Phytomedicine 25 (2017) 25–28

Contents lists available at ScienceDirect

Phytomedicine

journal homepage: www.elsevier.com/locate/phymed

Correlation between the cellular metabolism of quercetin and its glucuronide metabolite and oxidative stress in hypertrophied 3T3-L1 adipocytes

María Herranz-López[a,1], Isabel Borrás-Linares[b,1], Mariló Olivares-Vicente[a], Julio Gálvez[c], Antonio Segura-Carretero[b], Vicente Micol[a,d,*]

[a] Institute of Molecular and Cell Biology (IMCB), Miguel Hernández University (UMH), Elche 03202, Alicante, Spain
[b] Center for Research and Development of Functional Food (CIDAF), Science Technology Park Health, Armilla, Granada, Spain
[c] CIBER-EHD, Department of Pharmacology, Center for Biomedical Research (CIBM), University of Granada, Avenida del Conocimiento s/n 18016, Armilla, Granada, Spain
[d] CIBER, Fisiopatología de la Obesidad y la Nutrición, CIBERobn, Instituto de Salud Carlos III (CB12/03/30038), Spain

ARTICLE INFO

Article history:
Received 23 August 2016
Revised 14 November 2016
Accepted 15 December 2016

Keywords:
Quercetin
Quercetin-3-O-β-D-glucuronide
Adipocytes
Cellular metabolism
HPLC-DAD-ESI-TOF
ROS

ABSTRACT

Background: Quercetin (Q) is one of the most abundant flavonoids in human dietary sources and has been related to the capacity to ameliorate obesity-related pathologies. Quercetin-3-O-β-D-glucuronide (Q3GA) is supposed to be the main metabolite in blood circulation, but the intracellular final effectors for its activity are still unknown.
Hypothesis/purpose: To identify and quantitate the intracellular metabolites in hypertrophied adipocytes incubated with Q or Q3GA and to correlate them with the intracellular generation of oxygen radical species (ROS).
Methods: Cytoplasmic fractions were obtained and quercetin metabolites were determined by liquid chromatography coupled to a time-of-flight mass detector with electrospray ionization (HPLC-DAD-ESI-TOF). Intracellular ROS generation was measured by a ROS-sensitive fluorescent probe.
Results: Both Q and Q3GA were absorbed by hypertrophied adipocytes and metabolized to some extent to Q3GA and Q, respectively, but Q absorption was more efficient (1.92 ± 0.03 µg/µg protein) and faster than that of Q3GA (0.12 ± 0.0015 µg/µg protein), leading to a higher intracellular concentration of the aglycone. Intracellular decrease of ROS correlated with the presence of the most abundant quercetin metabolite.
Conclusion: Q and Q3GA are efficiently absorbed by hypertrophied adipocytes and metabolized to some extent to Q3GA and Q, respectively. The intracellular decrease of ROS in a hypertrophied adipocyte model treated with Q or Q3GA is correlated with the most abundant intracellular metabolite for the first time. Both compounds might be able to reach other intracellular targets, thus contributing to their bioactivity.

Introduction

Plant-derived polyphenols have demonstrated the potential to improve some disease states by a multitargeted mode of action (Barrajon-Catalan et al., 2014; Joven et al., 2012; Joven et al., 2014). Quercetin (3,3′,4′,5,7-pentahydroxyflavone) (Q) derivatives are the most abundant flavonoids in human dietary sources; ubiquitously present in fruits and vegetables (Ahn et al., 2008), they are converted into aglycone on the cell surface of intestinal epithelial cells and bacteria. Upon absorption, Q is subjected to different types of metabolism, with quercetin-3-O-β-D-glucuronide (Q3GA) as the major metabolite circulating in the bloodstream. The localization of Q3GA in macrophages, atherosclerotic lesions, brain and immune cells and lipid droplets of the liver by using specific antibodies has been reported (Joven et al., 2012; Kawai et al., 2008).

