ARTICLE
Journal of Cellular Biochemistry 106:103–108 (2009)

Journal of Cellular Biochemistry

# Lipocalin-2 Is Induced by Interleukin-1β in Murine Adipocytes In Vitro

Grit Sommer,[1] Sebastian Weise,[1] Susan Kralisch,[1,2] Ulrike Lossner,[1] Matthias Bluher,[1] Michael Stumvoll,[1] and Mathias Fasshauer[1,2*]

[1]*Department of Internal Medicine III, University of Leipzig, 04103 Leipzig, Germany*
[2]*Interdisciplinary Center for Clinical Research (IZKF) Leipzig, 04103 Leipzig, Germany*

## ABSTRACT

Lipocalin-2 (Lcn2) has recently been isolated as an adipocyte-secreted acute phase reactant that plays a role in insulin resistance, obesity, and atherosclerotic disease. In the current study, we determined regulation of Lcn2 by the proinflammatory and insulin resistance-inducing cytokine interleukin (IL)-1β in 3T3-L1 and brown adipocytes by relative real-time reverse transcription-polymerase chain reaction. Interestingly, IL-1β dramatically induced Lcn2 mRNA in both adipocyte models. Furthermore, Lcn2 protein secretion was dramatically upregulated in 3T3-L1 adipocytes after 24 h of IL-1β treatment. Experiments using pharmacological inhibitors indicated that IL-1β-induced Lcn2 expression is mediated via nuclear factor κB and janus kinase 2. Taken together, our results show an upregulation of Lcn2 by IL-1β in fat cells implicating a potential role of this adipocyte-secreted acute phase reactant in the development of insulin resistance, obesity, and associated disorders including cardiovascular disease. J. Cell. Biochem. 106: 103–108, 2009. 

**KEY WORDS:** 3T3-L1 ADIPOCYTE; ADIPOKINE; INTERLEUKIN-1β; LIPOCALIN-2

Obesity is associated with dysregulation of adipocyte-secreted factors so-called adipokines. Furthermore, these bioactive molecules have been suggested to link insulin resistance, obesity, and cardiovascular disease (CVD). Among those, the cytokines tumor necrosis factor (TNF) α and interleukin (IL)-6 induce insulin resistance [Hotamisligil et al., 1993; Fasshauer and Paschke, 2003], whereas the insulin-sensitizer adiponectin is an anti-atherogenic fat-secreted factor [Yamauchi et al., 2001; Ouchi et al., 2003].

Recently, lipocalin-2 (Lcn2) has been introduced as a novel adipokine which might contribute to obesity, insulin resistance, and associated CVD [Wang et al., 2007; Yan et al., 2007]. Lcn2, also termed SIP24/24p3 [Flower et al., 1991; Liu and Nilsen-Hamilton, 1995] and neutrophil gelatinase-associated lipocalin [Kjeldsen et al., 1993], belongs to the large family of lipocalins which show high affinity for small hydrophobic ligands such as steroids and pheromones [Flower et al., 1993; Akerstrom et al., 2000]. It is interesting to note in this context that retinol-binding protein-4, another member of the lipocalin superfamily, has recently been characterized as an adipocyte-secreted protein that impairs glucose metabolism and insulin sensitivity [Yang et al., 2005]. Wang et al. [2007] detected elevated Lcn2 plasma concentrations and mRNA expression in adipose tissue and liver of obese mice as compared to lean controls. In accordance with these findings, upregulation of Lcn2 was shown in multiple rodent models of obesity by Yan et al. [2007]. Circulating Lcn2 concentrations in humans positively and significantly correlated with adiposity, hypertriglyceridemia, hyperglycemia, and insulin resistance, whereas a negative correlation was found with high density lipoprotein cholesterol [Wang et al., 2007]. Increased Lcn2 levels in humans were normalized after rosiglitazone treatment in vivo [Wang et al., 2007] and downregulation of Lcn2 by thiazolidinediones could also be demonstrated in fat cells in vitro [Yan et al., 2007]. Most interestingly, forced reduction of Lcn2 by retrovirus-delivered short hairpin RNA significantly improved insulin action in 3T3-L1 adipocytes [Yan et al., 2007]. Furthermore, Yan et al. [2007] identified Lcn2 as a factor dramatically induced by glucocorticoids and TNFα in 3T3-L1 cells indicating that Lcn2 might mediate insulin resistance-inducing effects of both hormones. Besides insulin sensitivity, Lcn2 might

G. Sommer and S. Weise contributed equally to this work.

Grant sponsor: Deutsche Forschungsgemeinschaft (DFG); Grant sponsor: KFO 152 "Atherobesity"; Grant numbers: FA476/4-1 (TP4), BL833/1-1 (TP3); Grant sponsor: IZKF Leipzig; Grant number: Project B25; Grant sponsor: Deutsche Diabetes Stiftung.

