Accumulated High-intensity Interval Training Protocol: A New Approach to Study Health Markers in Wistar Rats

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Abstract

High-Intensity Interval Training (HIIT) and accumulated exercises are two time-efficient programs to improve health in humans and animal models. However, to date, there are no studies on whether HIIT performed in an accumulated fashion is as effective as a traditional HIIT performed with single daily sessions in improving health markers. This paper presents the effects of a new HIIT protocol, called accumulated HIIT, on body weight gain, maximal oxygen consumption (VO₂max), and cardiac hypertrophy in young Wistar rats.

Sixty-day-old male Wistar rats were assigned to three groups: untrained (UN; n = 16), HIIT performed with single daily sessions (1-HIIT; n = 16), and HIIT performed with three daily sessions (3-HIIT; n = 16). Body weight and VO₂max were recorded before and after the training period. The VO₂max measurements were taken using a metabolic analyzer at the maximal running velocity (V_{max}). The training was performed for both HIIT groups five days per week over eight weeks with the same weekly progression of the exercise intensity (85-100% V_{max}). The 1-HIIT group performed single daily sessions (6 bouts of 1 min interspersed with 1 min of passive recovery). The 3-HIIT group performed three daily sessions (2 bouts of 1 min interspersed with 1 min of passive recovery with an interval of 4 h between bouts). After the last VO₂max test, the rats were euthanized, and their hearts were harvested and weighed.

The results showed that 3-HIIT had similar beneficial effects to 1-HIIT in preventing body weight gain, improving VO₂max, and inducing cardiac hypertrophy. These findings reveal for the first time the efficacy of an accumulated HIIT protocol on the

health markers of young Wistar rats. This new HIIT protocol may be more feasible than traditional HIIT protocols as exercise can be split into very short sessions throughout a day in this new approach.

Introduction

A sedentary lifestyle is one of the main risk factors for the development of non-communicable chronic diseases¹. However, even with solid evidence for the multiple beneficial effects of exercise on health, a large section of the global population is still sedentary^{2,3}. Regular physical exercise has many positive effects on cardiometabolic^{4,5} and mental health^{6,7,8,9}. Recently, different time-efficient physical exercise programs¹⁰, such as HIIT¹¹ and accumulated exercise¹², have been proposed to increase physical exercise adherence and improve health.

HIIT is a time-efficient approach characterized by short periods of high-intensity physical exercise interspersed with periods of low-intensity physical exercise (active recovery) or rest (passive recovery)⁴. Several studies have demonstrated that HIIT has similar or even superior health effects than the traditional long-duration Moderate-Intensity Continuous Training (MICT), with the advantage of greater adherence by its practitioners^{4,12,13}. Accumulated exercise has also been proposed as an exercise modality to increase adherence and improve health. In this approach, low-to-moderate intensity exercises can be split into two or more short bouts over a day (e.g., two or three bouts of 5-10 min daily)¹⁴. Costa Pereira et al.¹⁴ showed that an accumulated exercise protocol (three daily sessions with a 4 h interval between sessions, 10-20 min/session, at 50-60% of maximal capacity, five days/week, during eight weeks) had greater positive effects for health markers in young Wistar rats than a traditional MICT

(the same exercise protocol but performed with single daily sessions of 30-60 min).

However, to date, studies have focused on the physiological consequences of accumulated exercise performed with short bouts of low-to-moderate intensity. Thus, there is a lack of evidence examining whether HIIT performed in an accumulated fashion (i.e., with multiple short bouts of high-intensity exercise throughout the day) is as effective as a traditional HIIT performed with single daily sessions in improving physiological markers of health.

We decided to use controlled laboratory rats to overcome methodological pitfalls in studies with free-living individuals with distinct daily physical activities and food intake. Moreover, the use of experimental animals allows researchers to perform invasive analyses (e.g., muscular and cardiac biopsy), which are difficult or even impossible in human studies.

We hypothesized that if rats exercise with an accumulated HIIT protocol (performed with three short daily sessions), they might improve cardiorespiratory fitness similarly to rats that exercise with a traditional HIIT protocol (performed with single daily sessions). Thus, this study aimed to investigate and compare the effects of HIIT performed with single daily sessions versus HIIT performed with three shorter daily sessions on VO₂max and cardiac hypertrophy of Wistar rats.

Protocol

All procedures used in the present study followed the "Principles of Laboratory Animal Care" in agreement with the ARRIVE guidelines and were approved by the Committee on Ethics and Animal Use in the *Universidade Federal dos Vales do Jequitinhonha e Mucuri (UFVJM)*-protocol number 031/2016). Refer to the **Table of Materials** for details about all materials used in this protocol.

