General Comments:

Zhao et al. investigated whether hydrogen-rich gas inhalation prior to exercise training attenuated the reduction in nitric oxide (NO) levels, its production pathways, oxidative damage, and inflammation in male professional rugby players. I think the scope of the study is good. Specifically, this study was conducted in a real training environment rather than an experimental setting, was double-blinded, and well-controlled, with participants undergoing the same training and consuming similar diets, making it a highly valuable study. However, there are several areas where explanations are insufficient. The points listed below highlight these issues, while acknowledging that certain problems may be inherent to field-based studies. Therefore, it is important to clearly indicate these limitations and assert the implications of the findings accurately and comprehensively for both scientific and practical applications in competitive sports.

Specific Comments:

Major:

1. Title: The term "nitric oxide signaling" suggests a focus on NO signaling pathways post-production, which does not appear to be analyzed in this study. It seems more appropriate to describe the study as an investigation of the NO bioavailability pathways. The title should be adjusted to accurately reflect the content and attract the correct audience. Additionally, as mentioned in the limitations section, if the study targets only male athletes, this demographic information should be included in the title.

2. Introduction (Overall): Why did the authors focus on “blood” NO levels? It is unclear how blood NO levels relate to training effects and performance in athletes, specifically in rugby players. Without clarifying this, interpreting the physiological significance of increased NO production due to H2 gas is challenging. The authors mentioned the effectiveness of H2 gas on beneficial adaptation in skeletal muscle but later discussed the potential of H2 gas to promote vascular adaptation. Is there a basis for similar effects in both skeletal muscle and blood vessels? If not, introducing their previous research into the current study's introduction would seem less effective.

3. Introduction (line 38-49): While the argument is understandable, the study does not quantitatively evaluate the training effects (performance) of athletes, making this section inappropriate for the introduction.

4. Method (line 97-103): Details of the training regimen should be provided as supplementary materials. The study evaluated only blood indicators, but whether these responses were to the same exercise load or if the H2 gas condition involved more (or less) exercise significantly affected the interpretation. Given that previous studies indicate H2 can improve maximal sprint ability even with transient intake (Botek et al., Nutrients, 2022, PMID: 35276867), assessing changes in exercise load is crucial. In team sports like rugby, it is advisable to measure total distance covered with GPS, heart rate, blood lactate levels, and subjective exercise intensity during each training session. Providing data supporting the overload principle, as discussed in the introduction, enhances credibility.

5. Method (Line 106-107): Why did the authors choose to administer hydrogen gas inhalation 20 minutes before training? Considering that ROS is produced more post-exercise and the rapid diffusion of hydrogen in the body, as well as blood sampling 1 hour post-exercise on D6, post-exercise inhalation, as in Shibayama et al. 2020 (Med Gas Res, PMID: 33380581), could also be considered. The rationale behind preconditioning with hydrogen gas and the 20-minute inhalation duration should be explained.

6. Method (Line 103): While stretching and physiotherapy are included in the training program, what about controlling care activities outside training, such as icing or supplement intake? Icing is known to reduce inflammation and oxidative stress, and rugby players are likely to use it.

7. Method (Line 112-113): Why did the authors evaluate at D1, D6, and D7, and why was

blood sampled 1 hour post-exercise on D6? A rationale for this should be provided.

8. Method (Line 114-119): Why did the authors choose hydrogen gas inhalation instead of hydrogen-rich water, which has already proven effective and seems more applicable for team sports interventions? The advantages of using hydrogen gas should be discussed.

9. Results (overall): While accurately describing the Two-way ANOVA results is good, presenting them similarly for all items is somewhat redundant. Including statistical results in figure captions and focusing on significant changes in the text would facilitate reader understanding.

10. Results (overall): If I am not mistaken, although the figures indicate the results, the text (line 156-318) does not mention the multiple comparison test results between conditions. Given that the purpose of this study was to detect differences between placebo and H2 gas intake, this information should be included in the text.

11. Results (overall): The figures suggest no differences between conditions at D1, making the statistical analysis valid; however, individual variations at D1 seem large. Comparing changes or rates of change from D1 rather than absolute values might clarify the differences.

12. Results (overall): How about conducting a correlation analysis with NO as the dependent variable and other indicators as independent variables? Showing that hydrogen's suppression of inflammation and oxidative damage contributes to increased NO production would clarify the authors' claims.

13. Discussion (line 327-331 Figure 1): Why did NO levels increase at D7 despite no hydrogen gas inhalation? This should be explained.

14. Discussion (line 341-344): This study did not evaluate training adaptations, so this statement is an overreach. As mentioned in Comment 2, the relationship between blood NO levels and athletic performance should be discussed, suggesting that increased NO levels might contribute to long-term performance improvement.

15. Discussion (line 369-377): Discussing the significance and reasons for the different responses of oxidative stress markers to hydrogen gas would strengthen the argument for hydrogen gas efficacy. For example, could the timing of hydrogen gas exposure (before, during, or after exercise) influence these responses?

16. Discussion (line 398-403 limitations): As noted in Comment 4, if exercise load is not regulated or monitored, this is a major limitation. Similarly, if icing or supplement intake is not controlled for, it should be stated as a limitation. Additionally, discussing whether these findings are applicable to athletes other than rugby players would be valuable.

17. Conclusion (line 407-409): This is an over-speculation and is not quite a conclusion of this study.

Minor:

1. Abstract-Results (Line 26-27): Abbreviations such as D6 and D7 are not defined and are unclear to readers at the abstract stage.
2. Method (line 88-113): The double-blinding method should be described in detail.
3. Method (line 128-138): Simply listing oxidative markers is unclear. Their relevance should be explained.
4. Results (overall): Before presenting the statistical analyses, an introductory text on the figures should be provided.
5. Results (overall): Numeric data need not be textually shown if presented in the figures. Showing absolute values is meaningful only if physiological stress levels are below

specific NO levels; otherwise, it is unnecessary at this stage.

6. Discussion (overall): Please remove the figure mentions from the discussion (e.g., "Figure 1"), as they are not needed in this section.