Q3GA is proposed to be deconjugated by β-glucuronidase into hydrophobic Q aglycone in cells, such as macrophages or vascular tissue, which in turn may improve intracellular pathological conditions (Ishisaka et al., 2013; Kawai et al., 2008; Menendez et al., 2011). Nevertheless, no deconjugation has been demonstrated in other cell models.

Abbreviations: Q, quercetin; Q3GA, quercetin-3-O-β-D-glucuronide; ROS, radical oxygen species; UGT, UDP-glucuronosyltransferase; PPAR, peroxisome proliferator-activated receptor; H2DCF-DA, 2′,7′-dichlorodihydrofluorescein diacetate.

* Corresponding author.
E-mail addresses: mherranz@umh.es (M. Herranz-López), vmicol@umh.es (V. Micol).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.phymed.2016.12.008
0944-7113/

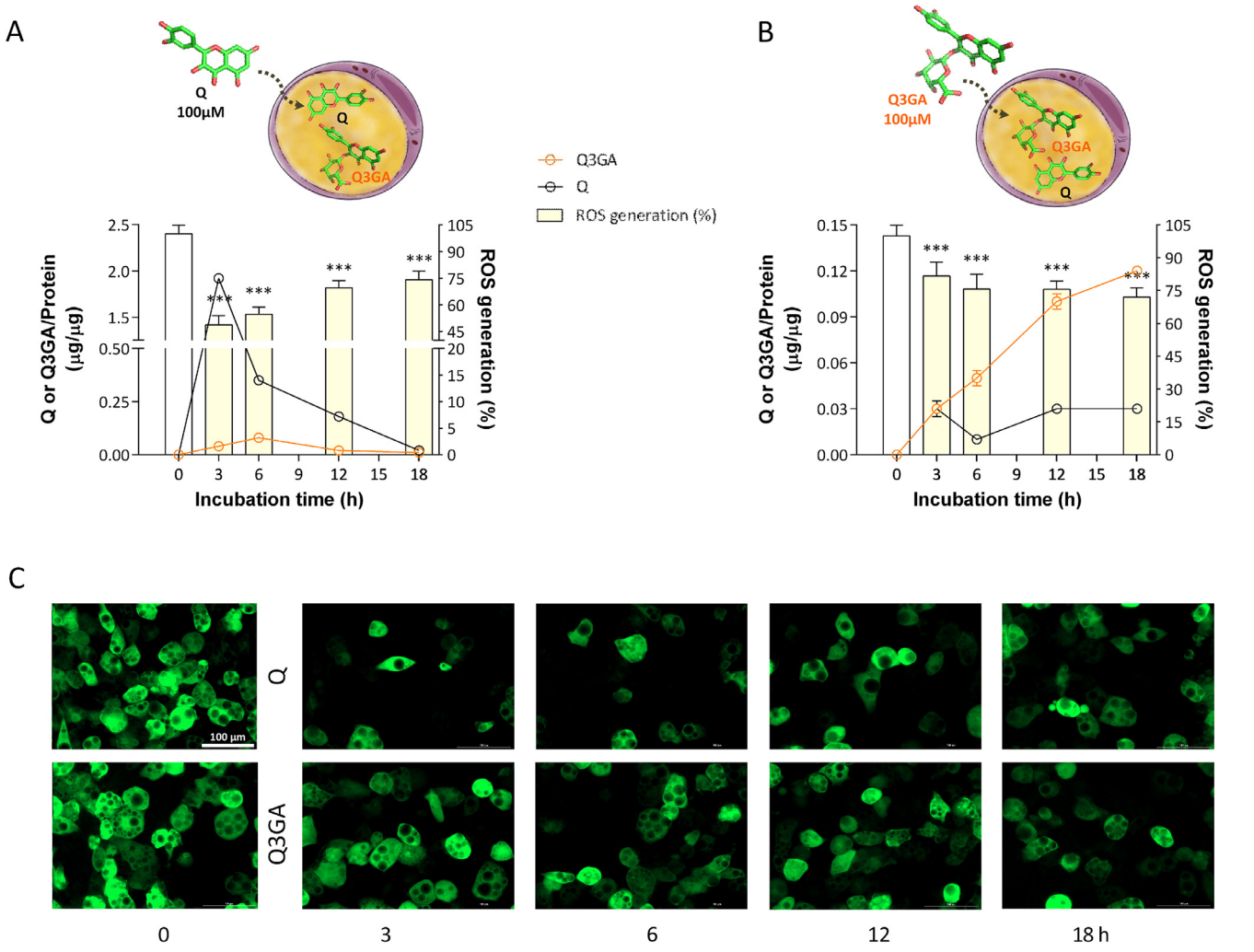

Fig. 1. Cellular metabolism of Q and Q3GA in hypertrophied adipocytes and the concomitant intracellular ROS decrease.
Hypertrophic adipocytes were treated with 100 µM Q (A) or 100 µM Q3GA (B) for 0, 3, 6, 12 and 18 h, and the cellular levels of both the aglycone and glucuronide metabolites were determined by HPLC-ESI-TOF-MS (black and orange circles, respectively). An inverse correlation was observed between intracellular ROS generation, determined by using the ROS-sensitive fluorescent probe H2DCF-DA (yellow bars) and the cytoplasmic levels of Q (A) and Q3GA (B). Representative photomicrographs of intracellular ROS after incubation of adipocytes with Q or Q3GA for 3, 6, 12 and 18 h (C). The intensity of green fluorescence represents the level of intracellular ROS compared to the control (0 h). All data were analyzed by using one-factor ANOVA and Tukey test for multiple comparisons. The results were expressed as the mean ± standard deviation. Differences showing $p < 0.05$ were considered statistically significant (n = 6).

