Regulation of CD163 mRNA and soluble CD163 protein in human adipose tissue in vitro

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Abstract

CD163-positive macrophages are highly expressed in the human adipose tissue (AT) particularly from obese individuals. However, little is known about the regulation of CD163 mRNA and the protein level of sCD163 in human AT. We aimed to examine the regulation of CD163 and sCD163 in AT. Human s.c. AT samples (n = 5) were stimulated with dexamethasone (DEX; 200 nmol/l), lipopolysaccharide (LPS; 100 ng/ml), or DEX + LPS for various time periods up to 24 h. Gene expressions of CD163, ADAM17, IL10, and TNFA (TNF) were measured by RT-PCR. Protein levels of sCD163, IL10, and TNFα (TNF) were measured by ELISA. Furthermore, AT was separated into stromal and adipocyte fraction. We found that CD163 mRNA was strongly expressed in the stromal vascular fraction but hardly detectable in the isolated adipocytes. Incubating whole AT with DEX significantly up-regulated CD163 (P < 0.001), whereas incubation with LPS had no effects on CD163 (P > 0.05). By contrast, the protein level of sCD163 was not affected by DEX (P > 0.05), but LPS significantly increased the level of sCD163 and TNFα (P < 0.05). This might be due to the concomitant LPS stimulation of ADAM17, which is known to mediate shedding of the extracellular domains of sCD163 and TNFα. Finally, DEX significantly reduced the LPS-induced TNFα release to the incubation medium but had no effects on sCD163. We conclude that the expression of CD163 and the release of sCD163 are differentially regulated in human AT. Moreover, similar to studies on differentiated blood monocytes, TNFα and sCD163 are concomitantly released in human AT by LPS, which also up-regulate ADAM17.

Key Words
- adipose tissue
- macrophages
- CD163
- soluble CD163
- tumor necrosis factor α
- ADAM17

Introduction

In obesity, there is an increased amount of macrophages in the adipose tissue (AT), which is associated with low-grade inflammation and insulin resistance (Weisberg et al. 2003, Dandona et al. 2004). CD163 is a membrane-bound protein primarily expressed on monocytes and macrophages (Moller et al. 2002a). CD163-positive macrophages are found, e.g., in the liver, lung, spleen, and the AT (Van den Heuvel et al. 1999). The AT macrophages highly express CD163 mRNA (Shakeri-Manesch et al. 2009), and it is found that CD163-positive AT macrophages phenotypically resemble anti-inflammatory macrophages, which are macrophages present in the down-regulatory phase of acute inflammation (Zeyda et al. 2007). CD163 is involved in the clearance of haptoglobin–hemoglobin complexes by mediating endocytosis, thus preventing the toxic and oxidative effects of free hemoglobin
The gene expression of CD163 was found to be regulated by different pro- and anti-inflammatory mediators. In vitro studies have demonstrated that the expression of CD163 in peripheral blood monocytes isolated from whole blood is up-regulated by glucocorticoids and IL10 (Hogger et al., 1998, Buechler et al., 2000, Sulahian et al., 2000) and down-regulated by lipopolysaccharide (LPS), interferon gamma, and tumor necrosis factor alpha (TNFalpha) (Buechler et al., 2000).

A soluble variant of the receptor is found in the plasma (Moller et al., 2002b). Soluble CD163 (sCD163) is the extracellular part of the receptor, and it is cleaved by the metalloproteinase 'TNFalpha-converting enzyme' (ADAM17) (Etzerodt et al., 2010). In vitro studies have shown that sCD163 is shed, if cells are activated via Toll-like receptor by, e.g., LPS, phorbol 12-myristate 13-acetate or oxidative stress (Droste et al., 1999, Hintz et al., 2002, Timmermann & Hogger 2005). In vivo studies have shown that injection of LPS in humans induces a rapid and simultaneous release of sCD163 and TNFalpha with levels of both proteins peaking after 1.5 h. The concentration of TNFalpha is normalized after 3 h, while sCD163 has a longer half-life and remains elevated after 24 h (Hintz et al., 2002, Etzerodt et al., 2010). Small amounts of sCD163 are constantly shed into the circulation, and the normal range is between 0.7 and 3.9 mg/ml in healthy individuals; however, it can be several fold higher in inflammatory conditions, e.g., sepsis, acute liver failure, chronic kidney disease, and rheumatoid arthritis (Moller, 2011). The function of sCD163 is not yet clear, but a recent study has found that sCD163 can bind to the bacteria Staphylococcus aureus and promote recognition and phagocytosis (Kneidl et al., 2012). We and others have found that the level of sCD163 and the gene expression level of CD163 in the AT are significantly elevated in obesity (Axelsson et al., 2006, Sporrer et al., 2009, Zanni et al., 2011, Fjeldborg et al., 2013, 2014, Kračmerová et al., 2014). Furthermore, there is a significant association between the insulin resistance measured by HOMA-IR and the level of sCD163 in AT samples (Zanni et al., 2011, Fjeldborg et al., 2013, 2014, Kračmerová et al., 2014). Finally, sCD163 is found to be strongly associated with the development of type 2 diabetes (Parkner et al., 2012).

