Resveratrol regulates human adipocyte number and function in a Sirt1-dependent manner¹⁻³

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ABSTRACT

Background: Caloric restriction leads to retardation of the aging processes and to longer life in many organisms. This effect of caloric restriction can be mimicked by resveratrol, a natural plant product present in grapes and red wine, which is known as a potent activator of sirtuin 1 (silent mating type information regulation 2 homolog 1 (Sirt1)).

Objectives: One main effect of caloric restriction in mammals is a reduction of body fat from white adipose tissue. We sought to identify the effects of resveratrol on fat cell biology and to elucidate whether Sirt1 is involved in resveratrol-mediated changes.

Design: Human Simpson-Golabi-Behmel syndrome preadipocytes and adipocytes were used to study proliferation, adipogenic differentiation, glucose uptake, de novo lipogenesis, and adipokine secretion. Sirt1-deficient human preadipocytes were generated by using a lentiviral small hairpin RNA system to study the role of Sirt1 in resveratrol-mediated changes.

Results: Resveratrol inhibited preadipocyte proliferation and adipogenic differentiation in a Sirt1-dependent manner. In human adipocytes, resveratrol stimulated basal and insulin-stimulated glucose uptake. De novo lipogenesis was inhibited in parallel with a down-regulation of lipogenic gene expression. Furthermore, resveratrol down-regulated the expression and secretion of interleukin-6 and interleukin-8. Sirt1 was only partially responsible for the regulation of resveratrol-mediated changes in adipokine secretion.

Conclusions: Taken together, our data suggest that resveratrol influences adipose tissue mass and function in a way that may positively interfere with the development of obesity-related comorbidities. Thus, our findings open up the new perspective that resveratrol-induced intracellular pathways could be a target for prevention or treatment of obesity-associated endocrine and metabolic adverse effects. Am J Clin Nutr 2010;92:5–15.

INTRODUCTION

Obesity is one of the main public health problems in developed countries. The abnormal increase in body fat is accompanied by severe health problems resulting in an increased morbidity and mortality (1). Via its secretion products, adipose tissue itself participates in the development of obesity-related disorders, which include components of the metabolic syndrome such as cardiovascular disease, insulin resistance, and type 2 diabetes. However, the risk of other age-related diseases such as cancer and inflammatory disorders also increases with obesity. It is well established that weight loss in obese patients will lead to a significant amelioration in obesity-related disorders (2). Energy homeostasis is regulated by the balance between caloric intake and expenditure. The reduction of caloric intake induces weight loss. Calorie restriction has been shown to delay age-related diseases and to extend life span in numerous species including mammals (3–5). It has long been assumed that the extension of life span by calorie restriction is mediated in a passive way by lowering oxidative stress, DNA damage, and apoptosis (6). Ongoing studies now show that the response to calorie restriction is a highly regulated process, and Sir2 has been identified as the key molecular player (7).

In higher organisms, calorie restriction results in a reduction of body fat from white adipose tissue (WAT), an increase in insulin sensitivity, and a decrease in body temperature (summarized in ref. 8). However, WAT size seems to be a major factor affecting life span because genetically engineered mice with reduced WAT display an increased longevity (9). Adipose tissue is an endocrine organ, and the life-extending effect of reduced WAT may be related to changes in hormones that are secreted from WAT in proportion to fat mass.

A certain natural plant product, resveratrol, can mimic the effect of calorie restriction (10, 11). Resveratrol is a sirtuin-activating compound that naturally occurs in grapes and red wine (12). Resveratrol may protect against diet-induced obesity and...
by activating sirtuin 1 [silent mating type information regulation 2 homolog 1 (Sir2)], the mammalian homolog of yeast Sir2 (13, 14). Likewise, transgenic mice overexpressing Sirt1 are protected from metabolic damages induced by a high-fat diet (15, 16), which implicates Sirt1-based therapies of obesity-related disorders. Indeed, small molecule activators of Sirt1 have been obtained from metabolic diseases such as hepatic steatosis and insulin resistance by activating sirtuin 1 [silent mating type information regulation 2 homolog 1 (Sirt1)], the mammalian homolog of yeast Sir2 (13, 14). Likewise, transgenic mice overexpressing Sirt1 are protected from metabolic damages induced by a high-fat diet (15, 16), which implicates Sirt1-based therapies of obesity-related disorders. Indeed, small molecule activators of Sirt1 have been obtained from metabolic diseases such as hepatic steatosis and insulin resistance. Picard et al (8) showed in mice that Sirt1 promotes fat mobilization in white adipocytes by repressing the peroxisome proliferator-activated receptor (PPARγ). Sirt1 is expressed in human white adipose tissue, and its expression is up-regulated by caloric restriction (19).

