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Resveratrol and genistein as adenosine triphosphate–depleting agents in fat cells

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Abstract

Resveratrol and genistein are plant-derived compounds known to exert pleiotropic effects in many cell types, including adipocytes. However, the effects of these compounds on the energetic status of fat cells are unknown. The present study aimed to determine whether resveratrol and genistein influence adenosine triphosphate (ATP) levels in freshly isolated rat adipocytes. To determine the effects of resveratrol and genistein on adipocyte ATP content, cells were exposed to insulin and glucose or insulin and alanine without tested compounds or with 6.25 to 50 μmol/L resveratrol or genistein. Resveratrol substantially reduced glucose- and alanine-derived ATP in adipocytes. This was not due to the inhibition of glucose transport because the influence of the test compound on insulin-stimulated glucose uptake by adipocytes appeared to be stimulatory. Moreover, resveratrol reduced both alanine oxidation and mitochondrial membrane hyperpolarization. It was also demonstrated that preincubation of cells with resveratrol slightly diminished ATP levels despite the withdrawal of the tested compound from the buffer. The genistein effect was accompanied by attenuation of the mitochondrial membrane hyperpolarization. The compound failed to significantly affect insulin-stimulated glucose uptake by fat cells. Similarly to resveratrol, preincubation of adipocytes with genistein slightly reduced ATP in cells exposed to glucose and insulin. Results of the present study revealed the potent ability of resveratrol to reduce ATP in rat adipocytes, whereas genistein appeared to be less effective. It is suggested that both tested compounds diminish adipocyte ATP via attenuation of the metabolic activity of mitochondria. Because numerous cellular events are strongly ATP dependent, the ATP-depleting effects of resveratrol and genistein may have pleiotropic consequences for adipocyte functions.

1. Introduction

Resveratrol and genistein are naturally occurring plant-derived compounds exerting beneficial effects in the organism. Resveratrol is known to induce anticancer [1], antioxidant [2,3], and anti-inflammatory effects [4,5]. This compound is present in high concentrations in red wine and is considered to be one of the wine components responsible for the French paradox, that is, low mortality due to coronary heart disease despite a high-fat diet and smoking habits [6-8]. The cardioprotective activity of resveratrol has been indeed demonstrated in different experimental animal models [9-11]. The most recent studies revealed the favorable effects of resveratrol in some metabolic disorders. It was found that mice on a high-fat diet consuming resveratrol remained lean and had improved muscle parameters. Moreover, resveratrol protected these animals against insulin resistance [12,13]. Deng et al [14] revealed that resveratrol partially attenuated hormonal and metabolic disturbances in rats on a high–cholesterol-fructose diet. Numerous benefits of resveratrol ingestion were also observed in obese Zucker rats [15]. According to results obtained by Barger et al [16], resveratrol is able to retard some aspects of aging. Interestingly, the positive effects induced by resveratrol were similar to changes evoked by a calorie-restricted diet. It is also worth noting that resveratrol may be useful in diabetes (for recent review, see Szkudelska and Szkudelski [17]) because insulin-like effects of this compound were observed in diabetic rats [18,19]. On the other hand, in animals with hyperinsulinemia, resveratrol diminished blood insulin concentrations [12-15]. Moreover, the direct inhibitory effect of resveratrol on insulin

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secretion from pancreatic islets of healthy rats is well documented [20-22]. Resveratrol was also found to decrease blood concentrations of adipocyte-derived hormone leptin in animals with hyperleptinemia [12,15] and to reduce leptin secretion from isolated adipocytes [23]. It is well established that resveratrol activates NAD^+-dependent protein deacetylase, Sirt1; and some long-term beneficial effects evoked by resveratrol are thought to be mediated through activation of this enzyme [13,24].

Genistein, present in particularly high concentrations in soy and soy-derived products, has been shown to exert antiatherogenic [25,26], cardioprotective, and anticancerogenic [27-30] effects and to reduce osteoporosis and some other postmenopausal problems [31,32]. Genistein was found to decrease blood leptin concentrations [33]. The beneficial effects of genistein on blood lipid parameters are also well known [34,35]. Numerous in vitro studies revealed the ability of genistein to increase insulin secretion [36]; however, in vivo genistein induces rather hypoinsulinemic effects [37]. It is known that adipocyte exposure to genistein results in reduced glucose conversion to lipids. The phytoestrogen was also demonstrated to enhance epinephrine-induced lipolysis and to counteract the antilipolytic action of insulin in isolated adipocytes [38,39].

It is well established that adipocytes play a crucial role in the regulation of energy balance in the whole organism. Moreover, their endocrine function is well recognized. Dysregulation of metabolism and endocrine function of fat cells may lead to obesity, insulin resistance, and type 2 diabetes mellitus. In the light of these data, it seems that the effects of different dietary components on adipocyte functions should be well elucidated.

Resveratrol and genistein were mainly studied in the context of their influence on the adiposity, adipogenesis, lipogenesis, lipolysis, and the secretory function of adipocytes. Data from the literature indicate that both these compounds reduce adipose tissue depots [13,40,41] and directly influence the metabolism of fat cells [23,33,38,39,42-45]. The antiadipogenic effect of resveratrol and genistein has been also documented [46-49]. However, despite the statement that resveratrol and genistein are able to induce different effects in adipocytes, the influence of these compounds on the energetic status of fat cells is unknown. This question seems to be pivotal because the majority of cellular processes is energy dependent. The present study aimed to determine whether resveratrol and genistein affect ATP contents in the isolated rat adipocytes.