Despite a high amount of CD163-positive macrophages in the AT, little is known about the regulation of the gene expression of CD163 and the protein level of sCD163 in the AT. The purpose of our study is to examine how CD163 and sCD163 are regulated in human AT.

Materials and methods

AT samples

S.c. AT samples were collected in relation to liposuction performed at the Department of Plastic Surgery at the private hospital Teres (Aarhus). We collected s.c. AT samples from five healthy females (age 46 ± 6.7 years, BMI 26 ± 2.2 kg/m²) deriving from the hip (n=3) and the abdomen (n=2). Immediately after the removal, the AT was transported to the laboratory where it was rinsed several times in isotonic NaCl. Visible vessels and connective tissue were carefully removed, and finally the AT was placed in medium 199 supplemented with penicillin/streptomycin (100 IU/ml), antipain/leupeptin (20 μl/ml), and 1 nmol/l insulin (1 μl/100 ml medium) but without BSA. We used 500 mg AT per 5 ml incubation medium per tube. All subsequent procedures were carried out under a laminar airflow hood chamber and under sterile conditions. The AT was pre-incubated in a humidified incubator (37 °C) in an atmosphere of 5% CO₂ for 18 h. The AT was either pre-incubated in medium 199 only, or medium 199 with dexamethasone 200 nmol/l (DEX). After pre-incubation, the medium was replaced with the incubation medium 199, and the AT was incubated for 0, 2, 4, 8, or 24 h in medium 199 either with LPS 100 ng/ml in order to activate an inflammatory response or only with medium 199 (control). At the end of the incubations, the AT and medium were harvested and snap frozen in liquid nitrogen and kept at −80 °C. All incubations were performed in duplicate. The concentrations of DEX and LPS were based on a dose–response study (data not shown). AT was pre-incubated with DEX in concentrations from 100 to 500 nmol/l for 18 h, and, based on these findings, DEX 200 nmol/l was used in the time course study. Similarly, incubations were performed with LPS in concentrations from 1 to 500 ng/ml, and a submaximal stimulatory concentration of LPS at 100 ng/ml was used in the following incubations.

The study was approved by the local ethics committee in the county of Aarhus, Denmark, and followed the principles of the Declaration of Helsinki.

Stromal vascular fraction and isolated adipocytes

Adipocytes were isolated by collagenase digestion (0.15 mg/g AT) of AT fragments in 10 mmol/l HEPES buffer for 45–60 min at 37 °C. The isolated adipocytes were washed three times in the medium, and then snap frozen in liquid nitrogen and kept at −80 °C for later RNA
extraction. The stromal vascular fraction (SVF) was centrifuged for 15 min at 6300 \(g\), re-suspended in 9 ml buffer, and filtered through a nylon mesh. This procedure was repeated three times after which the supernatant was removed, and the SVF was snap frozen in liquid nitrogen and kept at \(-80^\circ C\) for later RNA extraction.

**Determination of sCD163**

Soluble CD163 was quantified in the incubation medium using a highly sensitive in-house ELISA kit, modified from that described previously (Moller et al. 2002c). Briefly, rabbit anti-CD163 antibodies were coated onto micro-titer wells and samples (diluted 1:2) were added and incubated for 1 h at RT. The biotinylated monoclonal anti-CD163 antibody (Mac2-158, 1.9 \(\mu g/ml\)) was incubated for 1 h at 37\(^\circ C\), washed, and kept at \(-20^\circ C\) for later RNA extraction. The stromal vascular fraction (SVF) was re-suspended in 9 ml PBS and filtered through a nylon mesh. This procedure was repeated three times after which the supernatant was removed, and the SVF was snap frozen in liquid nitrogen and kept at \(-80^\circ C\) for later RNA extraction.