We hypothesized that resveratrol might directly act on adipose tissue by several mechanisms affecting fat cell differentiation, fat cell metabolism, or secretion profile. Using human preadipocytes and adipocytes, we therefore studied the effects of resveratrol on proliferation, differentiation, glucose uptake, de novo lipogenesis, and adipokine secretion.

MATERIALS AND METHODS

Materials

Cell culture medium, fetal bovine serum, and antibiotics were obtained from Life Technologies (Karlsruhe, Germany). Rosiglitazone (BRL 49653) was a kind gift from Smith Kline Beecham (London, United Kingdom). Recombinant human insulin was kindly provided by Novo Nordisk (Gentofte, Denmark). [Methyl-3H]-thymidine was purchased from Amersham Life Sciences (Buckinghamshire, United Kingdom). Other chemicals and reagents were purchased from Sigma Chemical (Taufkirchen, Germany).

Cell culture and lentiviral transduction

Human Simpson-Golabi-Behmel syndrome (SGBS) preadipocytes were used as a model system. SGBS cells are characterized by a high capacity for adipogenic differentiation in vitro and functionally behave like human primary adipocytes (20). They were cultured in DMEM/Ham’s F12 (1:1) containing 33 μmol/L biotin, 17 μmol/L pantothenate, antibiotics (serum-free, basal medium), and 10% FBS as described earlier (20).

A specific small hairpin RNA (shRNA)-targeting Sirt1 sequence at 1395 5′-gaagttgacctcctcattg-3′ (21) and a nonsense sequence, which was directed against a sequence with no corresponding part in the human-genome (5′-gatcgttagatgcctcagg-3′) were cloned into the pENTR/H1/TO entry vector by using the BLOCK-IT Inducible H1 RNAi Entry Vector Kit (Invitrogen, Karlsruhe, Germany) and subsequently transferred into the pLenti4/BLOCK-IT-DEST expression vector by targeted recombination by using the BLOCK-IT Inducible H1 Lentiviral RNAi System (Invitrogen) according to the manufacturer’s protocol. The pLenti expression constructs and pLenti-enhanced green fluorescent protein (Invitrogen) were cotransfected with packaging plasmids by Lipofectamine 2000 (Invitrogen) in the 293FT producer cell line by using ViraPower Lentiviral Expression Systems (Invitrogen) according to the manufacturer’s protocols. Three days after transfection, the viral supernatant fluid was harvested, filtered through a 0.45-μmol/L polyvinylidene fluoride filter, and stored at −80°C. SGBS cells were infected at MOI = 1 in the presence of 1 μg/mL polybrene. Fluorescence microscopy and flow cytometry of SGBS cells infected with pLenti-enhanced green fluorescent protein was performed 72 h postinfection. Stable bulk cultures were generated by selection with 0.2 mg/mL zeocin for 14 d, and down-regulation of Sirt1 protein was controlled by Western blot. Experiments were performed with cells from 3 independent lentiviral infections.

Proliferation assay

[3H]-thymidine incorporation

Preadipocytes were seeded in 24-well plates at low density (3.3 × 10^3 Cells/cm²). Cells were incubated for 60 h in basal medium with 10% FCS with either vehicle (dimethyl sulfoxide), maximal dose corresponding to 100 μmol/L resveratrol) or increasing doses of resveratrol. [3H]-thymidine (1 μCi/well) was added for another 12 h. After harvesting cells, incorporation of [3H]-thymidine into DNA was measured with a β-counter. Three independent experiments were each performed in triplicate.

Microscopic cell counting

Preadipocytes were seeded in 12-well plates at low density (3.3 × 10^3 Cells/cm²). Cells were treated with vehicle or increasing doses of resveratrol. After 24, 48, and 72 h, the number of adherent cells was determined by direct counting by using a net micrometer. Three independent experiments were performed in triplicate.

Differentiation assay

Induction of adipose differentiation

Adipogenic differentiation was induced after reaching near confluence. Unless otherwise noted, cells were washed 3 times with PBS and cultured in serum-free basal medium supplemented with 10 μg iron-poor transferrin/mL, 10 nM insulin, 200 pmol triiodothyronine/L, and 0.1 μmol/L cortisol. For the first 4 days, 2 μmol/L rosiglitazone (BRL 49653), 250 μmol/L isobutyloxanthine, and 25 nL dexamethasone were added. The medium was changed every 4 d. Morphologically differentiated adipocytes were obtained after 14 d. The number of differentiated cells was estimated in the monolayers by direct counting by using a net micrometer. Three independent experiments were performed in triplicate.

