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Zinc-α_2-glycoprotein, a lipid mobilizing factor, is expressed and secreted by human (SGBS) adipocytes

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Abstract Zinc-α_2-glycoprotein (ZAG), a lipid mobilizing factor, is expressed in mouse adipose tissue and is markedly upregulated in mice with cancer cachexia. We have explored whether ZAG is expressed and secreted by human adipocytes, using SGBS cells, and examined the regulation of ZAG expression. ZAG mRNA was detected by RT-PCR in mature human adipocytes and in SGBS cells post-, but not pre-, differentiation to adipocytes. Relative ZAG mRNA levels increased rapidly after differentiation of SGBS cells, peaking at day 8 post-induction. ZAG protein was evident in differentiated adipocytes (by day 3) and also detected in the culture medium (by day 6) post-induction. The PPARγ agonist rosiglitazone induced a 3-fold increase in ZAG mRNA level, while TNF-α led to a 4-fold decrease. Human adipocytes express and secrete ZAG, with ZAG expression being regulated particularly through TNF-α and the PPARγ nuclear receptor. ZAG is a novel adipokine, which may be involved in the local regulation of adipose tissue function.

Keywords: Adipokine; Human adipocyte; PPARγ; TNF-α; Zinc-α_2-glycoprotein

1. Introduction

Zinc-α_2-glycoprotein (ZAG) is a 43 kDa soluble glycoprotein first isolated from human plasma [1] and subsequently found in secretory epithelia cells of liver, breast, the gastrointestinal tract, and sweat glands [2]. ZAG is overexpressed in certain malignant tumors such that it may serve as a cancer marker [3,4]. The biological functions of ZAG are largely unknown, but it has been shown to act as a lipid-mobilizing factor [5,6]. Treatment with ZAG stimulates lipolysis in isolated mouse and human adipocytes, and selectively reduces body fat in both normal and *ob/ob* mice [6]. The lipolytic effect of ZAG in rodents has been attributed to the activation of β_3-adrenoreceptors and upregulation of cAMP [7].

White adipose tissue (WAT), conventionally considered simply as a site of fuel storage, is now recognized as a key endocrine organ [8–10]. It secretes a wide range of protein factors termed adipokines, including leptin, adiponectin, TNF-α, and IL-6, which act locally and/or distantly in the regulation of energy balance and other physiological processes such as insulin sensitivity and the inflammatory response. The secretory function of adipocytes, together with the lipolytic effect of ZAG, led us to consider that this protein might be produced directly by WAT, and we have recently shown that ZAG is expressed by mouse WAT as well as by murine 3T3-L1 adipocytes [11]. Moreover, ZAG mRNA and protein levels were increased substantially in WAT of mice bearing a cachexia-inducing tumor that causes a profound loss of body weight and fat mass [11]. These findings suggest that adipocyte-derived ZAG may modulate adipose tissue metabolism locally. Interestingly, overexpression of ZAG in 3T3-L1 adipocytes has been reported to lead to an increase in adiponectin mRNA levels [12].

Although ZAG appears to be expressed in WAT of obese subjects [11], it is not known whether the protein is synthesized in human adipocytes or whether it is secreted as an adipokine. In this study, we have used the SGBS (Simpson-Golabi-Behmel syndrome) cell strain to study ZAG synthesis in human adipocytes; SGBS cells have a high capacity for differentiation to mature cells that are functionally similar to human adipocytes [13]. We demonstrate that human adipocytes express the ZAG gene and that ZAG is secreted from SGBS cells. In addition, we show that ZAG expression is regulated particularly through the PPARγ receptor and by TNF-α.

2. Materials and methods

2.1. Human adipose tissue

Abdominal subcutaneous and omental adipose tissue were collected during gastroplasty from six obese male patients, aged 35–48 years, with body mass index >43; the subjects did not exhibit any other ongoing disease. The study was approved by the Sefton Ethics Committee and all patients gave their informed consent. After removal, samples were immediately frozen in liquid nitrogen and stored at −80 °C until analysis.

