Mask wearing and oxyhemoglobin saturation effects during exercise

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SUMMARY
Wearing face masks has become a common occurrence in everyday life and during athletics due to the spread of diseases. Hemoglobin transport is a key process that occurs when oxygen is carried to surrounding organs and tissues. During exercise, hemoglobin increases but the amount of oxygen may not increase which is why percent saturation of hemoglobin (SpO₂) drops. We had 30 subjects run on a treadmill at 6 miles per hour 3 separate times, with no mask, a cloth mask, and an elevation mask. They were separated into 3 groups based on fitness, and their SpO₂ levels were measured 3 times throughout the run. We wanted to see if masks would affect blood percent saturation of hemoglobin (SpO₂). To analyze our data, we used Statistical Analysis Software (SAS), and compared data with an F-test (specifically, General Linear Models). Our data analysis showed that mask type, time, and the interaction of mask type and time were significant. Although, the impacts of fitness group, and the interaction of fitness and mask type were not significant. Our results allow athletes to understand the differences between training and competing with and without a mask. In summary, our study found that masks heavily impede oxygen transport in the blood, regardless of physical ability.

INTRODUCTION
After the beginning of the COVID-19 pandemic, new laws were implemented about mask wearing, including mandatory mask wearing while competing and practicing sports, or exercising in the gym. We suspected that this could cause decreased SpO₂ levels. During exercise, hemoglobin transport is a vital process that allows your body to function during low and high intensity workouts. Hemoglobin is the largest protein of the red blood cells and is responsible for oxygen transport from the respiratory system to different tissues in the body. When an individual participates in a cardio-related sport, their muscles need more oxygen because oxygen enables muscle function. With unrestricted breathing, blood-oxygen levels increase when performing aerobic exercise (Eroglu et al., 2018). However, when less oxygen is readily available to breathe, the body goes into a “survival state.” In this state, the amount of hemoglobin in the bloodstream increases (Thiel et al., 2011). This would cause SpO₂ levels to lower if oxygen availability is restricted, because oxygen cannot increase as hemoglobin increases, so the ratio of fully saturated hemoglobin (SpO₂) lowers. This is due to the body’s process of acclimatization when the body is exposed to less oxygen.

The purpose of our experiment was to examine if masks, acting as hypoxic environments, had effects on blood oxygen (percent saturation of hemoglobin - SpO₂) levels during physical activity. The critical SpO₂ for any human is 94% (Mayo Clinic Staff et al., 2018). Critical SpO₂ refers to the point at which the SpO₂ drops low enough to affect an individual’s ability to achieve peak athletic performance. We expected to see hemoglobin saturation levels decrease as subjects used more restrictive masks, increasing exercise difficulty. Contrary to other studies we choose to use cloth masks rather than disposable masks because disposable masks can have more areas of loose fit meaning they do not provide proper protection against Covid-19. To avoid having faulty fitting for each test subject, we choose an adjustable cloth mask so we could adjust it to every individual and avoid areas of leak. We also expected that this effect would be more intense on individuals who did not exercise very often as compared to those who do exercise often. We hypothesized that time would influence SpO₂. We also hypothesized that the interaction of mask type and time, and the interaction of fitness level and mask type would have a significant effect on SpO₂. With the following hypotheses in mind, we found that cloth masks and elevation masks cause an athlete’s SpO₂ to enter the critical zone. In other words, both the cloth mask and elevation mask could cause an athlete’s performance to be affected negatively because the SpO₂ levels dropped below 94%, and blood oxygen below this value impedes the cardiovascular system.

RESULTS
As with any facial covering, oxygen flow can be impacted, resulting in a decrease in SpO₂. When asking subjects if they found trials to be difficult or not, most subjects stated that the trials where they wore a cloth mask or elevation mask were difficult, but that trials that involved not wearing a mask were not difficult. However, no statistical analyses of these data were performed. Statistical analysis of subjects’ heart rates was not performed because we did not have a complete data set for all 30 subjects. We did, however, have SpO₂ data from
all 30 subjects, so it is reasonable to assume that the data is approximately normal. In the preceding paragraphs, cross examination is mentioned heavily: cross examination means that we are showing the combined impact of two different variables. When comparing them, we looked at their impact on SpO₂ together but as one significance test.

First, we looked at the three different fitness groups (Figure 1) to see if their SpO₂ were statistically significant. In this analysis, data from all 3 trials were combined (ex. Mask, no mask, and elevation mask data were combined and then separated into fitness groups). The three fitness groups consisted of groups who worked out 0 days a week, 1-3 days a week, and 4-7 days a week, respectively. The group names are as follows: 0 days a week was Group 1, 1-3 days a week was Group 2, and 4-7 days a week was Group 3. We found that there was not a significant difference in the fitness groups. Using the General Linear Models test, we calculated a p-value of 0.8819 (p = 0.8819) with a F-value of 0.13 (f = 0.13). Fitness groups were not statistically significant or in other words the impact of fitness groups on SpO₂ did not differ to the point where different amounts of exercise impacted SpO₂. The first time interval measured was taken before the individual started running, the second being when the individual reached the 2.5-minute mark, and the final interval measured at the 5-minute mark. Note that the 5-minute mark was measured as they were still running and not measured when they completely stopped.