Oil red O staining

After washing cells twice with PBS and fixation in 10% formalin for 2 h at 4°C, cultures were incubated with oil red O staining solution (0.5% in 60% isopropanol) for 1 h at room temperature. Cells were washed twice with water to remove unbound dye, and images were captured with a CC12 digital camera (Soft Imaging System, Münster, Germany) by using AnalySIS 3.1 Software (Soft Imaging System).
Glucose uptake

After washing twice with PBS, in vitro-differentiated SGBS adipocytes on day 10 of adipogenic differentiation (differentiation rate >80%) were incubated in serum-free medium for 24 h with increasing doses of resveratrol. Cells were stimulated with 10^{-10} \text{mol/L} insulin for 15 min, and then 2-deoxy-D-[14C]-glucose was added for another 15 min. Afterward, cells were washed twice in ice-cold PBS and harvested with 100 \text{mmol NaOH/L}. Incorporation of 2-deoxy-D-[14C]-glucose was measured on a β-counter. Three independent experiments were performed in triplicate.

Insulin-stimulated lipogenesis

After washing twice with PBS, in vitro-differentiated SGBS adipocytes on day 10 of adipogenic differentiation (differentiation rate >80%) were incubated for 24 h in serum-free basal medium supplemented with increasing doses of resveratrol. Insulin and D-[14C]-glucose (0.1 μCi/well) was added for another 16 h. Cells were washed twice in ice-cold PBS and harvested with 100 mmol NaOH/L. Cellular lipids were extracted by adding a scintillation cocktail. Incorporation of D-[14C]-glucose into lipids was measured on a β-counter. Three independent experiments were performed in triplicate.

Sirt1 activity assay

Cells were washed twice with PBS and incubated for 2 h in serum-free basal medium supplemented with resveratrol or vehicle control. The “Fluor de Lys” Sirt1 activity assay (Enzo Life Sciences, Lörrach, Germany) was performed according to the manufacturer’s protocol.

Reverse transcriptase–polymerase chain reaction

Total RNA was prepared at different time points during adipogenic differentiation (4 independent experiments) or from preadipocytes and adipocytes treated with resveratrol by using RNeasy Lipid tissue kit (3 independent experiments) (Qiagen, Hilden, Germany). cDNA synthesis and block polymerase chain reaction (PCR) was performed as described previously (22).

Quantitative PCR (qPCR) on specific genes including interleukin-6 (IL-6), interleukin-8 (IL-8), Sirt1, PPARγ, Glut-4, fatty acid synthase (FASN), and acetyl-CoA carboxylase (ACC) was carried out on a Roche lightcycler. The mRNA amounts of these genes were normalized to those of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) by using the ΔCt method. Fold changes of gene expression were calculated by the 2^{-ΔΔCt} method. The sequences of gene-specific primers are available on request.

Measurement of cytokines in medium supernatant fluid

Secretion of IL-6 and IL-8 in medium supernatant fluid (n = 3 performed in triplicate) was measured by commercial enzyme-linked immunosorbent assays. Sensitivity for the IL-6 assay (Biosource, Nivelles, Belgium) was 2 pg/mL with an inter-/intraassay variability <8%/<6%. Sensitivity for the IL-8 assay (Biosource) was 0.7 pg/mL with an inter-/intraassay variability of <4.3%/<4.9%.

Western blot analysis

Western blot analysis was performed as described previously (23) by using rabbit PPARγ monoclonal antibody (1:1000, New England Biolabs, Frankfurt, Germany), rabbit FASN monoclonal antibody (1:1000, New England Biolabs), mouse GLUT4 monoclonal antibody (1:200; R&D Systems, Wiesbaden, Germany), mouse Sirt1 monoclonal antibody (1:500; Upstate, Lake Placid, NY), and mouse anti-β-actin monoclonal antibody (1:5000; Sigma, Steinheim, Germany) followed by goat anti-mouse IgG or goat anti-rabbit IgG conjugated to horseradish peroxidase (1:5000; Santa Cruz Biotechnology, Santa Cruz, CA). Enhanced chemiluminescence was used for detection (Amersham Bioscience, Freiburg, Germany). All Western blots shown are representative of at least 3 independent experiments.

Statistical analysis

If not otherwise stated, data from 3 independent triplicate experiments were expressed as means ± SDs. For statistical comparison, Student’s 2-tailed paired t test was used (Winstat, version 2006.1, Springer Germany, Heidelberg, Germany). P < 0.05 was considered statistically significant in comparison to the respective control cells.