1. Experimental design

1. Use 48 male, 60-day-old Wistar rats weighing 250 g, on average.
2. Randomly divide the rats into three groups: untrained (UN; n = 16); HIIT performed with single daily sessions (1-HIIT; n = 16); and HIIT performed with three daily sessions (3-HIIT; n = 16).
3. House the rats in individual cages in a controlled environment (22-23 °C, 50% humidity) with an inverted 12 h:12 h light-dark cycle (lights on at 6:00 p.m.) with free access to standard laboratory food pellets and water.
4. Familiarize the rats with the treadmill one week prior to initiating the exercise training protocol.
5. Weigh the rats using a precision electronic digital weighing scale 48 h before and 48 h after the training protocol.
6. Perform the VO₂max test with the rats of all groups 48 h before the initialization of the HIIT protocols, at the end of the 4th week of training (to adjust the running velocity), and 48 h after the HIIT protocols.

7. Euthanize the rats 24 h after the last VO₂max test by i.p. injection of 400 I.U. heparin, and remove and weigh the hearts and left ventricles (see step 5).

2. Familiarization with the treadmill equipment

1. Set the inclination of the treadmill to 0° (flat).
2. Turn on the treadmill.
3. Adjust the treadmill knob to the speed of 6 m/min.
4. Adjust the treadmill shock switch to 0.25 mA.
5. Hold the animal by the tail and place it on the treadmill for 10 min at 8:00 a.m.
6. Repeat this procedure for five consecutive days.

3. VO₂max test

1. Software adjustments (**Figure 1**)
 1. Open the Metabolism software.
 2. Click **file** to open a new record.
 3. In the **kind of cages** parameter, select **Treadmill, 1 cage**, and in **switching time**, select **2** (min).
 4. In **experiment**, select **oxymetry | new**.
 5. Press **on** to enter the number identification and the weight of the animal. Identify the project data. Go to the treadmill controller and set the test parameters.
 1. Start the test with a velocity of 5 m/min.
 2. Adjust the treadmill shock switch to 0.25 mA. Press **start**.
 6. Click on **accept**.
 7. Close the treadmill's acrylic cover.
 8. Enter the calibration gas reference values.

NOTE: The gas reference values are 0 mmHg (CO₂) and 20 mmHg (O₂). These values are indicated by the manufacturer.

9. Wait for 10 min.

2. Test

1. Turn on the treadmill.
2. Adjust the treadmill knob to the speed of 3 m/min.
3. Adjust the treadmill shock switch to 0.25 mA.
4. Set the inclination of the treadmill to 0° (flat).
5. Hold the animal by the tail and place it on the treadmill and close the treadmill's acrylic cover.
6. Increase the treadmill speed by 3 m/min every 2 min until exhaustion (established when the animal touches the bottom of the bay five times within 1 min).
7. Remove the animal from the treadmill and place it in its cage.
8. Record the maximal velocity (V_{max}) reached by the animal.

NOTE: For the VO₂max calculation, the software calculates the difference between inhaled O₂ and exhaled CO₂ by the animal at the V_{max}, and the final calculation is performed according to the individual body weight of each rat.

4. HIIT protocols (Figure 2)

1. Turn on the treadmill.
2. Adjust the treadmill knob to the speed of 50% V_{max} (warm-up).
3. Adjust the treadmill shock switch to 0.25 mA.

4. Set the inclination of the treadmill to 0° (flat).
5. Hold the animal by the tail and place it on the treadmill for 3 min.
6. Remove the animal from the treadmill and adjust the treadmill knob to the corresponding velocity of the week (week 1: 85% V_{max}; weeks 2-3: 90% V_{max}; weeks 4-5: 95% V_{max}; weeks 6-8: 100% V_{max}).
7. Place the animal on the treadmill for 1 min.
 1. Ensure that both HIIT groups exercise 5 days/week for 8 weeks: the 1-HIIT group must perform single daily sessions of 6 bouts of 1 min interspersed with 1 min of passive recovery, and the 3-HIIT group must perform three shorter daily sessions of 2 bouts of 1 min interspersed with 1 min of passive recovery with an interval of 4 h between the bouts.
 2. For the 3-HIIT group, perform the training sessions at 8:00 a.m., 12:00 p.m., and 4:00 p.m.
 3. Divide the 1-HIIT group into three groups starting the training sessions at the same hour (i.e., 8:00 a.m., 12:00 p.m., and 4:00 p.m.) as the 3-HIIT group to eliminate any possible circadian effect on exercise training adaptations.
 4. Ensure that the UN group is placed on the treadmill one day per week for 10 min at a speed of 10 m/min in the same room where the HIIT groups are exercising.

