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Royal St. George's College

The Young Researcher

2019 Volume 3 | Issue 1

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Recommended Citation

Kim, N. (2019). The impact of carnosine on myosin for a possible intervention or prevention of systolic heart failure. *The Young Researcher*, 3 (1), 89-101. Retrieved from <http://www.theyoungresearcher.com/papers/kim.pdf>

ISSN: 2560-9815 (Print) 2560-9823 (Online) Journal homepage: <http://www.theyoungresearcher.com>

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The Impact of Carnosine on Myosin for a Possible Intervention or Prevention of Systolic Heart Failure

Nari Kim

Heart failure is a common but serious condition that affects approximately 5.7 million adults in the United States and costs the economy \$30.7 billion each year. More specifically, systolic heart failure, otherwise known as heart failure with reduced ejection fraction (HFrEF), is the most common form of heart failure that occurs when the muscle of the left ventricle loses its ability to contract, preventing enough oxygen and blood from circulating throughout the body. Although a myriad of treatment options have emerged in recent years for the treatment of heart failure and left ventricular systolic heart dysfunction, there is yet to be an established and definite treatment for this condition. Thus, it is paramount to develop a more effective therapeutic treatment by specifically targeting the carnosine protein. Through analysis of the ATP activity of myosin with and without carnosine, it was discovered that myosin activity increases in the presence of carnosine. This is a significant discovery as carnosine allows for contractions of the heart to increase. As heart contractions increase from an increase in myosin, increasing actin activity through a directly proportional relationship, it allows for an increased amount of blood to be pumped from the heart. Thus, the hypothesis was supported as the carnosine protein can be seen to enhance muscle contractions of the heart and allow for the treatment or possible prevention of systolic heart failure, which can be caused by any condition that impairs the heart muscle's ability to pump blood.

Keywords: carnosine, myosin, myosin ATPase activity, systolic heart failure, contractile function

Introduction

Heart failure is a serious condition that affects approximately 5.7 million adults in the United States and costs the economy \$30.7 billion each year (Division for Heart Disease and Stroke Prevention, 2016). It occurs when the heart is not pumping enough blood to meet all of the body's needs, leading to a loss in vital functions, and can affect both left and right sides of the heart (Nic, 2012). The reasons for this reduced pumping of blood lie in cardiac enlargement, or the enlargement of the heart, and when heart muscles become too stiff to properly contract (McMullen and Jennings, 2007). More specifically, systolic heart failure is the most common form of heart failure and occurs when the muscle of the left ventricle of the heart loses its ability to contract, preventing enough oxy-

gen and blood from circulating throughout the body ("Heart Failure Signs and Symptoms," n.d.). Systolic heart failure is otherwise known as heart failure with reduced ejection fraction (HFrEF) and ejection fraction is an important measurement of how well a heart is pumping, which helps to classify the certain type of heart failure. Those possessing heart failure with reduced ejection fraction have 40% and below of the blood in the left ventricle of the heart pumped out to the rest of the body, which is below the normal ejection fraction of more than 55% (Pai et. al, 2017).

Although a myriad of treatment options have emerged in recent years for the treatment of heart failure, such as the use of Beta blockers or Angiotensin-converting enzyme (ACE) inhibitors, there is yet to be an established and definite treatment for this condition (McConaghy & Smith, 2004). This is due to

the fact that even though current treatment options are effective to some extent in serving as a base-line treatment, it is rather recommended by Becher, Blankenberg, and Westermann from the World Journal of Cardiology that future treatments focus on “augmentation of heart rate in order to increase cardiac output,” or the amount of blood pumped by the left ventricle of the heart in one minute (Becher et. al, 2015). With this, a promising approach to the development of an effective intervention of heart failure lies in a dipeptide that has been reasoned to increase cardiac contractility (Black et. al, 1997). The dipeptide, carnosine, is made up of B-alanine and histidine and is found in high concentrations in the skeletal and heart muscle. B-alanine and histidine are two amino acids that constitute carnosine; b-alanine is naturally produced by the body while histidine is not naturally produced and needs to be obtained through food. The researchers hypothesized that if the amount of carnosine is increased, heart contractions can increase as well but they were unsure of how this could occur and what the connections were between carnosine and heart contractions.

Using this study by Black and other researchers as a basis, it was interesting to see if carnosine could increase heart contractions by affecting one of the proteins responsible for heart contractions: myosin. Myosin is an adenosine triphosphatases (ATPase) and slides past another protein actin to form the muscle contractions (Valberg, 2008) using chemical energy it converts into these movements (Rayment, 1996).

Review of Relevant Literature

Carnosine has been seen to improve exercise performance, including beneficial effects towards “cycling capacity, ventilatory threshold, and [longer] time [until] exhaustion” (Culbertson, 2010). This is because B-alanine supplementation has recently been shown to increase intramuscular carnosine levels, which leads to improved athletic performance by reducing the lactic acid accumulation in the muscles during exercise (Cronkleton, 2018). Additionally, carnosine possesses antioxidant properties, meaning it neutralizes dangerous and toxic substances called free radicals, and acts as a fundamental buffer in maintaining a normal acidity balance in muscles. Ensuring acid levels re-

main low is crucial as muscles reduce work capacity by reducing its ability to contract during periods of high-intensity exercise when acid levels get too high. By improving exercise performance, carnosine allows for the maintenance of a healthy oxygen supply that can help reduce the impacts of aging as it prevents the hardening of connective tissue and the shrinking of muscle fibers (Muscle Metabolism, n.d.).

