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Metabolism Clinical and Experimental 55 (2006) 309–316

Metabolism
Clinical and Experimental

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Effect of troglitazone on tumor necrosis factor α and transforming growth factor β expression and action in human adipocyte precursor cells in primary culture

Thomas Skurk*, Michael Birgel, Yu-Mi Lee, Hans Hauner

Else Kröner-Fresenius-Center for Nutritional Medicine, Technical University Munich, D-85350 Freising/Weihenstephan, Germany

Received 15 January 2005; accepted 7 September 2005

Abstract

Troglitazone is a member of the class of thiazolidinediones that are known to act as insulin-sensitizing agents. Administration of these compounds ameliorates insulin resistance in type 2 diabetic patients, but may also promote weight gain. The main site of action is adipose tissue, where troglitazone binds to and activates the nuclear receptor peroxisome proliferator–activated receptor γ2. The aim of this study was to investigate whether troglitazone is able to affect the adipose expression and function of tumor necrosis factor α (TNF-α) and transforming growth factor β (TGF-β). Both TNF-α and TGF-β blocked adipose differentiation in vitro and led to a marked reduction in glycerol-3-phosphate dehydrogenase activity, a marker enzyme of adipose differentiation, by 69% ± 11% and 75% ± 15%, respectively. Addition of 2 μmol/L troglitazone significantly reduced this inhibitory effect of both cytokines on glycerol-3-phosphate dehydrogenase activity. Peroxisome proliferator–activated receptor γ messenger RNA (mRNA) was reduced by TNF-α in freshly isolated adipocytes. This effect was completely counteracted by troglitazone, whereas TGF-β had no immediate effect on peroxisome proliferator–activated receptor γ mRNA. Moreover, troglitazone alone promoted adipose differentiation in a time- and dose-dependent manner. Troglitazone treatment was found to result in a marked reduction of TNF-α mRNA expression in human preadipocytes to 54% ± 13% compared with untreated cultures. Furthermore, troglitazone was observed to partially antagonize the inhibitory effect of TNF-α on insulin-stimulated 2-deoxy-glucose uptake in newly differentiated human fat cells. In conclusion, troglitazone exerts a potent adipogenic activity in human preadipocytes, which may be mediated by suppression of the endogenous production of TNF-α and by counteracting the antiadipogenic effect of TGF-β. In addition, troglitazone improved insulin-stimulated glucose uptake in differentiated fat cells.
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1. Introduction

Troglitazone belongs to the class of thiazolidinediones and was recently reported to improve insulin resistance in obese and type 2 diabetic subjects [1-3]. The exact mechanisms of action of these antidiabetic drugs are currently unknown, but it is now well established that thiazolidinediones bind to and activate specific nuclear receptors, the peroxisome proliferator–activated receptor γ (PPAR-γ) [4]. Activation of PPAR-γ results in a modulation of the expression of genes that are involved in the regulation of both fat cell development and glucose and lipid metabolism [5].

Recent studies in rodents and humans with obesity and/or type 2 diabetes mellitus have demonstrated that TNF-α expression is increased in adipose tissue [6-9] and may be an important mediator of insulin resistance [10]. Several mechanisms of action have been proposed on how TNF-α can cause insulin resistance including suppression of GLUT-4 expression [11,12] and interference with the insulin signaling pathway [13-16]. In previous studies, we were able to demonstrate that TNF-α inhibits fat cell formation, blocks glucose uptake in response to insulin, and stimulates lipolysis in cultured human preadipocytes and adipocytes, respectively [17,18].

Transforming growth factor β (TGF-β) is another proinflammatory cytokine that is produced by adipose tissue and may exert local effects on adipocytes, for example, stimulation of the synthesis and release of plasminogen activator inhibitor 1 [19]. We have recently reported that

* Corresponding author. Tel.: +49 8161 712001; fax: +49 8161 712097.
E-mail address: nutritional.medicine@wzw.tum.de (T. Skurk).

0026-0495/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2005.09.004

TGF-β is also a potent inhibitor of adipose differentiation in human adipocyte precursor cells, indicating that this cytokine is also involved in the local regulation of adipose tissue growth [20].

