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Proanthocyanidins in health and disease

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Abstract

Proanthocyanidins (PAs) are the most abundant flavonoids in the human diet. Several epidemiological studies connect PA consumption and health benefits and the designation of PAs as healthy compounds started at the early stages of the twentieth century. The beneficial health properties of PAs are attributed to their conjugated and colonic metabolites. Therefore, gut microbial compositions can determine the effectiveness of PAs. Reciprocally, dietary polyphenols can act as prebiotics. Recently, it has also been described that PAs modulate the circadian rhythm. Biochemical and epigenetic mechanisms, including the modulation of microRNAs, allow PAs to modulate cell functionality. PA effects in metabolic diseases are also reviewed.

Key words: polyphenols, flavonoids, molecular mechanisms, metabolic diseases, microbiota, circadian rhythms

1. Historical view

Proanthocyanidins (PAs), also known as condensed tannins, are the most abundant flavonoids in the Western diet. They are a group of phenolic compounds that have received increasing attention in recent years due to the high number of beneficial properties attributed to their consumption. Currently, these compounds may be considered trendy, as confirmed by the continuously increasing number of functional foods incorporating them in their formulation to claim beneficial effects. However, to find the origins of research on this family of compounds, we have to look back almost one hundred years ago, to the early stages of the last century [(1)].

One of the most important scientists of the Twentieth Century, Albert Szent-Györgyi, awarded with a Nobel Prize in physiology and medicine in 1937, discovered flavonoids while looking for a cure for bleeding gums [(2)]. He prepared an extract from lemon fruits to help a friend who had this problem; after the extract was consumed the friend improved. Szent-Györgyi first attributed this effect to vitamin C (also discovered by Szent-Györgyi) but when the problem returned and he gave his friend a purified version of the same extract that was richer in vitamin C, the condition did not improve. Based on these results, Szent-Györgyi hypothesized that something required for the beneficial effect of the previous extract had been removed during the purification process. It was only when he treated his friend with an isolated fraction containing a mixture of flavonoids that the condition was resolved. He isolated them and, in light of the results, stated that these compounds were essential to avoid certain healthy problems. Thus, he baptized them under the denomination of "vitamin P" due to their effects on permeability by promoting good blood circulation in capillaries and thereby preventing petechia (bleeding and bruising caused by the breakdown of capillaries) [(3)]. However, flavonoids were not classified as vitamin for very long as further research did not demonstrate that these compounds were essential for the health of organisms; they were subsequently removed from the list of vitamins.

Although Szent-Györgyi never gave up his interest in these compounds, it was not until the studies by Maschelier that PAs received attention again. Following the diaries of an ancient explorer (Jacques Cartier) in the 1950's, Maschelier began to study an extract from pine tree bark in attempt to isolate bioactive compounds, which turned out to be oligomeric PAs complexes (OPC). Among the beneficial properties exerted by these compounds were strengthened capillaries and a reduction of swelling in the legs and ankles. Later, in 1970, he

obtained an extract with a similar composition from grape seeds; since this work was published, grape seeds have been considered the best source for these compounds for research[4].

The acceptance of PAs as healthy compounds resulted from the combination of the research carried out by scientist using this family of compounds and some epidemiological studies that linked the consumption of these molecules with a reduced occurrence of some diseases such as hypertension and cardiovascular disease (CVD). One of the most important of these was the study carried out by French epidemiologists in the 1980s which gave origin to the so called French paradox [5]. The conclusion from the study was that low CVD death rates are declared in France, despite the high intake of dietary cholesterol and saturated fat. After analyzing the nutritional pattern of the French, it was observed that a higher consumption of red wine may be the responsible for the low number of CVD diseases in the population. This study boosted the popularity of PAs which are present in high concentration in red wine.

Another important epidemiologic study was conducted by Hollenberg et al [6] in indigenous peoples from Kuna, an island in Panama. This population is characterized by low blood pressures (BP) even at older ages. In trying to identify whether this fact was due to a genetic factor, the researchers discovered that Kuna people living off the Island lost this health benefit and developed hypertension. After some research, they discovered that the low BP levels were due to a nutritional factor, the high consumption (5 cups a day) of a cocoa beverage that is rich in PAs. This findings contributed to the perceived health benefit from this family of compounds and increased their popularity over the last decade[7].