Gaps in Current Literature

Carnosine was first discovered in 1900 and became the central focus of cardiovascular research in the late 20th century as researchers noticed its benefits to areas such as exercise and became interested in researching its effects in relation to the heart. Despite the fact that carnosine was discovered 119 years ago and has been found to be beneficial towards certain areas, the actual physiological function of carnosine still remains unknown (Hipkiss, 2013). Currently, no papers have been published on carnosine and its effect on myosin in respect to heart failure, although several studies, including studies from the *Chemistry Central Journal* and *Journal of Applied Physiology*, address that numerous questions on the properties of carnosine remain unanswered and its role in “contractile function of various types of muscle fibers in vivo is poorly understood” (Kaczmarek, et.al, 2016; Artioli, 2018; Black, 1997; Hipkiss, 2013; Taylor, 2009). Roberts and Zaloga, authors of several studies published in the *American Journal of Physiology* on carnosine, have even mentioned in their study that “the specific functions [of carnosine]” and “an exact physiologic function for carnosine in muscle [are] unknown”(Roberts & Zaloga, 2000).

In addition to the fact that there has been little to no research conducted on the relations between carnosine and heart failure with myosin in particular, there have been only a few attempts in trying to develop pharmaceutical drugs using carnosine as it poses difficulties with a lack of a standard method for doing so (Boldyrev, 2012). It is vital to study carnosine now although it was discovered back in 1900 because in recent years, there has been an increase in studies that have showed there were benefits to taking carnosine supplements, such as “[improved] oxygen delivery to tissues for use during exercise” (Greene, 2017). Al-

though this is the case and the study by Black and other researchers should have opened more discussion into the development of heart failure treatments with carnosine, “to date, clinical studies evaluating the utility of carnosine in cardiac disorders appear to be lacking” (McCarty & DiNicolantonio, 2014). Therefore, it is the purpose of this study to look at the effect of carnosine on myosin. This will aim to address the lack of information on carnosine and take a step towards forming a new understanding behind the molecular mechanisms of carnosine on the improvement of cardiac function during heart failure. This leads to the research question: How can carnosine have an impact on myosin for a possible intervention or prevention of systolic heart failure?

In order to form this new understanding, it is essential to discover the molecular mechanisms by which this is to occur using two assumptions: the first being that carnosine can improve heart contractions and the second being that myosin can still be an indicator of muscle contraction activity by increasing actin to form the muscle contractions even when actin is not analyzed in this study. With this new understanding, it can facilitate the development of therapeutic treatments by specifically targeting the carnosine protein. This is because if it is seen that carnosine has a directly proportional relationship with myosin, then it can serve to further corroborate recent findings that carnosine improves cardiac function during heart failure by increasing heart contractions and leading to increased blood flow. The development of therapeutic treatments targeting carnosine will be seen to contribute immensely to a condition that has no current cure and will show to help the scientific community take one step closer to finding a cure for the condition.

Thus, as myosin is one of the proteins responsible for heart contractions and carnosine has been shown to increase the pumping of the heart, it is hypothesized that if carnosine and myosin are analyzed, then there will be a directly proportional relationship between the two with carnosine increasing myosin when there are increasing amounts of carnosine present in the body.

Methods

As there is still a lack of information about carnosine, there was not a fair amount of studies employing experiments that could form the basis of this study. However, there was one key study by Tyska and other researchers from Harvard Medical School who looked to see if a certain mutation of myosin related to a type of heart failure increased or decreased heart contractions (Tyska et. al, 2000). To do this, they first used an extraction and purification experiment to extract the mutated myosin from mice with this heart failure. This experiment was used in this study as it was similar to the purpose of this study to first remove normal myosin from normal mice hearts.

Experiment 1: Myosin Extraction and Purification

The first step for this research was the protein purification of the myosin protein from the hearts of mice around a year old. In order to purify the myosin protein, it was first extracted from the mouse heart. The mouse had 0.12 mL of pentobarbital injected into them to induce sleep, in accordance with the anesthesia guidelines established by the Institutional Animal Care and Use Committee (Office of Animal Resources- Institutional Animal Care and Use Committee, 2017). Mice hearts are ideal for this study as mice hearts and human hearts are anatomically similar with respect to the partitioning of the cardiac chambers. In both mice and human hearts, the partitioning of the cardiac chambers both follow the same sequence of events and both the maturation of the cardiac valves and myocardium are similar (Wessels & Sedmera, 2003). In essence, the structure of mice and human hearts are almost identical.

For the extraction of the myosin protein from the mice heart, which was conducted by an undergraduate student, the stomach of the mouse was sprayed with 70% ethanol and wiped with an alcohol prep wipe after the mouse had fallen asleep. Subsequently, the tail was tested to see if there was any movement to ensure the mouse had fallen into a deep sleep. The stomach was then opened and the still-beating heart was removed. After the heart was removed, it was wiped on a towel to remove excess blood. This was

in order to receive a more accurate measurement of its weight before homogenizing the heart in high salt buffer in a 1:5 ratio of wet tissue to volume. In order to compare the ratios in vitro to those existing in living muscle, weight per volume was more practical to use than weight per weight ratios. The heart was weighed and placed on ice after one single edge blade was used to cut the heart until it weighed approximately 30 mg. For future trials, the rest of the heart was cut into pieces weighing 30 mg, placed in 1.7 mL tubes, and stored in the freezer at -80 degrees Celsius. The materials for this procedure were thrown away in the biohazard box.

One 30 mg piece of the heart was used for the subsequent experiments. However, before this, the high salt buffer consisting of 0.3 mol/L KCl, 0.15 mol/L K₂HPO₄, 0.01 mol/L Na₄P₂O₇, 0.001 mol/L MgCl₂, and 0.002 mol/L DTT was made with a final pH at 6.8. After the high salt buffer was made, it was used to homogenize the 30 mg heart in a 1:5 wt:vol for 20 mins. 150 mL of high salt buffer was drawn up with a pipette and into a homogenizer tube with the heart which was pushed to the bottom of the tube. To homogenize the heart, a homogenizer rod was continuously twisted for 20 minutes to mix the buffer and heart together.

Following the homogenization, the homogenized heart was placed in a polycarbonate centrifuge tube and taken to the ultracentrifuge to be centrifuged at 65,000 RPM and 4 degrees Celsius for an hour to separate out proteins and particles from one another from the muscle tissue. During this time, the purification buffer was prepared. The purification buffer consisted of 2 mmol/L DTT and was used to precipitate the myosin, or cause the myosin protein particles to consolidate together in solid form. When the proteins consolidate together, this includes myosin as it is found in the heart muscle tissue which makes up the heart. Subsequently, further ultracentrifugation was performed with the pellet at 38,000 RPM and 4 degrees Celsius for 20 minutes.

