The Effects of Cryostimulation on Inflammatory Cytokines and Hematological Parameters

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Abstract: Cryostimulation is used frequently in sports medicine to increase recovery time and to decrease inflammation. Some of the key factors in this response are inflammatory cytokines and hematological parameters. Currently, there is no baseline value on the treatment time (number of minutes) for the most effective cryostimulation in different settings. The current study is going to evaluate the optimum time of cryostimulation required for the most effective treatment. This study was performed via systematic literature review of peer-reviewed papers that focus on inflammatory cytokines and hematological parameters. The data collected showed an increase in IL-10 and IL-1**β**, while IL-6, TNF-**α**, CK, Hb, and RBC all decreased in the blood after cryostimulation. This data suggests that cryostimulation promoted a decrease in inflammation. The greatest differences in the inflammatory cytokines were seen when cryostimulation is performed for 3 minutes which indicates that is the optimum cryostimulation should be performed for 3 minutes.

Key Terms: *cryostimulation, cryotherapy, inflammation, cold therapy, inflammatory cytokines, hematological parameters, cryochamber, recovery, cold exposure, and hemolysis*

Introduction

The cryostimulation process consists of a whole-body exposure of an individual for 2-3 minutes, to liquid nitrogen vapors at -110°C to -200°C, wearing minimal clothing in a cryochamber (Figure 1; Lombardi et al., 2013). Cryostimulation is performed in a cryochamber similar to the one in figure 1. The person would stand in the chamber for 2-3 minutes and rotate for that time to evenly distribute the liquid nitrogen around them. It has been effective in reducing the inflammatory process and the concomitant redox unbalances, a leading cause of cell damage. By increasing anti-inflammatory cytokines and decreasing pro-inflammatory cytokines it reduces inflammation significantly (Lubkowska et al., 2011). Cold is known to affect leukocyte mobilization, and cold exposure can initiate changes in cytokine expression associated with a nonspecific acute phase reaction that could be the effect of multiple interactions between the cytokines (Lubkowska et al., 2011). H. V. Allington is generally thought to have been first to use liquid nitrogen, in 1950. He recognized that properties of liquid nitrogen were very similar to those of liquid air and oxygen. He used a cotton swab for treating various benign lesions but poor heat transfer between swab and skin meant this method was insufficient for tumor treatment (Cooper & Dawber, 2001). Cryostimulation is a relatively new tool for the symptomatic treatment of inflammatory and painful conditions of subjects affected by inflammatory, auto-inflammatory, immune-mediated conditions as well as in healthy and physically fit individuals (Banfi et al., 2010). It is frequently used in sports medicine, however, its application in medicine is based on only a few randomized trials to support it.

Figure 1: Cryomed Pro Cryosauna
Source: www.criosauna.ro

Cold water immersion (CWI) and cryostimulation, significantly reduce skin temperature. However, cryostimulation had a greater decrease compared to CWI (Costello et al., 2012). The cooling

efficiency is almost the same, but cryostimulation has been shown to be more effective than CWI (ice baths). This is due to several factors: i) greater discomfort in CWI compared to cryostimulation; ii) head exposure to cold; iii) airways exposure to cold and consequent activation of the airway-associated immune system (Bleakley et al., 2014). Cryostimulation is more expensive than CWI, costing about $60-100 per treatment. CWI is more time consuming for therapists because the bath is filled with ice which melts and cannot be reused. But cryostimulation is performed in a cryochamber which is confined and reusable. Patients have to be in the ice baths for 6-8 minutes. Whereas, patients only have to do cryostimulation for less than half the time, 2-4 minutes (Ziemann et al., 2013).

Currently, there are a variety of different predictions and results for how many minutes cryostimulation should be done. The research purpose is to figure out the optimal number of minutes to obtain positive results on inflammatory cytokines and hematological factors. This study will collect data through systematic literature review. For the systematic literature review, by analyzing peer-reviewed papers on the effects of cryostimulation on symptoms of inflammation, inflammatory cytokines, and hematological parameters. The peer-reviewed papers date range will be from 2006 to 2018. The peer-reviewed papers' data will be analyzed to gather data to find the ideal number of minutes per cryotherapy session needed to see results. Previous research has used from 2 to 4 minutes, and this study would find a conclusion on what number of minutes is most effective.

This study is important so people understand how cryostimulation works and how it is effectively reducing their inflammation. People should know how long of sessions they will need. Research on cryostimulation could be crucial to demonstrate that athletes and non-athletes can recover faster and have to spend less time and money on physical therapy, CWI, or other less effective recovery methods. Cryostimulation is performed all over the world but there is no baseline value for the number of minutes it

should be performed for. This research study would provide the optimum number of minutes needed to be the most effective in reducing inflammation.

Background

Cryostimulation can be used in sports medicine to help prevent inflammation or help with injury recovery. In Northern Europe, it is performed by the general population (e.g., 10 sessions yearly) for wellness. There have been significant differences in the inflammatory status before and after cryotherapy. Cryostimulation decreases the serum concentration of pro-inflammatory cytokines and increases the anti-inflammatory cytokines (Banfi et al., 2009). The pro-inflammatory and anti-inflammatory cytokines in this study are Interleukin-6 (IL-6), Interleukin-10 (IL-10), Interleukin-1Beta (IL-1**β**), and Tumor Necrosis Factor-Alpha (TNF-**α**). In figure 2, it shows that IL-10 is an anti-inflammatory cytokine, and IL-6, TNF-**α**, and IL-1**β** are pro-inflammatory cytokines. But IL-6 is also at the top of the figure on both sides, it can act as both a pro-inflammatory cytokine and an anti inflammatory cytokine (Lubkowska et al., 2011), but its primary role in this study specifically is a pro-inflammatory cytokine. In order for cryostimulation to be affected significantly then it would be ideal for the pro-inflammatory cytokines to decrease and for the anti-inflammatory cytokines to increase.