2.2. Cell culture

SGBS cells were maintained and cultured as previously described [13]. In brief, cells were cultured at 37 °C in a humidified atmosphere of 5% CO_2/95% air. SGBS preadipocytes were maintained in DMEM/Ham's F12 (1:1) medium (Invitrogen) containing 10% (v/v) fetal calf serum (FCS; Sigma) and antibiotics (penicillin/streptomycin). Differentiation of the cells was initiated 24 h after confluence by incubation for 4 days in FCS-free medium containing 0.25 μM dexamethasone, 500 μM 3-isobutyl-1-methyl-xanthine, 10 nM insulin, 200 pM triiodothy-

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ronine (T_3), 1 μM cortisol (Sigma) and 2 μM rosiglitazone (GlaxoSmithKline). Differentiation into adipocytes was visualized under the microscope from the accumulation of lipid droplets and by Oil Red O staining. The cells were maintained in feeding medium (containing insulin, cortisol and T_3) for up to 25 days and collected every 1–3 days in the case of the time course study of ZAG expression during differentiation, or for 10 days for studies on the regulation of ZAG expression. For the study on the secretion of ZAG during adipocyte differentiation, culture medium was collected every 3 days; the medium was centrifuged at 1000 rpm for 10 min and the supernatant stored at −20 °C until analysis.

For studies on the regulation of ZAG expression, cells collected at day 10 were pre-incubated for 24 h with cortisol-free feeding medium or insulin-free feeding medium (for the study on the effect of insulin). Cells were then incubated for 24 h with medium containing each of the following agents: noradrenaline (Fluka), BRL 37344 (Tocris), isoprenaline, insulin, dexamethasone, TNF-α, interleukin-6 (IL-6), lipopolysaccharide (LPS) (Sigma), and rosiglitazone. The control cells for each study were maintained in pre-incubation medium, which was renewed every 3 days. After completion of the treatment, cells were collected in 700 μl of Trizol (Invitrogen).

Zen-Bio human preadipocytes and adipocytes differentiated in culture (Zen-Bio) were provided by Dr. Bohan Wang. cDNAs from mature adipocytes and stromal-vascular cells isolated by collagenase digestion of human subcutaneous and omental adipose tissue were provided by Dr. Stuart Wood.

2.3. RT-PCR

Total RNA was extracted from tissues (50–120 mg) and adipocytes with Trizol. RNA samples used for real-time PCR were treated with a DNA-free kit (Ambion) to remove any genomic DNA. The RNA concentration was determined from the absorbance at 260 nM. 1 μg of total RNA of each sample was reverse transcribed to cDNA in a final volume of 20 μl by using a Reverse-iT™ first strand kit (ABgene). 1 μl of each cDNA sample was then amplified in a PCR mixture containing 0.02 mM of each primer and 1.1×Reddy Mix PCR Master Mix (ABgene) in a final volume of 25 μl. Human β-actin was used as a housekeeping gene. The primer pair used and the PCR cycling conditions were as follows.

Human ZAG (250-bp product): 5′-CTT GGC TCA CTC AAT GAC CT-3′ (sense); 5′-CTC CGC TGC TTC TGT TAT TC-3′ (antisense); annealing temperature (T_A) 54 °C; 33 cycles.

Human β-actin (281-bp product): 5′-GTG GCA TCC ACG AAA CTA CCT T-3′ (sense); 5′-GGA CTC GTC ATA CTC CTG CTT G-3′ (antisense); T_A 57 °C; 23 cycles.

Human leptin (422-bp product): 5′-GAA CCC TGT GCG GAT TCT TG-3′ (sense); 5′-CAC CTC TGT GGA GTA GCC TGA A-3′ (antisense); T_A 56 °C; 33 cycles.

Human adiponectin (352-bp product): 5′-ATG CTG TTG CTG GGA GCT GTT C-3′ (sense); 5′-CCA CAC TGA ATG CTG AGC GGT A-3′ (antisense); T_A 60 °C; 31 cycles.

PCR was performed on a thermal cycler (Hybaid) with an initial denaturation at 94 °C for 2 min followed by cycles consisting of denaturation at 94 °C for 20 s, annealing at the specified temperature for 25 s, and extension at 72 °C for 59 s; the final step was an extension at 72 °C for 5 min. PCR products were separated on a 1% agarose gel stained with ethidium bromide. The PCR products were sequenced commercially to confirm their identity (MWG Biotech).

2.4. Real-time PCR

Relative ZAG mRNA levels were quantified using real-time PCR with an ABI Prism 7700 Sequence Detector (Applied Biosystems). Human β-actin mRNA levels were similarly measured and served as the reference gene. Primers and Taqman probes were designed using Primer Express software (Applied Biosystems). The sequences of primers and Taqman probes were as follows.

Human ZAG: 5′-ACG ACA GTA ACG GGT CTC ACG TA-3′ (forward); 5′-TCC TTT CCA TCA TAG TAA TAT TTC CAG AA-3′ (reverse); 5′-FAM-CAG GGA AGG TTT GGT TGT GAG ATC GAG AAT AAC-TAMRA (probe).