2. Materials and methods

2.1. Reagents

Transresveratrol, genistein, D-glucose, L-alanine, bovine serum albumin (BSA, fraction V), collagenase (EC 3.4.24.3, from *Clostridium histolyticum*, type II), insulin (from bovine pancreas), phloretin, kits used to determine ATP, rhodamine 123, thiazolyl blue tetrazolium bromide (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide; MTT), and all reagents used to prepare Krebs-Ringer buffer were from Sigma (St Louis, MO). Hyamine hydroxide was from Perkin Elmer (Boston, MA), 2-deoxy-D-[1-^{3}H]-glucose (specific activity, 296 GBq/mmoL) was from GE Healthcare (Buckinghamshire, United Kingdom), and L-[U-^{14}C]alanine (specific activity, 132 mCi/mmol) was from Hartmann Analytic (Brunswick, Germany). Dimethyl sulfoxide was from MP Biomedicals (Solon, OH). The composition of Krebs-Ringer buffer was the following (in millimoles per liter): 118 NaCl, 4.8 KCl, 1.3 $CaCl_2$, 1.2 KH_2PO_4, 1.2 $MgSO_4$, and 24.8 $NaHCO_3$.

2.2. Adipocyte preparation

Male Wistar rats that weighed 240 to 280 g and obtained from Brwinow (Poland) were used in all experiments. Animals were maintained in cages in an air-conditioned room at constant temperature (21°C ±1°C) with a 12:12-hour dark-light cycle. They were fed ad libitum a standard laboratory diet (Labofeed B; Morawski, Kcynia, Poland) containing cereals, cereals by-products, soybean meal, linseed meal expeller, dried brewers' yeast, potato protein concentrate, milk and whey powder, macro- and microelements, vitamins; crude protein 17.4%, crude fat 3.2%, crude fiber 0.7%, starch 29%, metabolizable energy 12 MJ/kg. Moreover, animals had free access to tap water. The experiments were performed according to rules and protocols accepted by the Local Ethical Commission for Investigations on Animals.

Adipocytes were isolated according to the Rodbell method [50] with minor modifications [38]. The animals were decapitated by guillotine, and the epididymal fat tissue was collected. The tissue was rinsed with 0.9% NaCl, cut into small pieces, and incubated with gentle shaking for 90 minutes at 37°C in Krebs-Ringer buffer containing 3% bovine serum albumin, 3 mmol/L glucose, 10 mmol/L HEPES, and 2 mg/mL collagenase. The buffer was gassed for 20 minutes with a carbogen (95% O_2/5% CO_2), and pH was adjusted to 7.4. The isolated cells were filtered through a nylon mesh, rinsed with warm Krebs-Ringer buffer, and counted under the microscope with a Bürker-Türk counting chamber.

2.3. Adipocyte incubations and measurement of ATP

To determine the effects of resveratrol and genistein on ATP content in adipocytes, cells (5×10^5/mL) were incubated in plastic tubes with Krebs-Ringer buffer containing 10 nmol/L insulin with 5 or 20 mmol/L glucose or 10 mmol/L alanine. Incubations were made without the tested compounds or in the presence of 6.25, 12.5, 25, or 50 μmol/L resveratrol or genistein. In addition, the effects of resveratrol on ATP reserves were compared with changes induced by 2.5 or 5 μmol/L rotenone. In these

experiments, cells were incubated for 2 hours at 37°C with gentle shaking. Resveratrol, genistein, and rotenone were dissolved in dimethyl sulfoxide; and stock solutions were added into the buffer (5 μL/995 μL).

Moreover, adipocytes were incubated in tubes with Krebs-Ringer buffer containing 5 mmol/L glucose and 10 nmol/L insulin with or without 25 μmol/L resveratrol for 15, 30, 45, and 60 minutes.

To determine whether the effects of resveratrol and genistein on ATP are reversible, fat cells were preincubated for 60 minutes with the tested compounds (50 μmol/L) in Krebs-Ringer buffer containing 3 mmol/L glucose. Afterward, adipocytes were washed to remove the tested compounds; and cells were incubated for 60 minutes in the buffer containing 5 mmol/L glucose and 10 nmol/L insulin.

At the end of each experiment, the lysis reagent was added, the tubes were vortexed and left in room temperature for a few minutes, and the upper phase was removed. Afterward, ATP was determined by a luminometric method with a kit containing firefly luciferase and luciferine (ATP Bioluminescent Assay Kit, Sigma) [51].

2.4. Glucose transport

The effect of resveratrol and genistein on glucose uptake by isolated adipocytes was measured using 2-deoxy-D-[1-^{3}H]-glucose. In these studies, fat cells (10^6/mL) were preincubated for 10 minutes at 37°C in plastic tubes with Krebs-Ringer buffer containing 0.5 mmol/L glucose without the tested compounds or in the presence of 6.25, 12.5, 25, or 50 μmol/L resveratrol or genistein. Afterward, 10 nmol/L insulin was added; and incubations were performed for 20 minutes. After this time, 1 μCi of 2-deoxy-D-[1-^{3}H]glucose was added. The reaction was stopped after 3 minutes by addition of ice-cold Krebs-Ringer buffer with phloretin (3 mmol/L). The silicone oil was then added, and tubes were centrifuged. In the next step, adipocytes were transferred into scintillation vials containing scintillation cocktail; and the radioactivity was measured using β-counter (Wallac 1409; Turku, Finland). The results were corrected for simple diffusion and nonspecific uptake by measuring glucose uptake by a separate group of cells that was pretreated with 1.5 mmol/L phloretin before the addition of 2-deoxy-D-[1-^{3}H]glucose.