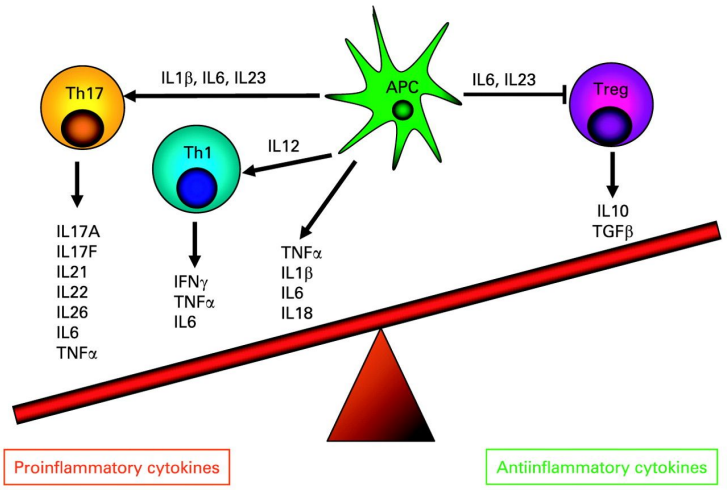

Figure 2: Anti-Inflammatory and proinflammatory cytokine diagram
Source: Selfhacked.com

Two minutes of cryostimulation was effective in reducing inflammation without inducing any negative effects (Banfi et al., 2009; Banfi et al., 2010). Three minutes of cryostimulation was also shown to have positive effects (Costello et al., 2012). There are many studies that did different numbers of

minutes but most have the conclusion that cryostimulation effective at reducing inflammation. Cryostimulation also decreased proinflammatory cytokine (tumor necrosis factor alpha) and pleiotropic cytokine (interleukin 6) increased (Ziemann et al., 2012). IL-6 is one of the pro-inflammatory cytokines, it has a pleiotropic effect on inflammation, immune response, and hematopoiesis (Tanaka et al., 2014). After IL-6 is synthesized in the initial stage of inflammation, it moves to the liver through the bloodstream, followed by the rapid induction of an extensive range of acute phase proteins (Tanaka et al., 2014). Interleukin-6 is not only involved in inflammation, but also in the regulation of metabolic, regenerative, and neural processes. Interleukin-6 stimulates target cells via a membrane bound interleukin-6 receptor, which upon ligand binding associates with the signaling receptor protein (Scheller et al., 2011). Cryostimulation is exposure to an extremely low temperature this can trigger muscle shivering, which can result in an the increase in IL-6 (Ziemann et al., 2012).

IL-10 is also known as human cytokine synthesis inhibitory factor (CSIF), it is an anti-inflammatory cytokine and it is encoded by the IL10 gene. It is a cytokine with potent anti-inflammatory properties that has a central role in limiting immune response to pathogens, which prevents damage to the host due to the rapid activation of IL-10 (Iyer & Cheng, 2012). The immune system has evolved parallel anti-inflammatory mechanisms to repress the production of pro-inflammatory molecules this limits tissue damage and helps to maintain or restore tissue homeostasis (Iyer and Cheng, 2012). Hypothermia constricts the inflammatory response, which contributes to its beneficial role in organ protection (Banfi et al., 2009). In previous studies, the anti-inflammatory mechanism, IL-10, has been discussed in studies on cell culture, which reported a decreased in a wide spectrum of pro-inflammatory cytokine genes (Ziemann et al., 2013).

IL-1**β** in pain and inflammatory processes is a tremendously complex system. IL-1β is a pro-inflammatory cytokine that has been associated with pain, inflammation and autoimmune conditions (Ren & Torres, 2009). Interleukin-1β is a prototypic proinflammatory cytokine that has pleiotropic effects on a variety of cells and plays a key role in acute and chronic inflammatory disorders. Cryostimulation can also be used to treat inflammatory diseases and not just regular inflammation. The overproduction of IL-1β is implicated in the pathophysiological changes that occur during different disease states, such as rheumatoid arthritis, neuropathic pain, inflammatory bowel disease, osteoarthritis, vascular disease, multiple sclerosis, and Alzheimer's disease (Ren & Torres, 2009). TNF-**α**, is one of the pro-inflammatory cytokine of this study. Cryostimulation enhanced the inverse correlation between TNF-**α** and cardiorespiratory fitness. In previous published studies, cryostimulation has been shown to effectively reduce TNF-**α** concentrations in athletes (Ziemann et al., 2013).

Cryostimulation has been shown to have positive effects on muscular enzymes, like creatine kinase (CK). This enzyme indicates muscular involvement during exercise and they indicate that cryostimulation facilitates athletic recovery. Within the cell, creatine and its associated enzyme CK facilitate the high energy phosphates in the form of phosphocreatine (PCr) between sites of ATP generation (Kitzenberg et al., 2016). The CK circuit is tightly linked to mitochondrial structure and energetics. This mitochondrial coupling reduces reactive oxygen (Kitzenberg et al., 2016). A decrease in CK concentration can cause rapid recovery from muscle damage, this is also a characteristic marker of exertional rhabdomyolysis, it can be used to measure how cryostimulation affects workload, recovery and possible overtraining (Banfi et al., 2009). Cryostimulation can stimulate muscle fiber repair by reducing cell membrane breakdown or increased cell permeability produced during physical exercise (Banfi et al., 2010). Exercising in a cold environment means there is a simultaneous increase in CK levels (Banfi et al., 2009).