Human adiponectin: 5′-CCC AAA GAG GAG AGA GGA AGC T-3′ (forward); 5′-GCC AGA GCA ATG AGA TGC AA-3′ (reverse); 5′-FAM-TTC CCA GAT GCC CCA GCA AGT GTA AC-TAMRA (probe).

Human IL-6: 5′-GGT ACA TCC TCG ACG GCA TCT-3′ (forward); 5′-GTG CCT CTT TGC TGC TTT CAC-3′ (reverse); 5′-FAM-TGT TAC TCT TGT TAC ATG TCT CCT TTC TCA GGG CT-TAMRA (probe).

Human β-actin: 5′-GGA TGC AGA AGG AGA TCA CTG-3′ (forward); 5′-CGA TCC ACA CGG AGT ACT TG-3′ (reverse); 5′-FAM-CCC TGG CAC CCA GCA CAA TG-TAMRA (probe).

Amplification was performed in a 96-well plate using a master mix made from qPCR™ core kit (Eurogentec), with 300 nM forward (900 nM in the case of β-actin) and 900 nM reverse primers, 225 nM probe and 1 μl of cDNA in a final volume of 25 μl. Each sample was run in triplicate for ZAG and in duplicate for β-actin, adiponectin, and IL-6. The PCR parameters were as follows: initial 2 min at 50 °C, denaturation at 95 °C for 10 min followed by 40 cycles of denaturation at 95 °C for 15 s and combined annealing and extension at 60 °C for 1 min. Data were recorded and analyzed with Sequence Detector software (Applied Biosystems). ZAG mRNA levels were normalized to the values of β-actin and the results expressed as relative fold changes using the $2^{-\Delta\Delta Ct}$ method [14].

2.5. Western blotting

Adipose tissue samples were homogenized in a buffer containing 250 mM sucrose, 1 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), and 0.2 mM EDTA. The homogenate was centrifuged at $12000 \times g$ for 15 min at 4 °C and the supernatant containing soluble proteins collected. In the case of SGBS cells, Trizol extraction for the isolation of proteins was performed according to the manufacturer's instructions. For the isolation of protein from culture medium, samples of medium were incubated with 50% trichloroacetic acid for 3 h on ice, followed by centrifugation at 12000*g* for 30 min at 4 °C. The supernatant was discarded and the protein pellet dissolved in 1% sodium dodecyl sulfate (SDS). The protein concentration was determined using the bovine serum albumin assay [15].

Samples containing 10–20 μg of protein were separated by SDS gel-electrophoresis on 12% polyacrylamide gels. The proteins were then transferred to nitrocellulose membranes (Hybond C; Amersham Pharmacia) and immunoblotting performed overnight at 4 °C. A mouse monoclonal antibody to human ZAG (Santa Cruz Biotechnology) was employed at a 1:1000 dilution in Tris buffered saline (pH 7.4) containing 0.1% Tween 20 and 5% dried milk. Blots were then incubated for 1 h at room temperature with a rabbit anti-mouse secondary antibody conjugated to horseradish peroxidase (Santa Cruz Biotechnology) at a 1:1000 dilution. Signals were detected by the enhanced chemiluminescence system (ECL; Amersham Pharmacia).

2.6. Statistical analysis

Data are presented as mean values ± S.E.M. Differences between groups were analyzed by Student's unpaired *t* test and were considered to be statistically significant when $P < 0.05$.

3. Results

3.1. ZAG mRNA and protein in human WAT

In the initial experiments, the presence of ZAG mRNA in human WAT, both subcutaneous and omental depots, was confirmed by RT-PCR (Fig. 1A), as in our previous work [11]. Sequence analysis of the 250-bp product revealed 100% homology with the corresponding region of human ZAG cDNA. ZAG protein was also detected in both subcutaneous and omental fat depots by Western blotting (Fig. 1B). RT-PCR analysis of human subcutaneous and omental adipose tissue fractionated by collagenase digestion indicated that the ZAG gene was expressed primarily in mature adipocytes; a weak signal was, however, also obtained with cells of the stromal-vascular fraction (Fig. 1C).