Adipocyte hypertrophy compromises cell function, initiating an oxidative stress-related inflammatory process leading to metabolic disorders associated with obesity (Furukawa et al., 2004). Our findings strongly support that polyphenols from *Hibiscus sabdariffa* L. (Malvaceae) may become an alternative way to alleviate the metabolic disturbances associated with obesity through the modulation of energy management and inflammation pathways (Barrajon-Catalan et al., 2014; Beltran-Debon et al., 2010; Herranz-Lopez et al., 2012). Evidence in animal models leads us to propose that Q and Q3GA, among other flavonols, are the major blood metabolites accounting for these effects (Fig. 1, Supplementary information) (Fernandez-Arroyo et al., 2012; Joven et al., 2012). Nevertheless, the main quercetin metabolites reaching adipocyte intracellular targets are yet to be discovered. In the present work, a comparative study of the cellular metabolism of Q and its glucuronide metabolite was carried out by high performance liquid chromatography coupled to a time-of-flight mass detector with electrospray ionization (HPLC-DAD-ESI-TOF) in hypertrophied adipocytes, and the intracellular metabolites were correlated with the generation of ROS in the cytosol.

Material and methods

Chemicals

LC-MS grade formic acid for mobile phase preparation and the standards Q, Q3GA and naringenin (used as internal standard) were purchased from Fluka, Sigma-Aldrich (Steinheim, Germany). LC-MS grade acetonitrile and analytical reagent grade methanol and ethanol were obtained from Fisher Scientific (Madrid, Spain). Stock solutions containing these analytes were prepared in methanol and stored at −80 °C until use. 3T3-L1 cells and all tissue culture reagents were purchased as reported (Herranz-Lopez et al., 2012). Briefly, 3T3-L1 cells were purchased from the American Type Culture Collection (Manassas, VA, USA). Dexamethasone, 3-isobutyl-1- methylxanthine and insulin were obtained from Sigma–Aldrich (Madrid, Spain). Dulbecco's modified Eagle's medium, calf serum, fetal bovine serum, and an antibiotic mixture (penicillin–streptomycin) were purchased from PAA Laboratories (Linz, Austria). Sodium pyruvate and trypsin–EDTA were obtained from Invitrogen (Carlsbad, CA). Polyvinyldifluoride (PVD) filters, 0.22 µm, were obtained from Millipore (Bedford, MA).

