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# Citrus bioactive phenolics: Role in the obesity treatment

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ABSTRACT

Adipose tissue performs many functions in the body, being considered an endocrine organ due to substances secreted, called adipokines. The excess of adipose tissue is called obesity, and it is associated with a state of chronic subclinical inflammation. Various strategies and products have been evaluated in an attempt to prevent and treat obesity, standing out the importance of polyphenols from citrus fruits. This paper aims to review studies developed to evaluate the role of these compounds in obesity and some general trends can be highlighted. The *in vitro* studies indicate that citrus polyphenols could assist in the management of obesity, since they cause a reduction in adipocyte differentiation, lipid content in the cell and adipocyte apoptosis. The biological assays are not entirely consistent; however, most of them indicated a reduction in adipose tissue; increased genes expression indicating a stimulus to β-oxidation; improved lipid profile and glycemia; as well as some evidence of improvement in inflammatory status. Several clinical trials have demonstrated the positive effect of citrus flavonoids in the reduction of proinflammatory cytokines in humans, being beneficial to alleviate the complications present in obesity. However, there are few clinical trials developed to examine its role in reducing adiposity.



## 1. Introduction

Adipose tissue has long been considered only as a site of energy storage, however it is now known that it performs many functions in the body. This tissue is considered an endocrine organ due to paracrine substances secreted, called adipokines (Grundy, Brewer Jr., Cleeman, Smith, & Lenfant, 2004). Also there are other cells present in adipose tissue, besides the adipocytes, that release active substances involved in metabolic pathways, such as macrophages (Weisberg et al., 2003). In parallel, the adipose tissue has receptors for afferent signals emitted by other endocrine systems, enabling a communication with the central nervous system. This network interaction explains the coordinating activity of adipose tissue in energy metabolism, neuroendocrine and immune function (Kershaw & Flier, 2004).

Obesity is a disease characterized by excess body weight, associated with a state of chronic subclinical inflammation, caused by an increased secretion of adipokines that modulate certain responses in the body (Balistreri, Caruso, & Candore, 2010). Overall, the vast majority of adipokines studied have a role in the development of chronic diseases associated with obesity causing insulin resistance, increased blood pressure, abnormal blood lipids, increased inflammatory response, and thrombus formation (Grundy et al., 2004).

In addition to many complications associated with obesity, the high prevalence of the disease made it a public health problem. Accordingly, various strategies and products have been evaluated in an attempt to prevent and treat excessive body weight. Among the compounds studied, stands out the importance of polyphenols in plant food.

A source of polyphenols widely studied is citrus fruits. This group of fruits is important source of bioactive compounds, mainly flavonoids, being target of many studies concerning the adipose tissue and obesity. Therefore, this paper aims to review studies developed to evaluate the role of these compounds in the obesity and associated changes.

## 2. Phenolics in citrus fruits

Phenolic compounds or polyphenols refers to a group of molecules found in plants, that exert photoprotection function, defense against microorganisms and insects, being responsible for pigmentation and some food organoleptic characteristics (Escarpa & Gonzalez, 2001). Among the various classes that comprise the phenolics, flavonoids are considered important for human consumption due to its wide distribution in plant foods.

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0023-6438/

The flavonoid structure is based on the flavylium nucleus, which consists of three phenolic rings (Fig. 1). The first benzene ring (A) is condensed with the sixth carbon of the third ring (C), which in the 2-position carries a phenyl group (B) as a substituent (Aherne & O'Brien, 2002).

The biochemical activities of flavonoids and their metabolites depend on their chemical structure, which may vary with one or more hydroxyl substituents, including derivatives. Flavonoids and isoflavones commonly occur as esters, ethers or derivatives glycosides, or a mixture of them. Except the group of leucoanthocyanins, other flavonoids occur in plants usually accompanied by carbohydrates thus receiving the name of glycosylated flavonoids. The glycidic substituents include: D-glucose, L-rhamnose, glucose-rhamnose, galactose and arabinose (Birt, Hendrich, & Wang, 2001). When the flavonoid is free of carbohydrates, the structure is called aglycone.

Citrus fruits are rich in various nutrients, such as vitamins A and C, folic acid and dietary fiber. Furthermore, these fruits are source of bioactive compounds, as flavonoids, coumarins, limonoids and carotenoids (Ding et al., 2012; Turner & Burri, 2013).

Among the flavonoids, citrus present considerable amounts of flavanones, flavones, flavonols and anthocyanins, however the main flavonoids are the flavanones (Benavente-García, Castillo, Marin, Ortuño, & Del Río, 1997). In this class of compounds, the most frequent ones are hesperidin, narirutin, naringin and eriocitrin (Ghasemi, Ghasemi, & Ebrahimzadeh, 2009; Sun et al., 2013). Other phenolics often found in citrus are p-coumaric, ferulic, caffeic and sinapic acids (Manthey & Grohmann, 2001; Sun et al., 2013).

Gattuso, Barreca, Garguilli, Leuzzi, and Caristi (2007) reviewed the flavonoid composition of citrus, and some of their results are summarized at the Table 1.

The genus *Citrus* comprises several orange species – *Citrus sinensis* (sweet orange), *Citrus aurantium* (sour oranges), *Citrus reticulata* (tangerine or mandarin) – and their hybrids – tangors, which are orange-tangerine hybrids, and tangelos, which are tangerine-grapefruit or tangerine pummelo hybrids. Many of these species or hybrids can have different varieties (Gattuso et al., 2007).

In general, the data where the specific *C. sinensis* variety analyzed is reported show that different varieties present approximately the same flavonoid composition pattern. Commercial orange juices present a similar composition to freshly squeezed ones, with the appearance of some unexpected compounds. Naringin and diosmin hint at the possibility that some of the samples analyzed are not pure orange juices, or, as in the case of hand-squeezed juices, the presence of PMFs in variable quantities suggests that they could be essentially derived from the flavedo and confirm that the amounts of polymethoxyflavones found in industrial juices are a consequence of the pressing process used (Gattuso et al., 2007).

It is also important to consider that the flavanones in citrus can be glycosylated or aglycone. The glycosylated forms are also divided into neohesperidosides that contain a neohesperidose (ramnosil-α-1,2 glucose) and have a bitter taste; and rutinosides that contain a flavanone and a disaccharide residue, and do not have taste (Macheix, Fleuriet, & Billot, 1990). Naringin, neohesperidin and neoeriocitrin are examples of neohesperidosides; while hesperidin, narirutin and didymin are examples of rutinosides (Tripoli, Guardia, Giammanco, Majo, & Giammanco, 2007). Naringenin and hesperetin are the most common aglycones, often found in trace concentrations.

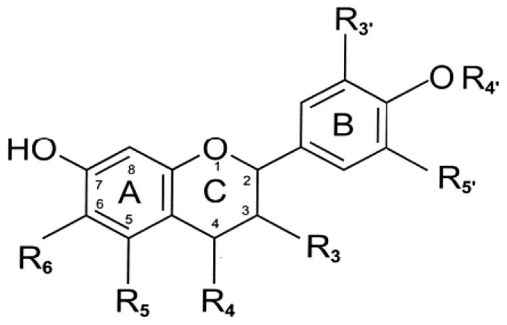



**Fig. 1.** General structure of food flavonoids.

Concerning the quantity of the compounds, Miller and Rice-Evans (1997) detected the presence of hesperidin (141 ± 49 μmol/L) and narirutin (62 ± 16 μmol/L) in longlife orange juice. Klimczak, Małecka, Szlachta, and Gliszczyńska-Świgło (2007) also evaluated longlife orange juice, verifying the presence of some hydroxycinnamic acids as caffeic (8.2 mg/L), p-coumaric (0.5 mg/L), ferulic (0.6 mg/L) and sinapic (0.7 mg/L). However, as mentioned above, the flavanones were found in greater quantity, being detected the presence of narirutin (70.2 mg/L), hesperidin (76.9 mg/L) and dydymin (9.9 mg/L). Of the flavanones analyzed, naringin and neohesperidin were not detected.