*Correspondence to: Mathias Fasshauer, Ph.-Rosenthal-Str. 27, 04103 Leipzig, Germany.
E-mail: mathias.fasshauer@medizin.uni-leipzig.de

Received 21 April 2008; Accepted 1 October 2008 • DOI 10.1002/jcb.21980 • 2008 Wiley-Liss, Inc.
Published online 13 November 2008 in Wiley InterScience (www.interscience.wiley.com).

also influence atherosclerotic disease. Thus, Hemdahl et al. [2006] reported a co-localization of Lcn2 with matrix metalloproteinase-9, an important mediator of vascular remodeling and plaque instability in atherosclerosis, with macrophages in atherosclerotic plaques, as well as with areas showing high proteolytic plaque activity. In addition, convincing evidence has been presented that Lcn2 serum levels are elevated in patients with coronary heart disease as compared to controls [Choi et al., 2008].

Recently, IL-1β has been introduced as a novel adipokine impairing insulin sensitivity and inducing proinflammatory gene expression in various tissues similar to TNFα [Lagathu et al., 2006; Jager et al., 2007]. However, the effect of this cytokine on expression of Lcn2 in fat cells has not been elucidated so far. Therefore, we determined regulation of Lcn2 by IL-1β in adipocytes in the current study.

## MATERIALS AND METHODS

### MATERIALS

Cell culture reagents were obtained from Life Technologies, Inc. (Grand Island, NY) and PAA (Pasching, Austria), oligonucleotides from MWG-Biotech (Ebersberg, Germany). IL-1β, insulin, and isobutylmethylxanthine were purchased from Sigma Aldrich (Steinheim, Germany). AG490, BAY 11-7082, LY294002, MG-132, parthenolide, and PD98059 were from Calbiochem (Bad Soden, Germany). Anti-mouse Lcn2 antibody was obtained from R&D Systems (Wiesbaden-Nordenstadt, Germany) and anti-mouse adiponectin antibody was from Cell Signaling Technology (Danvers, MA).

### CULTURE AND DIFFERENTIATION OF 3T3-L1 AND BROWN ADIPOCYTES (BAT)

3T3-L1 cells (American Type Culture Collection, Rockville, MD) were cultured as previously described [Fasshauer et al., 2004a]. Furthermore, the same protocol was used to culture immortalized brown preadipocytes [Fasshauer et al., 2000]. In brief, preadipocytes were grown to confluence in DMEM containing 25 mM glucose (DMEM-H), 10% fetal bovine serum, and antibiotics (culture medium). After this period, cells were induced for 3 days in culture medium further supplemented with 1 μM insulin, 0.5 mM isobutylmethylxanthine, and 0.1 μM dexamethasone. Subsequently, they were grown for 3 days in culture medium with 1 μM insulin and for additional 3–6 more days in culture medium. Various effectors were added to cells starved in DMEM-H only for the indicated periods of time. At the time of the stimulation experiments at least 95% of the cells had accumulated fat droplets.

### ANALYSIS OF Lcn2 PROTEIN EXPRESSION

Detection of Lcn2 secretion by Western blotting was performed essentially as described previously [Fasshauer et al., 2001b]. Briefly, equal amounts of 3T3-L1 culture medium supernatant were directly solubilized in Laemmli sample buffer, boiled for 3 min, separated by 14% SDS–PAGE, and transferred to nitrocellulose membranes. Membranes were blocked in TBS (10 mM Tris, 150 mM NaCl, 0.05% Tween, pH 7.2) containing 3% bovine serum albumin for 30 min and incubated with Lcn2 antibody overnight at 4°C. Specifically bound primary antibodies were detected with enhanced chemiluminescence. The same blots were re-probed with anti-mouse adiponectin antibody.

