated transcription factors such as Aryl Hydrocarbon Receptor (AhR), Nuclear factor erythroid-2-related factor (Nrf2), Pregnane X receptor (PXR), Constitutive androstane receptor (CAR), Peroxisome proliferator-activator receptors (PPAR), Farnesoid X Receptor (FXR), Liver X Receptor (LXR).

The UDP-glucuronosyltransferases enzymes reside in the luminal part of the endoplasmic reticulum, which is the principal protein-folding organelle for secretory and membrane proteins [14-16].

In the case of PXR, pregnane X receptor, it regulates the cytochrome P4503A4 expression by binding to several specific elements in the 5’ upstream regulatory region of the gene. PRX is a transcriptor activator (= transcription factor - a protein that increases transcription of a gene or set of genes) of xenobiotic- and drug-inducible expression of certain key genes that encode components in phase I and II metabolic enzymes and drug transporters [17-19]. The obtained compounds play roles in the three phases of the hepatic metabolism and transport: phase I monooxygenation, phase II conjugation, and phase III transporters that include multidrug resistance-associated proteins and organic anion transporting polypeptides [20,21].

LXR is a sterol sensor that promotes lipogenesis (fat build-up is determined by the balance between lipogenesis and lipolysis/fatty acid oxidation) [22], whereas PPARα controls a variety of genes in several pathways of lipid metabolism. The glucuronide products are water-soluble and readily excreted.

PPAR has been suggested to downregulate lung inflammation, to promote vascular function, and to act as tumor suppressor in lung and other carcinomas [23]. Farnesoid X Receptor is transcriptionally activated by farnesol metabolites such as farnesol itself and, to a small degree, farnesal, farnesyl acetate, farnesoic acid or geranylgeraniol.[24] These are precursors in the mevalonic secondary metabolism chemical reactions pathway in vegetables [25].

For this conjugation reactions, during the approximate phase II 12h duration, aminoacids or proteins and glutathione and albumin are needed to be consumed in the form of watery beverages. The skin plays also a role in this phase by eliminating the water soluble toxins by sweating. A sauna section is a good resource for this goal at this timing.

Phase III - Elimination

Phase III is almost concomitant with Phase II and they both need some hours to be completed. This Phase III is carried out by transport proteins, taking the final water-soluble obtained products away from the cell. The in and out transportation through the cellular membrane of various molecules is a crucial biochemical process for the homeostasis. These exporter and importer transport proteins in action in Phase III Detox are called ABC (ATP-biding cassette) transporters and do so by coupling transport of a substrate across the membrane taking energy from the hydrolysis of the phosphate bond between the γ- and the β-phosphate of ATP. They are found in the brain, intestines, liver and kidneys. ABC transporters proteins are called primary transporters as they are capable of generating the driving force to overcome the selective lipid bilayer for charged/polar molecules to break through. This driving force is achieved by an enzymatic reaction with - adenosine triphosphate - ATP, when the energy released when this ATP molecule is converted into ADP and orthophosphate (around -50 kJ/mol) can be used to accumulate the transported substrates inside or to remove them out the cells (Figure 5). For that matter ABC transporters are also called transport ATPases [26-29].

Excretion of liver toxicity is highly dependent on bile (gallbladder) production and its normal flow. The main organs involved in this phase are liver, kidneys, intestine, skin (sweating) and lungs.

Mitochondria - powerhouse of the cell - and ATP

Mitochondria are responsible for the sustainment of life. Without their proper functioning metabolic diseases can develop and ultimately, death. Not always properly addressed for their crucial role in any form of life specially in Nutrition studies and Health Therapies, mitochondria are oxygen dependent and by breathing this oxygen, they produce water and energy [30]. The carrier of this chemically produced energy is a molecule with three phosphorus bond, the ATP (Figure 5). When these bonds break they release energy. Mitochondria contain their own genome, as the best theory up to date is that a bacteria integrated in our eukaryotic cells - endosymbiotic theory, and they are inherited by the mother's side X chromosome [31].

Mitochondria have a double membrane structure, mitochondrial inner and outer membranes, both containing selective and nonselective ion channels and transporters. Impaired metal ion homeostasis at the cellular level is linked to mitochondrial dysfunction [32].

Mitochondria (Figure 1) are our source of energy. If you are lacking energy, it would probably be related to a mitochondria disfunction or lack of body oxygenation. The energy source is ATP, which cannot be stored and need to be produced or recycled from ADP, every single second, every single day. The brain is the most requiring energy organ, consuming around 70% of all ATP produced. So, even the brain, lacking in energy, will loose its proper functioning by mitochondria dysfunction and neurodegenerative diseases may appear [33-37].

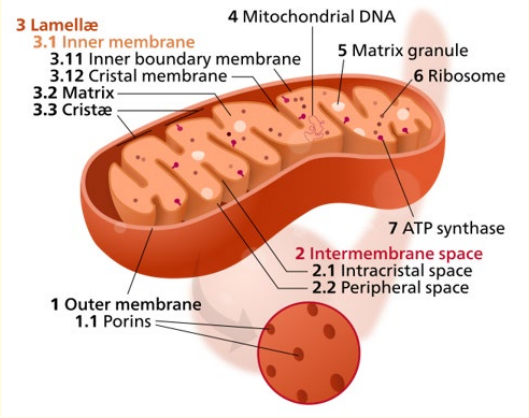


Figure 1: *A labelled diagram of the structure of a mitochondrion. (Courtesy of Kelvinsong, CC0, via Wikimedia Commons) access 19th December 2023.*

ATP biosynthesis has its origins in the citric acid cycle represented in Figure 2.

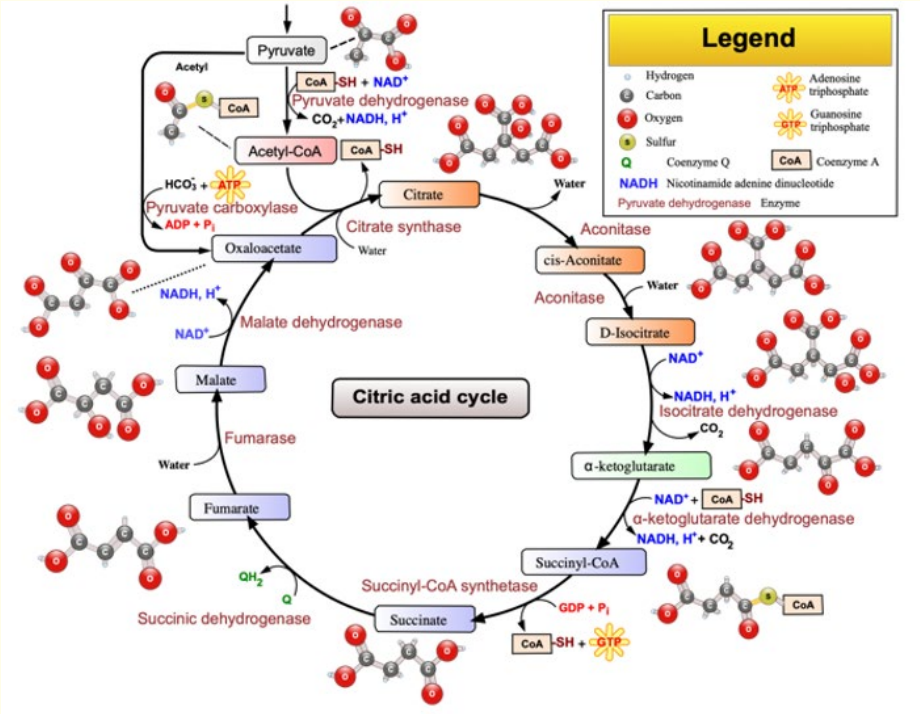


Figure 2: *Citric acid or Krebs cycle. For every NADH molecule, 3 molecules of ATP are generated in this oxidative phosphorylation biosynthesis. (Courtesy of: Narayanese, WikiUserPedia, YassineMrabet, TotoBaggins - http://biocyc.org/META/NEW-IMAGE?type=PATHWAY&object=TCA. Image adapted from Image: Citric acid cycle noi.svg, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=6217701) access 8th January 2024.*

Mitochondria are the major cellular source of NADH (Nicotinamide adenine dinucleotide reduced form) and is where parts of the pyrimidine and lipid biosynthetic pathways happens [31]. Although Figure 2 depicts a well known subject for many, it is always very important to remark the complexity and number of steps and the whole set of reagents needed to achieve the biosynthesis of ATP. Many of them, especially enzymes, are key, without them there will be no accomplishment of final products. Coenzyme A (CoA) and coenzyme Q (CoQ) - taken as examples, have many important functions apart from being essential in the citric acid cycle.