**mRNA isolation and RT-PCR analysis**

RNA was isolated using TRIzol reagent (GIBCO-BRL Life Technologies), and cDNA was synthesized using random hexamer primers using the Verso cDNA kit (Applied Biosystems). All analyzes were performed simultaneously and the mRNA levels of the target genes were expressed relative to the housekeeping gene LRPI0. The following primer pairs were used for amplification: CD163 (5’–3’), CGG CTG CCT CCA CCT CTA AGT and ATG AAG ATG CTG GCG TGA CA (NM_004244.5); IL6 (5’–3’), TTT TGT ACT CAT CTG CAC AGC and GGA TTC AAT GAG GAG ACT TGC (NM_000600.3); MCP1 (5’–3’), GTC TTG aTC ACA ACA GCT TCT TTG G and AGC CAG ATG CAA TCA ATG CC (NM_002982.3); TNFA (TNF) (5’–3’), TTG AGG GTT TGC TAC AAC ATG GG and GCT GCA CTT TGG AGT GAT CG (NM_000594.2); ADAM17 (5’–3’), ATC TGA ACA AGC ACA CCT GCT G and AAG GAC TGT TCC TGT CAC TGC AC (NM_003183.4); IL10 (5’–3’), TCA AGG CCC ATG TGA ACT C and AGG GAA GAA ATC GAT GAC AGC (NM_000572.2); and LRPI0 (5’–3’), AGG TTG CCC AGC AGC ACT GAT TTA TC and TGC CAT CCC ACC TGT AGA ACA C (NM_014145.3). The PCRs were performed in duplicate using the KAPA SYBR FAST qPCR kit (Kapa Biosystems, Inc., Woburn, MA, USA) in a LightCycler 480 (Roche Applied Science) using the following protocol: one step at 95\(^\circ C\) for 3 min, then 95\(^\circ C\) for 10 s, 60\(^\circ C\) for 20 s, and 72\(^\circ C\) for 10 s. The increase in fluorescence was measured in real time during the extension step. The relative gene expression was estimated using the default ‘Advanced Relative Quantification’ mode of the software version LCS 480 1.5.0.39 (Roche Applied Science). The primers were designed using the QuantPrime software (Arvidsson et al. 2008). The specificity was tested and amplification efficiency determined (between 1.9 and 2.0). Before analysis of target genes, the housekeeping gene was tested for stability and found to be stable displaying comparable number of \(C_T\) cycles.

**Statistical analysis**

Differences between isolated adipocytes and SVF were analyzed using an unpaired \(T\)-test. Differences between stimulated and unstimulated AT and media were measured using a paired \(T\)-test or a Wilcoxon signed-rank test for those variables, which were not normally distributed. Changes in gene expressions and protein levels over time and overall changes among control, DEX, DEX + LPS, and only LPS at 8 and 24 h were determined by one-way ANOVA and post-hoc pairwise analysis was Holm–Sidak corrected for multiple comparison. The protein levels of sCD163, TNF\(\alpha\), IL10, MCP1, and IL6 after 24 h of incubation were measured by the area under the curve (AUC). Descriptive statistics are expressed as mean \(\pm\) S.D. The chosen significance level was a two-tailed \(P\) value of \(<0.05\). The statistical software package SPSS was used for all calculations. Graphs were plotted using the SigmaPlot software.
Results

Expression of CD163 in isolated adipocytes and in the SVF

To sort out whether isolated adipocytes express CD163 mRNA, we divided s.c. AT into the SVF (which contains the macrophages) and isolated adipocytes and measured the level of CD163 mRNA. The gene expression level of CD163 mRNA was significantly elevated in the SVF compared with the isolated adipocytes ($P<0.001$), in which almost no expression of CD163 was determined (Fig. 1).

Regulation of CD163 and sCD163 in AT

Figure 2 illustrates the interaction between DEX and LPS on CD163 mRNA (Fig. 2A) and on sCD163 released to the medium after 8 and 24 h of incubation (Fig. 2B). The expression of CD163 was significantly elevated when stimulating the AT with DEX (both at 8 and 24 h, $P<0.001$), but stimulation with LPS had no significant effect on CD163 mRNA compared with control (both at 8 and 24 h, $P>0.05$). The expression of CD163 was slightly reduced when stimulating the AT with DEX together with LPS when compared with only DEX at 8 h of incubation ($P<0.05$, Fig. 2A).