Studies in obese rodents and 3T3-L1 adipocytes have provided some evidence that thiazolidinediones may reduce TNF-α expression and are able to partially antagonize the inhibitory effects of TNF-α on adipose differentiation [21-23]. To date, it is unknown whether troglitazone is able to interfere with TGF-β expression and action in human adipose tissue.

It was therefore the aim of this study to investigate the potential of troglitazone to modulate the inhibitory effect of TNF-α and TGF-β on adipose differentiation as well as to influence the expression of both cytokines in human adipocyte precursor cells. In addition, we also wanted to examine whether troglitazone is able to affect the inhibitory effect of TNF-α on glucose transport in newly differentiated adipose cells in primary culture.

2. Materials and methods

Triiodothyronine, human transferrin, pantothenate, bovine serum albumin (BSA), human recombinant TNF-α, and TGF-β were from Peprotech (London, UK). Collagenase was purchased from Serva (Heidelberg, Germany), and culture media and fetal calf serum were from Biochrom (Berlin, Germany). Human insulin and cortisol were from Sigma (Taufkirchen, Germany), and troglitazone was kindly donated by Sankyo Europe (Düsseldorf, Germany). All other chemicals were from Roche (Mannheim, Germany) or Merck (Darmstadt, Germany). Sterile plasticware for tissue culture was purchased from Corning (Wiesbaden, Germany). 2-Deoxy-D-[1-^{3}H]glucose (15 Ci/mmol) and [α-^{33}P]dATP were from Amersham (Braunschweig, Germany).

2.1. Subjects

Adipose tissue samples (50-100 g wet tissue) were obtained from mammary adipose tissue of subjects undergoing elective mammary reduction (age, <45 years; body mass index, <27 kg/m^2) or from subjects undergoing elective plastic surgery in the abdominal region (age, <45 years; body mass index, <32 kg/m^2). All subjects were free of metabolic or endocrine diseases as assessed by routine clinical examination and laboratory tests. The procedure for obtaining human adipose tissue has been approved by the Ethical Committee of the Heinrich-Heine-University Düsseldorf.

2.2. Cell culture

Stromal cells from human adipose tissue were prepared as described previously [24]. Briefly, after removing all fibrous material and visible blood vessels, adipose tissue samples were cut into small pieces (approximately 10-20 mg) and digested in 10 mmol/L phosphate-buffered saline (PBS) containing 250 U/mL crude collagenase and 20 mg/mL BSA (pH 7.4) for 90 minutes in a shaking water bath. After short centrifugation at 200g, the floating fat cells and the incubation solution were aspirated and discarded. The sedimented cells were resuspended in an erythrocyte lysing buffer consisting of 154 mmol/L NH_4Cl, 5.7 mmol/L K_2HPO_4, and 0.1 mmol/L EDTA for 10 minutes to remove contaminating red blood cells. The dispersed material was filtered through a nylon mesh with a pore size of 150 μm. After short centrifugation, the sedimented cells were resuspended in Dulbecco modified Eagle/Ham F-12 medium (DMEM/F12) (vol/vol, 1:1) supplemented with 10% fetal calf serum and inoculated into culture dishes at a density of approximately 3×10^4/cm^2. After a 16-hour incubation period for cell attachment, cells were washed with PBS and refed with a serum-free DMEM/F12 medium supplemented with 15 mmol/L $NaHCO_3$, 15 mmol/L HEPES, 33 μmol/L biotin, 17 μmol/L pantothenate, 10 mg/mL human transferrin, and 0.1 g/L gentamicin. To induce adipose differentiation, cells were exposed to 66 nmol/L insulin, 100 nmol/L cortisol, and 0.2 nmol/L triiodothyronine. The medium was changed every 2 to 3 days.