While the pellet was being centrifuged, the myosin buffer was prepared. The myosin buffer consisted of 0.025 mol/L imidazole, 0.004 mol/L MgCl₂, 0.01 mol/L DTT, 0.001 mol/L EGTA, and 0.3 mol/L KCl at an overall pH of 7.4. Once the ultracentrifugation was completed after 20 minutes, the pellet was suspended in the myosin buffer to stabilize the myosin proteins.

Experiment 2: Bradford Protein Assay

Subsequently, a Bradford protein assay was conducted using BioRad DCTM Protein Assay Reagents A, B, and S to measure the myosin concentration. This is because the Bradford protein assay allows for the exact protein concentration of a sample to be determined with a series of dilutions of known protein concentrations, such as BSA. BSA (bovine serum albumin) is often used as a standard for protein assays and other quantitative assays for “its stability to increase signal in assays, its lack of effect in many biochemical reactions, and its low cost” (Ascoli, C. et. al, n.d.) The known protein concentrations are then compared alongside unknowns, such as the purified myosin from the heart as the amount of myosin present in the heart was not known. From 1 µg/ µL BSA standard, 0, 5, 10, 15, 20, and 25 µL were drawn up and added to six different tubes. 25, 20, 15, 10, 5, and 0 µL of distilled water were added to each tube respectively for a total volume of 25 µL per tube. Using the purified myosin from the protein purification experiment, 5 µL and 15 µL were mixed with 20 µL of distilled water and 10 µL of distilled water respectively to find the current total protein concentration of the myosin. Following this, 1000 µL of Reagent A and 20 µL of Reagent S were mixed together. 125 µL of the mixed reagent was added to six of the tubes with the standard and two of the tubes with myosin samples. After adding 1000 µL of Reagent B to the eight tubes, the tubes were mixed, poured into cuvettes, and taken to a UV-Visible Spectrophotometer to be read.

Experiment 3: SDS-PAGE

SDS-Page was then conducted to separate and isolate the myosin protein based on its molecular weight. This was the third step that was used to ensure the protein was fully purified as the SDS- Page is commonly used to “determine the success of a protein purification scheme,” and allows for the total protein, total enzyme activity, specific activity, yield, as well as purification level to be determined (Berg et. al, 2002). It is essential to have a good protein purification scheme to ensure there is a high degree of purification and a high yield since a low yield will “leave protein with which to experiment” at a low degree of purification. This allows for many contaminants to be left

which will “complicate the interpretation of experiments” (Berg et. al, 2002).

Using the readings from the UV-Visible Spectrophotometer, the myosin concentration from the Bradford protein assay was determined and used to prepare 4X loading buffer with DTT. The loading buffer was prepared by adding 4 μg of DTT to 100 μL of a 4X Bio Rad Laemmli Sample Buffer. 50 μL of the myosin sample used for protein estimation was added to 12.5 μL of the loading buffer and the mixture was heated for 5 minutes at 95 degrees Celcius. While the mixture was heated, 1X running buffer was prepared. The running buffer consisted of 6g Tris, 28.8g glycine, 2g of SDS (sodium dodecyl sulfate), and distilled water. Once the running buffer was stirred with a stir rod and the mixture was finished heating, the buffer and mixture were placed on ice for 15 minutes. Following this, Criterion™ TGX™ Precast Gel was inserted into an electrophoresis tank, which was connected to a Power Pac 300 machine, before the running buffer was poured over the gel. 20 μL of Precision Plus Standard Protein was loaded in the ladder beside the lanes of the gel to serve as the control, 25 μL of the mixture of myosin and the loading buffer was loaded into the first lane, and 35 μL of the mixture was loaded into the second lane. The gel was then left running for an hour and a half at a voltage of 120 watts. Afterwards, the gel was taken out and left to incubate in a container on a rocking platform with Coomassie Blue to be stained overnight. Coomassie Blue is a commonly used dye in SDS-PAGE as it detects as little as 0.1 μg of protein (Protein Science, 2012). Subsequently, the Coomassie Blue was poured out and the destaining buffer was poured on the gel to destain the gel overnight. The next day, the gel was read by a Thermo Scientific MyECL imager.

Experiment 4: ATPase Assays

Once the protein purification, Bradford protein assay, and SDS-Page were completed, two ATPase assays were conducted: one without carnosine and one with carnosine. This was another method used by Tyska & the other Harvard researchers. This assay is significant as it tests the amount of Pi released from the myosin. This is important because during a power stroke, or when myosin and actin slide past one another to create the muscle contractions, both adenosine di-

phosphate (ADP) and inorganic phosphate (Pi) are released from the myosin (Freundenrich, 2001). The third assumption is that the amount of Pi is an indicator of myosin activity, which corresponds to muscle contractions, as it is released from the interactions of myosin and actin which are proteins responsible for heart contractions.

The assay was conducted with an incubation time period of 5 minutes and in fourteen wells of a 96-well plate. Six of the wells include the phosphate standards needed for the assay and eight of the wells include duplicates of myosin with carnosine, without carnosine, and each of their respective background blanks. Background blanks are needed for the assay in order to correct for high levels of phosphate which have the potential to result in a sample background. Sample backgrounds are not ideal as this is where phosphates are freely floating around in the sample and will hinder the result as this assay is testing for the ATP reaction by testing only the phosphates released by the myosin, not the free phosphate.

To start, six phosphate standards were prepared. After diluting a 1 mM Phosphate Standard with distilled water, a 50 μM Phosphate Standard Solution was formed. Six increasing concentrations of this were plated in six wells of a 96-well plate. Once these were diluted again by distilled water, six standards with concentrations of 0, 12.5, 25, 31.25, 37.5, and 50 μM were formed.

