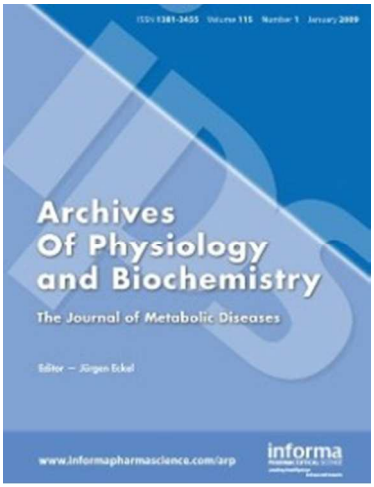


# PCR array and protein array studies demonstrate that IL-1β (interleukin-1β) stimulates the expression and secretion of multiple cytokines and chemokines in human adipocytes

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# PCR array and protein array studies demonstrate that IL-1β (interleukin-1β) stimulates the expression and secretion of multiple cytokines and chemokines in human adipocytes

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**RUNNING TITLE**: IL-1β stimulates inflammatory adipokines in human adipocytes

**Keywords**: Adipose tissue, gene expression, inflammation, obesity

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**Abstract**

The role of IL-1β in regulating the expression and secretion of cytokines and chemokines by human adipocytes was examined. Adipocytes were incubated with human IL-1β for 4 or 24 h. The expression of a panel of 84 cytokine/chemokine genes was probed using PCR arrays. IL-1β stimulated the expression of >30 cytokine/chemokine genes on the arrays; 15 showed >100-fold increases in mRNA at 4 or 24 h including *CSF3*, *CXCL1*, *CXCL2*, *CXCL12* and *IL8*. *CSF3* exhibited a 10,000-fold increase in mRNA at 4 h. *ADIPOQ* was among the genes whose expression was inhibited. Protein arrays were used to examine the secretion of cytokines/chemokines from adipocytes. IL-1β stimulated the secretion of multiple cytokines/chemokines including MCP-1, IL-8, IP-10, MIP-1α and MCP-4. The most responsive was IP-10, which exhibited a 5,000-fold increase in secretion with IL-1β. IL-1β is likely to play a substantial role in stimulating the inflammatory response in human adipocytes in obesity.

## Introduction

Obesity is defined by an expansion in white adipose tissue mass, and changes in the tissue are considered to underlie the development of several obesity-associated diseases including insulin resistance and the metabolic syndrome (Kopelman, 2000; Hotamisligil, 2006; Rosen & Spiegelman, 2006; Bluher, 2009). White adipose tissue is a major endocrine and secretory organ, with white adipocytes releasing a multiplicity of factors in the form of diverse adipokines, as well as fatty acids and other lipid moieties (Frühbeck *et al.*, 2001; Trayhurn & Beattie, 2001; Rajala *et al.*, 2003; Trayhurn, 2013). A number of adipokines are associated with the immune system and inflammation, and the expression and secretion of some of these protein signals and factors rise in obesity (Rajala *et al.*, 2003; Hotamisligil, 2006; Trayhurn, 2013); examples include TNFα (tumour necrosis factor α), IL-6 (interleukin-6) and leptin. A notable exception is the hormone adiponectin, with its anti-inflammatory and insulin sensitising actions (Ouchi *et al.*, 1999; Yokota *et al.*, 2000; Berg *et al.*, 2001; Yamauchi *et al.*, 2001), the production and secretion of which falls as fat mass expands (Arita *et al.*, 1999; Hotta *et al.*, 2000). The establishment of an inflammatory state in adipose tissue in obesity, which includes the recruitment of activated macrophages and other immune cells (Weisberg *et al.*, 2003; Xu *et al.*, 2003; Pond, 2005; Bertola *et al.*, 2012; Brestoff *et al.*, 2015), is widely considered to underpin the development of the diseases associated with the obese state.