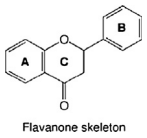
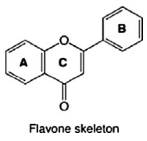
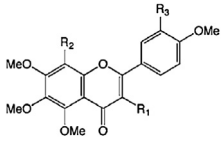
Stuetz, Prapamontol, Hongsibsong, and Biesalski (2010) evaluated the polyphenol content of *C. reticulata* Blanco cv. Sainampueng, to verify the difference between hand-pressed juice and the peeled fruit. The peeled fruit had low content of polymethoxyflavones, while the hand-pressed juice presented high content of tangeritin (5.99–31.8 mg/L), nobiletin (5.49–28.2 mg/L) and sinensetin (0.30–2.00 mg/L). The authors observed that the polymethoxyflavones were present in the peel of the fruit, and a simple squeezing can cause the transfer of these compounds from the peel to the juice. Besides this class of polyphenol, it was also detected the presence of the flavanones didymin (4.44–9.50 mg/L), narirutin (17.7–43.4 mg/L) and hesperidin (123.3–206.7 mg/L) in the hand-pressed juice. On the other side, the peeled fruit had high content of didymin (45–112 mg/kg), narirutin (181–600 mg/kg) and hesperidin (841–1898 mg/kg).

Some researchers also study the peels and peels extract of citrus fruits, as Ramful, Bahorun, Bourdon, Tarnus, and Aruoma (2010) that evaluated orange, clementine, mandarine, tangor, tangelo and pamplemousses peels. The flavonoids detected in this matrix were poncirin (2.49–18.85 mg/g FW), rhoifolin (4.54–10.39 mg/g FW), didymin (3.22–13.94 mg/g FW), rutin (8.16–42.13 mg/g FW), diosmin (4.01–18.06 mg/g FW), isorhoifolin (1.72–14.14 mg/g FW), neohesperidin (3.20–11.67 mg/g FW), hesperidin (83.4–234.1 mg/g FW), neoeriocitrin (8.8–34.65 mg/g FW) and narirutin (5.05–21.23 mg/g FW). Naringin (19.49 mg/g FW) was only detected in mandarine.

Londoño-Londoño et al. (2010) identified using HPLC-MS the presence of hesperidin, neohesperidin, diosmin, nobiletin and tangeritin in orange peel; hesperidin and neohesperidin in tangerine peel; and hesperidin, neohesperidin and diosmin in lime peel. Reinforcing the information above, none of the peels presented the aglycone hesperitin in their composition.

Ghasemi et al. (2009) evaluated the total polyphenol and flavonoid content of peels and tissues from three varieties of *C. sinensis*, three of *C. reticulata*, three of *Citrus unshiu*, one of *Citrus limon*, one of *Citrus paradisi* and two of *C. aurantium*. For most citrus analyzed the total polyphenols content was higher in the peel (104.2–223.2 mg gallic acid equivalent/g of extract powder) in comparison to tissue (66.5–396.8 2 mg gallic acid equivalent/g of extract powder), excepting all *C. reticulata* varieties, and one *C. sinensis* variety (var. Washington Navel). The total flavonoid content was also higher in the peel (0.3–31.1 mg quercetin equivalent/g of extract powder) in relation to tissue (0.3–17.1 mg quercetin equivalent/g of extract powder) in most of the samples, excepting four varieties (*C. sinensis* var. Sungin, *C. unshiu* var.

**Table 1**
Reviewed flavonoid composition of some citrus juices.

| | Mean | SD | Median | MIN | MAX |
|---|---|---|---|---|---|
| **Flavonoid composition of *C. sinensis* (sweet orange) juice (mg/100 mL)** | | | | | |
| *Flavanones* |  Flavanone skeleton | | | | |
| Didymin | 1.89 | 0.92 | 1.60 | 0.80 | 3.10 |
| Eriocitrin | 0.31 | 0.18 | 0.29 | 0.11 | 0.67 |
| Hesperidin | 28.6 | 11.9 | 28.0 | 3.51 | 55.2 |
| Narirutin | 5.2 | 3.1 | 4.2 | 0.55 | 14.2 |
| *Flavones* |  Flavone skeleton | | | | |
| Neoeriocitrin | 0.59 | – | – | – | – |
| Poncirin | 1.04 | 0.78 | 1.04 | 0.49 | 1.59 |
| 6,8-di-*C*-Glu-Apigenin | 5.72 | 2.02 | 5.00 | 4.15 | 8 |
| 6,8-di-*C*-Glu-Diosmetin | 0.35 | 0.14 | 0.35 | 0.25 | 0.45 |
| Rhoifolin | 0.05 | – | – | – | – |
| Isorhoifolin | 0.07 | – | – | – | – |
| Diosmin | 0.09 | – | – | – | – |
| Neodiosmin | 0.08 | – | – | – | – |
| *Polymethoxyflavones* |  | | | | |
| Nobiletin | 0.33 | 0.19 | 0.33 | 0.19 | 0.46 |
| Sinensetin | 0.37 | – | – | – | – |
| Tangeretin | 0.04 | 0.04 | 0.04 | 0.01 | 0.07 |
| **Flavonoid composition of *C. clementina* juice (mg/100 mL)** | | | | | |
| *Flavanones* | | | | | |
| Hesperidin | 39.9 | 29.4 | 34.9 | 5.21 | 86.1 |
| Naringin | 0.08 | 0.03 | 0.08 | 0.05 | 0.12 |
| Narirutin | 4.64 | – | – | – | – |
| *Flavones* | | | | | |
| 6,8-di-*C*-Glu-Apigenin | 0.5 | – | – | – | – |
| 6,8-di-*C*-Glu-Diosmetin | 0.2 | – | – | – | – |
| Diosmin | 1.25 | 0.51 | 1.26 | 0.67 | 2.12 |
| **Flavonoid composition of *C. limon* (lemon) juice (mg/100 mL)** | | | | | |
| *Flavanones* | | | | | |
| Eriocitrin | 16.7 | 10.3 | 16.55 | 1.67 | 39.1 |
| Hesperidin | 20.5 | 12.4 | 18.85 | 3.84 | 41 |
| *Flavones* | | | | | |
| 6,8-di-*C*-Glu-Apigenin | 1.17 | 0.25 | 1.05 | 1 | 1.45 |
| 6,8-di-*C*-Glu-Diosmetin | 4.95 | 0.88 | 5 | 4.05 | 5.8 |
| 7-*O*-Rut-Luteolin | 3.93 | 2.14 | 3.5 | 1.5 | 6.5 |
| Diosmin | 3.12 | 1.66 | 3.65 | 0.51 | 5.1 |
| *Aglycones* | | | | | |
| Luteolin | 0.08 | – | – | – | – |
| **Flavonoid composition of *C. paradisi* (grapefruit) juice (mg/100 mL)** | | | | | |
| *Flavanones* | | | | | |
| Didymin | 0.30 | 0.04 | 0.30 | 0.27 | 0.33 |
| Eriocitrin | 0.41 | 0.19 | 0.41 | 0.27 | 0.54 |
| Hesperidin | 0.93 | 0.58 | 0.87 | 0.25 | 1.79 |
| Naringin | 23.0 | 12.8 | 21.9 | 4.5 | 60.2 |
| Narirutin | 7.60 | 5.80 | 7.70 | 2.50 | 17.0 |
| Neohesperidin | 1.21 | 0.35 | 1.28 | 0.67 | 1.58 |
| Neoeriocitrin | 0.32 | 0.02 | 0.32 | 0.30 | 0.33 |
| Poncirin | 1.26 | 0.35 | 1.30 | 0.85 | 1.58 |
| *Flavones* | | | | | |
| Rutin | 3.26 | – | – | – | – |
| Rhoifolin | 0.28 | – | – | – | – |
| *Polymethoxyflavones* | | | | | |
| Heptamethoxyflavone | 0.06 | 0.07 | 0.06 | 0.01 | 0.11 |
| Nobiletin | 0.15 | 0.04 | 0.15 | 0.12 | 0.17 |
| Tangeretin | 0.12 | – | – | – | – |
| *Aglycones* | | | | | |
| Hesperetin | 0.74 | – | – | – | – |
| Naringenin | 2.70 | 2.68 | 1.70 | 0.98 | 8.00 |
| Taxifolin | 0.16 | – | – | – | – |
| Quercetin | 0.19 | 0.03 | 0.19 | 0.17 | 0.21 |

Adapted from Gattuso et al., 2007.