### ANALYSIS OF Lcn2 MRNA

Lcn2 mRNA was quantified by relative real-time reverse transcription-polymerase chain reaction (RT-PCR) in a fluorescent temperature cycler (ABI Prism 7000, Applied Biosystems, Darmstadt, Germany) as described previously [Fasshauer et al., 2001a]. Briefly, total RNA was isolated from both 3T3-L1 and brown adipocytes with TRIzol reagent (Invitrogen, Life Technologies, Inc.) and 1 μg RNA was reverse transcribed using standard reagents (Invitrogen, Life Technologies, Inc.). Two microliters of each RT reaction was amplified in a PCR with a total volume of 26 μl. After initial denaturation at 95°C for 10 min, 40 PCR cycles were performed using the following conditions: 95°C for 15 s, 60°C for 1 min, and 72°C for 1 min. The following primer pairs were used: Lcn2 (accession no. NM_008491), 5′-ATCCCTGCCCCATCTCTGCTC-3′ (sense) and 5′-GTACCACCTGCCCCGGAACTGAT-3′ (antisense); 36B4 (accession no. NM_007475) 5′-aagcgcgtc ctggcattgtct-3′ (sense) and 5′-ccgcaggggcagcagtggt-3′ (antisense) as described previously [Laborda, 1991; Yan et al., 2007]. SYBR Green I fluorescence emissions were monitored after each cycle and synthesis of Lcn2 and 36B4 mRNA was quantified using the second derivative maximum method of the ABI Prism 7000 software (Applied Biosystems). In brief, crossing points of individual samples were determined by an algorithm identifying the first turning point of the fluorescence curve. 36B4 was used as internal control due to its resistance to hormonal regulation [Laborda, 1991], and Lcn2 expression was calculated relative to 36B4. Specific transcripts were confirmed by melting curve profiles (cooling the sample to 68°C and heating slowly to 95°C with measurement of fluorescence) at the end of each PCR and the specificity of the PCR was further verified by subjecting the amplification products to agarose gel electrophoresis.

### STATISTICAL ANALYSIS

Results are shown as mean ± SE. Differences between various treatments were analyzed by Mann–Whitney *U*-tests with *P* values $<0.01$ considered highly significant and $<0.05$ considered significant.

## RESULTS

### Lcn2 PROTEIN SECRETION IS INDUCED BY IL-1β

Lcn2 protein secretion was quantified in supernatants of differentiated 3T3-L1 cells in the presence and absence of 20 ng/ml IL-1β for 8 and 24 h. Interestingly, IL-1β induced Lcn2 but not adiponectin protein secretion dramatically after 24 h of treatment as compared to control conditions (Fig. 1).

### Lcn2 mRNA EXPRESSION IS INDUCED TIME- AND DOSE-DEPENDENTLY BY IL-1β

Treatment with 20 ng/ml IL-1β rapidly increased Lcn2 mRNA expression in a time-dependent manner in both differentiated 3T3-L1 adipocytes (Fig. 2A) and BAT (Fig. 2B). Thus, in 3T3-L1 adipocytes significant 23-fold upregulation of Lcn2 mRNA

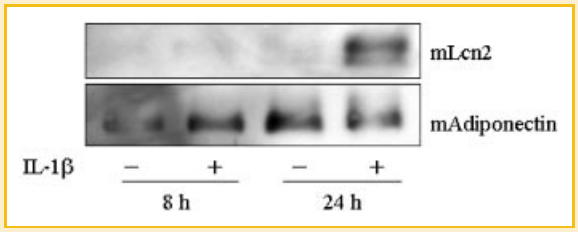


Fig. 1. Stimulation of Lcn2 protein secretion by IL-1β. Fully differentiated 3T3-L1 adipocytes were serum-deprived overnight before IL-1β (20 ng/ml) was added for the indicated periods of time. Equal amounts of supernatant were directly solubilized in Laemmli buffer, separated and immunoblotted with anti-mouse Lcn2 antibody as described in Materials and Methods Section. The same blots were re-probed with anti-mouse adiponectin antibody. A representative blot of four independent experiments is shown.

synthesis was seen after 2 h of IL-1β stimulation ($P < 0.01$) and maximal more than 1,500-fold induction after 24 h of effector addition ($P < 0.01$) (Fig. 2A). Furthermore, IL-1β upregulated Lcn2 gene expression in BAT with significant maximal 153-fold stimulation detectable 24 h after effector addition ($P < 0.05$) (Fig. 2B).