Analysis of Q and Q3GA accumulation in hypertrophied 3T3-L1 adipocytes, sample processing and intracellular ROS measurement

The 3T3-L1 preadipocyte line was propagated and differentiated (Green and Kehinde, 1975). Hypertrophied adipocytes were obtained (Herranz-Lopez et al., 2012) and were treated with 100 µmol/l of Q or Q3GA in serum-free medium. At 0, 3, 6, 12 and 18 h of incubation, cultures were washed with PBS (pH 7.4) twice. Fractionation of cytoplasmic (pooled cell supernatant) and membrane fractions (precipitate) was achieved as reported elsewhere (Borrás-Linares et al., 2015). Briefly, for fractionation of the cells, 1 ml of 0.1% ascorbic acid solution (pH 4) was added to the cells. After three cycles of freezing and thawing, cells were harvested by repetitive pipetting. Pooled mixtures were sonicated three times in an ice-cold bath sonicator (VWR Aquasonic 75D; West Chester, PA) for 10 min each at level 7, then centrifuged at 16,000 x g for 15 min at 4 °C. Pooled supernatant and precipitate fractions are referred to as the cytoplasmic and membrane fractions, respectively. Protein concentration in the cell lysates was analyzed by the Bradford method according to the manufacturer's protocol (Bio-Rad, Hercules, CA). Intracellular ROS generation was measured in hypertrophied adipocytes using 2′,7′-dichlorodihydrofluorescein diacetate (H2DCF-DA, Sigma-Aldrich, Spain) as reported elsewhere (Herranz-Lopez et al., 2015) (see Supplementary information).

For HPLC analysis, the cytoplasmic fractions were spiked with 30 µg/ml of naringenin (methanol solution) used as an internal standard for the analysis. These samples were subjected to protein precipitation using methanol:ethanol (50:50, v/v) at a proportion of 1:5 (sample-solvent), vortex-mixed for 10 s, kept at −20 °C for 2 h and centrifuged at 17,900 x g for 15 min at 4 °C. Finally, the supernatants were collected and evaporated in a vacuum concentrator (Eppendorf Concentrator Plus, Eppendorf AG, Hamburg Germany), dissolved in 100 µl of methanol and stored at −80 °C until their analysis.

HPLC-DAD-ESI-TOF analysis and quantitation

The analyses of the cytoplasmic fractions were carried out using an Agilent 1200 RRLC system (Agilent Technologies, Palo Alto, CA, USA) of the Series Rapid Resolution coupled to a microTOFTM mass analyzer (Bruker Daltonik, Bremen, Germany) via an ESI interface (model G1607A, Agilent Technologies, Palo Alto, CA, USA) operating in negative ionization mode. The chromatographic column, separation conditions, mobile phases, DAD detection and standard calibration data are detailed in Supplementary information (Supplementary Materials and Supplementary Table 1).

Results and discussion

H. sabdariffa polyphenols inhibit triglyceride accumulation, oxidative stress and inflammation in cell models (Herranz-Lopez et al., 2012) and prevent hepatic steatosis in hyperlipidemic mice through the regulation of glucose and lipid homeostasis pathways (Joven et al., 2012). These effects are concomitant with the presence of Q3GA in immune cells and lipid droplets of the liver. Bioavailability studies have demonstrated that Q and Q3GA are the major metabolites found in plasma of rats fed *H. sabdariffa* polyphenols (Fernandez-Arroyo et al., 2012) (Fig. 1, Supplementary information).