A reaction mix was first made with 20 μL of assay buffer added to 10 μL of 4 mM ATP. 4 mM was chosen as the concentration for ATP as researchers Avena and Bowen found “the influence of carnosine on the splitting of ATP by myosin...most effective” at 4 mM out of 0.5 mM, 2.0 mM, and 4.0 mM ATP (Avena & Bowen, 1969). The assay buffer consisted of 40 mM Tris, 80 mM NaCl, 8 mM MgAc₂, and 1 mM EDTA at a pH of 7.5. In two of the wells containing duplicates of myosin without carnosine, 5 μL of purified myosin, 5 μL of the assay buffer, and 30 μL of the reaction mix were added. However, in two of the wells containing duplicates of myosin with carnosine, 5 μL of purified myosin, 5 μL of 1 mM carnosine, and 30 μL of the reaction mix were added. A concentration of 1 mM carnosine was chosen as it falls near the range of 2 mM to 10 mM carnosine tested by Yun and Parker, who found “near maximum [ATPase] activation achieved at 0.002 M” (Yun & Parker, 1965). As Yun and Parker

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(Yun & Parker, 1965) determined maximum carnosine activation at a concentration of 0.002 M that was lower than the 0.10 M carnosine concentration found by Avena and Bowen (Avena & Bowen, 1969), it was reasoned that a lower concentration of 0.001 M should be used.

Once the reaction mix was added to the four wells, the timer began for 5 minutes. During the 5 minutes, the last four wells were prepared as an incubation period of 5 minutes at room temperature was not needed for the background blanks. For two of the wells that served as background blanks for the duplicates of myosin without carnosine, 5 μ L of myosin buffer, 5 μ L of the assay buffer, and 30 μ L of the reaction mix were mixed together. For the last two wells that served as background blanks for myosin with carnosine, 5 μ L of myosin buffer, 5 μ L of distilled water, and 30 μ L of the reaction mix were mixed together. Once 5 minutes had passed, 200 μ L of a malachite green reagent was added to all 8 wells, as well as to the phosphate standards, and the wells were left to incubate for an additional 30 minutes at room temperature to terminate the enzyme reaction. The incubation period of 30 minutes was also needed for the generation of a calorimetric product that results when the malachite green reagent forms a dark green color with the free phosphate released from the enzymes. After 30 minutes, the plate was taken to be read by the Gen5 spectrophotometric multiwell plate reader at an absorbance

of 620 nm. Using the generated data from the plate reader, Microsoft Excel was used to plot a standard curve with values from the 6 phosphate standards and to calculate the concentration of phosphate generated in the sample wells, which was used to determine the enzyme activity in units/L. This was repeated two more times for a total of three trials. A paired t-test was then performed to determine if the differences in the myosin activity values in the absence and presence of carnosine were simply due to random chance.

Results

Experiment 1: Extraction and Purification of the Myosin Protein

The myosin protein was successfully extracted and purified from the hearts of mice around a year old. The purified myosin from this experiment was used for the second experiment: the Bradford Protein Assay.

Experiment 2: Bradford Protein Assay Using Purified Myosin from First Experiment

The data shown in Table 1 depict the five BSA standards used for protein estimation in the Bradford Protein Assay, excluding the sixth one which

Table 1. Concentration (mg/mL) and Readings for BSA Standards in Bradford Protein Assay

Standard	Concentration (mg/mL)	Readings
Std 1	0.2	0.0611
Std 2	0.4	0.123
Std 3	0.6	0.1672
Std 4	0.8	0.2695
Std 5	1	0.36
	Calibration Equation	Abs = 0.37221 * Conc -0.02716
	Correlation Coefficient	0.97789

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served as a control. The concentration and readings obtained from the assay at an absorbance level of 750 nm were used to construct a standard curve, as seen in Figure 1, to which the unknown myosin concentrations could then be determined from through interpolation within the BSA standard calibration curve. These concentrations can be seen in Table 2. The correlation coefficient signifies a strong positive linear correlation between the concentration and readings,

meaning that the readings increase as the concentrations increase. This can be seen in Figure 1, where the R-squared value, or the coefficient of determination, shows that the graphed line is the best fit for the data and is close to the line of best fit represented as a dotted line, meaning the fitted values would almost always equal the observed values as most of the variance is accounted for.