2.5. The effect of resveratrol on alanine oxidation

To determine the effect of resveratrol on alanine oxidation, isolated adipocytes (5×10^5/mL) were incubated in Krebs-Ringer buffer containing 5 mmol/L alanine, 10 mmol/L HEPES, 3% BSA, 0.25 μCi L-[U-^{14}C]alanine, and 10 nmol/L insulin with or without resveratrol (6.25, 12.5, 25, and 50 μmol/L). 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 120 minutes at 37°C with gentle shaking. After this time, 200 μL of 1 N H_2SO_4 was added; and the capped tubes were left for an additional 60 minutes. Afterward, the blotting papers were transferred into the vials with scintillation fluid; and the radioactivity of CO_2 derived from L-[U-^{14}C]alanine was measured using the β-counter.

2.6. The effect of resveratrol and genistein on mitochondrial membrane potential

The changes in mitochondrial membrane potential were measured using rhodamine 123. The decrease in its fluorescence in cells exposed to glucose is due to hyperpolarization of the inner mitochondrial membrane and reflects metabolism of the sugar [52]. To determine the effect of resveratrol and genistein on mitochondrial membrane potential, adipocytes were isolated in Krebs-Ringer buffer without glucose. The obtained cells were loaded with rhodamine 123 for 10 minutes in Krebs-Ringer buffer at 37°C. Afterward, adipocytes were washed with the buffer without dye and were transferred on a plate and placed in a plate counter (Victor^2, Wallac) at 37°C. Fat cells were then exposed to 20 mmol/L glucose and 10 nmol/L insulin without the tested compounds or in the presence of 50 μmol/L resveratrol or 50 μmol/L genistein. In addition, one pool of cells was exposed to 20 mmol/L glucose and 10 nmol/L insulin in the presence of 10 μmol/L rotenone. Rhodamine 123 fluorescence was excited at 485 nm, and emitted light was measured using a 535-nm filter.

2.7. Adipocyte viability

Cell viability was determined by MTT assay [53] and trypan blue exclusion. Fat cells were incubated at 37°C in Krebs-Ringer buffer containing 5 mmol/L glucose, 10 nmol/L insulin, 3% BSA, and 10 mmol/L HEPES without the tested compounds (control cells) or in the presence of 50 μmol/L resveratrol or 50 μmol/L genistein. After 2 hours, all cells were rinsed 4 times with Krebs-Ringer buffer. One pool of adipocytes was then suspended in trypan blue solution; and adipocytes incubated with resveratrol or genistein were compared with control cells under the microscope. The other pool of control adipocytes and cells preincubated with resveratrol or genistein was incubated for 60 minutes in Krebs-Ringer buffer containing 5 mmol/L glucose, 3% BSA, 10 mmol/L HEPES, and 0.5 mg/mL MTT at 37°C. Isopropanol was then added to each tube; and after vigorous mixing, tubes were centrifuged, and the absorbance of formazan formed from MTT was read at 560 nm.

2.8. Statistical analysis

The means ± SEM from 3 independent experiments in quadruplicate were evaluated statistically using analysis of variance and Duncan multiple range test. In the case of mitochondrial membrane potential, means ± SEM obtained from 3 independent experiments were evaluated statistically using repeated measurements analysis of variance. Differences were considered significant at $P < .05$.

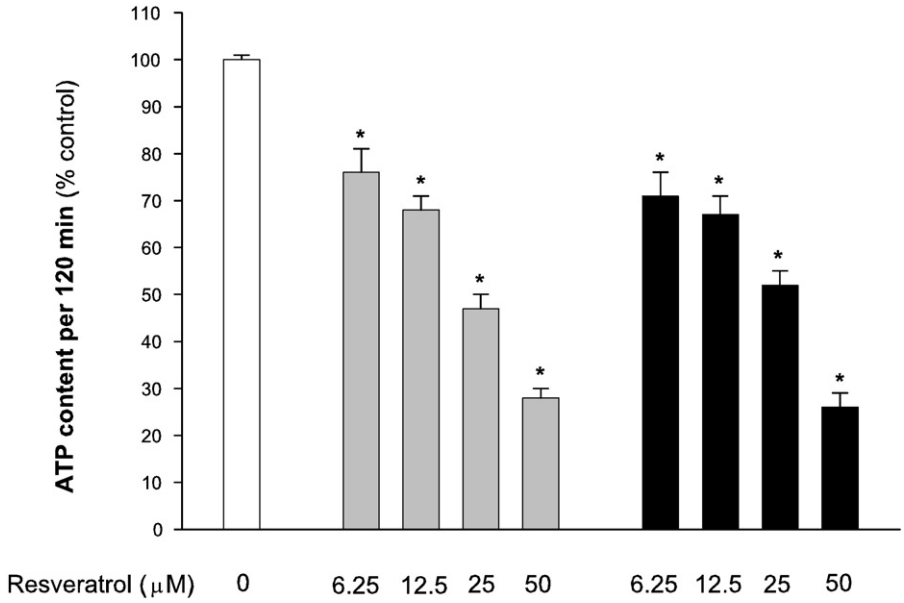

Fig. 1. The effect of resveratrol on ATP content in adipocytes incubated with 5 mmol/L glucose and 10 nmol/L insulin (gray bars) or 20 mmol/L glucose and 10 nmol/L insulin (black bars). Values represent means ± SEM of 12 determinations from 3 separate experiments. *$P < .05$ vs incubations without resveratrol (open bar).

