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Loma Linda University
School of Allied Health Professions
in conjunction with the
Faculty of Graduate Studies

Delayed Onset Muscle Soreness in People with Diabetes; Biomarkers
and Nutritional Supplementation

by

Hani H. Al-Nakhli

A Dissertation submitted in partial satisfaction of
the requirements for the degree
Doctor of Philosophy in Rehabilitations Science

December, 2011

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Each person whose signature appears below certifies that this dissertation in his/her opinion is adequate, in scope and quality, as a dissertation for the degree Doctor of Philosophy.

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ACKNOWLEDGMENTS

No research project is ever the product of one person's efforts, and certainly this one is no different. It would never have become reality without the help and suggestions of many supportive faculty and colleagues. My biggest gratitude goes to Dr. Jerrold Petrofsky. This dissertation would never have been accomplished without him. He not only served as my chairperson and supervisor, but also encouraged and challenged me throughout my academic program. He guided me through the research process, never accepting less than my best efforts. Thank you for all your time, energy, and sacrifice that you have invested in me.

I would also like to acknowledge the debt I owe to all my committee members, particularly Dr. Grenith Zimmerman; I have learned much from working with her, especially as her graduate teaching assistant. Not only did she give me valuable feedback throughout my research, but she also highlighted many of my unknown weaknesses and supported me in overcoming them. Of the many people who have been enormously helpful in the preparation of this dissertation, I am especially thankful to Dr. Lee Berk, who provided great balance between careful guidance and allowing creative autonomy. I would also like to warmly acknowledge Dr. Richard Hubbard, and Dr. Mike Laymon, for investing time and effort to provide me with necessary additional details regarding my research topic.

I would like to extend my heartfelt gratitude to my wife Eman not only for her willingness to be home to raise our children, but also for her patience and wisdom, which always kept me going forward through every step of this process. I would also like to

direct an enormous thank you to my Mother, Father, Family, and Friends. Words alone cannot express what I owe them for their encouragement and motivation.

A very special recognition is given to the Saudi Arabian Ministry of Higher Education (MOHE), in collaboration with the Saudi Arabian Cultural Mission (SACM), for granting me this opportunity to complete my post-graduate studies, and for making it a reality.

I would also like to thank my research support team for their assistance in recruiting and supervising the subjects; Darshita Dalal (MPT), Inayat Kaur (MPT), Vinit Dubey (MPT), and Swetha Regula (MPT). And finally, I would like to thank God for providing me this undeserved opportunity, and making all things possible.

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ABBREVIATIONS

BCAA	Branched Chain Amino Acids
DOMS	Delayed Onset Muscle Soreness
T2DM	Type 2 Diabetes Mellitus
IR	Infra Red
EAA	Essential Amino Acids
WHO	World Health Organization
IDF	International Diabetes Federation
HbA1c	Hemoglobin A1c
NSAID	Non-Steroidal Anti-Inflammatory Drugs
VAS	Visual Analog Scale
SF-MPQ	Short Form McGill Pain Questionnaire
MS	Muscle Strength
RM	Resistance Maximum
EMG	Electromyography
RROM	Relaxed Range of Motion
FFQ	Food Frequency Questionnaire
ANOVA	Analysis of Variance

ABSTRACT OF THE DISSERTATION

Delayed Onset Muscle Soreness in People with Diabetes; Biomarkers and Nutritional Supplementation

by

Hani H. Al-Nakhli

Doctor of Philosophy, Graduate Program in Rehabilitation Science
Loma Linda University, December 2011
Dr. Jerrold Petrofsky, Chairperson

Exercise is important for controlling hemoglobin A1c, and maintaining proper glycemic control in people with diabetes. Exercise also increases the diabetics overall insulin sensitivity, and decreases their dependency on diabetes medication. However, people with diabetes are faced with metabolic and endothelial impairment, which could result in a prolonged sensation of muscle soreness following exercise. This would make it difficult for these people to sustain exercise regimes. Delayed-onset muscle soreness (DOMS) is a common problem in healthy individuals and in people who have diabetes. DOMS is a painful sensation experienced by individuals who have been recently inactive and then over-exercise.