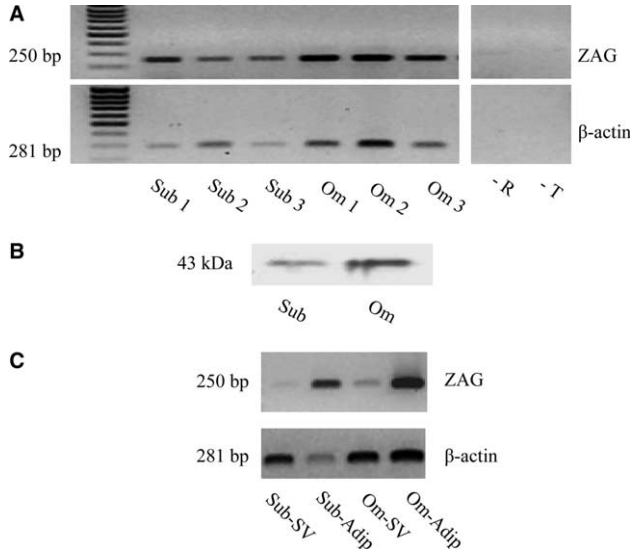

Fig. 1. ZAG gene expression and ZAG protein in human adipose tissue. (A) Detection of ZAG mRNA by RT-PCR in three individual subjects (1–3). −R, no RT control; −T, no template control. (B) Detection of ZAG protein in adipose tissue homogenates by Western blotting. (C) ZAG mRNA detection by RT-PCR in human adipocytes (Adip) and stromal-vascular cells (SV) isolated from omental and subcutaneous fat depots by collagenase digestion. Sub, subcutaneous; Om, omental.

3.2. ZAG gene expression in SGBS adipocytes

SGBS cells in culture were employed to examine the expression of ZAG by human adipocytes. ZAG gene expression was first assessed by RT-PCR in SGBS cells before and after the induction of differentiation to adipocytes. No signal for ZAG mRNA was detected in the cells before induction (Fig. 2A); however, a signal was detected at day 1 post-induction and thereafter (Fig. 2A). Expression of the adiponectin and leptin genes was examined for comparison with ZAG, and the mRNA for both these adipokine hormones was detected in SGBS adipocytes post-differentiation, from days 2 and 5, respectively (Fig. 2A).

To assess the quantitative changes in ZAG gene expression during the maturation of SGBS adipocytes, real-time PCR was employed. ZAG mRNA levels increased gradually from day 1 post-induction and reached a peak at day 8 of >100 times that at day 2 (Fig. 2B). Further confirmation that ZAG mRNA occurs only in mature human adipocytes was obtained from another human primary cell culture system (Zen-Bio cells). Consistent with the results of SGBS cells, no signal for ZAG mRNA was detected by RT-PCR in these preadipocytes at day 0, while a clear signal was present at day 6 after the induction of differentiation (Fig. 2C).

3.3. ZAG protein expression and secretion by SGBS adipocytes

Western blotting was performed to establish whether ZAG protein was present in SGBS adipocytes. During the time course of the differentiation of SGBS cells to adipocytes, ZAG protein was detected at day 3 post-induction and thereafter; no signal was obtained prior to this (Fig. 3A).

Western blotting was then performed on the culture medium to determine whether ZAG is released by adipocytes. ZAG protein was detected in the medium and as with the mRNA and protein measurements in the cells it was present only on differentiation, with a faint signal appearing by day 6 post-induction with a much stronger signal thereafter (Fig. 3B). The signal for ZAG protein occurred at the same position as the purified ZAG protein (from human serum; kindly provided by Dr. ST Russell) (Fig. 3B).