Coenzyme A (CoA) multi-step biosynthesis requires among others pantothenate (vitamin B5) which is produced by the ATP-dependent condensation of β-alanine and pantoate by pantothenate synthetase. The last step of CoA biosynthesis is catalyzed by dephospho-CoA kinase that adds an ATP-derived phosphate group to the 3′-hydroxyl of dephospho-CoA (Refer to Figure 3) [38].

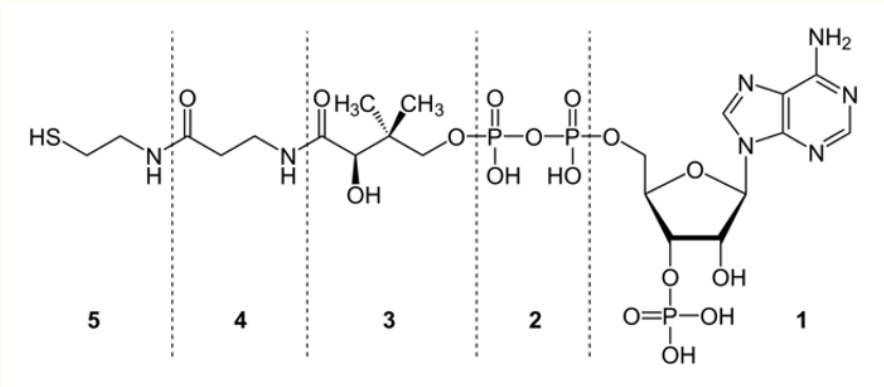


Figure 3: *Planar representative structure of coenzyme A. Part 1: 3'-phoshoadenosine. Part 2: diphosphate, organophosphate anhydride. Part 3: pantoic acid. Part 4: β-alanine. Part 5: β-cystamine. 1+2: 3'-phosphoadenosine-5'diphosphate. 3+4: pantothenic acid. 3+4+5: Pantetheine. (Courtesy of Wikimedia commons, public domain, 2007, work by NEUROtiker) access 8th January 2024.*

Coenzyme ubiquinone Q_n (CoQ), have key participation as electron carrier in the mitochondrial respiratory chain, in extra-mitochondrial electron transport, in the endogenously synthesized lipid soluble antioxidants, in the regulation of mitochondrial permeability transition pores, in the activation of mitochondrial uncoupling proteins and in the regulation of physicochemical properties of membranes, amongst others [39]. The free radicals that are formed in the cells are responsible for the oxidative stress, and are able to damage lipids (lipid peroxidation), included here the double lipid bilayer, and proteins and DNA. In the DNA of mitochondria these damages are far worse than nuclear DNA, as mitochondria lack protective histones contributing to less capability of repair [31].

CoQ (Figure 4) is produced in the mevalonate pathway starting with Acetyl-Coenzyme A (Acetyl-Co-A), and ending up with Farnesyl Pyrophosphate (FPP). FPP gives origin to squalene, cholesterol, dolichol and CoQ, among other compounds. Biosynthesis of CoQ starts with 4-hydroxybenzoate. The biochemical steps will add to the main bone structure (Figure 4b), replacing R, n units in the isoprenoid chain (Figure 4c for n = 10).

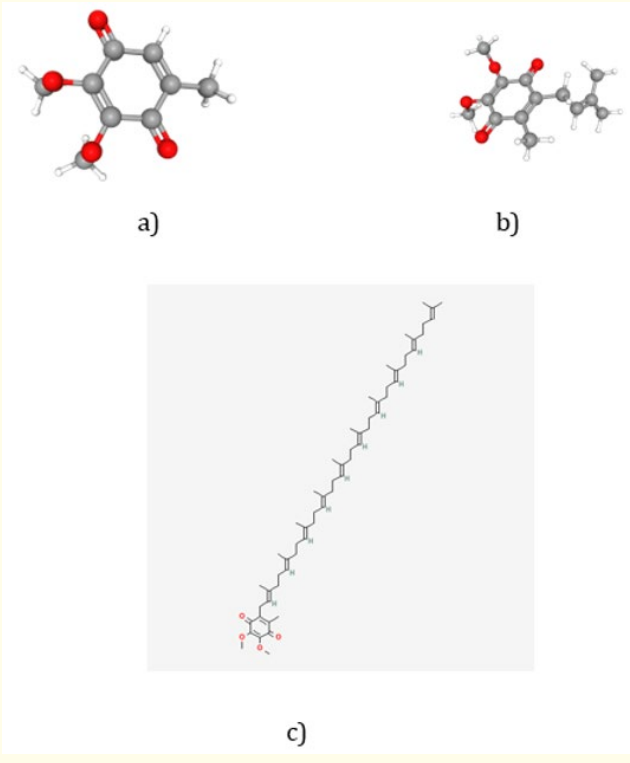


Figure 4: *a) 3D representation of coenzyme $Q_{(n=0)}$ (ubiquinone Q0) chemical structure, <https://pubchem.ncbi.nlm.nih.gov/compound/69068> and b) 3D representation of the chemical structure of Coenzyme Q(n=1), (ubiquinone Q1) <https://pubchem.ncbi.nlm.nih.gov/compound/4462> c) planar representation of Coenzyme $Q_{n=10}$ (ubiquinone Q10; ubidecarenone) <https://pubchem.ncbi.nlm.nih.gov/compound/5281915>. Courtesy of PubChem - NIH, National Library of Medicine, access 22nd Dec 2023.*

The oxidative phosphorylation pathway that the mitochondria produces ATP uses synthase and electron transport chain (ETC), a series of complexes in inner membrane that transport the electrons (Refer to Figure 5) [40].

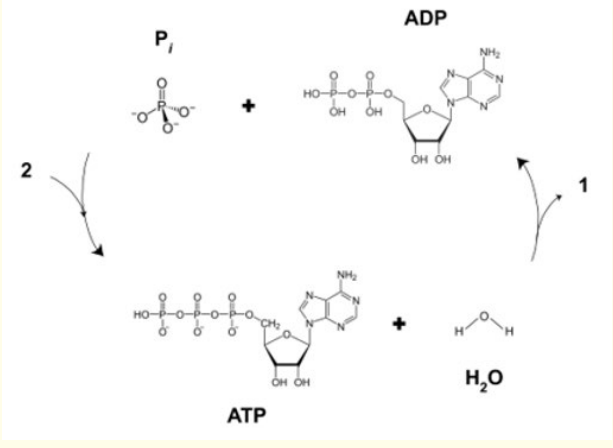


Figure 5: *The cycles of synthesis and degradation of ATP; 2 and 1 represent input and output of energy, respectively. (Courtesy of By Butrboy - Own work, Public Domain, <https://commons.wikimedia.org/w/index.php?curid=79504120>) access 25th December 2023.*

The mitochondria biochemistry is very sensitive to metal ions. Apart from the essential metal ions for its functioning, mainly Na^{+}, K^{+}, Ca^{++}, Zn^{++}..., the heavy metals are the ones posing a lot of damages to their integrity and therefore, to their performance. Heavy metals alter mitochondrial membrane permeability, generating increased amount of ROS. ROS induce changes in the structure of antioxidant enzymes minimizing their capability to counter attacking this nefarious oxidative activity [41].

