Published in final edited form as:

Cytokine. 2013 January ; 61(1): 71–77. doi:10.1016/j.cyto.2012.08.027.

Obesity and IL-6 interact in modulating the response to endotoxemia in mice

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Abstract

Obesity is associated with elevated levels of IL-6. High IL-6 is prognostic of mortality in sepsis, while controversial data link obesity to sepsis outcome. We used Lean and Diet-Induced Obese (DIO) WT and IL-6 KO mice to investigate the interaction between obesity and IL-6 in endotoxemia. Circulating levels of IL-6 were significantly higher in WT DIO vs WT Lean mice receiving LPS (2.5 µg/mouse, ip). Obesity lead to greater weight loss in response to LPS, with IL-6 deficiency being partially protective. Plasma TNFα, IFNγ, Galectin-3 and leptin were significantly elevated in response to LPS and were each differentially affected by obesity and/or IL-6 deficiency. Plasma Galectin-1 and adiponectin were significantly suppressed by LPS, with obesity and IL-6 deficiency modulating the response. However, LPS comparably increased IL-10 levels in each group. Leukopenia with relative neutrophilia and thrombocytopenia developed in each group after injection of LPS, with obesity and genotype affecting the kinetics, but not the magnitude, of the response. Hepatic induction of the acute-phase protein SAA by LPS was not affected by obesity or IL-6 deficiency, although baseline levels were highest in WT DIO mice. Injection of LPS significantly increased hepatic mRNA expression of PAI-1 in Lean WT and Lean KO mice, while it suppressed the high baseline levels observed in the liver of DIO WT and DIO KO mice. Thus, both IL-6 and obesity modulate the response to endotoxemia, suggesting a complex interaction that needs to be considered when evaluating the effect of obesity on the outcome of septic patients.

Keywords

Inflammation; cytokines; endotoxemia; obesity; acute-phase response

1. Introduction

Obesity is associated with a state of chronic inflammation, resulting in elevated production of several inflammatory mediators, mostly by macrophages and other leukocytes that infiltrate the expanded adipose tissue [1]. In addition to cytokines and other classical mediators of inflammation, some members of the galectin (Gal) family, particularly Gal1 and Gal3, are also elevated in obesity, in both humans and experimental animals [2, 3]. The excessive and chronic production of inflammatory mediators likely contributes to the

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increased risk of metabolic, cardiovascular and neoplastic disease of obese individuals [1, 4].

Interleukin-6, a cytokine that is elevated in obesity, is one of the most important inducers of the acute-phase response, a coordinated set of physiological processes that occurs after the onset of infection, trauma and other conditions [5]. The acute-phase response is chronically activated in obesity, partly in an IL-6-dependent manner, as manifested by increased production of acute-phase reactants, such as C reactive protein and serum amyloid A (SAA) by the liver as well as hematopoietic changes, particularly leukocytosis [6–8]. Elevated IL-6 in obesity not only participates in activation of the acute-phase response, but also modulates glucose metabolism and susceptibility to chronic diseases, including cancer [9].

Sepsis and septic shock are important causes of morbidity and mortality worldwide [10]. Endotoxin (lipopolysaccharide, LPS), a component of the membrane of gram negative bacteria, is a critical mediator of the systemic inflammatory response of sepsis through induction of inflammatory mediators and the associated acute-phase and hematological response [11]. Substantial evidence indicates that IL-6 is a prognostic marker of mortality and participates in the pathogenesis of sepsis [11]. On the other hand, controversial data exist about a connection between obesity and adverse outcomes in patients with sepsis [12] with data ranging from indications that obesity may be protective [13] to results demonstrating increased risk of death in obese bacteremic patients compared to non-obese subjects [12].

Obesity is associated not only with increased baseline levels of IL-6, but also with heightened and sustained production of this cytokine in response to several inflammatory stimuli. Thus, for example, both diet-induced and genetic obesity have been associated with elevated IL-6 production in response to systemic LPS in mice, although opposite results have been reported in an LPS-induced model of lung injury [14–16]. Elevated and sustained production of IL-6 has also been also reported in obese patients and experimental animals with acute pancreatitis, where this cytokine plays a major role in delaying recovery from pancreatic inflammation [17–19]. Thus, obesity and IL-6 interact in determining disease severity and duration.

In the present study, we used the murine model of diet-induced obesity (DIO) to investigate the interaction between obesity and IL-6 in modulating the inflammatory, hematological and acute-phase response to systemic administration of LPS.

