Mitochondria As Potential Targets of Flavonoids: Focus on Adipocytes and Endothelial Cells*

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Abstract: Obesity is a major public health problem, resulting from an excess of energy storage and/or a default of energy expenditure leading to the increased occurrence of cardiovascular risk factors that favour the development of vascular complications. As a consequence, many studies are interested to find novel therapeutic chemical including flavonoids that appear to be promising natural compounds to treat obesity and its complications. Several *in vitro* studies addressed the mechanisms involved that might explain their beneficial effects, on adipocytes and endothelial cells, two cell types that play major role in obesity and its vascular complications. Besides the well-described antioxidant properties of flavonoids, at least a part of their beneficial effects on these cell types might be explained by their action on the regulation of mitochondrial function. In this review, we will therefore focus on the pathophysiological role of mitochondria in regulating endothelial and adipocyte functions. In addition, we will present some of the more promising flavonoids, important in human diet, including flavanols, flavonols, isoflavones, anthocyanins, flavanones and flavones; and their potential effects to improve endothelial or adipocyte functions *via* the mitochondria.

Keywords: Adipocyte, apoptosis, endothelial cell, flavonoids, mitochondria, obesity, ROS.

INTRODUCTION

The worldwide incidence of obesity rapidly increased in the last two decades. According to a World Health Organization report, obesity has been classified as a growing epidemic, and if immediate action is not taken, millions will suffer from an array of serious weight-related disorders. Obesity arises when there is an imbalance between energy intake, principally stored as triglycerides in adipose tissue (food consumption), and energy expenditure (basal metabolic rate and biochemical processes). When adipose tissue function is compromised during obesity, the excessive fat accumulation predisposes the individual to the development of metabolic changes that increase overall risks of morbidity [1, 2]. Moreover, obesity is a complex trait influenced by diet, developmental stage, age, physical activity, and genes [3] and also represents a significant risk factor for major diseases including type 2 diabetes, vascular complications including coronary heart disease and hypertension, and certain forms of cancer. Here, we describe recent insights into events that may lead to obesity and its vascular complications, with a focus on the role of mitochondria, and discuss flavonoids that may improve its function.

PATHOPHYSIOLOGY OF OBESITY AND OBESITY ASSOCIATED VASCULAR DISEASES

Intense research on adipose tissue has contributed to increased knowledge on the management of lipid stores and the physiological adaptations when facing a nutrient overload. White adipose tissue constitutes the main energy supply in the body, which is mobilized according to body needs, implying a permanent communication with other organs [4]. At the cellular level, the development of obesity is characterized by changes in adipocyte properties including an increase in their number (hyperplasia), size (hypertrophy) or both [5]. While hyperplasia involves the recruitment, proliferation and differentiation of precursor preadipocytes, hypertrophic enlargement is due to the increase in lipid content in adipocytes. Moreover, obesity induces changes in the structure and function of adipose tissue that affects the secretory activity of adipocyte. Indeed, obesity is associated with increased secretion of proinflammatory adipokines, which may act at both local (autocrine and/or paracrine) and systemic (endocrine) levels. These factors include cytokines (interleukin 6 (Il6), tumor necrosis factor alpha (TNFα)), growth factors, adiponectin, leptin and components of the renin–angiotensin system [6]. The dysegulated production of these factors is clearly related to the development of obesity-associated pathologies, including vascular diseases [7, 8].

A growing body of evidence suggests the existence of a reciprocal interplay between adipose tissue and the vasculature. In adipose tissue, vascularization plays a crucial role in determining adipocyte differentiation and growth, as well as its physiological function, by supplying nutrients, growth factors and circulating stem cells [9]. Conversely, adipose tissue exerts profound effects on vascular tone in particular endothelium-dependent vasodilatation, inflammation and remodeling [10]. Indeed, endothelial dysfunction, considered as the first step in the progression of vascular diseases, has been reported in obese patients and is induced by TNFα through a down-regulation of endothelial nitric oxide (NO) synthase (eNOS) expression [11]; or by leptin, whose circulating levels are closely related to body fat mass [12, 13] and hypertension [14].

Finally, several lines of evidence point to a possible deleterious role of oxidative stress. In healthy volunteers, a lipid infusion induced a rise in plasma free fatty acids levels and produced an increase in plasma-free radical concentrations (indicating oxidative stress) [15]. In cultured adipocytes, it has been shown that free fatty acids activate NADPH oxidase and induce reactive oxygen species (ROS) production, and this oxidative stress results in dysregulated production of adipokines from adipose tissue [16].

ROLE OF THE MITOCHONDRIA IN OBESITY AND OBESITY ASSOCIATED VASCULAR DISEASES

Mitochondria control many cellular functions in adipocytes and endothelial cells, such as adipocyte differentiation or angiogenesis. Also, mitochondrial dysfunction, such as dysregulated metabolism, or excessive cell death and ROS production, are often associated with adipocyte and endothelial dysfunction. Consequently, mitochondria emerged as an essential organelle involved in the pathophysiology of obesity and its vascular complications.

Actually, recent evidence suggests that adipocyte mitochondrial dysfunction may have an important role in obesity [17]. Indeed, the transcriptional co-activators peroxisome proliferator-activated receptor gamma (PPARγ) co-activator-1 alpha (PGC1α) and beta (PGC1β) which regulate mitochondrial biogenesis, are found to be decreased in adipose tissue from animal models of obesity [18]. Conversely, studies performed on 3T3-L1 adipocytes have reported that over-expression of these factors improves insulin sensitivity, mitochondrial function and the resistance to oxidative stress [19]. Mitochondria are a physiologically important source of cellular ROS [20, 21]. While high levels of ROS are cytotoxic and

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genotoxic [22], low levels of ROS have an important role in cell signaling [23]. Oxidative stress and mitochondrial ROS are involved in mature adipocyte pathophysiology. Indeed, adipocyte differentiation is affected by mitochondrial ROS. It has been reported that ROS generated from complex III decreased adipocyte differentiation by increasing the expression of transcriptional factor CHOP-10, an inactive analog of CCAAT/enhancer-binding protein (C/EBP), a key transcriptional factor for adipocyte differentiation [24]. Interestingly, it was recently demonstrated that this ROS source was necessary to initiate adipocyte differentiation emphasizing the need to maintain physiological ROS [25]. Mitochondrial ROS are also implicated in the regulation of adipokine secretion [26, 27]. On one hand, the modulation of mitochondrial ROS production, and specifically as a consequence of the action of uncoupling protein-2, controls adiponectin gene expression, and represents a physiological mechanism by which the adipose tissue energetic status may determine the extent of adiponectin release [26]; on the other hand mitochondrial superoxide overproduction during intermittent high glucose exposition induced the aberrant production of adiponectin (decreased production) and resistin (increased production) [27].

Mitochondria also play a role in endothelial dysfunction associated with obesity. Mitochondria are involved in the regulation of cell death, and vascular diseases that are often associated with alterations of endothelium integrity. Indeed, several stimuli often observed in obesity, including TNFα [28], or oxidized-low density lipoprotein (oxLDL) [29, 30] have been reported to promote endothelial cell death. Moreover, besides their implication in endothelial apoptosis, mitochondria play an essential role in endothelial dysfunction through a significant ROS production [31]. For example, it has been shown in endothelial cells that diabetic complications are associated with an enhancement of ROS production, an activation of protein kinase C (PKC), an increase of the formation of glucose-derived advanced end products (AGE) and an activation of nuclear factor kappa B (NFκB). The activation of these pathways is fully prevented by normalizing the production of mitochondrial ROS using a complex II inhibitor, Mn-superoxide dismutase (MnSOD), or uncoupling protein (UCP)-1 [32]. Moreover, in obese patients, TNFα induces expression of the receptor of advanced glycation end products (RAGE) through an increase of mitochondrial ROS production and activation of NFκB [33].

THERAPEUTIC POTENTIAL OF FLAVONOIDS TARGETING MITOCHONDRIA AGAINST OBESITY AND OBESITY ASSOCIATED VASCULAR DISEASES

Various epidemiological studies reported an inverse correlation between a flavonoid enriched-diet and reduced risk of both cardiovascular [34, 35] and metabolic [36] diseases. Flavonoids represent the major class of polyphenols and are widely distributed in the plant kingdom. They are often involved primarily to plant defensive response against stress such as ultraviolet radiation, pathogens and physical damages [37] and they are consumed regularly in the human diet. Over 8,000 structurally unique flavonoids have been identified in plant sources particularly in citrus fruits, vegetables, nuts, seeds, herbs, spices, stems, flowers, as well as in tea and red wine [38].

The chemical structure of flavonoids varies from a simple phenol core to a complex molecule with a high molecular weight and a high degree of polymerization [39] (Table **1**). The main groups of flavonoids are (1) flavanols (e.g., epigallocatechin gallate (EGCG)), which are abundant in green tea, red wine, and chocolate; (2) flavonols (e.g., rutin, quercetin), which are found in onions, leeks, and broccoli; (3) isoflavones (e.g., daidzein, genistein), which are mainly found in soy and soy products; (4) anthocyanidins (e.g., delphinidin, pelargonidin, cyanidin, malvidin) which sources include red wine and berry fruits [40]; (5) flavanones (e.g., hesperetin, naringenin, isoxanthohumol), which are mainly found in citrus fruit and tomatoes and (6) flavones (e.g., apigenin), which are found in parsley and celery.

Flavonoids exist in plants and plant derived foods predominantly as glycoside conjugates rather than as a free form. For example, onions are a rich source of quercetin-4'-O-glucoside and quercetin-3,4'-O-diglucoside, and tomato juice is rich in quercetin-3-O-rutinoside [41, 42]. After ingestion, glycoside flavonoids are metabolized into their aglycone by β-glucosidase and may then cross the intestinal cell membrane by passive diffusion. Glysoside flavonoids can also cross the intestine barrier by active transport, and be transformed into aglycone inside epithelial cells. Once inside epithelial cells, aglycones are metabolized into their β-D-glucuronide and sulfonate esters and secreted into the blood. Therefore, very low amounts of flavonoids in their free forms are excreted into the bloodstream [43]. For example, after ingestion of 500 ml of green tea, the maximal concentration of free form EGCG in the plasma reached 25 nM, whereas epigallocatechin-O-glucuronide, 4'-O-Methyl-epigallocatechin-O-glucuronide and 4'-O-Methyl-epigallocatechin-O-sulfates reached 126, 46 and 79 nM respectively [42]. Flavonoids and their metabolites that are not absorbed in the small intestine can be absorbed in the large intestine where they undergo changes due to the colonic microflora. For instance, the isoflavone daidzein is transformed by bacterial transformation into equol, which reaches the bloodstream. Finally, flavonoids may also be transformed when they reach peripheral tissues. For example, the circulating glucuronide form of quercetin has been shown to be transformed into its aglycone form in smooth muscle cells by β-glucuronidase enzyme [44].

The flavonoids have long been recognized to possess anti-inflammatory [45], anti-allergic [46], hepatoprotective [47], anti-thrombotic [48], and anti-carcinogenic activities [49].

Furthermore, flavonoids are typical phenolic compounds with antioxidant properties and, therefore, act as potent metal chelators and free radical scavengers [50, 51]. Redox reactions taking place in the cytosol are essential for the maintenance of the metabolic competence of the cell and the integrity of cellular components. Glutathione (GSH) is present in large amounts in the cytosol where it plays pivotal roles in redox homeostasis through thiol–disulfide exchange reactions with cysteine-containing proteins, and also as an electron carrier for many enzymes involved in ROS reduction. Many redox enzymes located in the cytosol have been described, examples include non-thiol based enzymes (catalase and superoxide dismutases) and thiol-based enzymes (thioredoxin-dependent or GSH-dependent thiol peroxidases, and thioredoxin-dependent alkyl peroxidases).

The antioxidant capacities of many flavonoids are stronger than those of vitamins C and E. Moreover, they can act synergistically with the redox buffer.

- Catechins, such as EGCG, have been reported to possess chelating ability, and show antioxidant properties in a number of *in vitro* and chemical-based assays. EGCG scavenges a host of oxygen, nitrogen, and chlorine radical species [52], but is also able to induce many endogenous redox systems. Indeed, EGCG increases expression of heme-oxygenase-1 and superoxide dismutase in human mammary epithelial cells. These effects were reduced by small-interfering RNA (siRNA)-mediated disruption of Nrf2, suggesting a role for this pathway in the effect of EGCG *via* the induction of endogenous antioxidant systems [53]. Moreover, in coronary artery endothelial cells, increased expression of both NADPH oxidase and xanthine oxidase has been reported [54].

- Quercetin seems to be the main antioxidant flavonoid, because it is able to both directly scavenge free radicals by hydrogen atom donation [55] and to regulate redox enzymes activities. Recently, quercetin has been shown to modulate Nrf2 and GSH-related

defenses through a p38-dependent pathway in HepG2 [56]. These findings corroborate previous studies that showed an interaction of quercetin with cellular defense systems such as NADPH oxido-reductase, xanthine oxidase, heme-oxygenase-1 [57-59]. Furthermore, quercetin can induce glutathione S-transferase and UDP-glucuronosyl transferase that can also protect against oxidative stress [60].