Yet, because people with diabetes usually have neuropathies, they may not feel this soreness appropriately, leading to premature return to exercise and causing further injuries. Therefore, this investigation assessed the differences in DOMS between people with diabetes, and healthy individuals, at 2 different body regions. However, DOMS is mainly measured with subjective scales, but we wanted to establish a new objective measure. Infra-red (IR) thermal imaging was used as one of the biomarkers in this assessment, and after expanding on this technique, it was considered a valid and reliable

tool for detecting and quantifying delayed onset muscle soreness after an intense exercise session.

Once muscle soreness in people with diabetes was determined, and a new novel biomarker was established, another focus of this dissertation was to examine whether DOMS could be attenuated by ingesting a nutritional supplement. Branched Chain Amino Acids (BCAA) have been shown to be effective in promoting muscle recovery following exercise; however, the effects of this supplement have not been investigated amongst diabetic individuals.

The results of this experiment showed that people with diabetes get sorer than healthy individuals. It was also found that IR thermal imaging may be a valuable technique for identifying which muscles are sore after exercising. Thus, thermal imaging would be an efficient and painless way of quantifying DOMS in both healthy individuals and in people with diabetes. Furthermore, this investigation showed that BCAA significantly reduced muscle soreness and enhanced healing in subjects with diabetes. However, in the healthy control group this supplement had minimal effects.

CHAPTER ONE

INTRODUCTION

Diabetes and Exercise

The diabetes epidemic is on the rise in both developing and developed countries.^{1,2} According to the World Health Organization (WHO) and recent estimates, the disease now affects approximately 345 million people worldwide and is expected to affect around 440 million by 2030, representing almost 8% of the global adult population.^{3,4} Also, the International Diabetes Federation (IDF) determined that Type 2 diabetes mellitus (T2DM) accounts for almost 90% of all diabetes cases.⁵

Lifestyle characteristics such as obesity, a sedentary lifestyle, and lack of physical activity are regarded as the most important risk factors, both independently associated with diabetes and diabetes related co-morbidities.^{6,7} Co-morbidities associated with diabetes are considered an even bigger concern than being diagnosed with the disease itself. This is because diabetes has been ranked one of the leading causes of death by disease;⁸ the WHO has estimated that deaths from diabetes will almost double between 2005 and 2030.⁹

Exercise is considered a major cornerstone of diabetes management. Studies have verified the usefulness of exercise as part of the treatment and prevention of T2DM.^{6,10,11} Even though aerobic exercise has beneficial effects for managing and treating T2DM, it has been found that resistance-type exercise is also effective in controlling blood glucose, and in reducing Hemoglobin A1c (HbA1c).^{10,12-14} It would actually be more beneficial

for individuals with diabetes to participate in a combination of both aerobic and resistance exercises to properly manage their blood glucose. A combined exercise program has been shown to have more pronounced effects on glycemic control, muscle strength, and insulin sensitivity.^{10,12,15} An important finding in a study done by Church and colleagues¹² showed that a combination training group decreased their diabetes medication dependency, when compared to a control group who had increased their use of diabetes medications. It would be imperative then to include resistance training as a primary step in the exercise schedule of a diabetic patient. This is particularly important for obese individuals who have been inactive for a while, since they face the challenge of participating in aerobic exercises due to their low fitness levels.

Resistance training then, would be better for individuals with diabetes to begin with, as it contributes to the recruitment of previously inactive muscle fibers. This has been found to enhance the quality of the muscle, which eventually leads to a substantial gain in muscle mass.^{10,16,17} As a result, the body's insulin sensitivity will be amplified and glucose tolerance levels improved, thereby improving the body's glucose disposal capacity and giving the diabetic individual a better chance to cope with the disease.^{6,10,16}