RESULTS

Resveratrol inhibits human preadipocyte proliferation

To investigate the influence of resveratrol on preadipocyte proliferation, we grew human SGBS preadipocytes in serum containing basal medium supplemented with increasing doses of resveratrol (Figure 1A). Microscopic counting of adherent cells revealed a dose-dependent, robust inhibitory effect of resveratrol on preadipocyte proliferation (Figure 1A). After 4 d, the number of adherent cells increased by ≈3.8-fold in medium or vehicle (dimethyl sulfoxide)-treated cells, whereas only a ≈1.6-fold increase in cell number was observed in cultures treated with 100 μmol/L resveratrol. In parallel, resveratrol profoundly inhibited incorporation of [3H]-thymidine into DNA (Figure 1B).

The strong effect of resveratrol on [3H]-thymidine incorporation suggested a loss of cells by apoptosis. However, flow cytometry measuring the content of hypodiploid DNA revealed that there is only very low induction of apoptosis by resveratrol under serum-containing conditions (data not shown).

Resveratrol inhibits adipogenic differentiation

The effect of resveratrol on adipogenic differentiation was assessed by morphologic counting of lipid-laden cells (Figure 2). Preadipocytes were treated with increasing doses of resveratrol for the first 4 d of adipogenic differentiation. In cultures treated with vehicle alone, ≈95% of cells underwent adipogenic differentiation as assessed by staining intracellular lipids with oil red O on day 14 of adipogenic differentiation (Figure 2A). An inhibition of adipogenic differentiation was observed at concentrations >10 μmol/L (≈6%) with a maximal inhibitory effect at 100 μmol/L by ≈45% (Figure 2B). These findings were further confirmed by measuring the incorporation of the lipophilic dye Nile red into cellular lipids (Figure 2C). The morphologic observations were accompanied by changes in gene expression...
Resveratrol stimulates basal and insulin-stimulated glucose uptake but inhibits de novo lipogenesis

We then studied whether resveratrol influences important metabolic pathways such as glucose uptake and lipogenesis in mature, lipid-laden fat cells (Figure 3). After pretreatment with resveratrol for 24 h, incorporation of radioactively labeled deoxy-glucose was measured basally or after stimulation with insulin for 30 min (Figure 3A). Basal glucose uptake was stimulated by \( \approx 1.5 \)-fold at 10 \( \mu \text{mol/L} \) compared with vehicle-treated cells. This was comparable to stimulation with \( 10^{-6} \text{ mol/L} \) insulin, which stimulated glucose uptake by \( \approx 1.6 \)-fold. Pretreatment with resveratrol significantly enhanced insulin-stimulated glucose uptake from \( \approx 1.5 \)- to \( \approx 2.1 \)-fold at 10 \( \mu \text{mol/L} \). For studying de novo lipogenesis, fat cells were pretreated with resveratrol for 24 h, and incorporation of radioactively labeled glucose into cellular lipids was stimulated with insulin for another 16 h. At stimulation with \( 10^{-8} \text{ mol/L} \) of insulin, an inhibition of lipogenesis by resveratrol was observed at 50 \( \mu \text{mol/L} \) (inhibition by \( \approx 25\% \)) (Figure 3B). Basal lipogenesis was not affected by resveratrol treatment. The inhibition of lipogenesis might by due to changes in expression of genes involved in lipogenesis. In fact, after incubation of adipocytes with resveratrol for 24 h, the lipogenic genes Glut-4, FASN, and ACC were significantly down-regulated (Figure 3C).

Resveratrol influences the secretion profile of preadipocytes

Adipose tissue is now well recognized as an endocrine organ. It secretes a wide range of different factors that might contribute to the development of obesity-related disorders, particularly type 2 diabetes and cardiovascular disease. Serum concentrations of inflammatory cytokines such as IL-6 or IL-8 are increased in obesity and contribute to the development of its comorbidities (24). In human SGBS cells, IL-6 and IL-8 mRNA is most abundant in the preadipocyte state and is down-regulated by 80% during the process of adipogenic differentiation (Figure 4A and B). We studied whether resveratrol influences the expression and secretion of both interleukins in preadipocytes (Figure 4). Resveratrol treatment of preadipocytes for 24 h resulted in a robust, dose-dependent down-regulation of IL-6 and IL-8 mRNA (Figure 4, C and D). In parallel, a significant, dose-dependent reduction of IL-6 and IL-8 protein was observed in the culture medium. At 100 \( \mu \text{mol/L} \) for 24 h, IL-6 secretion into the culture medium was inhibited by \( \approx 72\% \); IL-8 secretion was inhibited by \( \approx 80\% \) (Figure 4, E and F).