The dietary essential ω-6 linoleic acid (LA) is converted in the body to ω-6 arachidonic acid. Arachidonic acid is not an essential fatty acid, becoming then when linoleic acid level in the body is deficient. Metabolism of arachidonic acid gives rise to hydroxyeicosatetraenoic acids and epoxyeicosatrienoic acids through cytochrome P450 (CYP) ω-hydroxylases and CYP epoxygenases, respectively. The CYP-mediated arachidonic acid metabolites is undoubtfully related to the pathogenesis of cardiac hypertrophy. Hydroxyeicosatetraenoic acids in general are pro-inflammatory, while epoxyeicosatrienoic acids have anti-inflammatory and cardioprotective properties. Several mechanisms are implicated in inflammation-induced cardiac hypertrophy, including the modulation of NF-κB (NF-κB induces the expression of various pro-inflammatory genes, including those encoding cytokines and chemokines) and MAPKs (mitogen-activated protein kinases) [42-44].

Arachidonic acid levels influence electron transport chain causing dysfunction of this mitochondrial electron transport, also generating substantial ROS production through cyclooxygenase and lipoxygenase action. This arachidonic acid levels (can also inhibit the activity of mitochondrial respiratory chain, and cause cytochrome oxidase system dysfunction) impaired electron transmission function, produce a large number of superoxide anions and hydrogen peroxide radical. Heavy metals also activate protein kinase C and NADPH oxidase, resulting in a massive generation of intracellular ROS [42].

For instance, cadmium [1] after absorption, is transported to the liver, bound to albumin and up taken by hepatocytes. Cytoplasmic Cd^{2+} is complexed with the glutathione leaving this thiol tripeptide less available to scavenge other toxics affecting the liver [45].

Mitochondria reproduces by fusion and division, where which one of the two will be done according to levels of nutrients and other criteria. The liver mitochondrial half-life can be 17 days. The liver in the presence of ethanol undergoes many needed detox functions: a) structural - accumulation of fat till cell death by oxidative modification in mitochondria - b) functional - a disfunction in its ribosome leading to a decrease in the mitochondrial protein synthesis and/or inactivation of mitochondria macromolecules - and c) different state of health - reaching even a critical decrease in ATP synthesis, even only after a few weeks of ethanol ingestion. In addition to the decrease in ATP synthesis, exposing liver mitochondria to ethanol, acutely or chronically, produces high levels of reactive oxygen species (ROS), thus oxidative stress [46-48].

Although short lived and more prone to act more locally, ROS (reactive oxygen species) when attacking polyunsaturated fatty acids (PUFAs), produce lipid peroxidation within the cell. The produced compounds (mainly aldehydes) have longer half-lives than ROS and also the potential to diffuse to reach distant intracellular and extracellular targets. The higher the quantity of double bonds in PUFAs, their rate of peroxidation increases exponentially. Peroxidation of mitochondrial membrane similar components increases cellular oxidative stress complicating the scenario even inside the mitochondria [49].

As complex as it may seem at first glance, all the biosynthesis involved in the health of the power house of the cells and the health of the mainly Detox organs, one should take for granted that they are intertwined, that their habits shapes the body's homeostasis and that the assurance of an adequate evacuation of all toxins produced in the body must be made.

Mitochondria diseases [50] are so widespread nowadays that is alarming. Many of them are mainly due to life style, nutrition quality and nutrient deficiencies, accumulation and production of toxins paving way to oxidative damages, smoking and alcohol consumption habits [46].

Liver diseases - fat liver, hepatic steatosis, liver cancer - 3 steps to chaos in the homeostasis

Mitochondria within the hepatocytes are key to hepatic energy metabolism [51]. Liver demands lots of oxygen due to possessing >20% of mitochondria of the total liver volume (and 25% of total volume of a cell). Disruption of mitochondrial β-oxidation and the carnitine shuttle that imports medium- and long-chain fatty acids into mitochondria are the frequent causes of hepatic steatosis or even more severe hepatic injury. Some synthetic drugs like antiviral compounds can interfere with replication of mitochondrial DNA, leading to deficiencies of 13 essential proteins of oxidative phosphorylation and a fulminant and irreversible hepatic failure [48,52].

A fat liver (hepatic steatosis, or steatotic liver disease - NAFLD) is when too much inadequate fat is build up is this organ. The first consequences of these metabolic alterations are inflammation and wounding. Every day consumption of alcohol or even occasionally binge drinking can start this disease named alcohol-related liver disease (ARLD). But also non-alcoholic fatty steatohepatitis (NASH) can happen as is the case of regular consumption of high fructose corn syrup for instance. They all depend on the eating habits and life style. These are stage 1 in the process of reaching a liver cancer. Stage 2 is fibrosis (where although the liver is still able to function normally, persistent inflammation causes scar tissue around the liver and nearby blood vessels). Stage 3 - cirrhosis, (after years of inflammation the liver shrinks and becomes scarred and lumpy; this damage is permanent and can lead to liver failure) and finally the person will end up in stage 4 - liver cancer. As the liver has no innervation for a person to feel direct pain on its steady failure, other symptoms may reveal this very inconvenient disease at already a late stage (Figure 6) [49,53].

In fat livers the pro-oxidant substances (ROS) are singlet oxygen molecules, superoxide anions, hydrogen peroxide, and hydroxyl radicals. Some of the consequences of increased ROS include the ability to trigger a cascade of oxidative reactions including or leading to protein unfolding and DNA damages as double-strand breaks, depletion of ATP and nicotinamide dinucleotide, the destruction of membranes via lipid peroxidation, and the release of proinflammatory cytokines. Human livers with NAFLD have increased levels of by-products of lipid peroxidation, a clear sign of an increase in oxidative stress caused by this condition [49].

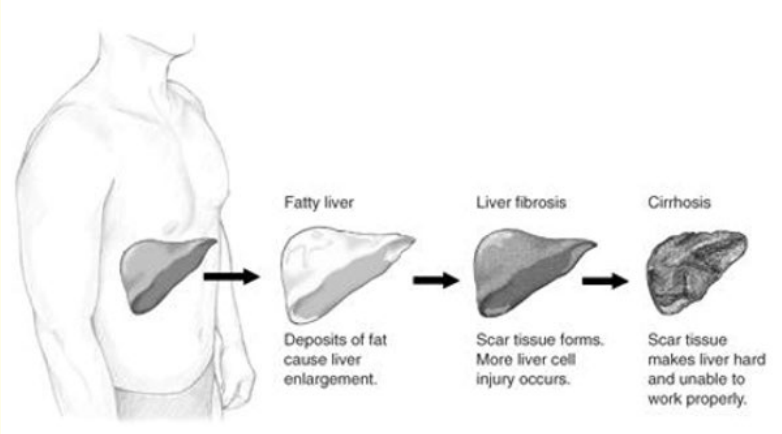


Figure 6: *Different stages of liver damage. (Courtesy of Wikimedia Commons, public domain, 2007) access 24th December 2023.*

Permeable intestine and leaky gut

In a Detox process, when the body is going to evacuate toxins, either direct and derived metabolic substances, the emunctories must be in perfect health. One can imagine the huge damage after a complete cleaning for some days (around 3) when all waste generated will go through a leaky gut, easily returning to the blood circulation in huge concentrations.

Fasano (2020) [54] has defined "leaky gut" as one of many chronic inflammatory diseases (CID). Seen as a recent epidemic, in his work he states that the "zonulin family, a group of proteins modulating gut permeability, is implicated in a variety of CIDs, including autoimmune, infective, metabolic, and tumoral diseases" and even as a biomarker of these diseases. The consequences of these metabolic conditions are autoimmune disorders of all kinds, starting with an irritable bowel syndrome, that at a late step, will develop a leaky gut (Figure 7).

