Author’s response to reviews

Title: Sepsis induced changes of adipokines and cytokines. Septic patients compared to morbidly obese patients

Authors:

Andreas Hillenbrand (Andreas.Hillenbrand@uniklinik-ulm.de)
Uwe Knippschild (uwe.knippschild@uniklinik-ulm.de)
Manfred Weiss (manfred.weiss@uniklinik-ulm.de)
Hubert Schrezenmeier (h.schrezenmeier@blutspende.de)
Doris Henne-Bruns (doris.henne-bruns@uniklinik-ulm.de)
Markus Huber-Lang (markus.huber-lang@uniklinik-ulm.de)
Anna M Wolf (anna-maria.wolf@uniklinik-ulm.de)

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Author’s response to reviews: see over
Dear Madam or Sir,

Thank you for consideration of our manuscript "Sepsis induced changes of adipokines and cytokines – septic patients compared to morbidly obese patients", authors: A. Hillenbrand, U. Knippschild, M. Weiss, H. Schrezenmeier, D. Henne-Bruns, M. Huber-Lang, AM Wolf, for publication in your journal.

White adipose tissue has been increasingly recognized as an important endocrine organ playing an important role in inflammation.

You asked for a new version with marked changes. Since most parts of the manuscript were completely reworked, initially we sent only the manuscript without indicated changes. Please find enclosed

- the manuscript without indicated changes,
- the manuscript with indicated changes, and
- below, a detailed report to the reviewers comments
We have reviewed the manuscript according to your reviewer’s comments. Please do not hesitate to contact me in case of any question arising. Thank you very much indeed for your efforts.

Yours faithfully

Andreas Hillenbrand

Reviewer number: 1
This study by Hillenbrand et al. describes changes in serum concentrations of adipose tissue derived factors in 33 septic patients, compared to 60 healthy blood donors.

• In the now submitted version we compare additionally morbid obese patients.

1. The manuscript suffers from a lack of a clearly stated hypothesis. Is it, as put forward in the beginning of the introduction, to investigate the ‘insulin resistant syndrome’ in sepsis? Or describing the levels of some adipokines?

• We hopefully stated a clear hypothesis in at the end of introduction. The studies aim is to determine a serum adipocytokine profile in critically ill patients. Further, comparing profile of these patients with the profile of morbidly obese patients and a healthy control group. Further, we investigated, if concentrations of adipocytokines are shifted in the same direction in critically ill septic patients as in morbidly obese patients compared to healthy controls.

2. The authors indicate in the introduction that septic patients are characterized by marked increases in proinflammatory cytokines. Thus presenting levels of these markers are not novel information. The novelty of the data on the cytokines comes more from their possible relation to the studied adipokines resistin, adiponectin and leptin. However, the reported correlations are not informative as no correlation coefficients are added. This makes it very difficult to evaluate the
significance of the observed correlations.

- Correlation coefficients were added.

3. The result section contains an overflow of numbers which are already stated in the figures or tables, and sometimes even both. The authors also refer to a table 3 which is not added to the manuscript.

- Result section was completely revised and restructured. Table 3 is removed.

4. In table 2, changes between groups are indicated by arrows, but when no significant change is observed (e.g. leptin and IL1alpha) no arrow should be displayed.

- Arrows at no significant changes in Table 2 were removed.

5. How certain are the authors that they are comparing their septic patients to proper controls? The age between the two groups is different, and, more importantly, no BMI data are given for the healthy controls. It is clear from the literature that BMI and adipokine levels are related. Also the samples of the healthy controls were not taken in a fasted state, which might explain the lack of effect on leptin levels.

- The control group is a limitation of our study. In the healthy blood donor group, we did not investigate hidden or not yet clinically manifest side diagnoses. Further, we have no direct information regarding the BMI of controls. Since mean leptin levels are in normal range, controls could be supposed to have a BMI in normal range, since BMI and leptin levels are related. Our results of unchanged leptin levels are in line with recent studies. As our study, Vachharajani V (Pathophysiology 2008) reported unchanged leptin levels, independently of fasting state. However, leptin has also been shown as an acute phase reactant with an immunomodulatory activity during the infectious stress response with correlation CRP (Maruna, Physiol. Res; 2001). These and further limitations of control group is mentioned in discussion.
6. Another shortcoming in the manuscript is that no discussion is made on the previous extensive literature of adipokines in sepsis. For instance Arnalich (J Infect Dis 1999), Bornstein (JCEM 1998), Tzanela (In Vivo 2006) found Maruna (Physiol Res 2001), Venkatesh (Critical Care 2009), Langouche (Critical Care 2009), Uji (Surgery 2009).