Ishikawa, *C. reticulata* var. Clementine, *C. reticulata* var. Page). These results indicate that considerable losses occur with the peel removal before consumption or in industrial process. And besides the information about the content of total polyphenol and flavonoid, several studies have already shown the positive effects of peel extracts in the treatment of chronic non-communicable diseases (Ding et al., 2012; Fukuchi, Hiramitsu, Okada, Hayashi, & Nabeno, 2008; Kang et al., 2012; Kim et al., 2012; Lee et al., 2011; Raasmaja et al., 2013).

A factor to consider when talking about flavonoids is whether the compound is in the glycosylated or aglycone form. It has been shown that in rats, after oral consumption of naringin, the sulfate and glucuronate conjugates forms of naringenin are found in the organism, indicating that for naringin absorption the glycoside needs to be released to the formation of the aglycone naringenin, thus depending on one glucosidase for its absorption (Wang et al., 2006). The better absorption of the aglycone form in relation to glycosylated in citrus flavonoids has also been observed in humans using glycosylated forms of eriocitrin and hesperidin compared with the corresponding aglycones eridictyol, homoeridictyol and hesperetin (Miyake et al., 2006). Therefore, the polyphenol structure can modify its bioavailability to the body. This might be the reason why many researchers chose to evaluate the aglycone potential, seen that this form is detected in the organism tissues and blood, and it has higher bioavailability.

The flavonoids found in citrus species act as antioxidants and may protect against oxidative stress-related to inflammation process, thus reducing the risk of macromolecules damage caused by the action of reactive species, conferring protection against several neurodegenerative diseases and reducing the risk of developing cardiovascular disease and cancer (Benavente-García et al., 1997).

## 3. Citrus phenolics effect on cell cultures models of obesity

*In vitro* studies are useful to understand the mechanisms of action, and guide the decision of which products should be further studied in biological assays and clinical trials. Besides that, they are an alternative when the product is in its early development phase, a moment that the yield is generally low.

Many *in vitro* studies are being conducted with citrus phenolics to evaluate its effects on obesity. One of the mechanisms proposed has been the role of these compounds in the adipocytes apoptosis, because it was observed that the addition of polymethoxyflavones analytical standard (5-hydroxy-3,6,7,8,30,40-hexamethoxyflavone (5-HxMF OH) 3,5,6,7,8,30,40-heptamethoxyflavone (HpMF); 5,6,7,30,40-pentamethoxyflavone (PMTCT), and 30-hydroxy-5,6,7,40-tetramethoxyflavone (30-OH-TtMF)) of citrus (100 μM) caused an increase in intracellular calcium, which induced the increase of calpain and caspase-12, two proteins associated with programmed cell death (Sergeev, Li, Ho, Rawson, & Dushenkov, 2009). The reduction in the number of adipose cells due to apoptosis could assist in maintaining weight loss, avoiding the weight cycling.

Another study evaluated the effect of nobiletin analytical standard in 3T3-L1 adipocytes (0–100 μM). The treatment of these cells with the citrus phenolic reduced, in a dose-dependent manner, the expression of C/EBPβ and PPARγ, transcription factors that are associated with differentiation of pre-adipocytes into mature adipocytes. Reinforcing this result, it was also observed lower lipid accumulation in cultured cells when the flavonoid was added (Kanda et al., 2012).

Nowadays, it is considered the importance of toll-like receptors (TLRs) on the association between obesity and other chronic non-communicable diseases, and it is recognized the fact that TLRs are responsible for the activation of inflammatory pathways (Sabroe, Parker, Dower, & Whyte, 2008). In a study evaluating the treatment of pre-adipocytes, adipocytes during its differentiation, and differentiated 3T3-L1 cells treated with naringenin analytical standard (100 μM), it was observed an inhibitory effect of the flavonoid on the expression of TLR 2, only during adipocyte differentiation (Yoshida et al., 2013), indicating a possible effect on the phase in which the individual is in the process of gaining body fat.

During the obesity development, it is known that in addition to the increase in adipose cells, there is an increase in the macrophages number in adipose tissue (Ramalho & Guimarães, 2008; Weisberg et al., 2003). Considering this information, Yoshida et al. (2013) conducted a test with 3T3-L1 adipocytes and macrophages RAW 264 in co-culture. The co-culture showed increased expression of TLR 2, and treatment with naringenin inhibited this increased expression observed. Furthermore, the expression of TLR 2 was increased with TNF-α addition to the culture of mature adipocytes, however naringenin added to this medium was able to inhibit TNF-α-induced TLR 2 expression by inhibiting JNK and NF-κB pathways. However, naringenin appears to reduce the expression of TLR 2 via increased activation of PPARγ, a nuclear transcription factor that could cause greater differentiation of pre-adipocytes into mature adipocytes and increase lipid accumulation in these cells, exactly as was observed on the experiment (Yoshida et al., 2013).

Also, Yoshida et al. (2010) found that in 3T3-L1 adipocytes cell culture, hesperetin and naringenin analytical standards showed anti-inflammatory effect by inhibiting the activation of NFκB through TNF-α, with a consequent reduction in the secretion of interleukin-6 (IL-6); and anti-lipolytic effect by inhibit ERK (extracellular signal regulated kinase) pathway causing a decreased activation of hormone sensitive lipase (HSL); contributing to reduce the insulin resistance (Fig. 2).

Other studies, one with orange peel flavonoids ethanol extract rich in hesperidin (13.79 mg/g), narirutin (7 mg/g) and naringin (262.5 mg/g) (Jung, Jeong, Park, Park, & Hong, 2011), and another with *C. aurantium* flavonoids extract that contained naringin, hesperidin, poncirin, isosiennsetin, sineesytin, tetramrthnl-o-isoscutellaeein, nobiletin, heptamethoxyflavone, 3-hydoxynobiletin, tangeretin, hydroxypentamethoxyflavone, and hexamethoxyflavone (Kim et al., 2012), also observed a stimulus in lipolysis and lower triglyceride accumulation in 3T3-L1 adipocytes. Still, extracts caused a lower accumulation of total lipids and reduced the expression of C/EBPα, C/EBPβ, PPARγ, aP2 (activating protein 2) and FAS (fatty acid synthase), being the last two ones target genes of C/EBPβ and PPARγ (Kim et al., 2012). The extracts have also generated a smaller amount of Akt (serine/threonine kinase) and phosphorylated GSK3β. The phosphorylated Akt, promotes the phosphorylation of GSK3β, and this phosphorylates C/EBPα and C/EBPβ, which become activated (Kim et al., 2012), then acting in adipocyte differentiation.

