Flavonoids, Obesity and Metabolic Syndrome

Subjects: Others

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Obesity is a medical condition, defined by WHO as an abnormal or excessive accumulation of fat that can compromise health. Energy imbalance in which energy intake is greater than energy expenditure is the primary cause of visceral or central obesity, as excess energy is stored as triglycerides within adipocytes, which increase in size (hypertrophy phynotype), and number (hyperplasic phenotype), or both. By accumulating excess energy, adipocytes become hypertrophic, which causes the release of free fatty acids into the circulation (lipotoxicity), adipocytes change their immunological balance, which promotes, with adipose tissue resident immunes cells (macrophages), the production and the circulating levels of proinflammatory cytokines and decreases the concentration of anti-inflammatory adipokines, such as adiponectin. These changes in adipose tissue lead to the development of chronic state of low-grade inflammation that secondarily generates obesity-related complications, commonly known as metabolic syndrome. This syndrome includes insulin resistance, hyperglycemia type 2 diabetes mellitus (T2DM), cardiovascular diseases, dyslipidemia (decreased concentration of cholesterol and triglycerides), steatosis, fibrosis, hypertension, heart attack. The literature strongly suggests that flavonoids demonstrate an important biological effect on obesity, as demonstrated by their ability to lower fat mass, lipid droplets in the liver, and total triglycerides/cholesterol in both in vitro and in vivo models.

zebrafish flavonoids polyphenols obesity inflammation obesity models natural compounds

1. Effects of Flavonoids on Lipid Accumulation

Lipids accumulate is caused by an increase in the size of adipocyte (hypertrophy) and an increase in their number (hyperplasia). Hyperplasia is regulated by the differentiation of multipotent mesenchymal stem cells into preadipocytes that, under appropriate stimulation, can differentiate terminally into mature adipocytes. These adipocytes are capable to storing excess energy as cytoplasmic neutral lipid droplets of different sizes which can exceed 100 micrometers [1][2]. In this regard, it has been reported that flavonoids have great potential. They deal with lipid accumulation through numerous mechanisms, including the inhibition of adipocyte differentiation, primarily caused by the reduced expression of important regulatory adipogenic transcription factors, decreased lipogenesis, and induction of adipocyte apoptosis [3]. Thus, quercetin, a plant flavonol found in a wide variety of vegetables and fruits, reduces the lipid accumulation through decreasing preadipocyte differentiation, lipogenesis, and induction of adipocyte apoptosis [4][5]. The inhibition of adipogenesis was regulated by the downregulation of central transcriptional regulators of adipogenesis (SREBP-1, C/EBPα, and PPARγ) and FAS, a key adipogenic enzyme. Quercetin increasing apoptosis involved mitogen-activated protein (MAP) kinases, specifically the

decrease in extracellular signal-regulated kinases (ERKs). Thus, inhibition of the extracellular signal-regulated kinases enhances apoptosis [5]. Kaempferol, another flavonoid, negatively regulates adipogenesis by downregulating PPARγ, aP2, and SREBP-1C [6] and CCAAT-enhancer-binding protein alpha [7] in 3T3-L1 adipocytes. In zebrafish, kaempferol has anti-adipogenic properties that regulate early adipogenic factors (KLF5, KLF4, KLF2, and C/EBPβ) [6]. Upregulation of lipolysis regulatory enzymes (Adipose triglyceride lipase (Pnpla2)) is one of the anti-adipogenic mechanism of kaempferol [7]. In addition, baicalein, a type of flavonoid originating from *Scutellaria baicalensis*, has a significant capacity to decrease lipid accumulation in zebrafish in a dose-dependent manner [8]. In 3T3-L1 cells, baicalein inhibit lipid accumulation during adipogenesis by arresting cell cycle in the G0/G1 phase through cyclin downregulation, suppressing the mRNA expression of early adipogenic factors. The above-mentioned factor shortage leads to the downregulation of late adipogenic factors, negatively regulating the m-TOR signaling pathway, involved in lipid accumulation during adipogenesis and decreased p-p38 MAPK and pERK levels in adipocytes [8][9]. *Puerariae Lobatae* radix flavonoids and puerarin successfully limited lipid accumulation in the abdomens of zebrafish larvae in a dose dependent manner [10].