- Discussion is rewritten with consideration of above mentioned literature. Papers of Maruna (Physiol Res 2001) and Venkatesh (Critical Care 2009) are included in discussion besides further actual literature.

7. The discussion could merit from a reduction in size. What is the main conclusion? Are the described changes cause or consequence of the insulin resistant state? What is the mechanism leading to the observed changes in the adipokines? Are they related to any measured changes in insulin sensitivity and/or mortality outcomes? What is a possible explanation for the fact that leptin, in contrast to other adipokines was not influenced by sepsis.

- Discussion was reduced in size and focused to the results. Main conclusion was precised. We determined a sepsis specific adipocytokine profile in critically ill patients and compared patients profile with healthy control group and morbidly obese patients. We found increased levels of proinflammatory cytokines and altered levels of adipokines; this possibly underlines a contribution in the development of insulin resistance in critically ill patients. The underlying mechanisms are still controversial debated and not topic of the presented paper.

Reviewer number: 2

1. The authors’ idea was to give a profile of the above mentioned parameters 24 hours after onset of sepsis. However, several publications on this topic exist in PubMed. The discussion on the role of adiponectin in critical ill patients has been raised by the finding of hypoadiponectinemia in those patients. The nature of this phenomenon, however, remains to be elucidated (Crit Care 2009;13:174). Pronounced elevation of resistin was found to correlate with severity of disease in severe sepsis and septic shock (Crit Care Med 2007 Jun;35:1536). The topic
is therefore of current interest. However, the concentrations of cytokines (IL-1, IL-6, TNFa) in sepsis and critical ill patients have long been described. The cytokine profile described by Hillenbrand confirmed these publications. Some parameters of the control group were missing (e.g. BMI) and this was addressed by the researches.

- We added morbidly obese patients, so we compared septic patients to healthy controls and morbidly obese patients. Of course, IL-1, IL-6, IL-8, and IL-10 are well described for septic patients. However, interleukins seems to influence adipokine profile (Kern Am J Physiol Endocrinol Metab2001) or are produced by adipose tissue; therefore, we included this interleukins in our paper. The limitations of our study (BMI …) is addressed in the discussion of the paper.

2. The data were well presented. However, the presentation resembled more a pilot study as many parameters were determined without adjustment and clear criteria for selecting were missing. The manuscript would be improved if clear reasons for parameter selection were given. Correlation analysis was done but correlation coefficients were not given. It is impossible to judge on relevance of correlation without knowing the factors. The authors should add these.

- We explained in introduction our criteria for parameter selecting.
- Correlation coefficients were added, so relevance of correlation could be judged.

3. The discussion is a description of previous findings and the results found were in conformity with those. The conclusion drawn is confirmation.

- Most parts of results and discussion is rewritten.

4. Patients' assessment using SAPS II and SOFA without Glasgow coma scale is very appropriate and well done. The reviewer appreciated.

- Patients' assessment using SAPS II and SOFA without Glasgow coma scale was not changed compared to the previous version.
5. A lot of parameters and complicated pathophysiological mechanisms are described. However, sepsis is very heterogenous and it is difficult to draw significant conclusions from the study (blood was drawn once). Cytokine profiles of obese subjects, diabetes type 2, and septic patients were described and the authors concluded that their data could help explain insulin resistance in critical ill patients. This is not very specific. If the authors concentrated on one issue this would focus the discussion significantly.

- We hopefully addressed our new version to these points.

6. The manuscript adheres to relevant standards for reporting and data deposition.

- We hopefully also addressed in our new version to these points.

7. The title gives the impression that the measured cytokines derive from the adipose tissue. This is true for adiponectin as it seems to be exclusively produced in adipose tissue. It could be assumed for leptin as well as major source is white adipose tissue although liver, stomach, bone, and other tissues can produce this as well. However, cytokines like IL-6, TNFa, IL-1, and others are secreted from macrophages as well and it is not possible to quantify the relative production and contribution of adipose tissue. The title should therefore be changed. The abstract is correct but conclusion weak and could be focused.

- According to the suggestion we have changed the title from “Adipose tissue derived hormones in septic patients” to “Sepsis induced changes of adipokines and cytokines – septic patients compared to morbidly obese patients”.
- The abstract was rewritten, conclusion was focused on results.

