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Proanthocyanidins modulate triglyceride secretion by repressing the expression of long chain acyl-CoA synthetases in Caco2 intestinal cells

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ABSTRACT

In this work we determined the ability of grape seed proanthocyanidins (GSPE) to modulate triglyceride (TG) secretion by enterocytes in post-prandial and fasting states, using Caco2 cell line. GSPE did not modify TG secretion in the post-prandial state, even though it modulated the long chain acyl-CoA synthetase (ACSL) 5 and the carnitine-palmitoyl-transferase-1a gene expression. On the contrary, GSPE decreased TG secretion in the fasting state, significantly repressing ACSL3, ACSL5, I-FABP and PPARalpha gene expression. Intestinal cells can use two different pathways to carry out TG synthesis. Fatty acids (FA) delivered by ACSL3 and by ACSL5 would be directed towards the monoacylglycerol and glycerol-3-phosphate pathways, respectively. Therefore, proanthocyanidins repress the supply of FA towards the monoacylglycerol pathway in the post-prandial state, whereas they repress the supply of FAs towards both pathways in the fasting state, suggesting that the feeding state is a key factor regarding the effectiveness of proanthocyanidins to reduce triglyceridaemia.

1. Introduction

The disturbances of triglyceride (TG) metabolism are considered to be a substantial risk factor for obesity, atherosclerosis, insulin-dependent diabetes, breast and colon cancers (Alipour, Elte, van Zaanen, Rietveld, & Castro Cabezas, 2008). Elevated levels of TG-rich lipoproteins (TRL), especially chylomicron (CM) remnants and very low density lipoprotein (VLDL) remnants, accumulate in the blood flow, and are strongly associated with an increased of the risk of myocardial infarction, ischaemic stroke and early death (Fujioka & Ishikawa, 2009).

The small intestine is the second most important source of plasma TRL, just after the liver (Levy, Mehran, & Seidman, 1995). The intestine secretes several different lipoproteins, CM and VLDLs are the major ones (Mu & Hoy, 2004). VLDLs are the predominant lipoproteins during the fasting state and their assembly occurs constitutively (Hussain, 2000). VLDLs may serve to transport lipids derived from the bile and fatty acids of the plasma (Hussain, 2000). In the post-prandial state, CM secretion is induced after fat ingestion and is impaired by the absence of bile acids (Hussain, 2000).

Most dietary TGs are absorbed by the enterocytes as fatty acids (FA) and monoacylglycerides (MAG). FA and MAG require reassembly to produce TG on the endoplasmic reticulum by, predominantly, the progressive acylation of MAG *via* the monoacylglycerol pathway (Levy et al., 1995; Mansbach & Gorelick, 2007). Additionally, TG can also be synthesised by a separate route, by means of the acylation of glycerol-3-phosphate (G-3-P) (Mansbach & Gorelick, 2007). The MAG pathway would predominate in the post-prandial period while the G-3-P pathway is the main one in the interprandial and fasted period (Petit, Niot, Poirier, & Besnard, 2007/3). Oleate entering from the apical membrane is preferentially shunted to the MAG pathway to form TG, whereas oleate entering from the basolateral membrane *via* the circulation, is shunted to the G-3-P acylation pathway. The required enzyme activating the FA prior to its incorporation into MAG or G-3-P is one of the five members of the acyl-CoA synthetase long chain family (ACSL) (Mansbach & Gorelick, 2007). Of these ACSLs, only ACSL3 and 5 are significantly expressed in the intestine (Mansbach & Gorelick, 2007). In this proposed scenario, oleate-CoA delivered by ACSL5 would be directed towards the MAG pathway and that delivered by ACSL3 would be directed towards the G-3-P pathway (Mansbach & Gorelick, 2007).

Proanthocyanidins (PA), the most abundant polyphenols in grapes, apples, red grape juice, red wine and chocolate (Crozier, Jaganath, & Clifford, 2009; Mink et al., 2007) have been shown to reduce post-prandrial hypertriglyceridaemia in animal models (Blade, Arola, & Salvado, 2010) and improve plasma lipids in humans (Zern et al., 2005). The hypolipidaemic action of proanthocyanidins is attributable to a reduction of plasma levels TRL both in normolipidaemic (Del Bas et al., 2005) and dyslipidaemic rats (Quesada et al., 2009). Plasma TG levels are the result of the balance between the TRL secretion by the intestine and the liver and their uptake by the extrahepatic tissues through the

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lipoprotein lipase (LPL). Several studies demonstrated the role of the liver in the hypotriglyceridaemic response triggered by PA (Del Bas et al., 2008) and established the molecular mechanisms by which PA modulated lipid and lipoprotein metabolism in the liver (Del Bas et al., 2009). However, the molecular mechanism by which PA modulates lipid and lipoprotein metabolism in the intestine is largely unknown. Thus, the aim of this study was to gain further insights into the role that the intestine plays in the hypotriglyceride action of PA. Intestinal cells can use two different pathways to carry out the TG synthesis, the MAG pathway in the post-prandial state and the G-3-P pathway in the fasting state. Thus, we evaluated the effects of grape seed proanthocyanidins extract (GSPE) on TG secretion and gene expression, by using the Caco2 cell line cultured in specific media simulating the two different feeding states.

2. Methods

2.1. Proanthocyanidin extract

GSPE was kindly provided by Les Dérives Résiniques et Terpéniques (Dax, France).