3. Results

3.1. The effect of resveratrol on ATP content in adipocytes

Incubations of freshly isolated rat adipocytes with resveratrol demonstrated the clear-cut ATP-depleting effects of the tested compound. It was found that in adipocytes exposed for 2 hours to 6.25, 12.5, 25, or 50 μmol/L resveratrol, concentrations of glucose-derived ATP were substantially reduced. These effects were irrespective of glucose concentrations; similar diminution of ATP reserves was noticed at 5 and 20 mmol/L glucose (Fig. 1).

Analysis of the time course of changes in adipocyte ATP levels demonstrated that 25 μmol/L resveratrol dramatically reduced ATP already after 15 minutes of exposure. Similar

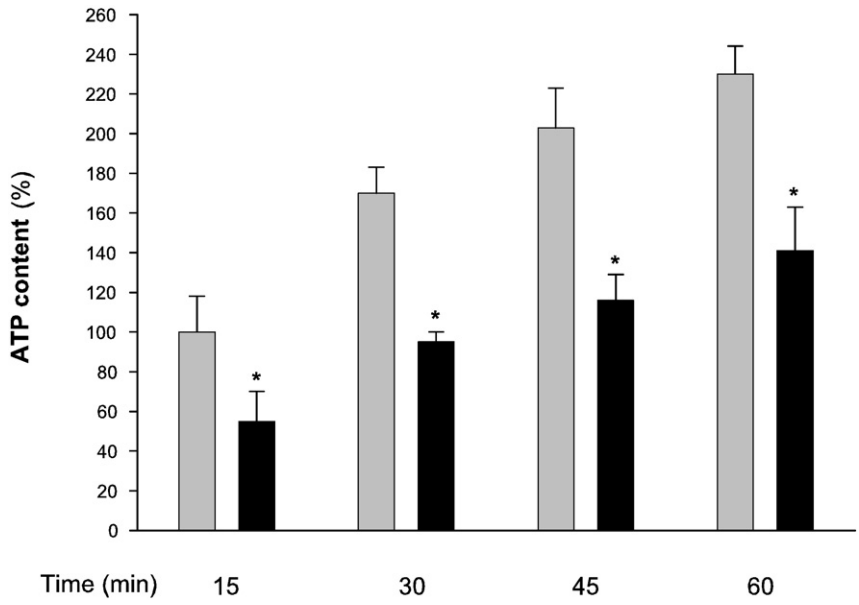

Fig. 2. The effect of resveratrol on ATP content in adipocytes in different time intervals. Cells were incubated with 5 mmol/L glucose and 10 nmol/L insulin (gray bars) or with 5 mmol/L glucose, 10 nmol/L insulin, and 25 μmol/L resveratrol (black bars). The ATP content in adipocytes incubated for 15 minutes with 5 mmol/L glucose and 10 nmol/L insulin was taken as 100%. Values represent means ± SEM of 12 determinations from 3 separate experiments. *$P < .05$ vs incubations without resveratrol.

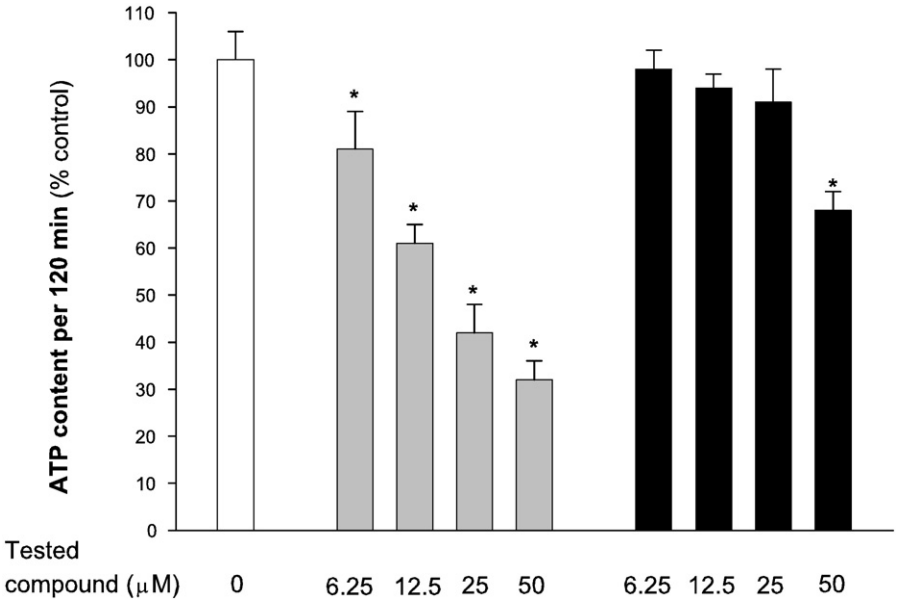

Fig. 3. The effect of resveratrol (gray bars) or genistein (black bars) on ATP content in adipocytes incubated with 10 mmol/L alanine and 10 nmol/L insulin. Values represent means ± SEM of 12 determinations from 3 separate experiments. *$P < .05$ vs incubations without tested compound (open bar).

effects were found in 30, 45, and 60 minutes of incubation with resveratrol compared with incubations without this compound (Fig. 2).