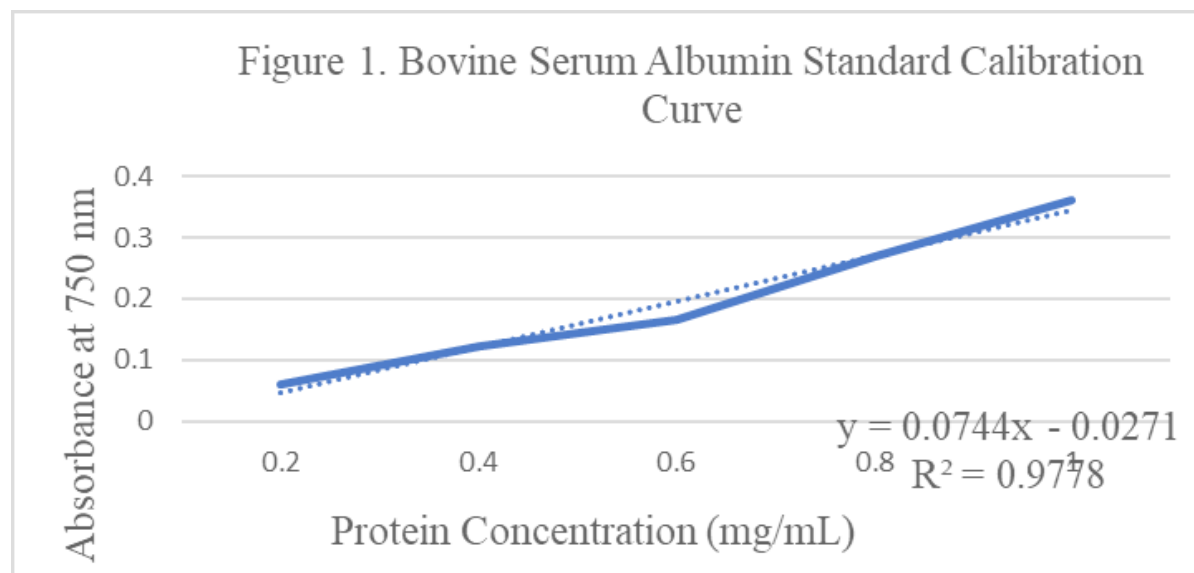


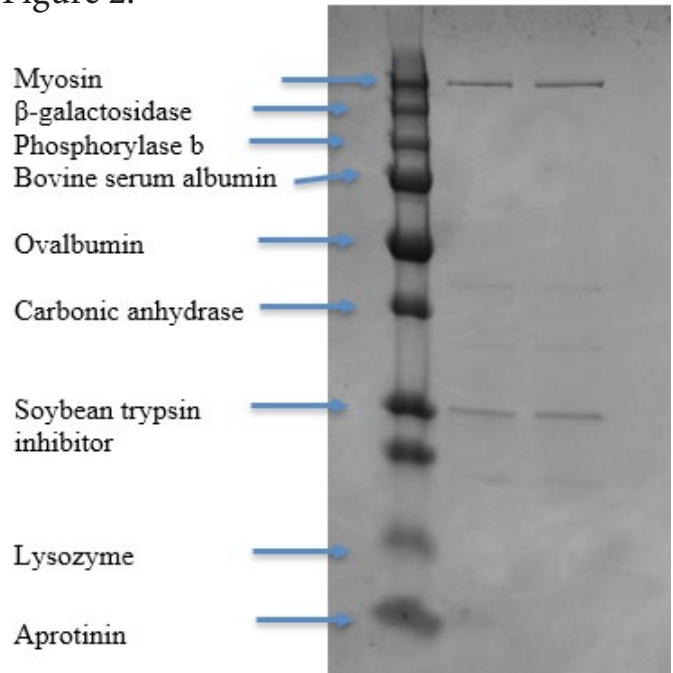
Table 2. Concentrations (mg/mL) of 5 μ L Myosin and 15 μ L Myosin

Sample	Concentration (mg/mL)	Readings
Sample 1 (5 μ L)	0.1361	0.0235
Sample 2 (15 μ L)	0.2846	0.0788

Experiment 3: SDS-PAGE

The image of the gel in Figure 2 displays the results from the SDS-PAGE. From this, a dark band can be seen at myosin, corroborating the fact that myosin was purified from the mouse heart. This also correlated with a common pattern found in SDS-PAGE where dark spectrin bands representing proteins with a molecular weight of more than 200 kDa are found at the top of the image of the gel. In this, dark bands can be seen at the top of the image next to myosin, which possesses a molecular weight of 210 kDa. This image demonstrated that only the myosin protein was extracted from the mouse heart and properly purified, which allows confirmation for the myosin concentration determined in the second experiment to be used for the fourth experiment.

Figure 2.



Experiment 4: ATPase Activity Assays With and Without Carnosine

Figures 3, 4, and 5 depict the standard phosphate curves for each of the three trials of the ATPase activity assay, which were used to calculate the myosin ATPase activity values seen in Table 3. Each of the standard phosphate curves were produced from the concentration and readings of the six phosphate standards from the ATPase activity assay. Interpolation within each of the standard phosphate curves produced the concentration of the generated phosphate in the assay that served as the phosphate control. This was then compared with the averages of the duplicates and background blanks for myosin with and without carnosine that measured the released phosphate. These values produced the myosin ATPase activity values seen in Table 3, which depicts the data for three trials conducted for myosin with and without carnosine. This table shows an increase in myosin activity when carnosine is present. When carnosine was pres-

ent, approximately double the amount of ATP were released from myosin per minute compared to when carnosine was not present. This means myosin activity was enhanced, indicating heart contractions increased in the presence of carnosine. When a paired t-test was conducted, it revealed the myosin ATPase activity values in the absence and presence of carnosine was statistically significant with a p-value of 0.033. This depicted a significance in that the differences between the values of myosin ATPase activity in the presence and absence of carnosine were not simply due to random chance which allows for the conclusion that the findings can support the hypothesis. This was that myosin values were increased in the presence of carnosine and there is a directly proportional relationship between the two.