2.3. Suspension culture of human adipocytes

In some parallel experiments, mature adipocytes were isolated. In contrast to the isolation of preadipocytes, a shorter collagenase digestion of 60 minutes in Krebs-Ringer buffer (pH 7.4) containing 4% BSA was performed. Cells were carefully centrifuged and washed twice with Krebs-Ringer buffer, supplemented with 0.1% BSA. After a filtration step through 250-μm nylon mesh (VWR International, Darmstadt, Germany), cells were allowed to recover from preparation for 24 hours in DMEM/F12. After this period, 400-μL packed adipocytes were cultured in a total volume of 4 mL DMEM/F12 and supplemented with either TNF-α (1 nmol/L) or TGF-β (20 pmol/L) for 24 hours. Cells and the buffer medium were separately stored at -20°C for later analyses.

2.4. Glucose transport

2-Deoxy-D-glucose uptake was determined as a functional parameter of the glucose transport system. Assays were performed at different time points as indicated in Results. Twenty-four hours before the assay, the glucose concentration was reduced to 5 mmol/L, and insulin was completely removed from the medium. For assessment of the stimulatory effect of insulin, dishes were incubated with 100 nmol/L human insulin for 15 minutes before the assay. ^{3}H-Labeled 2-deoxy-D-glucose (1 μCi per dish; concentration, 4-5 μmol/L) was added to the medium, which already contained 5 mmol/L of unlabeled glucose. Hexose uptake was measured for 20 minutes at 37°C and terminated by transferring the dishes to an ice bath. Cells were repeatedly washed with ice-cold PBS and incubated for another 20 minutes in PBS containing 0.1% sodium dodecyl sulfate for cell lysis. The radioactivity of the cell material was counted in a liquid scintillation counter (Beckman, Munich,

Germany). Values were corrected for the unspecific uptake as described recently [25].

2.5. Preparation of RNA, complementary DNA generation, and quantitative polymerase chain reaction

Total RNA was extracted with the NucleoSpin RNA II kit from Machery & Nagel (Düren, Germany) according to the instructions of the manufacturer including DNA digestion. Complementary DNA synthesis was performed using iScript synthesis kit from Bio-Rad (Hercules, CA). Afterward, complementary DNA was diluted 1:10 in H_2O, and polymerase chain reaction (PCR) was carried out using an equivalent of 7.5 ng RNA. Quantitative PCR was performed using the ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Foster City, CA). TaqMan Universal PCR Master Mixes and Assay-on-Demand Gene Expression Probes (Applied Biosystems) were used to determine the target sequence. The relative standard curve method was used to calculate amplification differences between treated samples and untreated controls. Reference sequences for these assays were obtained from the manufacturer: PPAR-γ, TTCTCAGTGGAGACCGCCCAGGTTT; TGF-β, AACCCACAACGA-AATCTATGACAAG; and TNF-α, ATGTTGTAGCAAACCCTCAAGCTGA, respectively. Target sequences were normalized to the abundantly expressed 18S RNA as internal standard (reference sequence, TGGAGGGCAAGTCTGGTGCCAGCAG).

2.6. Other biochemical assays

Glycerol-3-phosphate dehydrogenase (GPDH) was determined as marker enzyme of adipose differentiation and fat cell function according to an established method [26]. Trichloroacetic acid (TCA) precipitation was used to avoid lipid interference before measuring the protein content of the cultures according to a modified method of Lowry et al [27]. Glycerol-3-phosphate dehydrogenase activities are presented as specific activities.

2.7. Statistical analysis

Results are expressed as mean ± SD of at least 3 experiments in triplicate. For comparison, analysis of variance was used. P values of less than .05 were considered as statistically significant.

3. Results

3.1. Effect of troglitazone on adipose differentiation and PPARγ messenger RNA expression

In the first set of experiments, we studied the effect of troglitazone on adipose differentiation of human adipocyte precursor cells. In the presence of the adipogenic factors insulin and cortisol, troglitazone exhibited an additional stimulatory effect on differentiation, depending on concentration and exposure time. Glycerol-3-phosphate dehydrogenase activity was increased on day 16 by 1.3-, 3.6-, and 22-fold after 0.02, 0.2, and 2 μmol/L troglitazone added for the initial 4 days (data not shown). After the course of differentiation for 16 days, a continuous increase of GPDH activity was detectable with maximum levels of 1480 ± 400 mU/mg when measured on day 16 (Fig. 1A). However, when troglitazone was added at a later stage of differentiation, that is, between days 9 and 16, no stimulatory effect on GPDH activity was detectable (data not shown). Comparable effects of troglitazone on adipose differentiation were observed in the absence of cortisol, when insulin was the only adipogenic hormone added to the cells. However, under these conditions the increase of differentiation did not exceed 30% to 40% (data not shown).