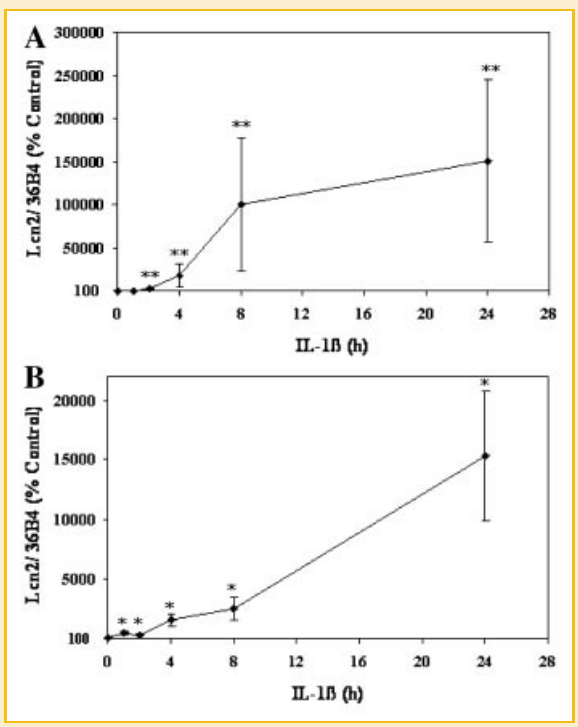


Fig. 2. Time-dependent stimulation of Lcn2 gene expression by IL-1β. Fully differentiated (A) 3T3-L1 adipocytes and (B) BAT were serum-deprived overnight before IL-1β (20 ng/ml) was added for the indicated periods of time. Total RNA was extracted and subjected to relative real-time RT-PCR determining Lcn2 mRNA levels normalized to 36B4 expression relative to untreated control cells which are set 100% as described in Materials and Methods Section. Results are the means ± SE of at least (A) six and (B) four independent experiments. $^{**}P < 0.01$, $^{*}P < 0.05$ comparing IL-1β-treated with non-treated cells.

In addition, IL-1β induced Lcn2 synthesis in a dose-dependent manner after 16 h of treatment in 3T3-L1 adipocytes (Fig. 3). Here, significant 27-fold stimulation was observed at IL-1β concentrations as low as 0.02 ng/ml ($P < 0.01$), and maximal 193-fold ($P < 0.01$) upregulation was seen at 6.7 ng/ml effector (Fig. 3).

## NUCLEAR FACTOR κB AND JANUS KINASE 2 MEDIATE THE EFFECT OF IL-1β ON Lcn2 EXPRESSION

We elucidated which molecules implicated in IL-1β signaling might mediate the positive effect of IL-1β on Lcn2 expression. To this end, 3T3-L1 cells were treated for 1 h with specific pharmacological inhibitors of janus kinase (Jak) 2 (AG490, 10 μM), nuclear factor κB (NFκB) (parthenolide, 50 μM), p44/42 mitogen-activated protein (MAP) kinase (PD98059, 50 μM), and phosphatidylinositol (PI) 3-kinase (LY294002, 10 μM) before IL-1β (20 ng/ml) was added for 16 h. Treatment of 3T3-L1 adipocytes with AG490 and PD98059 for 17 h significantly downregulated basal Lcn2 expression to 86% ($P < 0.01$) and to 88% ($P < 0.05$) of control levels, respectively, while parthenolide and LY294002 did not significantly influence basal Lcn2 mRNA synthesis (Fig. 4A). Again, Lcn2 expression was significantly increased 880-fold after 16 h of IL-1β treatment ($P < 0.01$) (Fig. 4A). This induction was significantly reversed by more than 92% and 99% in cells pretreated with the Jak2 inhibitor AG490 and the NFκB inhibitor parthenolide ($P < 0.01$), respectively (Fig. 4A). In contrast, PD98059 and LY294002 did not significantly influence IL-1β-stimulated Lcn2 expression (Fig. 4A).

Furthermore, experiments with two additional NFκB inhibitors (MG-132, 50 μM and BAY 11-7082, 100 μM) were performed. Here, treatment of 3T3-L1 adipocytes with MG-132 for 17 h significantly suppressed basal Lcn2 expression to 24% of control levels ($P < 0.01$) (Fig. 4B). In contrast, treatment of 3T3-L1 adipocytes with BAY 11-7082 for 17 h did not significantly influence basal Lcn2 mRNA synthesis (Fig. 4B). Again, Lcn2 expression was increased by more

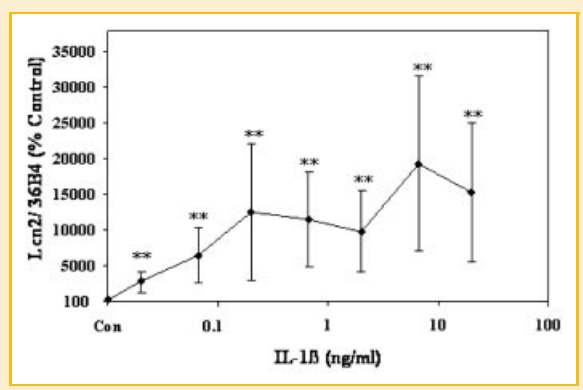


Fig. 3. Dose-dependent stimulation of Lcn2 mRNA expression by IL-1β. 3T3-L1 cells were serum-starved for 6 h before various concentrations of IL-1β were added for 16 h. Total RNA was extracted and subjected to relative real-time RT-PCR to determine Lcn2 mRNA levels normalized to 36B4 expression as described in Materials and Methods Section. Data are expressed relative to untreated control (Con) cells which are set 100%. Results are the means ± SE of at least six independent experiments. $^{**}P < 0.01$ comparing IL-1β-treated with non-treated cells.