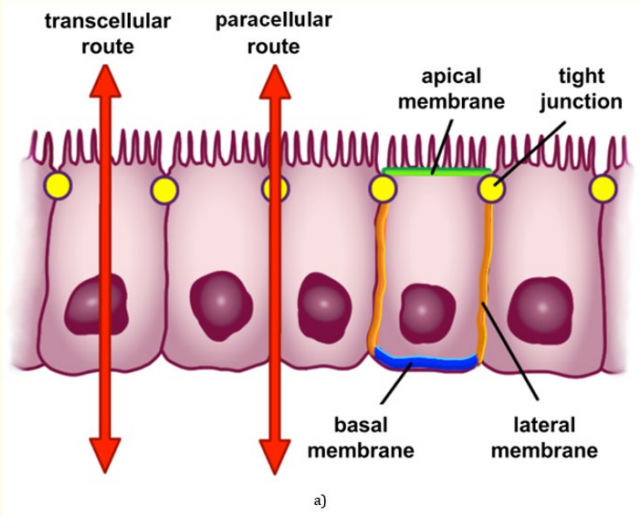


Figure 7: *a) Scheme of selective permeability routes of epithelial cells (red arrows). The transcellular (through the cells) and paracellular routes (between the cells) control the passage of substances between the intestinal lumen and blood. (Courtesy of and licensed under the Creative Commons Attribution-Share Alike 4.0 International license, by Ballenablanca, 2016); b) Normal tight intestinal membrane junctions (left) and the leaky and inflamed ones (right). (Courtesy of stockfreeimages.com) access 26th December 2023.*

Much has been attributed to this health condition and further studies are required, but one of the main causes of this inflammatory process in the intestine mucosa up to now is gluten. Gliadin (in gluten) is among the several potential intestinal luminal stimuli to zonulin release, also small exposure to large amounts of bacteria (bacteria overgrowth). These two conditions have been identified as the two most powerful triggers to the leaky gut [54].

Mold, parasites and acidosis

Molds are a serious threat to humans because they can produce several mycotoxins [55]. Mold spores are the size of a virus, and can go to the deepest inner parts of the respiratory tract, and when reaching the lungs can colonize them and produce their mycotoxins. These substances can cause a variety of adverse health effects, bringing an unhealthy condition that could go from acute toxicity to long-term diseases like autoimmune diseases, neurological disorders to even cancer. Mycotoxins are normally excreted by the kidneys and the gut. This mold condition if present, must be addressed before starting any Detox protocol.

Concerning parasites, many parasitic infections in the human body are derived from consumption of animal meat, contaminated soil and water among other minor sources. The parasites can install in almost every human body organs, even the brain, where there are many known parasitic infections. Some examples are Neurocysticercosis, Echinococcosis and Schistosomiasis. Parasitic infection is strongly linked to the composition of the gut microbiome [56]. However, the main problem generated by parasitic infections in someone doing a Detox program is that these parasites can generate the irritable bowel syndrome that could, most of the times, degenerate into a leaky gut. Parasites must be addressed and completely exterminated before any Detox program should be implemented. More about the leaky gut in the section 2.3.2.

Acidosis definition according to The Merck Manual is: "*Acidosis is* caused by an overproduction of acid that builds up in the blood or an excessive loss of bicarbonate from the blood (metabolic acidosis) or by a buildup of carbon dioxide in the blood that results from poor lung function or depressed breathing (respiratory acidosis)" [57]. Acidosis can also be due to metabolic disfunctions [58].

Acute metabolic acidosis can be due to serious illnesses or hospitalizations, and is generally caused when the body produces an excess amount of organic acids (in general mainly lactic acid), or worse, the result of impaired kidney function (chronic kidney disease) and/or bicarbonate wasting (Figure 8a). If the kidneys are not working properly, the evacuation of the toxins in the Detox process is not going to be successful and will probably compromise even more these organs.

Three blood buffers (equations 1 to 3) are in place to strictly maintain the blood homeostatic pH value. The risk of death is hanged by a strict small difference of 0,1 pH values (pH values are in logarithmic base 10 numbers).

One of these 3 buffers is the bicarbonate buffer, following equation 1 below:

$H^+ + HCO_3^- = H_2CO_3 = CO_2 + H_2O$ (1).

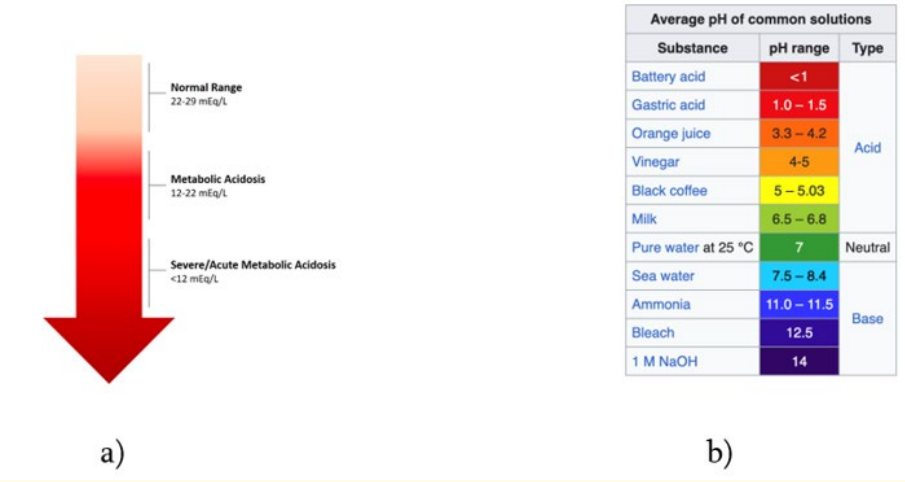
Even the respiration and the cell accumulation of CO_2 can be responsible for a consumption of H_2CO_3 and increasing acidosis in the blood.

The second is the phosphate buffer system, following the equation of the second deprotonation of phosphoric acid, equation 2, which happens around pH 7.2:

$H_2PO_4^- \rightleftharpoons HPO_4^{-2} + H^+ \; pK_{a2} = 7.20$ (2).

All sodas and beverages containing phosphoric acid or phosphate derivatives on a daily basis consumption or even scarcely consumed can highly contribute to the installation of a dangerous permanent acidosis condition.

Last but not least, is the third buffer composed of plasma proteins. Made up of amino acids (positively charged amino groups and negatively charged carboxyl groups), nearly all proteins can function as buffers. Blood buffering by proteins accounts for two-thirds of the total buffer power and most of the buffering within cells. The charged regions of these molecules can bind hydrogen and hydroxyl ions, and thus function as important blood buffers to maintain this very narrow permitted range of pH variation in the healthy blood.



Constitution of normal blood

Parameter	Value
Hematocrit	45 ± 7 (38–52%) for males 42 ± 5 (37–47%) for females
pH	7.35–7.45
base excess	–3 to +3
PO_2	10–13 kPa (80–100 mm Hg)
PCO_2	4.8–5.8 kPa (35–45 mm Hg)
HCO_3^-	21–27 mM
Oxygen saturation	Oxygenated: 98–99% Deoxygenated: 75%

c)

Figure 8: *a) The level of bicarbonate in the blood determines the severity of metabolic acidosis. Courtesy of by Metabolic Acidosis Expert, 2019); b) Average pH of common solutions; c) constitution of hematocrit and inorganic substances and pH of normal blood. (Courtesy of Creative Commons Attribution-Share Alike 4.0 International license and Wikimedia). Access 2nd January 2024.*

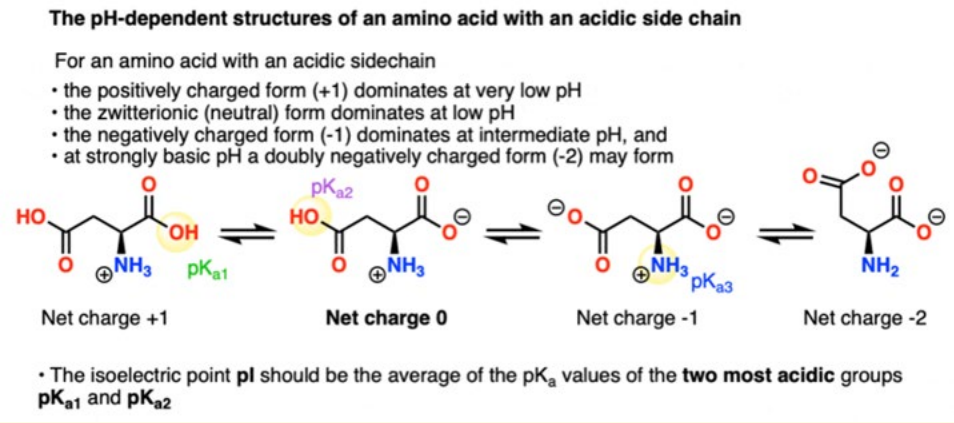


Figure 9: *Examples of pH-dependent amino acid structures and how they influence the uptake and release of H+ in the blood and cells. (Courtesy of <https://www.masterorganicchemistry.com/2023/02/09/isoelectric-point-calculation/>) access 28th December 2023.*

Discussion

Some edible substances (always organically grown) aiding in general the homeostasis, and particularly to induce [59] each of the 3 phase process of detoxification

The whole health state of the person in the process of detoxification is of utmost importance and some important topics need to be addressed first of all. Considering all the aspects leading to homeostasis discussed above, it is important to safely address them before attempts on using any Detox protocol is made. First of all, a complete deworming protocol must be made. One example is the use of artemisinin's, using *Artemisia annua* [60] or *Artemisia absinthium* [61].