Troglitazone is a well-known ligand to PPAR-γ, a member of the family of peroxisome proliferator–activated nuclear receptors that act as regulators of the transcription of adipose-related genes [5]. To study whether the adipogenic action of troglitazone involves a modulation of PPAR-γ, we investigated the effect of this compound on PPAR-γ messenger RNA (mRNA) in human preadipose and adipose cells. After an initial incubation of the cells with 2 μmol/L

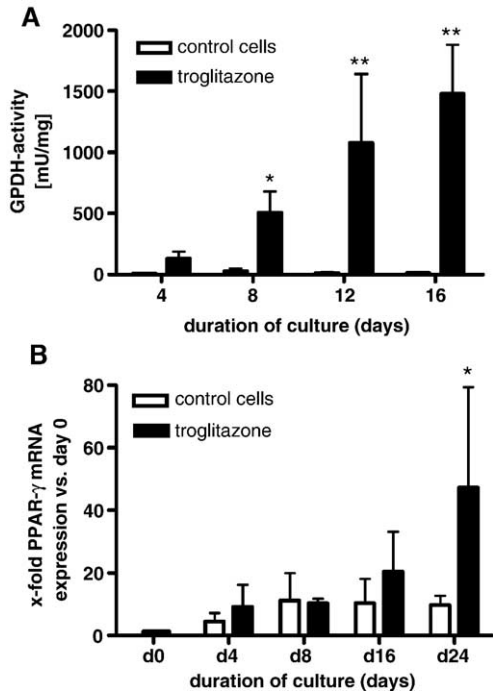

Fig. 1. Effect of 2 μmol/L troglitazone on the time course of adipose differentiation in human adipocyte precursor cells under serum-free culture conditions (black bars) compared with untreated control cultures (white bars). Cells were stimulated to undergo differentiation in the absence or presence of 2 μmol/L troglitazone for the initial 4 days. A, GPDH activity was measured on day 16 as described in Materials and Methods. Data are expressed as mean ± SD of 4 experiments in duplicate. *P < .05; **P < .01. B, Effect of troglitazone on the course of PPAR-γ mRNA expression in cultured human preadipocytes compared with undifferentiated cells. Peroxisome proliferator–activated receptor γ mRNA at baseline was defined as 1. Data are given as mean ± SD of 4 independent experiments in triplicate. *P < .05.