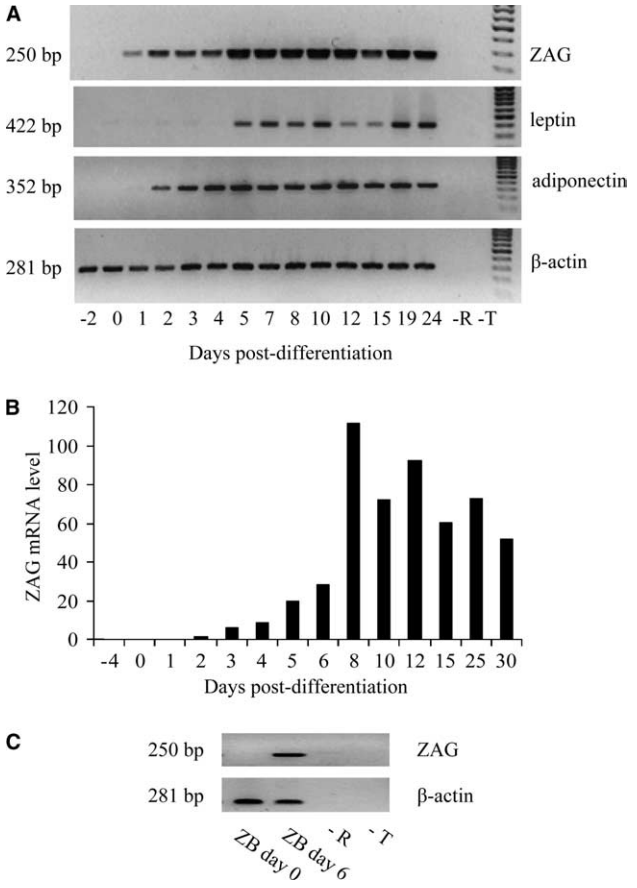

Fig. 2. ZAG gene and protein expression during the differentiation and development of SGBS cells to adipocytes. (A) ZAG, leptin and adiponectin mRNA assessed by RT-PCR in SGBS cells; differentiation was induced on day 0. (B) Relative ZAG mRNA levels (representative) measured by real-time PCR in SGBS adipocytes. (C) Detection of ZAG mRNA in human adipocytes (Zen-Bio) by RT-PCR at days 0 and 6 after the induction of differentiation. −R, no RT control; −T, no template control.

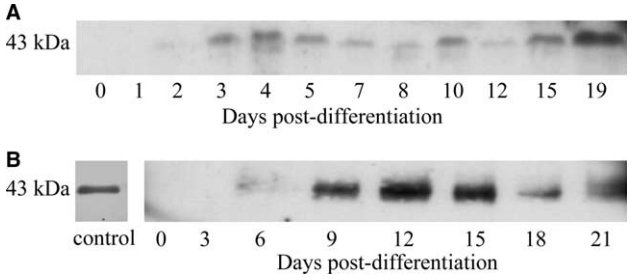

Fig. 3. ZAG protein in SGBS adipocytes and ZAG release into the culture medium. (A) Western blot of ZAG in SGBS cells before and after the induction of differentiation. (B) Western blot of ZAG in the culture medium of SGBS cells before and after the induction of differentiation. Positive control, 10 ng of purified human ZAG protein.

3.4. Regulation of ZAG gene expression in SGBS adipocytes

The regulation of ZAG gene expression in SBGS adipocytes was investigated using real-time PCR to quantify the relative ZAG mRNA levels. SGBS cells were treated at day 11 for 24 h with different agents, at two dose levels. Treatment with noradrenaline at either low or high doses had no effect on ZAG mRNA level (Fig. 4A). The addition of a selective β_3-adrenoceptor agonist, BRL 37344, led to a dose-dependent increase in ZAG mRNA level, but this was only statistically significant ($P < 0.001$) with the higher dose which increased the mRNA level 2.5-fold (Fig. 4B).

Insulin had no significant ($P > 0.05$) effect on ZAG mRNA level (Fig. 4C). However, treatment with dexamethasone led to a modest increase in ZAG mRNA at the higher dose, a 2-fold increase being observed (Fig. 4D). The largest increase in ZAG mRNA was observed with rosiglitazone, which resulted in a 3-fold rise in level at both low ($P < 0.01$) and high doses ($P < 0.001$) (Fig. 5A). To assess the specificity of the effect of rosiglitazone on ZAG expression in SGBS adipocytes, mRNA levels of two other adipokines, adiponectin, and IL-6, were measured. Adiponectin mRNA level increased 2.5-fold ($P < 0.001$ at the higher dose) with rosiglitazone

