



IL-33, a recently identified interleukin-1 gene family member, is expressed in human adipocytes

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ABSTRACT

Inflammation occurs in adipose tissue in obesity. We have examined whether IL-33, a recently identified IL-1 gene family member, and its associated receptors are expressed in human adipocytes. IL-33, IL-1RL1 and IL-1RAP gene expression was observed in human visceral white fat, in preadipocytes and in adipocytes (SGBS cells). Treatment with TNF α for 24 h induced a 6-fold increase in IL-33 mRNA level in preadipocytes and adipocytes. Time-course studies with adipocytes showed that the increase in IL-33 mRNA with TNF α was maximal (>55-fold) at 12 h. This response was markedly different to IL-1 β (peak mRNA increase at 2 h; 5.4-fold) and IL-18 (peak mRNA increase at 6 h; >1500-fold). Exposure of adipocytes to hypoxia (1% O₂, 24 h) did not alter IL-33 mRNA level; in preadipocytes, however, there was a 3-fold increase. Human adipocytes and preadipocytes express IL-33, but the various IL-1 family members exhibit major differences in responsiveness to TNF α .

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Introduction

Increased adipose tissue mass is the characteristic phenotype of obesity. It is now widely recognised that the obese state is accompanied by chronic, low-grade inflammation which may be the underlying cause of several of the associated metabolic disorders, particularly type 2 diabetes and the metabolic syndrome [1–3]. White adipose tissue produces a multiplicity of adipokines which can influence a range of biological functions, especially energy balance, lipid metabolism and insulin sensitivity. A major group of adipokines are inflammation-related, including TNF α , IL-1 β , IL-6, IL-8, and MCP-1 [3,4]. The source of these inflammation mediators can be both the adipocyte and non-adipocyte (pre-adipocytes, and macrophages) components of the tissue.

Members of the interleukin (IL)-1 family, namely IL-1 β , IL-1 receptor antagonist (IL-1Ra) and IL-18 are reported to exhibit elevated circulating levels in obese subjects [5,6]. Furthermore, IL-18 levels are modulated with weight change [5]. IL-1 β has been recognised as an adipokine for several years [6,7], and more recently IL-18 was shown to be expressed in human WAT and in adipocytes [8,9]. IL-18 gene expression in human adipocytes in culture was found to be dramatically increased following treatment with the

pro-inflammatory cytokine TNF α . However, although evidence for IL-18 secretion from adipocytes has been reported [8] the levels were at the limit of detection of the immunoassay employed and we were unable to detect any release of IL-18 even in adipocytes treated with TNF α [9].

A recent addition to the IL-1 gene family is IL-33 which is closest in similarity to IL-18 [10]. IL-33 is synthesised as a 30 kDa peptide which is cleaved by caspase 1 to form an active 18 kDa mature peptide. IL-33 is widely expressed in tissues, but specifically located within cell types such as epithelial linings and smooth muscle cells [10]. The cytokine mediates its effects through binding to the membrane bound receptor, IL-1RL1 (ST2) [10] and the accessory protein, IL-1RAP which is common to IL-1 β [11,12]. In contrast to IL-1 β and IL-18, IL-33 is thought to have anti-inflammatory properties and essentially drives T_H2 responses [10], although a T_H1 response has been reported [13]. A pro-inflammatory role has also been described in inflammatory arthritis [14]. IL-33 is known to be associated with areas of inflammatory tissue in Crohn's disease and rheumatoid arthritis [15]. An additional, dual-role has been proposed for IL-33 [15] in which it may act as a nuclear signal, and this is similar to that first identified for IL-1 α [16].

Since adipocytes express the key IL-1 gene family members IL-1 β and IL-18, we have investigated whether the IL-33 gene and the genes encoding its receptor complex are expressed in human WAT and in isolated adipocytes and preadipocytes. Furthermore,

Abbreviations: WAT, white adipose tissue; HIF-1 α , hypoxia-inducible factor 1 α ; POLR2A, RNA II polypeptide A.

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whether IL-33 expression is modulated by TNF α has been examined.

Materials and methods

Human adipose tissue. Omental white fat was obtained from 3 obese subjects (body mass index 43 ± 6) undergoing gastroplasty (provided by Dr. Jon Pinkney, University of Liverpool); the subjects did not exhibit any ongoing disease (e.g., infections, cancer). After removal, the fat samples were frozen in liquid N₂ and stored at -80°C . Informed consent for the removal of fat samples was obtained from the patients and ethical approval granted through the Sefton Ethics Committee.

