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Resveratrol, a naturally occurring diphenolic compound, affects lipogenesis, lipolysis and the antilipolytic action of insulin in isolated rat adipocytes

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ABSTRACT

Resveratrol is a naturally occurring diphenolic compound exerting numerous beneficial effects in the organism. The present study demonstrated its short-term, direct influence on lipogenesis, lipolysis and the antilipolytic action of insulin in freshly isolated rat adipocytes. In fat cells incubated for 90 min with 125 and 250 μM resveratrol (but not with 62.5 μM resveratrol), basal and insulin-induced lipogenesis from glucose was significantly reduced. The antilipogenic effect was accompanied by a significant diminution of CO_2 release and enhanced production of lactate. The inhibition of glucose conversion to lipids found in the presence of resveratrol was not attenuated by activator of protein kinase C. However, acetate conversion to lipids appeared to be insensitive to resveratrol.

In adipocytes incubated for 90 min with epinephrine, 10 and 100 μM resveratrol significantly enhanced lipolysis, especially at lower concentrations of the hormone. However, the lipolytic response to dibutyryl-cAMP, a direct activator of protein kinase A, was unchanged. Further studies demonstrated that, in cells stimulated with epinephrine, 1, 10 and 100 μM resveratrol significantly enhanced glycerol release despite the presence of insulin or H-89, an inhibitor of protein kinase A. The influence of resveratrol on epinephrine-induced lipolysis and on the antilipolytic action of insulin was not abated by the blocking of estrogen receptor and was accompanied by a significant (with the exception of 1 μM resveratrol in experiment with insulin) increase in cAMP in adipocytes. It was also revealed that resveratrol did not change the proportion between glycerol and fatty acids released from adipocytes exposed to epinephrine.

Results of the present study revealed that resveratrol reduced glucose conversion to lipids in adipocytes, probably due to disturbed mitochondrial metabolism of the sugar. Moreover, resveratrol increased epinephrine-induced lipolysis. This effect was found also in the presence of insulin and resulted from the synergistic action of resveratrol and epinephrine. The obtained results provided evidence that resveratrol affects lipogenesis and lipolysis in adipocytes contributing to reduced lipid accumulation in these cells.

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1. Introduction

Resveratrol (3,5,4′-trihydroxystilbene) is a naturally occurring phytoalexin present in more than 70 plant species, among others in *Polygonum cuspidatum*—which roots are the richest source of the compound [1,2]. The considerable amounts of resveratrol were found in red wine [3]. Moreover, the pure compound is now available in tablets and is recommended as a dietary supplement. It exerts numerous beneficial effects in the organism. The anti-cancer [4–6], antioxidant [7], anti-inflammatory [8] and cardioprotective [9,10] properties of this compound were found in vitro and in vivo. However, the experimental data do not allow to conclude that resveratrol derived from natural sources can evoke such clear effects, and the health benefits of resveratrol in humans are still not proven.

It is well known that resveratrol binds estrogen receptor and, due to this ability, is numbered to phytoestrogens. The affinity of resveratrol to estrogen receptor is much lower – about 7000-fold – than estradiol [11], however, some effects of resveratrol were found to be estrogen receptor dependent.

In 1982, Arichi et al. [12] demonstrated that in livers of rats fed lipid emulsion, resveratrol and piceid (resveratrol-3-O-D-glucoside) reduced lipogenesis from [1-^{14}C]palmitate. The most recent data point out other effects of resveratrol on metabolic and hormonal status of the organism. The experiments with streptozotocin-induced diabetic rats showed that resveratrol evoked several beneficial effects including restricted body weight loss, reduced polyphagia and polydipsia and decreased blood glucose and triglycerides [13,14]. The effects of resveratrol on insulin secretion [15–17] and blood insulin concentrations in both normal

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and diabetic rats were also described [14,17–19]. Interestingly, in the latter animals, resveratrol delayed the onset of insulin resistance and evinced an insulin-like activity [14,19]. The beneficial health modifications brought about by resveratrol were observed in mice fed a high-fat diet. The compound decreased mortality, increased insulin sensitivity and improved motor functions [20]. In the experiment of Lagouge et al. [21], resveratrol improved muscle parameters in mice consuming a high-fat diet and also protected them against insulin resistance. Importantly, the animals consuming resveratrol remained lean despite high-fat diet and their white adipose tissue depots were significantly diminished. It is well established that increased adiposity and disturbed adipocyte metabolism contribute to decreased insulin sensitivity, to develop type 2 diabetes and to some other metabolic disorders. However, the direct effect of resveratrol on adipocyte metabolism had never been studied. In the present report, the short-term influence of resveratrol on lipogenesis, lipolysis and the antilipolytic action of insulin in isolated rat adipocytes was investigated.

2. Materials and methods

2.1. Animals

Male Wistar rats that weighed 200–250 g were obtained from Brwinow, Poland. The animals were fed a standard laboratory diet (Labofeed B, Kcynia, Poland) and had free access to tap water. The rats were maintained in cages in an air-conditioned animal room with a 12:12-h dark–light cycle and a constant temperature of $21 \pm 1\,^{\circ}C$ and were killed by decapitation. In each experiment, the epididymal (metabolically very active) adipose tissue was taken from a few rats. The protocols of experiments were reviewed and approved by the Local Ethical Commission for Investigation on Animals.

