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Trans-resveratrol induces a potential anti-lipogenic effect in lipopolysaccharide-stimulated enterocytes

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Abstract

A DNA microarray analysis was conducted in Caco-2 cells to analyse the protective effects of *trans*-resveratrol on enterocyte physiology and metabolism in pro-inflammatory conditions. Cells were pre-treated with 50 µM of *trans*-resveratrol and, subsequently, lipopolysaccharide (LPS) was added for 48 h. The microarray analysis revealed 121 genes differentially expressed between resveratrol-treated and non-treated cells (B> 0, is the odd that the gene is differentially expressed). Inhibitor of DNA binding 1 (*ID1*), histidine-rich glycoprotein (*HRG*), NADPH oxidase (*NOX1*) and sprouty homolog 1 (*SPRY*), were upregulated by LPS treatment, but significantly blocked by *trans*-resveratrol pre-treatment (p_{adj}< 0.05, after adjusting for Benjamini-Hochberg procedure). Moreover, genes implicated in synthesis of lipids (z-score= -1.195) and concentration of cholesterol (z-score= -0.109), were markedly downregulated by *trans*-resveratrol. Other genes involved in fat turnover, but also in cell death and survival function, such as transcription factors Krüppel-like factor 5 (*KLF5*) and amphiregulin (*AREG*), were also significantly inhibited by *trans*-resveratrol pre-treatment. RT-qPCR-data confirmed the microarray results. Special mention deserves acyl-CoA synthetase long-chain family member 3 (*ACSL3*) and endothelial lipase (*LIPG*), which were downregulated by this stilbene and have been previously associated with fatty acid synthesis and obesity in other tissues. This study envisages that *trans*-resveratrol might exert an important anti-lipogenic effect at intestinal level under pro-inflammatory conditions, which has not been previously described.

Key words: Gut, resveratrol, *in vitro*, lipid metabolism, inflammation, enterocytes.

Introduction

Our intestine is the first defence barrier located between the host and the luminal environment (1). The intestinal epithelium controls the passage of nutrients and fluids, but also protects the organism from the permeation of external antigens into the intestinal mucosa and circulatory system (2). Scientific evidence has demonstrated the implication of intestinal barrier integrity impairment in the development of abnormal inflammatory response (3). Intestinal inflammation is a continuous and protective process that aims to maintain gut integrity and normal functionality (4). For this purpose, a crosstalk between different cell types from the gut is required, which results in the regulation of the secretion of a range of cytokines and growth factors. However, when a dysregulation of one of these components happens, an inappropriate inflammatory stimulus could lead to several diseases such as inflammatory bowel disease, including ulcerative colitis and Crohn's disease (5), celiac disease, food allergies, inflammatory bowel syndrome and metabolic diseases (6). Moreover, although controversial results have been reported about the occurrence of alterations in gut barrier integrity in obese animals (7), emerging data corroborate the presence of an intestinal inflammatory condition (8). Importantly, the presence of a chronic low-grade inflammatory response that leads to metabolic dysfunctions is well established in obesity (9) and it has been reported that obesity-related comorbidities, such as type 2 diabetes and atherosclerosis, are usually accompanied by higher circulating levels of pro-inflammatory cytokines (10).

The administration of natural compounds (i.e. polyphenols) to fight against inflammation-related metabolic diseases is under research (11). In this context, *trans*-resveratrol (*trans*-3, 5, 4'-trihydroxystilbene), is a stilbene that has been extensively studied for its antioxidant, anti-adipogenic and anti-lipogenic properties (12). In addition, beneficial properties of resveratrol on cardiovascular system have been widely studied (13). Accordingly, within the mechanisms involved in the anti-atherogenic effects of resveratrol, modulation of lipid metabolism has been reported (14). Besides, the anti-inflammatory role of the stilbene seems to be implicated in the protection against the development of cardiovascular risk factors (14) and the action of this molecule against acute inflammation at intestinal level has been demonstrated (15).