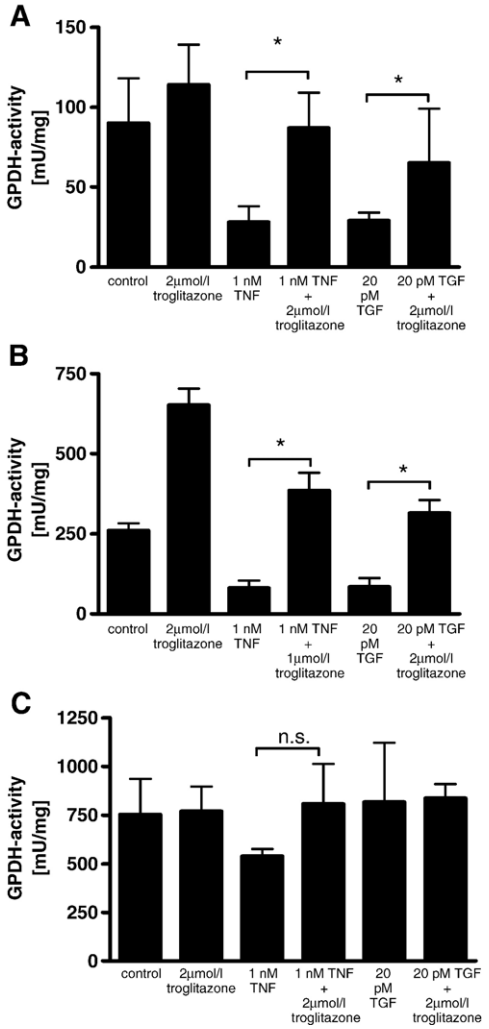

Fig. 2. Effect of troglitazone on TNF-α– and TGF-β–induced inhibition of adipose differentiation of in vitro cultured human preadipocytes. A, Human preadipocytes were exposed to 1 nmol/L TNF-α or 20 pmol/L TGF-β for the initial 4 days of the differentiation period with or without 2 μmol/L troglitazone. After 4 days under serum-free culture conditions, GPDH activity was determined as marker of differentiation. B, Human adipocyte precursor cells were cultured as described in A, but harvested on day 16 for GPDH measurement. C, Preadipocytes were allowed to differentiate and then exposed to the agents as indicated for the following 4 days. All results are given as mean ± SD of 3 to 4 independent experiments. *$P < .05$.

troglitazone for 4 days, PPAR-γ mRNA increased dramatically during the whole differentiation process. As assessed by real-time quantitative reverse transcriptase–PCR, PPAR-γ mRNA was low in undifferentiated cells and increased in the presence of troglitazone by approximately 45-fold in the course of differentiation compared with day 0 ($P < .05$) (Fig. 1B).

3.2. Effect of troglitazone on the inhibition of adipose differentiation by TNF-α and TGF-β

As already demonstrated in previous studies [17,20], exposure of human adipocyte precursor cells to TNF-α and TGF-β, respectively, results in a marked reduction of adipose differentiation. Addition of 1 nmol/L TNF-α for 4 days at the beginning of the differentiation process caused a significant reduction of GPDH activity by 69% ± 12% when measured on day 4 ($P < .01$). When 2 μmol/L troglitazone was concomitantly added to the culture medium, there was an attenuation of the inhibitory effect of TNF-α. Total GPDH activity was approximately doubled in the presence of troglitazone compared with TNF-α alone ($P < .05$), but did not reach the levels in the control cultures. Similarly, when cells were exposed for 4 days to 20 pmol/L TGF-β, GPDH activity was reduced by 75% ± 15% ($P < .01$). The inhibitory effect of TGF-β was also significantly reduced when cultures were at the same time exposed to 2 μmol/L troglitazone ($P < .05$) (Fig. 2A).

Similar results were found when TNF-α and TGF-β were added on day 0, but cells were harvested on day 16 for GPDH measurement. Addition of troglitazone to TNF-α– or TGF-β–treated cultures also significantly retained differentiation capacity to a similar extent as if added on day 0 (Fig. 2B).

In contrast, when TNF-α and TGF-β were added after 16 days of differentiation for 72 hours, only a minor effect of TNF-α with a reduction of 30% ± 12% ($P < .05$), but no effect of TGF-β, was detectable (Fig. 2C).

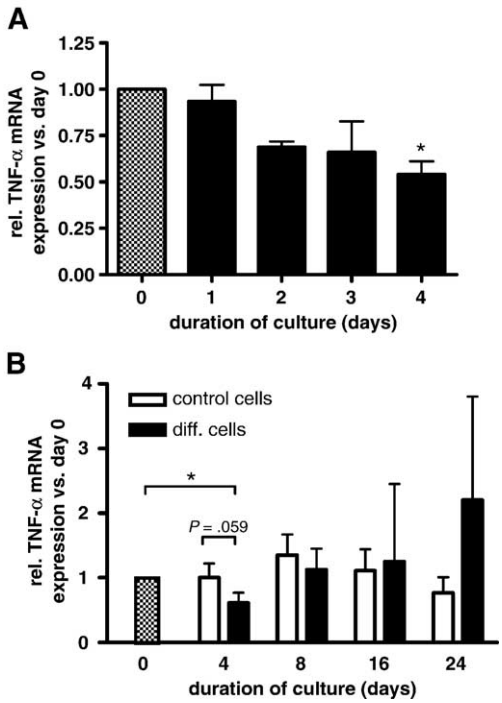

Fig. 3. Effect of 2 μmol/L troglitazone on TNF-α mRNA expression in human preadipocytes during the first 4 days of culture (A). Time course of TNF-α mRNA expression in cultured human adipocyte precursor cells during differentiation compared with control cultures (B). Troglitazone (2 μmol/L) was added for the first 4 days (black bars). Cells were harvested at the time points indicated for the measurement of TNF-α mRNA as described in Materials and Methods. Data are given as mean ± SD of 3 to 4 independent experiments. *$P < .05$.