Figure 3. Standard Phosphate Curve for First Trial

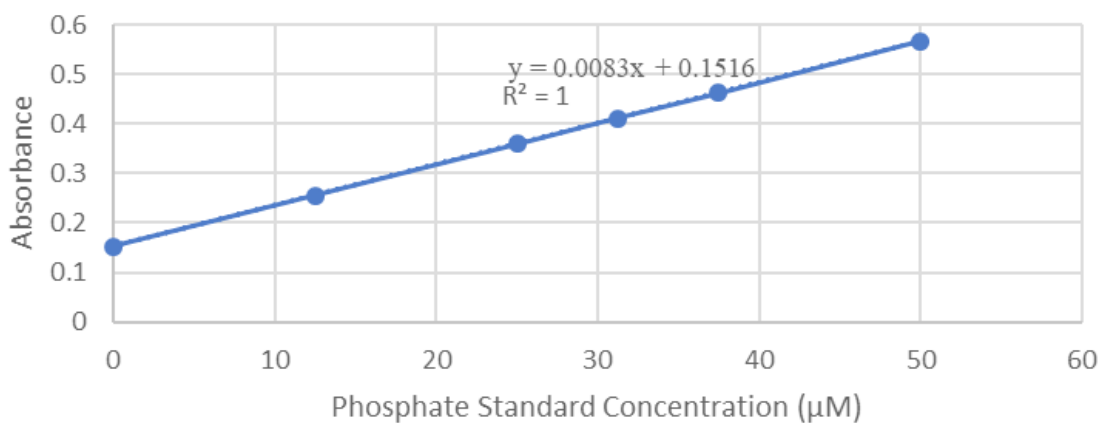


Figure 4. Standard Phosphate Curve for Second Trial

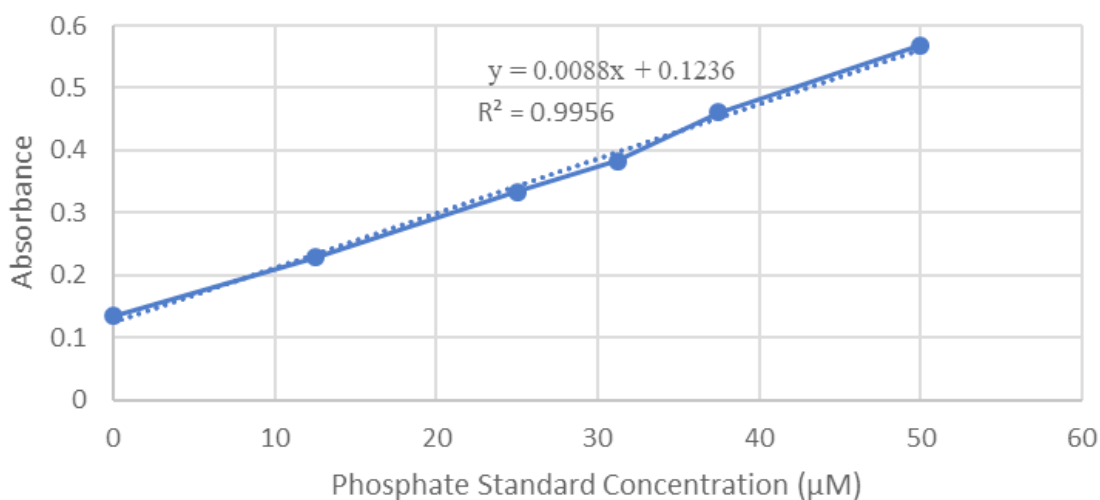


Figure 5. Standard Phosphate Curve for Third Trial

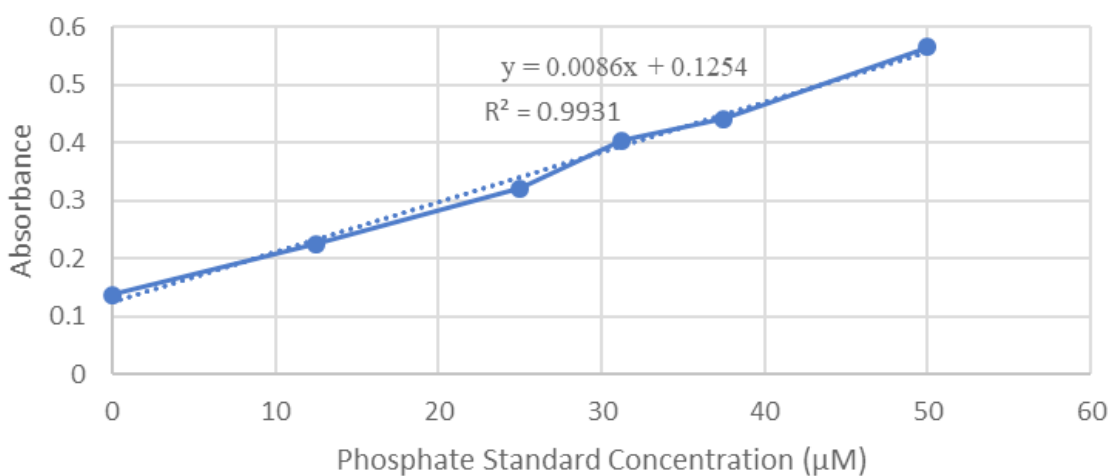


Table 3. Data for Three Trials on The Amount of ATP that Catalyzes the Production of 1 μM of Free Phosphate Per Minute

	- Carnosine	+ Carnosine
Myosin ATPase Activity ($\mu\text{M}/\text{min}$)	16.75181	27.93253
Myosin ATPase Activity ($\mu\text{M}/\text{min}$)	18.345456	33.254544
Myosin ATPase Activity ($\mu\text{M}/\text{min}$)	15.64651163	21.87906977
	T-Test Value:	0.033236954

Discussion/Conclusion

The results of this study suggest that myosin activity is enhanced in the presence of carnosine. Experiment 2 ensured the results for the myosin concentration in the protein estimation are precise with a correlation coefficient of 0.97789, meaning the BSA sample standards in the experiment were close to the standard concentrations. With Experiment 3, it further corroborated that myosin was properly purified from the mouse heart, which allowed for the myosin concentrations in Experiment 2 to be used for Experiment 4. Experiment 4 suggests that the myosin activity was increased in the presence of carnosine after a reaction time of 5 minutes as, on average, 27.689 μM of ATP was released from myosin per minute. Myosin ATPase activity was measured as myosin releases ADP and Pi to rebind to the actin filament and return to its initial position, causing the muscle contractions (Cooper, 2000). In other words, reaction of myosin ATPase is the immediate source of free energy that drives the muscle contractions. With an increase in myosin activity, it portrays an increase in the amount of free energy that can lead to increased muscle contractions. Current data indicates ATPase activity was enhanced in the presence of carnosine. This is in

agreement with several studies that have indicated that carnosine activates myofibrillar ATPase activity, which corresponds to the speed of muscle contraction (Briggs et. al, 1959; Yun & Parker, 1965; Avena & Bowen, 1968).

It was hypothesized that if carnosine and myosin are analyzed, then there will be a directly proportional relationship between the two with carnosine increasing myosin when there are increasing amounts of carnosine present in the body. This was supported by the results as myosin activity increased when carnosine was present compared to when carnosine was not present. As myosin activity corresponds to the activity of heart contractions and there was an increase in myosin activity, it can be understood that heart contractions are increased. With a directly proportional relationship between carnosine and myosin, as well as between myosin activity and heart contractions, there is a relationship between carnosine and heart contractions.