Caco-2 cells are a human intestinal epithelial cell model that has been previously used to investigate the intestinal absorption and metabolism of *trans*-resveratrol (16) and the impact of the stilbene on intestinal barrier function (17). Lipopolysaccharide (LPS), a Gram-negative bacterial outer membrane constituent, is known to interact with intestinal epithelial cells and to induce stimulation of transcription and translation of pro-inflammatory mediators (18). In this sense, high LPS levels are considered to be an important factor in the pathophysiology of intestinal inflammatory diseases, has been postulated as one of the leading causes of obesity (19) and has been associated with increased risk of atherosclerosis in humans (20, 21). Thus, induction of

inflammation in enterocytes by LPS administration is considered an appropriate *in vitro* model to mimic intestinal inflammation (22).

Accordingly, this investigation sought to analyse the molecular functions and pathways that might be affected by *trans*-resveratrol in LPS-treated enterocytes, a model that mimics the low-grade inflammatory condition usually present in metabolic diseases, such as obesity and atherosclerosis (19).

Materials and methods

Cell culture and treatment

Caco-2 cells were maintained in an incubator set at 37°C and 5% carbon dioxide and 90% of relative humidity. The cells were cultured in Dulbecco's modified Eagle's medium with GlutaMax (DMEM, Gibco, Rockville, MD, USA) containing 10% fetal bovine serum (FBS, Gibco), 1% of non-essential amino acids (NEAA, Lonza, Basel, Switzerland), 1% penicillin (10000 U/mL)-streptomicyn (10000 µg/mL) (Gibco) and 1% amphoterizin B (250 µg/mL, Gibco). The culture medium was changed every 2 days. Once cells reached 80% confluence, confirmed by microscopic observance, they were dissociated with 0.05% trypsin-EDTA solution and subcultured on a 75 cm^2 flasks at a density of 250000 cells per cm^2. The cells were seeded in 6-well cell culture plate at 30,000 cells per cm^2. Culture medium was replaced every 2 days until the day of the experiment. Experiments were conducted 15-19 days post-seeding.

For the experiments, cells were treated with standard LPS from *E. coli* K12 strain- TLR4 ligand (InvivoGen, San Diego, CA, USA). Concentrations of LPS (1 µg/mL) and time of exposure (48h) were established based on previous studies (23). Before LPS stimulation, some samples were pre-treated with 50 µM of *trans*-resveratrol, dissolved in ethanol, kindly provided by Prof. María Puy Portillo (Nutrition and Obesity group, University of the Basque Country, Vitoria, Spain). This dose was reported to be a realistic polyphenol concentration commonly found in the gut following the intake of 500 mg of polyphenols (24). Upon 1h of incubation at 37°C, cell cultures were stimulated with endotoxin as previously mentioned. Untreated cells were used as controls.

RNA isolation and microarray analysis

Total RNA was extracted from Caco-2 cells using TRIzol® reagent according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). As a last step of the extraction procedure, the RNA was purified with the RNeasy Mini-kit (Qiagen, Hilden, Germany). Before cDNA synthesis, RNA integrity from each sample was confirmed by using Agilent RNA Nano LabChips (Agilent Technologies, Santa Clara, CA, USA).

The sense cDNA was prepared from 300 ng of total RNA using the Ambion® WT Expression Kit (Thermo Fisher Scientific, Waltham, MA, USA). The sense strand cDNA was then fragmented and biotinylated with the Affymetrix GeneChip® WT Terminal Labeling Kit (PN 900671). Labeled sense cDNA was hybridized to the Affymetrix Human Gene 2.0 ST microarray according to the manufacturer protocols and using GeneChip® Hybridization, Wash and Stain Kit. Genechips were scanned with the Affymetrix GeneChip® Scanner 3000.