- Like others flavonoids, genistein has been shown to protect cells against ROS due to their free radical scavenging properties [61]. Few reports have addressed the molecular and cellular targets involved in the induction of antioxidant defense induced by genistein. Low concentrations of genistein increase heme-oxygenase 1 expression in artery smooth muscle cells [62]; whereas, high concentrations induce catalase mRNA expression without affecting Cu/Zn SOD activity in Caco-2 cells [63]. In EaHy.926 endothelial cells, genistein increases the expression and the cytosolic accumulation of Nrf1 and Nrf2 and the expression and activity of glutathione peroxidase [64].

- In addition to the higher superoxide-scavenging activity and peroxynitrite-scavenging activity observed with anthocyanins [65], a study performed on peripheral blood mononuclear cells obtained from healthy patients who received berry juice enriched on anthocyanin revealed decreased DNA damage (determined by the comet assay) and an increased level of glutathion [66]. Interestingly, a recent study conducted in rats confirmed that strawberry anthocyanin increased the expression of catalase and superoxide dismutase to attenuate oxidative stress induced by ethanol [67].

The *in vitro* antioxidant activity of flavonoids depends on the arrangement of functional groups on its core structure. Both the configuration and total number of hydroxyl groups substantially influence the mechanism of antioxidant activity [68]. According to these findings, quercetin seems to be the most powerful antioxidant because it has all the right structural features for free radical scavenging activities. Moreover, *in vitro* antioxidant activity can be increased by polymerization of flavonoid monomers. Thus, proanthocyanidins (also known as condensed tannins) and the polymers of catechins are excellent *in vitro* antioxidants due to the high number of hydroxyl groups in their molecules [69]. Another possible mechanism by which flavonoids act is through interaction with various antioxidant enzymes. In this last case, as described by Lee-Hilz *et al*. [60] the most effective inducers of NAD(P)H-quinone oxidoreductase or glutathione S-transferases were flavonoids containing a hydroxyl group at the 3-position (such as quercetin, EGCG, delphinidin), whereas flavonoids without this hydroxyl group (genistein) were low inducers of antioxidant enzymes.

FLAVONOIDS TARGETING ADIPOCYTE MITOCHONDRIA

Little information is available on nutritional requirements of adipose tissue to improve its lipid storage function, either under normal conditions or in obesity. Several mechanisms have been proposed for the treatment of obesity including decreased preadipocyte differentiation, induction of adipocyte apoptosis or regulation of lipid metabolism (decreased lipogenesis and increased lipolysis). Flavonoids have been reported to act on these parameters.

EFFECTS OF FLAVONOIDS ON ADIPOCYTE DIFFERENTIATION (TABLE 2)

Clonal mitotic expansion is an important step during adipocyte differentiation. This process is supported by several transcriptional factors including PPARγ, C/EBP, forkhead-box protein (Fox) O1, or even sterol regulatory element binding protein (SREBP) 1. Various mechanisms are involved in the regulation of clonal mitotic expansion. On one hand, intracellular ROS are reported to be increased along with differentiation of 3T3-L1 adipocytes, and to facilitate adipocyte differentiation by inducing clonal mitotic expansion [70]. On the other hand, AMP-activated protein kinase (AMPK) activation has been reported to inhibit clonal mitotic expansion [71]. Interestingly, ROS are described to be upstream molecules of AMPK activated signals [72], and potentially, those produced from mitochondria [73].

A number of studies show that flavonoids are able to regulate adipocyte differentiation. However, to the best of our knowledge, no data have been provided about the effect of flavonoids on mitochondria during adipocyte differentiation.

The flavanol EGCG, through its antioxidant property has been shown to inhibit adipocyte differentiation [74, 75]. On one hand, the antioxidant effect of EGCG suppresses adipocyte differentiation by reducing the transcriptional activity of FoxO1 and SREBP1 *via* the insulin signaling pathway [74] Fig. (**1A**). On the other hand, EGCG inhibits adipocyte differentiation by reducing the mRNA expression of PPARγ, C/EBPα and FoxO1 *via* the mitogen-activated protein kinase (MEK)/extracellular signal-regulated

Table 1. Chemical Structures and Common Sources of Flavonoids of Interest

Family	Flavonoids	R1	R2	R3	R4	R5	R6	Major Sources
Flavanol	EGCG	OH	OH	$C_7H_5O_4$	OH	OH	OH	Green Tea, Red Wine, Chocolate
Flavonol	Quercetin	OH	OH	-	OH	OH	-	Onions, Leeks, Broccoli
	Rutin	OH	OH	Rutinose	OH	-	-	
Isoflavone	Genistein	OH	OH	OH	-	-	-	Soy, Soy Products
	Daidzein	OH	-	OH	-	-	-	
Anthocyanidin	Cyanidin	OH	OH	-	OH	OH	-	Berry Fruits, Red Wine
	Delphinidin	OH	OH	OH	OH	OH	-	
	Malvidin	OH	OH	OCH_3	OH	OCH_3	-	
	Pelargonidin	OH	OH	-	OH	-	-	
Favanone	Hesperetin	-	OH	OH	OCH_3	OH	-	Citrus Fruit, Tomatoe
	Isoxanthohumol	C_3H_5	OH	OCH_3	OH	-	-	
	Naringenin	-	OH	OH	OH	-	-	
Flavone	Apigenin	OH	OH	OH	-	-	-	Parsley, Celery

Table 2. Modulation of Adipocyte Differentiation by Flavonoids

Flavonoids	Concentration	Cell Type	Adipogenesis	Effects	Reference
EGCG	100 µM	3T3-L1	↓	↓ROS, ↑MEK/ERK, ↑PI3K, ↑Akt	[75]
				↓PPARγ, ↓C/EBPα, ↓FoxO1, ↓SREBP1	[74, 75]
				↑AMPK	[72]
Quercetin	10-100 µM	3T3-L1	↓	↓PPARγ, ↓C/EBPα, ↓SREBP1	[76-78]
				↑AMPK	[77]
Genistein	50-200 µM	3T3-L1	↓	↓PPARγ, ↓C/EBPα and β	[79]
	100 µM		=	↓lipid accumulation,↑ROS, ↑AMPK	[72]
Delphinidin/Cyanidin/Pelargonidin	100 nM	3T3-L1	↓	↓lipid accumulation	[82]
Isoxanthohumol	75-100 µM	3T3-L1	↓	↓lipid accumulation	[83]

kinase (ERK) and phosphatidylinositol-3-kinase (PI3K)/Akt pathways [75] Fig. (**1A**). Furthermore, suppression of adipogenesis by EGCG has been shown to be associated with AMPK activation [72] Fig. (**1A**). However, the mechanisms involved in AMPK activation have not been reported in this last study.

