Commentary

Is physical performance (in mice) increased by *Veillonella atypica* or decreased by *Lactobacillus bulgaricus*

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1. Introduction

Regular exercise induces changes in the overall diversity and in the relative abundance of certain gut microbiota phyla and families in humans1,2 and in animal models.3,4 This relationship has attracted a great deal of interest because it is one of the mechanisms involved in the health benefits of regular exercise5 that could also influence performance.6 A recent publication by Scheiman et al.6 provides a multidimensional approach to the effects of acute exercise on gut microbiota composition in humans as well as on the influence of bacterial supplementation on performance in mice. Scheiman et al.6 conclude that *Veillonella atypica* (*V.* *atypica*) is a performance-enhancing microbe that functions via the utilization of lactate and the production of propionate.

The aim of this commentary is to discuss some of the controversial issues raised in the study by Scheiman et al.6 and to provide original data to support alternative explanations.

2. Main concerns

2.1. The relative abundance of *Veillonella* in human stool samples was not associated with training status or endurance performance

It is remarkable that Scheiman et al.6 found no significant differences in the relative abundance of *Veillonella* between runners and sedentary individuals. Also, the correlation between marathon performance and *Veillonella* abundance was not explored by Scheiman et al.,6 and the study did not investigate the effect of *Veillonella* administration on physical performance. Therefore, in light of these results, the *Veillonella* genus appears not to be a good biomarker of training status, and its influence on performance remains unexplored.

2.2. The native gut microbiota of gavaged mice and its response to exercise was not analyzed

Despite the fact that the results from the human study by Scheiman et al.6 did not support the idea of a performance-enhancing effect of *Veillonella*, the authors decided to gavage nontrained mice with the species *V.* *atypica* (isolated from runners’ samples) in order to analyze its potential effect on acute endurance performance. This species was selected for its high prevalence of enzymes involved in lactate metabolism. A control group was administered *Lactobacillus bulgaricus* (*L.* *bulgaricus*) due to its supposed incapacity to impact lactate metabolism.6

The authors administered these bacteria over the animals’ resident gut microbiota, whose composition was not analyzed. The native gut microbiota of mice shows a dynamic response to training,7 although the effect of acute exercise has not been explored. Furthermore, the relationship between performance improvements in response to training and gut microbiota composition are not associated with a single microbial population but with a set of changes in the microbiome7,8.

Thus, we have explored the effect on the gut microbiota composition of progressive treadmill endurance training for 5 days/week for 4 weeks in a group of 8-week-old C57BL/6N male mice. After 16S ribosomal RNA sequencing, α-diversity analysis of cecal samples showed a higher gut microbiota diversity in endurance-trained mice (*n* = 12) vs. sedentary control mice (*n* = 6) (Fig. 1A and Supplementary Table 1). However, considering the relative abundance at the family level in the phylum *Firmicutes* (Fig. 1B), where *V.* *atypica* and *L.* *bulgaricus*...
are included, we observed that the relative abundance of the *Lactobacillaceae* and *Veillonellaceae* families (Fig. 1C and Supplementary Table 2) were not significantly different between endurance-trained mice and nontrained control mice (*p* = 0.091 for *Lactobacillaceae*; *p* = 0.450 for *Veillonellaceae*).

2.3. The effect on performance of the supplementation with *V. atypica* is confounded by the use of *L. bulgaricus* as a control

As mentioned above, Scheiman et al. selected *V. atypica* from the human study based on its capacity for lactate catabolism which, in turn, produces propionate. The authors observed an association between propionate administration and improvements in run-to-exhaustion time in mice. On the contrary, the *Lactobacillaceae* family is characterized by high rates of lactate production. Specifically, *L. bulgaricus*, which was used as a control, is a homofermentative bacterium, generating 1 mol of lactate for each mol of glucose. This is particularly important considering the high carbohydrate content of standard rodent chow (usually more than 40% by weight, excluding fiber). Furthermore, Scheiman et al. did not explore the mutual metabolic interactions between the supplemented bacteria and the native microbiota.