The orange peel flavonoids ethanol extract evaluated by Jung (2011) also caused a suppressive effect on the expression of perilipin, indicating another factor that may be associated with the positive effect of citrus flavonoids in obesity. This is a lipid-associated protein secreted only in adipocytes (Persson, Degerman, Nilsson, & Lindholm, 2007), that controls fatty acid release stimulated by HSL, because it binds and stabilizes lipid droplets in adipose tissue (Le Lay & Dugail, 2009).

The treatment of mature 3T3-L1 adipocytes with *Citrus sunki* peel ethanol extract that contained tangeritin (55.13 mg/g), nobiletin (38.83 mg/g), rutin (17.02 mg/g), hesperidin (17.11 mg/g), sinensetin (4.23 mg/g) induced LKB1, AMPK (AMP-activated protein kinase) and ACC (acetyl-CoA carboxylase) phosphorylation in a dose-dependent manner, and also caused an increase in mRNA levels of CPT-1a (carnitine palmitoyltransferase 1a) indicating the role of this extract to increase the β-oxidation. Furthermore, a lipolysis stimulation occurred 24 h after the extract addition to the cell culture, in a dose dependent manner. Associated with this result, the authors observed that the extract caused phosphorylation of PKA substrate (cAMP-dependent protein kinase) and HSL (Kang et al., 2012).

Besides the effect on adipose tissue, flavonoids can also act in the management of obesity by interfering in the control of hunger and satiety. In this context, hesperetin analytical standard (0.1–1.0 mM) has shown to cause an increase in the secretion of cholecystokinin (CCK) in STC-1 cells through increase in intracellular calcium concentration by the TRP (transient receptor potential) and TRP 1 ankirin channels. The addition of hesperidin analytical standard in the same model caused no effect, indicating that only the aglycone form influences hormone secretion (Kim, Park, Kim, Lee, & Rhyu, 2013). The increase in CCK would be interesting because this hormone, secreted from endocrine cells in the small intestine, assists in the control of food intake (Raybould, 2009).

Some products have been developed in order to assist in the obesity prevention and treatment, and it could be mentioned Sinetrol, a citrus-based fruits (juice, peels, seeds) extract obtained by physical treatment (crushing of fruits, cold pressure of juice, extraction, centrifugation, filtration, spray drying) of a specific varieties of red orange (*C. sinensis* L. Osbeck (*Blood group*)), sweet orange (*C. aurantium* L. var. sinensis), bitter orange (*C. aurantium* L. var. amara), grapefruit (*C. paradisi*) and guarana (*Paullinia cupana*), which contained 60% of polyphenol, 16.7% of flavanones, 2% of anthocyanins and 3.6% of caffeine, studied by Dallas, Gerbi, Tenca, Juchaux, and Bernard (2008). The researchers noted that this supplement (20 mg/mL) was able to stimulate lipolysis in human fat cells in a *in vitro* study, verified by the free fatty acids enhancement. The authors suggest that the compounds present in the supplement, especially naringin and cyanidin 3-glycoside, have an effect on the inhibition of cAMP-phosphodiesterase, and thus there is an increase in cAMP and subsequent stimulation of hormone-sensitive lipase (HSL), the enzyme that stimulates lipolysis in human.

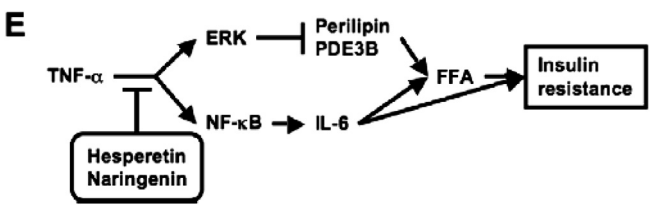



**Fig. 2.** Scheme proposed by Yoshida et al. (2010) for the action of hesperetin and naringenin on inhibition of ERK and NFκB pathways, resulting in the reduction of free fatty acids (FFA), and consequently improving insulin resistance.

## 4. Evaluation of citric polyphenol effect in biological assay

Despite the important information collected by *in vitro* assays, biological assay helps understand the bioactive compounds effects in the whole body, further illustrating the changes caused due to their consumption.

Alam, Kauter, and Brown (2013) evaluated the effect of supplementation with naringin analytical standard (approximately 100 mg/kg diet/day, corresponding to 0.01%) in male Wistar rats fed a diet rich in lipid and carbohydrate. They did not observe the effect of the flavonoid in weight gain. However, supplementation promoted a reduction in retroperitoneal abdominal fat deposition, a better serum lipid profile and oral glucose tolerance. The insulin concentration and pancreas wet weight in rats supplemented was similar to the control group, which received a standard diet, presenting lower values than the high carbohydrate, high fat diet-group without the flavonoid. A high carbohydrate, high fat diet promoted greater inflammatory cell infiltration and accumulation of fat droplets in the liver compared to the control group, however naringin supplementation decreased these two parameters.

In another study, naringenin analytical standard (1%) was supplemented in mice fed a high fat diet, and also no effect was observed in weight gain and food consumption. However, the supplementation improved hyperglycemia, reduced expression of TNF-α (tumor necrosis factor-alpha), MCP-1 (monocyte chemotactic protein-1), and TLR 2 in adipose tissue (Yoshida et al., 2013), promoting protection against chronicle non-communicable diseases.

In an experiment conducted with male Long-Evans adult rats fed a semi-purified experimental diet with 16% fat and 45.5% sucrose, supplementation with 0.012% naringenin analytical standard promoted less visceral fat accumulation, and lower triacylglycerol content in the tissue, compared to the control group that did not receive the flavonoid. However, no effect was observed in the total body weight. Food consumption did not differ between the groups. Also, the supplemented group had lower concentration of serum triglycerides, total and free cholesterol in plasma, and lower accumulation of triacylglycerol and cholesterol in the liver. The flavonoid intake caused an increased expression of PPARα, CPT1 (carnitine palmitoyltransferase 1) and UCP2 (uncoupling protein 2) protein, indicating the role of naringenin to increase lipid β-oxidation in animals (Cho, Kim, Andrade, Burgess, & Kim, 2011).

The administration of 0.05% coumarin analytical standard in C57BL/6J mice receiving a high fat diet also caused less accumulation of visceral fat, and yet caused reduction of total body weight compared to the high fat diet group without the supplement. Still, coumarin supplementation caused less accumulation of lipids, triacylglycerol and cholesterol in the liver; and reduced protein levels of SRBP-1c, FAS, ACC1, PPARγ and C/EBPα. Histological analyzes showed a minor adipocyte size by using the phenolic compound, indicating a contribution in the reduction of adipose tissue (Um, Moon, Ahn, & Youl Ha, 2013).

High fructose diets are used in animal experiments to induce hypertriglyceridemia and insulin resistance (Bezerra et al., 2000; Kelley, Allan, & Azhar, 2004). The supplementation of citrus polymethoxyflavones analytical standard (125 mg/kg body weight/day), mainly containing tangeritin and nobiletin, in hamsters subjected to this modified diet, reduced the weight gain, serum triglyceride, triglyceride in liver and heart, and improved adiponectin levels compared with the group receiving high-fructose diet without the flavonoid. Moreover, a positive effect was observed in the levels of some inflammatory cytokines, reducing TNFα and IFN-γ after the addition of polymethoxyflavones. In this experiment also occurred increased expression of hepatic PPARα and PPARγ as the effect of supplementation, which according to the authors, would be a major regulatory pathway of the effects observed (Li et al., 2006).