2.2. Reagents

Trans-resveratrol (the purity more than 99%), D-glucose, phorbol 12-myristate 13-acetate (PMA), bovine serum albumin (BSA), thiazolyl blue tetrazolium bromide (MTT), Trypan blue solution (0.4%, in 0.81% NaCl and 0.06% dibasic potassium phosphate), collagenase (from *Clostridium histolyticum*, Type II), insulin (from bovine pancreas), epinephrine, dibutyryl-cAMP, rotenone and all reagents used to prepare Krebs-Ringer buffer, to determine glycerol, fatty acids and lactate were from Sigma (St. Louis, MO). H-89 (N-[2-(p-bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide) and dimethyl sulfoxide (DMSO) were from ICN Biomedicals Inc. (Aurora, OH). ICI 182,780 was from Tocris (Ellisville, MO). Scintillation fluid (OptiPhase) was obtained from Fisher Chemicals (Loughborourgh, Leicester, UK). D-[U-^{14}C]glucose (specific activity 250 mCi/mmol) and hyamine hydroxide were from PerkinElmer (Boston, MA). cAMP kit (EIA) and [1-^{14}C]acetic acid (specific activity 56 mCi/mmol) were from Amersham (Buckinghamshire, UK).

2.3. Adipocyte isolation

Fat cells were isolated according to Rodbell method [22] with minor modifications [23]. The collected epididymal adipose tissue was cleaned, rinsed with warm 0.9% NaCl, cut with scissors into pieces and placed in a plastic flask with Krebs-Ringer buffer (118 mM NaCl, 4.8 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$ and 24.8 mM $NaHCO_3$) containing 3 mM glucose, 3% BSA, 10 mM HEPES and 2 mg/ml collagenase. The buffer was gassed with 95% O_2–5% CO_2 and its pH was adjusted to 7.4. The incubation with collagenase was carried out for 90 min with gentle shaking in a water bath at 37 °C. After incubation, isolated cells were filtered through a nylon mesh and rinsed four times with warm (37 °C) collagenase free Krebs-Ringer buffer. Adipocyte counts were performed using a microscope and a Bürker-Türk counting chamber.

2.4. Lipogenesis

To study the effect of resveratrol on glucose conversion to lipids, adipocytes ($10^6\,ml^{-1}$) were incubated in plastic tubes with Krebs-Ringer buffer containing 3 mM glucose, 0.5 μCi D-[U-^{14}C]glucose, 10 mM HEPES, 3% BSA (basal lipogenesis) and 1 or 10 nM insulin (stimulated lipogenesis). The effect of resveratrol on acetate conversion to lipids was studied in similar conditions, but the buffer contained 0.25 μCi [1-^{14}C]acetate instead of labeled glucose and was supplemented with 10 mM acetate. Cells were incubated without insulin or with 1 nM insulin. In each experiment, resveratrol was dissolved in DMSO and its final concentrations in the incubation medium were 62.5, 125 and 250 μM.

Additionally, experiments comparing the effect of 10 μM rotenone, a potent and specific inhibitor of mitochondrial electron transport, on lipogenesis from D-[U-^{14}C]glucose and [1-^{14}C]acetate were performed. In these studies, lipogenesis was stimulated by 1 nM insulin and cells were incubated under conditions similar to those used with resveratrol.

All incubations were maintained with gentle shaking for 90 min at 37 °C. After this time, the reaction was terminated by addition of 5 ml of cold Dole's extraction mixture [24] containing isopropanol–heptane–1N H_2SO_4 (40:10:1). The tubes were shaken and 2 ml of H_2O and 3 ml of heptane were added. The tubes were shaken once again and the upper phase containing extracted lipids was transferred into scintillation vials with scintillation fluid. The radioactivity of total lipids was measured using the β-counter.

To test whether the effect of resveratrol on insulin-stimulated glucose conversion to lipids may be affected by activation of protein kinase C, the isolated adipocytes were incubated for 90 min in Krebs-Ringer buffer containing 3 mM glucose, 0.5 μCi D-[U-^{14}C]glucose, 1 nM insulin in the presence of 250 μM resveratrol with or without 1 μM PMA. PMA was added 10 min before resveratrol. Afterwards, lipogenesis was determined as described above.

2.5. CO_2 release

The influence of resveratrol on CO_2 release was determined when isolated adipocytes ($10^6\,ml^{-1}$) were incubated for 90 min at 37 °C in Krebs-Ringer buffer containing 3 mM glucose, 10 mM HEPES, 3% BSA and 0.5 μCi D-[U-^{14}C]glucose under basal and insulin-stimulated (1 nM) conditions with or without resveratrol (62.5, 125 and 250 μM). The piece of blotting paper saturated with hyamine hydroxide was placed in each tube over the incubation mixture surface and the tubes were capped with rubber membranes. The experiments were performed for 90 min at 37 °C with gentle shaking. After this time, 200 μl of 1N H_2SO_4 was added and the capped tubes were remained for additional 60 min. Then, the blotting papers were transferred into the vials with scintillation fluid and the radioactivity was measured using the β-counter.

2.6. Lactate production

The effect of resveratrol on lactate production was performed in conditions similar to CO_2 release, but the buffer was without D-[U-^{14}C]glucose and tubes were not capped. After 90 min of incubation with 1 nM insulin, adipocytes were aspirated and aliquots of the incubation medium were mixed with two volumes of 10%

trichloroacetic acid. The tubes were centrifuged and lactate concentration in the supernatant was determined by the measure of NADH generated from NAD^+ in the presence of lactate dehydrogenase [25].