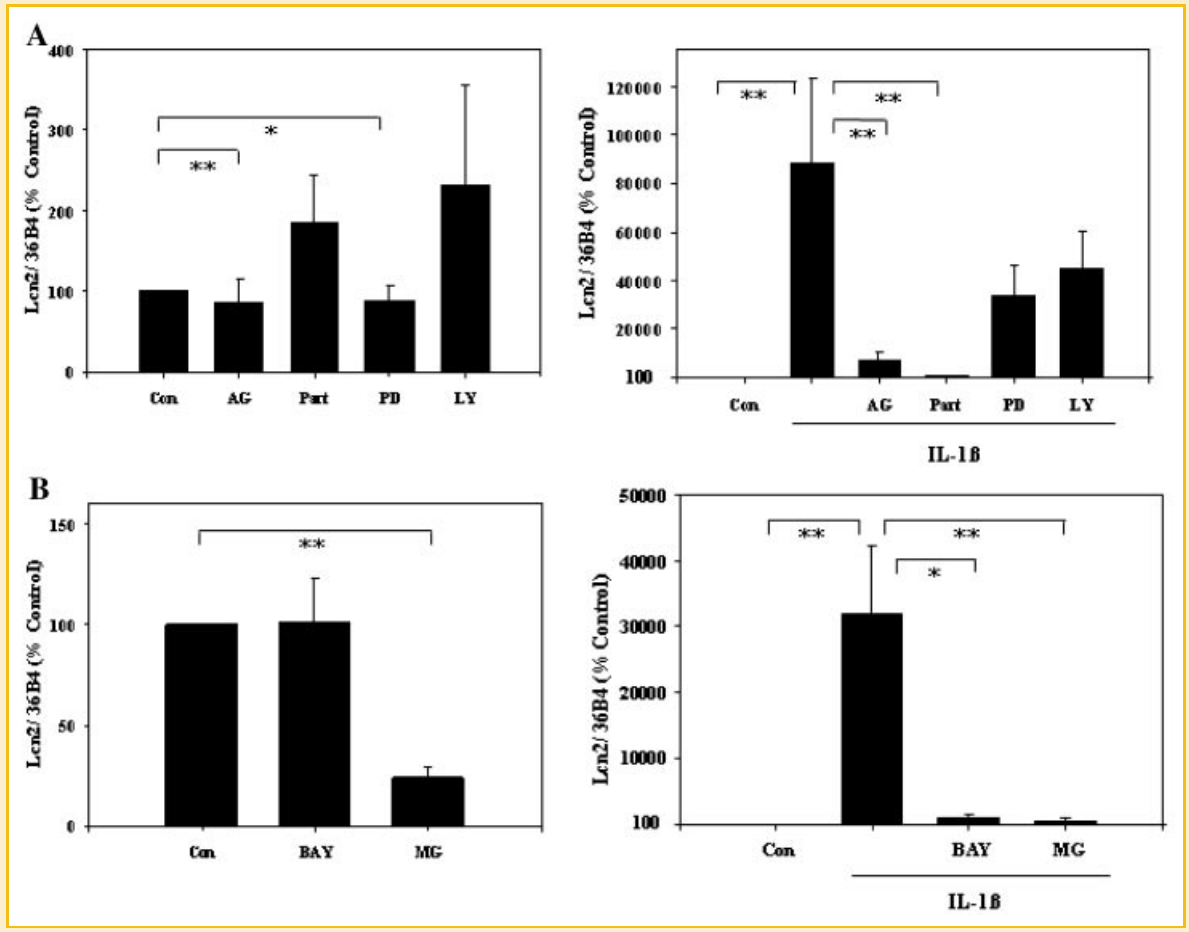


Fig. 4. Lcn2 mRNA induction by IL-1β is mediated via NFκB and Jak2. After serum-starvation, 3T3-L1 adipocytes were cultured in the presence or absence of (A) AG490 (AG, 10 μM), parthenolide (part, 50 μM), PD98059 (PD, 50 μM), or LY294002 (LY, 10 μM) and (B) MG-132 (MG, 50 μM) and BAY 11-7082 (BAY, 100 μM) for 1 h before IL-1β (20 ng/ml) was added for 16 h. Total RNA was extracted and subjected to relative real-time RT-PCR to determine Lcn2 normalized to 36B4 expression as described in Materials and Methods Section. Data are expressed relative to non-treated control (Con) cells which are set 100%. Results are the means ± SE of (A) 15 and (B) 5 independent experiments. $^{**}P < 0.01$, $^{*}P < 0.05$ comparing untreated with inhibitor-pretreated or IL-1β-treated cells, as well as comparing IL-1β-treated with inhibitor-pretreated adipocytes.

than 300-fold after 16 h of IL-1β treatment ($P < 0.01$) (Fig. 4B). This induction was significantly reversed by more than 96% and 98% in cells pretreated with BAY 11-7082 ($P < 0.05$) and MG-132 ($P < 0.01$), respectively (Fig. 4B).