The factors which stimulate the production of inflammation-related adipokines have been widely examined in cell culture studies on rodent and human white adipocytes. The pro-inflammatory cytokine TNFα has a substantial stimulatory effect, increasing the expression and release of other inflammation-related factors including IL-6, NGF (nerve growth factor), MCP-1 (monocyte chemoattractant protein-1), MMP1 and MMP3 (matrix metalloproteinases 1 and 3) (Wang *et al.*, 2005; O'Hara *et al.*, 2009). There has, however, been little focus on the extent to which another classical pro-inflammatory cytokine, IL-1β (interleukin-1β) – which is implicated in a wide range of inflammatory diseases in other cells and tissues (see (Fietta *et al.*, 2014; Lopalco *et al.*, 2015)) - is important in stimulating the inflammatory response in adipocytes. Macrophage-conditioned medium has a major stimulatory effect on gene expression in human adipocytes and preadipocytes (Lacasa *et al.*, 2007; O'Hara *et al.*, 2009), particularly of matrix metalloproteinases and this effect on MMP expression and secretion is mimicked by IL-1β (O'Hara *et al.*, 2012). IL-1β has also been shown to stimulate the expression and release of several cytokines and chemokines, including IL-6 and MCP-1, and these changes are accompanied by down-regulation of the production of proteins in the insulin signalling pathway

(Gao *et al.*, 2014). In the case of human pre-adipocytes, IL-1β has been identified as a key regulator of IL-6 production (O'Hara *et al.*, 2012).

IL-1β is predicted to have extensive effects on the inflammatory response in human fat cells and this proposition has been examined in the present study. PCR arrays were used to measure the effect of IL-1β on the expression of a panel of 84 cytokine and chemokine genes, while protein arrays have been employed to determine the effects on the secretion of key inflammation-related adipokines.

## Materials and Methods

### *Cell culture*

Human fibroblastic preadipocytes (C-12730) and the requisite proprietary culture media were obtained from PromoCell (Germany). The cells had been isolated from the subcutaneous white adipose tissue of an obese Caucasian female, aged 59 years. The pre-adipocytes (5,000 cells/cm$^2$) were plated in 12-well plates and incubated in pre-adipocyte growth medium (C-27410); this medium contains 5% fetal calf serum, epidermal growth factor (10 ng/ml), hydrocortisone (1 µg/ml) and heparin (90 µg/ml). When the cells had reached confluence (6-7 days), growth media was removed and the cells were incubated in pre-adipocyte differentiation medium (C-27436) to induce differentiation. The differentiation medium is supplemented with insulin (0.5 µg/ml), dexamathasone (400 ng/ml), IBMX (44 µg/ml), thyroxine (9 ng/ml) and ciglitazone (3 µg/ml), and does not contain fetal calf serum. After 72 h the cells were transferred to Adipocyte Nutrition Medium (C-27438); this medium contains 3% fetal calf serum, insulin (0.5 µg/ml) and dexamethasone (400 ng/ml). The medium was changed every 2/3 days and the cells were used at 12 days after the induction of differentiation, by which time they contained multiple lipid droplets. The presence of lipid droplets was confirmed by Oil Red O staining.

Human recombinant IL-1β (Sigma, UK) was added to the wells at a dose of 2 ng/ml which previous studies have demonstrated provides a maximal, or near-maximal, response in human fat cells (Gao & Bing, 2011). Groups of adipocytes were treated with IL-1β for either 4 h or 24 h (from previous pilot studies, a near maximal response was evident at 4 h); control cells were incubated without IL-1β. After incubation, the medium was removed and the adipocytes washed with ice-cold PBS and frozen in TRI Reagent (Sigma UK) and stored at -80°C. The medium was stored at -20°C until required for analysis at which point it was thawed and kept on ice during the plating of samples; the medium was not centrifuged prior to analysis so the presence of vesicular structures containing soluble and membrane-associated cytokines cannot be excluded. Six sets of cells were taken for each group.

***RNA extraction***

Human adipocytes were homogenized in TRI Reagent (Sigma, UK) and total RNA isolated using the RNeasy® Micro Kit (SABiosciences, Qiagen, UK). The purity and concentration of RNA was quantified with a UV-Vis spectrophotometer (NanoDrop 1000, USA) in 1 µl samples; all samples had 260/280 nm and 260/230 nm average absorbance ratios of 2.0. The integrity of the RNA was analysed by an Agilent 2100 bioanalyser (Agilent Technologies, Germany). The RNA Integrity Number for RNA samples was equal to 10.