Another experiment with polymethoxyflavones was performed with *Citrus depressa* Hayata peel methanol extract that contained nobiletin and tangeritin (1.5%), in ICR mice consuming a high fat diet. The addition of the extract caused less weight gain, lower weight of white adipose tissue, reduced adipocyte size, and lower serum levels of triglycerides and leptin. There was also a decrease in ACC1, SCD1 (esteroil-CoA desaturase), FATP (transport protein fatty acid), aP2 and DAGT1 (diacylglycerol acyltransferase 1) mRNA in white adipose tissue. All the genes cited are involved in the synthesis of fatty acids and triacylglycerols. Despite the positive effects observed, there was no effect on serum adiponectin nor in the mRNA levels of SREBP1 (binding protein sterol regulatory element 1), FAS and ACC1 in the liver (Lee et al., 2011).

In a study developed by Lee et al. (2013), the administration by gavage of 100 mg/kg of purified nobiletin extracted from *C. depressa* peel, to male C57BL/6J mice fed a high fat diet, caused less overall weight gain, lower weight of white adipose tissue and serum triglycerides. There was no effect of the extract on hepatic triacylglycerol levels and serum adiponectin. Controversially, there was an increase in the expression of PPARγ and PPARα, as well as their target genes SREBP-1c, FAS, SCD-1; and CPT-1, UCP2; respectively. These results indicate that the extract induced lipid accumulation and fatty acid oxidation at the same time, however with a greater catabolic effect seen the less weight gain when compared to the group receiving high fat diet without the extract. The extract had the positive effect of reducing TNF-α and MCP-1 (Lee et al., 2013), which helps improve insulin sensitivity, as it is known that TNF-α causes a reduction in expression and translocation of GLUT4, the glucose transport protein in insulin-dependent cells (Hotamisligil, Shargill, & Spiegelman, 1993). The authors observed an increase in expression of IκBα after the flavonoid use, indicating that the anti inflammatory effect is possibly through NFκB pathway inactivation (Lee et al., 2013).

The addition of lemon peel polyphenol ethanol extract (0.5%), containing greater amounts of eriocitrin, hesperidin and narirutin; also promoted less total weight and white adipose tissue gain after consumption of a high fat diet in male C57BL/6J mice. Note that polyphenols also caused increased hepatic PPARα mRNA level, and acyl-CoA oxidase in the liver and white adipose tissue, indicating increased peroxisomal fatty acid oxidation (Fukuchi et al., 2008).

Another extract that showed positive effects of citrus polyphenols was *Citrus ichangensis* peel ethanol extract that contained naringin (8.12 mg/g), hesperidin (0.84 mg/g) and poncirin (1.33 mg/g), administered to female mice fed standard (control), high-fat or high-fat diets supplemented with 1% extract. The weight gain in the group that received the high-fat diet alone was greater than the control, and the addition of the extract in the high-fat diet caused less weight gain, being similar to control group. The extract caused a lower fasting glucose and improved glucose tolerance. Also, there was less accumulation of triacylglycerol and cholesterol in the liver due to the extract administration. Moreover, this has caused lower expression of PPARγ mRNA and lower levels of this transcription factor target genes, including FAS, acyl-CoA oxidase and UCP 2 (Ding et al., 2012); in agreement with the *in vitro* studies presented previously in this paper.

Kang et al. (2012) also tested in mice a high-fat diet supplemented by gavage with an immature *C. sunki* peel extract (150 mg/kg body weight/day), source of the flavonoids tangeritin (55.13 mg/g), nobiletin (38.83 mg/g), rutin (17.02 mg/g), hesperidin (17.11 mg/g), sinensetin (4.23 mg/g). Likewise, the authors found that supplementation reduced weight gain caused by the consumption of a high-fat diet, in addition to promoting lower weight of perirenal and epididymal adipose tissue, as well as smaller size of adipocytes

in epididymal tissue. Another positive effect of the supplementation can be observed in the serum levels of total cholesterol and triglycerides, that were lower compared to the group that received only the high-fat diet. Still, lipid accumulation in the liver was lower, comparable to the control group that received a standard diet. It was also observed increased expression of proteins related to β-oxidation when the extract was added, along with a greater expression of adiponectin gene.

In another study, a water and alcohol extract of *Citrus grandis* whole fruits containing 19% naringin was tested in genetically obese Zucker rats fed with high-fat/high-cholesterol diet. No effect was observed in the body weight, however serum cholesterol and triglyceride were improved when 600 mg/kg of the extract was administered by intragastric gavage for four weeks (Raasmaja et al., 2013).

Salamone et al. (2012) evaluated the effect of *Moro* orange juice, rich in anthocyanins (85 mg/L) in mice C57BL6/J fed a high-fat diet. The juice consumption was *ad libitum*, resulting in a mean intake of 4.1 ± 0.75 mL/day and consequent anthocyanin consumption of about 0.34 mg per day. The group that received the juice had lower levels of triacylglycerol and total lipids in the liver. In addition, there was increased expression of PPARα and acyl-CoA oxidase, and lower of LXR (liver X receptor), FAS, HMG-CoA reductase; indicating a potential effect in stimulating lipid oxidation and reduction of lipogenesis.

A limitation in the comparison of the studies is the phenols administration form, in some cases the compounds are administered as a dietary ingredient and other by gavage. In studies in which the product is incorporated into the diet, consumption data are not always available, providing only the concentration in the diet; on the other hand in studies that used gavage, the information provided is the total quantity consumed.

## 5. Evaluation of citric polyphenol effect in clinical trials

Despite the evidence observed in *in vitro* studies and biological assays, clinical trial is essential to the final conclusions, since it considers the influence of compounds bioavailability in the human body. Accordingly, Ameer, Weintraub, Johnson, Yost, and Rouseff (1996) ascertained the bioavailability of naringin (500 mg) consumed pure, naringin (500 mg) administered with hesperidin (500 mg), grapefruit juice (1250 mL) co administered with orange juice (1250 mL) and grapefruit (1 unit) consumed daily for 4 weeks. The presence of the aglycone hesperetin and naringenin were detected in urine after the consumption of pure hesperidin and naringin, and after consuming grapefruit and orange juice. After 4 weeks of consuming grapefruit, naringenin was present in plasma and urine. The authors conclude that the aglycone forms of the flavonoid were detected, as the glycoside linkages are not stable to the acidic environment of the stomach, in addition to the possible action of glycosides from intestinal bacteria cleaving the sugar residues.

Another important finding of Ameer et al. (1996) was the observation that the consumption of hesperidin associated with naringin does not affect the urinary recovery of the second, indicating that it does not disturb the bioavailability of the other. Furthermore, these results indicate that the absorption of these two flavonoids occurs in pure form, and also when consumed in a food matrix in the form of juice or fresh fruit. And, as the aglycone form is found after the consumption, maybe its bioavailability is higher since it does not require an enzyme to be absorbed. However, it should be noted that this study was conducted with only 4 volunteers, limiting extrapolation of the results to the general population, and indicating the need for more bioavailability studies in clinical trials.

In addition to assessing the effect of a supplement rich in citrus polyphenols *in vitro*, Dallas et al. (2008) conducted a double-blind placebo-controlled study evaluating the effect of the supplementation in overweight men, observing a greater weight and body fat loss in the group that consumed the supplement. However, the food habits were not controlled, being only mentioned that the volunteers were not supposed to modify it.

A variation of this supplement was studied in another group of humans. This new product was polyphenolic rich fruit extract (red orange, grapefruit, orange sweet and guarana), that contained at least 90% of polyphenols, at least 20% of flavanones and between 1 and 3% of natural caffeine. The authors reported a reduction in waist and hip circumferences; in markers of inflammation C-reactive protein and fibrinogen; and improved oxidative stress status, with the reduction in malondialdehyde (MDA) and increase in superoxide dismutase and glutathione levels. There were no adverse effects in liver and kidney. There was an increase in serum free fatty acids, but no change in the serum lipids levels (Dallas et al., 2013). However, it was not reported in the paper what types of polyphenols and flavonoids were offered with the supplement, and there was no mention about the food habits of the volunteers.