3.3. Effect of troglitazone on TNF-α and TGF-β mRNA expression

To address the question whether the effect of troglitazone was mediated by a modulation of cytokine expression, we measured specific mRNA levels of TNF-α and TGF-β in differentiating cell cultures. We have previously observed that the induction of adipose differentiation by the phosphodiesterase inhibitor isobutyl methylxanthine is accompanied by a decrease in TNF-α mRNA [28]. Likewise, exposure of human preadipocytes to 2 μmol/L troglitazone for the initial 4 days of culture resulted in a suppression of TNF-α mRNA (Fig. 3A). However, when cells were kept in culture for the full differentiation period in adipogenic medium, a reappearance of the endogenous TNF-α mRNA was observed, indicating that the effect of troglitazone was transient (Fig. 3B). In contrast, presence of 2 μmol/L troglitazone in the culture medium was associated with a rapid increase in TGF-β mRNA levels within 4 days by 68% ± 13% ($P < .05$) (Fig. 4A), which was followed by a rapid decline to control mRNA levels during the course of differentiation (Fig. 4B).

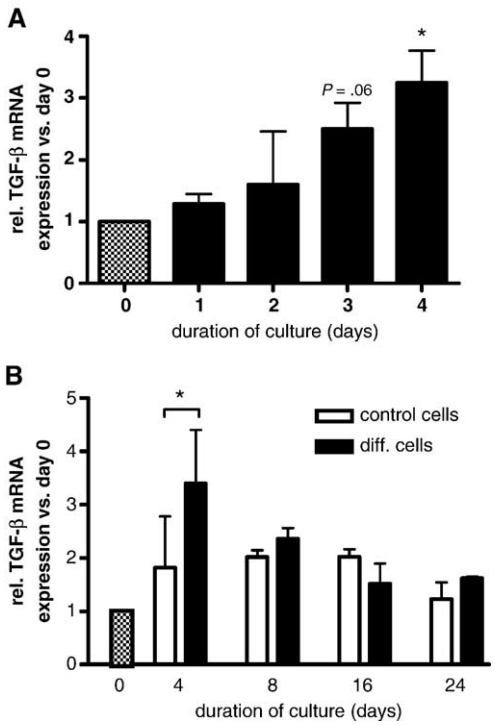

Fig. 4. Effect of 2 μmol/L troglitazone on TGF-β mRNA expression in human preadipocytes during the first 4 days of culture (A). Time course of TGF-β mRNA expression during differentiation compared with undifferentiated control cultures (white bars) (B). Cells were stimulated to undergo differentiation in the absence or presence of 2 μmol/L troglitazone for the initial 4 days. Cells were harvested at the time points indicated for the assessment of TGF-β mRNA. Data are given as mean ± SD of 4 experiments. *$P < .05$.

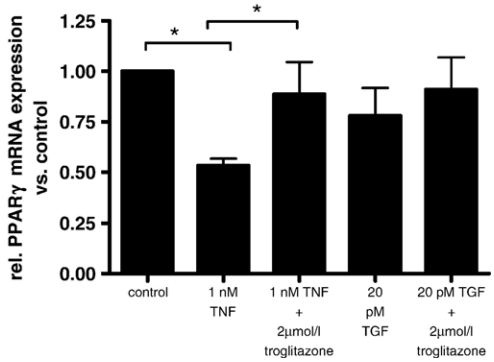

Fig. 5. Effect of 2 μmol/L troglitazone on PPAR-γ mRNA expression in TNF-α– and TGF-β–treated freshly isolated adipocytes in suspension culture. Cells were isolated as described in Materials and Methods and allowed to recover from isolation for 24 hours. Then, cells were treated with the compounds indicated for 24 hours and harvested for mRNA measurement. Data are given as mean ± SD from 3 independent experiments. *$P < .05$.