2.7. Lipolysis

In each experiment, the isolated adipocytes ($10^6\,ml^{-1}$) were transferred into plastic tubes with Krebs-Ringer buffer (pH 7.4) containing 3 mM glucose, 3% BSA and 10 mM HEPES. To study the effect of different concentrations of resveratrol on lipolysis stimulated by epinephrine and dibutyryl-cAMP, cells were incubated in the buffer containing 1 μM epinephrine or 1 mM dibutyryl-cAMP with or without 1, 10 and 100 μM resveratrol.

To ascertain the influence of resveratrol on lipolysis in the presence of different concentrations of lipolytic activators, adipocytes were incubated in the buffer containing 0.06, 0.125, 0.25, 0.5 and 1 μM epinephrine or 0.06, 0.125, 0.25, 0.5 and 1 mM dibutyryl-cAMP with or without 100 μM resveratrol.

The effect of resveratrol on the antilipolytic action of insulin and H-89 in isolated adipocytes was determined in experiments, in which cells were exposed to 0.5 μM epinephrine alone, epinephrine and 10 nM insulin or epinephrine and 50 μM H-89 with or without 1, 10 and 100 μM resveratrol.

It is known that estrogens can affect the lipolytic response of adipocytes to epinephrine. To determine whether effects of resveratrol on epinephrine induced lipolysis and on the antilipolytic action of insulin are mediated by estrogen receptor, isolated adipocytes were incubated with 0.5 μM epinephrine alone, with epinephrine and 10 nM insulin, with both hormones and 100 μM resveratrol with or without 1 μM ICI 182,780 (a blocker of the intracellular estrogen receptor). The proper experiments were preceded by 20 min preincubation with ICI 182,780.

The molar ratio of non-esterified fatty acids (NEFA) and glycerol released from isolated adipocytes was also determined. In these experiments, isolated cells were exposed to 0.5 μM epinephrine and epinephrine with 1, 10 and 100 μM resveratrol.

Moreover, the effect of preincubation with resveratrol on basal and epinephrine-stimulated lipolysis and on the antilipolytic action of insulin in the isolated cells was investigated. The cells were preincubated in Krebs-Ringer buffer with or without 100 μM resveratrol for 60 min. After this time, adipocytes were rinsed several times with the buffer without resveratrol and were incubated in the buffer alone, with 0.5 μM epinephrine or with epinephrine and 10 nM insulin.

In each lipolytic experiment, the final volume of the buffer in tubes with adipocytes was adjusted to 1 ml. The incubations were carried out for 90 min in a water bath at 37 °C with gentle shaking. Then, adipocytes were aspirated and the quantity of glycerol [26], and, in some cases, also fatty acids [27] released from cells to the incubation medium was determined.

2.8. cAMP measurement

To test the impact of resveratrol on cAMP concentrations, the isolated adipocytes were transferred to plastic tubes with Krebs-Ringer buffer (pH 7.4) containing 3 mM glucose, 3% BSA and 10 mM HEPES. The final amount of cells was 10^4 per 180 μl. The cells were incubated with 0.5 μM epinephrine and epinephrine with 1, 10 and 100 μM resveratrol. In the further investigations, adipocytes were incubated with 0.5 μM epinephrine and 10 nM insulin with or without 1, 10 and 100 μM resveratrol. After 30 min, 20 μl of the lysis buffer was added to each tube, the mixture was shaken and incubated for 10 min at room temperature. Afterwards, 100 μl of cell lysate was transferred to the assay plate and total cAMP was measured using non-acetylation EIA procedure according to the instruction enclosed by the manufacturer.

2.9. Cell viability

Adipocytes were incubated in Krebs-Ringer buffer (containing 3 mM glucose, 3% BSA and 10 mM HEPES) at 37 °C for 90 min without resveratrol or with 250 μM resveratrol. Then, cells were rinsed several times with Krebs-Ringer buffer without resveratrol. Afterwards, one pool of adipocytes was suspended in Trypan blue solution and cells preincubated with or without resveratrol were compared under a microscope. The second pool of cells preincubated with or without resveratrol was then incubated for 60 min in Krebs-Ringer buffer containing 3 mM glucose, 3% BSA, 10 mM HEPES and 0.5 mg/ml MTT at 37 °C. The reaction was terminated by addition of isopropanol. After vigorous mixing, tubes were centrifuged and the absorbance of isopropanol was read at 560 nm.

2.10. Statistical analysis

The means ± S.E.M. from three independent experiments in quadruplicates were evaluated statistically using analysis of variance and Duncan's multiple range test. Differences were considered significant at $p < 0.05$.

3. Results

3.1. Effect of resveratrol on viability of adipocytes

There was no significant difference in Trypan blue uptake between adipocytes preincubated for 90 min without resveratrol or with 250 μM resveratrol. Moreover, the absorbance of isopropanol was similar in the case of cells incubated for 90 min with or without 250 μM resveratrol (data not shown).

3.2. Effect of resveratrol on lipogenesis

Incubations of adipocytes with resveratrol revealed its inhibitory influence on glucose conversion to lipids. It was found that 125 and 250 μM resveratrol significantly diminished basal lipogenesis from glucose by 21% and 34%, respectively ($p < 0.05$), whereas 62.5 μM resveratrol was ineffective (Fig. 1). Similar inhibitory effect of 125 and 250 μM resveratrol was noticed when glucose conversion to lipids was stimulated by 1 nM insulin. Under these conditions, lipogenesis was diminished by 25% and 34%, respectively ($p < 0.05$; Fig. 1). In the further experiments, the concentration of insulin was increased to 10 nM. Glucose conversion to lipids stimulated by 10 nM insulin was attenuated in adipocytes exposed to 125 and 250 μM resveratrol by 15% and 25%, respectively ($p < 0.05$; Fig. 1). Further studies, employing acetate as a lipogenic substrate, demonstrated that neither basal nor insulin (1 nM)-induced lipogenesis from acetate was affected by resveratrol (data not shown). Incubations of adipocytes with 10 μM rotenone revealed its potent inhibitory effect on lipogenesis from glucose and acetate (lipogenesis was reduced by 90% and 60%, respectively; data not shown).