The purpose of this review is to compile relevant epidemiological data connecting PA consumption and health benefits. This review also focuses on the molecular and physiological mechanisms by which PAs modulate body functionality and confer protection against metabolic diseases.

2. Proanthocyanidin consumption and health benefits

2.1. Epidemiological studies linking proanthocyanidin consumption and health preservation

In addition to the studies discussed in the historical view, other epidemiological studies have reported an association between the consumption of PAs and the risk and/or incidence of CVD and cancer, which are two major public health problems found in Western countries.

For instance, several prospective cohort studies have revealed that the intake of PAs significantly decrease the risk of CVD[8]. Additionally, PA intake has been directly associated with a lower risk of death from CVD in a large prospective US cohort study with 7 years of follow-up [9]. Moreover, the European Prospective Investigation into Cancer and Nutrition (EPIC) study, which was conducted in 8 European countries, revealed an inverse association between the intake of PAs and the incidence of type 2 diabetes (T2D)[10], an important risk factor for CVD.

Interestingly, higher dietary intakes of PAs have also been associated with a lower risk of specific cancers. One study demonstrated an inverse relationship between PAs intake and high-grade prostate cancer, but not advanced prostate cancer, in a prospective US cohort of 43,268 men [11]. However, dietary PA consumption showed no association with colorectal adenoma recurrence[12] or esophageal cancer[13].

Therefore, although the protective action of PA consumption against CVD is clear, the evidence regarding PA intake and the prevention of cancers is more ambiguous. However, because the quantification of the actual PA intake by individuals may be inaccurate due to the incomplete data on PA content in foods, some epidemiological studies should be reconsidered.

2.2. Proanthocyanidin rich-foods and intake

Structurally, PAs are oligomers or polymers of monomeric flavan-3-ols and belong to the flavanol subclass. PA intake has been estimated to be in the range of 90 to 300 mg/day, with the levels varying significantly between countries[14,15]. PAs are mainly found in grapes, cocoa, chocolate, red wine, and green tea, but other fruits and vegetables also contain PAs. The "USDA Database" (http://www.ars.usda.gov/Services/docs.htm?docid=24953) and "Phenol-Explorer" (http://phenol-explorer.eu/) are two useful databases to check the PA content in numerous foods.

2.3. Interaction between proanthocyanidins and the microbiome

Once ingested, flavanol monomers and dimers are absorbed through the small intestine and recognized as xenobiotcs by the body. The flavanol monomers and dimers are subjected to phase-II metabolism by enterocyte or hepatocyte phase-II enzymes, such as uridin-glucuronil transferases (UGTs), sulfotransferases (SULTs) and/or catechol-O-methyl transferases

(COMTs), and form their respective glucuronidated, sulphated or methylated metabolites[16]. The glucuronidated forms are the predominant metabolites in plasma after the ingestion of PAs rich extracts, and their levels are substantially higher than those of the methylated and sulphated forms. However, free forms of unconjugated catechin, epicatechin and dimeric PAs are also detected in plasma. On the contrary, polymerized PAs that are larger than dimers are not detected in plasma. This finding indicate that PAs larger than dimers pass intact through the small intestine and reach the colon, where they are subjected to microbiota activity, which results in their degradation into smaller phenolic compounds that are subsequently absorbed and undergo phase-II metabolism[16].

The colon has a great diversity of microbial populations that consist of either obligatory or facultative anaerobes that are responsible for the degradation of the non-absorbed PAs and the conjugated forms excreted in the bile. Specific moieties of the conjugated forms are cleaved and the oligomeric forms undergo cleavage at the interflavanic link to produce monomers. Subsequently, a wide range of enzymes produced by gut bacteria can hydrolyse, reduce, dehydroxylate, decarboxylate and demethylate several of the polyphenolic functional groups. Thus, all PAs and conjugated forms are eventually converted into different low-molecular weight metabolites, such as valerolactone-related compounds, valeric acids, phenylpropionic acids, phenylacetic acids, benzoic acids and several conjugated phenolic acids [17]. These microbial metabolites can be absorbed by the colonocytes and reach the liver, where they are subjected to phase II metabolism before they enter the systemic circulation and are absorbed by different tissues or excreted through the urine. In a recent study with rats carried out by our group, the plasma concentration of conjugated forms of flavanols peaked 2 h after the administration of a PA-rich grape seed extract The colonic metabolites appeared in the plasma later than the conjugated forms of flavanols, thus indicating their gradual colonic biotransformation as valerolactone > phenylpropionic acids ≈ phenylacetic acids > benzoic acids[18].

