Resveratrol induces apoptosis and inhibits adipogenesis in 3T3-L1 adipocytes

Srujana Rayalam1, Jeong-Yeh Yang1, Suresh Ambati1, Mary Anne Della-Fera1ii and Clifton A. Baile1,2ii

1Department of Animal & Dairy Science, University of Georgia, Athens, GA 30602-2771
2Department of Foods and Nutrition, University of Georgia, Athens, GA 30602-2771

Resveratrol, a phytoalexin, has recently been reported to slow aging by acting as a sirtuin activator. Resveratrol also has a wide range of pharmacological effects on adipocytes. In this study, we investigated the effects of resveratrol on adipogenesis and apoptosis using 3T3-L1 cells. In mature adipocytes, 100 and 200 μM resveratrol decreased cell viability dose-dependently by 23 ± 2.7% and 75.3 ± 2.8% (p < 0.0001), respectively, after 48 h treatment, and 100 μM resveratrol increased apoptosis by 76 ± 8.7% (p < 0.0001). Resveratrol at 25 and 50 μM decreased lipid accumulation in maturing preadipocytes significantly by 43 ± 1.27% and 94.3 ± 0.3% (p < 0.0001) and decreased cell viability by 25 ± 1.3% and 70.4 ± 1.6% (p < 0.0001), respectively. In order to understand the anti-adipogenic effects of resveratrol, maturing 3T3-L1 preadipocytes were treated with 25 μM resveratrol and the change in the expression of several adipogenic transcription factors and enzymes was investigated using real-time RT-PCR. Resveratrol down-regulated the expression of PPARγ, C/EBPα, SREBP-1c, FAS, HSL, LPL and up-regulated the expression of genes regulating mitochondrial activity (SIRT3, UCP1 and Mfn2). These results indicate that resveratrol may alter fat mass by directly affecting cell viability and adipogenesis in maturing preadipocytes and inducing apoptosis in adipocytes and thus may have applications for the treatment of obesity. Copyright © 2008 John Wiley & Sons, Ltd.

Keywords: resveratrol; 3T3-L1 cells; apoptosis; adipogenesis; adipocyte specific genes; real-time RT-PCR.

INTRODUCTION

Resveratrol (3,5,4′-trihydroxystilbene), a naturally occurring phytoalexin found in red wines and grape juice, has been shown to reduce the synthesis of lipids in rat liver (Arichi et al., 1982) and 3T3-L1 adipocytes (Picard et al., 2004). Resveratrol decreased proliferation, induced apoptosis and cell-cycle arrest in various cell lines (Ferry-Dumazet et al., 2002; Liang et al., 2003). In addition, resveratrol was shown to increase the activity of sirtuins (silent mating type information regulator-2, sir2), which function in a wide variety of cellular processes, and a family of key enzymes in calorie restriction, Sir2-family histone deacetylases (Zhang, 2006). The activated SIRT1 further deacetylates the transcriptional coactivator Peroxisome proliferator-activated receptor gamma coactivator 1α (PGC-1α) at promoter regions to induce expression of genes involved in mitochondrial biogenesis and fatty acid oxidation (Gerhart-Hines et al., 2007).

Differentiation of preadipocytes and the induction of metabolic pathways related to lipid metabolism includes expression of several adipocytes-specific genes like peroxisome proliferator-activated receptor-gamma (PPARγ), and (CCAAT/enhancer binding protein-alpha (C/EBPα). (Lazar, 2002), sterol regulatory element binding proteins-1c (SREBP-1c) (Kim et al., 1998b), and fatty acid synthase (FAS) (Kim et al., 1998a). Lipoprotein lipase (LPL) (Awerx et al., 1992) and hormone-sensitive lipase (HSL) (Sztalryd et al., 1995) are the two major enzymes regulating the process of lipolysis.

The mitochondrial sirtuin deacetylase SIRT3 influences mitochondrial function by reducing membrane potential (Shi et al., 2005). The expression of SIRT3 was shown to be correlated with uncoupling protein 1 (UCP1), which resides on the mitochondrial inner membrane and mediates adaptive thermogenesis (Shi et al., 2005). Mitofusin 2 (Mfn2), a mitochondrial membrane protein that participates in mitochondrial fusion in mammalian cells, was shown to play an important role in glucose oxidation (Mingrone et al., 2005) and was also reported to have a role in the pathophysiology of obesity (Zorrano et al., 2004).