It was also demonstrated that in isolated adipocytes, 1 μM PMA failed to affect glucose conversion to lipids stimulated by 1 nM insulin. The exposure of cells to 1 μM PMA 10 min before resveratrol and together with 250 μM resveratrol did not attenuate the inhibitory effect of the tested compound on lipogenesis (data not shown).

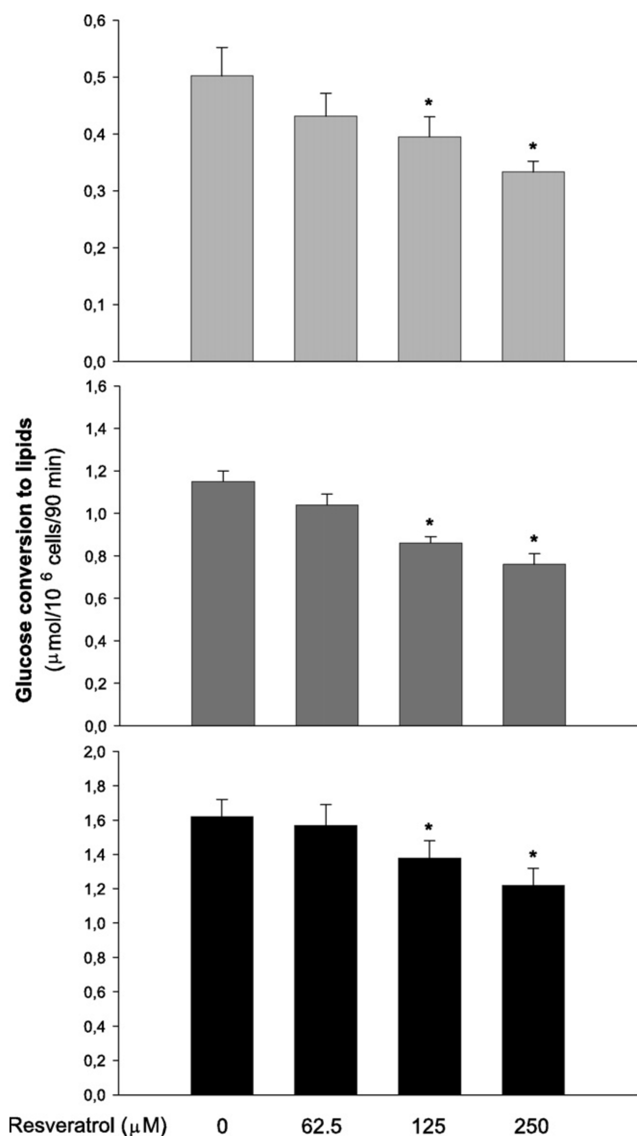

Fig. 1. The effect of resveratrol on basal (top) and insulin-stimulated (middle, 1 nM; bottom, 10 nM) lipogenesis from glucose in isolated rat adipocytes. Adipocytes were incubated for 90 min with 3 mM glucose and 0.5 μCi of D-[U-^{14}C]glucose with or without resveratrol. Each bar represents the mean ± S.E.M. of 12 determinations from 3 separate experiments. $p < 0.05$ vs. incubations without resveratrol (*).

3.3. Effect of resveratrol on CO_2 release and lactate production

The inhibitory influence of resveratrol on insulin (1 nM)-induced lipogenesis from glucose was accompanied by reduced release of CO_2. It was found that 125 and 250 μM resveratrol diminished CO_2 release by 16% and 25%, respectively ($p < 0.05$; Fig. 2). Moreover, in adipocytes incubated with 1 nM insulin and exposed to 125 and 250 μM resveratrol, production of lactate increased by 20% and 46%, respectively ($p < 0.05$; Fig. 2).

3.4. Effect of resveratrol on lipolysis and on the antilipolysis

Lipolysis stimulated by 1 μM epinephrine was not significantly affected by 1 μM resveratrol. However, in the presence of 10 and 100 μM resveratrol, epinephrine-induced lipolysis was enhanced by 15% and 17%, respectively ($p < 0.05$; Fig. 3). Lipolysis stimulated by 1 mM dibutyryl-cAMP, a direct activator of PKA, was unchanged in adipocytes incubated with 1, 10 and 100 μM