After finding fitness groups to be insignificant, we looked to see if time had a significant effect on subjects’ SpO₂. Each subject ran for at least 2.5 minutes for every trial. There were two cases where subjects could not complete their trials, but in every other trial, subjects completed the whole 5-minute trial. Using the same GLM procedure, a p-value of <0.0001 was calculated, with an F-value of 79.38 (f = 79.38) and degree of freedom 2. This means that time had a significant effect on SpO₂. The GLM procedure compares SpO₂ levels for all three time intervals at once. The lowest mean SpO₂ values were at the 5-minute mark, and the highest at the 0-minute mark (Figure 2).

Finally, mask type (no mask, cloth mask or elevation mask) was examined. The significance test yielded a p-value of 0.0001 (p = 0.0001) with an F-value of 9.32 (f = 9.32) and a degree of freedom of 2. This means that mask type had a significant impact on SpO₂ levels. There was a trend towards lowering SpO₂ levels with more restrictive mask types. The elevation mask (mask 3) had the lowest mean SpO₂ levels, cloth mask SpO₂ levels were in the middle, and no mask (mask 1) had the highest mean SpO₂ (Figure 2).

After examining each independent variable separately, two independent variables were cross examined at a single time. The two cross examinations we performed were mask type and time and fitness group and mask type. We cross examined mask type and time to determine if the three different mask types affected SpO₂ at various times. The second cross examination was performed to determine if the different mask types affected the fitness groups differently.

Before the results are explained, we would like to note that our alpha value was set to 0.05. This meant that any p-value that was calculated that was smaller than the alpha value was statistically significant because the interval in which your data could fall was within a smaller margin. If it were bigger, it would make it statistically insignificant. When examining mask type and time, the GLM procedure was used to calculate a p-value of 0.0228 with an F-value of 2.89 and degree of freedom of 4. This meant that the cross examination of mask type and time was statistically significant because the p-value was less than our alpha value of 0.05. This is to be expected because mask type and time (when examined separately) significantly affected SpO₂. This means that within the trials per each different mask type, SpO₂ levels were significantly different per each timestamp of when data was collected. The data followed the same trends as mentioned when these variables

Figure 1. Comparing SpO₂ regardless of masks between fitness groups. This graph shows the relationship between fitness groups and least-squares means for their SpO₂ levels, including all data from all trials (no mask, cloth mask, elevation mask).

Figure 2. Relationship between time and least-squares means for their SpO₂ levels. This graph shows the relationship between time since the start of a trial and mean SpO₂ levels measured, regardless of fitness group and mask type.
were analyzed individually. These independent variables were plotted on a graph (Figure 3) to visually see the relationship.

The last and final GLM procedure we used was to cross examine fitness groups and mask type. Using the GLM procedure for the cross examination of fitness group and mask type, a \( p \)-value of 0.53 was calculated, with an \( F \)-value of 0.79 and a degree of freedom of 4. This showed us that the cross examination between fitness group and mask type was statistically insignificant because the \( p \)-value was greater than the alpha value of 0.05. Aside from the numbers, this data tells us that each of our three fitness groups were all affected in the same way by the distinct types of masks and that athletic ability did not prohibit or benefit the individual’s SpO\(_2\).

DISCUSSION

Our experiment had a total of five hypotheses. Three of the hypotheses only involved examining one independent variable, but the other two involved cross examining two independent variables. Our hypotheses were: that fitness groups had a significant effect on SpO\(_2\); the amount of time had a significant effect on SpO\(_2\); and that mask type had a significant effect on SpO\(_2\). These are the hypotheses for just our independent variables (fitness, time, and mask type).

As shown above it was found that all three fitness groups had a similar drop in SpO\(_2\). The experiment had three different fitness groups with each exercising a different number of days. One problem with self-reporting of this data is that people might not provide accurate figures. A more objective assessment of physical fitness may have yielded more significant results. We also investigated a similar study that was performed a year or so prior (involving aerobic exercise), but they found no such correlation. However, that study had less participants and quite different methodology (4). A study with more subjects and a more controlled environment, but with mental stress rather than physical stress found that masks do impact SpO\(_2\) levels (5).

Mask type, time, and the interaction of mask type and time also have a significant impact on SpO\(_2\) due to multiple factors. For one, restrictive breathing conditions mean that the body must work harder than it normally would to complete the same physical activity. When the body starts working harder, it is not able to pull in as much oxygen. Therefore, the amount of oxygen bound to hemoglobin lowers since there is less to go around, and the SpO\(_2\) levels drop. Since time measurements begin before subjects have started running, it makes sense that SpO\(_2\) levels are high, and as they run, the effects of any mask they are wearing become apparent lowering oxygen levels and affecting SpO2 as previously stated. As they become more tired, their bodies work less efficiently and the effects of the masks only intensify, hence the lowest mean SpO\(_2\) values at the 5-minute mark (Figure 3).