In the experiments with alanine as the source of ATP, 6.25, 12.5, 25, or 50 μmol/L resveratrol profoundly reduced adipocyte ATP reserves. These effects were similar to results obtained in experiments using glucose (Fig. 3).

Moreover, the effect of preincubation of adipocytes with resveratrol on ATP was ascertained. It was demonstrated that preincubations of cells for 60 minutes with 50 μmol/L resveratrol significantly diminished ATP levels (by about 20%). There was no effect observed when cells were preincubated without tested compound (Fig. 4).

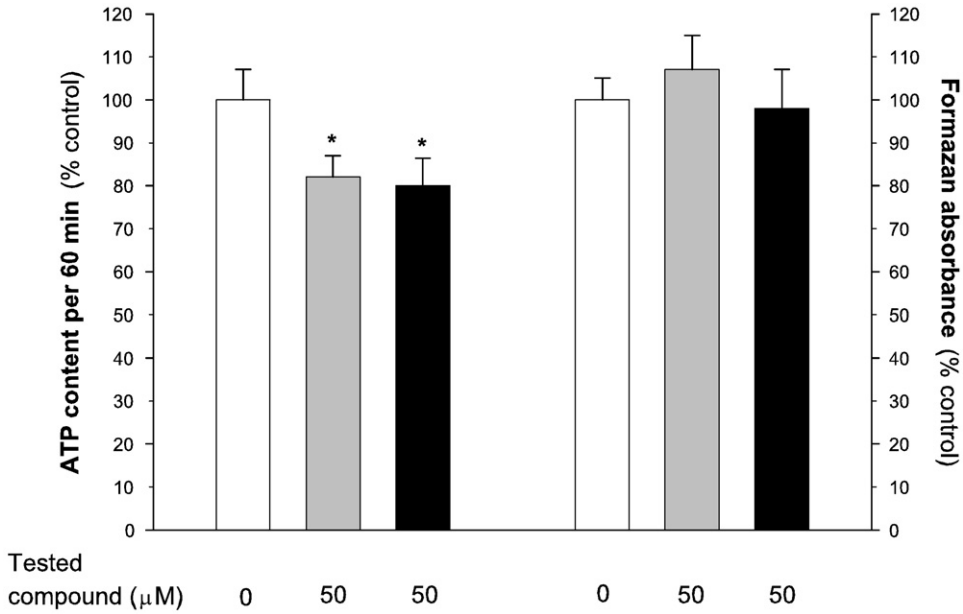

Fig. 4. The effect of preincubation with resveratrol (gray bars) or genistein (black bars) on ATP content (left bars) or formazan formation (right bars) in adipocytes. Cells were preincubated for 60 minutes without additives or with the tested compounds (50 μmol/L), and then all adipocytes were incubated for 60 minutes without tested compounds in the presence of 5 mmol/L glucose and 10 nmol/L insulin (ATP) or 5 mmol/L glucose and 0.5 mg/mL MTT (formazan). Values represent means ± SEM of 12 determinations from 3 separate experiments. *$P < .05$ vs incubations without tested compound (open bars).

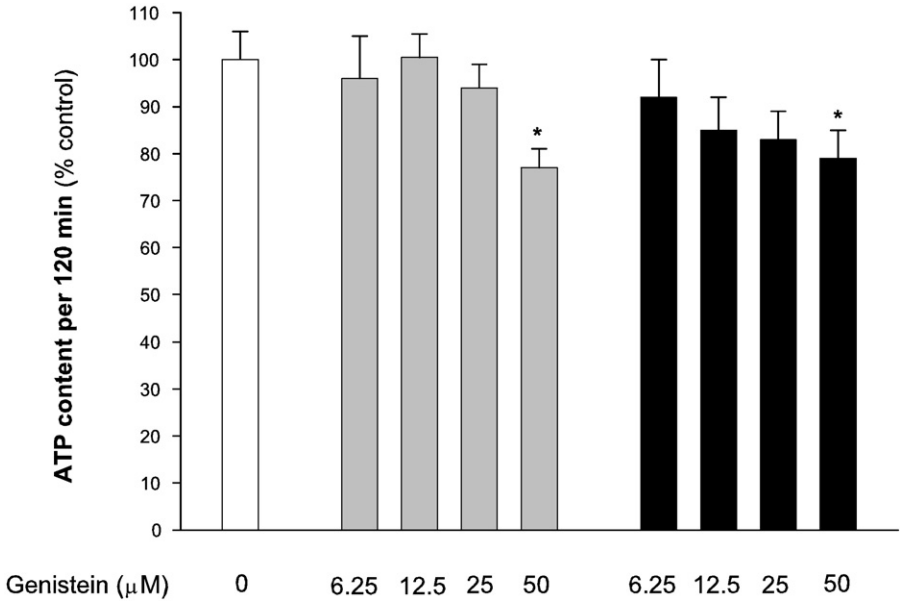

Fig. 5. The effect of genistein on ATP content in adipocytes incubated with 5 mmol/L glucose and 10 nmol/L insulin (gray bars) or 20 mmol/L glucose and 10 nmol/L insulin (black bars). Values represent means ± SEM of 12 determinations from 3 separate experiments. *$P < .05$ vs incubations without genistein (open bar).