Thus, the molecular forms of PAs that enter the peripheral circulation and tissues are metabolites derived from the PAs present in foods. Therefore, the beneficial health properties of flavanols are attributed to these metabolites. However, the complete catabolic pathway and all of the potential colonic metabolites of flavanols are not yet known due to several limitations, namely the difference in microbiota composition between subjects, especially in humans, and the limited number of identified human gut bacteria that are able to catabolize flavonoids.

The microbiota is composed of 500 to 1000 different species, but polyphenol catabolism has been only attributed to some of the species that are present. For instance, *Lactobacillus*, *Chlostridium*, *Eubacterium*, and *Bacteroides* species are able to catabolize flavonoids [19] and the conversion of epicatechin to specific forms of valerolactones has been reported in *Eggerthella lenta* and *F.Plautii* species[20] found in the human microbiome. Gut microbial compositions vary with diets [21] and disease states, such diabetes and obesity[22]. Thus, the generation of biologically active microbial metabolites and the subsequent beneficial effect of PAs could be controlled by the specific microbiota composition of each individual. In this sense, the bioconversion of polyphenols by human intestinal microbiota displays strong inter-individual variability [23]. Moreover, we have previously reported that the bioavailability of PAs and the kinetics and presence of specific bioactive metabolites in rat plasma depend on both the rat sex and the amount of ingested PAs[24].

In addition, a few studies have been dedicated to determining the role of dietary polyphenols as prebiotics by focusing on a single polyphenol and selected bacterial populations. These studies clearly suggest that polyphenols have the capacity to alter the gut microbiota composition by increasing the population of beneficial microflora in the gut[25]. Thus, the consumption of PAs could also confer health benefits as a result of their positive effect on the gut microbiota.

Although there is evidence for bidirectional effects in the relationship between gut bacteria and polyphenols, further studies focusing on the interactions PAs and gut microbiota and the capacity of the gut microbial to metabolize PAs are essential.

2.4. Modulation of circadian rhythms by proanthocyanidins

Coordinated daily rhythms are evident in human physiology and metabolism. These rhythms are driven by internal circadian clocks that separate incompatible cellular processes and optimize cellular and organism health. In fact, the disruption of the circadian clocks contributes to the pathophysiology of numerous diseases, such as obesity, DT2, neurological disorders and cancers [26].

The internal circadian clocks are synchronized with the external time by Zeitgebers, such as light, diet and feeding time. Interestingly, recent results from our group demonstrate that PAs can also act as a Zeitgeber. In this sense, an acute dose of PAs keeps nocturnal melatonin levels in the first 3 hours of the light phase and modifies the characteristic plasma fluctuation of some metabolites [27]. This phenotypic alteration was concomitant with the modulation of the oscillating expression of clock genes in the hypothalamus [27] , where the central clock resides. Moreover, PAs also modulate the liver clock [28], which suggests that PAs can regulate lipid and glucose metabolism by adjusting the circadian rhythm in the liver. Thus, PAs can improve body health thought the modulation of central and peripheral clocks.

3. Overview of molecular mechanisms involved in the health benefits of proanthocyanidins

Although bioavailability and metabolism data on PAs are still largely unavailable, several molecular mechanisms have been proposed to explain the biological actions of PAs on human health and disease. However, the actual molecular interactions of these compounds with biological systems remain mostly speculative.

Two major mechanisms have been proposed to explain the biological actions of PAs[29]: basic biochemical mechanisms, which focus on PAs' ability to bind strongly to proteins, and epigenetic mechanisms, which include histone modifications, DNA methylation and modulation of microRNAs (miRNAs). Both mechanisms allow PAs to modulate enzymatic activities, cell signaling cascades and gene expression, ultimately resulting in the modulation of cell functionality.

