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Can we use the epigenetic bioactivity of caloric restriction and phytochemicals to promote healthy ageing?

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Abbreviations: Acetyl-CoA: acetyl coenzyme A, ADP: adenosine diphosphate, ALDH2: aldehyde dehydrogenase 2, Akt: Protein Kinase B, AMPK: 5'-adenosine monophosphate-activated protein kinase, ATP: adenine triphosphate, DNMTs: DNA methyltransferases, CTA: citric acid cycle, CLOCK: Circadian Locomotor Output Cycles Kaput, eNoSC: energy dependent nucleolar silencing, HAT: histone AcetylTransferase, FoxO3a: forkhead box O3a, HDMs: histone demethylases, HDACs: histone deacetylases, KMTs: histone lysine methyltransferases, MS: Metabolic syndrome, mTOR: mammalian target of rapamycin, NAD: Nicotinamide adenine dinucleotide, NADP: Nicotinamide adenine dinucleotide phosphate, NAM: Nicotinamide, NAMPT: Nicotinamide Phosphoribosyl transferase, NR: Nicotinamide riboside, PARP: poly-ADP-ribose polymerases, PRC: Polycomb repressive complex , PRMTs: Arginine methyltransferases, RSV: Resveratrol, SAM: S-Adenosyl methionine, SIRTs: sirtuines. T2D: Type 2 Diabetes.

Abstract

In 2011, the Centers for Disease Control and Prevention reported that half of Americans are using food supplements, intended to provide them nutrients that they believed, they consume in insufficient quantities. Dietary supplements were repeatedly said unnecessary if one eats a balanced diet but within the booming "silver economy", sales of vitamins, minerals and supplements totaled nearly $23 billion in the U.S. last year (Euro monitor International). Seventy eight "antioxidants" supplements trials including 296,707 participants were reviewed by the "Cochrane Database System Review". It was concluded that beta-carotene and vitamin E supplements, instead of promoting longevity were increasing mortality and so may high doses of vitamin A. Several nutrition interventions with or without edible phytochemical, fasting regimens, and food supplements are now tried to prevent the development of many different types of tumors. Most were known as antioxidants and are now repurposed because they were shown to change the epigenome.

Epigenetic marks are enzymatic tags deposited on the genome which do not change the DNA sequence but changes gene expression. They are reversible and epigenetic dietary interventions are thus suggested for the prevention of ageing, ageing related diseases and for breaking the vicious circle of the trans-generational epigenetic inheritance of the metabolic disorders. It is a pressing issue because more and more children are exposed to parent's obesity and are at risk to develop type 2 diabetes.

Why is it relevant to propose epigenetic "Nutricures" to prevent diseases linked with ageing? To answer this question we produce this review whose scope is to underline the importance of the nutrition contribution for a correct functioning of Epigenetic. Almost all chromatin-modifying enzymes utilize co-factors that are crucial metabolites for core metabolism pathways. Because the cellular concentrations of these metabolites fluctuate as a function of the metabolic status of the cell, the activity of most epigenetic enzymes depends from the cell metabolism and thus from nutrition. Through one selected representative example: sirtuines and its metabolic co-factor: Nicotinamide, we illustrate the nutrition and epigenetic connections. Then we describe the epigenetic activities of some polyphenols. Finally we underline that with the globalization there is a continuing trend changing the variety of food items that we consume. Each may have bioactive effects which may combine with additional epigenetic effects of pollutants like pesticides.

We plead likewise that because they are bioactive "Nutricures" and food supplements should undergo better evaluation before marketing.

Introduction

Genome Wide Association Studies have not resolved the links between genetic susceptibilities and complex chronic diseases because they did not consider yet the epigenome, that changes gene expression that are not encoded into the DNA sequence, thus are reversible but are never the less passed to the progeny. When adding these studies, recent results have involved epigenetic to explain how the quality and the quantity of the diet in terms of consumed calories and phytochemicals, can have trans-generational epigenetic effects. In other words diets and bioactive food components consumed with or without the diet; can alter our own and our children's gene expression via epigenetic effects.

Epigenetic enzymes

It is beyond the scope of this review to achieve a comprehensive examination of all epigenetic enzymes and marks. However, in Table 1 are listed some families of epigenetic enzymes, the marks they produce and some references. Because of space limitations other epigenetic actors like microRNAs and chromatin modelers, are not detailed here.

Enzymes	Added/removed marks	Co-factors *and metabolism*	Inhibitors	Roles
DNMT1, 2 and 3a/b	Methyl group at C5 C	SAM *(1 C metabolism)*	Nucleosides Non nucleosides	Repression of gene expression (1-2)
DNMT4	Methyl group at C5 C	SAM *(1 C metabolism)*		RNA methylation
KMT1:A-F, KMT2: A-H, KMT3: A-G, KMT4, 6-7, KMT5: A-C KMT8: A-C	1 to 3 methyl groups on histone (H) lysine (K)	SAM *(1 C metabolism)*	SAM derivatives Lysine mimics	H3K4, 36 and 79 for gene activation H3K9 and 27 and H4K20 for gene repression (3)
PRMTs Type I: PRMT1, 3, 6,8 and CARM1 Type II: PRMT5, FBXO11 and HsI7 Type II/III: PRMT7 Type IV: PRMT2	Add 1 or 2 methyl groups on histone arginine (R) (symmetric or not)	SAM *(1 C metabolism)*	Methylated arginine Asymmetrical urea	Modulate R hydrogen bonding capacity and recognition of R methylation by Tudor-domain containing proteins. Activate or repress gene expression (4)
HDMs KDM1: A-B KDM2: A-B KDM3: A-C KDM4: A-D KDM5: A-D KDM6: A-B KDM7: A-C and **KDM8**	Remove 1 to 3 methyl groups on histone Nε-lysine (depending on specificity)	α-Ketoglutarate or Fe (II) or Flavin (*Oncometabolites inhibitors: succinate and 2-hydroxyglutarate*)	Alkynes Cyclopropylamines	Positive or negative modulation of gene expression depending on partners (5)

HATs **GCN5/PCAF, MYST including MOZ, Ybf2/Sas3 and Tip 60, p300/CBP and Rtt109**	Acetyl group on histone Nε-lysine and non-histone Nε-lysine	Acetyl coenzyme A (acetyl-CoA) *(Citric and pantothenic acids)*	Anacardic acid Acetyl CoA mimics	Type A HATs acetylates nucleosome histones. HAT1 acetylates nascent histones in the cytoplasm during the process of chromatin assembly. Gene activation (6)
HDACs **Class I: HDAC1–3,** and **8** **Class II: HDAC4–7** and **9–10** **Class IV: HDAC11**	Remove acetyl group from histone and non-histone Nε-lysine	Zinc and water	Short-chain carboxylic acids Hydroxamic acids Benzamides Sulphuryls Cyclic peptides ketones	Gene repression (7)
Sirtuines or class III HDACs 1 to 7	Remove acetyl group from histone and non-histone Nε-lysine	NAD (*Niacin*)	NAD mimics and peptidomimetics	Gene repression (also transfer of ADP-ribose to protein) (8)

Table 1: Main epigenetic enzymes: marks, co-factors, inhibitors and effects

Maternal nutrition epigenetic effects

Early epidemiological studies have demonstrated the impacts of maternal nutrition on the offspring health, more recent are showing that the health effects are driven by Epigenetic. The very first described the Dutch famine or "Hunger Winter "cohort. From 1944-1945 and because of the Second World War in occupied Netherlands, mothers suffered during their pregnancy of caloric restriction (CR) (down to 500 calories/day). An increased mortality was observed during the follow up of 408,015 Dutch male births born between 1944 and 1947. It was related to famine exposure in early pregnancy only and was accompanied with an increased risk for type 2 diabetes (T2D) in adulthood (9).

The starving prenatal environment seemed to set an offspring accelerated ageing process with both early cognitive decline and coronary artery diseases. Children of prenatally starved fathers, but not mothers, were also later, heavier and more obese than offspring of fathers and mothers who had not been undernourished prenatally (10). Individuals prenatally starved during the Hunger Winter had also, later, less DNA methylation of the imprinted Insulin Growth Factor 2 gene compared with their unexposed same-sex siblings (11). Others "famine" cohorts are confirming that CR promotes trans generational metabolic disease phenotypes transmitted through the mothers and affecting the sons and that Epigenetic might drive it (see the Chinese great leap (12) and the Biafra cohorts (13)).

Conversely, the study of the impact of maternal over nutrition, showed that both sex offspring exposed to maternal obesity, or diabetes have an increased risk, later in life, for obesity, hypertension and T2D (12). In children born to obese parents, several gene regulatory regions were found differently imprinted (change in DNA methylation) by comparison to children born to non-obese parents (13). High fat diets promoted progeny early adult onset of MS and obesity in both sex (16).

Thus, any "extreme" early-life nutritional exposure of a human fetus seems to "print" in his genome a "cellular mal programmed memory"; inducing in adulthood and for offspring's, a repetitive organism adaptive malfunctioning; giving long-term health deleterious effects. Epigenetic mechanisms at the roots of such diseases susceptibility are being now described (17). Thus it is relevant to believe that an "epigenetic orientated nutrition" of one generation could also prevent metabolic diseases and even unhealthy ageing in the following generations.

In the *Avy* mouse (agouti), the coat color is a direct readout of the methylation status of the *Avy* gene because the expression of the alleles varies with their DNA methylation levels. By

supplementing the diets of pregnant dams with high levels of folic acid as methyl donor it is possible to modify the *avy* phenotype or the coat color of the offspring. In an another mouse model, dietary methyl deficiency reducing folate or choline, and methionine was shown to alter hepatic DNA methylation patterns and induced liver cancer in the absence of a carcinogen with early re-feeding alleviating the defects (18).

These studies have proved the influence of maternal nutrition on the offspring epigenome and proved also that epigenetic marks established by dietary interventions can be passed to a successive generation via the female germ line. Finally, they have emphasized that correct timing must be considered for dietary interventions.

Caloric Restriction, metabolism and ageing

In mammal cells, the transformation of the chemical energy of fuel molecules (glucose, fatty acids, and amino acids) into energy is regulated. Cellular energy is mainly obtained from oxidation of glucose and is stored within cells in the form of adenosine triphosphate (ATP). The major delivering route is glycolysis, where glucose is converted into pyruvate (aerobic oxidation), which conversion is further extended up to NADH made from Nicotinamide (NAD+) as the acetyl coenzyme A (acetyl-CoA) is oxidized into the Krebs cycle (also known as the citric acid cycle or CAT). In anaerobic conditions, glycolysis produces lactate. Fats are catabolized by hydrolysis to energetic fatty acids and glycerol. Next glycerol enters glycolysis and the fatty acids are broken down by beta oxidation to release acetyl-CoA, which is then fed into the CAT cycle. Cells store excess glucose in skeletal muscle and liver as glycogen. Cells also regularly catabolize excess glucose to form acetyl-CoA for the fatty acid synthesis pathway used for long-term energy storage in liver and the adipose tissues.