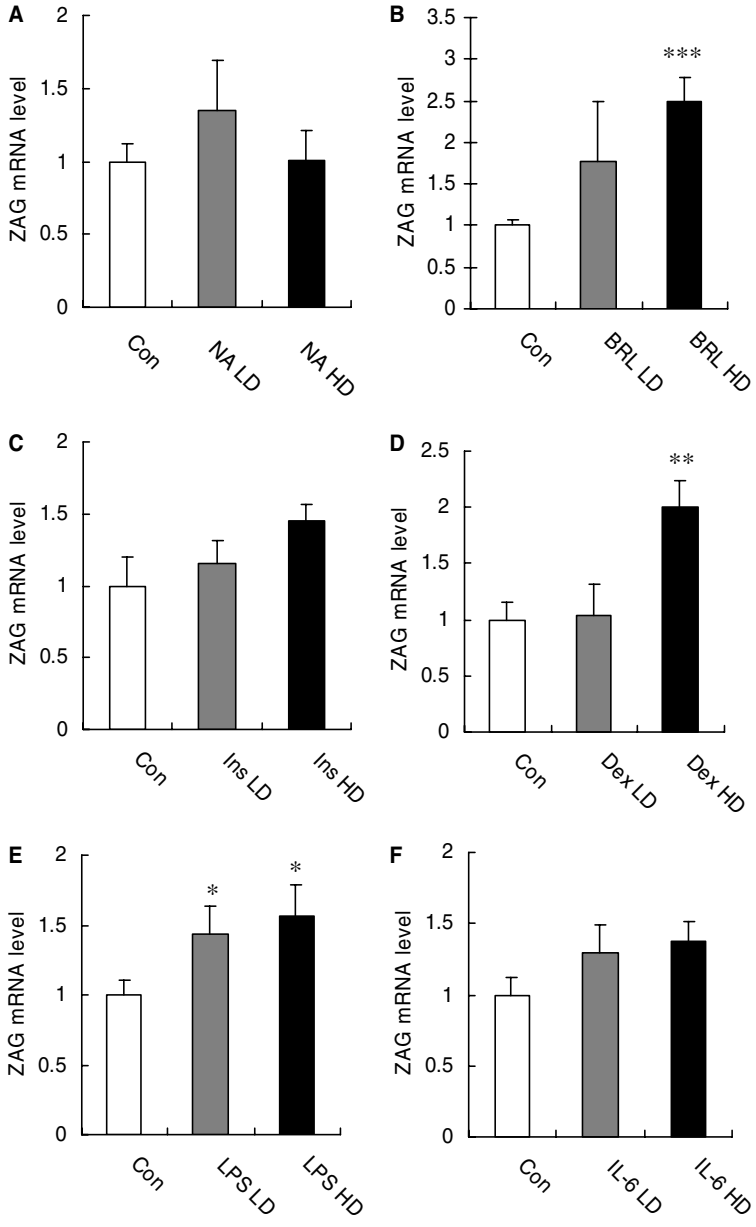

Fig. 4. Effect of noradrenaline (NA; A), BRL-37344 (BRL; B), insulin (Ins; C), dexamethasone (Dex; D), lipopolysaccharide (LPS; E), and IL-6 (F) on ZAG relative mRNA levels in SGBS cells. Cells were harvested at day 11 after the induction of differentiation and incubated for 24 h in a medium containing noradrenaline, BRL-37344, insulin, dexamethasome, LPS or IL-6 (LD, low dose; HD, high dose). No reagent was added to the medium of control cells. Cells were pre-incubated for 24 h in dexamethasone-free medium before treatment. NA LD, 100 nM; NA HD, 1 μM; BRL LD, 100 nM; BRL HD, 1 μM; Ins LD, 1 μM; Ins HD, 10 μM; Dex LD, 5 nM; Dex HD, 25 nM; LPS LD, 10 ng/ml; LPS HD, 100 ng/ml; IL-6 LD, 1 ng/ml; and IL-6 25, ng/ml. ZAG mRNA levels, measured by real-time PCR, are expressed relative to controls. Data are means ± S.E.M. for groups of 5–6. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$, compared with controls.