3.1. Basic biochemical mechanisms

For decades, PAs have been considered "simple" antioxidant molecules, a view supported by the presence of the phenolic OH groups that are able to reduce free radicals through a one-electron donation and the presence of aromatic structures that stabilize the resultant aroxyl radicals by resonance. Thus, antioxidant effects have been demonstrated in numerous assays of foods rich in PAs, such as grape seed and green tea extracts, wines and blueberry products. In some studies, PAs have promoted a direct decrease in ROS, reactive carbonyls derived from proteins or malondialdehyde from lipid oxidation. However, several authors have questioned the correlation between their direct antioxidant activity and their efficacy in health promotion due to the relatively low bioavailability of PAs, even after the consumption of foods rich in

these compounds[30]. The low bioavailability leads to a kinetically unfavourable condition with respect to other compounds that possess similar free radical scavenger properties and are present in blood or tissues in significantly higher concentrations. Thus, a potential action of PAs as free radical scavengers is unlikely to be physiologically relevant in most tissues, except for those exposed to a high PA concentration, such as that found in the gastrointestinal tract.

Nevertheless, most of the beneficial, chemopreventive and therapeutic properties associated with PAs appear to be due to specific interactions with proteins and enzymes. These specific interactions, which may be considered the physico-chemical hallmark of these oligomeric substances, are primarily due to hydrogen bonding, van der Waals and electrostatic interactions but may also include covalent bond formation[31]. The interactions between PAs and proteins will result in a biological effect determined by the function of the proteins involved, including the modification of enzymatic activities, binding of receptors and ligands, and transcription factors binding to their specific sites in DNA.

In this sense, PAs have robustly demonstrated the capacity to increase the expression and/or activity of several antioxidant enzymes, such as catalase, superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione-S-transferase (GST). In addition, we have demonstrated that grape seed PAs reduce the oxidized glutathione accumulation in Zucker rats, thus increasing the total GSH/oxidized glutathione hepatic ratio and consequently increasing the total antioxidant capacity of the cell[32].

Other studies reviewed by Martinez-Michaelo et al. [33] have reported the capacity of PAs to efficiently regulate the activity of nuclear factor-κB (NF-κB) and mitogen-activated protein kinase (MAPK), thus modulating the gene expression of pro-inflammatory factors, such as cyclooxygenase (COX), lipoxygenase (LOX), and several pivotal cytokines regulated pathways. It is worth mentioning that the regulation of NF-κB has demonstrated to play a role in cell proliferation and oncogenic processes. A number of studies have shown that PAs could exert significant anti-cancer effects through the suppression of NF-κB[34].

Moreover, PAs induce the transactivation of some nuclear receptors, such as the farnesoid X receptor (FXR)[35] and the retinoic acid-related orphan receptor alpha (RORα)[36], that regulate lipid homeostasis and the molecular clock in the liver.

Finally, PAs also have been reported to partially prevent cell apoptosis in both animal and cell culture models by attenuating endoplasmic reticulum (ER) stress and the unfolded protein response signature via modulation of the caspase-12 pathway [37].

Interestingly, under certain circumstances, the interaction between PAs and proteins may also be considered nonspecific. PAs interact with proline-rich proteins in a process that starts with a hydrophobic association between proline residues and aromatic phenolic rings, followed by the formation of small size aggregates, and ending with protein precipitation[38]. This mechanism is responsible, for example, for the astringent sensation noticed in the oral cavity during red wine drinking that result from the precipitation and subsequent denaturalization of the salivary proline-rich proteins.

PAs may also interact with phospholipid membranes by forming hydrogen bonds and hydrophobic interactions between the phospholipid OH groups and phenolic rings of the PAs. These interactions may indirectly affect cell function by modifying cell membrane structure and physical characteristics such as fluidity, density and electrical properties. These effects can be observed both when PAs are adsorbed on the membrane and when they are inserted into the bilayer [39].These modifications can result in functional events that may lead to the modulation of many membrane-dependent processes including the activity of membrane-associated enzymes, ion and/or metabolite fluxes, ligand-receptor interactions, and the direct modulation of signal transduction. Although the interactions of PAs with membranes can be considered to involve nonspecific mechanism of action, recent evidence suggests a selectivity of certain PAs for specialized areas of the membrane such as lipid rafts[40], which have a peculiar lipid and protein composition that is enriched in cholesterol and sphingolipids, and in areas containing proteins involved in membrane signaling and trafficking pathways.

