Reply to "Comments on: Acute aerobic exercise-conditioned serum reduces colon cancer cell proliferation in vitro through interleukin-6-induced regulation of DNA damage"

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Dear Editor-in-Chief,

We would like to thank Jiang and Yang[1] for their interest in our recent study published in *Int J Cancer*.[2] In their letter, Jiang and Yang[1] raise two important points: firstly, they suggest that molecules other than IL-6 may be responsible for the exercise-induced inhibition of colon cancer cell proliferation and DNA damage. We agree with this suggestion. Our findings demonstrated an increase in serum IL-6 following acute exercise, and directly exposing colon cancer cells to IL-6 reduced cell proliferation and DNA damage, mimicking the effect of exercise-conditioned serum.[2] These observations are consistent with existing evidence showing that acute aerobic exercise transiently increases serum IL-6[3] and that IL-6 reduces DNA damage in cancer cells following exposure to DNA damaging agents[4,5] and activates DNA repair enzymes after partial hepatectomy *in vivo*.[6] Thus, our data provide good evidence that IL-6 was at least partly responsible for the exercise-induced suppression of colon cancer cell proliferation and DNA damage. However, given the widespread effects of exercise on multiple organ systems, it is indeed possible that other humoral factors and signalling pathways were involved. We addressed this in the discussion section of our paper, stating: "while other serum markers were unaltered by exercise in our study, it is unlikely that the growth-inhibitory effect of exercise was driven exclusively by IL-6".[2]

We employed a targeted approach (immunoassays) to quantify exercise-induced changes in the serum concentration of seven cytokines, including IL-6, which were chosen based on previous research implicating them with exercise and cancer regression.[7,8] Of these cytokines, only serum IL-6 concentration changed from pre- to post-acute exercise.[2] Of course, this is not an exhaustive list of molecules that are modulated by acute exercise. Jiang and Yang's letter[1] refers to a study[9] that combined immunoassays with untargeted multi-omics techniques to demonstrate the rise and fall of thousands of molecules in plasma following an acute bout of aerobic exercise. While -omics technologies have huge discovery potential in this context, the ability of untargeted serum proteomics to detect small changes in exercise-regulated proteins which are of biological relevance (i.e., interleukins) is currently hindered by biological variability and the presence of high-abundance proteins.[10] It is noteworthy that the study[9] cited by Jiang and Yang was unable to detect any interleukins in plasma using untargeted mass spectrometry-based proteomics. Measuring a high number of serum analytes also increases the likelihood of making a type I error, potentially leading to lines of investigations based on false-positives.[11] Thus, assessing a panel of evidence-based, biologically-relevant cytokines via

immunoassays is a justifiable approach to exploring biological mechanisms underlying the effect of exercise-conditioned serum on colon cancer cell proliferation.

Secondly, the authors[1] propose that one more experiment is needed to confirm our findings; that is to investigate the effect of IL-6 signalling blockade on colon cancer cell proliferation and DNA damage following exposure to acute exercise-conditioned serum. We are in agreement with Jiang and Yang that further experimentation, such as blocking IL-6 signalling in exercise-conditioned serum via anti-IL-6 antibodies, is an important next step to potentially corroborate our findings. Many other experiments can also be undertaken to help corroborate or refine our results. For example, an ongoing study by our research group is investigating the effects of acute exercise-conditioned serum on adenoma organoid growth to determine whether our findings hold up in a pre-malignant model of colon cancer.[14] Nevertheless, our study showed a dose-response relationship between IL-6 and colon cancer proliferation and DNA damage and, applying the Bradford Hill criteria for causation,[12] this supports the presence of a causal effect.[13] Our conclusions are based on this evidence.

We encourage Jiang and Yang and other research groups to scrutinize our findings with further experiments, which will promote incremental scientific progress in this research area.

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