2. Effects of Flavonoid on Triglycerides

Triglycerides (TG) are important factors in fat accumulation during adipocyte differentiation. During lipogenesis, glycerol-3-phosphate acyltransferase converts glyceraldehyde-3-phosphate, a glucose metabolite, to lysophosphatidic acid (LPA); lysophosphatidic acid acyltransferase-θ (LPAATθ) converts LPA into phosphatidic acid (PA), a biosynthetic precursor of acylglycerols. PA is converted into diacylglycerol (DAG) by lipin1 and diglyceride acyltransferase-1 (DGAT1) catalyzing the conversion of DAG into TG [4]. Lipin1 is linked to low-density lipoprotein secretion and PPARγ expression. PPARγ expression is stimulated by m-TOR signaling, and m-TOR together with AKT (the upstream factor of m-TOR) stimulates TG synthesis. Available data suggest that flavonoid inhibited triglycerol levels in a dose-dependent manner [6]. The lipogenic factors involved in TG synthesis (LPAATθ, DGAT1 and lipin1) are dependent on adipogenesis. During adipogenesis, early adipogenic transcription factors, such as CCAAT/enhancer-binding protein-β(C/EBPβ), induce key adipogenic factors, such as CCAAT/enhancer-binding protein-α(C/EBPα) and peroxisome proliferator-activated receptor-γ(PPARγ). The expression of C/EBPα and PPARγ activates lipid synthetic proteins, including fatty acid-binding protein 4 (FABP4) and lipin1 [4]. Flavonoids have the capability to inhibit TG accumulation. This effect can be explained by the effect of flavonoids to reduce the levels of C/EBPβ, C/EBPα and PPAR γ protein in a dose-dependent manner, resulting in reduced levels of LPAATθ, DGAT1 and lipin1. Thus, quercetin inhibited TG accumulation by >40%, while curcumin, an antiadipogenic phytochemical, inhibited TG accumulation by ~25% [4]. Baicalein, kaempferol, and eriocitrin, flavonoids that have lately been reported, significantly suppressed the increase in TG in DIO-zebrafish [10][6][8].

3. Flavonoid and Cholesterol

The DIO zebrafish model, has a high cholesterol level. Many flavonoids, previously studied for anti-obesity effects, have the ability to reduce cholesterol levels. Puerariae Lobatae radix flavonoids and puerarin significantly downregulated the elevated mRNA levels of the 3-hydroxy-3-methylglutaryl coenzyme A reductase b (HMGCRB),

the key enzyme involved in lipid metabolism and cholesterol biosynthesis, in zebrafish larvae [10]. On the other hand, baicalein decreased the expression levels of SREBP1 and fatty acid synthase (fasn) and regulated the synthesis and desaturation of fatty acids, proteins and a master gene regulating cholesterol synthesis. Naringenin instead significantly induced a downregulation of the zebrafish larva mRNA of the fatty acid desaturase 2 (fads2), a dyslipidemia-related gene which influences the concentrations of total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol and triglycerides, fasn, enoyl-CoA hydratase, short chain 1 (echs1), Fatty acid-binding protein 10a (fabp10α), HMG coenzyme A reductase a (hmgcra) and hmgcrb [11].

4. Effects of Flavonoid on Inflammation

Obesity is a chronic state of low-grade inflammation. During the development of obesity, progressive hypertrophy of adipocytes promotes tissue hypoxia and macrophages infiltration, which induce the increased secretion of various proinflammatory mediators, such as tumor necrosis factor alpha (TNF-α), interleukin 6 (IL −6), plasminogen inhibitor 1 (PAI-1), C-reactive protein (CRP), and monocyte chemoattractant protein 1 (MCP-1), among others, besides the decrease in the concentration of anti-inflammatory adipokines, such as adiponectin, characterizing a chronic inflammation of low grade 6 [12].

Additionally, during obesity, adipose tissue produces a greater amount of reactive oxygen species (ROS) which causes oxidative stress. This stress in turn leads to the abnormal adipokines production (chronic low-grade inflammation), where it has been shown, for example, that adiponectin concentration is inversely related to the concentration of ROS [13].

There are multiple flavonoids that have been shown to be useful as anti-inflammatory agent in the low-grade inflammation that occurs in obesity. Quercetin, in particular, has been shown to have anti-inflammatory effects through the inhibition of MAPK signaling factors (ERK1/2, JNK and p38MAPK) and, consequently, to inhibit the secretion of inflammatory cytokines IL-1β and IL-6, MCP-1 and TNF-α in 3T3-L1 adipocytes and macrophages. In addition, quercetin has the ability to stimulate the amount of IL-10, a known inhibitory factor of cytokine synthesis and anti-inflammatory cytokine [4]. In studies conducted with Puerariae Lobatae radix flavonoids, puerarin, and Citrus sinensis flavonoids, they were observed to possess important anti-inflammatory activity, assured by the downregulation of inflammatory cytokine-related genes, such as IL-1β (interleukin-1β), IL-6 and TNFα (tumor necrosis factor-α) in a zebrafish model [10][14]. The flavonoid-rich extract of Withania somnifera leaf, essentially with kaempferol and agigenein, revealed the significant inhibition of TNFα in adult zebrafish [15].

In the DIO-zebrafish visceral adipose tissue, the ability of yuzu peel, vinaccia, and auraptene to upregulate the expression of adipokines, adiponectin negatively correlated with markers of inflammation and oxidative stress, has been demonstrated [16][17].