Metabolic syndrome (MS) is characterized by high blood pressure, dyslipidemia, and insulin resistance. MS leads to the increased risk of developing coronary artery disease, stroke, and DT2 which are also more frequent with ageing. DT2 is a chronic metabolic disorder characterized by increased blood sugar level resulting from unresponsiveness of target cells to insulin action.

Several examples of epigenetic disturbance in MS and T2D are documented. The methylome of diabetic pancreatic islets is different from the methylome of non-diabetic pancreatic islets (19). Glucagon activates Fork head box protein A2 (FOXA2) via Histone AcetylTransferase (HAT) driven acetylation; FOXA2 desacetylation is controlled by Sirtuine 1 (SIRT1) and HDACs (Histone deacetylases). FOXA2 regulates glucose metabolism and the differentiation of pancreas and liver (20). High fat diets induce chronic hyperglycemia promoting mitochondrial overproduction of reactive oxygen species ending into organism injury and further development of MS and TD2 (17-21

Caloric Restriction, Sirt1, Resveratrol and Ageing

For an aerobic given organism, the long term effects of its surrounding external and internal oxidative environment induces a vicious cycle in which stress damaged mitochondria produce increased amounts of reactive oxygen species that damage DNA, proteins and lipids inducing more damaging oxidative stress. Since it is thought that ageing results mostly from deleterious oxidative stress; nutritional and pharmacological strategies were sought to limit oxidative stress in order to prevent ageing and ageing related diseases like neurodegenerative diseases, MS, TD2, and cancers.

Caloric Restriction (CR) restricts calorie intake and delays ageing from yeast to mammals (8-20). When calorie restricted, mammals have reduced body weight and size, decreased blood insulin and improved insulin sensitivity. During CR and to get the most possible energy from the restricted nutritional resources there is a metabolic shift of the organism to oxidative metabolism with a concomitant higher resistance to oxidative stress via enhanced mitochondrial biogenesis (17-21).

SIRT1 is up regulated in CR and functions to preserve body energy by increasing fatty acid oxidation. Brain overexpression of SIRT1 is sufficient to promote longevity in mice (22). However SIRT1 over-expression has not yet been shown to result in lifespan extension in humans. In mice, SIRT1 up regulation protects against hepatic steatosis and hyperglycemia induced by high-fat diet (23). On the opposite and in response to high-fat feeding, deletion of SIRT1 results in hepatic

steatosis and inflammation (24). Thus SIRT1 activity is needed to adapt the organism responses to nutritional intake and its role in ageing needed investigation. Therefore screenings were realized to search for SIRT modulators.

Resveratrol (RSV) turned out as a SIRT1 activator (25). Like CR, RSV (3, 5, 4'-trihydroxystilbene), a polyphenol found in red wine, has been reported to extend lifespan in organisms ranging from yeast to mice. Most of its biological activities have been attributed to its *trans* isomer (26-28). RSV is found in many plant species and plant parts (peanuts, berries and grapes). It is at the root of the French paradox (high fat diets with moderate red wine consumption prevent ischemic heart diseases) and can mimic the effects of CR (29).

Several other plant-polyphenols such as flavones (quercetin), chalcones (butein), and anthocyanidins were claimed to activate also SIRT1 *in vitro* through an identical allosteric mechanism involving the lowering of the peptides substrates Km (see Table 3); RSV, being the most potent.

SIRT1 activation, *in vitro*, by RSV is substrate and sequence-selective (30). However besides SIRT1, RSV has several others targets: cyclooxygenases, lipo-oxygenases, kinases, ribonucleotide reductases, adenylyl cyclases, aromatase and DNA polymerases (25). Effectiveness of RSV is limited by poor systemic bioavailability and rapid metabolism. SRT501, a RSV formulation with improved bioavailability, has been shown to alleviate inflammation in a wide range of animal models but production has been arrested (31).

Recently synthetic small molecules, which activate SIRT1, like RSV, were claimed to have therapeutic potential to treat common diseases and ageing (32). Several have already entered clinical trials for sepsis, psoriasis, and ulcerative colitis because RSV cannot be approved to prevent ageing since ageing is not a disease.

In the USA, two-thirds of people who consume multiple dietary supplements consume also RSV to slow ageing and to increase sportive fitness (31). They might be disappointed by the recent observations that 30-days RSV supplementation induced CR-like effects on energy metabolism and metabolic profile in obese humans (32). However RSV did not improve metabolic function in non-obese women with normal glucose tolerance (33). A recent meta-analysis of 11 human clinical trials, confirms the beneficial effect of RSV consumption on fasting glucose, glycosylated hemoglobin values, and on insulin resistance but only for TD2 patients (34). Administration of tyrosol, a phenolic compound, abundant in olive oil and white wine, induced the expression of SIRT1 and activated SIRT1 target proteins helping mice to overcome heart ischemic stress (35).

A change in the concept of ageing has appeared with these studies: SIRT 1 and metabolism are linked with ageing. A certain amount of oxidative stress is needed for an efficient organism adaptation to CR; organism training by CR (up to 15 %) and/or intermittent controlled fasting could slow ageing decline.

Insulin metabolism, epigenetic, autophagy and ageing

RSV has ill-founded discussions about what is SIRT activation that neglected the importance of the other pathways that control lifespan in mammals. In yeast, flies, and mice lifespan is extended after disruption and/or down regulation of insulin signaling (36).

Low levels of insulin Growth factor 1 (IGF1) reduce growth and causes longer life in mice (37) inducing fat accumulation in humans (38). Most anti-ageing fasting regimens elicit a drop in circulating IGF1 (39).

Upon binding to their receptors, insulin and IGF1 induce a number of signaling pathways like the phosphatidylinositol 3-kinases class I (PI 3). PI 3-kinases regulate glucose uptake through a series of phosphorylation events, activate the serine/threonine kinase Akt (whose deletion in mice prolongs life) and activate the serine/threonine kinase mammalian target of rapamycin or mTOR (whose attenuation prolongs lifespan from yeast to mice). These pathways like Sirt1 control also the response to CR and cellular oxidative stress. Tumor suppressor phosphatase and tensin homologue on chromosome ten is a negative regulator of PI3K signaling (40-43). The AKT downstream effectors are the transcription factors: FOXOs.

The FOXOs family is involved in antioxidant response, gluconeogenesis, cell cycle control and cell survival. Multiple post translational modifications of FOXOs control their trafficking to the nucleus and thus their transcriptional activities. FOXOS are acetylated by HAT, deacetylated by SIRT1-2 and 3, and methylated by PMRT1 (44). For example, the activity of FOXO1 as a regulator of blood glucose is modulated by desacetylation by Sirt1 under conditions of cellular stress. Class IIa HDACs are positive regulators of hepatic FOXO1 in response to glucagon signaling during fasting (45).

Mutations in the mTOR pathway increase life span in invertebrates, but mutants no longer respond to CR. Attenuation of mTOR signaling is part of the downstream mechanism involved in the beneficial effects of CR (46) because it is one of the downstream targets of the 5'-adenosine monophosphate-activated protein kinase (AMPK). AMPK once activated by low energy status phosphorylates unique site on tuberous sclerosis complex (TSC2), switches on ATP-producing catabolic pathways such as fatty acid oxidation and glycolysis, and switches off ATP-consuming anabolic pathways such as lipogenesis. Thus pharmacological AMPK activation may reduce the risk of T2D, MS, and slow ageing. Any stress that depletes cellular ATP, such as oxidative stress, hypoxia, muscle contraction, CR but also leptin and adiponectin secretion activates AMPK directly or indirectly (40).

Insulin/IGF-1, mTOR, FOXOs, AMPK, and the SIRTs all play a role in ageing through their sensing and regulation of energy availability in times of cellular stress, epigenetic is prominent to regulate these pathways when controlling FOXOS localization (40).

Autophagy and ageing

Several of the resulting effects of these pathways are mediated in part by autophagy, a lysosome degradation process that degrades cell proteins and organelles. Activation of autophagy and lipophagy when nutrients are scarce allows cells to reutilize their own constituents. During the first fasting hours, autophagy-is dependent of proteolysis then activated lipophagy will predominate. Sirt1 induces autophagy via the desacetylation of FOXOs for the transcription of the specific autophagy genes (47). Genetic manipulations of the autophagy pathway have confirmed the tight connection between autophagy and life span; increased autophagy leading to longevity (40).

As organisms age the autophagy activity and the insulin sensitivity decline in almost all cells and tissues (40). Systemic infusion of the direct AMPK activator: 5-aminoimidazole-4-carboxamide riboside or AICAR, in normal and insulin-resistant obese rats decreased hepatic glucose production (48). Metformin and phenformin are indirect AMPK activators. Like them, quercetin, RSV, Green tea extracts, catechine, and curcumine were reported to possess similar anti-ageing activity (40).

Metformin is prescribed for the treatment of T2D. It does not modify insulin levels but decreases glycaemia and hepatic gluconeogenesis while increasing tissue insulin sensitivity (49). Metformin reproduces the CR lifespan extension gene expression profile in mice (50-52).

Rapamycin, known as an immunosuppressive drug and other rapalogs (everolimus and temserolimus) are strong inducers of autophagy. They extend the lifespan of middle-aged treated mice (53). In humans, only intermittent and low dose of Rapamycin is now considered for the prevention of T2D complications because of unwanted side effects but also in accordance with the concept of hormesis (weak doses of a product have more beneficial effects than high doses) (54).

Epigenetic connection with autophagy is also exemplified by the spermidine case. Spermidine promotes longevity in ageing yeasts by autophagy Induction. Spermidine triggers histone H3 desacetylation by inhibition of HAT, suppressing oxidative stress and necrosis. On the opposite, depletion of endogenous polyamines leads to chromatin hyper acetylation, generation of reactive oxygen species, early necrotic death and decreased yeast lifespan (55).

Polyphenols (PPs) found in red wine, like anthocyanin (oenin), stilbenoids (piceatannol) and flavonoids (catechin, epicatechin, quercetin, and myricetin), but also monophenols (caffeic and gallic acids) and glucosides (delphinidin, kuronamin, and peonidin) stimulate autophagy *in vitro* and a robust negative correlation could be established between autophagy induction and the acetylation levels of cytoplasmic proteins of the U2OS engineered cells used (56). Epigallocatechin gallate, RSV

and curcumin, have also been found to down regulate mTOR signaling *in vitro* (57). All could be used to slow ageing decline.