Cell culture. SGBS preadipocytes, derived from the subcutaneous WAT depot of a patient with Simpson–Golabi–Behmel syndrome, were a gift from Professor Martin Wabitsch (University of Ulm). The cells were cultured and treated as previously [9]. Additionally, preadipocytes were grown to full confluence and pre-treated with serum-free medium for 24 h prior to addition of human recombinant TNF α (5 ng/ml) for a further 24 h. For the hypoxia experiments, pre-adipocytes and adipocytes (day 15 post-induction) were placed in a MIC-101 modular incubator chamber (Billups–Rosenberg), which was flushed with 1% O₂/94% N₂/5% CO₂, sealed and placed at 37°C for 24 h as indicated. Control cells were cultured in normoxia (21% O₂/5% CO₂). Cells were recovered directly in 700 μl of TRI-reagent (Sigma) for isolation of RNA.

RT-PCR. First strand cDNA was synthesized from 1 μg of total RNA using Superscript II reverse transcriptase (Invitrogen) with 250 ng random hexamers (Invitrogen) and treated with DNase I (Ambion). Oligonucleotide primer pairs were used for PCR amplification (50 ng of template) with ThermoStart DNA polymerase (ABgene) and were of the following sequences: human IL-33—For:5'-CATCTGGTACTCGCTGCCTGTC-3', Rev:5'-CAACACCGTCACC TGATTCATT-3' (298 bp); IL-1RL1—For:5'-TCCCACTCAGGAAAGAA-3'; Rev:5'-CTCCGATTACTGGAAACA-3' (476 bp); IL-1RAP1—For:5'-AGAACCGCATTAGTAAGGAGAAA-3'; Rev:5'-CTTACAGTCAGAGTCC TGGTGAG-3' (431 bp); β -actin [9]. Amplicons were custom sequenced (MWG Biotech).

Real-time PCR. Relative quantification of gene expression was determined, as previously [9], by the $2^{-\Delta\Delta\text{Ct}}$ method using real-time PCR with a Mx3005P cyclor (Stratagene). Values were normalised to either POLR2A (TNF- α) or β -actin (hypoxia) and the results expressed as fold change relative to controls. A human IL-33 gene expression assay was obtained from Applied Biosystems (Hs00369211_m1). All other primer/probe sequences were designed using Beacon Designer software (Premier BioSoft Int) and synthesized commercially (Eurogentec). The sequences were: human IL-1 β , For:5'-TGGCCCTAAACAGATGAAGTGC-3'; Rev:5'-GTA GTGGTGGTCCGAGATTCG-3'; probe:5'-FAM-ACCTGGACCTCGCC CTCTGGATGG-TAMRA-3' (91 bp); IL-18, β -actin [9]; POLR2A [17].

RT was performed as described above. Real-time PCR was performed in 96-well plates using a qPCR Core Kit (Eurogentec), with 1/50th dilution for IL-33 gene expression assay mix or IL-1 β , IL-18, β -actin, POLR2A [9,17] for 40 cycles.

Immunodetection of IL-33. IL-33 protein was determined using a commercial human ELISA set (Apotech). Detection was enhanced using streptavidin peroxidase antibody (Jackson Immunoresearch) and developed using the substrate tetramethylbenzidine (Axxora Inc). The ELISA set sensitivity was 5 pg/ml. Culture medium from untreated and treated (TNF α or 1% O₂) cells was collected for analysis. Cell or tissue lysates were prepared in SHE buffer (sucrose, Hepes, EDTA, pH 7.4).

Statistics. The statistical difference between groups was assessed by Student's unpaired 't' test.

Results

Expression of IL-33 gene and receptors in white adipose tissue and human adipocytes

The presence of a band consistent with IL-33 mRNA was observed on PCR gels of cDNA isolated from human white adipose tissue (Fig. 1A). The tissue was also found to express the IL-33 related receptors, IL-1RL1 (ST-2) and IL-1 receptor accessory protein (IL-1RAP). Sequence analysis confirmed the identity of the PCR products. To determine whether IL-33 was expressed in adipocytes and preadipocytes a human cell culture system was used (SGBS cells). IL-33 mRNA, and the mRNAs encoding IL-1RL1 (ST-2) and IL-1, were found in both preadipocytes and adipocytes (day 15 post-induction of differentiation) by RT-PCR (Fig. 1B). IL-33 gene expression was also observed in THP-1 macrophages (cells treated with PMA), but interestingly not in untreated monocytes or KG-1 cells (positive control cells for IL-18); however, the corresponding receptor mRNAs were present in both PMA treated and untreated cells.