2. Materials and Methods

2.1 Animals and treatments

Four-week-old male WT and IL-6 KO C57BL6 were purchased from the Jackson Laboratories (Bar Harbor, ME). Mice were fed either a chow diet (10 Kcal% fat) or a high-fat diet (60 Kcal% fat, from Research Diets, Inc., New Brunswick, NJ) *ad libitum* for 20 weeks. At 24 weeks of age, LPS (*E. coli* O111:B1, Sigma Chemical Co., St. Louis, MO) was administered i.p. at 2.5 µg/mouse. Control mice received an injection of sterile saline. Blood was obtained from the retroorbital plexus in separate groups of mice at 2 h, 6 h, Day 1 and Day 7 after administration of LPS and at 1 day after administration of saline. The liver was collected at Day 1 and Day 7, immediately snap frozen in liquid nitrogen and stored at –70°C for subsequent processing. For evaluation of body weight loss, the same animals were studied longitudinally, with body weight evaluated before injection of LPS as well as at Day 1, Day 3 and Day 7. Protocols were approved by the Animal Care Committee of the University of Illinois at Chicago.

2.2 Miscellaneous Measurements

Hematological parameters were evaluated on peripheral EDTA-treated blood using the Hemavet 950 FS (Drew Scientific, Inc, Waterbury, CT). Levels of IFN γ , TNF α , IL-6 and IL-10 were measured by ELISA using kits from eBioscience (San Diego, CA). Levels of leptin, adiponectin, Gal1 and Gal3 were measured by ELISA using kits from R&D Systems (Minneapolis, MN). Blood glucose was measured with a glucometer and plasma Alanine Aminotransferase (ALT) levels with a kit from Teco Diagnostics.

2.3 RNA expression analysis

Total RNA was isolated from the liver using Trizol and reverse transcribed. Gene expression levels of SAA-1 and PAI-1 were assessed by quantitative RT-PCR using specific primers from Applied Biosystems (Foster City, CA). Relative expression was calculated using the $\Delta\Delta C_T$ method after normalizing for expression of GAPDH.

2.4 Statistical Analysis

ANOVA using Fisher's least significant difference was used to determine significance of differences between treatment and control groups. Differences were considered significant for $p < 0.05$. Data are expressed as mean $\pm$ SEM. Statistical analyses were performed using the MedCalc software (Mariakerke, Belgium).

43. Results

3.1 Body weight, glucose and ALT

As expected, mice in DIO groups had significantly higher body weight compared to mice in Lean groups, with no significant differences between WT and KO mice, in agreement with previous results from our group [8] (body weight before administration of LPS was 28.5 ± 0.6 , 27.7 ± 0.4 , 50.8 ± 0.6 , and 49.7 ± 0.7 grams in Lean WT, Lean KO, DIO WT and DIO KO, respectively; $p < 0.001$ in DIO WT and DIO KO *versus* Lean WT and Lean KO mice, $n=5-8$). Administration of LPS induced significant weight loss in each group at Day 1 (Fig. 1A; $p < 0.05$ for each group). However, DIO WT and DIO KO mice lost less weight compared to Lean WT and Lean KO mice at the Day 1 time point ($p < 0.05$ for DIO WT and DIO KO *versus* their respective Lean counterpart). Lean KO mice had completely recovered from LPS-induced weight loss by Day 3, whereas body weight of Lean WT mice was still significantly reduced at Day 3 and began to recover by Day 7. Despite a less dramatic reduction of body weight in DIO WT compared with Lean WT mice at Day 1, DIO WT mice continued to lose weight at Day 3, with no signs of recovery even by Day 7. A similar, albeit blunted, trend was observed in the DIO KO group. Quantification of the area under the curve indicated that Lean KO and DIO KO mice overall lost significantly less body weight compared to their respective WT groups over the 7-days time-course, while DIO WT mice lost the higher amount of body weight (Fig. 1B).

Baseline glucose and ALT levels were significantly elevated in the circulation of DIO WT and DIO KO mice compared to their lean counterpart, with no effect of genotype, in agreement with previous data [8] (Fig. 1C–D). Administration of LPS significantly reduced glycemia and increased ALT levels in each group at Day 1, with return to baseline by Day 3.

In summary, DIO was associated with overall increased weight loss in response to LPS, with IL-6 deficiency being partially protective.

