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High Intensity Exercise Protocol for Measuring Release of Interleukin-6.

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Abstract

It has been recently hypothesized that secretion of interleukin-6 (IL-6) by active muscles during and post exercise regulates carbohydrates and free fatty acid mobilization and their utilization by contracting muscle. While increased circulating IL-6 levels have been reported during, and especially after, a variety of exercise models, the extent to which high intensity exercise contributes is not well characterized. This project investigated the regulation of IL-6 secretion by high intensity, anaerobic cycling exercise. The purpose of this project was to characterize the difference between repeated high intensity bouts and one continuous bout on an absolute workload. 10 healthy, college-aged subjects completed the investigation. This study found that the high intensity and low intensity bouts were equal in workload, and that the continuous bout was an adequate control to examine the effects of this high intensity exercise bout.

Introduction

Interleukin (IL)-6 belongs to a group known as cytokines, which are proteins secreted from a variety of cells in the body in response to a stimuli⁸. IL-6 is often classified as a pro-inflammatory⁵, but also has been reported to partly mediate an anti-inflammatory response⁹. IL-6 has also been shown to increase the amount of blood glucose and free fatty acid availability, by increasing lipolysis in adipose tissue and glycogenolysis in the liver⁷. Because IL-6 is produced in and released from skeletal muscle during exercise, some have suggested that it should be called a "myokine." Skeletal muscle may now be seen in a novel role: an endocrine organ which produces and releases myokines in response to contractions due to exercise⁶.

Exercise intensity has been found to be an important factor in IL-6 concentrations⁴. Higher intensity levels elicit higher concentrations of IL-6 secretion, but the reason behind this is still unconfirmed. Peak levels of IL-6 are reached at the end of the exercise, or shortly thereafter¹. The IL-6 response to repeated bouts of high intensity exercise has not been studied, although this has been evaluated in endurance exercise^{10, 11}.

The purpose of this study is to characterize the response of IL-6 as a consequence of the intensity of exercise, rather than the duration. Therefore, it was important to create a protocol that can be used to measure this response.

Method

Ten college-aged individuals with characteristics in Table 1 completed one familiarization session in which subjects learned the procedures used, determined settings for the cycle ergometer (Velotron; RacerMate Inc.), and completed a maximal aerobic test on the cycle ergometer to determine their maximal oxygen consumption ($VO_{2\max}$), a quantitative measure of their cardio-respiratory fitness. VO_2 data was gathered with the use of a metabolic cart (TrueMax 2400 Metabolic Measurement System; ParvoMedics). Subjects pedaled the cycle ergometer at steadily increasing workloads, until maximum oxygen consumption was achieved. At the two subsequent testing sessions, subjects performed two different cycling tests. Both protocols used a 5 minute warm-up and cool-down at 50W and 20W respectively. The cycle exercise protocol was as follows:

1.) Four 30 s Wingate tests were performed with 4 min of recovery cycling at 20 W for total test duration of 14 min. Wingate test workload was 0.075 kpm/kg body weight. Protocol used was in accordance with Greer et al.².

2.) The total work performed during the first test was equally distributed over a 14 min exercise test duration.

An indwelling catheter was used to sample venous blood before, during, and after the exercise bouts at pre-test, 6.5, 11, and 15.5 minutes, as well as postexercise at 1, 15, 30, and 60 min for both protocols to determine the effects of intensity on blood IL-6 concentrations (Figure 2). Hemoglobin and hematocrit levels were determined to account for changes of blood plasma volume during exercise. Corrections due to blood plasma volume alterations were made to assess whether increased IL-6 concentrations are observed, or merely more concentrated due to loss of plasma. Hemoglobin and hematocrit were analyzed by cyanmethemoglobin and microcapillary techniques, respectively, while IL-6 will be measured by chemiluminescent immunoassay (Immulate analyzer; Diagnostic Products Corporation). Subjects' metabolic state was determined through blood glucose, lactate, and respiratory quotient, which will be used as a quantitative measure of substrate utilization during exercise.

Statistical Analysis

A two-tailed t-test was performed on the total work completed with $\alpha = .05$.

Results

Table 1. Subject Characteristics

Age	Height (in.)	Weight (lbs.)	VO ₂ Max (ml/kg/min)
20.9 ± .78	69.4 ± 4.05	161.1 ± 26.58	47.9 ± 9.05

All data are mean ± standard deviation. Subject pool was n = 5 each of males and females.

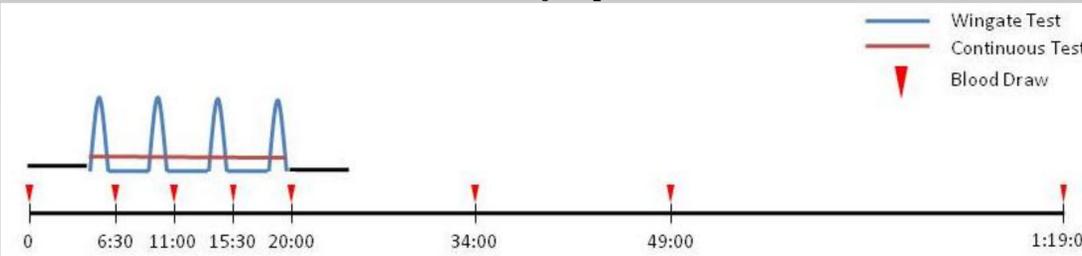


Figure 1. A timeline of the testing protocol outlining the work rates and blood draw times during both tests.