Expression of Sirt1 during adipogenic differentiation and generation of Sirt1-deficient preadipocytes

Resveratrol is known as a potent sirtuin-activating compound. In SGBS cells, Sirt1 is expressed in preadipocytes and further up-regulated during adipogenic differentiation (Figure 5A). To clarify whether Sirt1 mediates the above-described effects of resveratrol, we decided to target Sirt expression by an RNAi approach. To this end, we established a lentiviral system that allows the stable expression of shRNA in human preadipocytes. To clarify whether Sirt1 mediates the above-described effects of resveratrol, we decided to target Sirt expression by an RNAi approach. To this end, we established a lentiviral system that allows the stable expression of shRNA in human preadipocytes. We were able to transduce \( >90\% \) of the preadipocytes as shown by enhanced green fluorescent protein expression by using fluorescence microscopy and flow cytometry (Figure 5B). We then developed a lentiviral shRNA vector targeting Sirt mRNA at position 1347 (21). We used a nonsense RNAi sequence, which was directed against a sequence with no corresponding part in the human genome (nonsense shRNA) to control for the unspecific effects of shRNA. Infection of SGBS preadipocytes with the lentiviral construct containing shRNA-targeting Sirt1 resulted in undetectable levels of Sirt1 protein (Figure 5C).
Activation of Sirt1 by resveratrol was completely abolished in these cells as shown by a Sirt1 enzymatic activity assay (Figure 5D).

Resveratrol inhibits adipogenic differentiation and proliferation in a Sirt1-dependent manner

Picard et al (8) showed in 3T3-L1 cells that resveratrol inhibits adipogenic differentiation in a Sirt1-dependent manner. In human SGBS cells, 50 μmol/L resveratrol inhibited adipogenic differentiation in nonsense shRNA-transduced cells by ≈50%. In contrast, resveratrol had no significant effect on adipogenic differentiation in preadipocytes stably expressing Sirt1 shRNA (Figure 6, A–C). Next we investigated the effect of resveratrol on proliferation in these cells. Treatment with 50 μmol/L resveratrol inhibited the proliferation of nonsense shRNA-expressing cells by ≈80%. In contrast, there was no significant reduction of proliferation in preadipocytes stably expressing Sirt1 shRNA (Figure 6D).

Role of Sirt1 in resveratrol-stimulated metabolic and endocrine changes

In 3T3-L1 adipocytes, resveratrol stimulated the release of fatty acids on β-adrenergic stimulation, which was completely blunted by Sirt1 small interfering RNA (8). However, the role of Sirt1 for resveratrol-induced changes on glucose uptake and on de novo synthesis and storage of triglycerides has not been addressed. Resveratrol (50 μmol/L) induced an increase in basal glucose uptake in nonsense shRNA-expressing control cells, whereas there was no such stimulatory effect that was detectable in adipocytes deficient in Sirt1 (Figure 6E). In control cells,
Resveratrol treatment resulted in an increased insulin-stimulated glucose uptake. There was no significant increase of insulin-stimulated glucose uptake on Sirt1 knockdown.

The inhibition of lipogenesis by resveratrol was comparable in nontransduced and nonsense shRNA-expressing cells (parental, 25% compared with nonsense shRNA, 30%). The knockdown of Sirt1 partially abolished the inhibitory action of resveratrol (Figure 6F).

Finally, we studied the influence of Sirt1 knockdown on the expression of IL-6 and IL-8. In control cells (nonsense shRNA), resveratrol inhibited mRNA expression of IL-6 by 63%, and 50% of this effect was abolished by Sirt1 knockdown (Figure 6G). IL-8 mRNA expression was inhibited by 61% in control cells. Sirt1 deficiency was able to reverse this inhibition by 80% (Figure 6H).

DISCUSSION

Resveratrol first gained considerable attention because of its potential chemopreventive and anticancer properties as well as its antiinflammatory and cardioprotective effects (25, 26). In 2003, Howitz et al (10) showed that resveratrol mimics the effect of calorie restriction in yeast, which results in an extension of life span. Resveratrol has since been shown to promote longevity in many evolutionarily distant species, which include Caenorhabditis elegans, Drosophila melanogaster, and the vertebrate fish Nothobranchius furzeri (10, 11, 13, 27, 28). A recent study revealed that resveratrol improves survival in mice as well (13). According to the earlier findings that resveratrol mimics calorie restriction, the data from Baur et al (13) suggest that resveratrol shifts the physiology of mice on a high-calorie diet toward that of mice on a standard diet.