3.2 Cytokines, galectins and adipokines

To evaluate the interaction between DIO and IL-6 in mediating modulation of cytokines, galectins and adipokines, mice were injected with LPS or saline and plasma was prepared at

various time thereafter. Induction of IL-6 by LPS peaked at 2 h, with levels significantly higher in DIO WT compared with Lean WT mice (Fig. 2A) and no detectable IL-6 in either Lean KO or DIO KO mice, as expected.

As shown in Fig. 2B, plasma levels of TNF α peaked at 2 h in each group. Levels of TNF α were approximately 2.5-fold higher in DIO WT compared to Lean WT mice. A non-significant trend towards higher TNF α levels was also observed in DIO KO mice compared with Lean KO mice. Diet and genotype interacted in regulating production of TNF α in response to LPS, since DIO KO mice had significantly lower peak levels of TNF α compared to DIO WT mice, whereas no significant difference was observed between Lean WT and Lean KO mice.

Administration of LPS lead to significant induction of IFN γ in each group, with a peak observed at 6 h. Peak plasma levels of IFN γ were significantly higher in Lean KO and DIO KO mice compared with Lean WT and DIO WT groups, with no effect of diet (Fig. 2C). Plasma IL-10 was significantly induced by LPS in each group, with peak levels observed at 2 h and 6 h and no significant differences among groups due to either diet or genotype (Fig. 2D).

Circulating levels of Gal1 were significantly higher in saline-injected DIO WT and DIO KO mice compared to Lean WT and Lean KO mice, with no difference due to genotype (Fig. 2E). Administration of LPS induced a rapid decrease in plasma Gal1 levels in each group, with a significant nadir at 6 h post-LPS at 50–70% lower levels compared to saline-injected mice (Fig. 2E). There was a slower decline and a more rapid recovery of plasma Gal1 levels in LPS-injected DIO KO mice compared to each other group. Levels of Gal1 at Day 7 were significantly elevated compared to saline groups in LPS-injected Lean WT and Lean KO mice, but not in DIO WT or DIO KO groups. Because of differential regulation in lean *versus* DIO mice, circulating levels of Gal1 at Day 7 were no longer significantly different in lean *versus* DIO mice, even though at that time point DIO mice were still significantly obese.

Plasma levels of Gal3 were markedly elevated in saline-injected DIO WT and DIO KO mice compared to lean groups, without differences due to genotype (Fig. 2F). Administration of LPS did not significantly alter the elevated levels of Gal3 of DIO WT and DIO KO mice at any time point. In contrast with the lack of effect observed in DIO mice, administration of LPS lead to a rapid and significant increase in circulating levels of Gal3 in lean mice, with a peak at 2 h and comparable levels in Lean WT and Lean KO groups. In both Lean WT and Lean KO mice, Gal3 levels at 2 h post-LPS were comparable to those of DIO mice at each time point. In Lean mice, plasma Gal3 levels declined by 6 h post-LPS compared with levels observed at 2 h, but remained significantly elevated in LPS-injected Lean groups compared to saline controls up to Day 7.

As expected, baseline circulating levels of leptin were significantly higher in DIO WT and DIO KO mice compared to Lean groups and they remained so for the entire time course (Fig. 2G). Injection of LPS lead to a significant increase in plasma levels of leptin in each groups at 6h, with a return to baseline by Day 1, as previously demonstrated [20]. There was no influence of genotype on either baseline or LPS-induced plasma levels of leptin, again in agreement with previous reports [8, 20].

In agreement with previous results [21], baseline levels of adiponectin were comparable in Lean and DIO mice. Administration of LPS induced a significant reduction in circulating adiponectin levels in Lean WT and Lean KO mice at 6 h and Day 1, with a trend towards recovery, but levels still significantly lower compared to baseline, at Day 7 (Fig. 2H). In contrast, the suppressive effect of LPS on adiponectin did not reach statistical significance at

any time point in DIO WT or DIO KO mice, resulting in significantly lower adiponectin levels in Lean *versus* DIO groups at 6 h and Day 1.

Together, these results indicate that obesity and IL-6 modulate production of cytokines, galectins and adipokines in response to LPS, with differential effects on each individual mediators.