Oxidative stress is now recognized as a fundamental component of the aging process, leading to activation of pro-growth pathways like insulin/IGF-1 and mTOR, accumulation of cellular debris, and ultimately activation of cell death/survival pathways like autophagy. With the increasing resistance of aged cells to insulin, chronic over activation of autophagy could deregulate glucose homeostasis for further autophagy activation. This ''autophagy vicious circle'' may lead to MS and explains its higher prevalence with age. Several epigenetic modulators of autophagy are known: micro RNAs, not reviewed here and again acetylation/desacetylation of pro-autophagy genes.

As mammals age, their cells undergo a process termed cellular senescence, which is characterized by irreversible cell cycle exit, some telomeres shortening and the secretion of various pro-inflammatory molecules. MTOR converts cellular quiescence into senescence (58). Specific histone modifications are involved in regulating the expression of genes related to senescence in human embryonic lung fibroblasts-(59).

The sirtuines (see table 2)

SIRTs participate in the regulation of cellular homeostasis by desacetylating histone and non-histone proteins as described above. Most SIRTs desacetylate lysine residues of the histone tails H1, H3 and H4. The SIRTs induce likewise a better adhesion of the modified histone to the DNA, and thus, prevent the transcriptional machinery to access to the contiguous DNA whose expression is suppressed.

SIRTs can also desacetylate other proteins (to stabilize them?) such as transcription factors, chaperone proteins and effectors of DNA repair, signaling pathways and cellular metabolism (60). SIRT3 can desacetylate the majority of mitochondrial proteins: acetyl-CoA synthetase 2 and long-chain fatty acyl-CoA, to convert acetate and fatty acids, into acetyl-CoA (61). SIRT5 activates 3-hydroxy-3methylglutaryl CoA synthase 2 which is involved in ketogenesis; ornithine transcarbamoylase involved in the urea cycle; manganese superoxide dismutase which inhibits reactive oxygen species production; and ubiquinone 1 sub complex; a subunit of complex I in the electron transport chain (62). Absence of SIRT6 induces genomic instability and aging-like phenotype in mammals (63).

The activities of SIRT1, SIRT3, SIRT4, and SIRT6 are also relevant to the Warburg effect, in which cancer cells show a massive up-regulation of glycolysis and glutaminolysis and/or reduced transcription of CTA cycle genes with a decreased oxidative phosphorylation, even in the presence of sufficient oxygen. (64).

In addition, SIRT1 and suppressor of variegation 3-9 homolog 1 are members of the ribosomal DNA energy dependent nucleolar silencing complex (eNoSC) regulated by an NAD^+/NADH balanced energy-dependent pathway. SIRT1 desacetylates H3 then suppressor of variegation 3 to 9 homolog 1 KDM mediated K9 methylation leads to the silencing of the ribosomal DNA locus (64). In response to glucose starvation, eNoSC suppresses ribosomal RNA transcription and induces acetylation and accumulation of p53 which eventually leads to cell cycle arrest or to apoptosis.

The SIRTs are part of the HDACs class III. It includes 7 members which have a common catalytic core domain of 270 amino acids with a large (Nicotinamide Adenine Dinucleotide) or NAD+-binding domain and a smaller zinc-binding domain. They have also different C and N terminals with, therefore, different substrates preferences and different cellular locations (8-60).

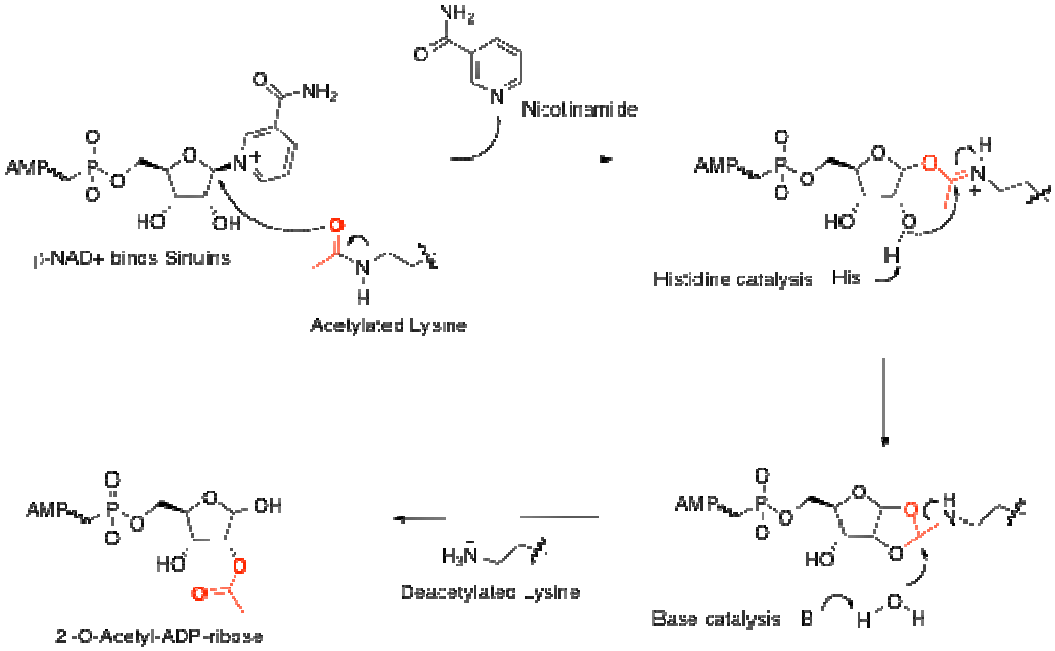

Figure 1: Proposed desacetylation by SIRTs consumes NAD^+ and involves an acetylated protein substrate to produce Nicotinamide (NAM) and 2' O-acetyl-adenosine diphosphate ribose in stoichiometric amounts and, the deacetylated substrate. NAM acts as an inhibitor of the reaction, and thus provides negative feedback inhibition of the SIRTs *in vivo*.

Name	Molecular Weight	Null mice Features	Localization	Activity	Functions
SIRT1	81.7 kDa	-/-: Perinatal deaths, abnormalities of the retina, heart and bones +/-mice have reduced plasma HDL-cholesterol and triglycerides when fed a normal diet	Nucleus and cytosol	Deacetylase	Metabolism adaptation, inflammation and cellular senescence (8-60)
SIRT2	43.2 kDa	Spontaneous tumor formation	Cytosol	Deacetylase	Apoptosis, motility and myelination (8-60)
SIRT3	43.6 kDa	Changed fatty oxidation, ATP levels and acetylated mitochondrial proteins levels Defect in the urea cycle and cardiac hypertrophy	Mitochondria	Deacetylase	Metabolism adaptation to fasting (8-61)
SIRT4	35.2 kDa	No physical abnormalities *but spontaneous Lung tumors*, increased mitochondrial glutamate dehydrogenase activity	Mitochondria	ADP-ribosyl transferase	Secretion of insulin and suppression of fatty acid oxidation and glutamine use (8-60)
SIRT5	33.9 kDa	No physical abnormalities,	Mitochondria	Deacylase	Malfunction of urea

		hyper ammonia during fasting or a high protein diet		Demalonylase Desuccinylase	cycle (8-62)
SIRT6	39.1 kDa	Premature female aging (Progeria like) and death. Low levels of insulin-like growth factor-1 and circulating glucose.	Nucleus	Deacetylase ADP-ribosyl transferase	Defects in the excision/repair system and genomic instability (8-63)
SIRT7	44.8 kDa	Cardiac hypertrophy and inflammation and premature death	Nucleolus	Deacetylase?	Enhances ribosomal RNA. Transcription (8-60)

Table 2: The sirtuines characteristics and roles

Intermittent fasting without CR and Epigenetic

Intermittent fasting extends lifespan in mammals and fasted mice (fed only at night) are resistant to diet-induced obesity (65). During fasting, muscle and liver stores of glycogen are depleted. Fatty acids are mobilized from adipocytes and transported to the liver for conversion to ketone bodies. Ketone bodies are produced in skeletal muscles after long fasting time, and metabolism changes from glucose to fatty acid oxidation. Circulating ketone bodies are then distributed to tissues where they are converted to acetyl-CoA to provide energy. Ketogenic diets are partial copies of CR through their effects on fatty acid metabolism. SIRT1 desacetylates peroxisome proliferative activated receptor gamma co-activator 1 alpha (PGC-1α) required for activating genes responsible for fatty acid oxidation (66). The nuclear hormone receptor Peroxisome proliferator-activated receptor alpha is a key activator of this hepatic ketogenic gene expression program in response to fasting (67). Butyrate**,** a short-chain fatty acid, is generated during the fermentation of dietary fibers in the large intestine. Butyrate is a class I and II HDAC inhibitor and is structurally close to β-hydroxybutyrate (βOHB) the major source of ketone body energy for mammals. Accumulation of βOHB in blood increases to 1 to 2 mM during fasting when the liver switches to fatty acid oxidation or even to higher levels in diabetic keto-acidosis. Inhibition of HDACs by βOHB may be the mechanism by which intermittent fasting confers health benefits (68). In the past, fasting has been used as an anticonvulsive therapy and we know now that low-carbohydrate diets that induce ketogenesis enhance resistance of neurons to oxidative damage (69).

The NAD world

In poor nutritional conditions such as fasting and CR, SIRTs orchestrate the programs that allow living organisms to survive. SIRT functioning has an absolute NAD requirement for desacetylation as well as for the transfer the ADP-ribose molecule to proteins by a yet less known mechanism (8-60). NAD is a metabolic co-factor that is present in cells either in its oxidized (NAD^+) or reduced (NADH) forms. It participates in redox cycling (NAD^+ accepts electrons and may become reduced into NADH, which donate electrons). NAD^+ is also consumed by several oxidases and for ADP-ribosylation. Synthesis of NAD^+ from Nicotinic acid (NA) or from NAmononucleotide (NAMN) involves a phosphoribosyl and then an adenylyl transfer. From Nicotinamide (NAM), the final product requires an additive ATP-dependent amidation step (70).

In mammals dietary forms of NAM are obtained from tryptophan (present in most proteins), NA (from plants) and NAD^+ (mainly in animal products). The mixture of NA and NAM is vitamin B3 or niacin. Tryptophan will generate NAD^+ in a complex pathway that is not regulated by the niacin status and which is minor in humans. NA is converted into NAD^+ in the intestine and liver and released into the bloodstream for uptake by extra hepatic tissues. The initial step is catalyzed by mononucleotide adenylyl transferase (NMNAT), which has 3 specialized isoforms either for the nucleus, the Golgi

apparatus, or the mitochondria. NAD^+ levels are reconstituted *de novo* or via the NAM salvage pathway which uses NAD^+ or NAM, the byproduct of NAD^+ consumption (Fig. 2). Some NAD^+ is converted into Nicotinamide adenine dinucleotide phosphate (NADP) by NAD^+ kinase, which phosphorylates NAD^+. $NADP^+$ is used in anabolic reactions, such as lipid and nucleic acid synthesis, which require NADPH as a reducing agent. NADPH provides the reducing equivalents needed for biosynthetic reactions and the oxidation-reduction involved in protecting against the toxicity of reactive oxygen species. NicotinAMide Phosphoribosyl Transferase (NAMPT) is the rate-limiting enzyme in the salvage pathway which, in the presence of ATP, catalyzes the synthesis of NMN (Nicotinamide MonoNucleotide) from NAM (71).