The hematological parameters being examined in this study are hemoglobin (Hb) and red blood cells (RBC). In regards to hematological parameters, cryostimulation causes a very slight decrease in RBC and Hb (Lombardi et al., 2013). RBCs carry O_2 from the lungs to the body and bring CO_2 back to the lungs. Hb is contained in RBCs and it is a "red" protein responsible for transporting O_2 from the respiratory organs to the rest of the body. Marked by bilirubin (a pigment created in the liver by the breakdown of hemoglobin) increase, hemolysis (destruction of RBC while membranes are broken) may be the cause behind the drop in RBC and Hb following the cryostimulation treatment. This would be a consequence of the cold-induced muscle contraction that constrains the blood vessels and causes RBC breaking (a phenomenon similar to that happening in the athletes during strenuous efforts). An increase in hemoglobin (Hb) concentration in plasma, and bilirubin are seen after cryostimulation treatments (Szygula et al., 2014). Hematological parameters go through changes during intense training (decrease in RBC and Hb) and the ferritin concentration will fall, mirroring a depletion of body iron stores (Lombardi et al., 2013). Changes in the hematological parameters due to reducing RBC counts and their hemoglobinization can occur after cryostimulation (Szygula et al., 2014). Vasoconstriction and muscle contraction (shivering) from cryostimulation can cause a large increase in RBC, which can be beneficial to stimulate RBC pool rejuvenation (Szygula et al., 2014).

The effect of cryostimulation on RBC and Hb can be affected by the type and intensity of physical training and, however, are only transitory since they promptly recover after multiple cryostimulation sessions. The kinetics of oxygenation potential-related parameters should be considered in scheduling the treatment protocol during the different phases of the season in order to avoid a paradoxical decrease of the performance. Many of the studies measure the factors that influence whether

inflammation is decreasing or not at (Szygula et al., 2014). Studies also discuss how many minutes cryostimulation is done for.

Purpose

There is no current optimum time of cryostimulation that the technician is expected to follow during the cryostimulation sessions. The purpose of this research is to gather data from other published papers on the effective cryostimulation time to significantly reduce inflammation. This research compares the different times (2-3 minutes) with how effectively it reduces pro-inflammatory cytokines and hematological parameters, or increases anti-inflammatory cytokines are either reduced or increase the production of these factors in the blood stream.

Research Question

What is the optimum cryostimulation time (minutes) to be performed to significantly reduce inflammation?

Alternate Hypothesis

The optimum time of cryostimulation to reduce inflammation significantly is 3 minutes per day.

Null Hypothesis

There is no difference between two or three minutes of cryostimulation with respect to inflammation.

Methods/Materials

Current research was conducted via systematic literature review. This involved obtaining peer-reviewed papers from various databases. The peer-reviewed papers' data was collected and analyzed to find the optimum number of minutes for cryostimulation. Similar papers have been published on this

topic but only a few focus specifically on the number of minutes. Surveys were not appropriate for this study because the study involves data collection through blood tests, which could not be obtained through surveys. Primary data analysis would also not be an appropriate method because such work needed a lab to work in. Secondary data analysis would also not have been accurate for this study because the data comes from multiple sources. A systematic literature review was the best method for collecting data from multiple sources and calculating averages followed by standard deviation and t-tests. These papers were focused on inflammatory cytokines and hematological parameters to determine an overall conclusion on which number of minutes (2 or 3) effects cryostimulation significantly. The selected research design is most accurate because it allows for a comparison of multiple factors from different studies to collect data and analyze it.

Explanation of Data Analysis

The peer-reviewed papers date ranged from 2006 to 2018. Any data outside of this time frame was outdated or did not fit the parameters of the study. The majority of articles came from online databases, the most frequently used databases were the Elsevier, ScienceDirect, Archives of Exercise in Health and Disease, Hindawi Publishing Corporation, Google Scholar, Plos One, and Springer. The literature is based on my searching terms of cryotherapy, inflammation, cryostimulation, cold therapy, cryokinetics, cytokines, hematological parameters, cryochamber, recovery, cold exposure, and hemolysis.

Two, three, and four minutes have all been tested in peer-reviewed papers, but this study only includes two and three minutes because four minutes is not used as frequently, so there is no data available on it. There would not be enough supporting documentation for all the factors included in this study, so there would not be as wide of a range of inflammatory cytokines and hematological parameters.

No data was collected through lab work, all through systematic literature review. All of the graphs were created and plotted in Microsoft Excel. The data collected was the averages of inflammatory cytokine or hematological parameters concentrations of 2-4 papers.

