CYANIDIN-3-O-GLUCOSIDE AMELIORATES PALMITATE-INDUCED INFLAMMATION AND INSULIN RESISTANCE IN MURINE ADIPOCYTES

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Introduction: Obesity and obesity-related disorders have become world-wide epidemics. Characteristic of these disorders, which include metabolic syndrome and type 2diabetes, are excess adipose tissue and local and systemic chronic low-grade inflammation that can predispose to insulin resistance. Obesity results in an increased flux of free fatty acids (FFA) into the circulation and subsequent uptake by myocytes, hepatocytes or adipose tissue, and it has been proposed that FFA can induce insulin resistance by promoting oxidative stress, inflammation, and hypertrophy in adipose tissue. Epidemiological evidences have shown that anthocyanins, natural polyphenols belonging to the flavonoids group, possess marked anti-obesity and insulin sensitizing effects. Therefore, the aim of this study was to investigate the in vitro protective effects of the anthocyanin cyanidin-3-O-glucoside (C3G) against hypertrophy, inflammation, and insulin resistance induced by high concentrations of the FFA palmitic acid (PA) in murine 3T3-L1.

Materials and methods: In all experiments fully differentiated 3T3-L1adipocytes were pretreated with C3G (5-10μM) for 24h, and then exposed to PA for the next 24h. Finally, in order to evaluate the effects on PA-induced insulin-resistance, cells were treated with insulin. In order to characterize the effects of PA and to demonstrate the protective effects of C3G in such conditions, lipid accumulation in adipocytes was evaluated by Oil Red O staining, while the effects on cellular signaling pathways involved in adipogenesis (PPAR-γ pathway), inflammatory process (NF-kB pathway), and insulin resistance (IRS-1/PI3K/Akt pathway), were evaluated by means of Western blot and real-time PCR.

Results: The results obtained on this model demonstrate that C3G pretreatment reduces lipid accumulation, the protein levels of the master transcriptional regulator of adipogenesis PPARγ, and gene expression of FAPB4, a key regulator of fatty acid uptake and lipid accumulation, induced by PA. Moreover, exposure to PA induces inflammation with activation of NF-κB pathway, increasing nuclear accumulation of p65, IKK phosphorylation, and IL-6gene expression, while C3G pretreatment dose-dependently inhibited the effect of PA on this pathway. Furthermore, adipocyte dysfunction associated with hypertrophy induces insulin resistance by affecting IRS1/PI3K/Akt axis. C3G pretreatment dose-dependently reverts these effects induced by PA, reducing phosphorylation of IRS at Ser307site, and restoring insulin ability to induce phosphorylation and activation of PI3K and Akt, as well as GLUT-1protein levels, and adiponectin gene expression.

Discussions and conclusions: The data of this study show that C3G is able to protect adipocytes from hypertrophy induced by elevated concentrations of PA by affecting the transcriptional factor PPARγ protein levels. Moreover, lipid overload, produced by PA exposure, induces chronic low-grade inflammation in adipocytes, as demonstrated by IKK phosphorylation and NF-κB nuclear translocation, while C3G pretreatment dose-dependently inhibited NF-κB pathway activation induced by PA. Finally, C3G ameliorates insulin resistance conditions by restoring IRS1/PI3K/Akt axis, which plays an essential role in mediating insulin-stimulated glucose uptake and gluconeogenesis suppression. Therefore, this study demonstrates the prevention potential of C3G against obesity comorbidities and reveals the molecular mechanisms involved, thus supporting a potential application of anthocyanins in the prevention and therapy of pathological conditions related to obesity.