To identify the intracellular compounds responsible for these effects, we carried out a comparative study of the uptake of Q and Q3AG by hypertrophied 3T3-L1 adipocytes after 3, 6, 12 and 18 h of incubation (Fig. 1, Supplementary Table 2). When adipocytes were incubated in the presence of Q, their glucuronide metabolite, Q3GA, appeared progressively in the cytoplasm, reaching its maximum concentration after 6 h of incubation (0.08 ± 0.0025 µg/µg protein) and then decaying throughout the assay (Fig. 1A, Supplementary Table 2). In contrast, the Q concentration sharply increased after 3 h of incubation up to a concentration of 1.92 ± 0.03 µg/µg (Fig. 1A). Upon incubation of hypertrophied adipocytes with Q3GA, the intracellular concentration of this compound steadily increased until it reached its maximum concentration at 18 h (0.12 ± 0.0015 µg/µg) (Fig. 1B). However, quercetin aglycone appeared quickly, reaching its maximum value after 3 h of incubation (0.03 ± 0.005 µg/µg) and showing a plateau that was maintained throughout the study.

The uptake of Q was approximately 16-fold higher and was much faster (3 h) compared to the maximum concentration of Q3GA achieved in adipocytes incubated with this metabolite (18 h), highlighting that Q was rapidly absorbed by passive diffusion. In contrast, Q3GA was less efficiently absorbed due to the higher polarity of glucuronic derivatives, which hampers their absorption through the cell membrane, as postulated for other cell lines (Spencer et al., 2003; Youdim et al., 2003). In agreement with our observation, it has been reported that Q-7- and Q-3-glucuronides, but not Q-4-glucuronides, can be absorbed and undergo turnover by intracellular β-glucuronidase activity in human liver hepatocellular cells, most likely by an influx transporter such as the organic anion transport polypeptide (OATP2) (O'Leary et al., 2003).

Interestingly, Q concentrations dropped sharply, approximately 5.5-fold, after 6 h of incubation compared with 3 h, which can be explained on the basis of metabolism/oxidation of Q leading to diverse degradation compounds (*o*-quinones), as reported in a human hepatocellular carcinoma cell line (Boyer et al., 2004). In our case, no degradation compounds were detected in the cytoplasmic fractions by HPLC-DAD-ESI-TOF. Alternatively, low quantities of Q3GA were found, indicating the presence of biotransformation most likely due to UDP-glucuronosyltransferase (UGT) activity.

Our results clearly indicate that biotransformation occurs in both directions in adipocytes, from Q to Q3GA and *vice versa*. Glucuronidation of several flavonoids has been observed in fibroblasts (Proteggente et al., 2003). Isoforms of UGT have been found in several cell lines (Barbier et al., 2003; Beaulieu et al., 1998), and UGT 1A9 has been implicated in the glucuronidation of the synthetic peroxisome proliferator-activated receptor (PPAR) activators (Prueksaritanont et al., 2002; Watanabe et al., 2002) and natural PPARα and PPARγ agonists (Barbier et al., 2003). Furthermore, treatment with PPAR activators, such as plant polyphenols (Encinar et al., 2015; Herranz-López et al., 2015; Wang et al., 2014), results in enhanced glucuronidation activity and UGT expression (Barbier et al., 2003). All these data suggest that glucuronidation reactions can occur *in vivo* in diverse tissues apart from the liver and small intestine and indicate that polyphenols may induce their own glucuronidation. In our hypertrophic adipocyte model, UGT activity seemed to be weaker than that of glucuronidase, since the conversion of Q3GA to Q seemed to be more efficient than the reverse process. According to our results, it has been reported that in cell conditions bearing high levels of inflammation or oxidative stress, as occurs in hypertrophic adipocytes, Q emerges by the action of β-glucuronidase (Terao et al., 2011).

The correlation between the biological effects of polyphenols and the intracellular presence of the responsible metabolites is hard to prove. Therefore, we aimed to study the effects of cytoplasmic metabolites on the evolution of concomitant oxidative stress in a hypertrophied adipocyte model (Han et al., 2007; Herranz-Lopez et al., 2012). Intracellular ROS levels were quantified in the same adipocyte model treated with Q or Q3GA using the fluorescent probe H2DCF-DA (Fig. 1). Adipocytes treated with Q or Q3GA during different times (3–18 h) exhibited a significant reduction of ROS generation compared with the controls (Fig. 1C). The highest reduction of ROS level achieved by Q incubation was obtained after 3 h of incubation (49% of oxidation) (Fig. 1A and C).