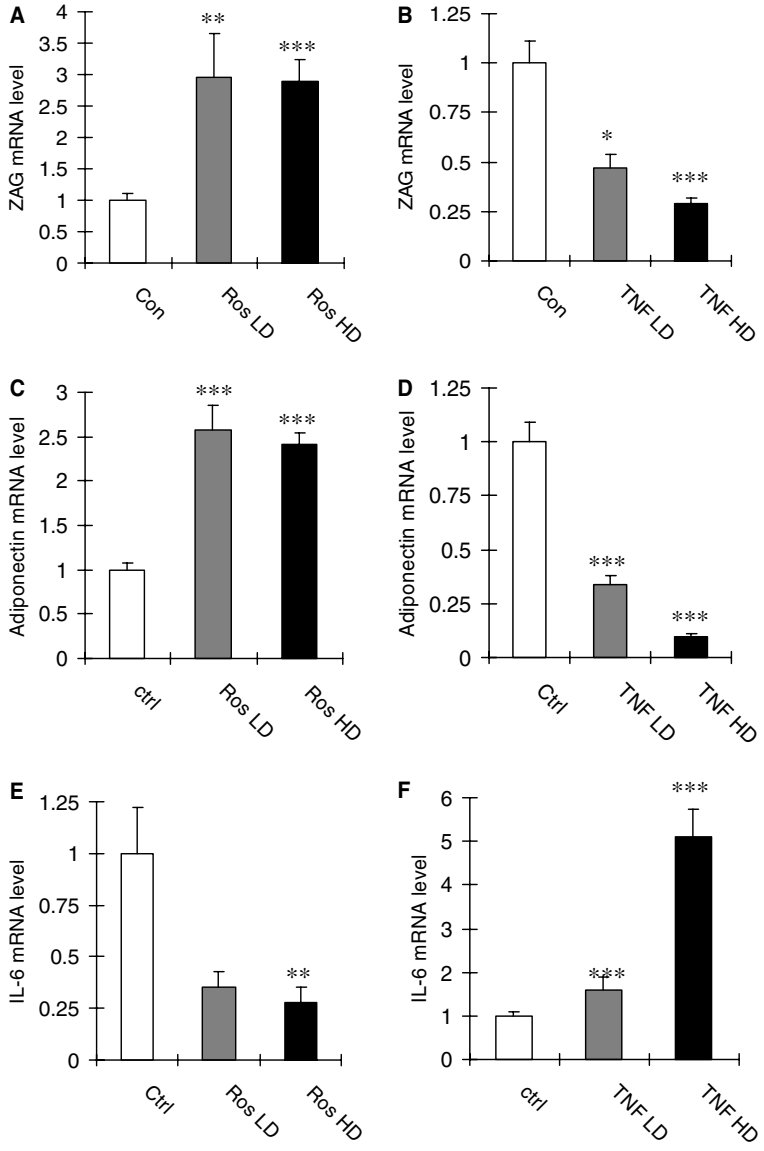

Fig. 5. Effect of rosiglitazone (Ros) and TNF- α on ZAG (A,B), adiponectin (C,D), and IL-6 (E,F) relative mRNA levels in SGBS cells. Cells were harvested at day 11 after the induction of differentiation and incubated for 24 h in a medium containing rosiglitazone or TNF-α (LD, low dose; HD, High dose). No reagent was added to the medium of control cells. Cells were pre-incubated for 24 h in dexamethasone-free medium before treatment: Ros LD, 100 nM; Ros HD, 1μM; TNF LD, 1 ng/ml; and TNF HD, 50 ng/ml. ZAG, adiponectin and IL-6 mRNA levels, measured by real-time PCR, are expressed relative to controls. Data are means ± S.E.M. for groups of 5–6. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$, compared with controls.

(Fig. 5C), while IL-6 mRNA was reduced 4-fold ($P < 0.01$; Fig. 5E).

Finally, the effects on ZAG gene expression of the inflammation-related agents, LPS, IL-6, and TNF-α were examined. LPS induced a very small (~50%), albeit statistically significant ($P < 0.05$), increase in ZAG mRNA level (Fig. 4E). Addition of IL-6 did not affect ZAG gene expression (Fig. 4F). In contrast, TNF-α induced a dose-dependent reduction in ZAG mRNA, the level decreasing 4-fold ($P < 0.001$) with the higher dose (Fig. 5B). The effect of TNF-α on ZAG expression was compared with that on adiponectin and IL-6. TNF-α induced a 10-fold reduction ($P < 0.001$) in adiponectin mRNA at the higher dose (Fig. 5D); in contrast, IL-6 mRNA level was increased 5-fold ($P < 0.001$) with the higher dose (Fig. 5F).

4. Discussion

The present study demonstrates, using SGBS cells in culture, that ZAG is expressed by human adipocytes, ZAG mRNA, and protein being present in differentiated adipocytes but not in preadipocytes. ZAG mRNA was also evident in freshly isolated human adipocytes, with only a very weak signal being found in cells of the stromal-vascular fraction (which could reflect expression in any component of that fraction, including macrophages and immature adipocytes). In SGBS cells the signal for ZAG mRNA appeared from day 1 after the induction of differentiation, with mRNA levels rising rapidly and reaching a peak at day 8 post-induction. ZAG protein was also detected in adipocytes at day 3 post-induction and thereafter. The presence of ZAG mRNA and

protein shortly after the induction of differentiation implies that ZAG is an early marker of differentiation in SGBS adipocytes.

Importantly, the present study indicates that ZAG, which contains a secretory signal sequence [4], is secreted by mature adipocytes and is therefore a novel adipokine. ZAG was detected in the medium from day 6 post differentiation, later than it was detected in the adipocytes (day 3). This difference may reflect a delay in the secretion of ZAG from adipocytes, or the dilution of the protein by the medium such that detection is compromised when the concentration is low; caution needs to be exercised, however, in the interpretation since the measurements of ZAG in the cells and the medium were not made at identical timepoints. ZAG could in principle function locally within WAT in an autocrine/paracrine manner, and/or contribute to the circulating pool of the protein to act distantly as an endocrine signal. The previously observed lipolytic effect of ZAG on adipocytes [6], together with the greatly elevated expression in WAT during the fat loss of cancer cachexia, suggests that there may well be a major local autocrine/paracrine action.