Results

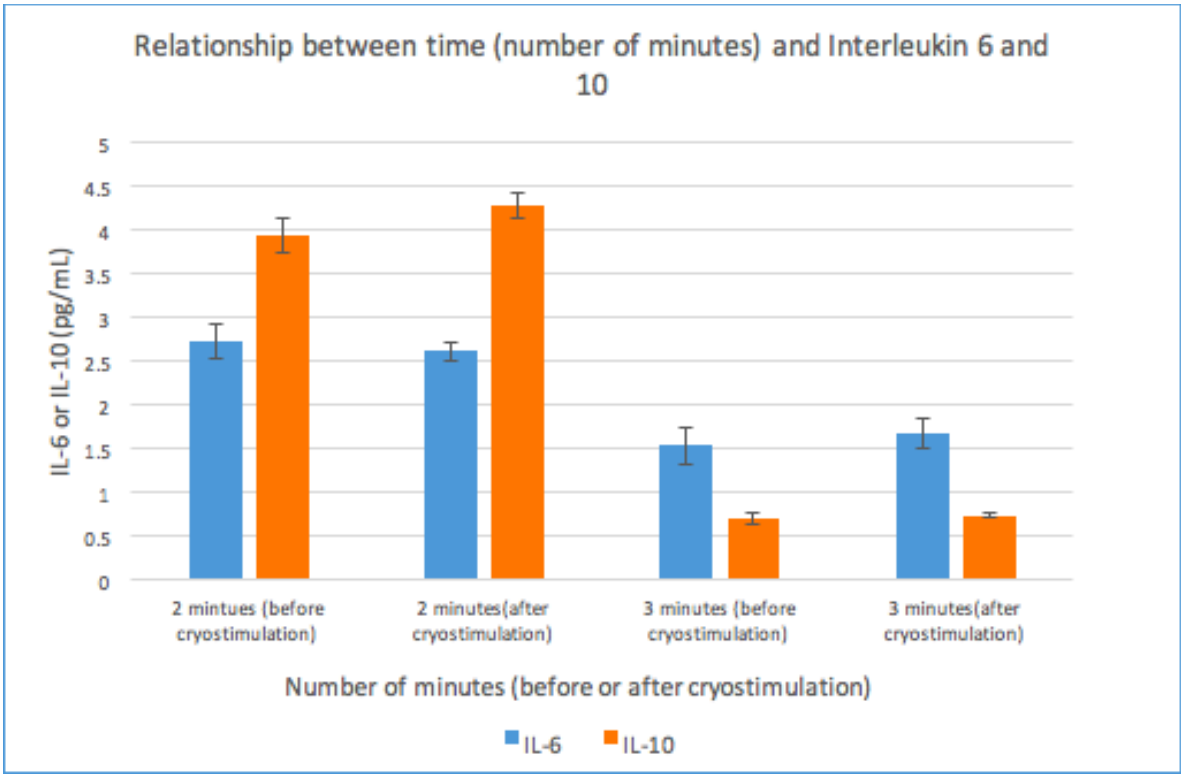

Figure 3: The concentrations of Interleukin 6 and 10(pg/mL) at 2 and 3 minutes before and after cryostimulation p=0.022136208

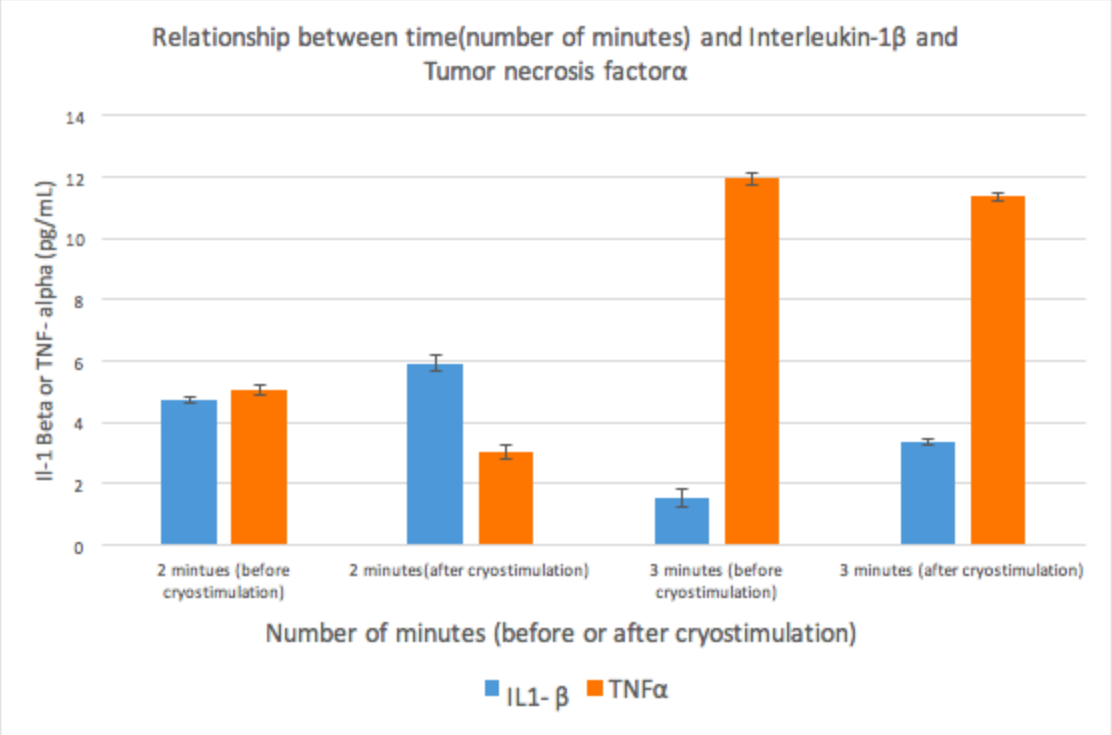

Figure 4: The concentrations of Interleukin 1-Beta and Tumor necrosis factor alpha (pg/mL) at 2 and 3 minutes before and after cryostimulation. P-value(IL-1**β**)=0.032309036 and P-value(TNF-**α**)=0.030070015