Second of all, having a health intestine lining. Many are the solutions to fix a leaky gut, for instance, by following a strict non gluten diet. L-glutamine [62] on a daily basis is one of the most efficient compound to heal a leak gut, nevertheless requiring several months to completely fix the joints in the intestine mucosa. The following references can be addressed for other options [63,64].

As the toxic compounds must get out of the cell, the goal of any Detox protocol, after Phase I they need to be made more water-soluble, accomplished in the conjugation step (Phase II). Also they must make way across the cell membrane lipid bilayers. For that, the cell membranes must be intact and in good shape. For the health of a membrane structure ω-3 fatty acids, glycerophospholipids, cholesterol and other specialized lipids are needed [65].

For the health of mitochondria regular consumption of L-carnitine, CoQ_{10} and ω-3 fatty acids are strongly desirable. L-Carnitine is essential for the transfer of long-chain fatty acids across the inner mitochondrial membrane for subsequent β-oxidation [52].

More details can be found below.

Phase I

Antioxidants

As discussed above, one of the main issues in Phase I human Detox process is the inevitable production of free radicals. For each toxin molecule metabolized there is the production of at least one free radical. It is imperative to take antioxidants, either as those present in plants or in the form of food complements.

The most important antioxidants considered in this review are α-lipoic acid (ALA) and N-acetyl cysteine (NAC), the last a biochemical precursor of reduced glutathione (γ-glutamylcysteinylglycine - GSH).

α-Lipoic acid (ALA) can be found in carrots, beets, spinach, broccoli, and potatoes. There are so far very few studies showing any contraindications in using ALA. However, it may be consumed with caution if someone has the following conditions/habits: Liver disease; consumption of large amounts of alcohol; diabetics (ALA is known to lower sugar blood); thyroid disorders, or thiamine deficiency according to the NIH [66].

NAC has a potent protective effect against oxidative stress and inflammation. Its complete metabolism can give way, after their complete metabolism in a human body, to cysteine, cystine, inorganic sulfate, and glutathione (GSH). Human biochemistry can synthesize GSH, involving two adenosine triphosphate-dependent steps. First, γ-glutamylcysteine is synthesized from L-glutamate and L-cysteine. This conversion requires the enzyme glutamate-cysteine ligase (= glutamate cysteine synthase - GCL). This reaction is the rate-limiting step in glutathione synthesis; second, glycine is added to the C-terminal of γ-glutamylcysteine. This condensation is catalyzed by glutathione synthetase. Then GSH is endogenously obtained [67,68]. The amino acids involved in this biosynthesis must be present in adequate concentrations for GSH to be obtained.

Garcinol or camboginol (polyisoprenylated benzophenone) extracted mainly from *Garcinia sp. (Garcinia indica* and *Garcinia cambogia*) fruit peel and leaves has antioxidant and anti-inflammatory properties. They both also present anticancer ability by promoting suppression of histone acetylation leading to cellular apoptosis [69,70].

The chemical arjunolic acid (Figure 10), a pentacyclic triterpenoid saponin, found in *Musanga cecropioides* (umbrella tree)*, Akebia quinata* (chocolate vine) and in *Terminalia arjuna* tree has been found in the literature to be an excellent antioxidant and anti-inflammatory compound among antidiabetic, anti-fungic, anti-bacterial, anticholinesterase, antitumor, antiasthmatic properties. *Terminalia arjuna*, largely used in the Ayurvedic medicine, provides many parts, mainly fruit and bark, to human consumption and is full of many bioactive constituents, like tannins, triterpenoid saponins (e.g. arjunolic acid), flavonoids, ellagic acid, gallic acid, oligomeric proanthocyanidins and phytosterols among others [71].

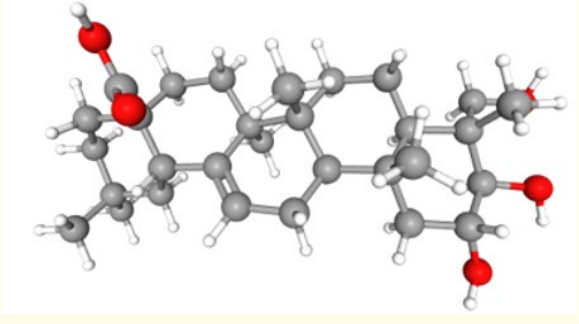


Figure 10: *3-D representation of arjunolic acid structure (IUPAC name: (4aS,6aR,6aS,6bR,8aR,9R,10R,11R,12aR,14bS)-10,11-dihydroxy-9-(hydroxymethyl)-2,2,6a,6b,9,12a-hexamethyl-1,3,4,5,6,6a,7,8,8a,10,11,12,13,14b-tetradecahydropicene-4a-carboxylic acid). C = gray; O= red; H = white balls. Courtesy of PubChem @ <https://pubchem.ncbi.nlm.nih.gov/compound/Arjunolic-acid> access 7th January 2024.*

In the *Baccharis* family, *Baccharis trimera* (known as carqueja in Brazil) has been proved to have antioxidant and anti-inflammatory abilities, among gastric and hepatic-protection, anti-microbial, anti-fungal and anti-parasitic properties. The aerial parts are mainly constituted by flavonoids, terpenes and chlorogenic acids [72]. In another work [73] using rats as models, cardiovascular alterations that were induced by the combination use of *narguilé* smoke, alcohol and energy drinks intake, the administration of *Baccharis trimera* extract was able to reverse these alterations although only at an early stage of the cardiovascular disfunction.

After the first 24h, during Phase I Detox, antioxidants must be provided either as liquids or complements in regular doses for the next 12h. Also, adequate levels of vitamins A, C, E, and the micronutrient ions selenium [74] (as part of selenoproteins, remarkable for their antioxidant capacity), copper, zinc and manganese are also needed in the antioxidant steps of the entire Detox process.

The organism undergoing a Detox protocol must have been previously provided with all these elements in adequate quantities before starting the protocol. This is in order for them not to be in low levels in the body during the Detox process. Also crucial is to have been supporting all the emunctories using the proposed herbs or foods for them to be prepared for the Detox task ahead of them.

Curcumin and its related compounds and examples of bioflavonoids

The flavonoid curcumin (diferuloylmethane - Figure 11) component of turmeric *Curcuma longa*, even presenting lipophilic affinity, have undisputable anti-inflammatory and antioxidant properties in both aqueous and fat-soluble extracts [75-77]. Along with that, antioxidant anti-inflammatory, neuroprotective, anticancer, hepatoprotective, cardioprotective, immunomodulatory, antimicrobial, antiallergic, antidermatophytic and antidepressant properties are also found in *Curcuma longa*.

Curcuma species, present hundreds of constituents, but in their rhizome the majority of them are terpenoids and polyphenols (of which curcuminoids represent 80%) [76].

Curcumin was studied as a natural chemoprotective agent once it was found that it increases the activities of Phase II detoxification enzymes of xenobiotic metabolism. Curcumin also is a Phase I enzyme inducer. It binds to the *Ah* receptor (Aryl hydrocarbon receptor), activates the transcription of the cytochrome P4501A1 gene, and elevates its enzyme activity [11].