IL-33 protein was detected by ELISA in both human WAT (0.17–1.99 ng/mg protein; $n = 4$) and in cell lysates of SGBS adipocytes (0.07–0.35 ng/ml; $n = 3$), indicating that the mRNA was transcribed.

Expression of IL-33 in response to TNF α

TNF α has a major stimulatory effect on the expression of a number of cytokines and other inflammation-related genes in human adipocytes [18]. To determine whether IL-33 expression in adipocytes is regulated by TNF α , SGBS cells were treated with the

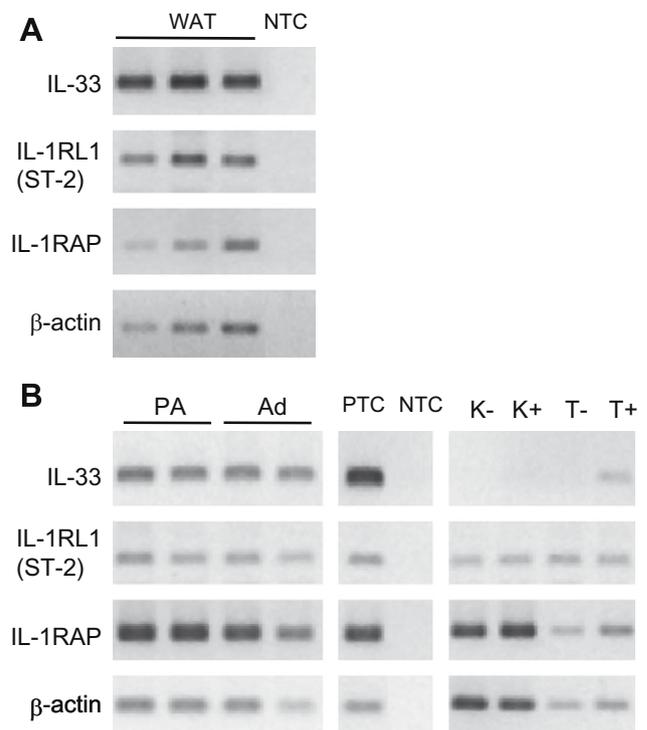


Fig. 1. RT-PCR of IL-33 and related genes in human adipose tissue and adipocytes. RT-PCR was performed and products visualised as described in the Materials and methods. Representative gels are shown for (A) isolated human adipose tissue; (B) cells in culture; PA, SGBS preadipocytes; Ad, SGBS adipocytes; WAT, white adipose tissue; NTC, no template control; PTC, positive template control (liver); K-, KG-1 cells untreated; K+, KG-1 cells TNF α treated; T-, THP-1 monocytes; T+, THP-1 macrophages (+PMA).

pro-inflammatory cytokine either as preadipocytes or when fully differentiated into mature adipocytes. Quantification of IL-33 and other IL-1 family mRNAs was performed by real-time PCR. As shown in Fig. 2A, incubation of SGBS adipocytes with TNF α for 24 h increased IL-33 mRNA level by 5.8-fold at a dose of 5 ng/ml (low dose) compared to the untreated controls, with no further increase with a higher dose (100 ng/ml) of the cytokine.

In contrast to IL-33, IL-1 β mRNA level decreased 2-fold with the low dose of TNF α , while with the high dose, no change in level was observed (Fig. 2B). IL-18 expression was also examined, and consistent with a previous study [9], TNF α treatment resulted in a substantial increase in IL-18 mRNA level (up to 100-fold) at both the low and high doses (Fig. 2C). Fig. 2D–F shows the response of the IL-33, IL-1 β , and IL-18 genes in preadipocytes to treatment with TNF α (5 ng/ml, 24 h). IL-33 mRNA (Fig. 2D) was increased by a similar amount (6.8-fold) to that observed with the mature adipocytes.