Microarray analysis

Both background correction and normalization were done using RMA (Robust Multichip Average) algorithm (25). After quality assessment, a filtering process was performed to eliminate low expression probe sets. Applying the criterion of an expression value greater than 16 in 3 samples for each experimental condition (CONTROL, LPS, LPS+RSV), 38959 probe sets were selected for statistical analysis. R and Bioconductor (26) were used for preprocessing and statistical analysis. LIMMA (Linear Models for Microarray Data) was used to find out the probe sets that showed significant differential expression between experimental conditions (27). Adjusted *p* value was calculated with Benjamini-Hochberg procedure. Genes were selected as significant using criteria of B> 0. The Log Odds or B value is the odds or probability that the gene is differentially expressed, meaning that a gene with B=0 has a 50% chance to be differentially expressed.

Functional enrichment analysis of Gene Ontology (GO) categories was carried out using standard hypergeometric test (28). The biological knowledge extraction was complemented through the use of Ingenuity Pathway Analysis (Ingenuity Systems, www.ingenuity.com), whose database includes manually curated and fully traceable data derived from literature sources.

Microarray data are accessible at the NCBI Gene Expression Omnibus website (http://www.ncbi.nlm.nih.gov/geo/) with the accession number of GSE73650.

Confirmatory real-time quantitative PCR

Some genes whose expression was affected by *trans*-resveratrol in the microarray analysis were validated using real-time quantitative polymerase chain reaction (RT-qPCR). For this purpose, RNA concentrations and quality were assessed by Nanodrop Spectrophotometer 1000 (Thermo Scientific, Wilminton, DE, USA). RNA (2 µg) were reverse-transcribed to cDNA using Moloney murine leukemia virus reverse transcriptase (MMLV, Invitrogen). Taqman® Universal Master Mix and the following pre-designed Taqman® assays-on-demand were used: *KLF5* (Krüppel-like factor 5 (intestinal), Hs00156145_m1; *ACSL3* (acyl-CoA synthetase long-chain family member -3), Hs00244853_m1; *LIPG* (lipase, endothelial), Hs00195812_m1; *AREG* (amphiregulin), Hs00950669_m1; *NOX1* (NADPH oxidase 1), Hs01071088_m1; *GAPDH* (glyceraldehyde-3-phosphate dehydrogenase), Hs02758991_g1 (Applied Biosystems, Foster City, CA, USA). Amplification and detection of specific products were conducted using ABI PRISM 7000 HT Sequence Detection System (Applied Biosystems). All samples were analysed in duplicate. The relative expression of each gene was calculated by the $2^{-\Delta\Delta Ct}$ method (29).

Statistical analyses

Results are expressed as the average mean ± standard error of the mean (SEM). Statistical significance between experimental groups was assessed by Student's *t* test. A probability of $p< 0.05$ was set up for determining statistically significant differences. SPSS 15.0 for

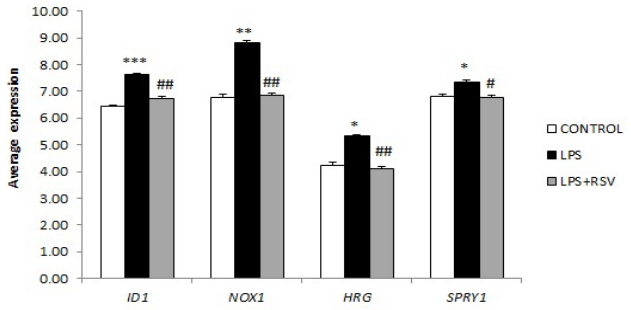

Figure 1. Average expression of genes that were downregulated by *trans*-resveratrol pre-treatment, according to Affymetrix Human Gene 2.0 ST DNA microarray analysis. Results are presented as mean ± SEM. Linear Models for Microarray Data was used to show probe sets with significant differential expression. *$p< 0.05$, **$p< 0.01$, ***$p< 0.001$ vs CONTROL group; $^{\#}p< 0.05$, $^{\#\#}p< 0.01$ vs LPS group. Adjusted p value was calculated with Benjamini-Hochberg procedure. SEM, standard error of the mean; LPS, lipopolysaccharide; RSV, *trans*-resveratrol; *ID1*, inhibitor of DNA binding 1, dominant negative helix-loop-helix protein; *NOX1*, NADPH oxidase 1; *HRG*, histidine-rich glycoprotein; SPRY1, sprouty homolog 1, antagonist of FGF signalling.

Windows (SPSS, Chicago, IL, USA) was used for statistical analyses.

Results

Differential gene expression profile in cells treated with trans-resveratrol

When comparing the gene expression profile of Caco-2 cells that were treated with LPS and those that were previously exposed to *trans*-resveratrol (50 μM), a total of 121 genes showed a B> 0 in the microarray analysis: 63 genes were downregulated and 58 genes upregulated (Supplementary table 1). From these, there were four that were upregulated by LPS, but were significantly reversed ($p_{adj}< 0.05$) by *trans*-resveratrol pre-treatment (Figure 1): the inhibitor DNA binding 1, dominant helix-loop-helix protein (*ID1*, log FC= -0.88 and $p_{adj}< 0.01$), histidine-rich glycoprotein (*HRG*, log FC= -1.24 and $p_{adj}=0.01$), NADPH oxidase 1 (*NOX1*, log FC= -1.94 and $p_{adj}=0.01$) and sprouty homolog 1, antagonist of FGF signalling (*SPRY1*, log FC= -0.57 and $p=0.03$).

Biologically relevant networks and pathways

Functional enrichment analysis with Ingenuity Pathway Analysis (IPA) software, detected altered (B> 0) molecular and cellular functions in the imported data set associated to *cell death and survival*; *infectious diseases*; *lipid metabolism*, *small molecule biochemistry*; *lymphoid tissue structure and development, tissue morphology*; *cellular development, cellular growth and proliferation*; *DNA replication, recombination and repair*. The genes involved in these pathways are listed in Table 1.

Networks analysed by the Ingenuity software describe the functional relationship between gene products based on known interactions reported in the literature. The most significant network that was affected by *trans*-resveratrol was *cell death and survival, cellular assembly and organization, cellular function and maintenance* (44 score). A second network was related to *hereditary disorder, neurological disease and organ morphology* (38 score). The third network was related to

Table 1. Classification of metabolic pathways and genes targeted by *trans*-resveratrol in LPS-treated Caco-2 cells.

Category	Diseases or functions annotation	*p* value	z-score	Genes
Cell Death and Survival				
	Necrosis	2.97E-03	1.804	*ABCB1,AHR,ANKRD1,AREG,BLNK,CLYBL,ENC1,EPHX2,HOXB9,HSPA8,ID1,IER3,IFI6,KLF5,KRT18,LUM,MT1X,MT2A,NEO1,NFE2L2,NOX1,PHB2,PKP2,RPS3,SLC20A1,SPTBN1,SSTR5-AS1,TOP1*
	Apoptosis	8.98E-03	1.174	*ABCB1,AHR,ANKRD1,ANXA4,AREG,BBS2,BLNK,ENC1,GLS2,HOXB9,HRG,HSPA8,ID1,IER3,IFI6,KLF5,KRT18,LUM,MT2A,NFE2L2,NOX1,PHB2,PKP2,RPS3,SLC20A1,SPTBN1,TOP1*
Infectious diseases				
	Infection of cells	2.06E-03	-1.140	*AREG,BMP2K,CRIM1,ENC1,KRT18,MT1X,MT2A,NOP56,OSBPL3,RPL5,SLC20A1,SPTBN1,ZBTB2*
	Viral Infection	3.35E-02	-0.595	*ABCB1,AHR,AREG,BMP2K,CRIM1,ENC1,FAM135A,IER3,IFI6,KRT18,MT1X,MT2A,NOP56,OSBPL3,RPL5,SPTBN1,ZBTB2*
Lipid metabolism, Small molecule Biochemistry				
	Synthesis of lipids	1.48E-02	-1.195	*ABCB1,ACSL3,ACSM3,AHR,AREG,EPHX2,HSPA8,KLF5,ME1,NOX1*
	Fatty acid metabolism	6.43E-03	-1.060	*ABCB1,ACSL3,ACSM3,AREG,EPHX2,HSPA8,KLF5,ME1,SC5D*
	Concentration of cholesterol	2.62E-02	-0,109	*AHR,EPHX2,LIPG,LPGAT1,SC5D*
Lymphoid tissue structure and development, tissue morphology				
	Quantity of lymphatic system cells	1.99E-02	-1.408	*ABCB1,AHR,BLNK,ID1,SLC20A1*
Cellular Development, Cellular Growth and Proliferation				
	Proliferation of tumor cell lines	3.29E-02	-1.625	*ABCB1,AHR,AREG,CLYBL,DDX21,ENC1,HOXB9,ID1,IER3,KLF5,MT2A,MTUS1,NEO1,NFE2L2,NFS1,TOP1*
DNA replication, Recombination and Repair				
	Metabolism of DNA	3.50E-02	1.996	*ABCB1,AHR,AREG,FAM135A,ID1,TOP1*