## DISCUSSION

Convincing evidence has been presented that blockade of IL-1 by anakinra improves glycemic control and reduces markers of systemic inflammation in humans [Larsen et al., 2007]. In the current study, we demonstrate for the first time that Lcn2 mRNA expression is significantly induced by IL-1β in 3T3-L1 adipocytes in vitro. Interestingly, IL-1β is also an inductor of Lcn2 mRNA synthesis in murine differentiated BAT. These results support the notion that IL-1β is a principal positive regulator of Lcn2 mRNA expression in fat cells. Recently, Yan et al. [2007] have shown that insulin resistance-inducing glucocorticoids and TNFα significantly upregulate Lcn2 in fat cells similar to results in our laboratory (data not shown). In the current study, we extend these findings by demonstrating that IL-1β is another strong inductor of Lcn2 in fat cells. Furthermore, our findings suggest that adipocyte-derived Lcn2 might contribute to IL-1β-induced insulin resistance and CVD. However, it has to be pointed out that other mechanisms probably also contribute. Thus, two recent studies demonstrate convincingly that IL-1β potently induces insulin resistance in human and rodent fat cells by interacting with insulin signaling molecules including insulin receptor, insulin receptor substrate 1, protein kinase B, and p44/42 MAP kinase [Lagathu et al., 2006; Jager et al., 2007]. Furthermore, reduced expression and secretion of insulin-sensitizing and anti-inflammatory adiponectin are found after treatment with IL-1β in differentiating 3T3-F442A cells, fully differentiated 3T3-L1 cells, as well as human adipocytes [Lihn et al., 2004; Lagathu et al., 2006]. Moreover, we have recently demonstrated that IL-1β is a potent inductor of the acute phase reactant, insulin resistance-associated protein serum amyloid A3 in murine adipocytes in vitro [Sommer et al., 2008].

The influence of IL-1β on Lcn2 has not been studied in adipocytes before. However, it has been shown that IL-1β is a positive regulator

of Lcn2 in other cell types. Thus, Cowland et al. [2003, 2006] demonstrate convincingly that IL-1β upregulates Lcn2 expression in the lung epithelium cell line A549 by induction of NFκB binding to the Lcn2 promoter. Furthermore, mRNA production and protein expression of Lcn2 are induced in a NFκB-dependent manner in vascular smooth muscle cells in response to IL-1β stimulation [Bu et al., 2006]. These results support the hypothesis that IL-1β is not only an important inductor of Lcn2 expression in fat cells but also in other cell types.

In the current study, we further determine by which molecules IL-1β induces Lcn2 synthesis. The main signaling pathways of IL-1β have been elucidated in more detail in recent years. Thus, IL-1β-mediated stimulation of the IL-1 receptor 1 leads to subsequent activation and phosphorylation of IL-1 receptor-associated kinase [Cao et al., 1996; Cooke et al., 2001]. Downstream signaling events include activation of NFκB, which, in turn, translocates into the nucleus leading to induction of target genes and production of proinflammatory cytokines and chemokines [Cao et al., 1996; Cooke et al., 2001]. We show that pharmacological inhibition of NFκB by parthenolide, MG-132, and BAY 11-7082 significantly reverses IL-1β-mediated upregulation of Lcn2 mRNA expression suggesting that this signaling molecule plays a major role in IL-1β-induced Lcn2 mRNA synthesis. Furthermore, the NFκB activator TNFα induces Lcn2 mRNA expression in 3T3-L1 adipocytes (data not shown) in accordance with previous findings [Yan et al., 2007].

Besides NFκB, IL-1β stimulates signaling molecules including Jak2, p44/42 MAP kinase, and PI 3-kinase [Doi et al., 2002]. Here, inhibition of Jak2 by AG490 significantly reverses IL-1β-induced Lcn2 mRNA expression indicating that this signaling molecule also plays a role in Lcn2 induction. In contrast, p44/42 MAP kinase and PI 3-kinase probably do not mediate IL-1β-stimulated Lcn2 expression since pharmacological inhibition of either molecule does not significantly influence IL-1β-stimulated Lcn2 synthesis. However, a minor influence of PD98059 and LY294002 on IL-1β-induced Lcn2 cannot be ruled out even after 15 independent experiments due to relatively high experiment-to-experiment differences. We have recently demonstrated in 3T3-L1 adipocytes that both 50 μM PD98059 and 10 μM LY294002, in fact, effectively block activation of p44/42 MAP kinase and the PI 3-kinase downstream target Akt, respectively [Fasshauer et al., 2004b].