3.3 Hematological alterations

A significantly higher number of leukocytes was present in the peripheral blood of saline-injected DIO WT mice compared with Lean WT mice, whereas no significant difference was observed between DIO KO and Lean KO mice (Fig. 3A), in agreement with our previous results [8]. Administration of LPS resulted in significant leukopenia in each group, with a nadir observed at 6 h. The decline in leukocyte counts was more rapid in DIO groups compared with lean groups, with a significant drop already observed at 2 h post-LPS in DIO WT and DIO KO mice compared to vehicle-injected groups. By Day 1 leukocyte counts had returned to levels comparable to those observed in vehicle-injected mice in each group. However, the difference between DIO WT and Lean WT mice was no longer significant at Days 1 and 7.

Although LPS-induced leukopenia in DIO mice was more rapid compared with that of Lean animals, these latter responded with development of relative neutrophilia and lymphopenia faster than DIO mice. In fact, the percentage of circulating neutrophils was already significantly increased and the percentage of lymphocytes significantly reduced at 2 h post-LPS in Lean WT and Lean KO mice, whereas significant changes were only observed at the later time point of 6 h in DIO WT and DIO KO mice (Fig. 3B and C). There were no significant changes in the percentage of circulating monocytes in any group at any time point (Fig. 3D).

Thrombocytopenia of comparable magnitude developed in each group, with a nadir at 6 h (Fig. 2E). Platelet counts were still significantly suppressed at Day 7 in DIO WT and DIO KO mice compared to their respective saline-injected groups, whereas they had completely recovered to baseline levels in Lean mice. No significant changes were observed in the erythropoietic compartment at any time point in any group (not shown).

To summarize, administration of LPS induced leukopenia, relative neutrophilia and thrombocytopenia in each group, with diet and genotype exerting selective effects on the kinetics, but not magnitude, of the response.

3.4 Acute-phase response

Previous evidence indicated that elevation of hepatic production of SAA-1 is dependent upon the presence of IL-6 in models of obesity, sterile inflammation, gram positive infections and acute pancreatitis, whereas induction of SAA-1 by LPS in Lean mice is only minimally affected by IL-6 deficiency [8, 18, 22, 23]. As shown in Fig. 4A, hepatic mRNA expression of SAA-1 was 10-fold higher in vehicle-injected DIO WT mice compared to Lean WT mice. IL-6 deficiency was associated with significantly lower hepatic expression of SAA-1 in both Lean KO and DIO KO mice receiving saline compared with their respective WT groups. However, DIO KO mice still had significantly higher hepatic expression of SAA-1 compared with Lean KO mice. Administration of LPS lead to a ~1,000-fold induction of SAA-1 expression in each group at Day 1, with no significant differences due to either diet or genotype. At Day 7 post-LPS, SAA-1 had returned to baseline levels in DIO groups, while still being significantly elevated compared to their respective saline-control groups in Lean WT and Lean KO mice (3–7-fold higher compared to saline-injected mice, $p < 0.05$).

PAI-1 is an IL-6-independent acute-phase reactant [24]. Accordingly, hepatic mRNA expression of PAI-1 was 10-fold higher in saline-injected DIO WT and DIO KO mice compared with Lean groups, with no effect of genotype (Fig. 4B). Administration of LPS induced an 8-fold increase in PAI-1 expression in Lean WT and Lean KO mice at Day 1, whereas PAI-1 expression in DIO groups decreased by approximately 50% in response to LPS. Regulation of PAI-1 in opposite directions in Lean and DIO mice injected with LPS resulted in hepatic PAI-1 expression being higher in Lean *versus* DIO groups at Day 1 post-LPS, the opposite of what observed in saline-injected groups. At Day 7, PAI-1 expression was still significantly induced in the liver of Lean mice and still significantly suppressed in DIO mice, resulting in comparable levels in each group.

Cumulatively, these findings indicate that the effects of IL-6 and obesity in modulating the magnitude and direction of the acute-phase response induced by LPS are protein-specific.

5. Discussion

In the present report we investigated the interaction between obesity and IL-6 in modulating the response to LPS. Our data indicate that selective parameters of the systemic response to LPS are influenced by either DIO, IL-6 deficiency or the combination of DIO and IL-6 deficiency.

The magnitude and kinetics of LPS-induced weight loss were altered by both DIO and IL-6 deficiency. Overall, DIO mice – particularly DIO WT mice – lost significantly more body weight compared with their Lean counterpart, with a delayed and prolonged response. Elevated production of TNF α may partly explain the increased weight loss of DIO compared to Lean mice, as neutralization of this cytokine prevents weight loss [25]. Furthermore, IL-6 deficiency was associated with overall reduced weight loss in both Lean and DIO groups, in agreement with data demonstrating that neutralization of IL-6 ameliorates LPS-induced weight loss [25]. Thus, DIO WT mice, the group with the highest production of both TNF α and IL-6, correspondingly had the highest amount of weight loss.