Functional enrichment analysis conducted by the Ingenuity Pathway Analysis.

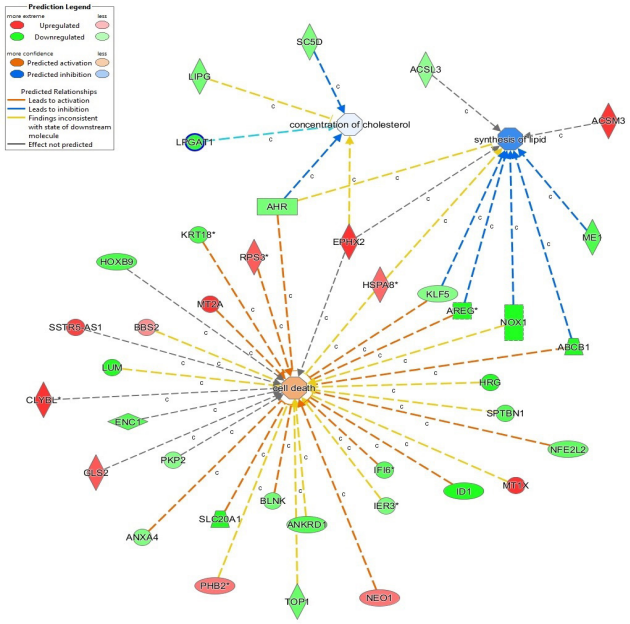

Figure 2. Integrated network analysis of upregulated and downregulated genes in Caco-2 cells pre-treated with *trans*-resveratrol and exposed to lipopolysaccharide. The node colour indicates the gene expression level.