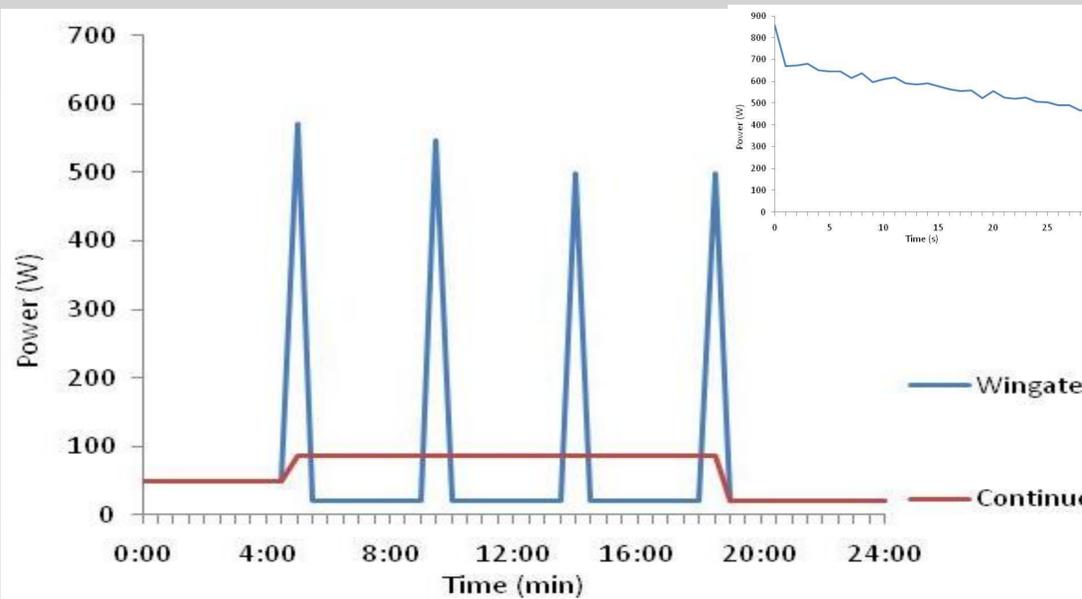


Figure 2. Average power performed during the continuous and wingate tests. The repeated wingate tests were of a higher intensity than the continuous test.

Figure 3 (inset). Average power during the course of one wingate test. The profile of all wingates ends at a slightly lower intensity than the beginning due to the strength and aerobic capacity needed to complete the work.

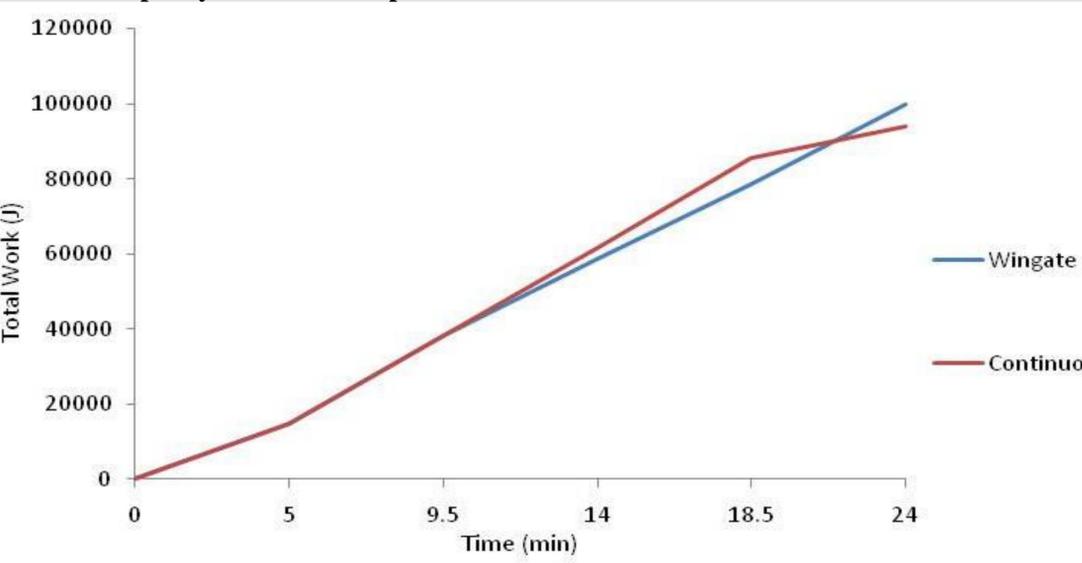


Figure 4. Total Work performed during the two tests was not significantly different with a P > .05. The continuous bout of exercise was a good control for the high intensity, repeated wingate test.

Discussion

The purpose of this study was to create a protocol which studies the effects of intensity of exercise on IL-6 release without the influence of duration of exercise or volume of exercise. The t-test shows that with $P > 0.05$, there was no significant difference between the total work done in each test, showing that the volume was equal for both tests. Since the tests lasted for the same length of time, duration was constant as well.

As stated before, IL-6 response to repeated bouts of high intensity exercise has not been studied, although this has been evaluated in endurance exercise^{10, 11}. These experiments that have looked at exercise that is at a higher intensity have not used a standardized protocol. Some studies have looked at resistance training³, while others have looked at running⁴. With such variance in the testing protocol, the values from the studies cannot be compared between different subject pools. The protocol used in this study can now be used as a standard bicycle ergometer protocol, eliminating variance so that any subject pool can be compared without outside factors affecting data.

Conclusion

The continuous bout of exercise was a good control for the high intensity, repeated wingate exercise bout since both tests accomplished the same amount of work. This allows the effects of intensity to be compared while factoring out duration of exercise.

Future Plans

It is planned to continue research into blood analysis, looking at IL-6 levels and hormone changes. When the final analysis is completed the data will be used in a senior thesis on the changes of IL-6 during high intensity exercise in college-aged males.

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