One of the main effects of calorie restriction in higher organisms is a reduction of body fat from WAT. Therefore, we investigated the biological effects of resveratrol on fat cell biology. Human SGBS cells were used as a model system. These cells are characterized by a capacity for adipogenic differentiation, and they behave like human primary adipocytes (29). Adipose tissue size is determined by both the volume and the number of adipocytes (24, 30). The number of adipocytes may increase due to proliferation of preadipocytes and subsequent adipogenic differentiation. We show here that resveratrol strongly inhibits human preadipocyte proliferation (>10 μmol/L) (Figure 1). This was already shown in other cell types, which include vascular smooth muscle cells (31), cardiac fibroblasts (32), and several malignant cell lines (33–36). In some cell types, resveratrol not only inhibited proliferation, but also induced apoptosis (31, 33, 36). In our studies, induction of apoptosis under serum-containing conditions was negligible. Thus, we conclude that the inhibition of 3H-thymidine incorporation is due to an
inhibition of cell replication rather than due to an additional loss of cells by apoptosis.

As described above, resveratrol is a potent activator of Sirt1 (10). Thus, we studied whether the effect of resveratrol on human preadipocyte proliferation is mediated by Sirt1. For this purpose, we generated human SGBS preadipocytes stably expressing Sirt1 shRNA. We achieved an almost complete knockdown of Sirt1 protein (Figure 5). In nonsense shRNA expressing control cells, proliferation was inhibited by treatment with resveratrol. Knockdown of Sirt1 almost completely abolished the inhibitory effect of resveratrol. Some members of the Sir2 family of proteins have been implicated in the regulation of proliferation and cell cycle progression (37, 38). We show here that Sirt1 is responsible for resveratrol-induced inhibition of proliferation in human preadipocytes.

Next we showed that resveratrol inhibited adipogenic differentiation in human preadipocytes (Figure 2). Thus resveratrol inhibited differentiation in murine cell lines (39–43). The inhibitory effect of resveratrol was abrogated by knockdown of Sirt1 in an RNAi approach, which clearly shows that the effect of resveratrol is mediated by Sirt1.

Picard et al (8) showed that Sirt1 attenuates adipogenesis by repressing PPARγ. PPARγ is a key element in the differentiation of precursor cells into adipocytes (44). In SGBS cells, expression of PPARγ is induced during the first few days of differentiation (20). Furthermore, adipogenic differentiation of this human preadipocyte cell strain is dependent on PPARγ activation, which is achieved by the use of a thiazolidindione, ie, rosiglitazone (20).

Treatment with resveratrol caused a down-regulation of PPARγ expression. In accordance with earlier findings, resveratrol treatment for the first 4 d of the differentiation procedure was sufficient to markedly inhibit adipogenic differentiation (Figure 2). This suggests an involvement of PPARγ downstream of Sirt1 in our human system (8). Knockdown of Sirt1 did not affect adipogenic differentiation capacity per se because differentiation rates were comparable in

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**FIGURE 4.** Resveratrol differentially regulates secretion of adipokines. A and B: Adipogenic differentiation was induced in Simpson-Golabi-Behmel syndrome (SGBS) preadipocytes. On days 1, 4, 7, 11, and 14, total RNA was prepared and reversely transcribed. Quantitative polymerase chain reaction (qPCR) was performed by using specific primer pairs for interleukin (IL)-6 and IL-8, and results were standardized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Data are expressed relative to GAPDH as means ± SDs (n = 4). *P < 0.01 (differentiated compared with undifferentiated control). C and D: SGBS preadipocytes (day 0) were incubated with increasing doses of resveratrol. After 24 h, mRNA expression of IL-6 (C) and IL-8 (D) was measured by qPCR. Data are expressed relative to GAPDH as means ± SDs (n = 3). *P < 0.02 (resveratrol compared with vehicle). Secretion of IL-6 (E) and IL-8 (F) into the culture medium was measured by enzyme-linked immunosorbent assays. Data are expressed as means ± SDs (n = 3 independent experiments each performed in triplicate). *P < 0.02 (resveratrol compared with vehicle).
Sirt1 shRNA, nonsense shRNA-expressing cells, and uninfected SGBS preadipocytes. Activation of Sirt1 decreased adipocyte differentiation in 3T3-L1 cells (8), which points to a role of Sirt1 in determining the lineage of mesenchymal stem cells. Accordingly, overexpression of Sirt1 attenuated adipogenic differentiation in 3T3-L1 cells (8). Another important factor that influences adipose tissue size is the volume of fat cells. Depending on whole-body energy balance, the volume of fat cells is determined by the competing processes of lipolysis and lipogenesis.

