ANTHOCYANIN RICH EXTRACT AMELIORATES PALMITIC ACID-INDUCED INFLAMMATION AND HYPERTROPHY IN 3T3-L1MURINE ADIPOCYTES

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Introduction: The World Health Organization established that obesity is a serious public health problem due to the associated risk factors that include type 2diabetes mellitus, hypertension, dyslipidemia and cardiovascular diseases. Obesity is often related to metabolic syndrome (MetS) and it is caused by an exorbitant and constant positive energy balance. Excessive lipid accumulation in adipose tissue leads to hypertrophic adipocytes which are associated with dysfunctional adipocytes and altered production of proinflammatory cytokines triggering lipotoxicity and an inflammatory state. In fact, obesity is related to a low-grade chronic inflammatory state, characterized by slow macrophage infiltration, which increases the local release of TNF-α and IL-6. In the last decades, anthocyanins, a subclass belonging to the flavonoid family, have been shown to be able to reduce the development of obesity and related diseases. However, the molecular mechanisms involved in their effects are not fully known. The aim of this study was to investigate the in vitro protect effect exerted by an anthocyanin rich extract (ACN), a standardized extract consisting of 17purified anthocyanins from bilberries (Vaccinium myrtillus) and blackcurrant (Ribes nigrum), against hypertrophy and inflammation induced by high concentrations of palmitic acid (PA) in 3T3-L1murine adipocytes.

Materials and methods: Fully differentiated 3T3-L1adipocytes were pretreated with different concentrations of ACN (10 – 20 μg/ml) for 24h and then exposed to high concentrations of PA (1mM) for 24h. The effect of ACN on lipid accumulation was evaluated through Oil Red O staining. Western blot and Real-time PCR techniques were used for the investigation of adipogenic PPAR-γ and NF-κB pathways.

Results: Results demonstrated that ACN pretreatment protects murine adipocytes from PA-induced excessive fatty acids storage through inhibition of adipogenesis process, as shown by reduced lipid accumulation and lower protein levels of the master transcriptional regulator of adipogenesis, PPARγ. In order to confirm PPARγ transcriptional activity, FABP4gene expression was evaluated, since FABP4is a target gene of this transcription factor. Our data confirmed that ACN pretreatment reduced FABP4mRNA levels induced by PA. In addition, ACN, at all the tested concentrations, have been shown to be able to reduce activation of the NF-κB inflammatory pathway triggered by high concentrations of PA. In fact, lower nuclear values of p65subunit (NF-κB) have been found in adipocytes pretreated with ACN. Furthermore, these data were confirmed at transcriptional levels since IL6gene expression was reduced by ACN pretreatment.

Discussions and conclusions: ACN showed its protective effect against PA-induced hypertrophy in adipocytes by affecting the transcriptional factor PPARγ and FABP4downstream gene. We further demonstrated that lipid overload, produced by PA exposure, induces chronic low grade inflammation in adipocytes as shown by NF-κB pathway activation that was reverted by ACN pretreatment. Interestingly, this study elucidated a PPARγ-indipendent mechanism in NF-κB modulation. In fact, our data showed that ACN reduced NF-κB pathway through mechanisms not involving PPARγ activation. In conclusion, these results clarify the molecular mechanisms underlying the protective effect of anthocyanins suggesting that the intake of these molecules could prevent pathological conditions related to obesity.