Significance

Currently, this is one of the only studies that assesses the impact of carnosine on myosin using mouse hearts as a way to determine if carnosine and myosin have a directly proportional relationship to increase blood flow in the heart. The results from the ATPase activity assay illustrates that in the presence of carnosine, myosin activity increases. For the first trial, myosin activity increased by approximately 11 $\mu\text{M}/\text{min}$. For the second trial, the myosin activity increased by approximately 15 $\mu\text{M}/\text{min}$, and the last trial illustrated that the myosin activity increased by approximately 6 $\mu\text{M}/\text{min}$. The findings are notable as it was found that in the presence of carnosine, myosin activity was increased, which would indicate increased muscle contractions and hypothetically lead to increased blood flow. With this, it can be assumed that myosin activity can increase with increasing amounts of carnosine. Thus, the carnosine protein enhances muscle contractions of the heart and allows for the treatment or possible prevention of systolic heart failure, which can be caused by any condition that impairs the heart muscle's ability to pump blood (Systolic Heart Failure, 2018). As previously established in the preceding section, there is a relationship between carnosine and heart contractions. The information from this study is significant as it provides a new understanding and lays the foundation for future treatments that have the potential to cure or prevent forms of heart failure by targeting the carnosine protein. Not only does this lay the foundation for future treatments for heart failure, it forms a new understanding in addressing the lack of information on the functions of carnosine since this study now shows that one of the functions of carnosine can be to increase heart contractions by affecting the myosin protein. This also corroborates the hypothesis of Black and others from the Bowman Gray School of Medicine who believed that carnosine increases cardiac contractility but had no definite proof. Furthermore, it provides support for the recommendation of Becher, Blankenburg, and Westermann for future treatments to augment cardiac output.

Limitations

Limitations include using three mice hearts for the three trials of the experiments conducted. This is a limitation as three mice hearts are not representative of all mice hearts; thus, the findings from this study could not yet be applied to human hearts. Another limitation is that this was an *in vitro* study because it would be more significant to see these results apply *in vivo* in mice themselves rather than in centrifuge tubes and well plates. Discrepancies in the data could have arisen due to issues with pipetting. This can be seen in Figure 1 with the line for readings. The line in Figure 1 seems to generally increase steadily with an exception to Standard 3 due to a slight dip in the readings. While the readings for Standards 1, 2, 4, and 5 seem to increase by approximately double the previous reading value, the reading value for Standard 3 only increased by a mere 0.03 from Standard 2.

Moreover, in Figure 2, it can be seen that a dull band is lightly highlighted to be soybean trypsin inhibitor. This can be attributed to an insufficient amount of β -Mercaptoethanol in the Laemmli Sample Buffer as a sufficient amount of β -Mercaptoethanol must be present to be able to reduce the disulfide proteins to allow for the protein to migrate properly to determine its molecular weight (2-Mercaptoethanol, n.d.). Disulfide proteins possess disulfide bonds that allow for the proteins to be stable with support for its protein structure. The lack of sufficient amount of the β -Mercaptoethanol most likely allowed for the presence of the line delineating the soybean trypsin inhibitor. If there was a sufficient amount, it would have reduced the disulfide bond, preventing the formation of a stable structure of the protein and a line delineating its presence in Figure 2.

Future Directions

Future directions will prioritize generalizing findings to be more representative of mice hearts, and subsequently, human hearts. These include experiments with more mice and looking at different proteins besides carnosine, such as anserine. This is because anserine and carnosine have been reported to prevent diseases related to oxidative stress (Arihara & Ohata, 2011) and oxidative stress has been seen to

be increased in heart failure (Sawyer, 2012). In the future, an in vivo study should also be conducted by testing carnosine in cardiac tissue in mice to see if it improves contractile function. If in vivo in mice is found to be significant, then carnosine could be tested in heart failure patients to see if it increases their heart rate or improves their blood flow.

References

- 2-Mercaptoethanol. (n.d.). Retrieved from <https://pubchem.ncbi.nlm.nih.gov/compound/2-mercaptoethanol#section=U-S-Production>
- Avena, R. M., & Bowen, W. J. (1969). Effects of Carnosine and Anserine on Muscle Adenosine Triphosphatases. *The Journal of Biological Chemistry*, 244(6), 1600-1604.1
- Arihara, K., & Ohata, M. (2011). Functional Meat Products. *Woodhead Publishing Series in Food Science, Technology and Nutrition*, 2, 512-533. doi:10.1533/9780857092557.3.512
- Artioli, G. G., Sale, C., & Jones, R. L. (2018). Carnosine in Health and Disease. *European Journal of Sport Science*, 1-10. doi:10.1080/17461391.2018.1444096
- Ascoli, C., Chimento, D., Zhang, Y., Heidemann, K. A., & Moncada, C. (n.d.). Bovine Serum Albumin. Retrieved from <https://rockland-inc.com/bovine-serum-albumin.aspx>
- Albumin. Retrieved from <https://rockland-inc.com/bovine-serum-albumin.aspx>
- Becher, P. M., Fluschnik, N., Blankenberg, S., & Westermann, D. (2015). Challenging aspects of treatment strategies in heart failure with preserved ejection fraction: “Why did recent clinical trials fail?” *World Journal of Cardiology*, 7(9), 544–554. <http://doi.org/10.4330/wjc.v7.i9.544>
- Berg JM, Tymoczko JL, Stryer L. (2002). Section 4.1: The Purification of Proteins Is an Essential First Step in Understanding Their Function. *Biochemistry* (5th ed.). New York: W H Freeman. Retrieved from <https://www.ncbi.nlm.nih.gov/books/NBK22410/>
- Black, K.W., Lin, M., Zapata-Sudo, G., Sudo, R.T., Zaloga, G.P., Nelson, T.E., & Roberts, P.R. (1997). Carnosine is a Novel Peptide Modulator of Intracellular Calcium and Contractility in Cardiac Cells, *American Journal of Physiology*. doi:10.1152/ajpheart.1997.272.1.H462
- Boldyrev, A. (2012). Carnosine: New concept for the function of an old molecule. *Biochemistry* (00062979), 77(4), 313–326. <https://doi.org/10.1134/S0006297912040013>
- Bovine Serum Albumin. (n.d.). Retrieved from <https://rockland-inc.com/bovine-serum-albumin.aspx>
- Briggs, F. N., Gergely, J., & Kaldor, G. (1959). Participation of a dialyzable cofactor in the relaxing factor system of muscle III. Substitution of pyrophosphate for the cofactor. *Biochimica Et Biophysica Acta*, 34, 224-227. doi:10.1016/0006-3002(59)90251-3
- Cooper, G. M. (2000). Actin, Myosin, and Cell Movement. *The Cell: A Molecular Approach*, 2. Retrieved from <https://www.ncbi.nlm.nih.gov/books/NBK9961/>.