Adiponectin acts as a negative acute-phase reactant in response to LPS or sepsis in lean animals [26, 27]. Our data support these previous results and demonstrate that the inhibitory effect of LPS on adiponectin in lean mice does not require IL-6 and is long-lasting, since plasma adiponectin levels were still significantly reduced 7 days post-LPS in both Lean WT and Lean KO mice. Surprisingly, only a non-significant trend towards lower adiponectin was observed in LPS-injected DIO WT or DIO KO mice, despite the elevated levels of TNF α -an adiponectin-inhibiting cytokine [28] - present in these mice. Circulating levels of adiponectin result from the combined production and output of this adipokine by visceral and subcutaneous adipose tissue. We previously demonstrated that the marked reduction in adiponectin mRNA expression of visceral adipose tissue of DIO mice is accompanied by elevated expression in the subcutaneous compartment [2]. Furthermore, data indicate that administration of LPS significantly increases production of adiponectin in subcutaneous adipose tissue, while suppressing it in epididymal (visceral) fat [29]. Thus, differential regulation of adiponectin in visceral *versus* subcutaneous adipose tissue in response to LPS likely explains our present findings.

Production of cytokines and galectins was altered by DIO and IL-6 deficiency in a selective manner. As mentioned above, obesity was associated with elevated induction of IL-6 and TNF α by LPS, confirming the concept that obesity acts as a priming factor for inflammatory responses [16, 30]. These data also indicate that the presence of systemic endotoxemia in DIO mice is not associated with development of endotoxin tolerance, which may result from chronic exposure to low doses of LPS [31]. Administration of LPS induced significantly higher levels of IFN γ in IL-6 KO compared with WT mice, irrespective of diet. These

results are in agreement with previous data indicating that IL-6 deficiency leads to excessive superantigen-induced IFN γ production by CD8 lymphocytes in a STAT-3-dependent manner [32].

We confirm here that DIO leads to elevated circulating levels of Gal1 and Gal3 [2] and demonstrate that this effect is independent of IL-6. Interestingly, administration of LPS lead to a profound suppression of plasma Gal1 levels in each group, with the kinetics of modulation being influenced by IL-6 deficiency in DIO mice. Galectin 1 exerts a variety of anti-inflammatory effects [33] and its downregulation by LPS may thus participate in modulating the magnitude of the response, as it has been reported for carrageenan-induced inflammation [34]. In contrast with the inhibitory effect on circulating Gal1, LPS markedly upregulated plasma levels of the pro-inflammatory Gal3 in Lean WT and Lean KO mice. However, LPS failed to alter the already elevated circulating levels of Gal3 observed in DIO WT and DIO KO mice. Although Gal3 exerts several pro-inflammatory activities, lean Gal3 KO mice have enhanced susceptibility to LPS-induced lethality and heightened TNF α production [35]. Thus, upregulation of Gal3 by LPS may contribute to balance the inflammatory response to LPS. Finally, it is important to note that galectins act both intra- and extra-cellularly [33] and it will therefore be important for future studies to evaluate regulation of both cell-associated and soluble Gal1 and Gal3 in response to obesity and LPS.

Although mice in each group developed leukopenia with relative neutrophilia as well as thrombocytopenia, DIO was associated with an altered kinetics of the response, whereas IL-6 deficiency did not significantly affect the outcome. Alterations in both the hematopoietic and adipose compartments of the bone marrow of DIO mice [36] likely account for the observed differences between Lean and DIO mice, since the bone marrow is the first target of LPS-induced hematological changes [37].

Evaluation of acute-phase proteins confirms that induction of SAA-1 by LPS is independent of IL-6 [22, 23], whereas elevation of this protein by obesity is partly dependent on IL-6 [8]. However, despite elevated baseline expression of SAA-1 in DIO WT mice, the magnitude and kinetics of induction was comparable to that of Lean groups. We also confirm that induction of PAI-1 in obesity is independent of IL-6 [8], but demonstrate a novel pattern of regulation for this acute-phase protein. Hepatic expression of PAI-1 was induced by LPS in Lean WT and Lean KO mice, as expected [38], reaching levels comparable to those observed in vehicle-injected DIO WT and DIO KO mice. In contrast, LPS lead to a significant downregulation of PAI-1 expression in DIO mice. Induction of PAI-1 by LPS is mediated by TNF α [38], whose levels are elevated in DIO mice, thus excluding this pathway as a possible explanation. To our best knowledge, this is the first observation on an inhibitory effect of LPS on PAI-1 expression, which requires further investigation, including a more detailed time-course study.