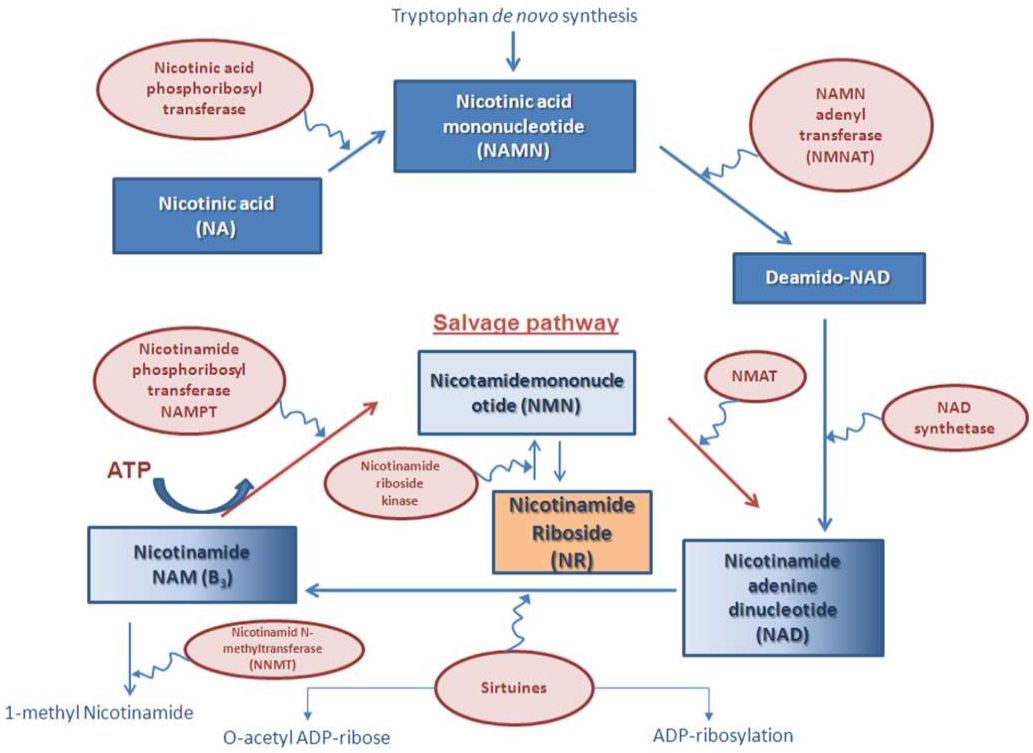

Fig. 2. The NAD biosynthesis pathway

Plasmatic NAMPT is very robust and converts NAM into NMN, which is uploaded by cells to be converted to NAD^+. Mature adipocytes synthesize and secrete through connexin 43 hemi channels, NAMPT into the bloodstream (72). Plasmatic NAMPT has discussed cytokine like functions. In cells, NAD^+ cannot directly reach the mitochondria because the salvage pathway is absent there. It requires first a conversion by cytosolic NAMPT in the cell cytoplasm. In mice, intra cellular NAMPT regulates the biosynthesis of NAD in a circadian-dependent oscillatory fashion through the rhythmic oscillation of NAMPT RNA and protein levels (73). For the moment such oscillations have not been shown for circulating NAMPT. In the mouse brain, SIRT1 governs circadian control by activating the transcription of the major circadian regulators (74). In aged wild-type mice, brain SIRT1 levels are decreased, giving rise to a longer intrinsic period and an inability to adapt to changes in light entrainment schedule (75). Young mice lacking brain SIRT1 develop these aging-dependent circadian changes. On the opposite, mice overexpressing SIRT1 in the brain are protected from the effects of aging (22-76). One of these circadian regulators is CLOCK which possesses an intrinsic HAT activity (77). SIRT1 regulates further the amplitude and the duration of the circadian gene expression through the desacetylation of CLOCK (78). A decrease in circulating NAMPT, reducing also SIRT1, is seen during cellular stress, nutritional deficiency and mice ageing. Mitochondria containing SIRT 3

and 4 are thought to synthetize NAD^+ and sequester it because NAD^+ may also diffuse between cytosol and nucleus through the nuclear pores, towards the nuclear enzymes.

Indeed and besides SIRTs, NAD^+ is a co-factor for: mono-ADP-ribose transferases, poly-ADP-ribose polymerases (PARP) and cyclic ADP-ribose synthases. During the time of the life course, all are more and more active to repair DNA ageing insults; thus they could compete with SIRTs to use NAD^+. In the concept of the NAD World (79), ageing is considered as the process in which organism gradually breaks down according to a hierarchal tissue susceptibility to NAD^+ availability. Brain and pancreas are exquisitely sensitive to NAD^+ bioavailability because intracellular NAMPT enriched in brown adipose tissue, heart, muscle, liver, and kidney is undetectable in the brain. Similarly, the pancreas has very low cellular levels of NAMPT and its cells' functioning is dependent on NAD^+ extracellular sources. It was recently shown that specific ablation of NAMPT in adult neural stem cells, recapitulated their aging functional defects (78). SIRTs activities are not know to oscillate like NAD^+'activities; however, via their modulation by cellular NAD^+ levels, nutritional perturbations could tick the SIRTs-dependent branch of the circadian clock towards accelerated aging.

Affinity for several NAD^+-derived metabolites like ADP-ribose, O-acetyl-ADP-ribose and poly-ADP-ribose has been demonstrated for 9 of the 16 macro domains found in mammalian proteins and especially into the chromatin histone H2A12 variant. MacroH2A1.1 binds O-acetyl-ADP-ribose. The family of macro domains includes some members with an enzymatic activity towards certain NAD^+ metabolites while others seem only binding modules. This suggests that MacroH2A could directly mediate transcriptional regulation in response to nutrient availability (80).

Shinichiro Imai is the father of the NAD World hypothesis postulated as a systemic regulatory network of metabolism and ageing (79). The latest research in association with this hypothesis provides convincing evidence for biochemical links of metabolism, biological rhythmicity and ageing in mammals. Metabolic regulation via epigenetic processes is the metabolism way to communicate with the transcriptional events in the nucleus (and vice versa).

Thus, the combination of SIRTs activation and NAD+ supplementation could be an effective anti-ageing intervention since modification of intracellular NAD metabolism may enhance the efficiency of this epigenetic therapy.

In eukaryotes, Nicotinamide riboside (NR) found in milk and yeast is used to synthesize NAD+ (81). NR supplementation in mammalian cells and mice increases NAD+ levels and activates SIRT1 and SIRT3 for enhanced oxidative metabolism and protection against high-fat diet induced metabolic abnormalities (82). In humans oral NAD+ can also significantly reduce actinic keratosis incidence (83). In humans, administration of NAM at supra physiological doses, raises intracellular NAD's level (84). In animals, NAM displays anti-inflammatory properties (85). In mammalian cells and tissues, NMN had the same effects as NR restoring higher levels of glucose-stimulated insulin secretion (86). Inhibition of NAMPT activity by FK866, a highly specific noncompetitive inhibitor of Nicotinamide Phosphoribosyl transferase, in rats did not affect the SIRT1 up regulation by CR but suppressed the CR-induced SIRT1 activity, attenuated the CR-induced SIRT3 activity, the CR-induced improvements of anti-oxidative activity, the mitochondrial biogenesis and the CR-induced decrease of oxidative stress. FK866 was able to block the CR-induced insulin sensitizing, Akt signaling activation, and endothelial nitric oxide synthase phosphorylation (87).

Chitooligosaccharide, a mixture of various lengths of glucosamine polymer, is an enzyme-digested product of chitosan which could activate Sirt1 through the elevation of the cellular NAD+/NADH ratio (88).

NAD deficiencies

Chronic reduced dietary intake of niacin causes pellagra, a skin inflammatory condition with sun sensitivity. Niacin is found in variety of foods, including liver, chicken, beef, fish, cereal, peanuts and legumes. Niacin is a precursor of NAD^+ and $NADP^+$. Both are cofactors for SIRTs and also for some

dehydrogenases. Alcohol abuse causes niacin deficiency with the development of fatty liver disease which is ameliorated by dietary NAD^+ supplementation in rats (89). Oxidation of ethanol and acetaldehyde by alcohol dehydrogenase alters the NADH/NAD^+ balance. In addition, chronic alcohol consumption interferes with the one-carbon (1C) metabolism and is causing disruption of DNA methylation by inhibiting methionine synthase, an enzyme that converts Homocysteine to methionine and leads to significant reduction in S-Adenosyl methionine (SAM) levels, thereby contributing to DNA hypo methylation (90).

Uncontrolled clinical studies have suggested that niacin therapy significantly reduces the risk of cardiovascular events in high risk subjects. Two clinical trials failed to confirm this benefit except for the familial hypercholesterolemia resistant to statin. However, the compliance to niacin is limited by its adverse effect: cutaneous flushing (reduced by niacin extended-release formulation). The use of niacin as a drug test evasion technique is highly championed on the Internet with documented acute hepatic toxicity in abusers (91).

We have illustrated the importance of NAD^+ for the functioning of the sirtuines. However other epigenetic enzymes consume metabolites such as SAM for DNA and histone methylation, ATP for histones phosphorylation, and acetyl-CoA for histones acetylation. Nutrients like Selenium, Folate, vitamin B-12, methionine, choline, and betaine can affect DNA and histone methylation through alteration of the 1-carbon metabolism. Citrate, a CTA metabolite, is necessary for histones and non-histones acetylation. Pantothenic acid is a part of CoA to form acetyl-CoA, which is the source of the needed acetyl group for histone acetylation. Biotin is a substrate of histone biotinylation.

Catabolites, but also onco-metabolites can inhibit epigenetic functioning. Mutations in genes encoding some TCA enzymes lead to the competitive inhibition of α-ketoglutarate–dependent KDMs and change the methylome of glioma (92).The world of the old metabolism players and its producing enzymes is opening again with the further complexity that all have genetic variants with different catalytic activities that renders our understanding of such matters difficult.

The path towards an epigenetic diet: the edible phytochemicals!

Epidemiologic and Intervention studies in humans and animals have found a positive association between diets rich in plants and protection against several diseases. In 128 out of 156 dietary studies, the consumption of fruit and vegetables was shown to protect against cancer development (93). Dietary flavonoid intake was significantly linked to reduced cardio vascular diseases (94). However the declarative nature of the nutrition facts complexifies a full apprehension of a told nutrition.