It has to be pointed out that the experiment-to-experiment differences in IL-1β-induced Lcn2 expression are high which is most probably due to low mRNA expression of this adipokine in 3T3-L1 adipocytes in the basal state. Since this basal state is set 100%, the extent of upregulation of the adipokine by IL-1β is quite different between independent experiments. Here, evaluation of the copy number of Lcn2 mRNA could help to define whether Lcn2 expression values are similar between independent IL-1β treatments. Similarly, Lcn2 protein secretion cannot be detected in the basal state whereas a dramatic upregulation is seen after 24 h of IL-1β treatment. However, it has to be pointed out that Lcn2 protein secretion per cellular protein reaches similar values from one experiment to another in IL-1β-stimulated cells in the current study.

Taken together, we show for the first time that proinflammatory IL-1β is a potent stimulator of Lcn2 expression in adipocytes in vitro and that this upregulation is mediated via NFκB and Jak2. Our findings support the notion that expression of Lcn2 in adipose tissue may constitute an important element in the pathophysiology of insulin resistance and associated metabolic, proinflammatory, and cardiovascular disorders. It needs to be determined in future studies whether IL-1β also increases circulating Lcn2 levels in vivo by upregulation of the adipokine in fat cells.

## ACKNOWLEDGMENTS

This study was supported by a grant from the Deutsche Forschungsgemeinschaft (DFG), KFO 152 ''Atherobesity'', project FA476/4-1 (TP4) to M.F. and project BL833/1-1 (TP3) to M.B., as well as by a grant from the IZKF Leipzig (Project B25) and the deutsche Diabetes Stiftung to M.F.

## REFERENCES

Akerstrom B, Flower DR, Salier JP. 2000. Lipocalins: Unity in diversity. Biochim Biophys Acta 1482:1–8.

Bu DX, Hemdahl AL, Gabrielsen A, Fuxe J, Zhu C, Eriksson P, Yan ZQ. 2006. Induction of neutrophil gelatinase-associated lipocalin in vascular injury via activation of nuclear factor-kappaB. Am J Pathol 169:2245–2253.

Cao Z, Henzel WJ, Gao X. 1996. IRAK: A kinase associated with the interleukin-1 receptor. Science 271:1128–1131.

Choi KM, Lee JS, Kim EJ, Baik SH, Seo HS, Choi DS, Oh DJ, Park CG. 2008. Implication of lipocalin-2 and visfatin levels in patients with coronary heart disease. Eur J Endocrinol 158:203–207.

Cooke EL, Uings IJ, Xia CL, Woo P, Ray KP. 2001. Functional analysis of the interleukin-1-receptor-associated kinase (IRAK-1) in interleukin-1 beta-stimulated nuclear factor kappa B (NF-kappa B) pathway activation: IRAK-1 associates with the NF-kappa B essential modulator (NEMO) upon receptor stimulation. Biochem J 359:403–410.

Cowland JB, Sorensen OE, Sehested M, Borregaard N. 2003. Neutrophil gelatinase-associated lipocalin is up-regulated in human epithelial cells by IL-1 beta, but not by TNF-alpha. J Immunol 171:6630–6639.

Cowland JB, Muta T, Borregaard N. 2006. IL-1beta-specific up-regulation of neutrophil gelatinase-associated lipocalin is controlled by IkappaB-zeta. J Immunol 176:5559–5566.

Doi M, Shichiri M, Katsuyama K, Ishimaru S, Hirata Y. 2002. Cytokine-activated Jak-2 is involved in inducible nitric oxide synthase expression independent from NF-kappaB activation in vascular smooth muscle cells. Atherosclerosis 160:123–132.

Fasshauer M, Paschke R. 2003. Regulation of adipocytokines and insulin resistance. Diabetologia 46:1594–1603.

Fasshauer M, Klein J, Ueki K, Kriauciunas KM, Benito M, White MF, Kahn CR. 2000. Essential role of insulin receptor substrate-2 in insulin stimulation of Glut4 translocation and glucose uptake in brown adipocytes. J Biol Chem 275:25494–25501.

Fasshauer M, Klein J, Neumann S, Eszlinger M, Paschke R. 2001a. Isoproterenol inhibits resistin gene expression through a G(S)-protein-coupled pathway in 3T3-L1 adipocytes. FEBS Lett 500:60–63.