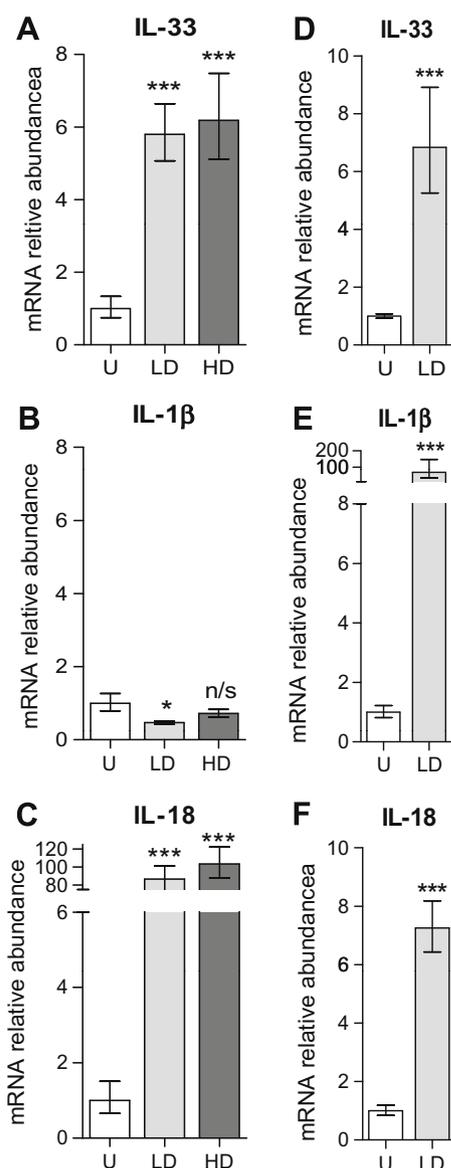


Fig. 2. Effect of TNF α on the relative mRNA abundance of IL-33 and the other IL-1 gene family members in human adipocytes and preadipocytes. mRNA levels in untreated and treated cells (24 h) was quantified by real-time PCR and the data normalised to Pol2A mRNA. (A–C) SGBS adipocytes; (D–F) SGBS preadipocytes. U, untreated cells; LD, low dose TNF α (5 ng/ml); HD, high dose TNF α (100 ng/ml). Results are mean values \pm SE for 5–6 sets of cells. * P < 0.05, *** P < 0.001, compared with untreated cells.

However, in marked contrast to adipocytes, in the case of IL-1 β (Fig. 2E) there was a dramatic increase (up to 70-fold) in the mRNA level in the preadipocytes. Although TNF α significantly increased the level of IL-18 mRNA (by 7.3-fold) in preadipocytes compared to the untreated cells (Fig. 2F), the increase was considerably less in preadipocytes than in the mature adipocytes. The inclusion of serum during TNF α treatment of the preadipocytes attenuated the response for both IL-1 β and IL-18 (14.4-fold and 1.7-fold, respectively). Surprisingly, the relative level of IL-33 mRNA was increased by 14.3-fold compared to the untreated controls (data not shown). Release of IL-33 into the culture media of untreated and TNF α treated cells was not detected.

Time course of response to TNF α

To further investigate the effects of TNF α on IL-33 and IL-1 β gene expression, SGBS adipocytes were exposed to the pro-inflammatory cytokine for time periods up to 48 h. As seen in Fig. 3A, IL-33 mRNA level was significantly increased by 4 h following the administration of TNF α with a peak increase (57.6-fold) by 12 h, the mRNA level returning to basal levels by 48 h. In contrast, the modest peak (5.4-fold increase) in IL-1 β mRNA level (see Fig. 3B) was seen as early as 2 h; the level returned to normal by 12 h. By 48 h IL-1 β mRNA level was significantly decreased relative to the untreated controls, there being a 4.5-fold reduction. IL-18 expression was examined for comparison and showed a pattern similar to our previous study [9]. Increased IL-18 mRNA levels were detected as early as 2 h (200-fold increase) with a further substantial rise (to 1600-fold) by 6 h, followed by a decline to lower but still elevated levels (67-fold) at 48 h (Fig. 3C).

Effect of hypoxia on IL-1 gene family expression

In the final set of experiments, SGBS cells were subjected to 1% O $_2$ for 24 h in order to examine whether expression of members of the IL-1 gene family is influenced by hypoxia. As shown in Table 1, both IL-33 and IL-18 mRNA levels in SGBS adipocytes did not change significantly compared to adipocytes under normoxia. However, IL-1 β mRNA level was decreased 2-fold. Expression of the glucose facilitative transporter, GLUT-1, regarded as a key marker for hypoxia, was measured as a reference gene and the mRNA level was found to increase 20-fold (data not shown). When preadipocytes were placed in 1% O $_2$, IL-18 and IL-1 β levels were unchanged. In contrast, IL-33 mRNA level increased by 3-fold compared to preadipocytes maintained under normal O $_2$ levels. GLUT-1 mRNA level increased 3-fold (data not shown) consistent with previous findings [19]. IL-33 protein was not detected in the culture media of either normoxic or hypoxia-treated adipocytes and preadipocytes.