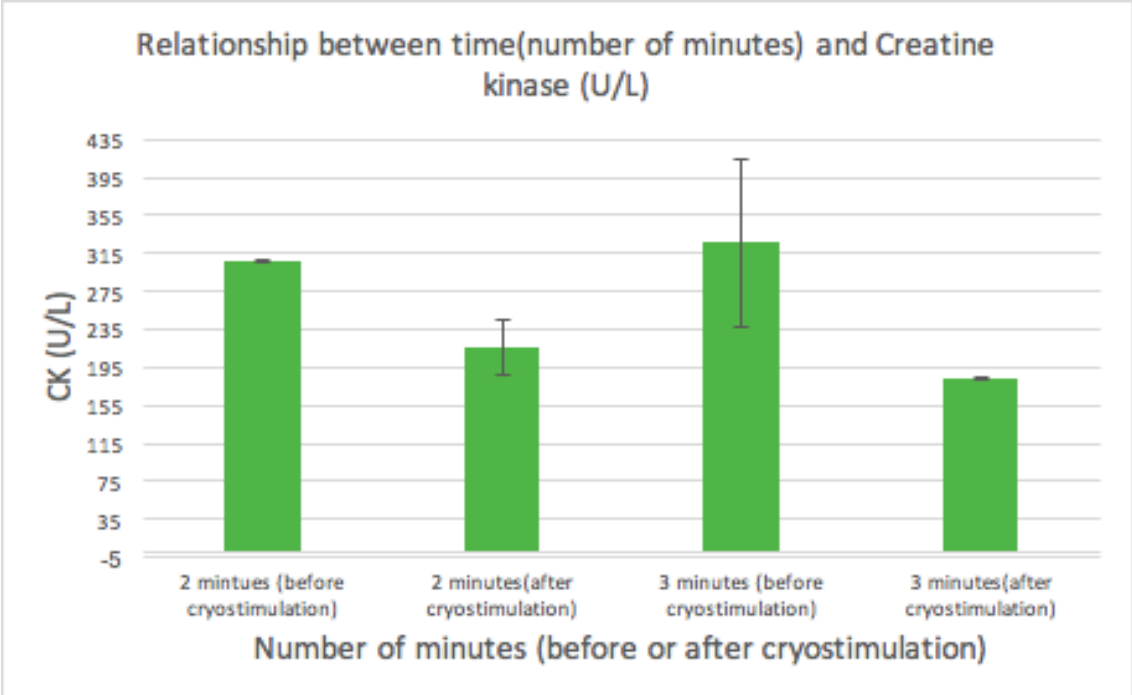

Figure 5: The concentration of Creatine kinase (U/L) at 2 and 3 minutes before and after cryostimulation. p=0.424390378

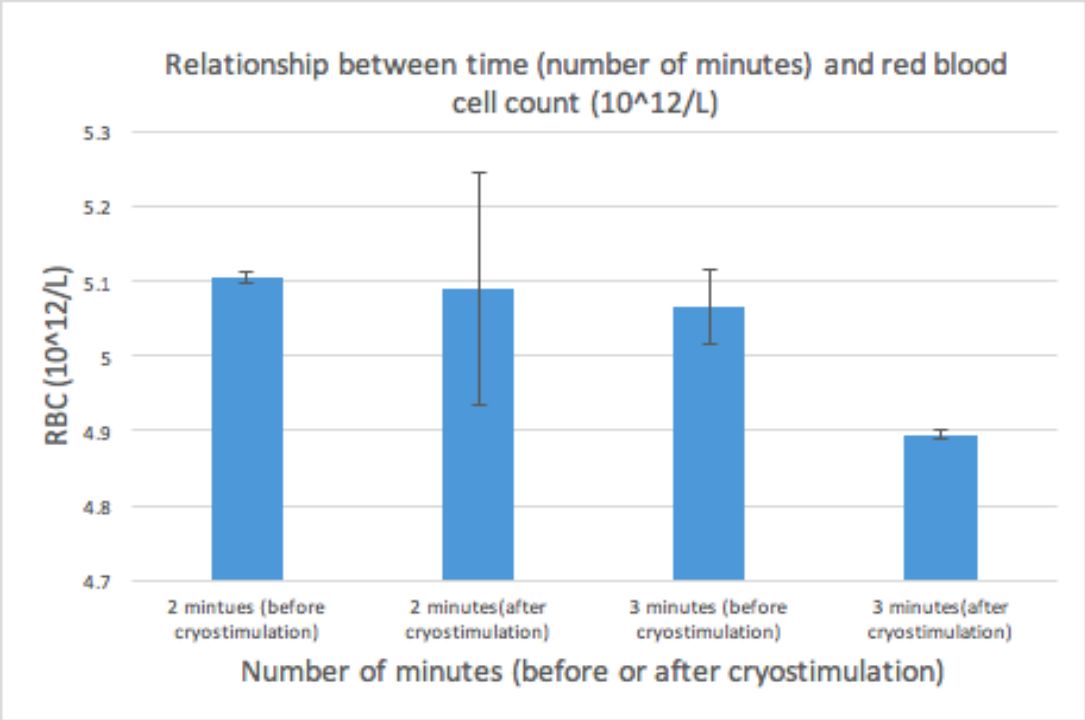

Figure 6: Shows he concentrations of red blood cells (10^12/L) at 2 and 3 minutes before and after cryostimulation p=0.23233428