The flavonol quercetin has also been shown to suppress adipocyte differentiation [76-78]. The mechanism implicates the down regulation of PPARγ, C/EBPα and SREBP1 mRNA expression. Potentiation of AMPK activation has also been reported for quercetin [77] Fig. (**1B**).

The isoflavone genistein (50-200 µM) is also described to inhibit adipocyte differentiation through a decrease of C/EBPα, C/EBPβ and PPARγ expression [79] Fig. (**1C**). Moreover, the antiadipogenic effect of genistein is also associated with increased ROS production and AMPK activation [72]. Unfortunately, the cellular origin of the ROS production induced by genistein was not determined in this study. Interestingly, genistein alone used at lower concentration (25 µM) does not affect adipocyte differentiation, but is reported to potentiate the antiadipogenic effect of resveratrol, another polyphenol compound [80, 81].

Others flavonoids such as anthocyanins (delphinidin, cyanidin or pelargonidin each used at 100 nM) [82] or flavanone (75-100 µM of isoxanthohumol) [83] have been described to inhibit adipogenesis through the diminution of lipid accumulation. However, the mechanisms sustaining these effects were not elucidated.

Many studies reported the antiadipogenic effect of flavonoids. One has to note that all the mechanisms leading to the antiadipogenic effect of flavonoids involve the regulation of clonal mitotic expansion. The potential implication of mitochondria in the antiadipogenic effect of flavonoids needs to be elucidated.

EFFECTS OF FLAVONOIDS ON ADIPOCYTE APOPTOSIS (TABLE 3)

Apoptosis is a genetically controlled cell death program, involved in physiological process such as elimination of excess or damaged cells. There are two main apoptosis pathway: an extrinsic pathway initiated by the activation of death receptors; and an intrinsic pathway characterized by the release of mitochondrial cytochrome c into the cytoplasm. These two mechanisms lead to the activation of caspases, which are essential proteases for apoptosis. The family of Bcl-2 proteins plays a crucial role in the mitochondrial dependent intrinsic pathway. Bcl-2 prevents cytochrome c release into the cytoplasm by controlling the permeability of the external mitochondrial membrane by inhibiting the permeability of transition pore. Apoptotic signals induce the translocation of pro-apoptotic molecules such as Bax or Bad, from the cytoplasm to the mitochondria where they inhibit Bcl-2. The subsequent activation of the transition mitochondrial pore leads to the release of cytochrome c into the cytoplasm where it interacts with cytosolic proteins to form the apoptosome. This multiprotein complex converts pro-caspase 9 into its active form, which in turn activates downstream caspases.

Flavonoids are reported to regulate apoptosis of preadipocytes and mature adipocytes, through various signaling pathways that converge to modulate the mitochondrial intrinsic pathway.

It has been reported that the same range of concentrations of EGCG (50-400 µM) induces apoptosis of preadipocytes [84, 85] and mature adipocytes [86] Fig. (**1A**). Then, EGCG induces apoptosis on murine preadipocytes through an increase of caspase-3 activity and a decrease of cyclin-cyclin dependent kinase (Cdk) 2 expression [84]. Moreover, in human preadipocytes, EGCG induces apoptosis through a reduction of NFκB activity, the phosphorylation of Akt and accumulation of the pro-apoptotic protein Bad [85]. However, the complete mechanisms involved in the pro-apoptotic effect of EGCG on mature adipocytes are not completely elucidated.

In human preadipocytes, quercetin induces apoptosis through a decreased expression of NFκB and an increase of the pro-apoptotic Bad protein [85]. Quercetin has also been shown to promote mature adipocyte apoptosis *in vitro* Fig. (**1B**). Indeed, quercetin (10 to 100 µM) induces apoptosis of 3T3-L1 adipocytes in a concentration-dependent manner [77, 78]. Quercetin stimulates the activation of caspases, increases the expression of pro-apoptotic proteins such as Bax and Bak and decreases expression of anti-apoptotic proteins such as Bcl-2 [77] with an enhancement of cytochrome c release into the cytosol [78]. Furthermore, the pro-apoptotic properties of quercetin are associated with the activation of AMPK [77], which has been reported to promote apoptosis [87]. The pro-apoptotic effect of quercetin on adipocytes also involves the modulation of the ERK and c-Jun N terminal kinase (JNK) pathways [77].

In vitro studies demonstrate the pro-apoptotic properties of genistein on mature adipocytes [72, 80, 88] Fig. (**1C**). Genistein (at concentrations of 100 µM to 400 µM) is able to promote adipocyte apoptosis, whereas lower concentrations had no effect. The *in vitro* pro-apoptotic effects of genistein are associated with the activation of AMPK [72]. Genistein has been also used *in vivo*, by sub-cutaneous injection (80-200 mg per kg of body weight per day during 21 days) [89], or by diet supplementation (1500 mg per kg of body weight per day during 21 days) [88] in ovariectomized mice. Genistein treatment led to a decrease in body fat mass [88, 89], through the induction of adipocyte apoptosis [88]. These effects involve a mechanism dependent on the estrogen receptor alpha (ERα) since genistein fails to decrease body fat mass in mice lacking this receptor [89].