In summary, PAs could exert their biological activities as 1) unabsorbed complex structures with local effects in the gastrointestinal tract, 2) absorbed PAs (probably low molecular-weight forms) and 3) absorbed metabolites derived from colonic metabolism. There is sufficient evidence to suggest that PAs possess a wide range of biochemical mechanisms of action with the putative capability to modulate pathways related to chronic inflammation, lipid homeostasis, energy metabolism, apoptosis and cell cycle arrest.

3.2. Epigenetic mechanisms

Epigenetics refers to heritable characteristics that are not encoded in the DNA sequence itself, but play an important role in the control of gene expression. In mammals, epigenetic mechanisms include changes in DNA methylation, histone modifications and non-coding RNAs. Although epigenetic changes are heritable in somatic cells, these modifications are also

potentially reversible, which makes them attractive for novel preventive and therapeutic strategies in several human diseases. Recently, we have begun to understand that diet and environmental factors directly influence epigenetic mechanisms in humans [41]. Notably, PAs may exert their biological activities, at least in part, by modulating various components of the epigenetic machinery in humans. In the following paragraphs, we briefly summarize the current knowledge regarding the effects of PAs or their metabolites on DNA methylation, histone modifications and regulation of expression of miRNAs in various *in vitro* and *in vivo* models.

The silencing of tumour suppressor genes via hyper-methylation constitutes a frequent epigenetic defect that is found in many human cancers. Reversing this hyper-methylation, mainly by inhibiting DNA methyltransferase (DNMT) activity in cancer cells, is a plausible strategy for developing epigenetic drugs, but the available DNMT inhibitors are toxic and nonspecific. However, dietary polyphenols have been shown to directly inhibit DNMT to partially reverse hypermethylation status without the associated toxicity [42]. In this regard, the significant demethylation and activation of several genes by green tea PAs have been documented in human cancer cells[43], but these results remain controversial, and further studies are needed to conclusively demonstrate these effects.

In addition to their ability to induced changes in DNA methylation, evidence indicates that PAs can regulate gene expression through changes in histone modifications. In this regard, green tea flavanols are known to possess potent histone acetyltransferase (HAT) and histone deacetylase (HDAC) inhibitory activities[44]. Interestingly, we have recently observed that PAs regulate the hepatic class III HDACs, which are often called sirtuins (SIRT1-7), in a dose-dependent manner, which was associated with significant protection against hepatic triglyceride and cholesterol accumulation in healthy rats (unpublished data). Additionally, we have determined that SIRT1 mediates the antihypertensive effect of PAs (unpublished data). Our data suggest that grape seed PAs could be a valid tool to enhance SIRT1 activity and could potentially provide a therapeutic approach to prevent or treat hepatic fat accumulation and hypertension.

miRNAs are small, gene-silencing RNAs that regulate mRNA translation by blockage or degradation. A number of miRNAs appear to play important regulatory roles in cell differentiation, insulin action and fat metabolism in both adipocytes and hepatocytes. Recent studies have demonstrated that miRNA deregulation is involved in fatty liver disease in obese mice and humans [45]. Notably, we demonstrated the ability of flavonoids to interact directly

with miR-33a and miR-122 *in vitro* using $^{(1)}$H NMR [46]. These data suggest that the specific and direct binding of polyphenols to miRNAs may be a new posttranscriptional mechanism by which PAs modulate metabolism. However, further studies are needed to elucidate the effects of PAs on miRNAs and the metabolic pathways affected by these small molecules.

4. Proanthocyanidin effects in metabolic diseases

4.1. Proanthocyanidin effects on cardiovascular diseases

Diet clearly impacts multiple CVD risk factors, including high BP, obesity and elevated blood lipids. In fact, epidemiological studies strongly suggest that PAs protect against CVD [8,9] based on their antioxidant and anti-inflammatory properties.