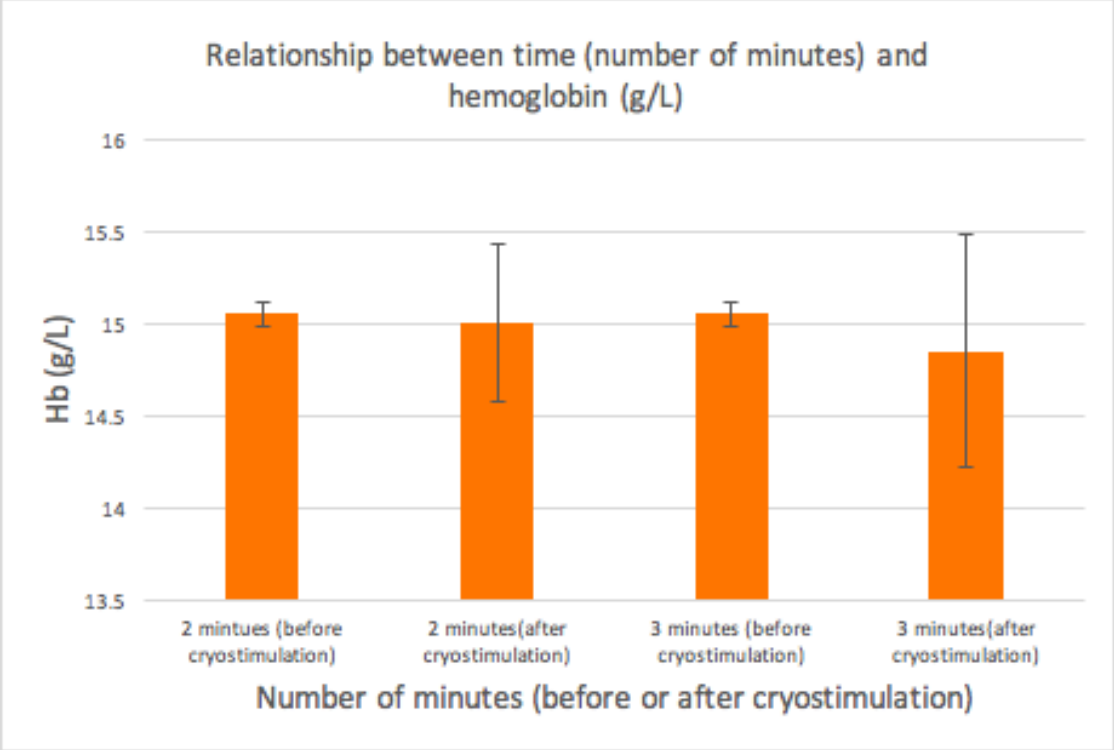

Figure 7: The concentration of hemoglobin (g/L) at 2 and 3 minutes before and after cryostimulation p=0.25

Figure 3 shows increases in IL-10, an anti-inflammatory cytokine, after cryostimulation. This chart also showed decreases in IL-6, a pro-inflammatory cytokines after cryostimulation. These trends are seen in both 2 and 3 minutes but, for 2 minutes the change in cytokine concentration is significantly greater. The increase in IL-10 and the decrease in IL-6 for 2 minutes are about 10% greater than the ones for 3 minutes. Figure 4 showed an increase in IL-1**β**, a pro-inflammatory cytokine, for both 2 and 3 minutes. It also shows a decrease in TNF-**α**, another pro-inflammatory cytokine. For 2 minutes of cryostimulation, there was a slightly more significant decrease in TNF-**α**, than the decrease at 3 minutes it was about 3% greater. There was also a less significant increase in IL-1**β**, the increase in concentration at 2 minutes was about 2% less than at 3 minutes.

Figure 5 showed the decrease in CK in both 2 and 3 minutes. In this muscle enzyme comparison, 3 minutes was more effective in reducing the concentration, which was the second of the data sets to favor 3 minutes compared to 2 minutes. There is only a small increase in the change in concentration of CK about 1.4% which is smaller than the other differences. In Figure 6, for both 2 and 3 minutes there was a decrease in RBC. There was a more significant decrease in RBC at 3 minutes when compared to 2 minutes, it was about 15% greater of a decrease. In figure 7, there is decreases Hb for both 2 and 3 minutes, but 3 minutes had about a 25% more significant decrease of Hb.

Discussion

In Figure 3, two minutes had a more significant effect on IL-6 and IL-10, when compared to 3 minutes. IL-6 can act as both a pro-inflammatory cytokine and an anti inflammatory cytokine (Lubkowska et al., 2011). But in figure 3, its primary role is a proinflammatory cytokine. Skeletal muscle contraction was shown to be a major source of IL-6 production. Thus, the exposure to an extremely low temperature through cryostimulation might have triggered muscle shivering, which could have resulted in an the decrease in IL-6 (Ziemann et al., 2012). But, these are only assumptions because through these

scientist's didn't access the source of IL-6, which requires advanced methods such as muscle biopsies and real-time polymerase chain reaction. The decrease in IL-6 may have also been caused by a change in the number of leukocytes after the cryostimulation process (Ziemann et al., 2012). IL-6 has a number of metabolic pathway so determining and interpreting its source is very challenging. IL-10 is an anti-inflammatory cytokine, and IL-6 can increase plasma IL-10 which can suppress other pro-inflammatory cytokines such as, IL-1**β** and TNF-**α**. Slight hypothermia is caused in the process of cryostimulation due to the extremely low temperatures, but it isn't significant enough to cause any permanent damage. But the hypothermia induced the expression of IL-10; the hypothermia constricts the inflammatory response, which contributes to its beneficial role in organ protection (Banfi et al., 2009). In previous studies, the anti-inflammatory mechanism, IL-10, has been discussed in studies on cell culture, which reported a decrease in a wide spectrum of pro-inflammatory cytokine genes, brought on by IL-10 (Ziemann et al., 2013). The increase observed in the level of the pro-inflammatory IL-1**β** might have been caused by the increased level of IL-10. Visfatin levels also correlated positively with IL-10. Visfatin is pre-B-cell colony-enhancing factor 1 (PBEF1) which is an enzyme that is encoded by the NAMPT gene. Visfatin may induce the expression of IL-10 and other anti-inflammatory cytokines.