The protein level of sCD163 was measured in the incubation medium after stimulating the AT with DEX and LPS. There was no change in the level of sCD163 when pre-incubating the AT with DEX compared with control ($P>0.05$), but the level of sCD163 was significantly elevated when stimulating the AT with LPS compared with control ($P<0.001$ at 8 h, and $P<0.05$ at 24 h, Fig. 2B), which is in contrast to the gene expression of CD163.

Furthermore, there was a tendency toward an even higher level of sCD163 in the medium at 24 h after stimulating with both DEX and LPS compared with only LPS (0.034 ± 0.02 vs 0.027 ± 0.01 mg/l, $P: 0.13$). The AUC of sCD163 during the incubation for 24 h was approximately twofold higher after LPS stimulation compared with control ($P<0.05$) (Fig. 3A). The level of sCD163 increased significantly within the first 2–4 h, then it reached a plateau at 8 h, and finally, at 24 h the level of sCD163 was continuously elevated by approximately sixfold compared with baseline levels (0.004 ± 0.001 vs 0.027 ± 0.011 mg/l, $P<0.001$) (Fig. 3A).

Release of TNFα in the AT

The release of TNFα was investigated in parallel with sCD163, as TNFα is released in a similar manner as sCD163 from the plasma membrane by the ADAM17 enzyme (Etzerodt et al. 2010). LPS had a pronounced stimulatory effect on the release of TNFα. The level of TNFα increased dramatically within the first 4 h of LPS stimulation, continued to rise reaching a plateau after 4–8 h, and after 24 h of incubation only a small decrement was observed ($P<0.001$) (Fig. 3C). TNFα (AUC) was significantly decreased when incubating the AT with LPS together with DEX compared with LPS alone ($P<0.001$, Fig. 3C).

Regulation of ADAM17 mRNA in AT

The metalloprotease ADAM17 is known to regulate the shedding of sCD163 and TNFα from the plasma membrane. In order to investigate the expression of ADAM17 in the AT, we measured the gene expression level in the SVF and isolated adipocytes. ADAM17 mRNA tended to be up-regulated in the SVF compared with the isolated adipocytes; however, the difference was not significant ($P: 0.08$). Pre-incubating the AT for 18 h with DEX down-regulated the level of ADAM17 compared with control ($P<0.05$). Furthermore, incubating the AT with LPS strongly up-regulated ADAM17 mRNA within the first 4 h, then the level reached a plateau at 4–8 h, and thereafter it slowly decreased to a level similar to the baseline level at 24 h ($P<0.001$) (Fig. 4A).

Expression of pro-inflammatory and anti-inflammatory genes at AT and protein levels

The expression levels of the pro-inflammatory genes MCP1, TNFA, and IL6 and the anti-inflammatory gene IL10 were measured. The gene expression of IL10 increased...
The changes in the gene expression of CD163 in the AT and (B) the protein level of sCD163 in the incubation medium after pre-incubating the AT with DEX. The protein level of IL10 increased significantly after LPS stimulation compared with control (AUC, both $P<0.05$), and incubating the AT with LPS for 24 h resulted in a 14-fold increase in the level of IL10 in the incubation medium (Fig. 3B).

**Discussion**

We examined the regulation of CD163 and sCD163 in human AT. Initially, the gene expression level of CD163 was measured in the SVF and isolated adipocytes. CD163 was only expressed in the SVF, which also contains the macrophages, indicating that the expression of CD163 in human AT may be due to the presence of mainly macrophages and not the adipocyte fraction.

It was found that the expression of CD163 in the AT was up-regulated by DEX, while the pro-inflammatory genes TNFA, MCP1, and IL6 were unchanged or slightly down-regulated compared with control (no DEX). Conversely, when stimulating the AT with LPS, the gene expression of TNFA, MCP1, and IL6 was strongly up-regulated compared with control, whereas the expression of CD163 was unaffected after 8 and 24 h of incubation. The changes in the gene expression of CD163 induced by DEX are consistent with the studies on differentiated peripheral blood monocytes (Buechler et al. 2000, Sulahian et al. 2000, Schaer et al. 2002). Buechler et al. (2000) found that LPS suppresses the gene expression level of CD163 in differentiated blood monocytes; however, we could not retrieve the unique suppression of CD163 mRNA by LPS compared with control in human AT, but only a tendency at one time point when comparing DEX with DEX + LPS (at 8 h).