8. Remove the animal from the treadmill and place it in its cage for 1 min (passive recovery).

5. Euthanasia and heart weight record

1. After an i.p. injection of 400 I.U. heparin, euthanize the rat by decapitation with a guillotine.

2. Carefully open the thorax of the rat, remove the heart, wash it with saline solution, weigh it, separate the left ventricle and weigh it.
3. Discard the animal's carcass in the appropriate freezer.

6. Statistical analysis

1. Express all data as mean $\pm$ standard deviation.
2. Check the normality of data using the Shapiro-Wilk test.
3. Analyze the data using one- or two-Way ANOVA followed by Tukey's post-hoc test; set the significance level at 5%.

Representative Results

Figure 3 presents the effects of 1-HIIT and 3-HIIT protocols on VO_{2max} (**Figure 3A**), body weight (**Figure 3B**), and food intake (**Figure 3C**). Before the training protocols, all groups presented similar VO_{2max} (UN: 52.19 ± 5.27 ; 1-HIIT: 48.32 ± 3.92 ; 3-HIIT: 51.24 ± 5.84 mL of $O_2 \cdot kg^{-1} \cdot min^{-1}$) body weight (UN: 242.62 ± 15.89 ; 1-HIIT: 259.06 ± 13.90 ; 3-HIIT: 251.43 ± 13.84 g) and food intake (UN: 118.26 ± 9.94 ; 1-HIIT: 116.51 ± 3.86 ; 3-HIIT: 119.01 ± 9.02 g/day).

After the training period, only the HIIT groups presented an increase in VO_{2max} (UN: 49.73 ± 3.13 ; 1-HIIT: 67.39 ± 4.22 ; 3-HIIT: 67.23 ± 2.86 mL of $O_2 \cdot kg^{-1} \cdot min^{-1}$; pretraining vs. posttraining: UN, $P = 0.99$; 1-HIIT, $P = 0.001$; 3-HIIT, $P = 0.001$), confirming the effectiveness of the training protocols.

After the training period, all groups showed an increase in body weight; however, when compared with UN, both HIIT groups presented a lower body weight (UN: 384.37 ± 27.25 ; 1-HIIT: 349.31 ± 14.49 ; 3-HIIT: 341.68 ± 23.78 g; UN vs. 1-HIIT, $P = 0.022$; UN vs. 3-HIIT, $P = 0.001$) despite having similar food intake (UN: 124.34 ± 3.70 ; 1-HIIT: 122.09 ± 8.68 ; 3-HIIT: 122.09 ± 5.40 g/day).

Figure 4 shows the effects of 1 and 3-HIIT protocols on cardiac hypertrophy. Compared with the UN group, both HIIT protocols induced cardiac hypertrophy as shown by the increase in heart/body weight (UN: 3.59 ± 0.24 vs. 1-HIIT: 4.82 ± 0.34 vs. 3-HIIT: 5.01 ± 0.50 mg/g; **Figure 4A**; UN vs. 1-HIIT, $P = 0.001$; UN vs. 3-HIIT, $P = 0.001$) and left ventricle/body weight ratios (UN: 1.76 ± 0.22 vs. 1-HIIT: 2.68 ± 0.51 vs. 3-HIIT: 2.85 ± 0.23 mg/g; **Figure 4B**; UN vs. 1-HIIT, $P = 0.001$; UN vs. 3-HIIT, $P = 0.001$).

Figure 1: Software adjustments for VO₂max test. (A) Recording of a new experiment. **(B)** Animal data. **(C)** Gases calibration. [Please click here to view a larger version of this figure.](#)

Figure 2: Schematic diagram of HIIT protocols. (A) 1-HIIT and **(B)** 3-HIIT protocols. Abbreviation: HIIT = high-intensity interval training; 1-HIIT = HIIT performed with single daily sessions; 3-HIIT = HIIT performed with 3 short daily sessions.

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Figure 3: Effects of 1-HIIT and 3-HIIT protocols on health markers. (A) VO₂max, (B) body weight, and (C) food intake.

Data are presented as mean $\pm$ SD; n = 16 for each of three groups. One or two-Way ANOVA followed by Tukey's test.

Different letters indicate statistical differences. P < 0.05. Abbreviations: HIIT = high-intensity interval training; VO₂max = maximal oxygen consumption; pre-tr = Pre-training; post-tr = Post-training; UN = untrained; 1-HIIT = HIIT performed with single daily sessions; 3-HIIT = HIIT performed with 3 short daily sessions. [Please click here to view a larger version of this figure.](#)

Figure 4: Effects of 1 and 3-HIIT protocols on cardiac hypertrophy. (A) Heart/body weight ratio; **(B)** left ventricle/body weight ratio. Data are presented as mean $\pm$ SD; $n = 16$ for each of three groups. One or two-Way ANOVA followed by Tukey's test. Different letters indicate statistical differences. $P < 0.05$. Abbreviations: HIIT = high-intensity interval training; UN = untrained; 1-HIIT = HIIT performed with single daily sessions; 3-HIIT = HIIT performed with 3 short daily sessions.