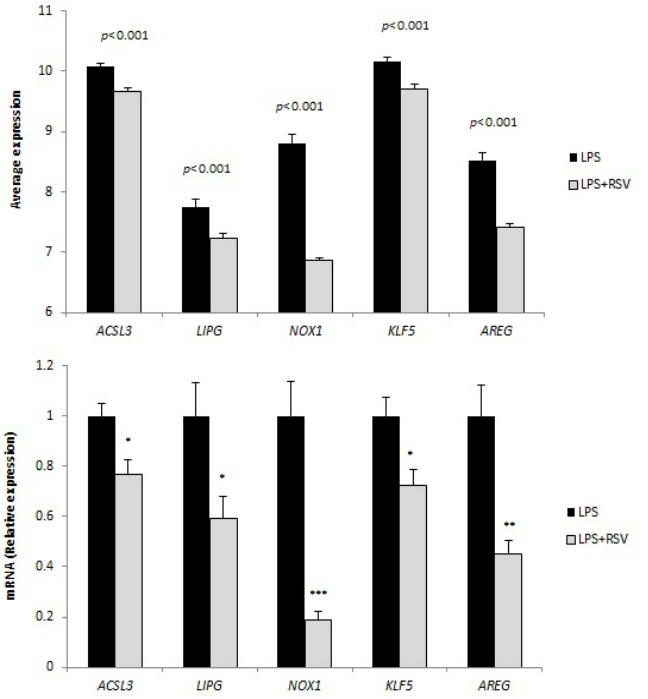

Figure 3. Average and relative expression of genes chosen for validation. A) Average expression of genes from microarray analysis in LSP-stimulated Caco-2 cells pre-treated with *trans*-resveratrol. B) Relative expression of genes following validation by RT-qPCR in LPS-stimulated Caco-2 cells pre-treated with *trans*-resveratrol**.** Statistical analyses were conducted with Student T-test, ***$p< 0.001$, **$p< 0.01$, *$p< 0.05$. LPS, lipopolysaccharide; RT-qPCR, quantitative real-time polymerase chain reaction.

lipid metabolism, molecular transport, small molecule biochemistry (31 score) and, finally, the forth and the fifth networks (28 score each) were associated with cell cycle. Figure 2 represents the integrated network analysis and shows the relationship between genes involved in the first network related to *cell death and survival, cellular assembly and organization, cellular function and maintenance* and those related to the third network, *lipid metabolism, molecular transport, small molecule biochemistry* -related network.

Validation of the expression of genes implicated in lipid metabolism

Genes that were selected for validation by RT-qPCR were implicated in both, *lipid metabolism* but also in *cell death and survival* categories (Table 1). Genes of interest were ACLS3, *LIPG*, *NOX1*, *KLF5* and *AREG*. All the genes selected for validation showed a B> 1. Due to the action of resveratrol as an anti-oxidant compound, *NOX1* was selected based on its relevance in oxidative stress processes. *ACSL3* and *LIPG* were chosen since they were genes belonging to the lipid metabolism pathway and were not implicated in other pathways. Finally, *KLF5* and *AREG* were selected since they were implicated in both lipid metabolism and in the first important pathway altered by resveratrol (*cell death and survival*).

The RT-qPCR findings were consistent with data of the microarray analysis (Figure 3).

In summary, the expression of key genes involved in synthesis of lipids (*ACSL3*, *AREG* and *KLF5*), cholesterol metabolism (*LIPG*) and control of reactive oxygen species (*NOX1*) was downregulated by *trans*-resveratrol in LPS-stimulated Caco-2 cells.

Discussion

The burden of chronic diseases related to inflammation and characterized by metabolic dysregulations is increasing (30, 31). In order to be able to prevent such disturbances or to find a treatment for these problems, it is essential to understand the pivotal cellular components and the specific tissues that participate in such metabolic impairments (32, 33). In the present research, Caco-2 cells were selected since enterocytes have been described to be a target site for *trans*-resveratrol action (16). Gene expression analysis conducted in this *in vitro* study revealed some of the previously reported data on the anti-proliferative role and apoptosis-promoting effect of resveratrol (34). In this context, in the current study, molecular pathways involved in *cell death and survival* (apoptosis) were enhanced (z-score= 1.174), while pathways related to *cellular development, cellular growth and proliferation* (proliferation of tumour cell lines) were inhibited (z-score= -1.625). Moreover, resveratrol has been described to affect all aspects of DNA metabolism (35). Accordingly, in our study, a suppressive action of *trans*-resveratrol in *DNA replication, recombination and repair* (metabolism of DNA) was detected (z-score= -1.996). This outcome might be produced through direct mechanisms, as for instance inhibition of genes related to tumorigenesis, but also through indirect mechanisms (35). *Trans*-resveratrol significantly repressed the expression levels of LPS-induced *ID1* and *HRG* genes, that particularly in the case of *ID1,* have been associated with tumorigenesis (36, 37). However, indirect mechanisms that involve the abrogation of endogenous reactive oxygen species (ROS) formation might also influence this molecular pathway, since the redox state of a cell has been reported to control the stability of genomic DNA (35). In this context, ROS formation might be avoided when the amide adenine dinucleotide phosphate (NADPH)