The Mediterranean diet is an Intangible Cultural Heritage of Spain, Morocco, Italy, Greece, Portugal, Cyprus and Croatia (http://www.wikipci.fr/page/Accueil). The Mediterranean diet is characterized by high consumption of olive oil, legumes, unrefined cereals, fruits, and vegetables, moderate to high consumption of fish, moderate consumption of wine and dairy products (mostly as cheese and yogurt), and low consumption of meat. Together they prevent MS (95). We will describe only some of the best known potential edible phytochemicals that could enter an 'epinutricure'.

After ingestion, edible phytochemicals absorption is a function of the associated food, the digestives enzymes and the intestinal degradation by the resident bacterial flora whose role is just beginning to be apprehended. Because they are further extensively metabolized, *in vivo* bioactive molecules are not those originally present in the food. In addition, the concentrations used for *in vitro* screening are not physiological doses. This limits the impact of the dose/effect relationship evidences obtained from *in vitro* assays. Finally, the obtained results may vary with the animal models used.

Fruits and vegetables consumption decreases mortality, cardiovascular diseases and cancers, but maybe not as antioxidants. For example, turmeric, soybeans, green tea, grapes, and cruciferous vegetables, have all been shown to possess polyphenols (PP), which can affect the epigenome. We report some of them in figure 3 and Table 3.

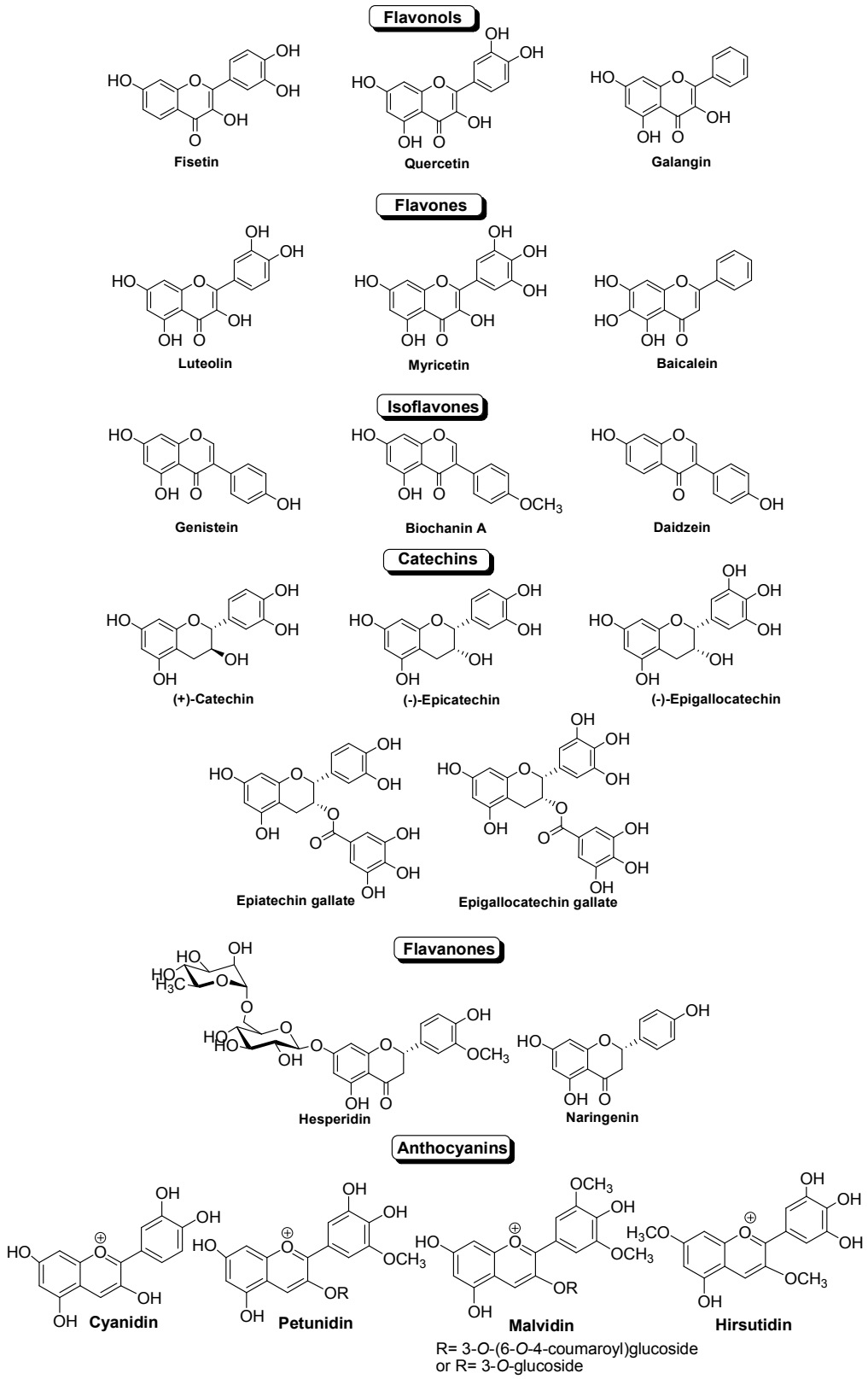

Figure 3. Chemical structures of different classes of polyphenols.

Plants PP are ultraviolet protectants, antioxidants and pest and insects dissuaders. The classification of plant PP is based on (i) the number of phenolic or benzene rings present and (ii) the

number and type of substituents present on the phenolic rings. The main classes include phenolic acids, flavonoids, stilbenes and lignans. Figure 3 illustrates the different groups of PP and their chemical structures. Phenolic acids are found abundantly in foods and divided into two classes: derivatives of benzoic acid and derivatives of cinnamic acid. The hydroxycinnamic acids are more common than the hydroxybenzoic acids and consist chiefly of *p*-coumaric, caffeic, ferulic and sinapic acids. Favonoids comprise the most studied group of PP. This group has a common basic structure consisting of two aromatic rings bound together by three carbon atoms that form an oxygenated heterocycle. Based on the variation in the type of heterocycle involved, flavonoids may be divided into six subclasses: flavonols, flavones, flavanones, flavanols, anthocyanins and isoflavones. Quercetin, myricetin and catechin are some of the most common flavonoids. Stilbenes contain two phenyl moieties connected by a two-carbon methylene bridge. One of the best studied, naturally occurring PP stilbene, is RSV.

Lignans are diphenolic compounds that contain a 2, 3-dibenzylbutane structure that is formed by the dimerization of two cinnamic acid residues. Several lignans, such as secoisolariciresinol, are considered to be phytoestrogens. The richest dietary source is linseed, which contains secoisolariciresinol (up to 3.7 g/kg dry weight) and low quantities of matairesinol (96).

The majority of PP in plants are polymers or are conjugated to sugars, the ‘‘glycone’’ part while the PP part is called the ‘‘aglycone’’. The aglycone is not normally found free in food; however, fermentation processing can increase its level (miso soup). Most PPs are O-glycosides, but some are C-glycosides. O but not C glucosides undergo hydrolysis in the lumen of the intestine, and the sugar residue is released upon the action of glucosidase from resident bacteria, setting the aglycone free. Flavonols and flavones occur in food usually as O-glycosides (97) whereas in vegetables quercetin glycosides predominate. D-glucose is the most usual sugar residue but other substitutions include arabinose, galactose, glucorhamnose, lignin, I-rhamnose, and xylose 4 (98).

Bacteria in the large intestine catalyze the conversion of aglycone by opening the heterocyclic B-ring and further cleavage gives absorbable metabolites which reach the circulation (99). The nature of the resident microbiome is therefore of paramount importance to ingest PPs.

The liver also metabolizes PPs, and circulating PPs are in the form of glucuronides and esters of sulfate with trace levels of aglycone. Most PPs absorption happens in the small intestine with further absorption occurring in the colon through both active and passive diffusions. Passive diffusion appears to contribute to the absorption of flavonoids with high log *P* values such as isoflavones and flavonones but it contributes little to those with a low log *P* value such as flavan3-ols (100). After their uptake by the intestinal cells, the PP, undergo metabolic biotransformation similar to the xenobiotics with the involvement of phase-I and phase-II enzymes (101). More than 4,000 flavonoids have been identified to date. Flavan-3-ols (e.g., catechin, epicatchin, epigallocatechin, epicatechin gallate, and apigallocatechin gallate), predominate in fruits and vegetables with quercetin glycosides being the most abundant in the diet (102).Non-flavonoid compounds contain an aromatic ring with one or more hydroxyl groups. This group includes stilben (e.g., resveratrol), phenolic acids (e.g., gallic acid), saponin (e.g., ginsenoside), and other polyphenols like curcumine and proanthocyanidin (or tannins) polymers of flavan-3-ols.

Data on the bio transformations and pharmacokinetics of dietary PPs were collected by J.A Rothwell et al and further stored in a publicly available web database. The Phenol-Explorer database is available online at http://www.phenol-explorer.eu. The oral delivery of flavonoids can be markedly improved by incorporation into lipidic or polymer-based nanoparticles as they can protect the molecule from degradation into the gastrointestinal tract or from the first liver bypass.

As said before, the importance of the digestive tract microbiome is beginning to be explored and for example, in laying hens, quercetin was found able to change the intestinal microbiome. This is an explored pathway to reduce the use of antibiotics in the poultry business (88, 89 and 90). Likewise transgenic fruits overproducing PPs are sought after.

On table 3 are mentioned some reported epigenetic PPs, this is not an exhaustive listing. Individual activities are described in the literature (103-133).

It is the same in table 4 which is not an exhaustive listing of all dietary interventions being actually assayed.

Conclusions

To prevent, the onset of cancer, The EU health authorities like the National Institutes of Health in the United States recommended a high fiber, low fat diet, consisting of more fruits and vegetables.

The question of the origin of edibles is important. Indeed vinclozolin a dicarboximide fungicide is an endocrine disruptor with anti-androgenic effects promoting imprinted gene DNA methylation changes in animal models. It is used against the pest of fruits. Like other environmental toxicants such as phtalate, fuel and dioxin, this pesticide may contaminate fruits and vegetables to promote diseases. It was recently not found in EU baby formula but genistein another undisputed endocrine disruptor in women was detected there.(111).

The presence of PPs in the diet is beneficial to health due to their antioxidant, anti-inflammatory, and vaso dilatators properties. In addition, to give to food taste and color; some have significant effects on the colonic flora providing a prebiotic effect. Even though the healthy properties of functional foods and fasting regimens still need to be fully elucidated, available data suggest that evaluated supplements, specific probiotic strains, and selected PPs as prebiotics, could be useful in MS prevention and thus promise a healthy ageing because they have also wanted epigenetic effects.