As expected, adipocyte exposure to rotenone substantially reduced ATP in these cells. The comparison of the ATP-depleting effects of resveratrol and rotenone demonstrated that the effects of 25 μmol/L resveratrol and 2.5 μmol/L rotenone were similar (reduction by about 60%; data not shown).

3.2. The effect of genistein on ATP content in adipocytes

Compared with resveratrol, genistein appeared to be less effective as an ATP-reducing agent. Adipocyte exposure to 6.25, 12.5, or 25 μmol/L genistein failed to affect glucose-derived ATP concentrations. It was found that only 50 μmol/L genistein was able to significantly reduce ATP in fat cells. The influence of genistein was similar at low and high glucose (Fig. 5).

In the incubations with alanine, 6.25, 12.5, or 25 μmol/L genistein appeared to be ineffective, whereas 50 μmol/L genistein significantly diminished ATP reserves in adipocytes (Fig. 3).

Preincubations of adipocytes with 50 μmol/L genistein significantly reduced (by about 20%) ATP levels compared with cells preincubated in the buffer containing no genistein (Fig. 4).

3.3. The effect of resveratrol and genistein on insulin-induced glucose uptake by adipocytes

Adipocyte exposure to 6.25 μmol/L resveratrol failed to influence insulin-stimulated glucose uptake. Moreover, it was found that 12.5 μmol/L resveratrol tended to enhance glucose uptake; but the effect was not statistically significant. It was also observed that resveratrol present in the incubation medium at concentrations 25 and 50 μmol/L significantly potentiated insulin-stimulated uptake of glucose by isolated rat adipocytes (Table 1).

Table 1
The effect of resveratrol and genistein on glucose uptake and the effect of resveratrol on alanine oxidation in isolated adipocytes

Concentration of the tested compound (μmol/L)	0	6.25	12.5	25	50
Glucose uptake (nmol/10^6 cells/3 min)					
Resveratrol	3.65 ± 0.2	3.72 ± 0.3	4.23 ± 0.2	4.34 ± 0.3*	4.38 ± 0.3*
Genistein	3.54 ± 0.2	3.36 ± 0.3	3.71 ± 0.4	4.00 ± 0.3	3.72 ± 0.4
CO_2 formation from alanine (nmol/10^6 cells/120 min)					
Resveratrol	50.4 ± 3	46.1 ± 3	44.0 ± 3*	41.5 ± 2*	40.5 ± 3*

To determine the effect of resveratrol or genistein on glucose uptake, cells were preincubated for 10 minutes with 0.5 mmol/L glucose alone, glucose with resveratrol, or glucose with genistein. Afterward, 10 nmol/L insulin was added; and incubations were performed for 20 minutes. After this time, 1 μCi of 2-deoxy-D-[1-^{3}H]glucose was added; and adipocytes were incubated for 3 minutes. To determine the effect of resveratrol on alanine oxidation, cells were incubated with 5 mmol/L alanine, 0.25 μCi L-[U-^{14}C]alanine, and 10 nmol/L insulin without resveratrol or in the presence of this compound. After 2 hours, alanine-derived CO_2 was trapped; and its radioactivity was measured. Values represent means ± SEM of 12 determinations from 3 separate experiments.

* $P < .05$ vs incubations without tested compound.

Genistein at all concentrations used, that is, 6.25 to 50 μmol/L, failed to significantly affect insulin-induced glucose uptake by fat cells (Table 1).

3.4. The effect of resveratrol on alanine oxidation

Experiments with freshly isolated rat adipocytes demonstrated the inhibitory effect of resveratrol on alanine-derived CO_2 released by cells, indicating reduced oxidation of alanine. The inhibition of alanine oxidation was statistically significant when cells were exposed to 12.5, 25, and 50 μmol/L resveratrol (Table 1).

3.5. The effect of resveratrol and genistein on mitochondrial membrane potential

Adipocyte exposure to 20 mmol/L glucose and 10 nmol/L insulin substantially diminished rhodamine 123 fluorescence. However, in the presence of 50 μmol/L resveratrol or 50 μmol/L genistein, the decrease in rhodamine 123 fluorescence appeared to be significantly blunted. As expected, 10 μmol/L rotenone almost completely suppressed the decrease in rhodamine 123 fluorescence in adipocytes incubated with glucose and insulin (Fig. 6).

3.6. The effect of resveratrol and genistein on adipocyte viability

Adipocyte exposure for 2 hours to 50 μmol/L resveratrol or 50 μmol/L genistein had no significant influence on trypan blue uptake compared with cells incubated without these compounds (data not shown). Moreover, the absorbance of formazan was similar in experiments in which fat cells were preincubated with 50 μmol/L resveratrol or 50 μmol/L genistein compared with incubations without tested compounds (Fig. 4).