oxidase or *NOX1* activity (35) is suppressed since it is considered one of the most relevant enzymes concerning intracellular ROS generation (38). Accordingly, in the current study, *NOX1* expression, significantly upregulated by LPS treatment was inhibited by *trans*-resveratrol in enterocytes. In this context, it has been previously found that resveratrol treatment inhibited LPS-induced *NOX1* expression and ROS generation in macrophages, which has been related to a suppression of LPS-induced foam cell formation (39). Nevertheless, another mechanism previously reported for other antioxidant molecules (i.e. ascorbic acid) might be plausible (40). This process is related to the capacity of resveratrol to avoid the activation of nuclear factor kappa β (NF-κβ) (23). NF-κβ is a transcription factor important in the regulation of immunity, inflammation, cell proliferation, cell transformation and tumour development and it has been demonstrated that ROS stress is a relevant stimuli that activates NF-κβ through the activation of inhibitors of kappa β kinase (IKK) (41). Since resveratrol has been reported to suppress nuclear translocation of p65 through the inhibition of IKK in LPS-stimulated Caco-2 cells, downregulation of *NOX1* by the stilbene observed in our study might be avoiding the NF-κβ signalling pathway, favouring an anti-inflammatory and apoptosis promoting effect of the stilbene in addition to its antioxidant function.

The main finding of this research work is the potential action of *trans*-resveratrol on the lipid synthesis process occurring at intestinal level. Indeed, the general molecular pathway associated with lipid synthesis was found to be inhibited (z-score= - 1.195). As far as we know, this is the first study conducted in intestinal cells showing an anti-lipogenic effect of resveratrol.

The small intestine synthesizes triglycerides (TG) through two main processes, the monoacylglycerol (MAG) pathway, which occurs in enterocytes after feeding, and the glycerol-3-phosphate (G-3-P) pathway, which is the *de novo* pathway for triglyceride synthesis (42). Under normal conditions, these processes have been reported to represent 20 to 80% of total TG levels in chylomicrons (43). In addition, in the absence of dietary fat, the contribution of the intestine to the total TG levels in plasma has been reported to be around 20% or more (44), while, *in vivo,* it might supply up to 40% in fasting conditions (45). Long-chain acyl-CoA synthetases (ACSL) are enzymes responsible for the activation process of fatty acids that precede their entrance to MAG or G-3-P pathway for TG synthesis.

Five members of ACSL family have been described but, in small intestine, the expression of two isoforms (ACSL3 and *ACSL5*), seems to predominate (46). ACSL3 is located in lipid droplets or endoplasmic reticulum, and it is supposed to play a key role in fatty acid uptake and lipid synthesis (47, 48). In this trial, an inhibitory action of *trans*-resveratrol on the expression of *ACSL3* gene was detected. In a study conducted by Bu et al. (47), it was observed that knockdown of ACSL3 significantly suppressed the gene activity of certain lipogenic transcription factors such as peroxisome proliferator activation receptor-γ (PPAR-γ), carbohydrate responsive element-binding protein (ChRBP), sterol regulatory element-binding protein-1c (SREBP-1c), and liver X receptor-α (LXR), as well as the expression of their target genes (47). In accordance, data from the current investigation showed that treatment of Caco-2 cells with 50 μM of *trans*-resveratrol, repressed the expression of *ACSL3*, but also inhibited the expression levels of certain transcription factors such as *KLF5* and *AREG*. Likewise, in relation to cholesterol metabolism, *trans*-resveratrol inhibited the expression of endothelial lipase (*LIPG*). This lipase belongs to the TG lipase gene family (49) and the members of this group show different substrate specificity. It has been stated that high density lipoprotein (HDL) is the main substrate for LIPG (50, 51). Interestingly, *LIPG* was implicated in the pathway related to concentration of cholesterol showing a z-score of - 0.109. It should be taken into consideration that the small intestine plays a role in cholesterol homeostasis (52). Remarkably, from human studies, a positive correlation between plasma levels of endothelial lipase and obesity-associated parameters (i.e. body mass index and waist circumference) have been demonstrated (53). From our data, it might be proposed that, apart from the already described inhibitory effect of *trans*-resveratrol on hepatic lipogenesis (54) and adipogenesis in 3T3-L1 adipocytes (55), the stilbene may affect TG synthesis and cholesterol metabolism in enterocytes.