In Figure 4, IL-1**β**, a pro-inflammatory cytokine, was increased by the cryostimulation process. Also there was a less significant increase at 3 minutes compared to 2 minutes in IL-1**β**. IL-1**β** decreasing in concentration is not ideal because this means cryostimulation was promoting inflammation. This inconsistency in the data could be because this value is an average from other peer-reviewed papers. Because this data was not directly collected through an experimental study, there is a high variability. Previous studies have shown the opposite of this data, showing a decrease in IL-1**β**, and it should be taken into consideration that even though there is an increase in IL-1**β** it was very slight. If the data would have been conducted through primary research done by this study then maybe these results would have been

more accurate because it would have been more controlled and could have been monitored more closely to ensure its accuracy. Whereas with these averages, there is a less chance of accuracy because there is a much higher variability because this study cannot guarantee these results. Although, several other studies have reported and indicated that plasma IL-1**β** does not change in response to exercise, while others observed that any slight increase was short-lived (Ziemann et al., 2013). Referring back to figure 4 again, TNF-**α**, a pro-inflammatory cytokine, decreased which shows that inflammation decreased. There was a slightly more significant decrease in TNF-**α** for 2 minutes compared to 3 minutes. TNF-**α** decreased after such a short period of cryostimulation. Although the anti-inflammatory effect of exercise has been well documented, the results strongly suggest that cryostimulation might have potentiated this impact of physical activity (Ziemann et al., 2013). The cryostimulation enhanced the inversely correlation between TNF-**α** and cardiorespiratory fitness. In previous published studies, this procedure has been shown to effectively reduce TNF-**α** concentrations in athletes (Ziemann et al., 2013).

In figure 5, CK decreases suggesting that inflammation is positively affected. In this case, 3 minutes was more effective in reducing the concentration when compared to 2 minutes. The significant decrease in serum total CK concentration suggested rapid recovery from muscle damage. Elevated serum CK is a characteristic marker of exertional rhabdomyolysis, it can be used to measure how cryostimulation affects workload, recovery and possible overtraining (Banfi et al., 2009). Acute exposure of the whole body to cold air can stimulate muscle fiber repair by reducing cell membrane breakdown or increased cell permeability caused by oxidant agents produced during physical exercise (Banfi et al., 2006). Exercising in a cold environment means there is a simultaneous increase in noradrenaline and CK levels, with the noradrenaline increase usually higher than that of CK (Banfi et al., 2009). Noradrenaline stimulation cannot completely explain the CK decrease in cryostimulation treatment, but it may have triggered a cascade of events that induced the decrease.

In Fig. 6, there was a slight decrease in RBC, it was a more significant of a decrease at 3 minutes when compared to 2 minutes. This favors cryostimulation in decreasing inflammation. Cryostimulation has shown no detrimental effect on hematological parameters. Generally, hematological changes during intense training (decrease in RBC and Hb) parallel the fall in ferritin concentration, mirroring a depletion of body iron stores (Lombardi et al., 2013). Cryostimulation induces some changes in the hematological parameters by reducing RBC counts and their hemoglobinization. In figure 7, there was a decrease in Hb and 3 minutes had a more significant decrease of Hb when compared to 2 minutes. The increased hemolysis of erythrocytes under the influence of cryostimulation is also suggested by high values of plasma hemoglobin (Szygula et al., 2014). Increases in plasma hemoglobin were also observed in blood taken after cryostimulation treatment, the concentration was much higher than before treatment. Vasoconstriction and muscle contraction (shivering) could be the cause of such a large increase in RBC, by losing the older erythrocytes it could be beneficial to stimulate RBC pool rejuvenation (Szygula et al., 2014). All in all, the decreases in RBC and Hb have a significantly positive effect on inflammation.

Conclusion

Overall, inflammation is more significantly affected by 3 minutes of cryostimulation because more of the hematological parameters and inflammatory cytokines have larger increases or decreases in concentrations, then when compared to 2 minutes. The increase in IL-10 and the decrease in IL-6 favor 2 minutes. For the decrease in TNF-**α**, it favors 2 minutes of cryostimulation. And for the increase in IL-1**β** concentration, it favored 3 minutes. The decrease in CK favors 3 minutes. There was a more significant decrease in RBC and Hb at 3 minutes when compared to 2 minutes. All in all, 4 of the hematological parameters and inflammatory cytokines favor 3 minutes, whereas only 3 of them favored 2 minutes. So as far as the data in this study can confirm, 3 minutes affects inflammation significantly. This study is

confirming the hypothesis and is rejecting the null hypothesis, there is no difference between two or three minutes of cryostimulation with respect to inflammation.

People who are using other methods of recovery that are not as effective as cryostimulation to prevent or heal injuries and to reduce inflammation should be aware of the more effective and faster way to recover or to reduce inflammation. People should also know the optimum number of minutes of cryotherapy they will need. This research on cryostimulation is crucial to demonstrate that athletes and non-athletes can recover faster and have to spend less time and money on physical therapy or other less effective treatment methods. This research paper may lead to a better understanding that 3 minutes of cryostimulation is more effective than other treatments.

Limations

The variability of the data was one limitation, because of the few papers written on each specific area being researched. Also the averages of other scientist's data means less accurate results. This study could only focus on 2 and 3 minutes because of the lack of research on 4 or longer and shorter minutes of cryostimulation. Primary data collection could have been performed, but under the constraints of this study it would not have been possible, even though it could have been more accurate than the current systematic literature review.