3.4. Effect of TNF-α and TGF-β on PPAR-γ expression in freshly isolated adipocytes

When PPAR-γ mRNA levels in freshly isolated adipocytes were investigated after the addition of 1 nmol/L TNF-α or 20 pmol/L TGF-β for 24 hours, a significant effect could only be observed for TNF-α with a reduction of PPAR-γ levels to 54% ± 13% compared with untreated controls. Again, addition of troglitazone completely inhibited the effect of TNF-α. Addition of TGF-β had no significant effect on PPAR-γ mRNA expression after 24 hours (Fig. 5).

3.5. Effect of troglitazone on glucose transport

A 24-hour exposure of newly differentiated fat cells to 2 μmol/L troglitazone showed a 24% ± 13% stimulatory

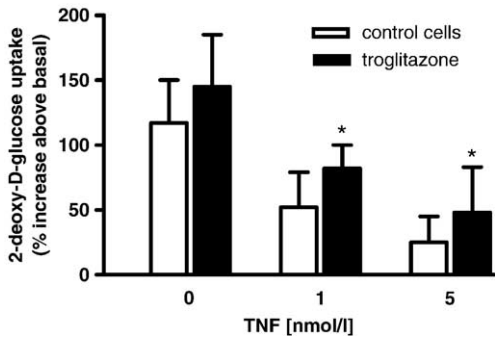

Fig. 6. Effect of 2 μmol/L troglitazone on insulin-stimulated 2-deoxy-D-glucose uptake in in vitro differentiated human adipocytes on day 16 of culture. Troglitazone and TNF-α, respectively, were present for 24 hours before the glucose transport assay. 2-Deoxy-D-glucose uptake of controls after a 15-minute preincubation with 10^{-7} mol/L insulin was defined as 100% (equivalent to 40 ± 6 fmol/mg protein per minute). Black columns represent troglitazone treatment. Data are given as mean ± SD of 4 experiments in triplicate. *$P < .05$.

effect on insulin-stimulated 2-deoxy-D-glucose transport. When newly differentiated human fat cells were preincubated on day 16 with 1 and 5 nmol/L TNF-α, respectively, for 24 hours, insulin-stimulated 2-deoxy-D-glucose uptake was reduced by 55% ± 22% and 76% ± 19%, respectively. Tumor necrosis factor α–treated fat cells that were simultaneously incubated with 2 μmol/L troglitazone showed a significantly higher insulin-stimulated glucose uptake compared with cells exposed to TNF-α alone. Thus, troglitazone was found to diminish the TNF-induced reduction of insulin-stimulated glucose transport (Fig. 6).

4. Discussion

The results of this study clearly show that troglitazone has some potential to antagonize the inhibitory effect of both TNF-α and TGF-β on the adipose differentiation of human adipocyte precursor cells. In addition, troglitazone was found to reduce the expression of TNF-α, but not of TGF-β in human preadipocytes, which may suggest that the inhibitory effect on TNF-α expression could be involved in the adipogenic activity of thiazolidinediones. Furthermore, troglitazone showed a potential to diminish the inhibition of insulin-stimulated glucose transport by TNF-α in newly differentiated fat cells.

Experimental investigations in 3T3-L1 preadipocytes and other cell lines from rodent origin have provided the first evidence that thiazolidinediones may promote adipose differentiation and may antagonize some of the antiadipogenic actions of TNF-α [21,23,29,30]. Our results hereby confirm the results of other recent studies on the effects of thiazolidinediones in human adipocyte precursor cells [31,32]. However, our data extend this observation by showing for the first time that administration of a thiazolidinedione is associated with a suppression of TNF-α mRNA in human adipose tissue, thereby providing evidence for another mechanism by which thiazolidinediones are able to promote differentiation. In addition, troglitazone also reduced TNF-α mRNA expression in freshly isolated fat cells in suspension culture, whereas no effect on TGF-β expression was observed (Fig. 5).

Although it is well known that thiazolidinediones act as ligands for the nuclear receptor PPAR-γ [4,5], exposure of human preadipocytes to troglitazone resulted in an elevation of PPAR-γ mRNA. This effect was rapid and persistent for the complete differentiation period, although troglitazone was added only for the first 4 days of culture. Therefore, it is obvious that thiazolidinediones not only activate PPAR-γ to form a heterodimer with the retinoid X receptor [5], but are also able to up-regulate its expression.

Increased adipose expression of TNF-α in human obesity was shown to be associated with the development of insulin resistance, which is a characteristic feature of the obese state [7-9]. The fact that troglitazone reduced TNF-α expression could explain the amelioration of metabolic disturbances commonly associated with obesity. Our study demonstrates for the first time that troglitazone is able to suppress TNF-α expression in cultured human preadipocytes. In contrast, in newly differentiated adipocytes, there was no significant effect of troglitazone on TNF-α mRNA even after treatment with the TNF-α inducer lipopolysaccharide (data not presented). This discrepancy between preadipocytes and adipocytes is striking, but is in agreement with the lack of troglitazone action on adipogenesis when the compound was added between days 9 and 16. Thus, it is tempting to assume that the troglitazone-induced reduction of endogenous TNF-α expression in preadipose cells may contribute to the adipogenic activity of the compound in earlier stages of adipose differentiation similar to observations made for the nonelective phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine, which also blocks TNF-α expression [28]. Another explanation may be found in the cellular components of adipose tissue, which are able to express TNF-α, for example, preadipocytes, adipocytes, and macrophages. For the latter cell type, a TNF-α–suppressing action of thiazolidinediones is known [33]. However, during differentiation, there is a relative shift from TNF-α production in the stromal vascular fraction to the lipid-containing adipocytes. Because of culture conditions, macrophage contamination can be excluded, at least at later stages of differentiation. Therefore, the possibility may arise that fat cells are responsive to troglitazone in this respect, but further experiments would be required to elucidate this difference in TNF-α expression.

From a physiological point of view, the overproduction of TNF-α in obesity may be a useful mechanism to limit adipose tissue expansion by the induction of insulin resistance as hypothesized by Kern et al [7]. It has been shown that subjects with high insulin sensitivity are more likely to gain body weight than insulin-resistant subjects [34]; therefore, insulin resistance appears to be associated with a lower rate of weight gain, as part of a self-regulatory closed-loop system. For this reason, it is not surprising that long-term treatment with thiazolidinediones of insulin-resistant obese humans results in increased body weight [35]. However, this weight gain is not solely due to expansion of adipose tissue mass, but may also be caused by fluid retention [35]. In addition, as long as energy balance remains unchanged, the risk of weight gain should be low. Nevertheless, this point requires further critical attention as most type 2 diabetic patients are obese and promotion of weight gain would induce or potentiate other unfavorable sequelae of obesity.

Another new observation was that TGF-β is expressed in human preadipocytes. It was surprising to see that treatment with troglitazone constantly increased TGF-β expression. In view of the potent antiadipogenic activity of TGF-β [20], this observation may suggest that the inhibitory effect of troglitazone on the antiadipogenic activity exerted by TGF-β is not related to a suppression of TGF-β expression. In addition, TGF-β is an important signal for the termination of inflammatory reaction and can suppress

release of proinflammatory mediators. This finding could explain the commonly observed anti-inflammatory properties of thiazolidinediones.

In conclusion, the results of our studies suggest that troglitazone exerts a differentiation-promoting effect in human adipose tissue that could be partially mediated by a reduction of the antiadipogenic effect, at least, of TNF-α. In this context, troglitazone was found to suppress TNF-α gene suppression in preadipocytes, whereas there was a surprising modest stimulatory effect on TGF-β mRNA. Furthermore, troglitazone was able to counteract the suppressing effect of TNF-α on insulin-stimulated glucose transport in human adipocytes, thereby reducing insulin resistance. These observations may contribute to a better understanding of the metabolic effects of thiazolidinediones, but further studies are required to examine the clinical relevance of these in vitro findings and to elucidate the mechanisms involved.

Acknowledgments

This study was supported by a grant from the Hochschulsonderprogramm of the Government of Nordrhein-Westfalen. The financial support by Sankyo Europe GmbH, Düsseldorf, Germany, is gratefully acknowledged.

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