The AhR, a ligand-activated transcription factor that controls adaptation to the cellular environment is relevant to autoimmune and metabolic diseases, among others. As an example, this receptor, capable of probing both endogenous factors (oxygen tension or redox potential…) and exogenous factors (such as polyaromatic hydrocarbons and environmental toxins…), adjusts biological processes applicable to maintaining tissue homeostasis [78].

Although many studies have shown the efficacy of curcumin in these above described properties, the oral consumption of powdered curcumin (powdered rhizome of turmeric plant - *Curcuma longa*) is known to a) have poor systemic bioavailability due to its low aqueous solubility (which supposedly prevents curcumin delivery to non enteric organs), b) have a poor absorption not sufficient to present a quick metabolism leading to extensive metabolic conversion and quick excretion (in other words, presents a poor pharmacokinetics) [79-81].

Once this absorption limitations are overcome, for instance by increasing systemic bioavailability by some transformation strategies [81-85], the results should increase the incredible potency of curcumin (bioactivity) in the protection and in the detoxification process of an organism.

Another approach in the literature is that the efficacy of curcumin might not be entirely due to the parent molecule, but in part, due to its metabolites. Therefore, to explain the observed successful of many pre-clinical and clinical studies involving curcumin itself [80]

(and references therein mainly [10] and [6]), after it was metabolized in the liver/intestine, there come the curcumin conjugates and degradation products.

Once in the blood, curcumin can undergo conjugation reaction (useful in the phase II) mainly in the liver such as sulfation and glucuronidation. In Figure 11, curcumin structure shows the easily accessible -OH and -OCH_3, that can be reversible changed by the action of enzymes - glucuronidases and sulfatases.

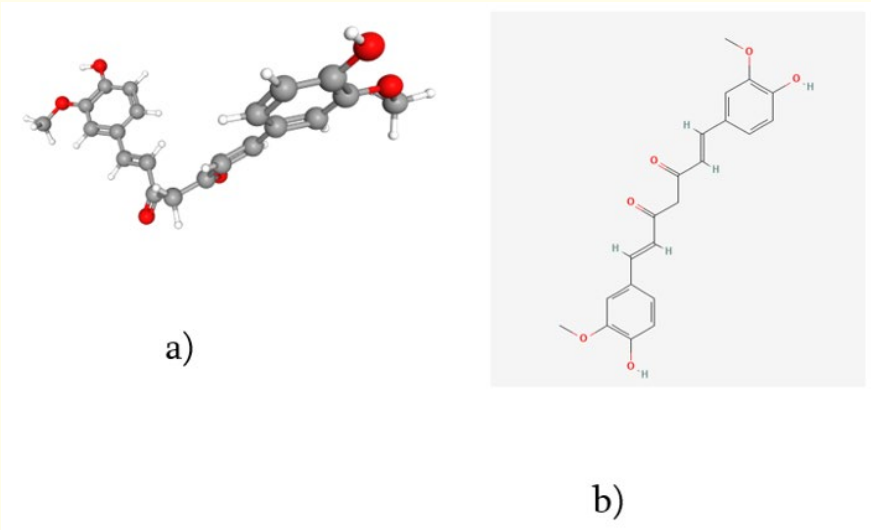


Figure 11: *a) 3-D and b) planar structure representation of curcumin (diferuloylmethane - active free form, curcuminoid), a poly-phenol (β-diketone) present in the roots of Curcuma longa. One or two of the -OCH3 groups substituted by -H group(s) - are the structures of demethoxycurcumin or bisdemethoxycurcumin. C = gray; O = red and white balls = H atoms. (Images kindly available by PubChem @: <https://pubchem.ncbi.nlm.nih.gov/compound/Curcumin#section=Drug-Labels> access 29th December 2023.*

In studies on a water dispersible formulation of curcuminoids with fenugreek dietary fibre rich in galactomannans, it was reported that the formulation exhibited improved tissue distribution and better Blood Brain Barrier permeability of all the three free curcuminoids - biologically relevant unconjugated forms of curcumin - with higher results in bioavailability and consequently, in superior clinical outcomes [82,83,86]. The main goal here is to provide the body with unconjugated curcuminoids for improved detoxification.

Two groups of compounds acting as classical Michael reaction acceptors examples are curcumin and β-diketones (dibenzoylmethanes, which lack direct kind of Michael reactivity). Curcumin (Figure 11) contains two Michael reaction acceptor functionalities in its molecule, inducing quinone reductase. Chemically speaking, the presence of two structural elements was found to be required for "higher induced potency" [11] in Phase II detoxification: *o*-OH groups in the aromatic rings and β-diketone functionality (Figure 12).

Also, tetrahydrocurcumin (Figure 12d) was found to be a very potent inducer of Phase II Detox *in vitro* with a proposed mechanism involving β-diketone moiety [11].

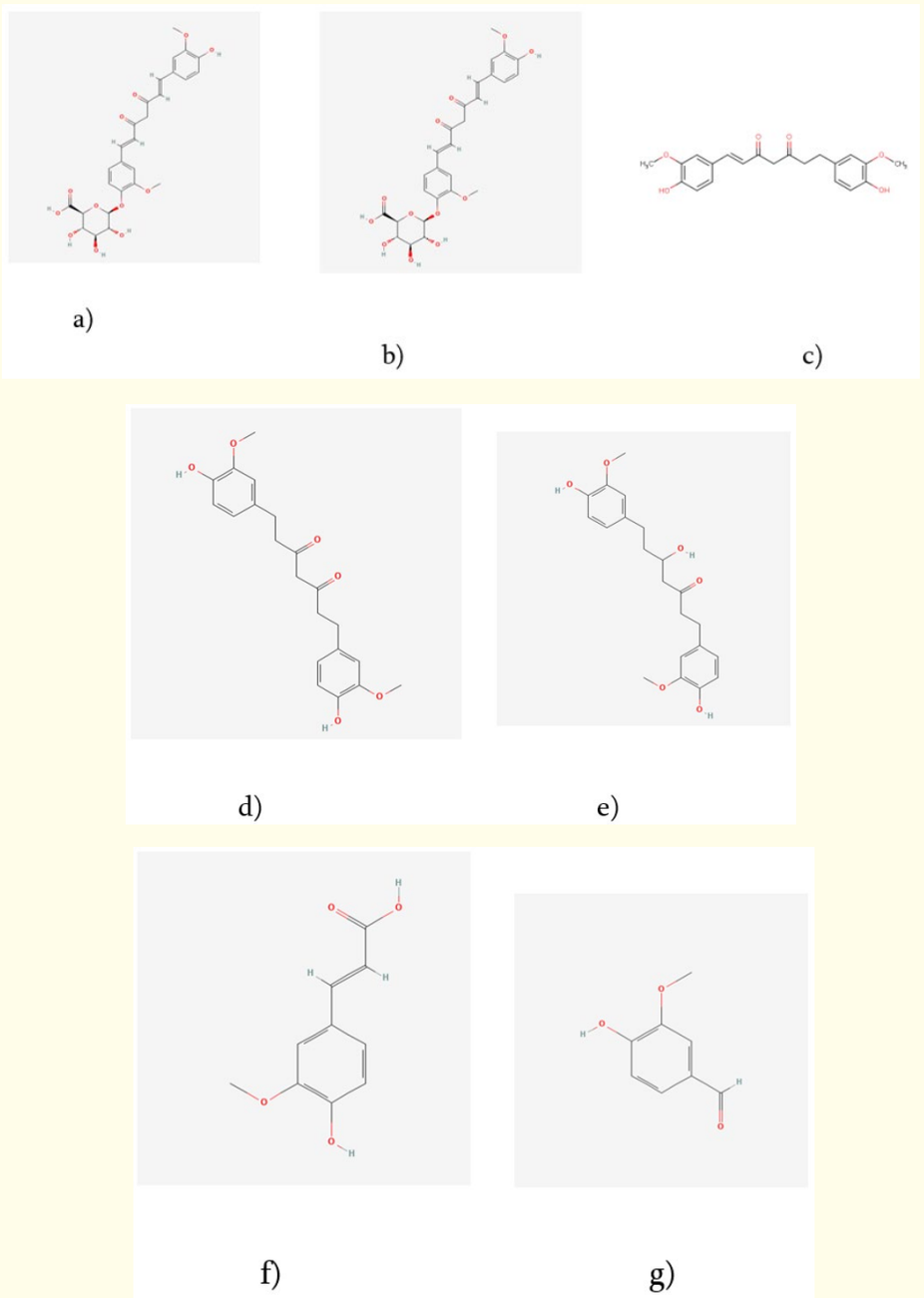


Figure 12: *Planar structure representation of curcumin after Michael reactions - in the presence of intestinal/liver enzymes - inactivated major fraction conjugated forms: a) curcumin glucuronide b) curcumin sulfate. Planar structure representation of reduced curcumin metabolites: c) dihydrocurcumin d) tetrahydrocurcumin e) hexahydrocurcumin; degradation products: f) ferulic acid g) vanillin, among others. Images kindly available by PubChem and ChEBI @ (access 15th November 2023):*