Further Work

This study can be used to establish a baseline for the number of minutes that cryostimulation is performed. Further studies should be done focusing on the other inflammatory cytokines like IL-3, IL-2

and IL-8 that this study does not investigate, but those could also affect inflammation. Also in the future more hematological parameters should be looked into, such as hematocrit, mean corpuscular value, mean corpuscular hemoglobin, and platelets. These other hematological factors have also been shown to affect inflammation in other previous studies. More research should be conducted on IL-1**β** because according to this data, it increased, while in most other studies it has been found to decrease. Further experimentation should examine the long-term effects of cryostimulation and how the number of sessions affects cryostimulation. More research should be conducted on cryostimulation being used as a treatment process for inflammatory diseases such as rheumatoid arthritis, neuropathic pain, inflammatory bowel disease, osteoarthritis, vascular disease, multiple sclerosis, and Alzheimer's disease. Further studies should be done for cryostimulation at 3 minutes to confirm that IL-10 increases, and IL-6, TNF-**α**, CK, Hb, and RBC all decrease.

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References

Banfi, G., Lombardi, G., Colombini, A., & Melegati, G. (2010). Whole-Body Cryotherapy in Athletes. *Sports Medicine*, 40(6), 509-517. doi:10.2165/11531940-000000000-00000

Banfi, G., Melegati, G., Barassi, A., & Deril, G. M. (2009). Beneficial effects of the whole-body cryotherapy on sport haemolysis. *Journal of Human Sport and Exercise*, 4(2), 189-193. doi:10.4100/jhse.2009.42.11

Banfi, G., Melegati, G., Barassi, A., Dogliotti, G., D'Eril, G. M., Dugué, B., & Corsi, M. M. (2009). Effects of whole-body cryotherapy on serum mediators of inflammation and serum muscle enzymes in athletes. *Journal of Thermal Biology*, 34(2), 55-59. doi:10.1016/j.jtherbio.2008.10.003

Bleakley, C., Bieuzen, F., Davison, G., & Costello, J. (2014). Whole-body cryotherapy: empirical evidence and theoretical perspectives. *Open Access Journal of Sports Medicine*, 25. doi:10.2147/oajsm.s41655

Cooper, S. M., & Dawber, R. P. R. (2001). The history of cryosurgery. *Journal of the Royal Society of Medicine*, 94(4), 196–201.

Costello, J. T., Culligan, K., Selfe, J., & Donnelly, A. E. (2012). Muscle, Skin and Core Temperature after −110°C Cold Air and 8°C Water Treatment. *PLoS ONE*, 7(11). doi:10.1371/journal.pone.0048190

Iyer, S. S., & Cheng, G. (2012). Role of Interleukin 10 Transcriptional Regulation in Inflammation and Autoimmune Disease. *Critical Reviews™ in Immunology*, 32(1), 23-63. doi:10.1615/critrevimmunol.v32.i1.30

Kitzenberg, D., Colgan, S. P., & Glover, L. E. (2016). Creatine kinase in ischemic and inflammatory disorders. *Clinical and Translational Medicine*, *5*, 31. http://doi.org/10.1186/s40169-016-0114-5

Lombardi, G., Lanteri, P., Porcelli, S., Mauri, C., Colombini, A., Grasso, D., . . . Banfi, G. (2013). Hematological Profile and Martial Status in Rugby Players during Whole Body Cryostimulation. *PLoS ONE*, 8(2). doi:10.1371/journal.pone.0055803

Lubkowska, A., Szyguła, Z., Chlubek, D., & Banfi, G. (2011). The effect of prolonged whole-body cryostimulation treatment with different amounts of sessions on chosen pro- and anti-inflammatory cytokines levels in healthy men. *Scandinavian Journal of Clinical and Laboratory Investigation,* 71(5), 419-425. doi:10.3109/00365513.2011.580859

Ren, K., & Torres, R. (2009). Role of interleukin-1β during pain and inflammation. *Brain Research Reviews*, 60(1), 57-64. doi:10.1016/j.brainresrev.2008.12.020

Scheller, Jürgen & Chalaris, Athena & Schmidt-Arras, Dirk & Rose-John, Stefan. (2011). The pro- and anti-inflammatory properties of the cytokine interleukin-6. *Biochimica et biophysica acta*, 878-88. doi:10.1016/j.bbamcr.2011.01.034.

Szyguła, Z., Lubkowska, A., Giemza, C., Skrzek, A., Bryczkowska, I., & Dołęgowska, B. (2014). Hematological Parameters, and Hematopoietic Growth Factors: Epo and IL-3 in Response to Whole-Body Cryostimulation (WBC) in Military Academy Students. *PLoS ONE*, 9(4). doi:10.1371/journal.pone.0093096

Tanaka, T., Narazaki, M., & Kishimoto, T. (2014). IL-6 in Inflammation, Immunity, and Disease. *Cold Spring Harbor Perspectives in Biology*, 6(10), a016295. http://doi.org/10.1101/cshperspect.a016295

Ziemann, E., Olek, R. A., Grzywacz, T., Antosiewicz, J., Kujach, S., Łuszczyk, M., Laskowski, R. (2013). Whole-body cryostimulation as an effective method of reducing low-grade inflammation in obese men. *The Journal of Physiological Sciences*, 63(5), 333-343. doi:10.1007/s12576-013-0269-4

Ziemann, E., Olek, R. A., Kujach, S., Grzywacz, T., Antosiewicz, J., Garsztka, T., & Laskowski, R. (2012). Five-Day Whole-Body Cryostimulation, Blood Inflammatory Markers, and Performance in High-Ranking Professional Tennis Players. *Journal of Athletic Training*, 47(6), 664-672. doi:10.4085/1062-6050-47.6.13