In conclusion, our data demonstrate that IL-6 and obesity regulate selective aspects of the response to LPS. Given the controversial findings on the effect of obesity on the outcome of septic patients, the complex interactions between obesity and IL-6 in modulating the inflammatory response during endotoxemia will need to be taken into consideration.

Acknowledgments

This study was supported by NIH grant DK083328 to GF.

Abbreviations

ALT alanine aminotransferase

DIO	diet-induced obesity
Gal	galectin
IFN	interferon
IL	Interleukin
LPS	lipopolysaccharide
PAI-1	platelet activator inhibitor-1
SAA	serum amyloid A
TNF	tumor necrosis factor

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Highlights

- Obese mice with endotoxemia produce higher IL-6 than lean mice.
- Exacerbated weight loss in endotoxemic obese mice is partially mediated by IL-6.
- Obesity and IL-6 deficiency modulate production of cytokines and adipokines.
- Induction of serum amyloid A by LPS is obesity- and IL-6-independent.
- LPS inhibits the high expression of PAI-1 in the liver of obese mice.

Figure 1. Body weight loss, glucose and ALT levels

Panel A: Grams of body weight loss by Lean WT (black squares), Lean KO (empty squares), DIO WT (black triangles) and DIO KO (empty triangles) were measured before administration of LPS, as well as Days 1, 3 and 7 post-LPS. Panel B: quantification of area under the curve for body weight loss. Panel C: blood glucose levels. Panel D: plasma ALT levels. Data are mean $\pm$ SEM (n=5–8). $^{\circ}$ p<0.05 vs respective control; *p<0.05 *versus* respective Lean group at same time point; $\dagger$ p<0.05 *versus* respective WT group at same time point.

Figure 2. Circulating levels of cytokines, galectins and adipokines

Plasma was obtained at 2h, 6h, Day 1 or Day 7 after administration of LPS or at Day 1 after administration of vehicle for measurement of circulating levels of IL-6 (A), TNFα (B), IFNγ (C), IL-10 (D), Gal1 (E), Gal3 (F), Leptin (G) and Adiponectin (H) in Lean WT (black squares), Lean KO (empty squares), DIO WT (black triangles) and DIO KO (empty triangles). Data are mean $\pm$ SEM (n=5–8). . $p<0.05$, ° $p<0.01$, °° $p<0.001$ vs respective control; * $p<0.05$, ** $p<0.01$, *** $p<0.001$ versus respective Lean group at same time point; † $p<0.05$ versus respective WT group at same time point.

Figure 3. Hematological changes

Blood was obtained at 2h, 6h, Day 1 or Day 7 after administration of LPS or at Day 1 after administration of vehicle for evaluation of total leukocytes (A), % neutrophils (B), % lymphocytes (C), % monocytes (D), and platelets (E) counts in Lean WT (black squares), Lean KO (empty squares), DIO WT (black triangles) and DIO KO (empty triangles). Data are mean $\pm$ SEM (n=5–8). .^{oo}p<0.01, ^{ooo}p<0.001 vs respective control; *p<0.05, **p<0.01 *versus* respective Lean group at same time point.

Figure 4. Acute-phase response

Total mRNA was extracted from the liver obtained at Day 1 or Day 7 after administration of LPS or at Day 1 after administration of vehicle for evaluation expression of SAA-1 (A) and PAI-1 (B) by qRT-PCR counts in Lean WT (black squares), Lean KO (empty squares), DIO WT (black triangles) and DIO KO (empty triangles). Data are mean $\pm$ SEM (n=5–8) and are expressed as fold change over Lean WT vehicle using the $\Delta\Delta^{ct}$ method after normalization by expression of GAPDH. $^{\circ}p<0.05$, $^{\circ\circ}p<0.01$, $^{\circ\circ\circ}p<0.001$ vs respective control; $^{*}p<0.05$ versus respective Lean group at same time point; $^{\dagger}p<0.05$ versus respective WT group at same time point.