We also examined the protein level of sCD163 in the incubation medium after pre-incubating the AT with DEX. Although DEX highly up-regulated the expression of CD163 in the AT, it did not increase the release of sCD163, as the level of sCD163 was constant up to 24 h and significantly when stimulating the AT with LPS compared with control ($P<0.05$) (Fig. 4B). The expression of IL10 reached a plateau after 4 h of incubation with an unaltered level observed after 24 h. Stimulating the AT with DEX did not have an effect on the level of IL10 mRNA compared with control ($P>0.05$) (Fig. 4B). The gene expression levels of TNFA, MCP1, and IL6 were either identical to control or slightly down-regulated by DEX ($P>0.05$) (TNFA: Fig. 4C).

When stimulating the AT with LPS, there was a strong up-regulation of MCP1, TNFA, and IL6 mRNA within the first few hours compared with control. The expression of TNFA mRNA decreased abruptly after 4 h (Fig. 4C), whereas the expression of MCP1 and IL6 continued to be elevated after 24 h of incubation compared with the baseline level (TNFA and MCP1 $P<0.001$, IL6 $P<0.05$).

The protein levels of MCP1 and IL6 were measured in the incubation medium and both were significantly elevated when stimulating the AT with LPS compared with control (AUC, both $P<0.001$). When stimulating the AT with DEX + LPS, the AUC of IL6 and MCP1 was suppressed compared with only LPS (both $P<0.01$). The protein level of IL10 increased significantly after LPS stimulation compared with control (AUC, $P<0.05$), and incubating the AT with LPS for 24 h resulted in a 14-fold increase in the level of IL10 in the incubation medium (Fig. 3B).
when pre-incubating with DEX. LPS is a well-known activator of the innate immune response, and it initiates an inflammatory response by activating the toll-like receptor 4 (TLR4), which is expressed, e.g., on the macrophages. Activation of TLR4 leads to induction or suppression of several genes involved in the inflammatory response. Thus, as observed in the AT incubations, LPS up-regulates the gene expression and increases the protein level of the pro-inflammatory cytokines. It has been demonstrated that LPS stimulation of peripheral blood monocytes induces shedding of sCD163 from the extracellular receptor by TLR activation (Hintz et al. 2002, Sulahian et al. 2004). We found a significant increase in the level of sCD163 after LPS stimulation; however, the expression of CD163 was not up-regulated, confirming that the gene and protein levels of CD163 are regulated differently not only in peripheral blood monocytes but also in the AT. In vitro and in vivo studies have demonstrated that, in addition to acute shedding of sCD163, the surface expression of CD163 recovers to levels that are significantly higher at 24–72 h after LPS stimulation compared with control (Hintz et al. 2002,
Weaver et al. 2007). Unfortunately, our incubations were stopped after 24 h; thus, we were not able to investigate whether the gene expression of CD163 was similarly increased in the period of 24–72 h after LPS stimulation. However, we found that the expression of CD163 was unaffected at 24 h compared with the baseline level, which could indicate that the change in the surface expression of CD163 by LPS may not involve the transcription or translation level but instead mobilization of CD163 from the intracellular compartment to the cell membrane. In accordance with this theory, a study by Tippett et al. (2011) showed that a significant proportion of CD163 in the mature macrophages are found intracellularly, which may also explain the rapid and continuous shedding of sCD163 in relation to LPS stimulation.

As DEX up-regulates the expression of CD163, we speculated that a combination of DEX + LPS would result in a higher protein level to be shed from the receptor by LPS activation; however, we only found a trend toward a higher level of sCD163 when stimulating the AT with DEX + LPS for 24 h compared with only LPS stimulation (Fig. 3A).

The metalloproteinase ADAM17 has been found to mediate the shedding of sCD163 and TNFα in a study by Etzerodt et al. (2010). We investigated the release of sCD163 and TNFα and found a similar increase in the level of sCD163 and TNFα with a sharp rise within the first hours of LPS stimulation. Moreover, a significant rise in the gene expression of ADAM17 was also observed within the first 2–4 hours of incubation with LPS. The gene expression level of TNFα also increased within the first 2 h of LPS incubation, but the gene expression of CD163 mRNA was unchanged. Although sCD163 and TNFα are regulated similarly via activation of ADAM17, the gene expressions of CD163 and TNFα are regulated by separate mechanisms. This is in agreement with the suggestion that TNFα has mainly pro-inflammatory effects and CD163 and sCD163 as suggested have mainly anti-inflammatory effects, which also seems to be the case for macrophages in human AT.