Several studies have demonstrated that PAs inhibit the oxidation of low-density lipoproteins (LDLs) [47] and that this antioxidant activity increases as the PA chain lengths increase [48]. Furthermore, PAs from grape seed have been reported to affect the activity and gene expression of antioxidant enzymes such as glutathione peroxidase, glutathione reductase, and glutathione S-transferase in cell cultures [49]. Recently, Fernández-Iglesias *et al*. [32] demonstrated that the administration of grape seed PAs to genetically obese Zucker rats reduced oxidised glutathione hepatic accumulation, thereby decreasing the activation of antioxidant enzymes and increasing the total antioxidant capacity of hepatocytes.

PAs can also protect against CVD due to their anti-inflammatory properties, as previously described[33]. PAs have been reported to target various mediators of inflammation such as cytokines (e.g., interleukins (ILs), tumour necrosis factor-α (TNF-α), interferon-γ (INF-γ), transforming growth factor-β (TGF-β)), nitric oxide (NO) synthesis, and both the MAPK and NF-κB pathways. Moreover, PAs modulate the arachidonic acid pathway by inhibition of eicosanoid-generating enzymes such as COX and LOXs.

Despite the antioxidant and anti-inflammatory properties of these flavonoids, one of the mechanisms by which PAs exert their cardiovascular protection is improving lipid homeostasis.

4.2. Proanthocyanidin effects in dyslipidaemias

The effects of PAs on human plasma lipids have yielded inconsistent results when evaluated by randomised controlled trials. Specifically, some meta-analysis showed no statistically

significant effects of grape seed PAs on the total cholesterol (TC), triglycerides (TGs), LDL-cholesterol (LDL-C), or HDL-cholesterol (HDL-C) levels[50], whereas other studies showed that these PAs significantly decreased TC and LDL-C[47]. However, PAs have been clearly demonstrated to have a hypolipidaemic effect in animal models [51]. For instance, an acute oral dose of grape seed PAs reduced plasma TGs and apolipoprotein B (apoB) and improved the atherosclerotic risk index in healthy rats[52]. A chronic treatment of these PAs corrected dyslipidaemia associated with rats fed a high-fat[53]. In fact, it has been suggested that grape seed PAs induce hypotriglyceridaemia by repressing lipoprotein secretion and not by increasing lipoprotein catabolism[54]. In addition, these PAs exert some hypolipidaemic effects by inhibiting the absorption of dietary lipids and diminishing chylomicrons (CM) secretion by enterocytes[55]. Furthermore, the repression of liver VLDL secretion due to a reduction in the bioavailability of TGs also appears to plays an important role in reducing plasma lipids[56]. Moreover, grape seed PAs improve lipid homeostasis by increasing the reverse transport of cholesterol and favouring cholesterol elimination from the body via bile[52].

The molecular mechanisms by which grape seed PAs modulate lipid metabolism have been thoroughly evaluated in liver. These studies showed that grape seed PAs decrease plasma TG concentrations by activating the nuclear receptor FXR, upregulating the nuclear receptor small heterodimer partner (SHP) and subsequently repressing the transcription factor SREBP1 in liver[35,56]. As previously mentioned, it has also been demonstrated that PAs may modulate lipid metabolism through miRNAs regulation, thereby repressing liver miR-33a and miR-122, which are two miRNAs controllers of lipid metabolism[57] .

4.3. Proanthocyanidin effects in hypertension

Reducing BP is one of the CVD risk factors on which PAs have been shown to have a clear effect. In fact, antihypertensive effects have been demonstrated for flavanol-rich food such as cocoa[58] or grape seed[59] PAs. The effects of PAs on BP have been evaluated in a meta-analysis study that showed that grape seed[50] and cocoa[60] PAs reduce systolic BP (SBP), with the reduction more significant with cocoa than with grape seed PAs. Others foods rich in flavanols, such as green or black tea, were shown to have no effect on BP[60], possibly due to the different flavanol composition.