After that, oxidative stress gradually increased to reach 74% oxidation after 18 h of incubation with the aglycone. Considering that Q was more abundant than Q3GA and that oxidation behavior correlated with the intracellular accumulation of Q, quercetin aglycone was proposed to be primarily responsible for the ROS decrease in this case. However, when adipocytes were incubated with Q3GA, the intracellular ROS production decreased steadily from 3 h (82% oxidation) up to the minimum achieved at the end of the assay (72% oxidation) (Fig. 1B and C). This result confirms a direct relation between the accumulation of Q3GA and the decrease of ROS. However, we cannot rule out that the total intracellular antioxidant activity is the result of the balanced contribution of all conjugate metabolites (Terao et al., 2011).

Conclusions

In conclusion, both Q and Q3GA were absorbed by hypertrophied adipocytes and metabolized to Q3GA and Q by glucuronosyltransferase and glucuronidase activity, respectively. Absorption was more efficient and faster for Q than Q3GA, leading to a higher intracellular concentration of the aglycone. Both metabolites Q and Q3GA were responsible for an intracellular decrease of ROS in hypertrophied adipocytes. These results support for the first time that both metabolites may account for the strong radical scavenging capacity observed in hypertrophied adipocytes treated with the polyphenolic extract of *H. sabdariffa* (Herranz-Lopez et al., 2012) and might also reach other intracellular targets that contribute to their bioactivity.

Conflict of interest

We confirm that there are no known conflicts of interest associated with this publication.

Acknowledgments

We are grateful for grant AGL2011-29857-C03-03 from MICINN; Grants AGL2015-67995-C3-1-R, AGL2015-67995-C3-2-R, AGL2015-67995-C3-3R, PTQ-13-06429 and PTQ-14-07243 from MINECO; PROMETEO/2012/007 and PROMETEO/2016/006, and ACIF/2016/230 to MOV from Generalitat Valenciana; CIBER (CB12/03/30038, Fisiopatologia de la Obesidad y la Nutricion, CIBERobn, Instituto de Salud Carlos III).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.phymed.2016.12.008.

References

Ahn, J., Lee, H., Kim, S., Park, J., Ha, T., 2008. The anti-obesity effect of quercetin is mediated by the AMPK and MAPK signaling pathways. Biochem. Biophys. Res. Commun. 373, 545–549.

Barbier, O., Villeneuve, L., Bocher, V., Fontaine, C., Torra, I.P., Duhem, C., Kosykh, V., Fruchart, J.-C., Guillemette, C., Staels, B., 2003. The UDP-glucuronosyltransferase 1A9 enzyme is a peroxisome proliferator-activated receptor α and γ target gene. J. Biol. Chem. 278, 13975–13983.

Barrajon-Catalan, E., Herranz-Lopez, M., Joven, J., Segura-Carretero, A., Alonso-Villaverde, C., Menendez, J.A., Micol, V., 2014. Molecular promiscuity of plant polyphenols in the management of age-related diseases: far beyond their antioxidant properties. Adv. Exp. Med. Biol. 824, 141–159.

Beaulieu, M., Lévesque, É., Hum, D.W., Bélanger, A., 1998. Isolation and characterization of a human orphan UDP-glucuronosyltransferase, UGT2B11. Biochem. Biophys. Res. Commun. 248, 44–50.

Beltran-Debon, R., Alonso-Villaverde, C., Aragones, G., Rodriguez-Medina, I., Rull, A., Micol, V., Segura-Carretero, A., Fernandez-Gutierrez, A., Camps, J., Joven, J., 2010. The aqueous extract of *Hibiscus sabdariffa* calices modulates the production of monocyte chemoattractant protein-1 in humans. Phytomedicine 17, 186–191.