The regulation of ZAG production in WAT is unknown, although an initial study in 3T3-L1 adipocytes suggested that at least in mice both dexamethasone and β_3-adrenoceptor agonists might be stimulatory [11]. The present study shows that several factors influence ZAG gene expression in human adipocytes. Although there was no effect of noradrenaline, some sympathetic involvement is suggested by the dose-dependent increase in ZAG mRNA following treatment with the β_3-adrenoceptor agonist, BRL-37344. While this is similar to previous observations with 3T3-L1 adipocytes [11], it is surprising since the abundance of β_3-adrenoceptors in adipose tissue is much lower in humans than in rodents [16] and further consideration may be needed.

Insulin had no significant effect on ZAG gene expression, but a small increase in ZAG mRNA level was observed with dexamethasone, suggesting some involvement of glucocorticoids consistent with 3T3-L1 adipocytes [11]. Dexamethasone has been shown to strongly induce ZAG mRNA in human breast cancer cells [17]. A very small stimulatory effect was also observed with the inflammatory agent LPS, although IL-6 had no significant effect on ZAG gene expression.

The most substantial effects on ZAG gene expression were obtained with rosiglitazone and TNF-α. Treatment with rosiglitazone, a selective PPARγ agonist, induced a 3-fold increase in ZAG mRNA level in SGBS cells, paralleling its effect on adiponectin mRNA, thus indicating that the PPARγ nuclear receptor is involved in the regulation of ZAG synthesis. PPARγ ligands have been implicated in the expression of several adipokines, repressing those linked to insulin resistance as well as the inflammatory response, such as resistin [18,19], TNF-α [20,21], and PAI-1 [22]. In contrast, PPARγ ligands stimulate adiponectin, an insulin-sensitizing and anti-inflammatory adipokine [23,24], as also observed in this study. Whether the stimulatory effect of rosiglitazone on expression implies a role for ZAG in insulin sensitivity or in mitigating the inflammatory response in adipose tissue is not clear.

The largest effect on ZAG synthesis was with the proinflammatory cytokine TNF-α. TNF-α led to a dose-dependent decrease in ZAG mRNA levels, with a 4-fold reduction at the high dose, and this again paralleled the changes in adiponectin mRNA. Several adipokines implicated in the inflammatory response of adipose tissue, such as leptin, IL-6, MCP-1, and nerve growth factor, are substantially stimulated by TNF-α in 3T3-L1 adipocytes [25–28]. Thus, the inhibitory effect of TNF-α on ZAG expression reported here suggests that ZAG is unlikely to be part of the pro-inflammatory response of human adipocytes.

TNF-α is also considered to be a cachectic mediator, and it has been shown to stimulate lipolysis in differentiated 3T3-L1 cells and human adipocytes [29–31]. Given that ZAG can function as a lipid-mobilizing factor, inhibition of ZAG expression in adipocytes by TNF-α indicates that the two cachectic mediators have different effects on lipid metabolism within adipose tissue. It has been suggested that the lipolytic effect of TNF-α may be dependent on inhibition of the lipid-droplet associated protein perilipin, since TNF-α-induced lipolysis in human adipocytes parallels a decrease in perilipin synthesis [32]. In contrast, the lipid-mobilizing effect of ZAG has been attributed to the activation of β_3-adrenoceptors and the cAMP pathway in rodents [7], although it is unclear whether this is also the case in humans.

TNF-α has been shown to inhibit adiponectin gene expression in 3T3-L1 adipocytes [33] and in human adipose tissue in organ culture [34]; as shown here, it also inhibits adiponectin expression in SGBS adipocytes. The inhibitory effects of TNF-α together with the stimulatory effect of rosiglitazone on ZAG synthesis in adipocytes mirrors the regulation of adiponectin by the two factors, which may point to a potential functional link between the two adipokines. Indeed, it has been reported that overexpression of ZAG in 3T3-L1 adipocytes leads to an increase in adiponectin mRNA, raising the possibility that ZAG might be a candidate gene in the regulation of body weight [12].

In conclusion, the work presented here demonstrates that human adipocytes express the ZAG gene and that ZAG protein is secreted by differentiated adipocytes. The study also indicates that both TNF-α and the PPARγ nuclear receptor may be important in the regulation of ZAG expression. ZAG appears to be a novel adipokine, which may be involved in lipid metabolism and other processes in human adipose tissue.

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