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Intensive Training and Sex Influence Intestinal Microbiota Composition: A Preclinical Approach †

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Abstract: Lifestyle including regular physical activity and dietary habits influences the microbiota composition. Although some studies have demonstrated changes in microbiota composition due to moderate or intense intensity training in athletes, the relationship between physical activity and changes in the intestinal bacteria is still a matter of discussion. In addition, as most studies are performed in males, the role of sexual dimorphism deserves to be explored. Therefore, the aim of this preliminary study was to ascertain the influence of an intensive training and the rat's sex on the caecal microbiota composition. For this purpose, female and male 4 week-old Wistar rats were submitted to a running training 4-weeks program. At the end, caecal samples were collected immediately after performing an exhaustion test to characterize the microbiota composition by 16S rRNA sequencing technique. Parallel groups of female and male sedentary (SED) rats (age matched) were included. The results showed that young female rats had a higher ability to run than males but no sex- or training-associated changes were observed on microbiota diversity and richness indexes among groups. However, the Actinobacteria, *Bifidobacteriaceae* and *Bifidobacterium* spp. proportions were significantly higher in male rats than in female when comparing SED groups ($p < 0.05$), whose proportions in males were decreased by the training program ($p < 0.05$ vs SED). On the contrary, female SED rats showed a higher proportion of *Odoribacteraceae* (belonging to Bacteroidetes), *Clostridiaceae* and *Eubacteriaceae* (both Firmicutes) than in the respective male group ($p < 0.05$), although *Eubacteriaceae* proportion decreased by running ($p < 0.05$ vs SED). However, training increased the proportion of *Paraeggerthella* genus (Actinobacteria) in female rats with respect to its sedentary counterpart ($p < 0.05$). Overall, caecal microbiota composition is modified by intensive training in young rats but there are also sex-based differences that should be considered for interventional studies.

Keywords: exercise; training; running; microbiota; dimorphism

1. Introduction

The modulatory role of the intestinal microbiome composition on physical activity has been described in some studies, which have shown an association with increased biodiversity and the presence of bacterial groups with beneficial functions [1]. However, other studies have not been able to find such correlation and for instance, a recent study in lean and overweight men showed the lack of effect of a short-term high-intensity interval training exercise on gut bacterial diversity or composition [2]. For this reason, the relationship between physical activity and changes in the

intestinal bacteria is still a matter of discussion [3]. In addition, the few studies performed aiming to stablish this link between exercise and microbiota is mainly performed in male athletes. Although diet is one of the strongest factors influencing gut microbiota composition [4], sex differences can also have an impact on that. Thus, as microbiota composition is suggested to be different in males and females [5], the differential response to exercise should be explored in both sexes.

In order to deep into the effect on microbiota composition of these two variables, exercise and sex, we used a preclinical approach based in an intensive training design in rats which has demonstrated in previous studies to have an impact on rat physiology [6,7]. Particularly, the acute exercise performance with increasing duration and speed induced some changes in body weight, intestinal features and the immune status of both innate and acquired immune response [6,7]. The aim of this study was to ascertain the influence of an intensive training and the rat's sex on the caecal microbiota composition.

2. Material and Methods

2.1. Animals and Training Program

For this purpose, 4-week-old males and females Wistar rats (n = 22) from Envigo (Blackthorn, UK) were housed in the animal facilities of the Faculty of Biology of the University of Barcelona (UB) in polycarbonate cages (2–3 rats per cage) in a controlled environment of temperature and humidity, in a 12/12 h light/dark cycle with free access to water and food. Sample size used in the study was established by the Appraising Project Office's program from the Universidad Miguel Hernández (Elche, Spain).

The training programme was achieved by means of a LE8700 treadmill (Panlab, Harvard, MA, USA) and an Exer3/6 treadmill (Columbus, OH, USA) with same length and width for each running lane following an exercise programme design previously described [6,7]. It included two periods: a 3 day-period of habituation on a turned-off treadmill and a 5 days-period of preselection in treadmills with increasing duration and speed (RUN group) (Figure 1). During the experiment, the age and sex matched sedentary animals (SED group) were placed also in the static treadmill receptacle. Thus, the four experimental groups were as follows: female RUN (n = 6), female SED (n = 5), male RUN (n = 6), male SED (n = 5). After selection, the RUN group began a period in which the duration and the speed was increased progressively (10 min/session at 5 m/min to a 25 min/session at 25 m/min). In the following 2 weeks, intensive training started and RUN animals ran twice a day (30 min at 30 m/min, 6 h between sessions), 5 days per week. After the daily exercise, rats from both RUN and SED groups received a 50% solution of condensed milk (100 μL/100 g body weight) as a reward.