Compounds	Plant sources	Validated Target(s)	*In vitro* model	*In vivo* models	*In vitro* activities
Anacardic acid	Cashew nuts	P300, PCAF, & Tip 60	Lung melanoma	Mice skin (	HATi
Baicalein	Scutellaria baicalensis Georgia		Breast	Mice	DNMTi
Anthocyanidine	Fruit red pigments Black berries		Colon	Human colon cancer Sedentary humans	DNMTi
Brazilin	Caesalpinia sappan		U266 Cells		HDACi
Biochanin A	Soy, red clover, peanuts & peas	P16, RARβ & MGMT	Esophageal Prostate	Daphnids	DNMTi
Caffeic acid	Coffee artichoke	RARβ,&P16	Breast		DNMTi (Comt)
Catechin	All teas, cacoa &	RARβ	Breast Prostate		DNMTi

	grapes				
Chlorogenic acid	Coffee	RARβ&P16	Breast		DNMTi (Comt)
Chalcones	Apples & plants		Breast		HDACi
Coumaric acid	Cinnamon		Esophageal	Mice	DNMTi SIRT1/2i
Curcumin	Turmeric & mustard	H3 P300	Esophageal Leukemia Breast colon	Diabetic rats Prostate	DNMTi, HATi KDMi, HDACDi
Cyanidin	Berries & grapes		Breast		DNMTi
Daidzein and Equol	Soy	P16, RARβ & MGMT	Esophageal Prostate	Mice	DNMTi
Ellagic acids	Strawberries, Walnuts & cranberries		Breast		DNMTi
Epicatechin	Green tea, apples, grapes, pears & chocolate		Esophageal Breast	Diabetic mices	DNMTi
Epicatechin gallate	Green tea	P16, RARβ,MGMT,HMLH1	Esophageal .		DNMTi (COMT)
Epigallocatechin	Green tea		Esophageal		DNMTi
Epigalocatechin-3-gallate	Green tea	P16, RARβ, MGMT, HMLH1* RECK* E-cadherin** PRC2 and PRC1	Esophageal & *Oral Prostate & Urinary Lung & Colon Leukemia, skin and Lymphoma **Breast .	Agouti mouse, Mouse models for: skin, prostate, colon & uterus cancers Human: gastric & oral cancers, Premenopausal women	DNMTi ++* HATi** KDMi
Fisetin	Poison ivy, fruit pigment, grapes, onions & chinese lacquer		Esophageal Breast		DNMTi SIRTa
Emodin	Rhubarb	H3Ser10, K27			HDACi

Galangin	Galangal root & propolis		Breast		DNMTi
Gallic acid	Mango, blackberry, tea & nuts	Histones			HATi
Hesperidin	Citrus		Esophageal		DNMTi
Genistein	Soy, Red clovers & peanuts	RARβ, MGMT, P16, GSTP1, HMGN5, BTG3 & TERT and ER	Esophageal Prostate	Daphnids, Agouti mice Women	DNMTi HDACi HATa
Luteolin	Parsley & celery, beets, artichoke & celery		Esophageal cervix		DNMTi SIRTa
lycopen	Tomato & fruits	RARβ & GSTP	breast		DNMTi
Myricetin	Berries, nuts & grapes		Esophageal Breast		DNMTi
Naringenin	Citrus		Esophageal		DNMTi
Phloretin	Apples		Breast		DNMTi
Polyvanilic acid	Fagara	Histones			HDACi
Piceatannol	Grapes blueberries		cervix	Drosophilia	DNMTi SIRTa
Plumbagine	Plumbago	Histones			HATi
Protocatechuric acid	Olives & tea		Breast		DNMTi
Quercetin	Citrus, tea, grapes, apples & buckwheat	P16	Esophageal Breast Colon	Drosophilia Rats Mice	DNMTi SIRTa HATi
Resveratrol	Grapes, wines, nuts, cocoa & eucalyptus		Breast Lung	Humans, mice (26-27)	SIRTa SIRTi
Rosmarinic acid	Rosemary, Laemicae, oregano, sage, peppermint & thyme	RASF1 & GSTP1	Breast		DNMTi
Sinapic acid	Sinapis		Breast		DNMTi

	(mustard)				HDACi
Syringic acid	Red grapes		Breast		DNMTi

Table 3: Examples of nutrients which can affect directly the epigenetic enzymes (**103-134**).

(I stands for inhibitors and a for activators). BTG3: B cell translocation gene, GSTP1: Glutathione S-Transferase pi, HMGN5: high mobility group nucleosome binding domain 5, HMLH1: human mutL homolog 1, human mutL homolog 1 MGMT: O 6 methyl guanine transferase, NFKb: nuclear factor kappa b, P16: or CDK2: cyclin-dependent kinase inhibitor 2A, RARβ: Retinoic Receptor beta, RECK: reversion-Inducing Cysteine Rich protein with Kazam motif, TERT: Telomerase reverse transcriptase.

Chemoprevention of Prostate Cancer, HDAC Inhibition and DNA Methylation. The objective of the study is to identify mechanisms by which compounds found in cruciferous vegetables alter gene expression via epigenetic modifications and may prevent prostate cancer development (NCT01265953T).

Pomegranate-Extract Pill in Preventing Tumor Growth in Patients With Localized Prostate Cancer Undergoing Active Surveillance. This randomized phase II trial studies pomegranate-extract pill in preventing tumor growth in patients with prostate cancer that is limited to a certain part of the body. (NCT02095145).

Effect of Quercetin on Green Tea Polyphenol Uptake in Prostate Tissue From Patients With Prostate Cancer Undergoing Surgery. This randomized pilot phase I trial will evaluate if quercetin enhances the uptake of green tea polyphenols in the prostate tissue of men taking green tea extract and undergoing radical prostatectomy (NCT01912820).

Green Tea Extract in Treating Patients With Low-Risk Prostate Cancer. This randomized phase II trial studies how well green tea extract works in treating patients with low-risk prostate cancer (NCT01928485).

Polyphenol E in Treating Patients With High-Risk of Colorectal Cancer. This phase II trial studies how well Polyphenol E works in treating patients with high-risk of colorectal cancer (NCT01606124).

Curcumin in Treating Patients With Familial Adenomatous Polyposis. This clinical trial studies curcumin in treating patients with familial adenomatous polyposis (NCT00641147).

Curcumin Biomarker Trial in Head and Neck Cancer. This study examines the short-term effects of supplementation with a turmeric extract, Curcumin C3 Complex®, on biomarkers of head and neck squamous cell carcinoma (NCT01160302).

Curcumin and Cholecalciferol in Treating Patients With Previously Untreated Stage 0-II Chronic Lymphocytic Leukemia or Small Lymphocytic Lymphoma. This phase II trial studies the efficacy (activity), and tolerability of curcumin and cholecalciferol combination in treating patients with previously untreated stage 0-II chronic lymphocytic leukemia or small lymphocytic lymphoma (NCT02100423).

Broccoli Sprout Extract in Treating Patients With Estrogen Receptor-Positive Breast Cancer. This randomized pilot trial studies broccoli sprout extract in treating patients with estrogen receptor-positive breast cancer (NCT01753908).

Cruciferous Vegetable Intake and Histone Status in Screening Colonoscopy Patients. This research study will assess cruciferous vegetable intake in patients presenting for screening colonoscopy and correlate intake with histone status and histone deacetylase (HDAC) expression in tissue biopsy

specimens and peripheral blood mononuclear cells (PBMCs) (NCT01344330).

Diet in Altering Disease Progression in Patients With Prostate Cancer on Active Surveillance. This randomized clinical trial is studying how well diet works in altering disease progression in patients with prostate cancer on active surveillance (NCT01238172).

Protein-Sparing Modified Fast Intervention for Weight Loss in Obese Endometrial Cancer Survivors. This pilot clinical trial studies protein-sparing modified fast (PSMF) intervention for weight loss in obese endometrial cancer survivors. The PSMF is a diet that is very low in carbohydrates and calories, designed to induce fast, safe weight loss (NCT02135562).

FLAX FX, A Research Study of the Effects of Flaxseed Lignans on Colon Health. Gut bacteria can convert the lignans into biologically active compounds that in animal models prevent the development of colon cancer. The investigators will study how these biologically active compounds affect colon cell-signaling pathways important to colorectal cancer risk (NCT01619020).

Short-Term Fasting Before Chemotherapy in Treating Patients With Cancer. This clinical trial studies short-term fasting before chemotherapy in treating patients with cancer (NCT01175837).

Ketogenic Diet With Concurrent Chemoradiation for Pancreatic Cancer. This study investigates if using a very low carbohydrate diet during combined chemotherapy and radiation therapy is safe and if it can be tolerated by patients NCT01419483.

Ketogenic Diet With Chemoradiation for Lung Cancer (KETOLUNG) This study investigates if using a very low carbohydrate diet during combined chemotherapy and radiation therapy is safe and if it can be tolerated by lung cancer patients: NCT01419587.

Ketogenic Diet Phase 1 for Head & Neck Cancer This study investigates if using a very low carbohydrate diet during combined chemotherapy and radiation therapy is safe and if it can be tolerated by patients : NCT01975766.

Low-Fat Diet and Fish Oil in Men on Active Surveillance for Prostate Cancer This randomized phase II trial will evaluate if a low-fat diet with fish oil has the potential to delay disease progression in patients with prostate cancer undergoing active surveillance: NCT02176902.

Table 4. Selected clinical trials of edible phytochemical or regimens with epigenetic activities. The ID trial begins with NCT. Details can be found at: http://www.cancer.gov/clinicaltrials.

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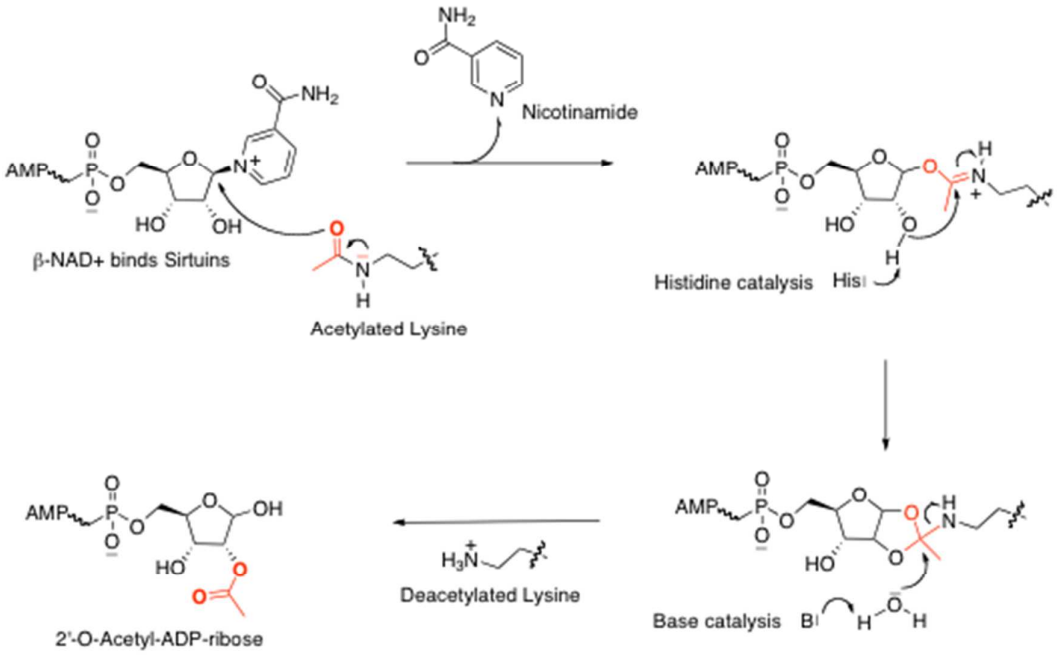

Figure 1: Proposed desacetylation by SIRTs consumes NAD^+ and involves an acetylated protein substrate to produce Nicotinamide (NAM) and 2' O-acetyl-adenosine diphosphate ribose in stoichiometric amounts and, the deacetylated substrate. NAM acts as an inhibitor of the reaction, and thus provides negative feedback inhibition of the SIRTs *in vivo*.