a) <https://pubchem.ncbi.nlm.nih.gov/compound/Curcumin-glucuronide>
b) <https://pubchem.ncbi.nlm.nih.gov/compound/66645351#section=2D-Structure>
c) <https://www.ebi.ac.uk/chebi/searchId.do?chebiId=CHEBI:67262>
d)<https://pubchem.ncbi.nlm.nih.gov/compound/Tetrahydrocurcumin#section=2D-Structure>
e) <https://pubchem.ncbi.nlm.nih.gov/compound/hexahydrocurcumin#section=2D-Structure>
f) <https://pubchem.ncbi.nlm.nih.gov/compound/Ferulic-acid>
g) <https://pubchem.ncbi.nlm.nih.gov/compound/Vanillin#section=2D-Structure>.

Silymarin and gale of the wind

Silymarin is the extract from the seeds of the plant *Silybum marianum* (Milk Thistle), containing a group of flavonoids used for hepatic disorders (flavonolignans, flavonoids {taxifolin, quercetin} and polyphenolic molecules). It is composed of approximately 50% silibinin (plus silychristin, and silidianin), which are considered the biologically active components of silymarin [87].

Silymarin extract main characteristic is antioxidant activity against ROS and lipid peroxidation (and therefore, lowers cytotoxicity), but also increases the endogenous concentrations of antioxidant enzymes such as glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase [88].

Silymarin can scavenge free radicals and controlling inflammatory cytokines. Consequently, it presents a notable anti-inflammatory effect, mainly by inhibiting the nuclear transcription factor NFκB and as a consequence, reducing the inflammatory cytokines in the hepatic parenchyma, and downregulating cyclooxygenase 2 [89].

The effect of silymarin in hepatic cells are reported in the literature as capable of revert steps 1, 2 and 3 of liver diseases (fat liver, liver fibrosis and liver cirrhosis, (Refer to Figure 6) and drug-induced liver injury, presenting a regenerative effect in hepatic cells. The treatment can be from 28 days to as long as 6 months, but many clinical results have shown that silymarin is capable of reversion of some liver disease cases [89].

Silymarin also present beneficial effects on the immune system, anti-inflammatory properties in the protection against viral infections modulating the membrane permeability - and therefore, preventing hazardous compounds from being absorbed specially in the phalloidin-transporting system, helping the treatment of antituberculosis, among others [89].

The aqueous extract of gale of the wind (*Phyllanthus niruri*) is known as an Indian ayurvedic herbal medicine for centuries, in Traditional Chinese Medicine and in Indonesian Jamu presenting ailments for treatment of bronchitis, anaemia, skin diseases, asthma, cough, liver, kidney and urinary tract disorders, dyspepsia, influenza, vaginitis, hyperglycaemia, jaundice, for removing kidney stones also presenting hepatoprotective properties, for treating hepatotoxicity, hepatitis B and bacterial diseases. Known as "erva quebra pedra in Brazil" it is used as an effective remedy for urinary and bladder disorders as well as hepatic disorders and hyperglycemia in Brazilian herbal medicine. Gale of the wind possessing many phenolic derived constituents in its aqueous extract of dried leaves, presents potent antioxidant and anti-inflammatory activities [90,91].

Main phytochemicals in gale of the wind are flavonoids, terpenes, coumarins, lignans, tannins, alkaloids and saponins [92]. Taking into account the small size of the plant its biochemistry is astonishingly successful.

Oligomeric proanthocyanidins, CoQ10 and thiol based natural sources

Oligomeric proanthocyanidin complexes are naturally occurring plant metabolites present in fruits, vegetables, seeds, nuts and bark. They must be consumed organically cultivated [88]. Pesticide residue in any edible food is highly hepatotoxic ultimately leading to unbalances in the metabolism and in the liver oxidative equilibrium. Plants used as foods must be fresh, free from mold and any signs of deterioration, special care in summer when they can easily rot [93].

Primarily known as antioxidants, oligomeric proanthocyanidin complexes were found to present antibacterial, antiviral, anticarcinogenic, anti-inflammatory, anti-allergic, vasodilatory actions, to inhibit lipid peroxidation, platelet aggregation, capillary permeability and fragility, and to affect enzyme systems including phospholipase A2, cyclooxygenase, and lipoxygenase, and presenting anticancer and anti-diabetic properties [94-96]. They are the catechins, leucoanthocyanidins, A-type proanthocyanidins, B-type proanthocyanidins found widely in human proper nutrition [97].

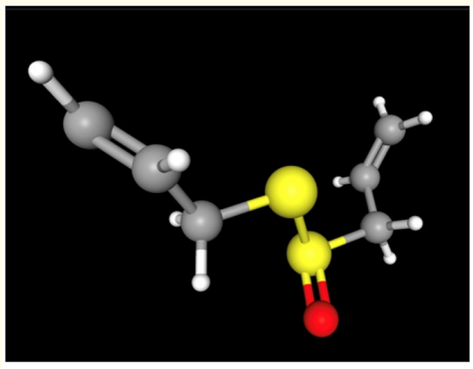
Coenzyme Q_{10} (ubiquinone, lipid soluble) main food sources are in the soybean oil and in the olive oil, followed by meat, fish, nuts and vegetables, fruits and cereals (Refer to Figure 4) [98].

Thiols, present in garlic, onions, shallots, chives, leeks (*Allium*-derived sulfur compounds, e.g. allicin = diallyl thiosulfate - Figure 13) and cruciferous edible vegetables are very supportive of the mitochondria and liver and even presenting anti-cancer properties [99-101]. All very antioxidant in nature, Glutathione (GSH) is one of the most important thiol based compound in the prevention of any cell damage and also for its capability of binding to toxic metal ions in human body [102]. Another one are the set of metallothionein's (glutathione related peptides - MTs - metallothionein-1 and metallothionein-2 present in all organs, metallothionein-3 expressed mainly in the brain, and metallothionein-4, in certain stratified tissues) [103], cysteine-rich proteins also capable of binding toxic and essential metals, playing a number of roles in the biology of cells. Some of the most potent inducers of MT-1/MT-2 biosynthesis are metal ions such as Zn^{2+} and Cd^{2+}. The cadmium induces MTs for the defense of the organism, to excrete this very toxic metal ion, whilst zinc is an inducer of GSH to be in high levels to protect the organism.

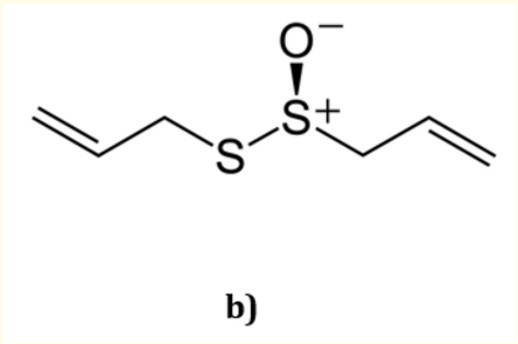
Zinc ion is necessary in many physiological processes, including enzyme catalysis, protein structure stabilization, and the regulation of many proteins. Examples of Zn^{2+} ions participation are zinc fingers, hormone receptors, metallothionein's and in DNA-binding proteins [104,105].