Fasshauer M, Klein J, Neumann S, Eszlinger M, Paschke R. 2001b. Tumor necrosis factor alpha is a negative regulator of resistin gene expression and secretion in 3T3-L1 adipocytes. Biochem Biophys Res Commun 288:1027–1031.

Fasshauer M, Klein J, Kralisch S, Klier M, Lossner U, Bluher M, Paschke R. 2004a. Serum amyloid A3 expression is stimulated by dexamethasone and interleukin-6 in 3T3-L1 adipocytes. J Endocrinol 183:561–567.

Fasshauer M, Kralisch S, Klier M, Lossner U, Bluher M, Klein J, Paschke R. 2004b. Insulin resistance-inducing cytokines differentially regulate SOCS

mRNA expression via growth factor- and Jak/Stat signaling pathways in 3T3-L1 adipocytes. J Endocrinol 181:129–138.

Flower DR, North AC, Attwood TK. 1991. Mouse oncogene protein 24p3 is a member of the lipocalin protein family. Biochem Biophys Res Commun 180:69–74.

Flower DR, North AC, Attwood TK. 1993. Structure and sequence relationships in the lipocalins and related proteins. Protein Sci 2:753–761.

Hemdahl AL, Gabrielsen A, Zhu C, Eriksson P, Hedin U, Kastrup J, Thoren P, Hansson GK. 2006. Expression of neutrophil gelatinase-associated lipocalin in atherosclerosis and myocardial infarction. Arterioscler Thromb Vasc Biol 26:136–142.

Hotamisligil GS, Shargill NS, Spiegelman BM. 1993. Adipose expression of tumor necrosis factor-alpha: Direct role in obesity-linked insulin resistance. Science 259:87–91.

Jager J, Gremeaux T, Cormont M, Marchand-Brustel Y, Tanti JF. 2007. Interleukin-1beta-induced insulin resistance in adipocytes through down-regulation of insulin receptor substrate-1 expression. Endocrinology 148:241–251.

Kjeldsen L, Johnsen AH, Sengelov H, Borregaard N. 1993. Isolation and primary structure of NGAL, a novel protein associated with human neutrophil gelatinase. J Biol Chem 268:10425–10432.

Laborda J. 1991. 36B4 cDNA used as an estradiol-independent mRNA control is the cDNA for human acidic ribosomal phosphoprotein PO. Nucleic Acids Res 19:3998.

Lagathu C, Yvan-Charvet L, Bastard JP, Maachi M, Quignard-Boulange A, Capeau J, Caron M. 2006. Long-term treatment with interleukin-1beta induces insulin resistance in murine and human adipocytes. Diabetologia 49:2162–2173.

Larsen CM, Faulenbach M, Vaag A, Volund A, Ehses JA, Seifert B, Mandrup-Poulsen T, Donath MY. 2007. Interleukin-1-receptor antagonist in type 2 diabetes mellitus. N Engl J Med 356:1517–1526.

Lihn AS, Bruun JM, He G, Pedersen SB, Jensen PF, Richelsen B. 2004. Lower expression of adiponectin mRNA in visceral adipose tissue in lean and obese subjects. Mol Cell Endocrinol 219:9–15.

Liu Q, Nilsen-Hamilton M. 1995. Identification of a new acute phase protein. J Biol Chem 270:22565–22570.

Ouchi N, Kihara S, Funahashi T, Matsuzawa Y, Walsh K. 2003. Obesity, adiponectin and vascular inflammatory disease. Curr Opin Lipidol 14:561–566.

Sommer G, Weise S, Kralisch S, Scherer PE, Lossner U, Bluher M, Stumvoll M, Fasshauer M. 2008. The adipokine SAA3 is induced by interleukin-1β in mouse adipocytes. J Cell Biochem 104:2241–2247.

Wang Y, Lam KS, Kraegen EW, Sweeney G, Zhang J, Tso AW, Chow WS, Wat NM, Xu JY, Hoo RL, Xu A. 2007. Lipocalin-2 is an inflammatory marker closely associated with obesity, insulin resistance, and hyperglycemia in humans. Clin Chem 53:34–41.

Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, Tsuboyama-Kasaoka N, Ezaki O, Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudo K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S, Tomita M, Froguel P, Kadowaki T. 2001. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat Med 7:941–946.

Yan QW, Yang Q, Mody N, Graham TE, Hsu CH, Xu Z, Houstis NE, Kahn BB, Rosen ED. 2007. The adipokine lipocalin 2 is regulated by obesity and promotes insulin resistance. Diabetes 56:2533–2540.

Yang Q, Graham TE, Mody N, Preitner F, Peroni OD, Zabolotny JM, Kotani K, Quadro L, Kahn BB. 2005. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. Nature 436:356–362.