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Discussion

This paper highlights the efficacy of an accumulated HIIT protocol for rats for the first time. The accumulated HIIT protocol was as efficient as a traditional HIIT protocol performed with single daily sessions in preventing weight gain, improving VO_{2max} , and inducing cardiac hypertrophy in young male Wistar rats. The improvement in VO_{2max} and cardiac hypertrophy development are the most well-known adaptations to aerobic exercise training and are important health biomarkers¹⁷.

Using an MICT protocol with Wistar rats of the same age as those used in the present study, Costa-Pereira et al.¹⁴ also found that an accumulated exercise protocol (three daily sessions with a 4 h interval between sessions, 10-20 min/session, at 50-60% of maximal capacity, five days/week over eight weeks) was as efficient as the exercise protocol performed with single daily sessions (single daily sessions, 30-60 min/session, at 50-60% of maximal capacity,

five days/week over eight weeks) in preventing weight gain and inducing cardiac hypertrophy¹⁴. Costa-Pereira et al. found a decrease of 5.12 and 9.85% in body mass and an increase of 16.22 and 18.61% in heart/body weight ratio in the accumulated and single daily sessions MICT groups, respectively, compared with the untrained control group.

Here, we found superior results using HIIT. The 1-HIIT and 3-HIIT groups presented a decrease of 9.12 and 11.10% in body mass, an increase of 35.51 and 35.19% in VO_{2max} , and an increase of 34.26 and 39.55% in heart/body weight ratio, respectively, compared with the untrained group. Some studies have demonstrated that traditional HIIT protocols are superior to MICT protocols in increasing red blood cell volume, maximal cardiac output (mainly through cardiac ejection and/or contractility), and skeletal muscle mitochondrial density¹⁸. These physiological adaptations seem to explain the superiority of traditional HIIT protocols (compared to MICT protocols) in increasing aerobic capacity and inducing physiological cardiac hypertrophy. The results

from the present study and the study by Costa Pereira et al.¹⁴ indicate that this superiority of HIIT vs. MICT can be observed when these modalities are performed with short bouts of exercise throughout a day.

The most important strength of this accumulated HIIT protocol is that its sessions last only 5 min, three times per day. Other strengths are the ease of treadmill setting adjustments and animal handling. Once the maximal velocity is obtained from the VO_2max test, the treadmill is set at a flat inclination. The velocity is the same for each bout according to the intensity prescribed for that specific week of training. Another positive aspect is that, during all training sessions, the bouts have the same number and duration: 6 bouts of 1 min interspersed with 1 min of passive recovery performed in single daily sessions for the 1-HIIT group; and 2 bouts of 1 min interspersed with 1 min passive recovery performed three times per day with an interval of 4 h between bouts for the 3-HIIT group. Finally, the choice of passive recovery is another facility as, after the end of each bout, the researcher removes the animal from the treadmill and places it in its cage until the initiation of the next bout. Thus, after the treadmill adjustments are set at the beginning of each exercise session, and no additional adjustments are necessary during the entire exercise session.

The present study also has some limitations. Not all laboratories have a gas analyzer to measure maximum oxygen consumption. However, this limitation can be overcome by using maximum physical capacity protocols that do not use metabolic treadmills to determine VO_2max for exercise intensity prescription, including a previously published HIIT protocol¹⁹. Another limitation is that, compared with protocols using low-to-moderate intensity, continuous exercise, the animals present more difficulty

maintaining the running velocity because it is already high at the beginning of each bout. Thus, the use of shock may be higher in this accumulated protocol than in an MICT protocol. This requires more attention from the researcher, as it is inappropriate to conduct an exercise session with five or more rats simultaneously.

Finally, this physical exercise protocol was designed for healthy young Wistar rats; future studies should test this protocol using rats of different ages and health conditions. Moreover, although a complete characterization of the physiological adaptations to the accumulated HIIT protocol is beyond the scope of the present study, it warrants future investigation. Taken together, these findings reveal, for the first time, the effects of an accumulated HIIT protocol on physiological adaptations to exercise training in young Wistar rats. This new HIIT protocol may be a more feasible strategy than traditional HIIT protocols as, in this new approach, exercise can be split into very short sessions throughout the day.

Disclosures

The authors have no conflicts of interest to declare.

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