***PCR arrays***

Total RNA (1.5 µg) was DNAse-treated and reverse transcribed using a $RT^2$ First Strand Kit (SABiosciences, Qiagen, UK). Human cytokine and chemokine $RT^2$ Profiler™ PCR arrays with SYBR® Green were performed to screen the expression of 84 genes in mature human adipocytes exposed to IL-1β; the strategy was similar to that employed previously using hypoxia signalling pathway arrays to investigate the effects of low $O_2$ tension on gene expression in adipocytes (Wang *et al.*, 2008). PCR amplification was performed using real-time PCR detection (ABI StepOneplus™, Applied Biosystems, USA) with a two-step thermal cycling: 95°C for 10 min, followed by 40 cycles (95°C for 15 sec, 60°C for 1 min). Data were analysed using the comparative $^{\Delta\Delta}$Ct method and expressed as fold-changes in the target gene normalised to the mean of endogenous control genes (*ACTB, B2M, GAPDH, HPRT* and *RPLP0)* in adipocytes treated with IL-1β, and related to the expression of the untreated samples (normalised such that the controls = 1).

***Protein arrays***

Selected cytokines and chemokines released into the cell culture medium were measured using MSD Cytokines and Chemokines assay kits (Meso Scale Discovery, USA). These are sandwich immunoassays and provide a rapid and convenient method for measuring the levels of protein targets in a small volume sample. MSD plates were pre-coated with capture antibodies on independent and well-defined spots. Ten multi-spot plates were used: V-PLEX Cytokine Panel 1 human Kit (Ref: K15050D), V-PLEX Chemokine Panel 1 human Kit (Ref: K15047D) and V-PLEX Pro-inflammatory Panel 1 human Kit (Ref: K15049D).

Briefly, 50 µl samples or calibrators were added per well in duplicate and the plate was sealed and incubated at room temperature with shaking (700-1000 oscillations/min) for 2 h. The plate was washed three times with 200 µl/well of wash buffer and 25 µl of detection antibody solution was added to each well. The plate was then sealed and incubated at room temperature with shaking (700-1000 oscillations/min) for a further 2 h. Finally, the plate was washed as described previously and 150 µl of reading buffer was added to each plate and read using a MSD

instrument (SECTOR Imager 2400). The data were analysed using Meso Scale software. The lowest limit of detection was for IL-8 (0.01 pg/ml).

***Statistical analysis***

The statistical significance of differences between groups was assessed using Student's 't' test.

## Results

***Gene expression***

The human adipocytes used here were employed at 12 days after the induction of differentiation from fibroblastic preadipocytes. At this stage, they contained multiple lipid droplets as demonstrated by Oil Red O staining. The adipocytes were incubated with IL-1β for either 4 or 24 h, in order to examine both acute and sustained responses to the cytokine. Gene expression in the cells was then probed with a pathway-specific PCR array for 84 cytokine and chemokine genes. Stringent criteria of a >2.0-fold increase in mRNA level at $P<0.05$ (or more), were employed to assess whether IL-1β had a significant effect on gene expression.

At 4 h of treatment with IL-1β, the expression of 33 genes was significantly increased relative to control cells, amounting to >one-third of the genes represented on the arrays (Table 1). The most strongly upregulated genes were *CSF3*, *CXCL2*, *CXCL1* and *CXCL10*, each of which showed >1,000-fold increases in mRNA level (Table 1). IL-1β mRNA level was also increased >1,000-fold at 4 h, indicating a marked autocatalytic effect on the expression of the cytokine. Other cytokine and chemokine genes whose expression was stimulated by IL-1β at 4 h included *IL6*, *IL7*, *IL8*, *IL15*, *CCL5*, *MCP1*, *MCP2*, *MCP3* and *BMP2*. In contrast to the substantial number of genes where expression was stimulated by IL-1β, the expression of only three genes on the arrays was decreased at 4 h; these were *SPP1*, *IL16* and *NODAL*, which encode secreted phosphoprotein 1 (osteopontin), interleukin-16 and pro interleukin-16, respectively.

The mRNA level of slightly fewer genes was increased at 24 h of treatment with IL-1β than at 4 h – 25 genes compared with 33 at 4 h (Table 1). However, the pattern of stimulated genes was broadly similar at the two times. *CSF3* was the most highly stimulated gene at 24 h, as at 4 h, with an increase in mRNA level of nearly 5,000-fold at the longer time-point. Other highly upregulated genes at 24 h were *CCL5*, *CCL20*, *CCL19* and *CXCL1*, each of which exhibited a more than 1,000-fold increase in mRNA level. The highly pronounced autocatalytic effect on IL-1β expression had, however, terminated by 24 h. Of the 25 genes whose expression was increased following 24 h treatment with IL-1β, only *CXCL12* exhibited an increase at 24 h, but not at 4 h.