In this study we investigated the effect of resveratrol on the expression of genes regulating adipogenesis and lipolysis and genes involved in mitochondrial activity to evaluate the anti-adipogenic and pro-apoptotic effects of resveratrol in 3T3-L1 cells.

MATERIALS AND METHODS

Reagents. Dulbecco’s modified Eagle’s medium (DMEM) was purchased from Gibco (BRL Life Technologies, Grand Island, NY). ApoStrand ELISA Apoptosis Detection Kit was purchased from BIOMOL (Plymouth...
Table 1. List of probes for different adipocyte specific transcription factors, enzymes and other genes used in real time RT-PCR

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Gene name</th>
<th>Probe sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>18S</td>
<td>Eukaryotic 18S rRNA</td>
<td>CATTGGAGGGCAAGTCTGGGC</td>
</tr>
<tr>
<td>PPARγ</td>
<td>Peroxisome proliferator activated receptor gamma</td>
<td>AGTTGGACAGCCAGGGTCTGGT</td>
</tr>
<tr>
<td>CEBPα</td>
<td>CCAAT enhancer binding protein (C/EBP), alpha</td>
<td>AGCCACCGCGCCACGGAGCC</td>
</tr>
<tr>
<td>SREBF1</td>
<td>Sterol regulatory element binding factor 1</td>
<td>ACATCGAAGATGCTCCAG</td>
</tr>
<tr>
<td>FASN</td>
<td>Fatty acid synthase</td>
<td>GTGGATGAGGTATCAGGACGGCT</td>
</tr>
<tr>
<td>LIPE</td>
<td>Lipase, hormone sensitive</td>
<td>GGGCCACAGGAGTTGGGTCC</td>
</tr>
<tr>
<td>LPL</td>
<td>Lipoprotein lipase</td>
<td>ATCTAGTGGATGGGTAAACG</td>
</tr>
<tr>
<td>SIRT3</td>
<td>Sirtuin 3 (silent mating type information regulation 2, homolog) 3</td>
<td>AGGGGAAGACATATGGGCTGAT</td>
</tr>
<tr>
<td>UCP1</td>
<td>Uncoupling protein 1, mitochondrial</td>
<td>AAAGTCCGGCTTCAGATCCAGG</td>
</tr>
<tr>
<td>MFN2</td>
<td>Mitofusin 2</td>
<td>TTTTTTGCAGGAGCAATGGGA</td>
</tr>
</tbody>
</table>

Results:

Effect of resveratrol on cell viability and apoptosis in mature adipocytes

As shown in Fig. 1a, resveratrol at 100 and 200 μM decreased cell viability dose-dependently by 23 ± 2.7%.
and 75.3 ± 2.8% (p < 0.0001) respectively after 48 h treatment, whereas lower concentrations of resveratrol showed no significant effect on decreasing viability. To determine whether the reduction in cell number was caused by apoptosis, ssDNA ELISA was used as a determinant of cellular apoptosis. As shown in Fig. 1b, resveratrol at 100 μM increased apoptosis by 76 ± 8.7% (p < 0.0001). An increase in apoptotic nuclei with 100 μM resveratrol treatment was also visualized with Hoechst staining (Fig. 1c).

**Effect of resveratrol on lipid accumulation and cell viability in maturing preadipocytes**

In maturing preadipocytes, preliminary experiments with a range of resveratrol concentrations (data not included) showed that concentrations of 100 μM and above were very potent and dose-dependent effects were not noticed. Therefore, lower concentrations were used in experiments with maturing preadipocytes. The results showed that resveratrol at 25 and 50 μM significantly decreased lipid accumulation by 43 ± 1.27% and 94.3 ± 0.3% (p < 0.0001), respectively (Fig. 2a). However, resveratrol also decreased cell viability by 25 ± 1.3% and 70.4 ± 1.6% (p < 0.0001) at 25 and 50 μM, respectively, in maturing preadipocytes, indicating that the effect on lipid accumulation is due not only to decreased adipogenesis but also to the effect on cell viability (Fig. 2b). Oil Red O staining to visualize lipid accumulation in cells after treatment show that resveratrol caused a greater reduction of lipid accumulation when compared to control cells (Fig. 2c).