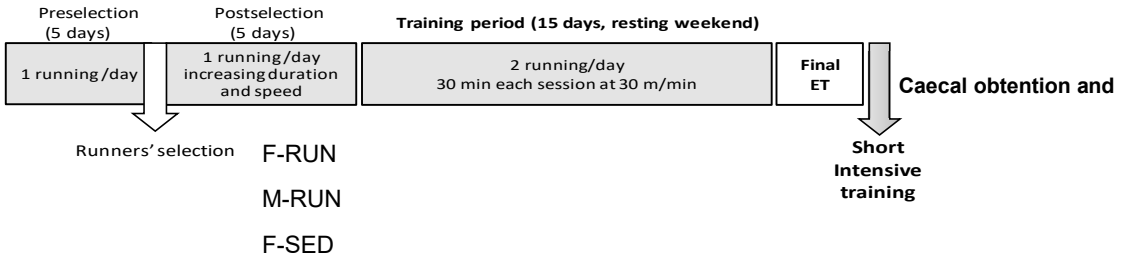

Figure 1. Experimental design of the intensive training program. Groups: female runners (F-RUN, n = 6), female sedentary (F-SED, n = 5), male runners (M-RUN, n = 6), male sedentary (M-SED, n = 5). At the end of the training RUN animals were subjected to a final exhaustion test (ET).

At the end of the 2 weeks, an exhaustion test (ET) was performed, starting with an initial speed of 5 m/min with a progressive increase of 1.8 m/min every min until exhaustion. Immediately after the final exhaustion test, animals were euthanized with ketamine (Merial Laboratories S.A., Barcelona, Spain) / xylazine (Bayer A.G., Leverkusen, Germany) and caecal samples were collected.

The experimental procedure was validated by the Ethical Committee for Animal Experimentation of the University of Barcelona and the Catalonia Government (CEEA/UB ref. 464/16 and DAAM 9257, respectively).

2.2. Microbiota Determination

Three samples from each group were selected to characterize the microbiota composition by 16S rRNA sequencing as in previous studies [8]. Briefly, genomic DNA was extracted from caecal samples (n = 3/group) using the DNeasy Blood and Tissue Mini kit (Qiagen, Madrid, Spain) and amplified following the 16S Metagenomic Sequencing Library Illumina 15044223 B protocol (Illumina Inc, San Diego, CA, USA). Image analysis, base calling and data quality assessment were performed in the MiSeq instrument. Sequences were merged and processed using Pair-End read merger (PEAR v 0.9.6, Exelixis Lab, Heidelberg, Germany) and Cutadapt v1.8.1, as previously describe [9]. The presence or absence of genera at qualitative level was represented in a Venn diagram. A bacterial group was considered as present by establishing a cutoff of 3 animals displaying proportions higher than 0.001%, while the bacterial groups not detected in any of the animals were regarded as absent.

2.3. Statistical Analysis

For statistical analysis, the Statistical Package for the Social Sciences (SPSS v22.0) (IBM, Chicago, IL, USA) was used. Homogeneity of variance and normality distribution were tested by the Levene's and Shapiro−Wilk tests, respectively. When data were homogeneous and had a normal behavior, Student t-test was used to analyze statistical differences. Otherwise, the Mann−Whitney U test was performed. Significant differences were established when $p < 0.05$.

3. Results and Discussion

The results showed no sex- or training-associated changes on microbiota diversity and richness indexes among groups. However, some changes in relative abundances were observed. At phylum level, only the Actinobacteria proportion was associated to sex or exercise condition. This change was in line with the changes in the family *Bifidobacteriaceae* and the genus *Bifidobacterium* spp. All these proportions were significantly higher in male rats than in female rats when comparing SED groups ($p < 0.05$), and the proportion in males were decreased by the training program ($p < 0.05$ vs. SED) (Figure 2).