## 6. Potential of citrus flavonoids produced by biotechnology

Bioprocessing strategies aiming the improvement of the bioaccessibility of phenolic compounds have been investigated in the last years. The use of α-L-rhamnosidases from *Aspergillus aculeatus* was investigated in the transformation of flavonoid rutinosides from fruit juices (orange and blackcurrant) and green tea into their flavonoid glucoside counterparts in a reaction at 30 °C for 10 h. Aliquots of the controls and the enzyme treated samples were taken at different time points and flavonoids rutinosides (anthocyanins in blackcurrant juice, flavanones in orange in juice, and flavonols in green tea) and glucosides were identified and quantified. Even with the assay conditions in each beverage being different, the enzyme was able to remove terminal rhamnosyl groups in the three beverages. Results showed a decrease in the flavonoid rutinoside and an increase in their flavonoid glucoside counterparts (González-Barrio et al., 2004).

The effects on the bioavailability of hesperitin was investigated in a double-blind, randomized, crossover study, in human subjects. The volunteers consumed orange juice with natural hesperidin (hesperitin-7-*O*-rutinoside), orange juice treated with the enzyme hesperidinase and orange juice fortified to obtain 3 times more hesperidin than naturally present. A significant improvement in the bioavailability of the aglycone hesperetin was observed after enzymatic modification of the orange juice. The peak plasma concentrations of the aglycone when subjects consumed the juice containing hesperetin-7-glucoside, generated after removal of the rhamnose by the hesperidinase, were 4-fold higher compared with the untreated juice and 1.5-fold higher than the fortified juice (Nielsen et al., 2006).

A study employing orange pomace as substrate for solid-state fermentation by *Paecilomyces variotii* to produce the enzymes tannase and phytase simultaneously, also evaluated the phenolic content and antioxidant capacity of orange pomace during fermentation. The fermentation medium was prepared with the orange pomace, a saline solution and 10% tannic acid and, after inoculation, was incubated at 30 °C for 120 h. In addition to tannase and phytase production at significant levels, results showed no difference in total phenolic content before and after the fermentation processes. However, the antioxidant capacity of orange pomace, tested against the free radical ABTS, increased approximately tenfold after fermentation, potentially enhancing the value of this residue (Madeira Jr., Macedo, & Macedo, 2012).

Enzymatic hydrolysis and fermentation appear to be an attractive mean to promote the biotransformation of phenolic glycosides and polymers and to increase the concentration of free phenolics in citrus fruits and agro-industrial wastes. The biotransformation of phenolics improved the antioxidant activity and bioaccessibility of these compounds. Further research is necessary to explore new substrates, enzymes and microorganisms and to evaluate the use of biotransformed products as natural antioxidants and as food supplements.

## 7. Conclusion

Despite the difficulties in the comparison of study results, due to the variety of methodologies and samples evaluated, some general trends can be highlighted.

The studies with cells culture indicate that citrus polyphenols could assist in the management of obesity, since they cause a reduction in adipocyte differentiation, lipid content in the cell and may also function in programmed cell death.

The results of biological assays are not entirely consistent, since in some cases the addition of citrus fruit polyphenols caused lower weight gain, and in other studies this effect was not noticed. However, most of them indicated a reduction in adipose tissue; increased expression of PPARα and its target genes, indicating a stimulus to β-oxidation; improved lipid profile and glycemia; as well as some evidence of improvement in inflammatory status due to a reduction in the proinflammatory cytokines levels. The effects on total body weight are more evident in the studies that used extracts instead of analytical standards, indicating a possible synergistic effect among the different phenolics found when using an extract. Furthermore, the higher cost of analytical standards limits their use in biological assay; meanwhile the extracts are generally made from fruits industrial wastes, a material that would be discarded.

Several clinical trials have demonstrated the positive effect of citrus flavonoids in the reduction of proinflammatory cytokines in humans (Bernabé et al., 2013; Buscemi et al., 2012; Devaraj, Jialal, Rockwood, & Zak, 2011; Iwamoto, Imai, Ohta, Shirouchi, & Sato, 2012; Morand et al., 2011), being beneficial to alleviate the complications present in obesity. However, there are few clinical trials developed to examine its role in reducing adiposity, indicating a research field still in expansion.

## References


Aherne, S. A., & O'Brien, N. M. (2002). Dietary flavonols: chemistry, food content, and metabolism. *Nutrition, 18*(1), 75–81.

Alam, M. A., Kauter, K., & Brown, L. (2013). Naringin improves diet-induced cardiovascular dysfunction and obesity in high carbohydrate, high fat diet-fed rats. *Nutrients, 5*(3), 637–650. http://dx.doi.org/10.3390/nu5030637.

Ameer, B., Weintraub, R. A., Johnson, J. V., Yost, R. A., & Rouseff, R. L. (1996). Flavanone absorption after naringin, hesperidin, and citrus administration. *Clinical Pharmacology and Therapeutics, 60*(1), 34–40. http://dx.doi.org/10.1016/S0009-9236(96)90164-2.

Balistreri, C. R., Caruso, C., & Candore, G. (2010). The role of adipose tissue and adipokines in obesity-related inflammatory diseases. *Mediators of Inflammation, 2010*, 1–19. http://dx.doi.org/10.1155/2010/802078.

Benavente-García, O., Castillo, J., Marin, F. R., Ortuño, A., & Del Río, J. A. (1997). Uses and properties of citrus flavonoids. *Journal of Agricultural and Food Chemistry, 45*(12), 4505–4515.

Bernabé, J., Mulero, J., Cerdá, B., García-Viguera, C., Moreno, D. A., Parra, S., et al. (2013). Effects of a citrus based juice on biomarkers of oxidative stress in metabolic syndrome patients. *Journal of Functional Foods, 5*(3), 1031–1038. http://dx.doi.org/10.1016/j.jff.2013.02.003.

Bezerra, R. M. N., Ueno, M., Silva, M. S., Tavares, D. Q., Carvalho, C. R. O., & Saad, M. J. A. (2000). A high fructose diet affects the early steps of insulin action in muscle and liver of rats. *The Journal of Nutrition, 130*, 1531–1535.

Birt, D. F., Hendrich, S., & Wang, W. (2001). Dietary agents in cancer prevention: flavonoids and isoflavonoids. *Pharmacology and Therapeutics, 90*(2–3), 157–177.

Buscemi, S., Rosafio, G., Arcoleo, G., Mattina, A., Canino, B., Montana, M., et al. (2012). Effects of red orange juice intake on endothelial function and inflammatory markers in adult subjects with increased cardiovascular risk. *American Journal of Clinical Nutrition, 95*, 1089–1095. http://dx.doi.org/10.3945/ajcn.111.031088.Am.

Cho, K. W., Kim, Y. O., Andrade, J. E., Burgess, J. R., & Kim, Y.-C. (2011). Dietary naringenin increases hepatic peroxisome proliferators-activated receptor α protein expression and decreases plasma triglyceride and adiposity in rats. *European Journal of Nutrition, 50*(2), 81–88. http://dx.doi.org/10.1007/s00394-010-0117-8.

Dallas, C., Gerbi, A., Elbez, Y., Caillard, P., Zamaria, N., & Cloarec, M. (2013). Clinical study to assess the efficacy and safety of a citrus polyphenolic extract of red orange, grapefruit, and orange (Sinetrol-XPur) on weight management and metabolic parameters in healthy overweight individuals. *Phytotherapy Research*, 1–7. http://dx.doi.org/10.1002/ptr.4981.