**Effect of resveratrol on the expression of adipocyte specific genes in maturing preadipocytes**

Resveratrol decreased PPARγ, C/EBPα, SREBP1 and FAS mRNA expression by 63 ± 4%, 53.4 ± 9.2%, 62.7 ± 3.4% and 70.5 ± 3.2% (p < 0.0001), respectively. However, the mRNA levels of all these genes in undifferentiated preadipocytes were significantly lower than in resveratrol treated cells (Fig. 3a). Further, resveratrol decreased HSL and LPL mRNA levels by 52 ± 7% and 53 ± 5% (p < 0.001), respectively, in maturing preadipocytes (Fig. 3b).

Interestingly, resveratrol increased mRNA expression of SIRT3, UCP1 and Mfn2 by 83 ± 10%, 167 ± 40% and 48 ± 4.8% (p < 0.05), respectively, (Fig. 3c). The probes used for all the genes are described in Table 1.

**DISCUSSION**

The results of this study demonstrate that resveratrol inhibited adipogenesis and induced apoptosis in 3T3-L1 adipocytes. Resveratrol was shown to inhibit cell viability in 3T3-L1 preadipocytes (Hsu and Yen, 2006), but to our knowledge this is the first study to report that resveratrol induced apoptosis in mature 3T3-L1 adipocytes. Resveratrol-induced apoptosis in colon cancer cells was associated with AMPK activation and reactive oxygen species (ROS) generation (Hwang et al., 2007). Further studies are needed to investigate the molecular mechanisms leading to apoptosis by resveratrol in mature adipocytes.

In an *in vitro* study, resveratrol was shown to decrease adipogenesis in pig primary preadipocytes (Pang et al., 2006). The decrease in adipogenesis was thought to be associated in part with resveratrol’s effect on increasing the expression of SIRT1 mRNA. Since resveratrol is a proven SIRT1 activator (Howitz et al., 2003) and SIRT1 was shown to promote fat mobilization by repressing PPARγ, our results on PPARγ expression are in agreement with this observation (Picard et al., 2004). Moreover, sirtuin activation by resveratrol was shown to promote nuclear translocation of forkhead box transcription factors, O subfamily (FoxO) (Frescas et al.,...
these enzymes depends on their phosphorylation. Further studies are required to investigate the degree of phosphorylation of HSL and LPL after resveratrol treatment.

Resveratrol was shown to improve mitochondrial function by activating SIRT1 in mice (Lagouge et al., 2006), and SIRT3, another mitochondrial sirtuin deacetylase, activates mitochondria functions by reducing membrane potential (Shi et al., 2005). We hypothesized that the

Figure 2. Effect of resveratrol on lipid content and cell viability of maturing preadipocytes. 2a. Lipid content was measured by using AdipoRed™ assay. 2b. Effect on cell viability after treatment was determined by MTS assay. The experiments were performed with at least 6 replicates per treatment and were repeated three times. abc: means that are not denoted with a common superscript are different, $p < 0.05$. 2c. Representative images of Oil Red O staining.

Figure 3. Effect of resveratrol on expression of adipocyte specific genes. 3a. Effect of resveratrol on expression of the adipocyte specific transcription factors, PPARγ, C/EBPa and SREBP1, and FAS. 3b. Effect of resveratrol on expression of the lipolytic enzymes, HSL and LPL. 3c. Effect of resveratrol on expression of genes regulating mitochondrial functions. The experiments were performed in at least 6 replicates per treatment. Data are expressed as means ± SEM for each group and, within each gene, means without a common letter are different. ($p < 0.05$).

2005) and FoxO1 in turn was shown to suppress PPARγ in adipocytes.

Lipolytic enzymes HSL and LPL were down-regulated with resveratrol treatment in maturing preadipocytes, indicating that the decrease in lipid accumulation by resveratrol is mediated by decreasing adipogenesis and might not be due to lipolysis. Moreover, the level of HSL and LPL mRNA expression does not necessarily predict an effect on lipolysis, because the activity of
RESVERATROL EFFECTS ON 3T3-L1 ADIPOCYTES


REFERENCES