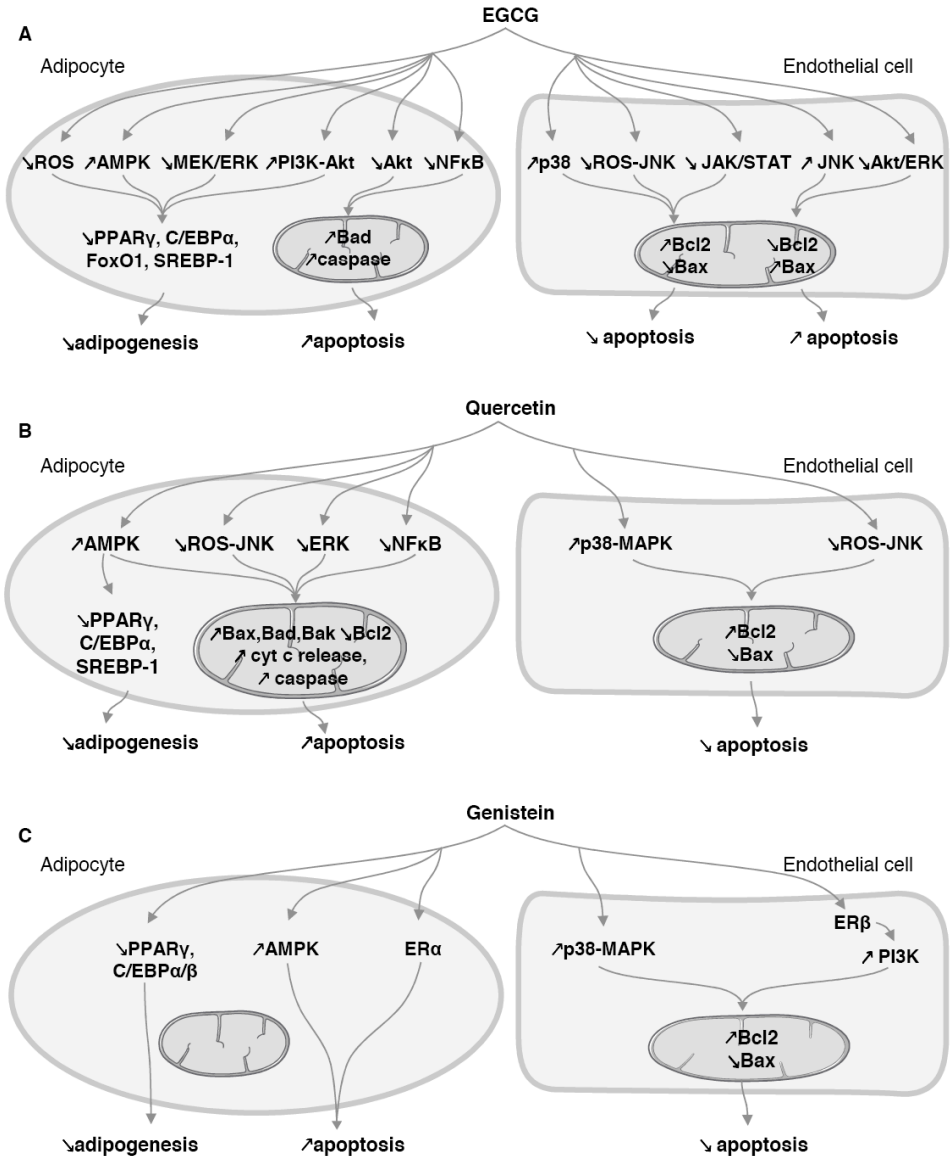

Fig. (1). Schematic representation of the well-studied flavonoids EGCG (A), quercetin (B) and genistein (C) on both endothelial cells and adipocytes. AMPK, AMP-protein kinase; C/EBP, CCAT/enhancer-binding protein; ER, estrogen receptor; ERK, extracellular-activated protein kinase; FoxO1, forkhead-box protein O1; JAK, janus kinase; JNK, cJun N terminal kinase; MEK, mitogen-activated protein kinase; NFκB, nuclear factor kappa B; PI3K, phsphatidylinositol-3-kinase; PPARγ, peroxisome proliferator-activated receptor gamma; ROS, reactive oxygen species; SREBP, sterol regulatory element binding protein; STAT, signal transducer and activator of transcription.

Finally, other flavonoids including isoxanthohumol (75 μM) have been reported to increase mature adipocyte apoptosis, through a decrease of the mitochondrial membrane potential, cytochrome c release into the cytosol and caspase activation [83]. Moreover, naringenin and hesperetin induce human pre-adipocytes apoptosis, through a decrease of anti-apoptotic protein expression (NFκB, Akt, Bcl2) and an increase of Bad expression [90].

EFFECTS OF FLAVONOIDS ON LIPID METABOLISM

A large number of studies report that flavonoids regulate lipid metabolism in adipocytes. Indeed, EGCG [91], quercetin [76], genistein [89] or even naringenin and hesperetin [90] have been shown to decrease lipid accumulation. However, no signaling pathways involving mitochondria are described to explain these properties.

Nonetheless, mitochondria represent a promising tool to regulate lipid metabolism. Indeed, the increase of mitochondrial content through the stimulation of mitochondrial biogenesis may potentially enhance lipolysis leading to a decreased fat content [92]. Moreover, increasing mitochondrial content can potentially decrease the excessive mitochondrial ROS production, and thus, prevent their deleterious effects on adipokine expression [93]. However, to the best of our knowledge, no study has investigated the effects of flavonoids on mitochondrial biogenesis in adipocytes, which represent a promising research topic.

EFFECTS OF FLAVONOIDS ON BODY WEIGHT AND FAT MASS MODULATION (TABLE 4)

Several studies have examined the effect of flavonoid-rich diet on body and/or fat mass weight variations in animal models of obesity and in some human clinical and epidemiological investigations (Table **4**).

Table 3. Modulation of Preadipocyte/Adipocyte Apoptosis by Flavonoids

Flavonoids	Concentration	Cell Type	Apoptosis	Effects	Reference
EGCG	50-200 μM	3T3-L1 Mature adipocyte	↑	-	[86]
	100-400 μM	3T3-L1 Preadipocyte		↓cyclin-cdk2, ↓caspase-3	[84]
	100 μM	Human Preadipocyte AML-I		↓NFκB, ↓pAkt, ↑Bad	[85]
Quercetin	100 μM	3T3-L1 mature adipocyte	↑	↑AMPK, ↓JNK, ↑caspase, ↑Bax, ↑Bak, ↓Bcl2	[77]
				↑cytochrome c release	[78]
				↓ERK	[77, 78]
		Human Preadipocyte AML-I		↓NFκB, ↑Bad	[85]
Genistein	50-100 μM	3T3-L1 mature adipocyte	↑	↑AMPK	[72]
	10-400 μM			-	[71]
	100 μM			-	[80]
	80-200 mg/kg body weight/day	Ovariectomized Mice, sub-cutaneous injection		↑adipose tissue apoptosis, ↓Body fat mass	[88]
	1500 mg/kg body weight/day	Ovariectomized Mice, diet supplementation		ERα-dependent, ↓Body fat mass	[89]
Isoxanthohumol	75 μM	3T3-L1 mature adipocyte	↑	↓mitochondrial membrane potential, ↑cytochrome c release, ↑caspase	[83]
Naringenin / Hesperetin	250-500 μM	Human Preadipocyte AML-I	↑	↓NFκB, ↓Akt, ↓Bcl2, ↑Bad	[90]

