Author’s response to reviews

Title: Insight in modulation of inflammation by gene, protein and metabolite profiling in overweight males: a human intervention study

Authors:

Marjan J van Erk (marjan.vanerk@tno.nl)
Suzan Wopereis (suzan.wopereis@tno.nl)
Carina Rubingh (carina.dejong@tno.nl)
Trinette van Vliet (trinette@lieke.net)
Elwin Verheij (elwin.verheij@tno.nl)
Nicole H.P. Cnubben (nicole.cnubben@tno.nl)
Theresa L. Pedersen (theresa.chem@gmail.com)
John W Newman (jwnewman@ucdavis.edu)
Age K Smilde (A.K.Smilde@uva.nl)
Jan van der Greef (jan.vandergreef@tno.nl)
Henk F.J. Hendriks (henk.hendriks@tno.nl)
Ben van Ommen (ben.vanommen@tno.nl)

Version: 3 Date: 4 December 2009

Author’s response to reviews:

MS: 1861417505290671

Research article

Insight in modulation of inflammation by gene, protein and metabolite profiling in mildly obese males: a human intervention study

Marjan J van Erk, Suzan Wopereis, Carina Rubingh, Trinette van Vliet, Elwin Verheij, Nicole H.P. Cnubben, Theresa L. Pedersen, John W Newman, Age K Smilde, Jan van der Greef, Henk F.J. Hendriks and Ben van Ommen

BMC Medical Genomics

To Dr. Danielle Burgess

Assistant Scientific Editor

Zeist, December 4 2009

Dear Dr. Burgess,

Thank you very much for sending us the further comments of the reviewers on our manuscript. We appreciate the comments and suggestions of the reviewers. The manuscript and the supplementary tables have been revised according to
the reviewers’ remarks. We sincerely hope that the manuscript is acceptable for publication in its present form.

Please find below our response to the reviewers’ comments and suggestions.

With kind regards, on behalf of the other authors,

Marjan van Erk

Response to the reviewers’ comments (November 2009)

Reviewer 1 (John Fain)

With respect to supplementary table 2:

1. Randomization of subjects to the placebo and the diclofenac group was restricted by CRP, BMI, fasting glucose and age. Despite these restrictions, certain parameters could differ between the two groups, specifically since levels of inflammatory markers can vary considerably between obese subjects and appeared to be variable over short time intervals, i.e. between the moment of pre-study analysis and day 0 of the intervention. In this study, a significant difference at day 0 between the two groups was only observed for CRP. The levels of ferritin and CEA at day 0 were not significantly different between the placebo and diclofenac group. The between-subject variation is the reason for studying within-subject effects of the intervention, as was also analyzed in this study. For three proteins in supplementary table 2 (MIP-1alpha, cancer antigen 19-9 and ENA-78) we have recalculated the average levels, excluding the subjects (one or two) in which the protein could not be measured above the detection limit.

2. The % change was calculated for each subject individually (day 9 vs day 0). Next, the median value for % change in the placebo group and in the diclofenac group is reported in the table. As the % change can vary considerably among the subjects, the reported median % change can differ from the average change that can be inferred from the average values for the protein reported in supplementary table 2. For example, TNF alpha levels were decreased in 6 subjects of the diclofenac group (values from -306% to -25%). In two subjects the levels did not change (0 and 8% change), in one subject levels increased by 438%. For all diclofenac responsive parameters, including TNF alpha, the individual changes are reported in figure 3.

3. We agree with the reviewer that the supplementary tables become easier to interpret when including only the relevant changes. Therefore, we have adjusted supplementary table 1 and 2, these now contain median % change if % change was >20 in 6 or more subjects in the group or if % change was <-20% in 6 or more subjects in the group (selection criterium as described in methods section).

With respect to main text:
Both the main text and the legend of figure 2 state that CRP levels change significantly in the placebo group. [Main text: “CRP levels were significantly reduced in the placebo group (2.05 ± 2.30 µg/mL (mean ± stdev) at day 9 compared to 4.03 ± 3.34 µg/mL at day0, p = 0.0062), but not in the diclofenac group”; figure legend: “In the placebo group, hsCRP levels were significantly different at day 9 compared to day 0 (P = 0.0062)”]. The paper does not state that CRP levels were different between placebo and diclofenac at day 9. On the contrary, these levels are almost equal (placebo: 2.05 vs diclofenac 1.95 µg/ml). The paper does state that the response of CRP over time differed between the placebo and diclofenac group, which is due to the fluctuations of CRP in the placebo group. Of course, the reviewer is correct that diclofenac treatment had no effect on CRP. As suggested by the reviewer, we have removed the paragraph on CRP network analysis from the results section, so that the results section only focuses on the treatment effects. Discussion on CRP related effects is now restricted to the discussion section.

Reviewer 2 (Yu Wang)

The focus of the present study was to investigate and provide insight into the modulation of obesity-associated inflammation, i.e. chronic low grade systemic inflammation. We realized that chronic low-grade inflammatory status in overweight subjects is difficult to quantify with a single inflammation marker as inflammation is complex process. Therefore, we have applied an ‘omics’ strategy, measuring profiles of gene expression, 79 inflammation related plasma proteins and metabolomics, to assess a broad range of known inflammatory and unknown markers. This study confirms: 1) the difficulty of good candidate biomarkers for inflammation, as we observed changes in CRP in the placebo group rather than in the intervention group and 2) that the complexity of modulation of inflammation may be detected using omics technology, as using our analysis approach we could distinguish between treatment-unrelated inflammatory fluctuations (correlated and biologically linked to CRP / acute inflammation) and changes in inflammatory markers related to diclofenac treatment. As such, we were able to corroborate anti-inflammatory effects of diclofenac (changes in PGE2, TNF alpha and annexin A2) and we were able to detect a broader set of inflammatory markers that specifically respond to diclofenac.

The manuscript has been revised to address and clarify these issues. We have improved the discussion section on page 16/17. We have adjusted the background section (page 3-4) to emphasize our aim and approach. We believe that at present there are no good markers for chronic systemic inflammation in overweight subjects; therefore it was our aim to provide insight into possible ways to quantify and modulate inflammation in overweight subjects, who often have chronic systemic inflammation. It was not our aim to find markers that correlate to chronic systemic inflammation.

Furthermore, to emphasize that we measured all or almost all known inflammatory markers we have added a full list of the 79 measured inflammation related proteins in plasma in additional file 1. With respect to TNF alpha, the
heatmap in figure 3 displays changes in each subject for all markers that are altered by the diclofenac treatment, including TNF alpha.