As mentioned previously, subjects waited a minimum of one day before beginning any other phase of the experiment. This turned out to be problematic because some subjects ran every other day while others ran a couple of days or even an entire week apart. Standardizing the wait times could have yielded more accurate results but could not be accomplished due to extraneous circumstances in the global pandemic. This could have given some subjects small advantages over others as they had more rest between phases of the experiment. There were also significant problems with the pulse oximeter. Multiple trials of subjects had to be redone when the oximeter did not get a reading. This caused us to have to perform more than three trials of the same phase for some subjects which caused our data collection period to extend much further than we had initially predicted. In the future, a different pulse oximeter would be used. However, a new pulse oximeter could not be used because of shipping problems. It is also possible that since each subject performed their trials in the same order (no mask, cloth mask, elevation mask), that there was a slight training effect. However, this is improbable due to the short length of the trials. The data could have been more reliable if trial order was randomized.

METHODS

Thirty local volunteers participated in this experiment. Individuals had to sign a waiver (IRB) before participating in the study. Due to the difficulty in finding subjects in the current pandemic, the sample could not be truly randomized. Subjects of varying athletic ability were recruited, to get an accurate sample of the population. Athletic ability was measured by the sorting of subjects into 3 groups, based on self-reported exercise frequency: Group 1, who exercised 0 days per week, Group 2 who exercised 1 to 3 days per week, and Group 3, who exercised 4 to 7 days per week. The youngest subject was 14, and the oldest subject was 55. From our observations, age did not seem to impact SpO\(_2\) but no statistical analyses of age versus SpO\(_2\) was performed. Testing was performed at the Williamston Barbell Club. All participants used the same treadmill. A pulse oximeter was chosen, as a simple
noninvasive method of determining oxygen levels in the blood. Sterilization of the pulse oximeter consisted of wiping down the device. Since the goal was to investigate the effect of mask wearing during exercise on SpO₂, an exercise was chosen that would be easily controlled and which would be easy to perform with a simple setup. A moderate speed was selected which allowed subjects to run normally throughout the entire length of each trial.

This allowed us to see percent saturation of hemoglobin (SpO₂) during non-sprinting running. During each trial, participants wore a pulse oximeter on one of their fingers. Before running, participants walked for a 2-minute warm up at 4 miles per hour to make sure the pulse oximeter provided accurate readings. Then, subjects ran for 5 minutes at 6 miles per hour on the treadmill. The procedure consisted of three phases for each person; phases were conducted at least 1 day apart to provide adequate rest time for subjects. Phase one had subjects run for 5 minutes on a treadmill at 6 miles per hour without a mask.

In phase 2, subjects performed the same run with a knitted mask made of 100% cotton (provided by us) that covered their mouth and nose. The same two masks were used with all subjects and were sterilized between each use. The third and final phase consisted of subjects running on the treadmill for 5 minutes at the same setting with an elevation mask, which was set to a medium elevation (level 6 of 8). An elevation mask is a mask with a filter whose permeability can be adjusted with a lever to control how much airflow is restricted for whomever is wearing the mask. We set the elevation mask to setting 6 after the mask on ourselves and extensively researching the effects of high and low altitude climates on the human body. Throughout their runs, participants’ heart rates and SpO₂ levels were recorded 3 times: immediately before they started, after they had been running for 2.5 minutes, and when they had been running for 5 minutes (right before their trial finished). This allowed us to analyze data from all subjects prior to running with any of the three masks, as well as when they were in the middle of their exercise, and when their body had 5 minutes to adjust to any mask they were wearing. If a subject could not finish any phase of testing, we recorded the time when they could not finish and wrote CNC (could not continue) in the time slot(s) where they did not finish. If a subject did not finish, we substituted data from the 2.5-minute mark to fill in the 5-minute mark. Following each trial participants informed us whether they found the run to be difficult or not. This added additional applicability to the data.

To analyze our data, we used Statistical Analysis Software (SAS), and compared data with an F-test (specifically, General Linear Models). We used SpO₂ as our dependent variable, and measurement times, and mask type and fitness group as our independent variables. The General Linear Models (GLM) procedure allowed us to examine each independent variable separately to see if that independent variable was statistically significant. In a GLM test, you can use 0.01, 0.05, and 0.10 as your significance level (also known as the alpha value). In our experiment, we set our alpha value equal to 0.05. In an GLM test, if the p-value you calculated is less than the alpha value, then you fail to reject the null hypothesis (meaning the data was statistically significant). If the calculated p-value is greater than the alpha value, we would reject the null hypothesis (meaning the data was not statistically significant). As seen in the hypothesis, we wanted to not only compare different independent variables, but we also wanted to cross compare independent variables. For example, it was desirable to cross compare mask type and time to see if, combined, they were significantly significant.

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