Borrás-Linares, I., Herranz-López, M., Barrajón-Catalán, E., Arráez-Román, D., González-Álvarez, I., Bermejo, M., Gutiérrez, A.F., Micol, V., Segura-Carretero, A., 2015. Permeability study of polyphenols derived from a phenolic-enriched *Hibiscus sabdariffa* extract by UHPLC-ESI-UHR-Qq-TOF-MS. Int. J. Mol. Sci. 16, 18396–18411.

Boyer, J., Brown, D., Liu, R.H., 2004. Uptake of quercetin and quercetin 3-glucoside from whole onion and apple peel extracts by Caco-2 cell monolayers. J Agric. Food Chem. 52, 7172–7179.

Encinar, J.A., Fernandez-Ballester, G., Galiano-Ibarra, V., Micol, V., 2015. In silico approach for the discovery of new PPARgamma modulators among plant-derived polyphenols. Drug Des. Dev. Ther. 9, 5877–5895.

Fernandez-Arroyo, S., Herranz-Lopez, M., Beltran-Debon, R., Borras-Linares, I., Barrajon-Catalan, E., Joven, J., Fernandez-Gutierrez, A., Segura-Carretero, A., Micol, V., 2012. Bioavailability study of a polyphenol-enriched extract from *Hibiscus sabdariffa* in rats and associated antioxidant status. Mol. Nutr. Food Res. 56, 1590–1595.

Furukawa, S., Fujita, T., Shimabukuro, M., Iwaki, M., Yamada, Y., Nakajima, Y., Nakayama, O., Makishima, M., Matsuda, M., Shimomura, I., 2004. Increased oxidative stress in obesity and its impact on metabolic syndrome. J. Clin. Invest. 114, 1752–1761.

Green, H., Kehinde, O., 1975. An established preadipose cell line and its differentiation in culture. II. Factors affecting the adipose conversion. Cell 5, 19–27.

Han, C.Y., Subramanian, S., Chan, C.K., Omer, M., Chiba, T., Wight, T.N., Chait, A., 2007. Adipocyte-derived serum amyloid A3 and hyaluronan play a role in monocyte recruitment and adhesion. Diabetes 56, 2260–2273.

Herranz-Lopez, M., Barrajon-Catalan, E., Segura-Carretero, A., Menendez, J.A., Joven, J., Micol, V., 2015. *Lemon verbena* (*Lippia citriodora*) polyphenols alleviate obesity-related disturbances in hypertrophic adipocytes through AMPK-dependent mechanisms. Phytomedicine 22, 605–614.

Herranz-Lopez, M., Fernandez-Arroyo, S., Perez-Sanchez, A., Barrajon-Catalan, E., Beltran-Debon, R., Menendez, J.A., Alonso-Villaverde, C., Segura-Carretero, A., Joven, J., Micol, V., 2012. Synergism of plant-derived polyphenols in adipogenesis: perspectives and implications. Phytomedicine 19, 253–261.

Ishisaka, A., Kawabata, K., Miki, S., Shiba, Y., Minekawa, S., Nishikawa, T., Mukai, R., Terao, J., Kawai, Y., 2013. Mitochondrial dysfunction leads to deconjugation of quercetin glucuronides in inflammatory macrophages. PLoS One. 8, e80843.

Joven, J., Espinel, E., Rull, A., Aragones, G., Rodriguez-Gallego, E., Camps, J., Micol, V., Herranz-Lopez, M., Menendez, J.A., Borras, I., Segura-Carretero, A., Alonso-Villaverde, C., Beltran-Debon, R., 2012. Plant-derived polyphenols regulate expression of miRNA paralogs miR-103/107 and miR-122 and prevent diet-induced fatty liver disease in hyperlipidemic mice. Biochem. Biophys. Acta. 1820, 894–899.