Table 4. Modulation of Body Weight and Fat Mass by Flavonoids

Treatment	Dose	Duration	Species	Body Weight	Fat Mass	Ref
FRF	100 mg/kg/day	21 days	Mice	↓	nd	[94]
EGCG	0.5-1% in diet	4 weeks	Mice	↓	↓	[95]
EGCG	85 mg/kg bw/day	5 days	Mice	↓	nd	[96]
EGCG	0.2-0.5% in fluid	11 months	Mice	↓	↓	[97, 98]
EGCG	0.32% in diet	16 weeks	Mice	↓	↓	[99]
EGCG	1% in diet	9 weeks	Mice	↓	nd	[100]
EGCG	0.32% in diet	17 weeks	Mice	↓	nd	[101]
EGCG	0.32% in diet	16 weeks	Mice	↓	nd	[102]
EGCG	0.32% in diet	6 weeks	Mice	↓	nd	[103]
GTE	1-2% in diet	6 weeks	Mice	↓	↓	[104]
GTE	0.2-0.5% in diet	10 weeks	Mice	=	↓	[111]
GTC	500-900 mg/day	90 days	Human	↓	nd	[105]
GTC	250 mg/day	8-12 weeks	Human	↓	nd	[106]
GTC	270-1200 mg/day	12 weeks	Human	↓	nd	[107]
GTC	690 mg/day	12 weeks	Human	↓	↓	[108]
GTC	530 mg/day	6 weeks	Human	↓	nd	[109]
GTC	1500 mg/day	8 weeks	Human	↓	↓	[110]
Quercetin	0.025% in diet	9 weeks	Mice	↓	↓	[112]
Quercetin	0.05% in diet	20 weeks	Mice	nd	↓	[113]
Quercetin	0.8 g/kg in diet	8 weeks	Rat	↓	↓	[114]
Quercetin + vitamin D	148 μM/kg	8 weeks	Rat	↓	nd	[115]
Quercetin +δtocotrienol	80-400-2000 mg/kg in diet	4 weeks	Chicken	↓	nd	[116]
Quercetin+vitamin C	500-1000 mg/day	12 weeks	Human	=	↓	[117]
Genistein	1500 mg/kg bw/day	12 days	Mice	nd	↓	[89]
Genistein	80-200 mg/kg bw/day	21 days	Mice	↓	↓	[88]
Genistein	0.1-0.4% in diet	12 weeks	Mice	↓	↓	[118]

(Table 4) contd…

Treatment	Dose	Duration	Species	Body Weight	Fat Mass	Ref
Genistein	250 mg/kg in diet		Mice	=	nd	[119]
Genistein	42 mg/kg bw/day	10 weeks	Rat	=	nd	[120]
Genistein	4-40-160 mg/kg bw	6 weeks	Rat	↓	nd	[121]
Genistein+vitamin D	64-256-1040 mg/kg in diet	8 weeks	Rat	↓	↓	[115]
Biochanin A	10 mg/kg bw	45 days	Rat	=	nd	[122]
Genistein/Daidzein	50 mg/day	8 weeks	Human	=	nd	[123]
Isoflavones	75 mg/day	1 year	Human	nd	↓	[124]
Cyanidin3-glucoside	2 g/kg of diet	5 weeks	Mice	=	=	[126]
Cyanidin3-glucoside	1 g/kg of diet	12 weeks	Mice	↓	↓	[129]
ABE	27 g/kg of diet	5 weeks	Mice	=	=	[125]
BE	4% in diet	8 weeks	Mice	=	=	[127]
BAE	2.9 mg/g of diet		Mice	↓	↓	[128]
Naringin	0.2 g/kg of diet	20 weeks	Mice	↓	↓	[101]
Naringin	1 or 3% in diet	4 weeks	Mice	↓	nd	[130]
Naringin	3% in diet	6 months	Mice	↓	nd	[131]

FRF, flavonoids rich fraction; EGCG, epigallocatechin gallate; GTE, green tea extract; GTC, green tea catechin; ABE anthocyanidin rich bilberry extract; BE, bilberry extract; BAE, blueberry anthocyanidin extract. Nd, non-determined; =, unchanged; ↓, decrease.

The effect of a diet contained flavonoid rich fraction composed of chlorogenic acid, rutin, luteolin-7-O-glucoside, isorhoifolin, apigenin-7-O-glucoside and quercetin was investigate in mice. Daily intake of this mixture, at a dose of 100 mg/Kg, causes a significant reduction of mice body weight [94].

Flavanols show a reduction of body weight in mice and human, independently from dose and duration of treatments [95-110], except for a green tea extract (at doses of 0.2 or 0.5% in diet for 10 weeks) [111]. Moreover, they are able to decrease fat mass [95, 97-99, 104-111].

Low doses of quercetin in mice reduce adipose tissue weight [112, 113]. In rat, 8 weeks of quercetin treatment, alone or in combination with vitamin D, reduces both body weight and fat mass [114, 115]. Moreover, concomitant consumption of quercetin and δ-tocotrienol in chicken shows that these compounds decrease total weight [116]. However, the intake of quercetin at two different doses associated with vitamin C is not able to modify body weight in humans [117].

Differential effects of isoflavones have been observed on rodent and human weight variations. Regarding total weight, these compounds show differential effects in rodent, probably due to species or different doses and durations used. They decrease fat mass whatever the model used [88, 89, 115, 118-122]. Isoflavones are not very effective in reducing body weight in postmenopausal women, although they did decrease fat mass in a long-term study [123, 124].

Anthocyanidins have no effect on weight in mice [125-127], except in two studies [128, 129] where a reduction of body weight and adipose tissue has been reported.

Finally, reports on favanone naringin have shown a decrease of total weight independently of the dose or the duration. However, it is not clear if the observed reduction is associated with a lowering of fat mass in mice [101, 130, 131].

In general, these *in vivo* investigations suggest that consumption of a flavonoid-rich diet may facilitate weight loss and prevent weight gain. However most of data have been obtained from animal models and their relevance to human needs further investigation. Furthermore, more studies are needed to determine the long-term consequences of high flavonoid intake.

FLAVONOIDS TARGETING MITOCHONDRIA IN ENDOTHELIAL CELLS

Flavonoids have been shown to preserve endothelial integrity and function by decreasing apoptosis, ROS production or by increasing NO availability. Since mitochondria are implicated in all of these processes, one could advance the hypothesis that these organelles may be a potential therapeutic target [132].

FLAVONOIDS TARGETING ENDOTHELIAL CELL APOPTOSIS (TABLE 5)

The effects of different class of flavonoids on the regulation of endothelial cell apoptosis have been described in many studies. Flavonoids can either prevent [29, 30, 133], or promote endothelial cell apoptosis [133-135], depending on their nature and on the apoptotic *stimuli* used.