8. Table 3 was missing (mentioned on page 9, gender-specific differences ...). Figures: Significant differences should be marked with a symbol.
Box-and-whisker plot are appropriate. Writing: The authors should not mention positive or negative correlation without the corresponding correlation factor. General comment: If it was the intention of the authors to compare (adipo)cytokine profiles of several populations the authors should clearly state this and add the profiles of obese patients and diabetic patients. This would tremendously improve the manuscript.

- Table 3 is removed from the text.
- The suggestion to mark significant differences on box-and-whiskers plots is good. However, we compare three different groups. This means, we must explain in every figure, which of the three different groups have a significant change. This makes figures complex, so we kept figures in original form.
- The correlation factor was added.
- We clearly stated in the background and conclusion the intention to compare / determine different.
- Profile of obese patients was added. Unfortunately we can not add a profile of diabetic patients.

9. There seem to be no ethical or competing interest issues.

Reviewer number: 3

1. The role of adipokines in septic patients is not fully understood. Several studies have been performed to examine the impact of adipokines on survival and various laboratory parameters.
The idea to demonstrate specific adipokine-profiles in septic patients and different clinical settings (e.g. medical vs. surgical patients, origin of septic shock) is interesting. The title of the manuscript should clearly state which population was evaluated in the study.

- According to the suggestion we have changed the title from “Adipose tissue derived hormones in septic patients” to “Sepsis induced changes of adipokines and cytokines – septic patients compared to morbidly obese patients”.

2. To my opinion, the main limitation of this study is the very small absolute number of 33 patients. Furthermore, the authors pointed out the heterogeneousness of the septic group. An a priori examination should have been performed to assure an adequate statistical power. Given the high heterogeneity of critically ill patients, this small study does not allow to draw any meaningful conclusions. A detailed report on the examined cohort should be given, including organ function. Some adipokines, e.g. resistin show a strong association to liver and renal function.

- The limitations including the small number of patients and the heterogeneousness of the septic patients group is stated under limitations of the study. Absolute number of 33 critically ill patients is small. A comparable paper by Venkatesh (Critical care 2009) presents only 23 critically ill patients.
- To find statistically significant differences in the three compared groups, size was sufficient.
- We tried to create a table with background and organ function of presented critically ill patients. However, due to the heterogeneousness of the septic group including patients with different cause of sepsis, side diagnoses, operations and organ failures, most tables were more confusing than helpful.
3.
An important methodical weakness of this study is the measurement of the adipokines on the day after admission to the ICU. In intensive care medicine extensive immediate therapy is crucial. So the collected data are most likely influenced by initial therapeutic interventions (e.g., fluid resuscitation, insulin administration, possibly steroids) and do probably not reflect the initial adipokine profile in critically ill patients. Laboratory investigations or blood sampling therefore should have been made at the time of admission to the ICU.

- Measurement of adipocytokines was not performed one day after admission to ICU, but on day after onset of severe sepsis. This is stated precise in the new version.

4.
The interpretation of the collected data is well, but conclusions suffer to my opinion from statistical and methodical limitations

- Conclusion is newly written.

Reviewer number: 4

The aim of this study was to determine serum adipokine profile in critically ill patients.

The conclusion is that adipokine levels are changed extensively in patients with severe sepsis and shock. These changes are shifted in the same direction as in obese subjects and patients with type 2 diabetes. The authors suggest that their result could help to explain insulin resistance in critically ill patients and patients with systemic inflammation response syndrome.

Major concern;
The conclusion of this manuscript is too speculative.

- Conclusion is newly written.

The authors claim that they demonstrate a sepsis specific adipokine profile in critically ill patients and all significant changes are shifted in the same direction
as in obese subjects and patients with type 2 diabetes. This could help to explain insulin resistance in critically ill patients and patients with systemic inflammation response syndrome, page 14.

It is well-known that acute illness or injury may result in insulin resistance, glucose tolerance and hyperglycemia (1-3) and many of the acute metabolic changes seen in critically ill patient are similar to those seen in patient with metabolic syndrome (4). Cuthbertson (5) introduced the concept of ebb and flow phases of metabolism after trauma and the ebb phase was characterized by a general fuel mobilization which shifted gradually into the flow phase. The latter is characterized by a state of catabolism which subsequently changes into anabolism and recovery. During the catabolic phase, energy expenditure is elevated, and a general breakdown of body tissues occurs. The changes occurring in the two phases are often reflected by typical alterations in circulating levels as well as turnover of different substrates, hormones and mediators.

Therefore, the described changes of adipokines in this manuscript are not exclusively demonstrating a septic specific adipokine profile.