However, we have observed a significant negative correlation between the relative abundance of the *Lactobacillaceae* family or *Lactobacillus* genus in trained animals and endurance capacity improvements in response to training (Fig. 1D...
and 1F, respectively, and Supplementary Table 3). A clear trend toward a significant negative correlation \((r = -0.565, p = 0.056)\) was also observed between the relative abundance of *Lactobacillus* genus and a post-training run-to-exhaustion test (Fig. 1F). These data indicate that the relative abundance of *Lactobacillus* in the native gut microbiota of trained mice is related to a lower endurance performance. On the other hand, no differences in the relative abundance of the Veillonellaceae family were observed between trained and untrained mice (Fig. 1C), and no relationship with physical performance was found (Fig. 1D and 1E). Therefore, it is possible that what Scheiman et al.\(^6\) actually observed was, in fact, a decrease in performance induced by *L. bulgaricus* administration.

Thus, the choice of *L. bulgaricus*-gavaged mice as a control against which comparisons were made may be a confounding factor due to the influence of *L. bulgaricus* on intensive lactate metabolism and its negative relationship with endurance performance.

### 2.4. The absence of a vehicle-treated group does not allow elucidation of the role of *V. atypica* administration on maximal endurance performance in nontrained mice when *L. bulgaricus* is used as control

To determine whether what Scheiman et al.\(^6\) observed was an increase in endurance performance due to *V. atypica* administration or a decrease associated with *L. bulgaricus*, it would have been necessary to have a new experimental group of mice to which only vehicle was administered, without bacteria supplementation. However, this group was not included in the study by Scheiman et al.\(^6\).

The need for this additional group is also evident considering that *V. atypica*-treated animals did not perform better in a run-to-exhaustion treadmill test than those previously described in the literature. In fact, both *V. atypica*- and *L. bulgaricus*-treated animals showed considerably lower performance than non-trained mice of the same strain and age under similar exercise protocols (Fig. 1G), as observed by our research group (Pretraining, and Supplementary Table 4 and Fig. 1H) and others.\(^10\)\(^\sim\)\(^12\)

These differences are even greater when compared to trained animals (Post-training) (Fig. 1H). A lower performance in the run-to-exhaustion tests would have been expected if Scheiman et al.\(^6\) had pretreated their animals with antibiotics, as previously described by Hsu et al.\(^3\). Therefore, these discrepancies with other studies, which could be explained by the differences in the run-to-exhaustion tests used, highlight the fact that the improvements in performance reported by Scheiman et al.\(^6\) may not be attributable to *V. atypica* but, instead, may have been determined by their experiment’s design.

In summary, it is not clear whether what Scheiman et al.\(^6\) report is an improvement in performance attributable to *V. atypica* administration or a decrease associated with *L. bulgaricus*. In the absence of a vehicle-treated group, both explanations are possible. Considering the wide pharmacological and dietary use of probiotics, particularly those containing members of the Lactobacillaceae family, additional research is needed to confirm the relationship between microorganism administration, gut microbiota composition, and performance.

### 3. Data availability

Sequence raw data have been uploaded to NCBI and SRA as Bioproject PRJNA558220. Other raw data are available as a Supplementary Tables file. Additional data are available on request from the authors. Methods are available as Supplementary Data.

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### Authors’ contributions

MFS and JF performed the experiments, analyzed the data, and wrote the manuscript; CTZ analyzed the data, prepared the figures, and wrote the manuscript; BFG designed and supervised the study; CJV analyzed the data; FL and EIG designed and supervised the study and wrote the manuscript. All authors have read and approved the final version of the manuscript, and agree with the order of the presentation of the authors.

### Competing interests

The authors declare that they have no competing interests.

### Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jshs.2020.02.005.

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