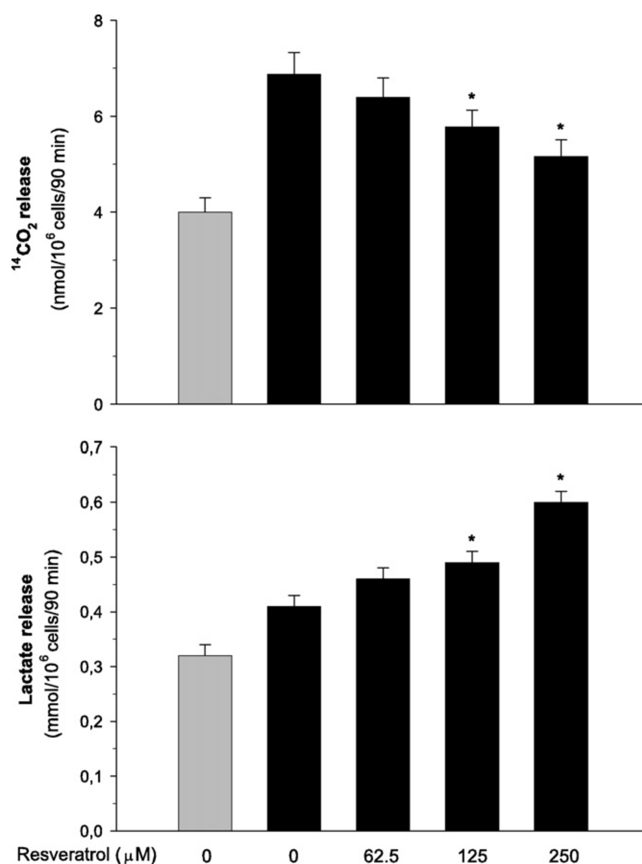

Fig. 2. The effect of resveratrol on basal (grey bar) and insulin-stimulated (black bars, 1 nM) release of $^{14}CO_2$ (top) and lactate (bottom) from isolated rat adipocytes. To test $^{14}CO_2$ release, adipocytes were incubated for 90 min with 3 mM glucose and 0.5 μCi of D-[U-^{14}C]glucose with or without resveratrol. Lactate release was determined under similar conditions, but the incubation buffer was without D-[U-^{14}C]glucose. Each bar represents the mean ± S.E.M. of 12 determinations from 3 separate experiments. $p < 0.05$ vs. insulin-stimulated incubations without resveratrol (*).

resveratrol (Fig. 3). Incubations of fat cells with 0.06, 0.125, 0.25, 0.5 and 1 μM epinephrine allow to demonstrate that the lipolytic response to this hormone was potentiated in the presence of 100 μM resveratrol by 163%, 99%, 43%, 20% and 16%, respec-

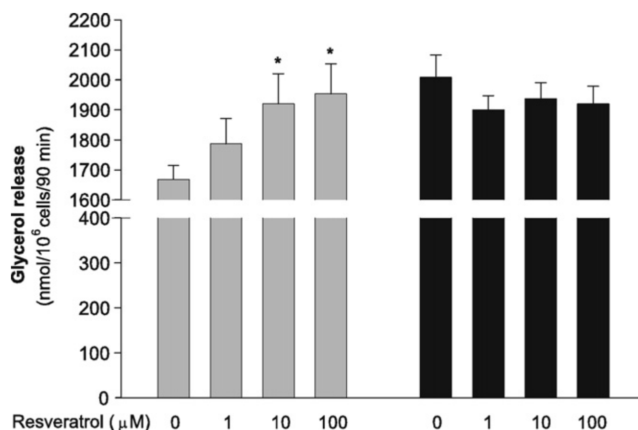

Fig. 3. The effect of resveratrol on lipolysis induced by 1 μM epinephrine (grey bars) and 1 mM dibutyryl-cAMP (black bars) in isolated rat adipocytes. Adipocytes were incubated for 90 min with or without resveratrol. Each bar represents the mean ± S.E.M. of 12 determinations from 3 separate experiments. $p < 0.05$ vs. incubations without resveratrol (*).

tively ($p < 0.05$; Fig. 4). Lipolysis induced by 0.06, 0.125, 0.25, 0.5 and 1 mM dibutyryl-cAMP was not significantly affected in cells exposed to 100 μM resveratrol (Fig. 4). It was also found that 100 μM resveratrol failed to change basal glycerol release (Fig. 4).

Lipolysis induced by 0.5 μM epinephrine was attenuated by 51% in cells exposed to 10 nM insulin ($p < 0.05$). However, in the presence of 1, 10 and 100 μM resveratrol, the release of glycerol increased by 14%, 28% and 69%, respectively, compared with adipocytes incubated with epinephrine and insulin without resveratrol ($p < 0.05$; Fig. 5). In the further experiments, the pancreatic hormone was replaced by H-89, a specific inhibitor of protein kinase A. The inhibition of the enzyme by 50 μM H-89 reduced epinephrine-induced lipolysis by 70% ($p < 0.05$). It was also found that 1, 10 and 100 μM resveratrol increased, by 20%, 39% and 94%, respectively, the amount of glycerol released from adipocytes incubated with epinephrine and H-89 without resveratrol ($p < 0.05$; Fig. 5).

To test whether resveratrol-induced changes in the lipolytic response to epinephrine and in the antilipolytic action of insulin are mediated by estrogen receptor, adipocytes were preincubated for 20 min with an estrogen receptor blocker (ICI 182,780; 1 nM) and then were incubated with the blocker, 0.25 μM epinephrine or 0.25 μM epinephrine and 1 nM insulin with or without 100 μM resveratrol. ICI 182,780 incubated with adipocytes did not affect the lipolytic response to epinephrine and the antilipolytic action of insulin. It was also demonstrated that resveratrol affected epinephrine-induced lipolysis and the antilipolytic action of insulin despite the presence of ICI 182,780 ($p < 0.05$; Fig. 7).