Dallas, C., Gerbi, A., Tenca, G., Juchaux, F., & Bernard, F.-X. (2008). Lipolytic effect of a polyphenolic citrus dry extract of red orange, grapefruit, orange (SINETROL) in human body fat adipocytes. Mechanism of action by inhibition of cAMP-phosphodiesterase (PDE). *Phytomedicine, 15*, 783–792. http://dx.doi.org/10.1016/j.phymed.2008.05.006.

Devaraj, S., Jialal, I., Rockwood, J., & Zak, D. (2011). Effect of orange juice and beverage with phytosterols on cytokines and PAI-1 activity. *Clinical Nutrition, 30*(5), 668–671. http://dx.doi.org/10.1016/j.clnu.2011.03.009.

Ding, X., Fan, S., Lu, Y., Zhang, Y., Gu, M., Zhang, L., et al. (2012). *Citrus ichangensis* peel extract exhibits anti-metabolic disorder effects by the inhibition of PPARγ and LXR signaling in high-fat diet-induced C57BL/6 mouse. *Evidence-Based Complementary and Alternative Medicine, 2012*, 1–10. http://dx.doi.org/10.1155/2012/678592.

Escarpa, A., & Gonzalez, M. C. (2001). An overview of analytical chemistry of phenolic compounds in foods. *Critical Reviews in Analytical Chemistry, 31*(2), 57–139. http://dx.doi.org/10.1080/20014091076695.

Fukuchi, Y., Hiramitsu, M., Okada, M., Hayashi, S., & Nabeno, Y. (2008). Lemon polyphenols suppress diet-induced obesity by up-regulating of mRNA levels of the enzymes involved in beta-oxidation in mouse white adipose tissue. *Journal of Clinical Biochemistry and Nutrition, 43*, 201–209. November.

Gattuso, G., Barreca, D., Garguilli, C., Leuzzi, U., & Caristi, C. (2007). Flavonoid composition of citrus juices. *Molecules, 12*, 1641–1673.

Ghasemi, K., Ghasemi, Y., & Ebrahimzadeh, M. A. (2009). Antioxidant activity, phenol and flavonoid contents of 13 citrus species peels and tissues. *Pakistan Journal of Pharmaceutical Sciences, 22*(3), 277–281.

González-Barrio, R., Trindade, L. M., Manzanares, P., de Graaff, L. H., Tomás-Barberán, F. A., & Espín, J. C. (2004). Production of bioavailable flavonoid glucosides in fruit juices and green tea by use of fungal alpha-L-rhamnosidases. *Journal of Agricultural and Food Chemistry, 52*(20), 6136–6142. http://dx.doi.org/10.1021/jf0490807.

Grundy, S. M., Brewer, H. B., Jr., Cleeman, J. I., Smith, S. C., & Lenfant, C. (2004). Definition of metabolic syndrome: report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Circulation, 109*, 433–438. http://dx.doi.org/10.1161/01.CIR.0000111245.75752.C6.

Hotamisligil, G. S., Shargill, N. S., & Spiegelman, B. M. (1993). Adipose expression of tumor necrosis factor-alfa: direct role in obesity-linked insulin resistance. *Science, 259*, 87–91. January.

Iwamoto, M., Imai, K., Ohta, H., Shirouchi, B., & Sato, M. (2012). Supplementation of highly concentrated β-cryptoxanthin in a satsuma mandarin beverage improves adipocytokine profiles in obese Japanese women. *Lipids in Health and Disease, 11*(52), 1–4. http://dx.doi.org/10.1186/1476-511X-11-52.

Jung, H. K., Jeong, Y. S., Park, C.-D., Park, C.-H., & Hong, J.-H. (2011). Inhibitory effect of citrus peel extract on lipid accumulation of 3T3-L1 adipocytes. *Journal of the Korean Society for Applied Biological Chemistry, 54*(2), 169–176. http://dx.doi.org/10.3839/jksabc.2011.028.

Kanda, K., Nishi, K., Kadota, A., Nishimoto, S., Liu, M.-C., & Sugahara, T. (2012). Nobiletin suppresses adipocyte differentiation of 3T3-L1 cells by an insulin and IBMX mixture induction. *Biochimica et Biophysica Acta, 1820*, 461–468. http://dx.doi.org/10.1016/j.bbagen.2011.11.015.

Kang, S.-I., Shin, H.-S., Kim, H.-M., Hong, Y.-S., Yoon, S.-A., Kang, S.-W., et al. (2012). Immature *Citrus sunki* peel extract exhibits antiobesity effects by β-oxidation and lipolysis in high-fat diet-induced obese mice. *Biological & Pharmaceutical Bulletin, 35*(2), 223–230.

Kelley, G. L., Allan, G., & Azhar, S. (2004). High dietary fructose induces a hepatic stress response resulting in cholesterol and lipid dysregulation. *Endocrinology, 145*(2), 548–555. http://dx.doi.org/10.1210/en.2003-1167.

Kershaw, E. E., & Flier, J. S. (2004). Adipose tissue as an endocrine organ. *The Journal of Clinical Endocrinology & Metabolism, 89*(6), 2548–2556. http://dx.doi.org/10.1210/jc.2004-0395.

Kim, H. Y., Park, M., Kim, K., Lee, Y. M., & Rhyu, M. R. (2013). Hesperetin stimulates cholecystokinin secretion in enteroendocrine STC-1 cells. *Biomolecules & Therapeutics, 21*(2), 121–125.

Kim, G.-S., Park, H. J., Woo, J.-H., Kim, M.-K., Koh, P.-O., Min, W., et al. (2012). *Citrus aurantium* flavonoids inhibit adipogenesis through the Akt signaling pathway in 3T3-L1 cells. *BMC Complementary and Alternative Medicine, 12*(31), 1–10. http://dx.doi.org/10.1186/1472-6882-12-31.

Klimczak, I., Małecka, M., Szlachta, M., & Gliszczyńska-Świgło, A. (2007). Effect of storage on the content of polyphenols, vitamin C and the antioxidant activity of orange juices. *Journal of Food Composition and Analysis, 20*(3–4), 313–322. http://dx.doi.org/10.1016/j.jfca.2006.02.012.

Lee, Y.-S., Cha, B.-Y., Choi, S.-S., Choi, B.-K., Yonezawa, T., Teruya, T., et al. (2013). Nobiletin improves obesity and insulin resistance in high-fat diet-induced

obese mice. *The Journal of Nutritional Biochemistry, 24*, 156–162. http://dx.doi.org/10.1016/j.jnutbio.2012.03.014.

Lee, Y.-S., Cha, B.-Y., Saito, K., Choi, S.-S., Wang, X. X., Choi, B.-K., et al. (2011). Effects of a *Citrus depressa* Hayata (shiikuwasa) extract on obesity in high-fat diet-induced obese mice. *Phytomedicine, 18*, 648–654. http://dx.doi.org/10.1016/j.phymed.2010.11.005.

Le Lay, S., & Dugail, I. (2009). Connecting lipid droplet biology and the metabolic syndrome. *Progress in Lipid Research, 48*, 191–195. http://dx.doi.org/10.1016/j.plipres.2009.03.001.

Li, R. W., Theriault, A. G., Au, K., Douglas, T. D., Casaschi, A., Kurowska, E. M., et al. (2006). Citrus polymethoxylated flavones improve lipid and glucose homeostasis and modulate adipocytokines in fructose-induced insulin resistant hamsters. *Life Sciences, 79*, 365–373. http://dx.doi.org/10.1016/j.lfs.2006.01.023.