Joven, J., Micol, V., Segura-Carretero, A., Alonso-Villaverde, C., Menéndez, J.A., Aragonès, G., Barrajón-Catalán, E., Beltrán-Debón, R., Camps, J., Cufí, S., Fernández-Arroyo, S., Fernández-Gutiérrez, A., Guillén, E., Herranz-López, M., Iswaldi, I., Lozano-Sánchez, J., Martin-Castillo, B., Oliveras-Ferraros, C., Pérez-Sánchez, A., Rodríguez-Gallego, E., Rull, A., Saura, D., Vázquez-Martín, A., 2014. Polyphenols and the modulation of gene expression pathways: can we eat our way out of the danger of chronic disease? Crit. Rev. Food Sci. Nutr. 54, 985–1001.

Kawai, Y., Nishikawa, T., Shiba, Y., Saito, S., Murota, K., Shibata, N., Kobayashi, M., Kanayama, M., Uchida, K., Terao, J., 2008. Macrophage as a target of quercetin glucuronides in human atherosclerotic arteries: implication in the anti-atherosclerotic mechanism of dietary flavonoids. J. Biol. Chem. 283, 9424–9434.

Menendez, C., Duenas, M., Galindo, P., Gonzalez-Manzano, S., Jimenez, R., Moreno, L., Zarzuelo, M.J., Rodriguez-Gomez, I., Duarte, J., Santos-Buelga, C., Perez-Vizcaino, F., 2011. Vascular deconjugation of quercetin glucuronide: the flavonoid paradox revealed? Mol. Nutr. Food Res. 55, 1780–1790.

O'Leary, K.A., Day, A.J., Needs, P.W., Mellon, F.A., O'Brien, N.M., Williamson, G., 2003. Metabolism of quercetin-7- and quercetin-3-glucuronides by an in vitro hepatic model: the role of human beta-glucuronidase, sulfotransferase, catechol-*O*-methyltransferase and multi-resistant protein 2 (MRP2) in flavonoid metabolism. Biochem. Pharmacol. 65, 479–491.

Proteggente, A.R., Basu-Modak, S., Kuhnle, G., Gordon, M.J., Youdim, K., Tyrrell, R., Rice-Evans, C.A., 2003. Hesperetin glucuronide, a photoprotective agent arising from flavonoid metabolism in human skin fibroblasts. Photochem. Photobiol. 78, 256–261.

Prueksaritanont, T., Tang, C., Qiu, Y., Mu, L., Subramanian, R., Lin, J.H., 2002. Effects of fibrates on metabolism of statins in human hepatocytes. Drug Metab. Dispos. 30, 1280–1287.

Spencer, J.P.E., Kuhnle, G.G.C., Williams, R.J., Rice-Evans, C., 2003. Intracellular metabolism and bioactivity of quercetin and its in vivo metabolites. Biochem. J. 372, 173–181.

Terao, J., Murota, K., Kawai, Y, 2011. Conjugated quercetin glucuronides as bioactive metabolites and precursors of aglycone in vivo. Food Funct. 2, 11–17.

Wang, L., Waltenberger, B., Pferschy-Wenzig, E.-M., Blunder, M., Liu, X., Malainer, C., Blazevic, T., Schwaiger, S., Rollinger, J.M., Heiss, E.H., Schuster, D., Kopp, B., Bauer, R., Stuppner, H., Dirsch, V.M., Atanasov, A.G., 2014. Natural product agonists of peroxisome proliferator-activated receptor gamma (PPARγ): a review. Biochem. Pharmacol. 92, 73–89.

Watanabe, Y., Nakajima, M., Yokoi, T., 2002. Troglitazone glucuronidation in human liver and intestine microsomes: high catalytic activity of UGT1A8 and UGT1A10. Drug Metab. Dispos. 30, 1462–1469.

Youdim, K.A., Dobbie, M.S., Kuhnle, G., Proteggente, A.R., Abbott, N.J., Rice-Evans, C., 2003. Interaction between flavonoids and the blood–brain barrier: *in vitro* studies. J. Neurochem. 85, 180–192.

دانشیاری؛ یار دانش پژوهان ایرانی

daneshyari.com