Discussion

Adipose tissue produces a large number of inflammation-related adipokines, including both pro-inflammatory (e.g. TNF α , IL-6) and anti-inflammatory (e.g. IL-10, adiponectin) factors as well as acute phase proteins (e.g. haptoglobin, serum amyloid A), the majority of which are elevated with increased adiposity; conversely adiponectin production is reduced [3,4]. The interleukin-1 gene family consists of 11 members [20] with the four most intensively studied, IL-1 α , IL-1 β , IL-1Ra, and IL-18 [6,8,9,21] also being expressed in adipose tissue.

In the present study, we have found from studies on SGBS cells that the latest member of the IL-1 gene family to be identified, IL-33, is expressed in both human adipocytes and pre-adipocytes. This was corroborated (data not shown) in human preadipocytes obtained from a commercial source (Zen-Bio). Moreover, IL-33

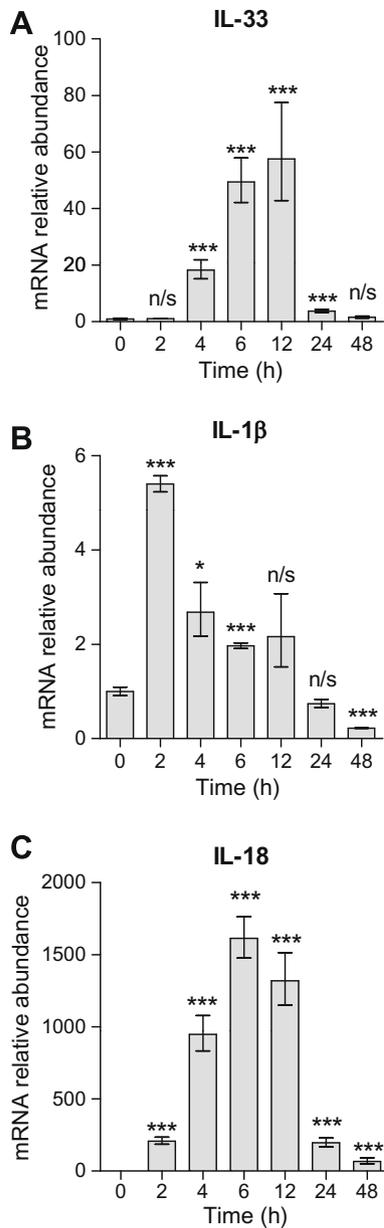


Fig. 3. Time course of effect of TNF α on the expression of IL-33, IL-18, and IL-1 β in human adipocytes. SGBS cells were treated with TNF α (25 ng/ml) for up to 48 h. Relative mRNA levels for IL-33 (A), IL-1 β (B), and IL-18 (C) were quantified by real-time PCR. Gene expression data was normalised to Pol2A mRNA. Results are mean values \pm SE for 5–6 sets of cells. * P < 0.05, *** P < 0.001, compared with time '0'.

expression was detected in human omental white adipose tissue. Expression of the known IL-33 receptors, interleukin-1 receptor

like-1 (IL-1RL1 or ST2) [10] and the interleukin-1 receptor accessory protein (IL-1RAP), was additionally detected; the latter is also involved in IL-1 β signal transduction [22]. The expression of both IL-33 related receptors in pre-adipocytes, adipocytes and white adipose tissue would imply a possible role for IL-33 signalling within the tissue via autocrine and/or paracrine routes.

Little is known of the interaction between the cytokine cascade networks operating within adipose tissue. It is, however, increasingly evident that cellular cross-talk exists between adipocytes, preadipocytes and macrophages (both resident and invading) in the tissue. These paracrine/autocrine interactions are a crucial factor in adipose tissue function. Circulating levels of the pro-inflammatory cytokine, TNF α , are elevated in obesity and this adipokine has been implicated as a causal agent in insulin resistance [23]. The treatment of adipocytes with TNF α has been shown to regulate the expression of a number of adipokines [18]; it induces a dramatic increase in IL-18 mRNA levels in human adipocytes [9].