Londoño-Londoño, J., Lima, V. R. D., Lara, O., Gil, A., Pasa, T. B. C., Arango, G. J., et al. (2010). Clean recovery of antioxidant flavonoids from citrus peel: optimizing an aqueous ultrasound-assisted extraction method. *Food Chemistry, 119*(1), 81–87. http://dx.doi.org/10.1016/j.foodchem.2009.05.075.

Macheix, J.-J., Fleuriet, A., & Billot, J. (1990). *Fruit phenolics*. Florida: CRC Press.

Madeira, J. V., Jr., Macedo, J. A., & Macedo, G. A. (2012). A new process for simultaneous production of tannase and phytase by *Paecilomyces variotii* in solid-state fermentation of orange pomace. *Bioprocess Biosystems Engineering, 8551*, 1–7. http://dx.doi.org/10.1007/s00449-011-0587-y.

Manthey, J. A., & Grohmann, K. (2001). Phenols in citrus peel byproducts. Concentrations of hydroxycinnamates and polymethoxylated flavones in citrus peel molasses. *Journal of Agricultural and Food Chemistry, 49*, 3268–3273.

Miller, N. J., & Rice-Evans, C. A. (1997). The relative contributions of ascorbic acid and phenolic antioxidants to the total antioxidant activity of orange and apple fruit juices and blackcurrant drink. *Food Chemistry, 60*(3), 331–337. http://dx.doi.org/10.1016/S0308-8146(96)00339-1.

Miyake, Y., Sakurai, C., Usuda, M., Fukumoto, S., Hiramitsu, M., Sakaida, K., et al. (2006). Difference in plasma metabolite concentration after ingestion of lemon flavonoids and their aglycones in humans. *Journal of Nutritional Science and Vitaminology, 52*, 54–60.

Morand, C., Dubray, C., Milenkovic, D., Lioger, D., Martin, J. F., Scalbert, A., et al. (2011). Hesperidin contributes to the vascular protective effects of orange juice: a randomized crossover study in healthy volunteers. *American Journal of Clinical Nutrition, 93*, 73–80. http://dx.doi.org/10.3945/ajcn.110.004945.

Nielsen, I. L. F., Chee, W. S. S., Poulsen, L., Offord-Cavin, E., Rasmussen, S. E., Frederiksen, H., et al. (2006). Bioavailability is improved by enzymatic modification of the citrus flavonoid hesperidin in humans: a randomized, double-blind, crossover trial. *The Journal of Nutrition, 136*, 404–408.

Persson, J., Degerman, E., Nilsson, J., & Lindholm, M. W. (2007). Perilipin and adipophilin expression in lipid loaded macrophages. *Biochemical and Biophysical Research Communications, 363*, 1020–1026. http://dx.doi.org/10.1016/j.bbrc.2007.09.074.

Raasmaja, A., Lecklin, A., Li, X. M., Zou, J., Zhu, G.-G., Laakso, I., et al. (2013). A water-alcohol extract of *Citrus grandis* whole fruits has beneficial metabolic effects in the obese Zucker rats fed with high fat/high cholesterol diet. *Food Chemistry, 138*, 1392–1399. http://dx.doi.org/10.1016/j.foodchem.2012.09.140.

Ramalho, R., & Guimarães, C. (2008). Papel do tecido adiposo e dos macrófagos no estado de inflamação crónica associada à obesidade – implicações clínicas. *Acta Medica Portuguesa, 21*, 489–496.

Ramful, D., Bahorun, T., Bourdon, E., Tarnus, E., & Aruoma, O. I. (2010). Bioactive phenolics and antioxidant propensity of flavedo extracts of Mauritian citrus fruits: potential prophylactic ingredients for functional foods application. *Toxicology, 278*(1), 75–87. http://dx.doi.org/10.1016/j.tox.2010.01.012.

Raybould, H. E. (2009). Mechanisms of CCK signaling from gut to brain. *Current Opinion in Pharmacology, 7*(6), 570–574. http://dx.doi.org/10.1016/j.coph.2007.09.006.

Sabroe, I., Parker, L., Dower, S., & Whyte, M. (2008). The role of TLR activation in inflammation. *The Journal of Pathology*, 126–135. http://dx.doi.org/10.1002/path.

Salamone, F., Li Volti, G., Titta, L., Puzzo, L., Barbagallo, I., La Delia, F., et al. (2012). Moro orange juice prevents fatty liver in mice. *World Journal of Gastroenterology, 18*(29), 3862–3868. http://dx.doi.org/10.3748/wjg.v18.i29.3862.

Sergeev, I. N., Li, S., Ho, C.-T., Rawson, N. E., & Dushenkov, S. (2009). Polymethoxyflavones activate Ca2+-dependent apoptotic targets in adipocytes. *Journal of Agricultural and Food Chemistry, 57*, 5771–5776. http://dx.doi.org/10.1021/jf901071k.

Stuetz, W., Prapamontol, T., Hongsibsong, S., & Biesalski, H.-K. (2010). Polymethoxylated flavones, flavanone glycosides, carotenoids, and antioxidants in different cultivation types of tangerines (*Citrus reticulata* Blanco cv. Sainampueng) from Northern Thailand. *Journal of Agricultural and Food Chemistry, 58*(10), 6069–6074. http://dx.doi.org/10.1021/jf904608h.

Sun, Y., Qiao, L., Shen, Y., Jiang, P., Chen, J., & Ye, X. (2013). Phytochemical profile and antioxidant activity of physiological drop of citrus fruits. *Journal of Food Science, 78*(1), C37–C42. http://dx.doi.org/10.1111/j.1750-3841.2012.03002.x.

Tripoli, E., Guardia, M. L., Giammanco, S., Majo, D. D., & Giammanco, M. (2007). Citrus flavonoids: molecular structure, biological activity and nutritional properties: a review. *Food Chemistry, 104*, 466–479. http://dx.doi.org/10.1016/j.foodchem.2006.11.054.

Turner, T., & Burri, B. J. (2013). Potential nutritional benefits of current citrus consumption. *Agriculture, 3*, 170–187. http://dx.doi.org/10.3390/agriculture3010170.

Um, M. Y., Moon, M. K., Ahn, J., & Youl Ha, T. (2013). Coumarin attenuates hepatic steatosis by down-regulating lipogenic gene expression in mice fed a high-fat diet. *The British Journal of Nutrition, 109*, 1590–1597. http://dx.doi.org/10.1017/S0007114512005260.

Wang, M.-J., Chao, P. L., Hou, Y.-C., Hsiu, S.-L., Wen, K.-C., & Tsai, S.-Y. (2006). Pharmacokinetics and conjugation metabolism of naringin and naringenin in rats after single dose and multiple dose administrations. *Journal of Food and Drug Analysis, 14*(3), 247–253.

Weisberg, S. P., Mccann, D., Desai, M., Rosenbaum, M., Leibel, R. L., & Ferrante, A. W., Jr. (2003). Obesity is associated with macrophage accumulation in adipose tissue. *The Journal of Clinical Investigation, 112*(12), 1796–1808. http://dx.doi.org/10.1172/JCI200319246.

Yoshida, H., Takamura, N., Shuto, T., Ogata, K., Tokunaga, J., Kawai, K., et al. (2010). The citrus flavonoids hesperetin and naringenin block the lipolytic actions of TNF-alpha in mouse adipocytes. *Biochemical and Biophysical Research Communications, 394*, 728–732. http://dx.doi.org/10.1016/j.bbrc.2010.03.060.

Yoshida, H., Watanabe, W., Oomagari, H., Tsuruta, E., Shida, M., & Kurokawa, M. (2013). Citrus flavonoid naringenin inhibits TLR2 expression in adipocytes. *The Journal of Nutritional Biochemistry, 24*(7), 1276–1284. http://dx.doi.org/10.1016/j.jnutbio.2012.10.003.