Resveratrol stimulated lipolysis and the release of free fatty acids in 3T3-L1 adipocytes in a Sirt1-dependent manner (8). We show here that resveratrol increased basal- and insulin-stimulated glucose uptake in human adipocytes. This finding is in agreement with several other studies showing that this phytoalexin stimulated basal- and insulin-stimulated glucose uptake in muscle cells (46–48). Interestingly, resveratrol inhibited insulin-stimulated de novo lipogenesis. This effect was accompanied by the down-regulation of key lipogenic molecules, such as Glut-4, FASN, and ACC (Figure 3). At first glance, stimulation of glucose uptake and parallel inhibition of lipogenesis seems contradictory because they are normally regulated coordinately. A differential regulation of these 2 pathways was described in brown adipocytes. Here, rosiglitazone stimulated de novo lipogenesis without altering glucose uptake (49). Resveratrol may act on different levels of cell signaling. It is conceivable that resveratrol influences glucose transport by stimulating GLUT4 transporter intrinsic activity (46). Furthermore, Deng et al (48) showed that resveratrol stimulation of muscular glucose uptake is mediated by Erk/p38 activation in the early phase and is shifted to p38/P38K activation in the late phase. On the other hand, our data suggest that the effects of resveratrol on lipogenesis are mediated by reducing the expression of lipogenic genes.

Stimulation of lipolysis (8) and the inhibition of lipogenesis suggest that resveratrol mimics a situation similar to caloric restriction in our human adipocyte model system. It mimics a shift from an anabolic situation, in which excess energy is stored, to a catabolic situation in which storage of energy in fat cells is prevented and mobilization of energy is triggered. The detected inhibition of adipogenic differentiation by resveratrol also fits in this model because the de novo recruitment of adipocytes from precursor cells would be reasonable only in a situation in which additional fat cells for the storage of excess energy are needed.

Resveratrol has been implicated in the so-called French paradox (50, 51) —that is, the lower prevalence of cardiovascular diseases in France compared with other central European countries due to a moderate consumption of red wine. In fact, prospective cohort studies show a significant inverse association between flavonoid consumption and cardiovascular risk (52). Likewise, cardiovascular risk is lowered by weight loss (2). Most importantly, a reduction in body fat normalizes the secretion pattern of hormones and other factors from adipose tissue, which thereby improves obesity-associated diseases such as the metabolic syndrome (2).

This led us to the hypothesis that resveratrol may exert its positive effects by acting on adipose tissue endocrine function. We show here that resveratrol regulates the expression and secretion of adipokines (Figure 4). We detected a robust and significant down-regulation of IL-6 and IL-8 from preadipocytes.