Medlar leaf, grape skin and acai puree are three major sources of flavonoids tested for their anti-atherosclerotic and anti-diabetic activity. These plant extracts have shown antioxidant, anti-inflammatory and anti-atherosclerotic activities [18].

5. Insulin Resistance

Insulin resistance (IR), defined as a decreased ability of cells to respond to insulin stimulation, is a crucial feature of prediabetes and is the first detectable abnormality in type 2 diabetes mellitus, a progressive metabolic disorder characterized by high blood glucose concentration, abnormalities in carbohydrate, lipid, and protein metabolism, [19][20][21]. In the early phase of IR, normal pancreatic β cells increase insulin production to compensate for IR and glucose utilization remains relatively normal. However, when IR continues, β cells gradually fail to secrete adequate amounts of insulin for metabolic compensation, leading to insulin insufficiency and impaired glucose tolerance. IR usually occurs in peripheral tissues, such as liver, adipose, and skeletal muscle [20][21]. Accumulating evidence shows that inflammation initiated by adipose tissue is a major contributor to the development of IR and T2D. Elevated levels of proinflammatory cytokines (tumor necrosis factor α (TNF-α), interleukin 6 (IL-6), interleukin 1β (IL-1β) and resistin) secreted by adipose tissue and macrophages infiltrating, as well as decreased levels of anti-inflammatory cytokines (interleukin 10 (IL-10) and adiponectin), have been reported in various diabetic and IR states [20][21]. The release of various cytokines and chemokines promotes the migration and activation of macrophages, which are recruited to the islet and further enhance the inflammatory environment by exacerbating the release of cytokines causing β-cell loss [21][22]. In the aforementioned section of our review, we extensively discussed the modulation of cytokines by flavonoids with an emphasis on therapeutic application against inflammation. Flavonoids, which showed anti-inflammatory properties, have been suggested as an excellent candidate to prevent hyperglycemia and the IR of zebrafish larvae by targeting inflammatory signals [19][4][18][21][23].

6. Flavonoid Effects on Non-Alcoholic Fatty Liver Disease

Non-alcoholic fatty liver disease (NAFLD), is an excessive accumulation of neutral lipids in the liver due to elevated hepatic lipogenesis, and low hepatic excretion of very low-density lipoprotein (VLDL) associated with metabolic syndrome, particularly obesity, insulin resistance, type 2 diabetes and cardiovascular disease and encompasses a spectrum of liver disorders, ranging from steatosis to non-alcoholic steatohepatitis (NASH), fibrosis, cirrhosis, and hepatocellular carcinoma [24][25][26][27]. In zebrafish, diet composition has a significant impact on the development of NAFLD. It is known that the excessive consumption of calories through overeating or a high fat or high sugar diet can induce obesity and hepatic steatosis [25][28].

Several flavonoids have been found to have positive effects on lipid metabolism, insulin resistance and inflammation, the most important pathophysiological pathways in NAFLD [24]. The effect of flavonoids on NAFLD was conducted through upregulation of PPARα which stimulates β-oxidation, mitigate inflammation and increases energy expenditure [24][16]. Another target identified in the treatment of NAFLD are fatty acid synthesis (FASN) associated genes and sterol regulatory element-binding protein 1 (SREBP-1C), a transcription factors that controls de novo lipogenesis through the induction of lipogenic enzymes that stimulate steatosis [24]. In zebrafish, several flavonoids, such as kaempferol and baicalein, reduce both SREBP-1c and FASN protein and gene expression [6][8]. Flavonoid-rich yuzu peel extract, increased mRNA expression of lipid oxidation markers (pparab, the zebrafish homolog of human *pparay*, and *acadm* in liver, *pparg* in adipose tissue, and *acox1* in both) causing lipid removal in the liver [24][16]. In addition, both flavonoid-rich extracts of medlar leaf, and acai puree, have anti-oxidant, anti-

inflammatory activity and reduce hepatic steatosis in a hypercholesterolemic zebrafish model [18]. Phytochemical studies revealed that *Salvia plebeia* mainly contains flavonoids, such as apigenin, hispidulin, homoplantaginin, nepetin, nepetin-7-glucoside, and luteolin [29]. A study conducted in zebrafish, proves the effect of the ethanolic extracts of *S. plebeia* to reduce fat vacuoles, lipid accumulation and hepatic steatosis by reducing the expression of lipid metabolism genes [30]. Eriocitrin significantly suppresses the increase in plasma TGs in DIO-zebrafish, reduced lipid droplets in the liver tissues by activating mitochondrial functions (upregulation of cox4i1 and atp5j), ATP synthesis (upregulation of cox4i1 and atp5j) and by upregulating the mRNA level of lipid metabolism genes, *pparab, acox1* and *acadm* [26]. Naringenin is another flavonoid known to reduce hepatic lipid accumulation in zebrafish, as well as in other models [11][31][32].

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