Differential effects of grape-seed derived procyanidins on adipocyte differentiation markers in different in vivo situations

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Introduction

Obesity is one of the main problems in industrialized societies and adipose tissue is a key target tissue of this disease. There is therefore much interest in studying the adipose metabolism and the mechanisms of adipocyte differentiation. Procyanidins (oligomeric forms of catechins) are the most abundant polyphenols in red wine, apple and chocolate. Previous studies by our group showed that grape seed derived procyanidins (GSPE) limit adipocyte formation by altering the gene expression profile during in vitro adipocyte differentiation [1]. GSPE also decreased the mRNA levels of PPAR γ 2 and HSL on fully differentiated 3T3-L1 adipocytes [2]. In this paper we analyze the effects of GSPE on adipocyte differentiation markers under different in vivo physiological conditions.

Materials and methods

Chemical

Grape seed procyanidin extract was provided by Les Dérives Résiniques et Terpéniques (Dax, France). According to the manufacturer, this procyanidin extract essentially contained monomeric (16.55%), dimeric (18.77%), trimeric (16%), tetrameric (9.3%), oligomeric procyanidins (5–13 units; 35.7%), and phenolic acids (4.22%).

Animal experimental procedures

For the acute treatment, we worked with 2-month-old male Wistar rats from Charles River Laboratories (Barcelona, Spain). Some of the animals were treated with streptozotocin to obtain animals with Type 1 diabetes mellitus [3]. On the day of the experiment, the rats were fed an oral gavage of GSPE in aqueous solution (250 mg/kg body wt). Five hours after treatment, the rats were killed by beheading and epididymal tissue was frozen immediately. For the chronic treatment, 2-month-old male fa/fa Zucker rats from Charles River (Barcelona, Spain) were chronically administered 19.5 mg/g day of GSPE. The treatment was oral gavage and the GSPE was dissolved in sunflower oil. After 30 days of treatment, the rats were killed by beheading and mesenteric and epididymal adipose tissues were excised and frozen immediately. All procedures were approved by the Animal Ethics Committee of the Rovira i Virgili University.

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Quantitative RT-PCR

Changes in the mRNA expression of selected adipocyte markers (PPAR γ 2, HSL, C/EBP α and Pref-1) [1] were analysed by quantitative PCR. Briefly, 1 mg of total RNA was reverse transcribed by the SuperScript II Rnase H₂ Reverse Transcriptase (LifeTechnologies). RT product corresponding to 20 ng initial RNA was amplified according to the protocols of Applied Biosystems. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was used as an endogenous control to normalize data. The following primers were used:

GAPDH	CAT GGC CTT CCG TGT TCC T (forward) CCTGCT TCA CCA CCT TCT TGA (reverse)
PPAR γ 2	CTG TTGACC CAG AGC ATG GT (forward) AGA GGT CCA CAG AGC TGA TTC C (reverse)
HSL	GGA GCA CTA CAA ACG CAA CGA (forward) AAT CGG CCA CCG GTA AAG AG (reverse)
Pref-1	TGC GCC AAC AAT GGA ACT T (forward) TGG CAG TCC TTT CCA GAG AAC (reverse)
C/EBP α	GGT GGA CAA GAA CAG CAA CGA (forward) CGT TGC GTT GTT TGG CTT TAT C (reverse)

Statistical analysis

Results are expressed as mean $\pm$ SEM. Effects were assessed using *t* test. All calculations were performed using SPSS software.

Results and discussion

In healthy Wistar rats, an acute high oral dose of GSPE decreased epididymal mRNA levels of early adipocyte markers such as PPAR γ 2, C/EBP α and Pref-1 but did not affect late adipocyte markers such as HSL. However, the same acute GSPE treatment induced almost opposite effects on streptozotocin-induced diabetic animals (Fig. 1). In this pathological model we also studied an animal group treated with an effective dose of insulin to evaluate the insulin-like effect of procyanidins. As we showed previously [4] when working with another functions targets of procyanidins, the GSPE did not totally mimic the insulin effect. The effects obtained in the healthy animals under acute treatment were similar to those obtained in the *fa/fa* Zucker rats after a chronic oral treatment with GSPE i.e. decreased mRNA levels of PPAR γ 2, C/EBP α and Pref-1, and minimal effect on HSL gene expression (Fig. 2A).

Fig. 1 GSPE effects on mRNA levels of adipose differentiation markers

Fig. 2 GSPE effects on mRNA levels of adipocyte markers in different adipose tissues

However, these effects were only identified in epididymal adipose tissue (Fig. 2B). The same analysis on mesenteric adipose tissue showed a decrease only in Pref-1 expression with no changes in the other adipocyte markers. In conclusion, these results show that GSPE modifies adipose differentiation markers differently depending on the physiological conditions and adipose tissue depot.

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