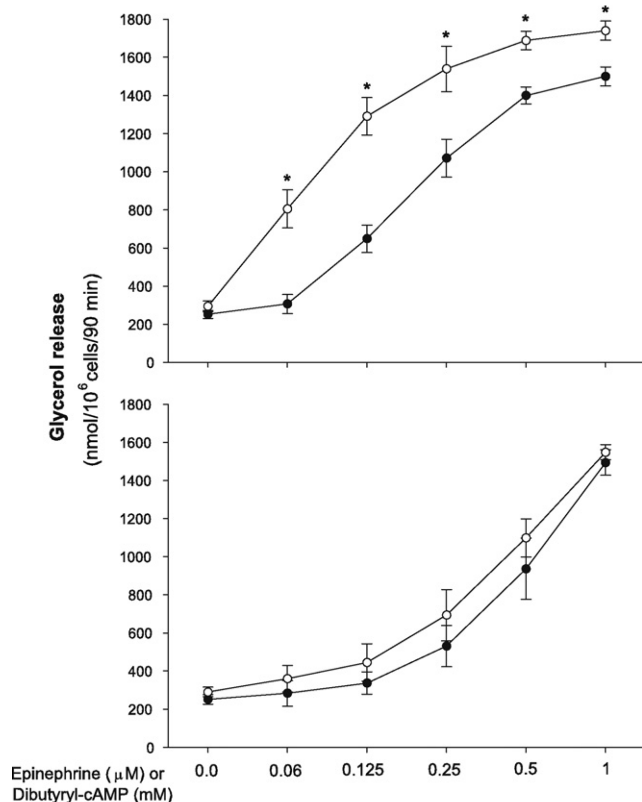

Fig. 4. The effect of resveratrol on lipolysis induced by different concentrations of epinephrine (top) or dibutyryl-cAMP (bottom) in isolated rat adipocytes. Adipocytes were incubated for 90 min without resveratrol (open circles) or with 100 μM resveratrol (black circles). Each bar represents the mean ± S.E.M. of 12 determinations from 3 separate experiments. $p < 0.05$ vs. incubations without resveratrol (*).

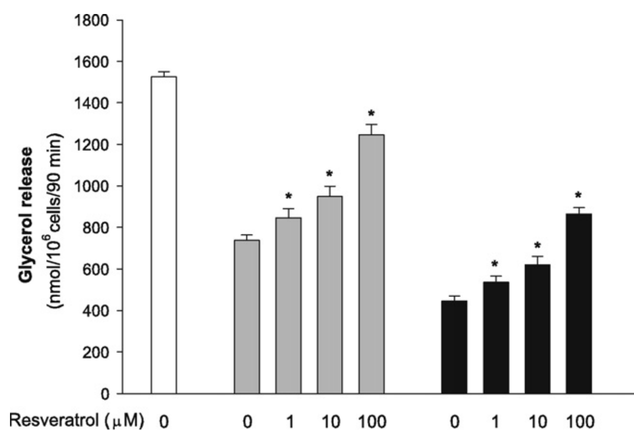

Fig. 5. The effect of resveratrol on the antilipolytic action of insulin (grey bars) and H-89 (black bars) in isolated rat adipocytes. Adipocytes were incubated for 90 min with 0.5 μM epinephrine alone (open bar), 0.5 μM epinephrine and 10 nM insulin (grey bars) or 0.5 μM epinephrine and 50 μM H-89 (black bars) with or without resveratrol. Each bar represents the mean ± S.E.M. of 12 determinations from 3 separate experiments. $p < 0.05$ vs. incubations without resveratrol (*).

Further experiments revealed that in adipocytes incubated with 0.5 μM epinephrine and exposed to 1, 10 and 100 μM resveratrol, the proportion between glycerol and fatty acids released to the buffer was unchanged (Table 1).

To test whether the effects of resveratrol on lipolysis are transient, adipocytes were preincubated for 60 min without resveratrol or with 100 μM resveratrol. Afterwards, cells were incubated without any hormones, with 0.5 μM epinephrine or with 0.5 μM epinephrine and 10 nM insulin. Preincubation of adipocytes with resveratrol failed to change basal lipolysis. However, lipolysis induced by epinephrine was significantly increased (by 27%) in fat cells preincubated with resveratrol ($p < 0.05$; Fig. 8). Similarly, in adipocytes exposed to epinephrine and insulin, glycerol release was greater (by 26%) when cells were preincubated with resveratrol ($p < 0.05$; Fig. 8).

3.5. Effect of resveratrol on cAMP concentrations

Incubations of adipocytes for 30 min with 0.5 μM epinephrine and 1, 10 and 100 μM resveratrol resulted in enhanced accumulation of cAMP compared with cells exposed to epinephrine alone.

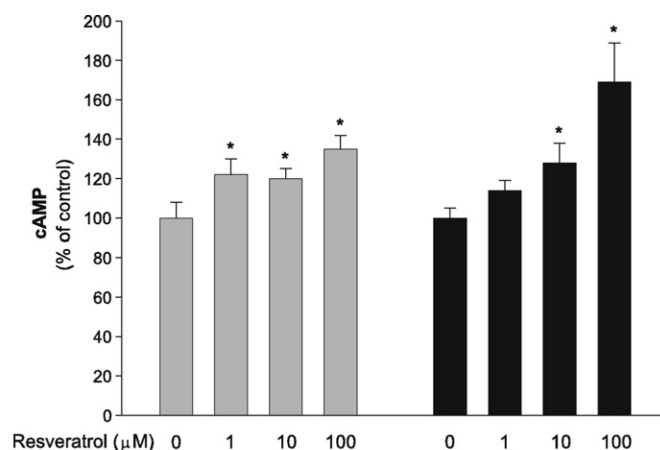

Fig. 6. The effect of resveratrol on cAMP concentrations in isolated rat adipocytes. Adipocyteswere incubated for 30 min with 0.5 μM epinephrine alone (grey bars) or 0.5 μM epinephrine and 10 nM insulin (black bars) with or without resveratrol. Each bar represents the mean ± S.E.M. of 12 determinations from 3 separate experiments. $p < 0.05$ vs. incubations without resveratrol (*).