4. Discussion

In the present study, resveratrol was found to diminish ATP content in freshly isolated rat adipocytes. This effect was concentration dependent and appeared already at very low concentrations of resveratrol. It was also demonstrated that resveratrol-induced ATP depletion was not overcome by high glucose. These results indicate the potent ability of the tested compound to diminish ATP in adipose cells at a broad range of glucose concentrations. The potency of resveratrol to reduce ATP concentrations was very high indeed as evidenced in experiments with rotenone, a potent and specific inhibitor of mitochondrial electron transport. In these experiments, the effects of 25 μmol/L resveratrol on adipocyte ATP reserves were comparable to those caused by 2.5 μmol/L rotenone.

It is known that reduction of intracellular ATP may be due to decreased synthesis of ATP and/or its increased utilization. Experiments with isolated rat adipocytes demonstrated resveratrol-induced effects leading to decreased ATP synthesis. Because glucose is the main source of energy in adipocytes, the pivotal effect whereby resveratrol induces ATP depletion may be the inhibition of glucose transport and/or metabolism. Results obtained in the present study strongly point to the latter possibility because the effect of resveratrol on insulin-stimulated glucose uptake by

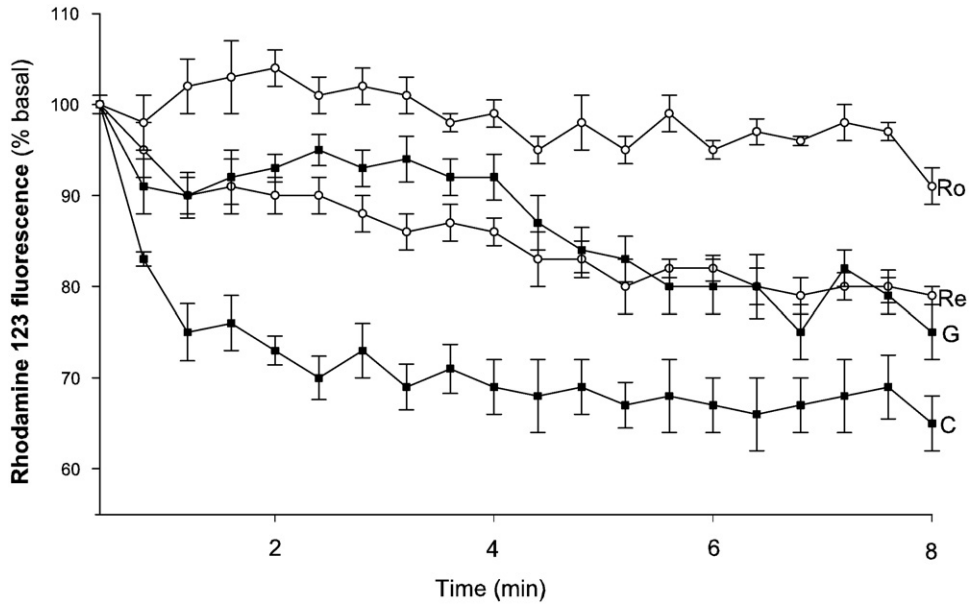

Fig. 6. The effect of resveratrol, genistein, and rotenone on mitochondrial membrane hyperpolarization. Adipocytes loaded with rhodamine 123 were exposed to 20 mmol/L glucose and 10 nmol/L insulin without the tested compounds (C) or in the presence of 50 μmol/L resveratrol (Re), 50 μmol/L genistein (G), or 10 μmol/L rotenone (Ro); and changes in rhodamine 123 fluorescence were measured. Values represent means ± SEM from 3 separate experiments. All values obtained in the presence of resveratrol, genistein, or rotenone are statistically significant ($P < .05$) vs incubations without these compounds.

adipocytes appeared to be potentiatory. Further evidence demonstrating the pivotal role of metabolic disturbances in the mechanism whereby resveratrol reduces ATP in adipocytes was provided by experiments in which glucose was replaced by alanine. It is known that ATP derived from glucose is produced by glycolysis and by mitochondrial metabolism of glycolytic products such as NADH and pyruvate, whereas in the presence of alanine, ATP is generated exclusively in the mitochondria. Therefore, in the case of adipocytes incubated with alanine, the potential inhibitory effects of the tested compound on glucose transport and/or glycolysis are bypassed. However, experiments using this amino acid revealed that resveratrol effectively diminished alanine-derived ATP. Importantly, our present studies also demonstrated reduced oxidation of alanine in adipocytes exposed to resveratrol. These outcomes clearly indicate that disturbances in mitochondrial metabolism are pivotal for resveratrol-induced ATP depletion in adipose cells. This assumption was confirmed by demonstration that resveratrol substantially attenuated hyperpolarization of the inner mitochondrial membrane in adipocytes incubated with glucose and insulin. Data from the literature also point to the influence of resveratrol on mitochondria. In our previous experiments on isolated rat adipocytes, resveratrol reduced secretion of leptin when this process was stimulated not only by insulin and glucose, but also by insulin and alanine [23]. In the other study, resveratrol inhibited the activity of ATP synthase and diminished oxygen consumption by mitochondria of rat brain and liver [54-56]. Moreover, in rat pancreatic islets, resveratrol exerted an insulin-suppressive effect when hormone secretion was induced by glucose or by mitochondrial fuels (leucine and glutamine); but insulin secretion stimulated without metabolic events was not affected by resveratrol [20,21]. Diminished oxidative metabolism of glucose in adipocytes exposed to resveratrol was also recently shown [45].