**FIGURE 5.** Expression of sirtuin 1 [silent mating type information regulation 2 homolog 1 (Sirt1)] during adipogenic differentiation and successful knockdown of Sirt1 by using lentiviral small hairpin RNA (shRNA). A: Adipogenic differentiation was induced in Simpson-Golabi-Behmel syndrome (SGBS) preadipocytes. On days 1, 4, 7, 11, and 14, total RNA was prepared and reverse-transcribed. Quantitative polymerase chain reaction was performed by using specific primer pairs for Sirt1, and results were standardized to glyceraldehyde 3-phosphate dehydrogenase. Data are expressed as means ± SDs (n = 3 independent experiments). *P < 0.02 (expression during adipogenic differentiation compared with undifferentiated cells). B: SGBS preadipocytes were infected with pLenti-enhanced green fluorescent protein (EGFP) as a positive control for transduction or left untreated. After 72 h, cells were analyzed by fluorescence microscopy (magnification ×100) and flow cytometry. One representative experiment of 3 performed is shown. C: SGBS preadipocytes were infected with lentiviral shRNA vectors containing either nonsense shRNA or a Sirt1 RNAi sequence. Protein expression of Sirt1 and α-tubulin in stable bulk cultures was assessed by Western blot. One representative experiment of 3 performed is shown. D: SGBS preadipocytes stably expressing nonsense shRNA or Sirt1 shRNA were stimulated with vehicle or 100 μmol/L resveratrol for 2 h. Sirt1 activity was determined by Fluor de Lys assay. Data are expressed as means ± SDs (n = 3 independent experiments each performed in triplicate). *P < 0.05 (resveratrol compared with vehicle). kDa, kiloDalton.
FIGURE 6. Sirtuin 1 [silent mating type information regulation 2 homolog 1 (Sirt1)] is involved in resveratrol-mediated changes in human preadipocytes and adipocytes. Simpson-Golabi-Behmel syndrome (SGBS) preadipocytes stably expressing nonsense small hairpin RNA (shRNA) or Sirt1 shRNA were studied in terms of adipogenic differentiation (A–C), preadipocyte proliferation (D), glucose uptake (E), de novo lipogenesis (F), and cytokine expression (G and H). A–C: Cells were treated with vehicle or 50 μmol/L resveratrol for the first 4 d of adipogenic differentiation. A: On day 14, cultures were stained with oil red O. B: Rate of adipogenic differentiation was determined as described in Materials and Methods. *P < 0.01 (resveratrol compared with vehicle). C: Lipid content was determined on day 10 with Nile Red. *P < 0.001 (resveratrol compared with vehicle). D: Cells were incubated in serum-containing medium with either vehicle or 50 μmol/L resveratrol. [3H]-Thymidine incorporation into DNA was measured after 72 h. Data are shown as the percentage of untreated control. *P < 0.001 (resveratrol compared with vehicle). E: SGBS adipocytes stably expressing nonsense shRNA or Sirt1 shRNA were incubated for 24 h with vehicle or 50 μmol/L resveratrol. Basal and insulin-stimulated (10^{-8} mol/L) glucose uptake was measured by incorporation of 2-deoxy-D-[14C]-glucose after stimulation with insulin for 30 min. *P < 0.01 (insulin-stimulated compared with vehicle control); **P < 0.05 (basal glucose uptake, resveratrol compared with untreated control); ***P < 0.05 (insulin-stimulated uptake, resveratrol compared with untreated control). F: SGBS adipocytes stably expressing nonsense shRNA or Sirt1 shRNA were incubated for 24 h with vehicle or 50 μmol/L resveratrol. Insulin-stimulated (10^{-8} mol/L) incorporation of D-[14C]-glucose into cellular lipids was measured on a β-counter. *P < 0.05 (resveratrol compared with vehicle). G and H: SGBS preadipocytes stably expressing nonsense shRNA or Sirt1 shRNA were incubated in serum-free medium with either vehicle or 50 μmol/L resveratrol. After 24 h, mRNA expression of interleukin (IL)-6 (G) and IL-8 (H) was measured by quantitative polymerase chain reaction. IL-6: *P < 0.01 (resveratrol compared with vehicle), **P < 0.01 (nonsense compared with Sirt1 shRNA); IL-8: *P < 0.01 (resveratrol compared with vehicle), **P < 0.01 (nonsense compared with Sirt1 shRNA). B–H: Data are expressed as means ± SDs (n = 3 independent experiments each performed in triplicate). Ins, insulin-stimulated; Res, resveratrol.
under resveratrol treatment. Interestingly, the knockdown of Sirt1 only partially abolished the inhibitory effect of resveratrol on IL-6 and IL-8 production, which suggests that other pathways are involved. Indeed, resveratrol inhibited TNF-α-activated nuclear transcription factor κB (NF-κB) signaling in murine adipocytes and, as a result, significantly reduced cytokine expression (53). Sirt1, in turn, is able to inhibit NF-κB signaling by physically interacting with the RelA/p65 subunit of NF-κB, which causes an inhibition of transcription by deacetylating RelA/p65 (54).

Resveratrol is a known antiinflammatory agent (26), and it inhibits expression of IL-6 or IL-8 in other cell types (55, 56). IL-6 and IL-8 are increased in obesity (57–60). IL-6 promotes insulin resistance (57, 59, 61), and IL-8 acts proatherogenically (60, 62). Thus, a down-regulation of inflammatory molecules such as IL-6 and IL-8 under resveratrol treatment would be very advantageous in preventing the development of insulin resistance and type 2 diabetes as well as atherosclerosis. However, our data also revealed a down-regulation of PPARγ and Glut-4, which is not preferable because these molecules are important regulators of insulin sensitivity (63, 64). Thus, doses of resveratrol need to be carefully established and its beneficial effects have to be weighed against adverse effects.

The concentrations of resveratrol used in our experiments are typically used in in vitro studies (10–100 μmol/L) (40–43). In mice, doses of 22 to 400 mg/kg of body weight were used (13, 14). These concentrations cannot be achieved in humans by drinking red wine because the concentration of resveratrol in red wine is too low. Today, there are many resveratrol supplements available. However, there is only limited data on the adverse effects of resveratrol and long-term safety. New synthetic activators of Sirt1 such as SRT1720 have been developed and are currently studied in terms of their therapeutic potential for the treatment of metabolic disorders (18).

Taken together, our in vitro studies suggest that resveratrol influences adipose tissue mass by inhibition of preadipocyte proliferation, inhibition of adipogenic differentiation, and inhibition of de novo lipogenesis in a Sirt1-dependent manner. Furthermore, we show that resveratrol influences the secretory profile of human preadipocytes in a way that may positively interfere with the development of obesity-associated comorbidities. Thus, our findings open up the new perspective that resveratrol-induced intracellular pathways could be a target for prevention or treatment of obesity-associated endocrine and metabolic adverse effects.

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