With some genes, such as *CSF3*, *IL8*, *CCL3*, *LIF* and *CXCL10*, the response to IL-1β was markedly lower at 24 h than at 4 h. For other genes, expression was higher at 24 h than at 4 h, and these include *CCL5*, *CCL19* and *CXCL5*. Examples of the overall pattern of responses to IL-1β are shown in Fig. 1. More genes were downregulated by IL-1β at 24 h than at 4 h – 11 as compared with 3 – and only the expression of *NODAL* was significantly inhibited at both time-points. Among the genes whose expression was decreased following 24 h treatment with IL-1β was *ADIPOQ* (Fig. 1), which encodes the adipocyte hormone adiponectin. The expression of one gene, *TGFB2,* was significantly raised at 4 h, but decreased at 24 h.

***Protein secretion***

The production and release of selected cytokines and chemokines in response to IL-1β was then examined in the culture medium employing protein arrays. The proteins were selected primarily on the basis of the PCR array data, including those where there was a strong response to IL-1β and where the Ct values implied that sufficient quantities of the protein were likely to be secreted. The measurements were made only on the 24 h samples, to maximise the likelihood of detecting proteins of low abundance, and the culture medium was screened for a total of nearly 30 proteins.

The release of 23 of the proteins examined was stimulated by IL-1β by at least 2-fold (Table 2). The proteins which showed the greatest concentration in the medium following treatment with IL-1β were IL-6, IL-8 and MCP-1, each of which was present at ng levels (between 2 and 8 ng/ml medium). The release of all three of these adipokines was substantially stimulated by IL-1β; this was also the case for most of the other cytokines and chemokines on the arrays (Table 2). Several proteins, including IP-10 (interferon gamma-induced protein 10), TNFα, IL-13, IL-4 and MIP-1β (macrophage inflammatory protein 1β), were either undetectable or barely detectable in the medium of control cells, secretion being only evident in the IL-1β treated adipocytes. This reflects the gene expression data where the mRNA was detected essentially only in treated cells. The overall pattern of responses to IL-1β are shown in Fig. 2.

The few examples of proteins where the release into the culture medium was not stimulated by IL-1β included IL-5, IL-16 and IL-1α. Indeed, IL-1α was the sole protein of those examined where secretion was strongly inhibited by IL-1β; although expression of the *IL1A* gene was stimulated at 4 h, at 24 h there was a decrease in mRNA level which was not, however, statistically significant.

## Discussion

The presence of inflammation in white adipose tissue in obesity, together with its link with the key diseases associated with the obese state (Hotamisligil, 2006; Rosen & Spiegelman, 2006; Bluher, 2009), has resulted in interest in the factors that underlie and modulate the inflammatory response in fat cells. A number of studies have examined the role of TNFα, a key pro-inflammatory factor, and of activators of PPARγ. TNFα has, for example, been shown to stimulate the expression and release of multiple inflammation-related factors including IL-6, MCP-1, VEGF (vascular endothelial growth factor) and NGF, while inhibiting the production of leptin (Peeraully *et al.*, 2004; Wang *et al.*, 2005; Wang & Trayhurn, 2006). TNFα is itself produced by adipocytes, being linked to the induction of insulin resistance (Hotamisligil *et al.*, 1993), and this cytokine was one of the first adipokines to be identified.

In a recent study using bead arrays, LPS (lipopolysaccharide) was shown to stimulate the expression and release of multiple cytokines and chemokines in human adipocytes (Meijer *et al.*, 2011). Adipocytes both exhibit immune cell functions and prime inflammation in a manner that it is independent of macrophages. Macrophages themselves not only participate directly in the inflammatory response in adipose tissue being recruited as tissue mass expands in obesity (Weisberg *et al.*, 2003; Xu *et al.*, 2003), but they also stimulate the production of inflammation-related factors in fat cells. Microarray studies on human adipocytes, for example, have demonstrated that conditioned medium from macrophages stimulates the expression of multiple genes (in excess of 1000) (O'Hara *et al.*, 2009). The major pathways modulated by macrophage-conditioned medium in adipocytes encompass those involved in inflammation, macrophage infiltration, glucose uptake and lipid metabolism. Among the most highly up-regulated genes in human fat cells are several matrix metalloproteinases, particularly *MMP1*, *MMP3* and *MMP10* (O'Hara *et al.*, 2009).