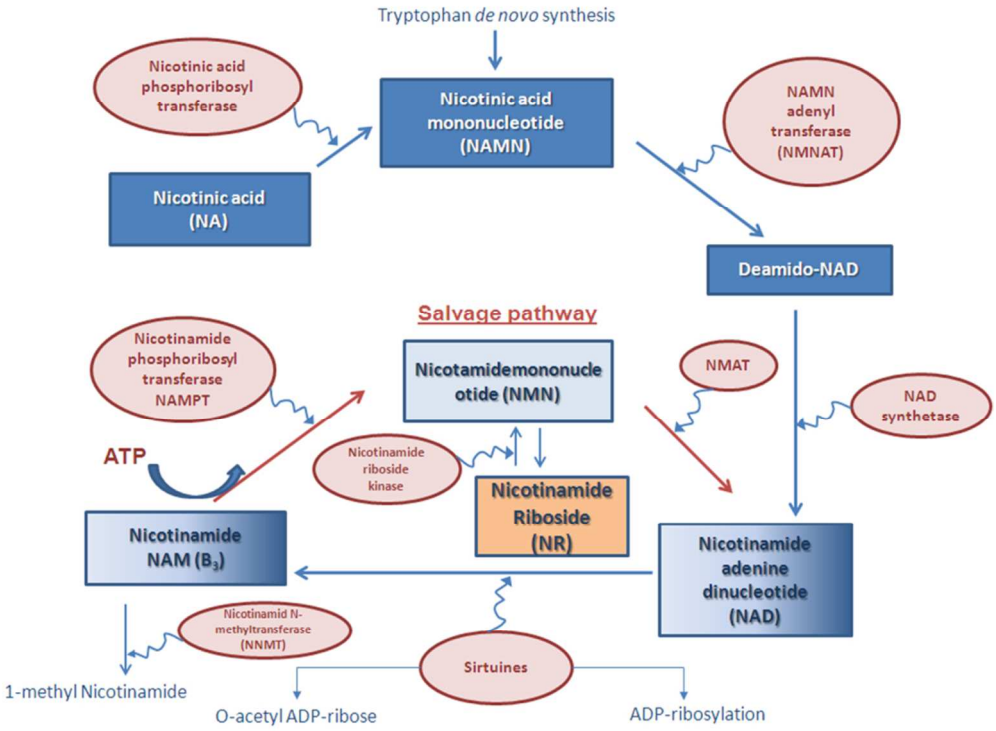

figure 2

Enzymes	Added/removed marks	Co-factors *and metabolism*	Inhibitors	Roles
DNMT1, 2 and 3a/b	Methyl group at C5 C	SAM *(1 C metabolism)*	Nucleosides Non nucleosides	Repression of gene expression (1-2)
DNMT4	Methyl group at C5 C	SAM *(1 C metabolism)*		RNA methylation
KMT1:A-F, KMT2: A-H, KMT3: A-G, KMT4, 6-7, KMT5: A-C KMT8: A-C	1 to 3 methyl groups on histone (H) lysine (K)	SAM *(1 C metabolism)*	SAM derivatives Lysine mimics	H3K4, 36 and 79 for gene activation H3K9 and 27 and H4K20 for gene repression (3)
PRMTs Type I: PRMT1, 3, 6,8 and CARM1 Type II: PRMT5, FBXO11 and HsI7 Type II/III: PRMT7 Type IV: PRMT2	Add 1 or 2 methyl groups on histone arginine (R) (symmetric or not)	SAM *(1 C metabolism)*	Methylated arginine Asymmetrical urea	Modulate R hydrogen bonding capacity and recognition of R methylation by Tudor-domain containing proteins. Activate or repress gene expression (4)
HDMs KDM1: A-B KDM2: A-B KDM3: A-C KDM4: A-D KDM5: A-D KDM6: A-B KDM7: A-C and **KDM8**	Remove 1 to 3 methyl groups on histone Nε-lysine (depending on specificity)	α-Ketoglutarate or Fe (II) or Flavin (*Oncometabolites inhibitors: succinate and 2-hydroxyglutarate*)	Alkynes Cyclopropylamines	Positive or negative modulation of gene expression depending on partners (5)
HATs GCN5/PCAF, MYST including MOZ, Ybf2/Sas3 and Tip 60, p300/CBP and Rtt109	Acetyl group on histone Nε-lysine and non-histone Nε-lysine	Acetyl coenzyme A (acetyl-CoA) *(Citric and pantothenic acids)*	Anacardic acid Acetyl CoA mimics	Type A HATs acetylates nucleosome histones. HAT1 acetylates nascent histones in the cytoplasm during the process of chromatin assembly. Gene activation (6)
HDACs Class I: HDAC1–3, and **8 Class II: HDAC4–7** and **9–10 Class IV: HDAC11**	Remove acetyl group from histone and non-histone Nε-lysine	Zinc and water	Short-chain carboxylic acids Hydroxamic acids Benzamides Sulphuryls Cyclic peptides ketones	Gene repression (7)
Sirtuines or class III HDACs 1 to 7	Remove acetyl group from histone and non-histone Nε-lysine	NAD (*Niacin*)	NAD mimics and peptidomimetics	Gene repression (also transfer of ADP-ribose to protein) (8)

Table 1: Main epigenetic enzymes: marks, co-factors, inhibitors and effects

Name	Molecular Weight	Null mice Features	Localization	Activity	Functions
SIRT1	81.7 kDa	-/-: Perinatal deaths, abnormalities of the retina, heart and bones +/-mice have reduced plasma HDL-cholesterol and triglycerides when fed a normal diet	Nucleus and cytosol	Deacetylase	Metabolism adaptation, inflammation and cellular senescence (8-60)
SIRT2	43.2 kDa	Spontaneous tumor formation	Cytosol	Deacetylase	Apoptosis, motility and myelination (8-60)
SIRT3	43.6 kDa	Changed fatty oxidation, ATP levels and acetylated mitochondrial proteins levels Defect in the urea cycle and cardiac hypertrophy	Mitochondria	Deacetylase	Metabolism adaptation to fasting (8-61)
SIRT4	35.2 kDa	No physical abnormalities *but spontaneous Lung tumors*, increased mitochondrial glutamate dehydrogenase activity	Mitochondria	ADP-ribosyl transferase	Secretion of insulin and suppression of fatty acid oxidation and glutamine use (8-60)
SIRT5	33.9 kDa	No physical abnormalities, hyper ammonia during fasting or a high protein diet	Mitochondria	Deacylase Demalonylase Desuccinylase	Malfunction of urea cycle (8-62)
SIRT6	39.1 kDa	Premature female aging (Progeria like) and death. Low levels of insulin-like growth factor-1 and circulating glucose.	Nucleus	Deacetylase ADP-ribosyl transferase	Defects in the excision/repair system and genomic instability (8-63)
SIRT7	44.8 kDa	Cardiac hypertrophy and inflammation and premature death	Nucleolus	Deacetylase?	Enhances ribosomal RNA. Transcription (8-60)

Table 2: The sirtuines characteristics and roles

Compounds	Plant sources	Validated Target(s)	*In vitro* model	*In vivo* models	*In vitro* activities
Anacardic acid	Cashew nuts	P300, PCAF, & Tip 60	Lung melanoma	Mice skin (	HATi
Baicalein	Scutellaria baicalensis Georgia		Breast	Mice	DNMTi
Anthocyanidine	Fruit red pigments Black berries		Colon	Human colon cancer Sedentary humans	DNMTi
Brazilin	Caesalpinia sappan		U266 Cells		HDACi
Biochanin A	Soy, red clover, peanuts & peas	P16, RARβ & MGMT	Esophageal Prostate	Daphnids	DNMTi
Caffeic acid	Coffee artichoke	RARβ,&P16	Breast		DNMTi (Comt)
Catechin	All teas, cacoa & grapes	RARβ	Breast Prostate		DNMTi
Chlorogenic acid	Coffee	RARβ&P16	Breast		DNMTi (Comt)
Chalcones	Apples & plants		Breast		HDACi
Coumaric acid	Cinnamon		Esophageal	Mice	DNMTi SIRT1/2i
Curcumin	Turmeric & mustard	H3 P300	Esophageal Leukemia Breast colon	Diabetic rats Prostate	DNMTi, HATi KDMi, HDACDi
Cyanidin	Berries & grapes		Breast		DNMTi
Daidzein and Equol	Soy	P16, RARβ & MGMT	Esophageal Prostate	Mice	DNMTi
Ellagic acids	Strawberries, Walnuts & cranberries		Breast		DNMTi
Epicatechin	Green tea, apples,		Esophageal Breast	Diabetic mices	DNMTi

	grapes, pears & chocolate				
Epicatechin gallate	Green tea	P16, RARβ,MGMT,HMLH1	Esophageal .		DNMTi (COMT)
Epigallocatechin	Green tea		Esophageal		DNMTi
Epigalocatechin-3-gallate	Green tea	P16, RARβ, MGMT, HMLH1* RECK* E-cadherin** PRC2 and PRC1	Esophageal & *Oral Prostate & Urinary Lung & Colon Leukemia, skin and Lymphoma **Breast .	Agouti mouse, Mouse models for: skin, prostate, colon & uterus cancers Human: gastric & oral cancers, Premenopausal women	DNMTi ++* HATi** KDMi
Fisetin	Poison ivy, fruit pigment, grapes, onions & chinese lacquer		Esophageal Breast		DNMTi SIRTa
Emodin	Rhubarb	H3Ser10, K27			HDACi
Galangin	Galangal root & propolis		Breast		DNMTi
Gallic acid	Mango, blackberry, tea & nuts	Histones			HATi
Hesperidin	Citrus		Esophageal		DNMTi
Genistein	Soy, Red clovers & peanuts	RARβ, MGMT, P16, GSTP1, HMGN5, BTG3 & TERT and ER	Esophageal Prostate	Daphnids, Agouti mice Women	DNMTi HDACi HATa
Luteolin	Parsley & celery, beets, artichoke & celery		Esophageal cervix		DNMTi SIRTa
lycopen	Tomato & fruits	RARβ & GSTP	breast		DNMTi
Myricetin	Berries, nuts & grapes		Esophageal Breast		DNMTi
Naringenin	Citrus		Esophageal		DNMTi