Table 1
The effect of resveratrol on the release of glycerol and non-esterified fatty acids (NEFA) and on NEFA/glycerol molar ratio.

Resveratrol (μM)	0	1	10	100
Glycerol (nmol/10^6 cells/90 min)	1400 ± 45	1499 ± 55	1615 ± 62*	1788 ± 49*
NEFA (nmol/10^6 cells/90 min)	2688 ± 125	2785 ± 110	3192 ± 99*	3377 ± 138*
NEFA/glycerol molar ratio	1.920 ± 0.110	1.858 ± 0.130	1.976 ± 0.110	1.889 ± 0.220

Adipocytes were incubated for 90 min with 0.5 μM epinephrine alone or epinephrine and resveratrol. Values represent the mean ± S.E.M. of 12 determinations from 3 separate experiments.
* $p < 0.05$ vs. incubations without resveratrol.

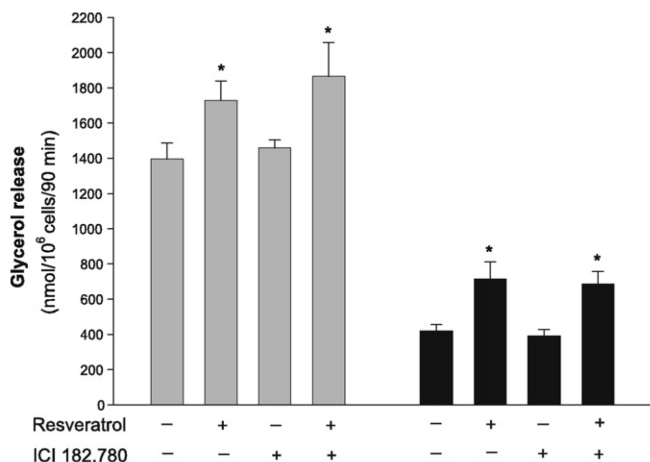

Fig. 7. The effect of resveratrol on epinephrine-induced lipolysis (grey bars) and on the antilipolytic action of insulin (black bars) in isolated rat adipocytes in the presence of ICI 182,780. Adipocytes were incubated for 90 min with 0.5 μM epinephrine alone, 0.5 μM epinephrine and 10 nM insulin, or with these hormones in the presence of 100 μM resveratrol, 1 nMICI 182,780 or both. ICI 182,780 was added 20 min before resveratrol. Each bar represents the mean ± S.E.M. of 12 determinations from 3 separate experiments. $p < 0.05$ vs. incubations without resveratrol (*).

The increase in cAMP was 22%, 20% and 35%, respectively ($p < 0.05$; Fig. 6). Similar effect was seen in the presence of insulin. It was found that 10 and 100 μM resveratrol significantly increased cAMP by 28% and 69%, respectively, in adipocytes incubated with 0.5 μM epinephrine and 10 nM insulin ($p < 0.05$). The influence of 1 μM resveratrol was not statistically significant (Fig. 6).

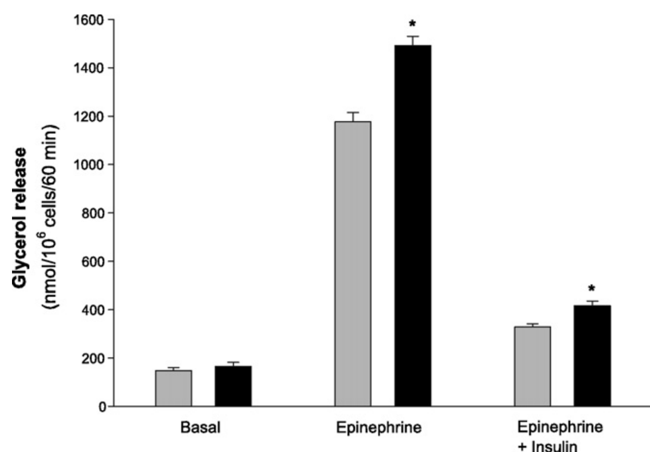

Fig. 8. The effect of preincubation with resveratrol on basal and epinephrinein-duced lipolysis and on the antilipolytic action of insulin in isolated rat adipocytes. Adipocytes were incubated without resveratrol (grey bars) or with 100 M resveratrol (black bars) for 60 min. After this time, all cells were rinsed with the buffer containing no resveratrol and were incubated for 90 min in the buffer alone (basal lipolysis), with 0.5 M epinephrine or 0.5 μM epinephrine and 10 nM insulin. Each bar represents the mean ± S.E.M. of 12 determinations from 3 separate experiments. $p < 0.05$ vs. preincubation without resveratrol (*).

4. Discussion

The present study was initiated to determine the effects of resveratrol on metabolism of adipocytes—cells responsible, among others, for lipid synthesis, release and storage. The obtained results provided evidence that resveratrol affects lipogenesis and lipolysis in adipocytes. One of the effects was reduced basal and insulin-induced glucose conversion to lipids. Zhang [28] demonstrated resveratrol-induced attenuation of insulin signaling pathway in some kinds of cells. However, the diminution of lipogenesis non-stimulated by insulin indicates that disturbances other than reduced insulin signaling are pivotal for reduction of glucose conversion to lipids in fat cells exposed to resveratrol.

It is known that, in some kinds of cells, resveratrol inhibits protein kinase C (PKC) [15,29,30]. Activation of this enzyme in adipocytes is involved in the insulin-induced stimulation of lipogenesis from glucose [31,32]. However, in fat cells exposed to PMA, an activator of PKC, the inhibitory influence of resveratrol on insulin-induced glucose conversion to lipids was not mitigated. This indicates that the inhibition of PKC in adipocytes, if any, does not play a crucial role in the reduction of glucose conversion to lipids found in the presence of resveratrol.