The secretion of MMPs from adipocytes is also strongly stimulated by macrophage-conditioned medium, consistent with tissue remodelling during inflammation. This effect on both gene expression and release of the encoded protein is mimicked by TNFα (O'Hara *et al.*, 2009). The extent to which other secreted factors from macrophages, particularly IL-1β, stimulates the production of inflammation-related factors by adipocytes is essentially unknown, despite the central role of this cytokine in inflammation and disease processes in other tissues (Fietta *et al.*, 2014; Lopalco *et al.*, 2015). Nevertheless, IL-1β has been shown to be a regulator of IL-6 production by human preadipocytes (O'Hara *et al.*, 2012), and to stimulate the expression and release of IL-6 and MCP-1 in human fat cells (Gao *et al.*, 2014). IL-1β, like TNFα, is an adipokine, being synthesised and secreted by adipocytes themselves. As such, both adipocytes

and macrophages are sources of IL-1β in adipose tissue, and the action of the cytokine on fat cells may be autocrine as well as paracrine.

The present study demonstrates that IL-1β has an extensive stimulatory effect on the expression and release of cytokines and chemokines in human adipocytes. Indeed, a wide range of these factors are clearly expressed and secreted by human fat cells, with production being markedly enhanced by IL-1β. Quantitatively, the most important factors released in response to IL-1β were MCP-1, IL-8, IL-6, VEGF, IP-10 and MIP-1α, with IP-10 showing the largest proportionate increase in secretion. Substantial responses to IL-1β were also evident for IL-4, GM-CSF (granulocyte macrophage-colony-stimulating factor), TNFα, IL12p70 (interleukin 12 subunit p70) and IFNγ (interferon γ), but the absolute amount of these factors released over the 24 h culture period was much lower. GM-CSF, which is coded by *CSF2*, exhibited the largest response at the gene expression level in terms of the fold increase in mRNA level.

It is, of course, important to recognise that high 'fold increases' can reflect what may be negligible gene expression or protein secretion in control, un-treated cells, leading to the denominator being minimal. In the case of the protein measurements, for a small number of cytokines/chemokines the levels observed in the medium from control adipocytes were at, or close to, the limit of detection of the arrays. Thus, in some cases the fold-change in response to IL-1β is likely to be an under-estimate. In practise, what is significant is that IL-1β induces a substantial stimulation of the expression and secretion of key cytokines and chemokines, rather than the precise scale of response. It should also be noted that as with a number of such studies, a limitation of the present work is that the adipocytes were derived from a single donor.

Stimulation of the production and release of some of these proteins by LPS (Meijer *et al.*, 2011), and also by TNFα, has been observed previously in human adipocytes, as noted above. This includes IL-6 and MCP-1 in the case of TNFα, while both IL-1β and LPS stimulate the secretion of factors such as IL-8, MIP-1β, IP-10, eotaxin and GM-CSF (Meijer *et al.*, 2011). There are, however, important differences in the response to IL-1β and LPS; for example, the secretion of IL-7 and IL-10 is reduced following treatment with LPS, while the expression and release of both these factors is stimulated by IL-1β. This reflects the different receptors (and intracellular signalling systems) for IL-1β and LPS – respectively, IL-R1 and Toll-like receptor 4 (TLR-4).

Similarly, there are some differences in the response of human adipocytes to IL-1β as found here, and to macrophage-conditioned medium as observed in an earlier study (O'Hara *et al.*, 2009). For example, most of the specific cytokines and chemokines on the PCR arrays whose expression was stimulated by IL-1β also exhibited a stimulation of expression on exposure to

macrophage secretions. However, the expression of several genes, including *CXCL10*, *CXCL11*, *CCL11*, *CCL19* and *IL15*, was not altered by macrophage-condition media, while their expression was stimulated by IL-1β. Macrophages release, of course, a range of factors, including TNFα. The difference between the response to macrophage-conditioned medium and IL-1β suggests that factors released from macrophages may inhibit, as well as stimulate, the expression of some inflammation-related genes, and in particular counteract the stimulatory effect of IL-1β.