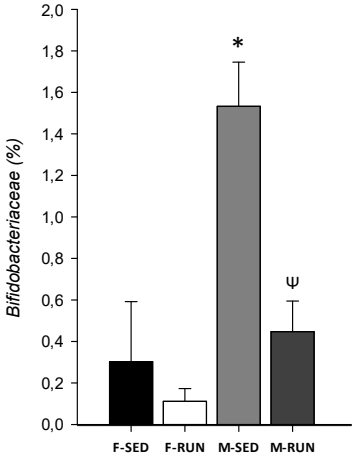

Figure 2. Relative abundance of the family *Bifidobacteriaceae* in caecal content at the end of the exhaustion test differentiating between sexes and exercise condition. Groups: female runners (F-RUN), female sedentary (F-SED), male runners (M-RUN), male sedentary (M-SED). Results are expressed as mean ± SEM (n = 3/group). Statistical significance: * $p < 0.05$ male vs. female with same exercise condition, $^{\Psi}$ $p < 0.05$ RUN vs. SED with same sex condition.

On the contrary, female SED rats showed a higher proportion of *Odoribacteraceae* (belonging to Bacteroidetes), *Clostridiaceae* and *Eubacteriaceae* (both Firmicutes) than in the respective male group ($p < 0.05$), although *Eubacteriaceae* proportion decreased by running ($p < 0.05$ vs. SED). However, training increased the proportion of *Paraeggerthella* genus (Actinobacteria) in female rats with respect to its sedentary counterpart ($p < 0.05$).

The analysis of the genera distribution in Venn diagrams at family level revealed that there was a core of 30–32 genera that persisted between all four experimental groups when considering both sexes and exercise condition separately (Figure 3).

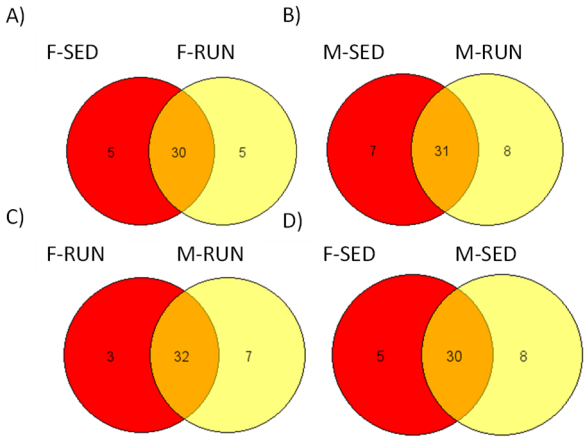

Figure 3. Venn diagrams showing the diversity in all families differentiating between sexes and exercise condition. Results derived from n = 3/group. Groups: female runners (F-RUN), female sedentary (F-SED), male runners (M-RUN), male sedentary (M-SED).

Moreover, when comparing the microbiota depending on the sex or the exercise, it could be observed that specific families were particularly associated to a certain condition. In this sense, the training and exhaustion test led to the colonization of new genera, such as *Chromobacteriaceae, Coriobacteriaceae, Methylococcaceae, Phyllobacteriaceae* and *Rhodobacteraceae* in the case of females (Figure 1A) and *Erwiniaceae, Intrasporangiaceae, Phyllobacteriaceae, Rhizobiaceae, Rhodanobacteraceae, Rhodobacteraceae, Spongiibacteraceae* and *Staphylococcaceae* in the case of males (Figure 1B). Regarding to sex differential presence of bacterial groups, *Aerococcaceae, Atopobiaceae, Chromobacteriaceae, Comamonadaceae, Coriobacteriaceae, Pasteurellaceae, Planococcaceae* and *Xanthomonadaceae* were exclusively present in males but not in females at sedentary conditions (Figure 1C), whereas *Atopobiaceae, Erwiniaceae, Intrasporangiaceae, Pasteurellaceae, Planococcaceae, Rhodanobacteraceae* and *Spongiibacteraceae* appeared in males but not in females after the training and exhaustion test (Figure 1D).

4. Conclusions

Overall, the present study evidenced that the caecal microbiota composition is modified by intensive training in young rats at a relative abundance and qualitative level. In addition, some sex-based differential response against the exercise were found. Thus, sex is a variable that should be considered for interventional studies in the future concerning physical activity.

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Conflicts of Interest: The authors declare no conflict of interest.

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