This proanthocyanidin extract contained essentially monomeric (21.3%), dimeric (17.4%), trimeric (16.3%), tetrameric (13.3%) and oligomeric (5–13 units) (31.7%) proanthocyanidins.

2.2. Materials

Taurocholate acid sodium salt (TC), fatty-acid-free bovine serum albumin (BSA), and monoolein were obtained from Sigma. Oleic acid (OA) was purchased from Merck.

2.3. Cell cultures

Caco2 cells (ATCC, Manassas, VA) were grown in Dulbecco's modified Eagle's medium (DMEM; BioWhittaker) supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin (BioWhittaker), 2 mM L-Glutamine (BioWhittaker) and 20% foetal bovine serum (BioWhittaker) in a 95% air, 5% CO_2 atmosphere at 37 °C. The growth medium was replenished every two or three days. For gene expression analysis, cells at passages 58–61 were seeded in 12-well plates at 2.5×10^5 cells per well. For experiments on the secretion of TG from Caco2 cells, the cells at passages 60–63 were seeded in 12-well Millicell Hanging Cell Culture Inserts (Millipore, Billerica, MA) at 1×10^5 per insert. The experiments were performed at 18–21 days post-seeding.

2.4. Preparation of feeding and fasting state

In order to perform a post-prandial state (Luchoomun & Hussain, 1999), the volume of the culture medium was 0.4 mL of DMEM without Phenol Red (BioWhittaker) supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin (BioWhittaker), 2 mM L-Glutamine (BioWhittaker), 0.8 mM OA, 0.5 mM TC and 0.4 mM monoolein on the apical side, and 1 mL of DMEM without Phenol Red (BioWhittaker) supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin (BioWhittaker), 2 mM L-glutamine (BioWhittaker) and 0.4 mM BSA on the basolateral side.

In order to perform a fasting state (Luchoomun & Hussain, 1999), the volume of the culture medium was 0.4 mL of DMEM without Phenol Red (BioWhittaker) supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin (BioWhittaker), 2 mM L-glutamine (BioWhittaker) supplemented with 0.4 mM BSA on the apical side, and 1 mL of DMEM without Phenol Red (BioWhittaker) supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin (BioWhittaker), 2 mM L-Glutamine (BioWhittaker), 0.8 mM OA and 0.4 mM BSA on the basolateral side.

Transepithelial electrical resistance (TEER) was measured just after removing the growth medium by using the Millicell-ERS system (Millipore). The TEER value of Caco2 cultured in each transwells chamber was around $308 \pm 3.5\ \Omega\ cm^2$ in the post-prandial state and $319 \pm 5.9\ \Omega\ cm^2$ in the fasted state indicating the formation of tight monolayers (Ho, Delgado, & Storch, 2002).

2.5. Triglyceride secretion

Cells were cultured for 24 h with the medium described above and treated with different concentrations of GSPE diluted in ethanol. In all the experiments GSPE was added into the apical side. The basolateral medium and cells were harvested after the treatments. The medium was ultracentrifuged with Amicon Ultra-4 centrifugal filter (Millipore) to concentrate it and the amount of TG secreted by the Caco2 cells was measured by using an enzymatic colorimetric kit (QCA, Amposta, Spain). Values were corrected per mg cell protein determined by colorimetric assay (Bradford, Sigma).

2.6. Gene expression analyses

Caco2 cells were cultured for an hour with the post-prandial or fasting medium; all of them were treated with different concentrations of GSPE diluted in ethanol. Total RNA was isolated by using an RNeasy Mini kit (Quiagen, Hilden, Germany) following the manufacturer's instructions. cDNA was synthesised from 2 μg of total RNA using High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA). A total 20 ng of cDNA was subjected to quantitative RT-PCR amplification using Taqman Master Mix (Applied Biosystems). Specific Taqman probes (Applied Biosystems) were used for different genes: microsomal triglyceride transfer protein (MTP: Hs00165177_m1), Long-chain acyl-CoA synthetase 3 (ACSL3: Hs00244853_m1), long-chain acyl-CoA synthetase 5 (ACSL5: Hs00212106_m1), diacylglycerol acyltransferase 1 (DGAT1: Hs00201385_m1), apolipoprotein A4 (ApoA4: Hs00166636_m1), carnitine palmitoyl transferase 1 (CPT-1a: Hs00157079_m1), peroxisome proliferator-activated receptor alpha (PPARα: Hs00223686_m1), intestinal-fatty acid binding protein (I-FABP: Hs00164552_m1). Cyclophilin (Ppia: Hs99999904_m1) was used as an endogenous control. Real-time quantitative PCR reactions were performed using the ABI Prism 7300 SDS Real-Time PCR system (Applied Biosystems).

2.7. Statistical analysis

The results are reported as the mean ± SEM of three independent experiments for the TG secretion and two independent experiments for the gene expression analyses. Each independent experiment was performed in triplicate. The means were calculated considering each well in the two or three independent experiments. Group means were compared with an independent-samples Student's *t*-test ($p \leqslant 0.05$) using SPSS software (SPSS Inc., Chicago, IL).

3. Results

3.1. GSPE treatment repressed the TG secretion in a fasting state but not in a post-prandial state in Caco2 intestinal cells

Caco2 cells 21 days post-confluence were cultured for 24 h with a post-prandial or a fasted medium in 12-well Millicell Hanging Cell Culture Inserts (Millipore) and treated with 25–100 mg/L GSPE always added into the apical side. As shown in Fig. 1, only 100 mg/L