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- Cronkleton, E. (2018, November 21). 6 Ways to Get Rid of Lactic Acid in the Muscles. Retrieved from <https://www.healthline.com/health/how-to-get-rid-of-lactic-acid>
- Culbertson, J. Y., Kreider, R. B., Greenwood, M., & Cooke, M. (2010). Effects of Beta-Alanine on \ Muscle Carnosine and Exercise Performance: A Review of the Current Literature. *Nutrients*, 2(1), 75-98. doi:10.3390/nu2010075
- Division for Heart Disease and Stroke Prevention. (2016, June 16). Retrieved September 4, 2018, from https://www.cdc.gov/dhdsp/data_statistics/fact_sheets/fs_heart_failure.htm
- Freundenrich, C. (2001, April 11). How Muscles Work. Retrieved from <https://health.howstuffworks.com/human-body/systems/musculoskeletal/muscle2.htm>
- Greene, E. (2017, June 27). Carnosine for Heart and Brain Health. Retrieved from <https://www.journalofnaturallon-gevity.com/carnosine-heart-brain-health/>
- Heart Failure Signs and Symptoms. (n.d.). Retrieved from https://www.ucsfhealth.org/conditions/heart_failure/signs_and_symptoms.html
- Hipkiss, A. R., Cartwright, S. P., Bromley, C., Gross, S. R., & Bill, R. M. (2013). Carnosine: can understanding its actions on energy metabolism and protein homeostasis inform its therapeutic potential? *Chemistry Central Journal*, 7(1), 1-9. <https://doi.org/10.1186/1752-153X-7-38>
- Kaczmarek, D., Łochyński, D., Everaert, I., Pawlak, M., Derave, W., & Celichowski, J. (2016). Role of histidyl dipeptides in contractile function of fast and slow motor units in rat skeletal muscle. *Journal of Applied Physiology*, 121(1), 164-172. doi:10.1152/jappphysiol.00848.2015
- McConaghy, J. R., & Smith, S. J. (2004, December 01). Outpatient Treatment of Systolic Heart Failure. Retrieved from <https://www.aafp.org/afp/2004/1201/p2157.html>
- McMullen, J. R., & Jennings, G. L. (2007). Differences Between Pathological and Physiological Cardiac Hypertrophy: Novel Therapeutic Strategies To Treat Heart Failure. *Clinical and Experimental Pharmacology and Physiology*, 34(4), 255-262. doi:10.1111/j.1440-1681.2007.04585.x
- Muscle Metabolism. (n.d.). Retrieved from <https://courses.lumenlearning.com/boundless-ap/chapter/muscle-metabolism/>
- Nic. (2012, November 12). Systolic Heart Failure: Causes, Symptoms & Treatment. Retrieved from <http://www.medicalhealthtests.com/articles/632/general-articles/systolic-heart-failure.html>
- Office of Animal Resources- Institutional Animal Care and Use Committee. (2017, January 11). Retrieved from <https://animal.research.uiowa.edu/iacuc-guidelines-anesthesia>
- Pai, R. K., Thompson, E. G., Gabica, M. J., Husney, A., & Fort, S. (2017, December 6). Heart Failure With Reduced Ejection Fraction (Systolic Heart Failure). Retrieved from <https://www.uofmhealth.org/health-library/tx4090abc>
- Protein Science: Coomassie. (2012, July 14). Retrieved from <https://www.protocolonline.com/protein-science/coomassie/>
- Rayment, I. (1996). The Structural Basis of the Myosin ATPase Activity. *Journal of Biological Chemistry*, 271(27), 15850-15853. doi:10.1074/jbc.271.27.15850
- Roberts, P. R., & Zaloga, G. P. (2000). Cardiovascular Effects of Carnosine [Review]. *Biochemistry*, 65(7), 856-861. Retrieved August 5, 2018, from http://www.protein.bio.msu.ru/biokhimiya/contents/v65/pdf/bcm_0856.pdf
- Sawyer, D. B. (2012). Oxidative Stress in Heart Failure: What are we missing? *The American Journal of the Medical Sciences*, 342(2), 120-124, 1-10. doi:10.1097/MAJ.0b013e3182249fcd.
- Systolic Heart Failure. (2018). Retrieved from <https://www.rightshape.com/systolic-heart-failure/>
- Taylor, S.L. (2009). *Advances in Food and Nutrition Research*. (Vol.57). London: Academic
- Tyska, M. J., Hayes, E., Giewat, M., Seidman, C. E., Seidman, J. G., & Warshaw, D. M. (2000). Single-Molecule Mechanics of R403Q Cardiac Myosin Isolated From the Mouse Model of Familial Hypertrophic Cardiomyopathy. *Circulation Research*, 86(7), 737-744. doi:10.1161/01.res.86.7.737
- Valberg, S. J. (2008). Skeletal Muscle Function. *Clinical Biochemistry of Domestic Animals (Sixth Edition)*, 459-484. doi:10.1016/B978-0-12-370491-7.00015-5
- Wessels, A., & Sedmera, D. (2003). Developmental Anatomy of the Heart: A Tale of Mice and Man. *Physiological Genomics*, 15(3), 165-176. Doi:10.1152/physiolgenomics.00033.2003
- Yun, J., & Parker, C. (1965). The effect of carnosine on myofibrillar ATPase activity. *Biochimica Et Biophysica Acta (BBA) - Enzymology and Biological Oxidation*, 110(1), 212-214. doi:10.1016/s0926-6593(65)80115-1