Conversely to lipogenesis from glucose, metabolism of acetate to lipids appeared to be insensitive to resveratrol. This difference can be explained by mitochondrial dysfunction. Other studies have indeed reported that resveratrol disturbed mitochondrial functions [16,33,34]. Moreover, our experiments demonstrated that the inhibition of mitochondrial electron transport in fat cells diminishes much more lipogenesis from glucose than from acetate. It is also known that in adipocytes glucose conversion to lipids involves mitochondrial metabolism of the sugar, whereas acetate is activated to acetyl-CoA in the cytoplasm. Disturbances in the respiratory chain result in reduced reoxidation of NADH generated during glycolysis, diminish glucose oxidation and restrain the formation of acetyl-CoA from glucose [35]. In our study, resveratrol was indeed found to disturb aerobic metabolism of glucose as evidenced by increased release of lactate. Simultaneously, glucose oxidation to CO_2 appeared to be reduced. All these data point to impaired mitochondrial metabolism of glucose as a main reason of decreased conversion of the sugar to lipids in fat cells incubated with resveratrol.

Results of the present study revealed enhanced lipolytic response to epinephrine in adipocytes exposed to resveratrol. The effect was substantially greater at lower (0.06–0.25 μM) than at higher (0.5–1 μM) epinephrine. Resveratrol failed to rise basal glycerol release indicating rather synergistic than additive action with the lipolytic hormone. The potentiatory effect of resveratrol on epinephrine-induced lipolysis seems to result, at least partially, from increased cAMP in adipocytes. The rise in cAMP is crucial for the stimulation of lipolysis (reviewed by [36]). The resveratrol's ability to enhance cAMP was previously shown in breast [37] and colon cancer cells [38]. Interestingly, resveratrol failed to significantly augment lipolysis induced by dibutyryl-cAMP, a direct activator of PKA. This indicates that the tested compound does not act synergistically with all lipolytic agents.

Results of our present study demonstrated the ability of resveratrol to modify the antilipolytic action of insulin since resveratrol increased the release of glycerol from cells incubated with epinephrine and insulin. The antilipolytic action of insulin is achieved mainly through activation of cAMP phosphodiesterase 3B resulting in reduced cAMP [39,40]. However, in resveratrol-treated cells, cAMP was increased despite the presence of insulin. To test whether the rise in lipolysis caused by resveratrol in cells incubated with epinephrine and insulin is due to deterioration of insulin action, in the further experiments insulin was replaced by the other antilipolytic agent—H-89, a direct inhibitor of PKA. It was found that the rise in lipolysis caused by resveratrol in cells stimulated by epinephrine was similar in the presence of insulin or H-89. The similar effects found in the case of two distinct antilipolytic agents indicate that, under these conditions, resveratrol did not augment lipolysis due to deterioration of insulin action and that the effect of resveratrol was not specific only for insulin. Moreover, the ability of resveratrol to enhance epinephrine-induced lipolysis in the presence of H-89 implies that the rise in cAMP is not the sole effect of resveratrol, because H-89 restricts lipolysis even despite the increased cAMP levels in adipocytes [41].

It is worth noting that in adipocytes incubated with epinephrine and resveratrol, insulin was able to reduce lipolysis compared with lipolysis in cells exposed to epinephrine alone. Therefore, the antilipolytic action of insulin was preserved, whereas the observed rise in glycerol release was due to the amplifying effect of resveratrol on the lipolytic action of epinephrine.

Lipolysis in adipocytes results in the release of both glycerol and fatty acids, but the proportion between these lipolytic products may vary [42–46]. Resveratrol was found to affect some metabolic processes in the cell, however, as demonstrated in the present study, the tested compound failed to change the proportion between non-esterified fatty acids and glycerol released from adipocytes stimulated by epinephrine.

Some cellular effects of resveratrol are mediated through estrogen receptor and may be suppressed by its blockade [11,47,48]. On the other hand, studies of receptor binding by estradiol displacement in MCF7 human breast cancer cells revealed the inability of resveratrol to displace estradiol from estrogen receptor [49]. Results of our study demonstrated that blockade of intracellular estrogen receptors did not attenuate the influence of resveratrol on epinephrine-induced lipolysis and on the antilipolytic action of insulin in adipocytes. This indicates that these effects are not mediated via interaction of resveratrol with estrogen receptor and that they are independent of differences in tissue sensitivity to estrogens.

It is worth noting that resveratrol is able to affect adipocyte metabolism after short-term exposure. Moreover, the influence of the compound on epinephrine-induced lipolysis and on the antilipolytic action of insulin was found to be preserved even after removal of resveratrol from the incubation medium. It seems, however, that the observed effects did not result from the injury of cells, as confirmed by MTT assay and Trypan blue exclusion. Results obtained in the present study demonstrated that lipogenesis was less sensitive to resveratrol than lipolysis since, in the latter case, concentrations of resveratrol required to induce changes were lower.

In conclusion, our results revealed the ability of resveratrol to affect lipogenesis and lipolysis in adipocytes of normal rats. The observed inhibition of glucose conversion to lipids, accompanied by reduced release of CO_2 and increased formation of lactate, was probably due to disturbed mitochondrial metabolism. Moreover, resveratrol was shown to exert synergistic action with epinephrine enhancing its lipolytic capacity, also in the presence of insulin. All these effects diminish lipid accumulation in fat cells and may contribute to reduced adiposity.

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