For instance, EGCG have been described to possess anti-apoptotic properties on endothelial cells stimulated either with oxLDL [29, 30] or hydrogen peroxide (H_2O_2) [133, 134] Fig. (**1A**). Indeed, EGCG (50 μM) prevents oxLDL-induced apoptosis of endothelial cells through a decrease of Bax expression and an increase of Bcl2 expression [29]. Other studies report that EGCG (25 μM) reduces oxLDL-induced apoptosis through the inhibition of the ROS-triggered activation of JNK, and the blocking of janus kinase (JAK) 2/signal transducer and activator of transcription (STAT) 3-dependent signaling pathway. These effects result in the reduction of caspase activation and consequently, in lowering apoptosis in response to oxLDL [30]. Furthermore, the effect of EGCG (50 μM) has also been studied on H_2O_2-induced endothelial apoptosis. EGCG enhances Bcl2 expression and inhibits Bax expression [133] through a mechanism that prevents ROS-triggered activation of JNK and p38 mitogen-activated protein kinase (MAPK)-dependent pathways and caspase activity. The anti-apoptotic properties of EGCG appear to be achieved essentially through its antioxidant capacity. Finally, in a context of *in vitro* ischemia/reperfusion injury, EGCG used at 10 or 100 μM promotes endothelial cell apoptosis through the inhibition of Akt and ERK

Table 5. Modulation of Endothelial Cell Apoptosis by Flavonoids

Flavonoids	Concentration	Cell Type	Apoptotic Stimuli	Apoptosis	Effects	Reference
EGCG	50 µM	HUVECs	oxLDL	↓	↑Bcl2, ↓Bax	[29]
	25 µM		oxLDL		↓ROS-induced JNK activation ↓JAK/STAT	[30]
	50 µM		H_2O_2		↑Bcl2, ↓Bax, ↓ROS-induced JNK activation, ↑p38-MAPK	[133]
	10-100 µM		*In vitro* ischemia/reperfusion	↑	↓Akt/ERK, ↑JNK	[135]
Quercetin	50 µM	HUVECs	oxLDL	↓	↑Bcl2, ↓Bax, ↓caspase	[29]
			H_2O_2		↑Bcl2, ↓Bax, ↓ROS-induced JNK activation	[133, 134]
	300 nM		High Glucose		↓ROS-induced JNK activation, ↑p38-MAPK	[136]
Genistein	5-10 µM	HAECs	TNFα	↓	↑Bcl2, ↓Bax, ↑p38-MAPK	[137]
	100 nM	HUVECs	H_2O_2		ERβ-dependent, ↑PI3K, ↑Bcl2, ↓Bax	[28]
Delphinidin	30 µM	BAECs	7-β-hydroxycholesterol	↓	↑NO, ↓cytochrome c release	[138]
	25 µM	BAECs	Peroxynitrite		↑PI3K, ↑mitochondrial membrane potential, ↑Bcl2	[140]
	100-600 µM	HUVECs	oxLDL		↓ROS, ↑Bcl2, ↓Bax	[139]
Apigenin	50 µM	HUVECs	H_2O_2	↑	↑ROS, ↑phospho-p53, ↑Bax, ↓Bcl2, ↑caspase	[133, 134]

expression and the subsequent enhancement of JNK activity, exacerbating ischemia/reperfusion-induced endothelial apoptosis [135]. According to these studies, the JNK-pathway appears to be an essential signaling pathway regulated by EGCG to control endothelial cell apoptosis. However, the cellular source of ROS leading to activation of the JNK pathway is not elucidated and remains to be clarified.

In the same way, the anti-apoptotic properties of quercetin (50 µM) have been studied on oxLDL- [29] or on H_2O_2-induced [133, 134] apoptosis in endothelial cells Fig. (**1B**). Quercetin prevents the increased expression of Bax, and promotes Bcl2 expression, associated with a reduction of caspase activation. In oxidant-induced apoptosis, quercetin prevents endothelial cell apoptosis through the regulation of the balance between Bax and Bcl2 [133] and blunts ROS-triggered activation of JNK and p38 MAPK [134]. Interestingly, a recent study shows that quercetin used at nanomolar concentrations (300 nm) is able to prevent endothelial cell apoptosis induced by high glucose through the inhibition of ROS-induced JNK activation [136].

Isoflavones have also been tested to prevent endothelial cell apoptosis Fig. (**1C**). For instance, genistein used at 5-10 µM protects endothelial cells against TNFα-induced apoptosis *via* regulation of Bax/Bcl2 expression. This effect involves the activation of p38 MAPK but not the ER pathways [137]. In another study, the protective effect of genistein is described on oxidative stress-induced endothelial cell apoptosis [28]. These authors show that genistein used at nanomolar concentrations (100 nM) protects against oxidative stress-induced apoptosis. The mechanisms involved an ERβ-dependent pathway, the modulation of the balance between the expression of Bcl2 and Bax, and the activation of cell survival signaling such as PI3K pathway [28].

A number of authors have studied the effects of anthocyanins on endothelial cell apoptosis [138-140]. First, delphinidin used at low concentration (30 µM), inhibited 7β-hydroxycholesterol-induced-apoptosis by preventing cytochrome c release from the mitochondria *via* a mechanism involving the NO pathway and regulation of calcium homeostasis [138]. Moreover, delphinidin reduces apoptosis of endothelial cells induced by peroxynitrite through the prevention of decreased mitochondrial membrane potential and the increase of Bax expression [140]. Finally, high concentrations of delphinidin (100-600 µM) prevent oxLDL-induced apoptosis through a significant decrease of oxidative stress and a modulation of Bcl2/Bax balance [139]. A number of mechanisms may participate in the antiapoptotic properties of delphindin depending on the *stimuli* and the concentrations used. As previously described by our team [141], the α isoform of ER is a target of this flavonoid, that may be involved in the regulation of endothelial cells apoptosis.

Finally, unlike others flavonoids, flavones, and most particularly apigenin, promote endothelial cell apoptosis [133, 134]. Apigenin amplifies the oxidative stress induced by H_2O_2, leading to increased phosphorylation of p53 and its translocation into the nucleus, the increased expression of Bax and decreased expression of Bcl2, and finally to caspase activation.

To conclude, flavonoids affect multiple signaling pathways to protect endothelial cells from apoptosis. The anti-apoptotic properties of flavonoids against factors known to play a key role in the development of metabolic and cardiovascular diseases may be of importance in the preservation of endothelial integrity. However, the *in vivo* relevance of the results obtained from *in vitro* cell culture needs to be verified *in vivo* in order to confirm the benefit of these polyphenolic compounds.

FLAVONOIDS TARGETING MITOCHONDRIAL ROS PRODUCTION IN ENDOTHELIAL CELLS

The protective effect of flavonoids in human health is partly explained by their antioxidant properties [142]. Mitochondria derived ROS are involved in physiological pathways, but their excessive production is deleterious. As a consequence, therapeutic strategy requires a careful balance between the "good"

(physiological) and "bad" (pathological) ROS. Flavonoids have been shown to induce mitochondrial ROS production for signal transduction [143, 144], or even can regulate their deleterious production in endothelial cells [145].