In accordance with a previous study (Rose-John 2013), we found significant up-regulation of ADAM17 by LPS compared with control. The level of ADAM17 mRNA reached a maximum after 4 h and, thereafter, it decreased to baseline levels. Moreover, the level of ADAM17 mRNA was suppressed by DEX compared with control. We also examined the gene expression level of ADAM17 in the SVF and isolated adipocytes and found a similar expression in the two cellular fractions, thus, confirming that ADAM17 is a general enzyme, which is expressed in all the cells of the body (Scheller et al. 2011).

The anti-inflammatory cytokine IL10 is associated with the regulation of CD163 (Buechler et al. 2000, Sulahian et al. 2000); therefore, we investigated the protein and gene expression levels of IL10 in the AT. We found that both IL10 mRNA and protein levels were significantly increased by LPS, which is similar to other studies (de Waal Malefyt et al. 1991, Brunet et al. 1998). In vitro studies have demonstrated that IL10 inhibits the production and down-regulates the expression of different pro-inflammatory cytokines; therefore, the increase in IL10 in relation to LPS stimulation could be a regulatory function of the inflammatory response. It has been demonstrated that IL10 up-regulates the expression of CD163 in differentiated peripheral blood monocytes (Buechler et al. 2000, Sulahian et al. 2000). We were not able to investigate the direct effect of IL10 on CD163; however, despite a strong increase in the level of IL10 by LPS in the incubation medium, there was no change in the gene expression of CD163 in AT stimulated by LPS compared with control.

It is well described that the visceral AT is more infiltrated by macrophages and more inflamed compared with the s.c. AT (Harman-Boehm et al. 2007). We have previously shown that CD163 mRNA is up-regulated in s.c. AT from obese subjects compared with lean subjects (Fjeldborg et al. 2014); moreover, CD163 mRNA is found to be up-regulated in visceral AT compared with s.c. AT in obese subjects (K Fjeldborg, SB Pedersen, B Richelsen, unpublished observations). Thus, it is a limitation to our study that we examined the regulation of sCD163 and CD163 only in s.c. AT and not in visceral AT, and that we used AT samples only from slightly overweight subjects and not from obese subjects. Future studies should focus on the regulation of sCD163 and CD163 in both s.c. and visceral AT in lean and obese subjects. Furthermore, it is a limitation to our study that we did not measure the surface expression of CD163. Finally, the duration of the incubations should have exceeded 24 h in order to determine whether there is a rise in the gene expression level of CD163.

In conclusion, we found that CD163 mRNA is expressed in the SVF exclusively, emphasizing that CD163 is mainly expressed in the AT macrophages. The expression of CD163 in human AT is up-regulated by DEX, but unchanged by LPS. By contrast, sCD163 is released by LPS activation but unaffected by DEX alone. Thus, the gene expression of CD163 and the shedding of sCD163 are regulated differently in the AT. The protein levels of sCD163 and TNFα increase rapidly within the first few hours of LPS stimulation. The level of TNFα was also increased within the first 2 h of LPS stimulation, but the gene expression of CD163 mRNA was unchanged. Although sCD163 and TNFα are regulated similarly via activation of ADAM17, the gene expressions of CD163 and TNFα are regulated by separate mechanisms. This is in agreement with the suggestion that TNFα has mainly pro-inflammatory effects and CD163 and sCD163 as suggested have mainly anti-inflammatory effects, which also seems to be the case for macrophages in human AT.

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hours of LPS stimulation and a similar increase is observed in ADAM17 mRNA, whereas the gene expressions of CD163 and TNFA seem to be regulated by separate mechanisms.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding
This study was supported by The Danish Council for Strategic Research (TRAIN 10-092797), the Novo Nordisk Foundation, and Aarhus University.

Author contribution statement
K F analyzed the data, searched the literature, generated the figures, wrote the initial manuscript, and interpreted the data together with S B P and B R. K F and S B P performed the laboratory work. K F, S B P, and H J M measured the gene expressions and the protein levels. All authors were involved in writing the final paper and had final approval of the submitted and published version.

Acknowledgements
The authors thank Lenette Pedersen, Pia Hornbæk, and Kirsten Bank Pedersen for their very skillful technical assistance. Finally, they thank the private hospital Teres and their patients for their cooperation.

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Received in final form 10 July 2014
Accepted 29 July 2014
Accepted Preprint published online 29 July 2014