The authors are measuring adipokines in serum in septic patients vs. controls and compare the results with changes of adipokines in obese subjects. On page 4 and 5 the adipose tissue is described, however, the connection between the circulating levels of adipokines compared with adipokines in the adipose tissue is not described and could be extended.

- Connection of adipose tissue and circulating adipokines is now described in the background part.

Leptin and adiponectin are secreted by the adipocytes, but 90% of adipokines are in fact produced by the stormal-vascular cells.

- This fact is described in the background part.

Also, secretion of adipokines varies between different regions of white adipose tissue. For example, IL-6 release is increased from the visceral adipose tissue, while leptin is mainly secreted by the subcutaneous. Changes of adipokines and insulin resistance in obese subjects can therefore differ for many reasons. Furthermore, it is
important to note, that only a limited number of adipokines are released into the bloodstream at levels that are detectable with current assays, resulting in increased circulating levels in the obese state. Some adipokines acting in a paracrine or autocrine manner may play an important role; thus, circulating levels of the adipokines may represent only spillover from WAT and may not be associated with the obese condition. Discussion of regulated adipokines is weak and could be extended.

• Discussion regarding adipokines was rewritten.

For example, the role of resistin in the pathophysiology of obesity and insulin resistance in humans is controversial. Several studies have shown positive correlations of circulating resistin levels with body fat mass (6, 7) or insulin resistance (8, 9), but other studies found no relationship between resistin gene expression and body weight or insulin sensitivity (10-12). The resistin levels in humans are thought to correlate more closely with inflammation than with insulin resistance, since resistin concentration is elevated in the patients with severe inflammatory disease (13). Also the role of TNFα in humans is controversial. Pfeiffer et al. showed that men with T2DM had higher TNFα concentrations compared with nondiabetic subjects (14). However, several studies reported no association between circulating levels of TNFα and insulin sensitivity (15,16). Circulating TNF has been reported to be associated with a soluble receptor that inhibits its biological activity (17), suggesting that the action of TNFα is primarily a local one. Therefore, it seems unlikely that the circulating levels of TNF reflect the insulin resistance state of the whole body.

• We have implemented parts of above very helpful suggestion in our new discussion.

On page 12, row 11; “resistin levels are generally more elevated with obesity”. On the same page, row 20; “unexpectedly, resistin did not correlate with BMI in the patients with sepsis”. Is this correlation performed with the mean value of BMI.
in the septic group (mean BMI= 26 kg/m2)? WHO classification of adults according to BMI gives that obese subject should have a BMI over 30 kg/m2.

- We initially expected a correlation of septic patients BMI with resistin levels. Paraphrase is changed in the new version.

From table 1, there is only 8 subjects with BMI over 30 kg/m2 (underweight = 1 subject, normalweight=10 subjects, overweight=14 subjects). If the correlation was performed between resistin and only those 8 obese subjects in the septic group, could this give another, more expected, result?

- Result was the same (not stated in the manuscript).

The manuscript seems to be in a working process since there are several details that should be corrected before submitting the manuscript, such as;
In conclusion, page 3, row 4 and page 14, row 8, there is missing, in serum. Adipokine levels in serum are changed… and serum levels of adiponectin in male / female.
Full-name and abbreviations of mentioned proteins are inconsistent in the manuscript. TNF-alpha and interleukins are not written at all in the text, just abbreviations. CRP are translated twice, page 2, row 6 and page 6, row 9 and this is the same with BMI, translated on page 6, row 12, page 8, row 18 and page 13 row 16. In the list of abbreviations, page 15, all of abbreviations are not there (table 1 includes a lot of abbreviations not listed on page 15).
There are two parenthesis on page 8, row 16 and there is no hyphen in the word IL-6 on page 13, row 23.
Tables are written inconsistent;
Tab 1, table 2 and additional file 1; Tabeller 1.doc
In table 1, there is a column with WBC, but there is no discussion around these values in the text.
The mark for significant P, should be written with capital letter and cursive (Page 7, last row and in Results page 8 and 9 and in Table 2).
In the result part, page 9, last 2 rows, there is information about seven of the included patients, (they had preexisting type 2 diabetes). This information should
be included in the Material and Method part, under Patients and controls, page 6. Since these seven patients showed no difference in any analyzed parameter, the authors should discuss around this lack of changes.

All values that are not significant should not be included, page 8, row 12-14 and row 16. When there are significant values, those should be included, page 8, row 21 and page 9, row 10-12).

There is a missing reference, page 5 row 13

- Paper was adjusted according the reviewers suggestions.

References