The antihypertensive properties of PAs are related to NO-mediated vasodilation[61], angiotensin converting enzyme (ACE) inhibition[62] and a reduction in oxidative stress[59]. Nonetheless, the blood pressure-lowering effect of flavanols is mainly mediated through the

NO pathway, and for grape seed PAs, it is also partially mediated by prostacyclin[63]. Other mechanisms that may be involved in the vasodilator effect of PAs include the inhibition of both phosphodiesterases (PDEs) 2 and 4, which catalyse the degradation of cAMP and cGMP, and PDE-5, which degrades cGMP[64].

4.4. Proanthocyanidin effects in obesity and type 2 diabetes

Obesity is defined as the abnormal or excessive accumulation of body fat that may impair health and has been described as a worldwide epidemic. Obesity is a multifactorial disorder that is associated with an increased risk of developing insulin resistance and T2D. The pleiotropic characteristic of PAs may present an opportunity to fight such multifactorial diseases.

PAs can combat obesity by acting at the gut level. *In vitro* and animal studies have shown that PAs inhibit intestinal lipase and amylase, thereby reducing the absorption of lipids and glucose. Furthermore, it has been postulated that unabsorbed PA can control satiety and food intake by an incretin like action, by stimulation of GLP-1/DPP4 activity[65], and by the regulation of gastrointestinal tract-brain signals[66].

Several animal studies have demonstrated the ability of PAs to reduce body weight and fat depots (revised in[66]). However, PAs were ineffective in reducing the body weight in other studies[67,68], which suggests that the PA doses, days of administration, animal species and the experimental approach can largely affect the capacity of PAs to significantly modulate body weight.

In addition to their net effect on body weight, PAs modulate the functionality of skeletal muscle, adipose tissue and the liver, thereby improving obesity-related pathologies. In this sense, cocoa PAs repress key lipogenic genes[69] and increase the expression of lipolytic genes[69]. PAs down-regulate lipogenic and adipogenic genes through the inhibition of both SREBP-1c and PPAR γ[69]. Conversely, the up-regulation of lipolysis by PAs has been attributed to a PPAR β/δ-dependent fatty acid oxidative genes via Prkaa1 gene activation in adipose tissue[69]. Cocoa[69] and grape seed [67,68] PAs also activate mitochondrial biogenesis, thus increasing the lipolitic power of cells.

Some of these effects have been attributed to the activation of AMPKa. Other studies have suggested that PAs increase plasma adiponectin levels and thus, cause the activation of AMPKa in skeletal muscle, liver, and adipose tissue[69]. The activation of AMPKa promotes the activity

of the proliferator-activated receptor c coactivator-1a (PGC-1a), mitochondrial biogenesis and the expression of uncoupling protein, which result in increased thermogenesis and energy expenditure in skeletal muscle, adipose tissue and liver[69,70]. The activation of AMPKa also inhibits lipogenesis and stimulates fatty acid oxidation in the liver and skeletal muscle[70].

In obese individuals, adipose tissue releases increased amounts of non-esterified fatty acids, glycerol, hormones, pro-inflammatory cytokines and other factors that are involved in the development of insulin resistance. However, although obesity is a major risk factor for diabetes, the two conditions are not always linked.

Several studies have focused on the role of PAs in glucose homeostasis and insulin resistance (revised in[71]) with controversial results. In this role, PAs improve glycemia and insulin sensitivity in fructose or high-fat induced insulin resistant models. However, PAs are ineffective in other animal models, such as genetically obese or cafeteria diet-induced insulin resistant models.

PAs modulate glycemia by targeting several tissues involved in glucose homeostasis, such as muscle and adipose tissue, where PAs activate glucose uptake and improve their oxidative/inflammatory state[71]. Moreover, PAs increase insulin secretion by the pancreas and β-cell mass[71]. Finally, some studies have also implied the gut in the antihyperglycemic effect of PAs through the modulation of the levels of active glucagon-like peptide-1 (GLP-1).

Conclusion

The potential health benefits of PAs have been widely studied in both animal models and humans. In total, the research suggests that these compounds confer beneficial health effects in cardiovascular and metabolic disorders and in some cancers. However, more studies are needed to reveal the specific compounds and/or metabolites responsible for the health benefits of PAs and their mechanisms of action.

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