Phloretin	Apples		Breast		DNMTi
Polyvanilic acid	Fagara	Histones			HDACi
Piceatannol	Grapes blueberries		cervix	Drosophilia	DNMTi SIRTa
Plumbagine	Plumbago	Histones			HATi
Protocatechuric acid	Olives & tea		Breast		DNMTi
Quercetin	Citrus, tea, grapes, apples & buckwheat	P16	Esophageal Breast Colon	Drosophilia Rats Mice	DNMTi SIRTa HATi
Resveratrol	Grapes, wines, nuts, cocoa & eucalyptus		Breast Lung	Humans, mice (26-27)	SIRTa SIRTi
Rosmarinic acid	Rosemary, Laemicae, oregano, sage, peppermint & thyme	RASF1 & GSTP1	Breast		DNMTi
Sinapic acid	Sinapis (mustard)		Breast		DNMTi HDACi
Syringic acid	Red grapes		Breast		DNMTi

BTG3: B cell translocation gene, GSTP1: Glutathione S-Transferase pi, HMGN5: high mobility group nucleosome binding domain 5, HMLH1: human mutL homolog 1, MGMT: O 6 methyl guanine transferase, NFKb: nuclear factor kappa b, P16: or CDK2: cyclin-dependent kinase inhibitor 2A, RARβ: Retinoic Receptor beta, RECK: reversion-Inducing Cysteine Rich protein with Kazam motif, TERT: Telomerase reverse transcriptase.

Table 3: Examples of nutrients which can affect directly the epigenetic enzymes (**103-134**).

(I stands for inhibitors and a for activators)

Chemoprevention of Prostate Cancer, HDAC Inhibition and DNA Methylation. The objective of the study is to identify mechanisms by which compounds found in cruciferous vegetables alter gene expression via epigenetic modifications and may prevent prostate cancer development (NCT01265953T).

Pomegranate-Extract Pill in Preventing Tumor Growth in Patients With Localized Prostate Cancer Undergoing Active Surveillance. This randomized phase II trial studies pomegranate-extract pill in preventing tumor growth in patients with prostate cancer that is limited to a certain part of the body. (NCT02095145).

Effect of Quercetin on Green Tea Polyphenol Uptake in Prostate Tissue From Patients With Prostate Cancer Undergoing Surgery. This randomized pilot phase I trial will evaluate if quercetin enhances the uptake of green tea polyphenols in the prostate tissue of men taking green tea extract and undergoing radical prostatectomy (NCT01912820).

Green Tea Extract in Treating Patients With Low-Risk Prostate Cancer. This randomized phase II trial studies how well green tea extract works in treating patients with low-risk prostate cancer (NCT01928485).

Polyphenol E in Treating Patients With High-Risk of Colorectal Cancer. This phase II trial studies how well Polyphenol E works in treating patients with high-risk of colorectal cancer (NCT01606124).

Curcumin in Treating Patients With Familial Adenomatous Polyposis. This clinical trial studies curcumin in treating patients with familial adenomatous polyposis (NCT00641147).

Curcumin Biomarker Trial in Head and Neck Cancer. This study examines the short-term effects of supplementation with a turmeric extract, Curcumin C3 Complex®, on biomarkers of head and neck squamous cell carcinoma (NCT01160302).

Curcumin and Cholecalciferol in Treating Patients With Previously Untreated Stage 0-II Chronic Lymphocytic Leukemia or Small Lymphocytic Lymphoma. This phase II trial studies the efficacy (activity), and tolerability of curcumin and cholecalciferol combination in treating patients with previously untreated stage 0-II chronic lymphocytic leukemia or small lymphocytic lymphoma (NCT02100423).

Broccoli Sprout Extract in Treating Patients With Estrogen Receptor-Positive Breast Cancer. This randomized pilot trial studies broccoli sprout extract in treating patients with estrogen receptor-positive breast cancer (NCT01753908).

Cruciferous Vegetable Intake and Histone Status in Screening Colonoscopy Patients. This research study will assess cruciferous vegetable intake in patients presenting for screening colonoscopy and correlate intake with histone status and histone deacetylase (HDAC) expression in tissue biopsy specimens and peripheral blood mononuclear cells (PBMCs) (NCT01344330).

Diet in Altering Disease Progression in Patients With Prostate Cancer on Active Surveillance. This randomized clinical trial is studying how well diet works in altering disease progression in patients with prostate cancer on active surveillance (NCT01238172).

Protein-Sparing Modified Fast Intervention for Weight Loss in Obese Endometrial Cancer Survivors. This pilot clinical trial studies protein-sparing modified fast (PSMF) intervention for weight loss in obese endometrial cancer survivors. The PSMF is a diet that is very low in carbohydrates and calories,

designed to induce fast, safe weight loss (NCT02135562).

FLAX FX, A Research Study of the Effects of Flaxseed Lignans on Colon Health. Gut bacteria can convert the lignans into biologically active compounds that in animal models prevent the development of colon cancer. The investigators will study how these biologically active compounds affect colon cell-signaling pathways important to colorectal cancer risk (NCT01619020).

Short-Term Fasting Before Chemotherapy in Treating Patients With Cancer. This clinical trial studies short-term fasting before chemotherapy in treating patients with cancer (NCT01175837).

Ketogenic Diet With Concurrent Chemoradiation for Pancreatic Cancer. This study investigates if using a very low carbohydrate diet during combined chemotherapy and radiation therapy is safe and if it can be tolerated by patients NCT01419483.

Ketogenic Diet With Chemoradiation for Lung Cancer (KETOLUNG) This study investigates if using a very low carbohydrate diet during combined chemotherapy and radiation therapy is safe and if it can be tolerated by lung cancer patients: NCT01419587.

Ketogenic Diet Phase 1 for Head & Neck Cancer This study investigates if using a very low carbohydrate diet during combined chemotherapy and radiation therapy is safe and if it can be tolerated by patients : NCT01975766.

Low-Fat Diet and Fish Oil in Men on Active Surveillance for Prostate Cancer This randomized phase II trial will evaluate if a low-fat diet with fish oil has the potential to delay disease progression in patients with prostate cancer undergoing active surveillance: NCT02176902.

Table 4. Selected clinical trials of edible phytochemical or regimens with epigenetic activities. The ID trial begins with NCT. Details can be found at: http://www.cancer.gov/clinicaltrials.

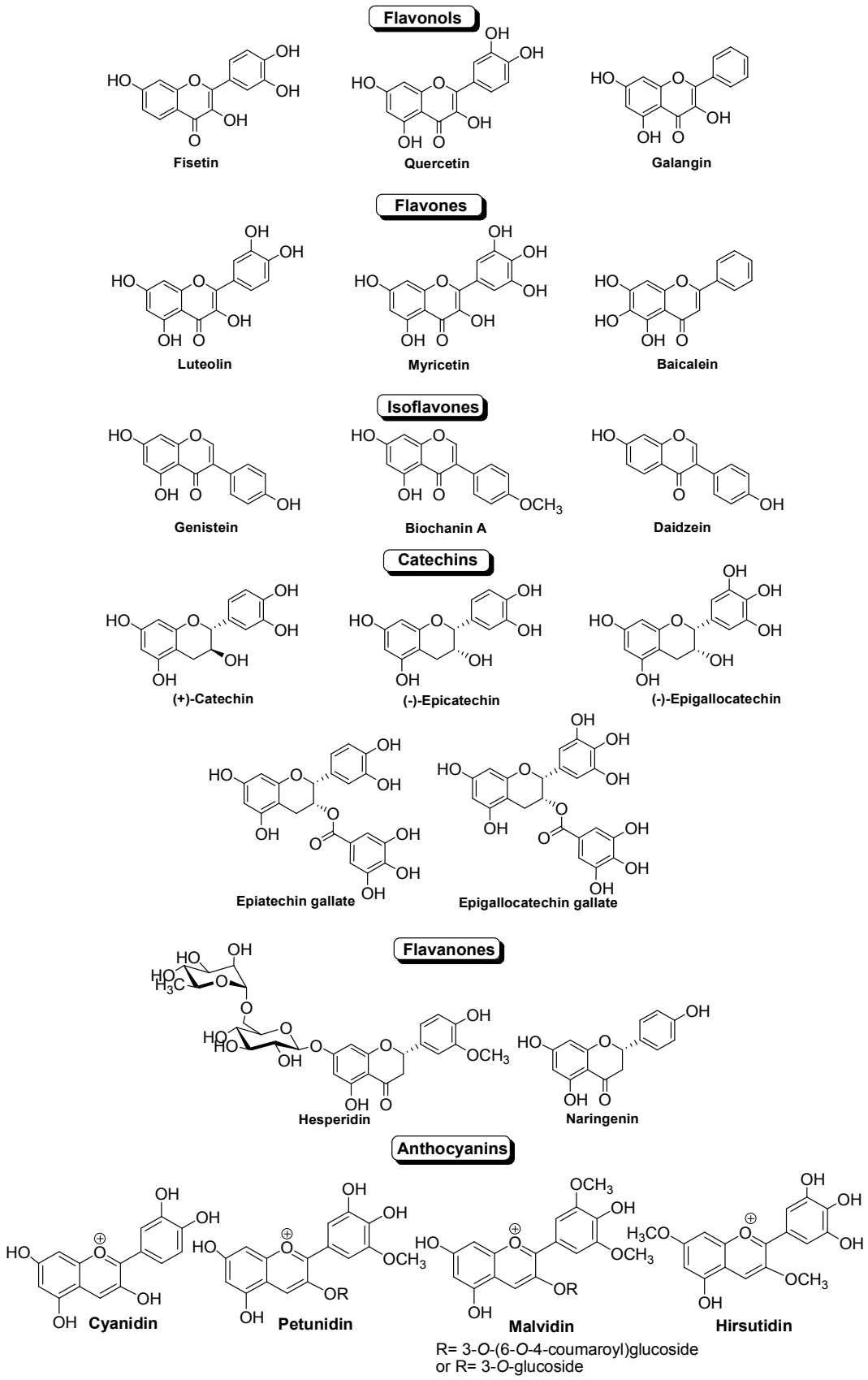

Figure 3. Chemical structures of different classes of polyphenols.