EGCG (25-100 μM) has been reported to upregulate expression of the cytoprotective enzyme heme oxygenase 1 (HO-1), in response to oxidative injury [143]. In this study, rotenone, an inhibitor of complex I activity, prevents HO-1 upregulation by EGCG. It has been hypothesized that EGCG may cause a mild increase in mitochondrial ROS production in order to trigger the signaling pathways that upregulate HO-1 gene expression [143].

Genistein has been reported to correct excessive mitochondrial ROS production and thus, prevents endothelial dysfunction. For instance, in aortic endothelial cells, 50 μM of genistein prevents the mitochondria-derived oxidative stress induced by leptin *via* inhibition of tyrosine kinase and stimulation of fatty acid oxidation. These findings suggest that genistein may block the progression of atherosclerosis through a preservation of endothelial function [145]. The effect of soy isoflavone equol (100 nM), a product derived from daidzein metabolism from intestinal gut microflora, has been reported to involve mitochondrial ROS as a key element for its signal transduction. Indeed, the inhibition of mitochondrial ROS abolishes equol-induced activation of Akt, ERK1/2, eNOS phosphorylation and NO production [144].

Another promising potential way to preserve endothelial function is to promote mitochondrial biogenesis to regulate mitochondrial ROS production. This is an attractive goal for preventing the reduction of mitochondrial mass, reported to be an early manifestation of endothelial dysfunction [146] and to increase the removal of cytosolic superoxide anion, since mitochondria are able to scavenge extramitochondrial superoxide anion [147]. Thus, many flavonoids including flavanols (EGCG [148]), flavonols (quercetin [148]), isoflavone (equol [144]) and anthocyanins (delphinidin [141]) have the capacity to improve endothelial function by stimulating the endothelial formation of NO by eNOS. NO is a key regulator of mitochondrial content due to its ability to regulate expression of mitochondrial biogenesis factors such as nuclear respiratory factors (NRF1 and NRF2) [149]. Consequently, flavonoids may have the potential to correct endothelial function by stimulating mitochondrial biogenesis. Indeed, many flavonoids including quercetin [150, 151], isoflavone (genistein, daidzein) [152] or anthocyannins [153] are reported to increase mitochondrial biogenesis in various cell type such as renal, muscular or neuronal cells. However, even if a role of flavonoids in the regulation of mitochondrial biogenesis is largely supported, the demonstration of such a link in endothelial cells needs to be confirmed.

PERSPECTIVES OF CLINICAL USE OF FLAVONOIDS AGAINST OBESITY AND OBESITY RELATED VASCULAR DISEASES

Although a large number of *in vitro* studies have demonstrated beneficial effects of flavonoids against adipocyte or endothelial dysfunction, the concentrations used in the majority of these *in vitro* studies (25 to 400 μM), seem to be higher than those observed in human plasma and tissues [41, 42, 154, 155]. For instance, after an infusion of green tea containing 112 mg of EGCG, the plasma concentration of EGCG varies from 19 to 262 nM [156]. Consequently, the possible *in vivo* extrapolation from these *in vitro* experiments requires caution.

Even though low concentrations of unconjugated flavonoids and their various metabolites are found in plasma, these do not necessarily associate with a lack of *in vivo* effect. Recently, the notion of the "flavonoid paradox" has been described. Thus, oral administration of flavonoids exerts biologically demonstrable systemic effects, while their circulating forms show weak activity *in vitro* [157]. Despite low concentration of flavonoids and their metabolites in plasma, flavonoids may accumulate in specific tissues, including liver, small intestine, and kidneys, where their concentration reached up to 17-fold the concentration observed in plasma [158]. Thus, it might be possible that such high concentrations can be reached in the target tissues. Moreover, β-glucuronidase activity was detected in some tissues, including kidneys, lung, muscle or vessels [44, 158], suggesting a possible deconjugation of flavonoids, and thus an *in situ* production of aglycone from its glucuronide form. Indeed, quercetin-3-O-glucuronide can act as molecule for the plasmatic transport of quercetin to the target tissues. Quercetin released from its glucuronidated metabolites could be responsible for its beneficial properties such as vasorelaxant and hypotensive effects [44, 159]. Finally, the hormesis concept has also been advanced referring to the phenomenon by which benefits can be obtained with "low doses" of external stressors whereas higher concentrations are noxious. It is now considered that several plant antioxidants exhibit hormetic properties, by acting as "low-dose stressors" that may prepare cells to resist to more severe stress [160].

Therefore, while flavonoids have been implicated in many aspects of cellular energy metabolism, *in vivo* studies regarding these compounds are limited [161]. Indeed, no *in vivo* studies have assessed the impact of flavonoids on the function and the mitochondrial biogenesis. However, clear effects on energy metabolism have been identified with EGCG [111]. Indeed, EGCG prevents diet-induced obesity in mice and rats [162] and reduces leptin levels in mice fed with high-fat diet [95]. A pilot study shows that EGCG has the potential to increase fat oxidation in men and may thereby contribute to the anti-obesity effects of green tea [163].

Many clinical studies have used a mixture of several polyphenols, and the active molecule(s) is (are) generally not known. The presence of related compounds in mixtures and extracts makes it difficult to determine the respective contribution of each in the overall effect. However, although many *in vitro* studies have shown the beneficial effects of flavonoids in metabolic diseases, caution is mandatory when attempting to extrapolate these observations to *in vivo* animal models and, most importantly, to humans, since none of these experimental features have yet been proved to occur in humans.

Flavonoids may contribute to the maintenance of health and the treatment of metabolic diseases. These studies are consistent with the epidemiological evidence that consumption of fruits and vegetables [164] prevent obesity and metabolic diseases [36]. However, the results of the studies described in this review are not unequivocal, which is not surprising considering the variety of study design, the lack of clinical studies and the diversity of flavonoids tested. Therefore, consistent results from well-designed, long-term studies with flavonoids would greatly facilitate the understanding of the benefits of flavonoid consumption.

CONCLUSION

There is traditional and widespread use of dietary flavonoids all around the world. While the epidemiological evidence has historically supported the idea of a link between varied diet and health, experimental evidence accumulated in recent years from various pre-clinical studies clearly supports the idea that flavonoids play a crucial role in the prevention of metabolic diseases such as obesity. Recent studies highlight the crucial role of mitochondria in the development of metabolic diseases that may be initiated by a combination of both reduced mitochondrial activity and increased mitochondrial ROS production. As discussed in this review, benefits of flavonoids on mitochondrial function have been established in both adipocytes and endothelial cells. Thus, the potential use of flavonoids in the preventing obesity is tremendous. However, the cellular and molecular action of flavonoids involved

in metabolic diseases needs further study. Because obesity has been classified as a growing epidemic and despite few strategies to effectively prevent or attenuate this disease, it is important to determine the potential beneficial effects of dietary polyphenols in the prevention and alleviation of such complications in humans.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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