Reviewer's report

Title: Inflammatory cytokine response to exercise in alpha-1-antitrypsin deficient COPD patients 'on' or 'off' augmentation therapy

Version: 2 Date: 2 February 2014

Reviewer: Harry Rossiter

Reviewer's report:

General Comments
The study investigates the exercise-related change in inflammatory cytokines in AATD patients and a range of controls. The data are collected by a world-leading group with huge experience in this area. The question is of interest, and the findings a pertinent addition to the literature. However, there are a number of concerns that reduce my enthusiasm for the manuscript as presented.

Major Comments

2. The question of whether pro-inflammatory cytokines increase with exercise in AATD (and/or are not offset by increases in anti-inflammatory cytokines) is a good one. But in AATD the concern is largely focused on the lung. Therefore the focus on the circulation and the skeletal muscle, and the omission of cytokine measurements in the sputum, is unfortunate. A more complete investigation would have included sputum measurements, which some believe to be a less variable measure of pulmonary cytokine expression. Are these data available?

3. The hypothesis section in the introduction is complicated. As there are two comparisons being made (rest and post-exercise) I recommend making two hypotheses specifically focusing on these two states. At least one of the hypotheses is not well justified by the introductory text: why would AATD PFTnorm have a similar cytokine profile to controls, when a1 antitrypsin itself is anti-inflammatory? This section could be simplified by first addressing resting hypotheses and then post-exercise hypotheses.

4. Why where PiMZ participants included in the control group? Surely controls should be limited to homozygous ‘normal’ for the gene of interest?
5. The rationale for taking a muscle biopsy is not clear to me. You have appropriately argued that exercise might be deleterious in AATD. But presumably the main concern, were exercise to increasing inflammatory state, would be in the lung. This is after all where a major aspect of the AATD pathology occurs. Therefore I and understand the arterial and venous blood sampling, but I do not understand why a muscle biopsy is needed (let alone two!).

6. There is a potentially important and glaring result that is largely ignored by the authors: The AATD patients with normal lung function have a far greater functional capacity (VO2max; single leg aerobic capacity; peak power) than the age and activity matched healthy controls, and markedly greater than other AATD patients. Does this suggest that aerobic capacity is protective for disease progression in AATD? Or is it self-selective? These data could hint at very important finding.

7. The v-a difference measurements in Fig 4 suggest muscle TNFa output in the AATD+AUG group. But this seems to be related to (an almost imperceptible) reduction in arterial TNFa (Fig 3), not an increase in venous output as suggested in the discussion. Also the reduction in intramuscular TNFa in this group (Fig 5) is not significant when comparing pre- to post-exercise. These relationships question the internal consistency of the data and the contention that muscle TNFa output in this group is really increased.

Minor comments (page, line)
2 Throughout the manuscript the work ‘level’ is used, where the authors mean ‘concentration’. Suggest revising.
2,7-11 There are a large number of patient groups, which makes the abstract a very complicated read. Seeing as the PFTnorm group is not mentioned in the results or conclusion, you could consider omitting them from the abstract? Perhaps the order of presentation of each group could be standardized between the methods and results to help the reader put all the information together? It is not stated whether the AATD-AUG and AATD+AUG groups both have abnormal lung function. You’ve defined the non-AATD-COPD as ‘COPD’ in the methods of the abstract. Suggest using this abbreviation throughout the abstract to simplify. The methods state that measurements were made “at rest and during exercise”. But you should state that the exercise was 60 minutes of KE at 50% KE max, and that blood and muscle samples were taken during and up to 4 hours following exercise.
2,12-18 I encourage the authors to provide the measured values for inflammatory cytokine concentration for the important variables in the abstract.
3,1 disorder that leads
3,2 among other complications
3,4 destruction, and which can occur
3,14 make clear that you are referring to COPD patient without AATD (if that is indeed what you mean)
3,20 what is the difference between influence and contribute? Are both terms needed here?


4,11 ‘boosting immune responses’ is not clear. Response to what – exercise? Please clarify

6,4 What questions were use to normalize for physical activity? Have these been validated as a reliable method for PA reporting compared to, say, pedometer or accelerometer measurements? Recommend reporting PA scores in Table 1.

6,6 How may pack years did the former smokers have? Where the former smokers distributed evenly through the groups?

7,2 Groningen (also the US distributor is not required)

7,3 and then performed an incremental exercise test to the limit of tolerance in which

9,8 What was the CV for these repeated measures?

10,7 ‘exercise capacity’: Do you mean ‘aerobic capacity’?

10,7 Is this referring to all patients with COPD (both AATD and non-AATD)? Please clarify.

10,10-13 the notion that the degree of sedentariness results in similar isolated leg aerobic capacity and BMI across groups is far too speculative. Suggest delete.

11,2 Again is this referring to all patients with COPD (both AATD and non-AATD)? Please clarify.

12,8 Are not these values also lower than your previously stated detection limit?

14,20 Consider showing this graphically (spirometry vs systemic inflammation in AATD).

16,21 individual’s maximal aerobic power, finds

17,6-13 You state that the KE is advantageous because all groups can complete the exercise. This is a true advantage from an experiment perspective – to normalize the exercise response. But it argues against the previous sentence that the exercise chosen represented a ‘real world’ stress. Clearly this is not the case if some patients cannot complete 60 minutes of bipedal exercise, and therefore have to be experimentally constrained to KE exercise.

Table 1. The ‘d’ symbol listed in the legend is not used in the table.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable
**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests