Author's response to reviews

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

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Research article

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

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BMC Medical Genomics

To Dr. Danielle Burgess

Assistant Scientific Editor

Zeist, October 1 2009

Dear Dr. Burgess,

Thank you very much for sending us the comments of the reviewers on our manuscript. We appreciate the thoughtful comments and suggestions of the reviewers. The manuscript has been revised according to the reviewers’ remarks. Also, we have adjusted the formatting of the manuscript to conform to the journal style. We hope that the manuscript is acceptable for publication in its present form.

Please find below our response to the reviewers’ comments.
Response to the reviewers’ comments

Reviewer 1 (John Fain)

1. Diclofenac and others NSAID are associated with risks in patients with cardiovascular disease (e.g. McGettigan and Henry, JAMA 2006). To minimize the risk of any side effects no subjects were included that have a medical history or current medical status that might increase the risk of side effects (e.g. subjects with a history of cardiovascular disease or stroke). This is now emphasized in the subjects and study design section (p.5) by putting this exclusion criterion first in the paragraph. To prevent irritation of the gastrointestinal tract (one of the main side effect associated with use of NSAID) gastric juice resistance coated capsules were used. A treatment period of 9 days was chosen as suitable treatment duration to both detect anti-inflammatory effect and avoid side effects as much as possible (these become more common with long-term use). The dose of 150 mg/day is a common dose prescribed in osteoarthritis or rheumatoid arthritis (e.g. Silverstein et al., 2000).

2. We agree with the reviewer that the relatively high CRP levels and subsequent decrease of these levels in three placebo subjects is an unexpected and confounding effect in our study. As the aim of our study was to investigate anti-inflammatory effects of diclofenac, this is our main focus in the manuscript. The first paragraph of the results section (p.12) was adjusted to clarify the findings in the diclofenac and the placebo group. The CRP values listed in the reviewer’s comment reflect the CRP levels in the placebo and diclofenac group at screening, not at the end of the study. At the end of the study, there was no difference in CRP levels in the placebo (2.05 ± 2.3 µg/mL) and the diclofenac group (1.95 ± 1.76 µg/mL), in contrast to the start of the study, where CRP levels were higher in the placebo group (4.03 ± 2.3 µg/mL) than in the diclofenac group (1.48 ± 1.12 µg/mL).

In addition to the main focus on diclofenac-induced effects, we were interested in the CRP change, as CRP is used as a marker for both acute inflammation or acute response and systemic inflammation (e.g. in relation to cardiovascular disease). The relatively high CRP levels in three subjects at the start of the study might be reflective of the role of CRP as acute phase response marker. The correlation analysis, combined with biological network analysis, was performed on all subjects, to investigate whether CRP response (across all subjects) was paralleled by changes in genes, proteins or metabolites with a closely related biological function. This analysis resulted in a network of genes and proteins involved in acute phase response that change in parallel to CRP (figure 5 in manuscript), which indicates that the CRP fluctuations are not an independent effect and therefore may not be noise. More importantly, in this study we were able to construct a network of anti-inflammatory effects in response to diclofenac despite the CRP fluctuations (figure 4).

3. The risk of associated chronic diseases increases with increased severity of

With kind regards, on behalf of the other authors,
Marjan van Erk
obesity (e.g. increased BMI). Our study population consisted of healthy, overweight males with BMI between 26 and 31 kg/m². We chose this population because we are interested in prevention or reversal of early signs of chronic disease development associated with obesity, and specifically the interaction between inflammation and metabolism at “mild metabolic stress” conditions. The term ‘mildly obese’ was replaced with ‘overweight’ throughout the manuscript.

4. We agree with the reviewer that the wording does not match with the data, so the word ‘strongly’ has been removed from the sentence describing the effect of annexin (discussion p. 16). To our experience, a 60% expression change (or 1.6 fold up-regulation) is quite substantive for a highly-controlled tissue like blood cells and for a short-term intervention. Also, annexin expression was consistently up-regulated (>=35%) in 7 out of 8 subjects receiving diclofenac.

5. The subtle differences induced by diclofenac in face of an oral glucose tolerance test are described in more detail in discussion on p.17.

6. Additional files 1 and 2 were adjusted and now included mean (± stdev) levels for all parameters together with median of percentage change in placebo and diclofenac group.

7. The paragraph on ‘(Combined) data analysis & interpretation’ on p.11 has been extended to include more information on biological network analysis (which resulted in figure 4 en 5).

The discussion on established inflammatory markers has been extended (p. 15). In addition to COX inhibition, diclofenac can suppress TNF-mediated NFκB activation. NFκB is an important regulator in inflammation with a wide range of target genes. Through NFκB and COX diclofenac could potentially exert a range of inflammatory effects. In this manuscript, the anti-inflammatory effects after 9 day intervention in mildly obese men have been discussed. The potential role of both NFκB and COX are now included in figure 4. We believe that this figure shows the added value of ‘omics’ technologies and analysis approaches using networks, as it provides a consistent story on top of the effects in conventional inflammatory markers.

Reviewer 2 (Yu Wang)

1. The reviewer asks for more details on proteomics analysis and for detailed information on identification and quantification of the proteins. A more detailed description of the proteomics analysis is now included on p.8.

2. The reviewer asks for changes in levels of common cytokines in placebo and diclofenac group. These data have been added to additional file 2. This supplementary table with the proteomics data now includes mean (± stdev) levels together with median of percentage change in placebo and diclofenac group (see also comment 6 by reviewer 1).

3. In this study, we detected a significant decrease of CRP levels in the placebo group between day 9 and day 0. This was due to three subjects that displayed relatively high CRP levels (>5 ug/mL) at day 0 that subsequently decreased to levels below 2 ug/mL at day 9. CRP levels did not change in the diclofenac treated group. As the aim of our study was to investigate anti-inflammatory
effects of diclofenac, this is our main focus in the manuscript. In addition, we explored whether fluctuation in CRP response as detected in this study was reflected or paralleled by changes in other genes, proteins and metabolites with related function across all subjects, see also point 2 for reviewer 1. CRP response was not used for correcting the other datasets; we aimed to investigate diclofenac induced effects independent of CRP changes that might be reflective of acute phase response. Levels and changes in other relevant inflammatory markers, as requested by the reviewer, were added to the manuscript, in additional file 2 (see also point 2 above).