While many of the cytokines and chemokines synthesised and secreted from human adipocytes in the present study have been noted before (Meijer *et al.*, 2011), MCP-4 has not been previously described as an adipokine. This factor has, nonetheless, been reported to be expressed in human adipose tissue (Hashimoto *et al.*, 2006). While secretion of both eotaxin-3 and GM-CSF is evident in this and a previous study on human fat cells in vitro (Meijer *et al.*, 2011), their expression in adipose tissue in vivo has not been noted. Cytokines and chemokines are part of the totality of protein signals and factors synthesised and released by white adipocytes. The adipokines, which proteomic studies suggest number several hundred distinct entities (Dahlman *et al.*, 2012; Rosenow *et al.*, 2013), are involved in a diverse range of metabolic and physiological processes. It is evident that a multiplicity of chemoattractants and other pro- and anti-inflammatory factors are synthesised and released by adipocytes as part of the inflammatory response in obesity.

Interestingly, a key inflammation-related adipokine whose production was down-regulated by IL-1β is the major adipocyte hormone adiponectin. Adiponectin has an anti-inflammatory action (Ouchi *et al.*, 1999; Yokota *et al.*, 2000), so inhibition of its synthesis would be expected to augment the pro-inflammatory effect of IL-1β. However, the secretion of other anti-inflammatory factors, such as IL-10, was markedly stimulated by IL-1β, and thus the overall effect of IL-1β on inflammation in adipose tissue is not uni-dimensional. In contrast to adiponectin, production of leptin, the other signature hormone of adipocytes, is stimulated by IL-1β (Sarraf *et al.*, 1997; Simons *et al.*, 2007).

IL-1β is widely implicated in the development of type 2 diabetes, both in relation to insulin resistance and to impaired insulin secretion, and IL-1β antagonism and blockade of the IL-1 receptor are potential targets for the treatment of type 2 diabetes (Donath, 2014; Bing, 2015). At the level of the adipocyte, IL-1β has recently been shown to inhibit insulin signal transduction, this involving reduced expression of signalling proteins (Gao & Bing, 2011). Insulin sensivity may also be impaired through the IL-1β-induced suppression of adiponectin production.

## Conclusions

IL-1β is a major factor in the regulation of the expression and secretion of a wide range of cytokines and chemokines in human adipocytes. As would be expected, there are in particular powerful stimulatory effects of IL-1β on the production of key inflammation-related signals from fat cells. Within adipose tissue, IL-1β may be derived from macrophages or from the adipocytes themselves – or indeed, from other cells in the tissue. Comparison with the response to macrophage-conditioned medium indicates that multiple factors from macrophages are likely to be implicated in the regulation of the inflammatory response in adipocytes.

## Declarations of interest

The authors acknowledge the Distinguished Scientist Fellowship Programme at King Saud University for financial support of this work. The authors also declare that they have no conflicts of interest in regard to this study.

**Table 1.** Genes on cytokine and chemokine PCR pathway arrays whose expression was up- or down-regulated in human white adipocytes by treatment with IL-1β

| Gene | Change (fold) with IL-1β at 4 h | Change (fold) with IL-1β at 24 h |
|---|---|---|
| | | |
| ***Upregulated: 4 and 24 h*** | | |
| *CSF3* | 9628 | 4649 |
| *CXCL2* | 1551 | 818 |
| *CXCL1* | 1537 | 1109 |
| *IL1B* | 1138 | 6.99** |
| *CXCL10* | 1089 | 36.8 |
| *IL8* | 759 | 310 |
| *CCL20* | 669 | 2277 |
| *CCL5* | 581 | 2433 |
| *CCL3* | 288 | 26.3 |
| *IL6* | 245 | 106 |
| *LIF* | 195 | 11.9 |
| *CXCL11* | 148 | 17.3** |
| *CCL19* | 124 | 1594 |
| *CCL2* | 119 | 136 |
| *CSF2* | 57.0 | 3.27* |
| *CCL7* | 51.4 | 9.56 |
| *CXCL5* | 45.2 | 148 |
| *IL1RN* | 36.3 | 53.0 |
| *IL7* | 22.0 | 10.5 |
| *CCL11* | 17.7 | 4.19 |
| *IL15* | 4.82 | 2.41 |
| *CX3CL1* | 4.64 | 2.27** |
| *CCL13* | 2.61 | 6.68 |
| *CSF1* | 2.57 | 2.39 |
| | | |
| ***Upregulated: 4 h only*** | | |
| *IL1A* | 23.1 | |
| *TNFA* | 21.9 | |
| *CCL8* | 9.04 | |
| *TNFRSF11B* | 7.90 | |
| *BMP2* | 5.12 | |
| *IL23A* | 5.06 | |
| *CCL1* | 3.19* | |
| *TGFB2* | 2.84 | |
| *IL12A* | 2.26** | |
| | | |
| ***Upregulated: 24 h only*** | | |
| *CXCL12* | | 4.07 |
| | | |
| ***Downregulated: 4 and 24 h*** | | |
| *NODAL* | 0.275** | 0.438* |
| *IL16* | 0.371 | 0.438 |
| | | |
| ***Downregulated: 4 h only*** | | |
| *SIPP1* | 0.400 | |