Numerous studies concerning the action of resveratrol in different kinds of cells focus rather on long-term effects such as induction of apoptosis, activation of sirtuins, and changes in gene expression [12,13]. However, our present study has provided convincing evidence that resveratrol is also able to exert short-term effects because a dramatic reduction in adipocyte ATP was noticed already within minutes.

Conversely to resveratrol, genistein used at similar concentrations appeared to be a weaker ATP-depleting agent because this compound reduced ATP only at the highest concentration used (50 μmol/L), whereas 6.25 to 25 μmol/L genistein was ineffective. These observations are in accord with previous results that demonstrated that both resveratrol and genistein are able to inhibit ATP synthase in rat brain and liver, but the potency of genistein is significantly lower compared with resveratrol [54]. Genistein was also found to inhibit basal and insulin-stimulated glucose transport in isolated adipocytes [57,58]. However, under our experimental conditions, insulin-stimulated glucose uptake by adipocytes was not significantly affected in the presence of genistein. This implies that other effects induced by the phytoestrogen are responsible for its ATP-depleting capacity. Because the reduction of ATP levels by 50 μmol/L genistein appeared to be similar in adipocytes incubated with glucose and alanine, changes in mitochondria are proposed to be responsible for this effect. This is supported by data demonstrating that 50 μmol/L genistein reduced glucose-induced hyperpolarization of the inner mitochondrial membrane in adipocytes. Moreover, Salvi et al [59] also revealed that 50 μmol/L genistein induced numerous changes in rat liver mitochondria.

In conclusion, results of the present study revealed the potent ability of resveratrol to decrease ATP content in rat adipocytes. It should be emphasized that this effect was noticed at very low concentrations of resveratrol. Genistein was also found to diminish ATP in fat cells; however, compared with resveratrol, genistein appeared to be much less effective. Despite the clear-cut differences between resveratrol and genistein in their potency to reduce ATP in adipose cells, the mechanism underlying this action seems to be similar for both tested compounds and is proposed to involve mitochondrial dysfunction, whereas the action via the inhibition of glucose transport and inhibition of glycolysis may be ruled out. Our experiments also revealed that preincubation of adipocytes with resveratrol or genistein slightly diminished ATP content despite the withdrawal of these compounds from the medium. This effect seems, however, not to be due to cell injury as demonstrated by MTT assay and trypan blue exclusion.

Because the majority of cellular processes are energy dependent, it can be hypothesized that the ATP-depleting effects of resveratrol and, to a lesser extent, genistein described in the present study have pleiotropic consequences for adipocyte functions and may change a number of adipocyte metabolic pathways—metabolism of glucose, lipid accumulation, fatty acids mobilization, insulin sensitivity, antilipolytic action of insulin, and adipokine secretion. Data from the literature confirm this assumption. It is known that the ATP to adenosine diphosphate ratio is a key regulatory factor of pyruvate carboxylase in mitochondrial matrix of white adipocytes [60,61]. Insufficient ATP decreases carboxylation of pyruvate leading to diminished fatty acid synthesis and, as a consequence, to reduced lipogenesis [60,62]. Both resveratrol and genistein were indeed reported as exerting antilipogenic effect [38,45], and depletion of ATP could be one of the reasons of this effect. In the case of resveratrol, there is another reason of decreased lipogenesis that may be connected with ATP. Resveratrol is an activator of adenosine monophosphate–activated kinase (AMPK), the enzyme that is a cellular sensor of energy and regulates energy balance [63,64]. The resveratrol-induced activation of AMPK may be direct or may be mediated by sirtuins [65,66]. When activated, AMPK switches off ATP-consuming processes, for example, lipogenesis [67].

Lipolysis is also known to be dependent on energy status in adipocytes. A decrease of ATP inhibits lipolysis stimulated by catecholamines [68]. On the other hand, the high rate of lipolysis and elevation of fatty acids in adipocytes act as mitochondrial uncouplers and decrease energy production [69]. Moreover, fatty acids are also partly reactivated into acyl–coenzyme A; and this process is also ATP dependent. In our previous experiments, resveratrol and genistein appeared to increase epinephrine-stimulated lipolysis [38,45]. Thus, the regulation of lipolysis by these phytochemicals seems to be more complex' and ATP depletion is not the key reason for their lipolytic activity.

The effect of resveratrol on ATP depletion in our experiment was very strong and appeared just after 15 minutes of incubation in the presence of glucose and insulin. In the other experiments [70,71], insulin signaling in fat cells was strongly dependent on intracellular ATP content. The 60% to 80% decrease in ATP content reduced insulin functions and impaired all insulin signaling proteins (IRS1/2, PI-3K, Akt1) as well as glucose transport system [70]. In our previous experiments, resveratrol and genistein counteracted the antilipolytic action of insulin in adipocytes [39,45], the process that may be limited by intracellular ATP because of impaired insulin signaling [72]. However, the mechanisms of this effect were probably different for both compounds; and the role of the ATP depletion in the attenuation of the antilipolytic action of insulin is not proven.

The reduced ATP level found in the presence of resveratrol and genistein may also influence the secretion of some adipokines because secretion of some adipokines was reported to be reduced by ATP-depleting agents [73]. In our previous study, genistein and resveratrol indeed decreased leptin secretion from isolated rat adipocytes; and this effect was proposed to be mainly due to diminished production of energy [23,33].

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