In our study, the expression of fatty acid synthase (*FASN*), which is considered one of the rate limiting enzymes in *de novo* lipogenesis, was not differentially expressed between both experimental groups (log FC= -0.34 when comparing the *trans*-resveratrol pre-treated Caco-2 cells and those exposed to LPS). However, FASN has been shown to be target of resveratrol and either the expression of the gene (56) or the activity of the enzyme (57) have been found to be affected by the stilbene. Importantly, regulation of *FASN* has been reported to take place mainly at transcriptional level, but also at post-transcriptional level (58, 59). In our study, *trans*-resveratrol also acted on the expression of *KLF5* and *AREG*. KLF5, also called basic transcription element-binding (BTEB) 2, is highly expressed in the gut (60) and has been reported to control proliferation of different cell types, including fibroblasts, smooth muscle cells, white adipose tissue and intestinal epithelial cells (61). Importantly, KLF5 plays a key role in the pathogenesis of cardiovascular diseases (61). KLF5 has been demonstrated to be a pivotal regulator in the control of fatty acid synthase (FASN) expression (the key lipogenic gene) through an interaction with SREBP-1 (61). On the other hand, *AREG* is a common ligand for the epidermal growth factor receptor (EGFR), which contributes to the growth of various cell types including intestinal epithelial cells (62). Ligands of EGFR, such as the EGF peptide, have been found to stimulate FASN expression mediated by SREBPs in certain cancer cell types (63). Besides, increases in protein expression of EGF (64) and EGFR (65) in the liver appeared to be related to cholesterol synthesis (66) and fatty acid synthesis (67). Accordingly, in human studies, a positive correlation between EGF ligands and serum cholesterol levels has been reported (68). Therefore, our data suggest that downregulation of *AREG* expression by *trans*-resveratrol might be linked somehow to alterations in lipid metabolism, including cholesterol levels and a reduction of lipogenesis.

Noteworthy, resveratrol is believed to modulate li-

poprotein metabolism, decreasing circulating levels of LDL cholesterol and reducing cardiovascular disease risk (13). Supplementation with high doses of resveratrol to healthy humans with mild hypertriglyceridemia has been demonstrated to reduce intestinal and hepatic triglyceride-rich lipoproteins (TRL) production, independent of its action on plasma TG concentrations (69). In this context, it might be hypothesized that the potential action of resveratrol at either intestinal level and also in the liver might be a mechanism to be taken into consideration when exploring the protective role of this stilbene against metabolic diseases characterized by a low-grade inflammatory status such as atherosclerosis. To the best of our knowledge, the inhibitory action of *trans*-resveratrol on lipid metabolism has not been previously described in enterocytes. Hence, results from this microarray and further validation analysis might be taken into consideration when evaluating the mechanisms implicated in the hypolipidemic effects of *trans*-resveratrol, particularly based on the interest of this compound owing to the potential health-promoting effects.

In summary, the microarray analysis performed in the current study evidenced that *trans*-resveratrol acts upon LPS-stimulated enterocytes activating genes related to cell death and repressing genes associated with cellular development, growth and proliferation, as well as on DNA replication, recombination and repair. Indeed, the main finding of this experimental trial is that pre-treatment of Caco-2 cells with *trans*-resveratrol before exposure to LPS significantly affects the expression of enzymes directly associated with lipid metabolism, particularly those involved in fatty acid synthesis and cholesterol turnover. Moreover, a significant influence of the stilbene was also demonstrated on the expression of several transcription factors, such as *KLF5* and *AREG* that, through their target genes, might impact on intestinal lipid metabolism and blood cholesterol and TG levels, contributing to further explain the beneficial effects of resveratrol.

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