| | | |
|---|---|---|
| ***Downregulated: 24 h only*** | | |
| *IL18* | | 0.466** |
| *IFNA2* | | 0.349* |
| *BMP4* | | 0.339 |
| *IL24* | | 0.305** |
| *TNFSF13B* | | 0.267 |
| *C5* | | 0.263 |
| *TGFB2* | | 0.248 |
| *ADIPOQ* | | 0.229 |
| *TNFSF10* | | 0.145 |
| | | |

Human adipocytes differentiated from preadipocytes in culture were incubated in the presence or absence of human recombinant IL-1β (2 ng/ml) and cytokine and chemokine gene expression probed with PCR pathway arrays. The genes listed are those where there was a statistically significant (*P<0.05 or more*) change in relative mRNA level of at least 2-fold between IL-1β treated and control adipocytes (controls normalised to = 1.0); all fold-changes are significant at *P<0.001*, except those marked * or ** where *P<0.05* or *P<0.01*, respectively. The fold-changes are derived from 5-6 groups of adipocytes at both 4 and 24 h.

**Table 2.** Effect of IL-1β on cytokine and chemokine protein secretion from human white adipocytes

| Protein | Control (pg/ml) | IL-1β treated (pg/ml) | Fold-change with IL-1β |
|---|---|---|---|
| | | | |
| IP-10 (interferon gamma-induced protein 10) | 0.05±0.02 | 251.2±28.9 | 5127 |
| IL-4 (interleukin-4) | 0.01±0.00 | 2.38±0.14 | 380 |
| IL-6 (interleukin-6) | 6.00±0.19 | 2184±19 | 364 |
| GM-CSF (granulocyte macrophage-colony-stimulating factor) | 0.04±0.01 | 12.64±0.60 | 330 |
| TNFα (tumour necrosis factor α) | 0.03±0.01 | 5.55±0.32 | 168 |
| IL12p70 (interleukin 12 subunit p70) | 0.04±0.01 | 5.38±0.53 | 129 |
| IL-8 (interleukin-8) | 54.68±2.02 | 6330±23 | 116 |
| IL-10 (interleukin-10) | 0.02±0.00 | 2.33±0.16 | 108 |
| IFNγ (interferon γ) | 0.09±0.03 | 9.45±0.32 | 104 |
| MIP-1β (macrophage inflammatory protein 1β) | 0.27±0.02 | 17.89±1.30 | 67.3 |
| MIP-1α (macrophage inflammatory protein 1α) | 1.90±0.20 | 105.9±3.9 | 55.7 |
| MCP-1 (monocyte chemoattractant protein 1) | 207.6±15.7 | 7000±152 | 33.7 |
| IL-13 (interleukin-13) | 0.47±0.06 | 13.56±0.29 | 28.7 |
| IL-2 (interleukin-2) | 0.09±0.02 | 2.54±0.09 | 27.9 |
| MDC (macrophage-derived chemokine) | 1.71±0.48 | 47.27±10.72** | 27.7 |
| Eotaxin | 0.47±0.13 | 11.62±0.92 | 24.7 |
| MCP-4 (monocyte chemoattractant protein 4) | 0.44±0.08 | 10.41±0.57 | 23.9 |
| Eotaxin-3 | 0.97±0.43 | 17.94±6.47* | 18.6 |
| TARC (thymus and activation regulated chemokine) | 0.58±0.08 | 7.43±1.35 | 12.8 |
| IL-7 (interleukin-7) | 0.82±0.05 | 5.66±0.21 | 6.93 |
| VEGF (vascular endothelial growth factor) | 204.8±2.6 | 648.4±17.1 | 3.17 |
| IL-15 (interleukin-15) | 0.29±0.01 | 0.67±0.03 | 2.30 |
| IL-16 (interleukin-15) | 0.07±0.05 | 0.14±0.05$^{NS}$ | 2.12 |
| IL-17 (interleukin-17) | 0.06±0.03 | 0.13±0.03$^{NS}$ | 2.11 |
| TNFβ (tumour necrosis factor β) | 0.03±0.01 | 0.05±0.01* | 1.81 |
| IL12p40 (interleukin 12 subunit p40) | 0.13±0.04 | 0.22±0.04$^{NS}$ | 1.60 |
| IL-5 (interleukin-5) | 0.10±0.02 | 0.11±0.02$^{NS}$ | 1.08 |
| IL-1α (interleukin-1α) | 0.42±0.40 | 0.03±0.01$^{NS}$ | 0.07 |
| | | | |
| *IL-1β (interleukin-1β)* | *0.03±0.00* | *280.3±4.1* | *10376* |
| | | | |