IL-33 expression is also increased in response to TNF α . Moreover, the relative response varies amongst the IL-1 gene family members in a dose and time-dependent manner. The extent of the IL-33 response to TNF α is similar in both adipocytes and preadipocytes. However, IL-1 β gene expression is induced to substantial levels whilst IL-18 gene expression is greatly attenuated in preadipocytes compared to adipocytes. The time-course of the stimulation of IL-33 expression in adipocytes when treated with TNF α was found to be different to the other two IL-1-like genes. The time profile of expression is much broader (4–24 h) for IL-33 compared with the very short (2–6 h) and small response with IL-1 β , while there was also a broad (2–48 h) response in the case of IL-18. Interestingly, IL-1 β mRNA levels were found to be significantly decreased by 48 h compared with the control adipocytes.

The reasons for these differences are unclear. It would suggest that signalling pathways may be differentially expressed with the various cellular components of adipose tissue during development and differentiation which may be critical to intercellular communication within the tissue. Collectively, the data implies that the IL-1 gene family exhibits a differential response to the TNF α inflammatory cascade within adipose tissue. The rapid induction of IL-1 β suggests that this may indeed be an upstream response, but it is not clear if any interactions exist between these three cytokines. However, a recent report found that synovial fibroblasts, which do not express detectable IL-33 in the resting state, could be induced to express this factor when treated with a combination of IL-1 β and TNF α [14]. We were unable to detect IL-33 protein in the culture medium following treatment of cells with TNF α . This suggests that if IL-33 is secreted from preadipocytes/adipocytes (at levels below the sensitivity of the assay) it is likely to act locally, a scenario similar to that of IL-18 [9]. Alternatively, IL-33 may act intracellularly [15]. Indeed, IL-33 immunoreactivity was observed in cell lysates of human WAT and SGBS adipocytes.

We have previously proposed that hypoxia within adipose tissue as a result of increased fat mass may be a key trigger for the induction

Table 1
Effect of hypoxia on mRNA abundance of the IL-1 gene family members in human SGBS cells.

Cell	Gene	Treatment	
		21% O ₂	1% O ₂
Adipocyte	IL-33	1.00 (+0.15; -0.13)	1.56 (+0.48; -0.37)
	IL-1 β	1.00 (+0.18; -0.15)	0.47 (+0.07; -0.06)***
	IL-18	1.00 (+0.24; -0.19)	1.00 (+0.29; -0.22)
Preadipocyte	IL-33	1.00 (+0.35; -0.26)	3.33 (+1.43; -1.00) [†]
	IL-1 β	1.00 (+0.25; -0.20)	1.24 (+0.26; -0.21)
	IL-18	1.00 (+0.18; -0.15)	1.02 (+0.13; -0.11)

mRNA levels in cells exposed to 21% O₂ or 1% O₂ for 24 h was quantified by real-time PCR and the data normalised to β -actin. Results are mean $2^{-\Delta\Delta Ct}$ values (\pm SE) for 5–6 sets of cells. * P < 0.05, *** P < 0.001, relative to controls maintained at normal oxygen levels (21% O₂).

of inflammation-related adipokines [3] and this concept has gained support [4,24]. Exposure of preadipocytes to a low O₂ environment resulted in a modest increase in IL-33 gene expression, but no changes in IL-1 β or IL-18 expression were observed. However, when adipocytes were rendered hypoxic, IL-33 expression was unchanged, as was that of IL-18. Surprisingly, IL-1 β showed a modest decline in relative gene expression. In a PCR array study using human adipocytes, caspase-1 (the enzyme that cleaves the propeptides of the IL-1 family) was found to be markedly down-regulated by hypoxia [25]. This implies that production of mature peptides would be compromised in the unlikely event of increased expression of the IL-1 family members. Collectively, the data suggests that there is no direct involvement of hypoxia on the IL-1 family over this time period. However, chronic hypoxia which is a more realistic environment within adipose tissue in obesity may have some influence.

A key question is the functional role of IL-33 in adipose tissue. The indication that IL-33 is an anti-inflammatory mediator, or indeed has protective cardiovascular roles [26], has parallels with other inflammation-related adipokines such as IL-10 and adiponectin [27,28]. The potential role of IL-33 as a nuclear signal is intriguing and may be relevant to the action of this factor in adipocytes. In conclusion, the present study expands the range of inflammation-related factors that are expressed by adipocytes.

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