Any organic zinc edible plant Zn^{2+} source (seeds in general, specially pumpkin seeds, cashews seed, and Brazil nuts [106], seafood, mainly oysters, among others) absolutely needs to be harvest from a rich Zn^{2+} soil or water. In the case of soil grown food, the geological soil composition knowledge is a very important asset. According to Noulas., *et al.* 2018 [107] "In Europe high Zn values occur in the Canary Islands (basalt), west-central Spain, the Pyrenees, the Poitou region in France, the southern Central Massif, the east part of northern Italy, Slovenia, Sardinia, Calabria, and Lavrion in Greece".

A poor Zn^{2+} ion soil (zinc ion deficiency in soil-crop systems is widespread nowadays and is common in calcareous, high pH, eroded and land-levelled soils) cannot provide this essential microelement, even if the plant is reputed to have the capacity of concentrating them [108].



a)



b)

Figure 13: *a) 3-D structure representation of Allicin-S-(Prop-2-en-1-yl) prop-2-ene-1-sulfinothioate, present in garlic. (C=grey; H=white; O=red; S=yellow balls); b) 2-D planar structure representation of allicin. Courtesy of PubChem, at <https://pubchem.ncbi.nlm.nih.gov/compound/Allicin#section=3D-Conformer>, access 26th December 2023.*

Phase II

After the first 24h of the DETOX process, and for 12h more, protein or aminoacids in liquid form should be provided to support Phase II in regular doses.

Glutathione-S-transferase

Glutathione-S-transferases (GSH-S-Ts) are divided according to their cellular localization into at least three major families of proteins, namely cytosolic GSH-S-Ts (alpha, zeta, theta, mu, pi, sigma and omega), mitochondrial GSH-S-Ts (kappa) and microsomal GSH-S-Ts (=MAPEG (membrane-associated proteins involved in eicosanoid and glutathione metabolism). GSH-S-Ts also possess non enzymatic functions, some like cell proliferation, DNA repair, and cell death [109].

When speaking of glutathione-S-transferase enzymes, is important to mention their role in catalyzing the conjugation of glutathione (GSH) to many different hydrophobic electrophiles - endogenous and exogenous substances - in the detoxification phase II. These hydrophobic electrophilic molecules generated in phase I or produced in the body can be carcinogens, having as origins therapeutic drugs as well as many products of oxidative metabolism (aflatoxins, lipoperoxidation-derived aldehydes, the highly reactive aldehyde acrolein, among others) [109,110].

A group of genes produce the whole number of GSH-S-T family proteins. Once the conjugation reaction ready, the toxins attached to the glutathione are neutralized and ready to be eliminated [111-113].

In order to endogenously increase the production of GSH-S-Ts, the consumption of organically grown sulfur-rich foods is highly indicated. Sulfur-rich foods are those composed of methionine and cysteine aminoacids, cruciferous vegetables, *Allium* family vegetables…). Selenium rich foods, (as this element is a GSH cofactor needed for GSH activity) are Brazil nuts, brown rice (depending on the soil as stated already elsewhere in this work), and last but not least, GSH rich foods are avocados, spinach, asparagus…

To promote bile production and facilitate its flow, the consumption of theobromine, theophylline (main alkaloids in *Theobroma cacao* (the raw cacao bean) and other plants) as well as kahweol and cafestol palmitates (green coffee components) is advised. These compounds also promotes the endogenously production and enhance the activities of glutathione-S-transferases [114].

Uridine diphosphate-glucuronosyltransferase

The conjugation reactions with human Uridine diphosphate-glucuronosyltransferase enzymes (UGT), a superfamily of 22 proteins, catalyse the glucuronic acid covalent bond from the cofactor UDP-glucuronic acid, to conjugate to a substrate with a suitable acceptor functional group. This glucuronidation process transforms the acceptor into a more hydrophilic compound, the main process of phase II xenobiotic biotransformation/conjugation. The liver mainly, the kidneys, gastrointestinal tract, among others, express some groups of these enzymes (UGT1A and UGT2B). UGTs are important in the metabolization of endogenous compounds, like steroids (e.g. β-estradiol, testosterone), thyroid hormone, bilirubin, and retinoic acid. The body performs these enzymatic reactions in order to obtain glucuronide metabolites, them presenting enhanced aqueous solubility compared to the substrates facilitating biliary and renal excretion [4,115].

UDP-glucuronic acid is utilized in biosynthetic reactions that involve condensation of glucuronic acid with a variety of molecules. These reactions use the high-energy bond between Uridine Diphosphate and glucuronic acid to provide the energy to form the new bond in the conjugated product. UDP is then hydrolyzed to Uridine Monophosphate and inorganic phosphate, ensuring the one way reaction equilibrium, thus forming stable conjugated products [116].

The role of UGTs in the maintenance of brain homeostasis has been reported to include neuronal protection against xenobiotics capable of crossing the Blood Brain Barrier (BBB). For instance, ethanol is primarily oxidized both in the liver and in the BBB. Polychlorinated

biphenyls (PCBs - man-made group of chlorinated compounds)) are also capable of crossing the BBB, among other examples [117].

Glucuronic acid form is mainly found in no added sugar (nor any sweetener) and no sugar left Kombucha drinks, and in gums - *Acacia* and xanthan gums.

Phase III

After 36h of the start of the DETOX program, and for another period of 12h, healthy fats and high levels of hydration with pure water must be provided to the body. Total suggested time for the 3 phases, 48h = (24+12+12)h. The Detox process is only accomplished in its entirety in the absence of solid food for this whole period of time.

Other important Phase III support nutrients

Yarrow, artichoke, cumin, coriander, ajowan, garlic and ginger

In phase III of the detoxifying process, at this point, all that it is needed is to evacuate all created compounds in the previous steps. For that well hydrated cells as well as providing support substances for kidneys, liver and intestines are very welcome. Infusions and water extract of some plants can really help in this last Detox phase - elimination. Some examples are below. In this specific phase also a good source of organic fat (e.g. olive oil, unrefined almond oil, nigella oil…) is recommended to support the emunctories.

Yarrow (*Achillea millefolium*) consumed as tea (flowering top aerial parts) or plant extract, is diuretic and kidney, gastrointestinal and liver supportive as well as blood purifier [118-120].

Artichoke (*Cynara cardunculus* var. *scolymus*), one of the richest sources of polyphenols, is a true bile flow stimulant, the edible immature flower heads of the plant itself or the extract [121].

Cumin and coriander seeds have beneficial effects in human gut microbiota increasing short chain fatty acid levels in the intestines [122].

Ajowan (*Trachyspermum ammi L.*) is very helpful in the treatment of kidney stones and also a strong disinfectant and digestive looking at its chemical composition [123]. So is ginger *(Zingiber officinale*) root, its tea or extract [124].

Conclusion

As it can be seen from the above, the process of Detox is not only a protocol to be careless followed, but a long time lifestyle. Providing support for this biochemical cleaning process to correctly take place and go smoothly in a natural way is a day to day job.

The body's Detox, comprised of 3 phases, needs certain nutrients and/or supplements to support the correct unfolding of each one of them at the right timing. The correct use of supplements and as much knowledge as possible to the understanding of the possible incompatibilities one may encounter with any of the chosen ones, are very desired to any person starting a Detox program. There are some description in the literature that some supplements association (even from edible plants) can trigger some further complications to those already presenting liver diseases [125].

The health of the liver, gallbladder (bile), the intestines, lungs, kidneys as well as their mitochondria, are very important before any Detox protocol be initiated. Ultimately, during all the process, hydration with pure water are a must and plant extracts or infusion are also key nutrients.

Another review to come will explore the many essential oils capabilities in doing/supporting the Detox process as well as in the health maintenance of the emunctories.

Conflict of Interest

The author declares that no whatsoever financial interest and/or conflict of interest exist.

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