Human adipocytes differentiated from preadipocytes in culture were incubated in the presence or absence of human recombinant IL-1β (2 ng/ml). Protein arrays were used to screen the effect of IL-1β on cytokine and chemokine secretion into the medium over 24 h. The differences between the IL-1β treated and control cells were statistically significant at *P<0.001*, except those marked * and ** where *P<0.05* and *P<0.01,* respectively. NS, not significant (*P>0.05*). The results are the means ± SE of 5-6 groups of adipocytes. IL-1β is shown to illustrate the very low basal levels of secretion of this cytokine. The lowest detection limit was for IL-8 (0.01 pg/ml).

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## Legends to Figures

**Figure 1.** Illustration of patterns of effects of IL-1β on cytokine and chemokine gene expression in human adipocytes. The results show the fold-changes in mRNA level for adipocytes treated with IL-1β compared with controls (controls normalised to 1) at 4 and 24 h. All differences shown reflect statistically significant changes (*P<0.001*) in mRNA level between IL-1β treated and control adipocytes, except for *ADIPOQ* at 4 h. The fold-changes are derived from 5-6 groups of adipocytes at both 4 and 24 h.

**Figure 2.** Examples of pattern of effects of IL-1β on cytokine and chemokine protein secretion by human adipocytes. Data, which are for 24 h, are means ± SE (bars) for 5-6 groups of adipocytes. Differences were statistically significant (*P<0.001*) between IL-1β treated and control adipocytes, except those marked NS (not significant, *P>0.05)*.

Figure 1

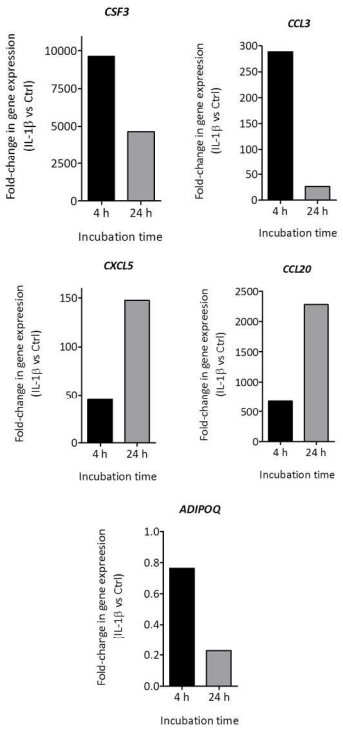


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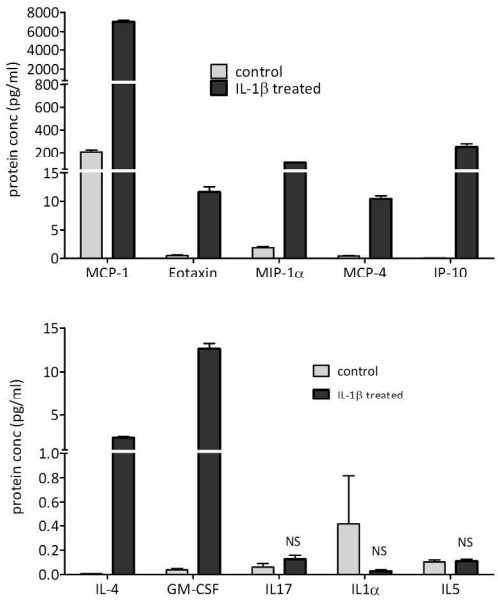
Figure 2

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