Delayed Onset Muscle Soreness

People with or without diabetes might get discouraged from exercising because of the related soreness. This type of soreness is called delayed onset muscle soreness (DOMS), and is a typical phenomenon that occurs in skeletal muscle as a result of novel or unaccustomed exercise.¹⁸⁻²⁰ The severity of the damage and the extent of discomfort is exacerbated over time, and occurs minutes to days after an acute exercise bout.^{18,19} The

intensity of the symptoms and discomfort associated with DOMS usually peaks between 24 to 72 hours post exercise, and can last for up to 10 days, especially when the exercise bout encompasses an eccentric component.²⁰⁻²² Activities that are comprised of repeated eccentric contractions have been shown to result in damage to the ultrastructure of skeletal muscle.^{23,24} This damage manifests itself as a temporary decrease in muscle function, restricted range of motion in the associated joint, increased muscle soreness, and swelling of the involved muscle group.^{23,25,26} Consequently, DOMS is considered one of the most common recurrent forms of injury, which can lead to further injuries if a premature return to exercise is attempted.¹⁹

The DOMS phenomenon has gained a considerable amount of interest lately, particularly amongst researchers and specialists in exercise physiology, sports, and rehabilitation.²⁷ However, DOMS has not been investigated in people with diabetes, and being able to objectively determine the extent of soreness using a reliable biomarker is sometimes a challenge.

Biomarkers and Thermal Imaging

DOMS is not an easy pathology to quantify, as there is a wide amount of variability between the measurement tools and methods used to quantify this condition.²⁷ No agreement has been made on one best evaluation measure for DOMS, which makes it difficult to identify the impact of a specific intervention in decreasing the symptoms associated with DOMS. Even though needle biopsies of the muscle and blood levels of myofiber proteins might be considered a gold standard,²⁷ large variations in some of these blood proteins have been documented.^{27,28} There are also high risks sometimes

associated with invasive techniques. Thus, it can be seen that muscle soreness, especially in diabetic individuals who have neuropathies is somewhat ambiguous, because many studies depend on measuring soreness using a subjective visual analog scale (VAS).²⁹⁻³³

Muscle soreness has various underlying mechanisms, but a main mechanism of soreness is related to blood flow and inflammation in the muscle.^{19,34} Thus, when there is damage to the muscle, the blood flow should increase because of the inflammatory process, which results in the increase of the skin temperature above it. Infrared (IR) thermography has been used, and found to be successful in detecting different types of diseases and infections since the 1950's.³⁵ There is ample evidence for this phenomenon. For example, a common technique for detecting some types of tumors is by using thermography.³⁶⁻³⁹ In many cases, the tumor has higher blood flows than the rest of the examined area, which warms up a spot under the skin and shows up on a thermal image.³⁶⁻³⁹ Consequently, a muscle that is sore should also show up on a thermal image because of this same phenomenon. The advantage of thermal imaging is that it would provide useful information relative to the damage in the muscle, unlike subjective pain measurements which can be altered by the individual's sensation of pain and the associated neuropathies from diabetes. Therefore, a noninvasive technique that detects DOMS in its early stages would be very beneficial for this population, as it could prevent them from premature return to exercise, thus decreasing the risks of further injuries.

On the other hand, people with diabetes might not be getting enough nutrients, vitamins, and amino acids from their diets, due to the fact that they have gastrointestinal and mal-absorption problems.⁴⁰⁻⁴² Interestingly, some studies have suggested that nutritional supplementation after exercising can reduce or even prevent DOMS.

Nutritional Supplementation and Amino Acids

Amino acids are the molecular building blocks from which proteins are made. There are essential and nonessential amino acids, and the difference between them is that the human body can synthesize the nonessential amino acids, but the essential amino acids (EAA) have to be taken through our diets.⁴³ Resistance exercise causes decreases of some of the EAA, including branched chain amino acids (BCAA), during the recovery period after exercise.^{44,45} Skeletal muscle however, remains in an overall catabolic state unless muscle protein synthesis surpasses muscle protein breakdown, and for this to take place, adequate nutritional intervention is necessary during this recovery period.^{46,47} Thus, when EAA are ingested in conjunction with an acute bout of resistance training, the hypertrophic response is enhanced, resulting in a net increase in muscle protein synthesis.^{25,48}

Among these EAA, exceptional results have been observed with BCAA.^{48,49} Studies have indicated that BCAA are mainly taken up by active skeletal muscles during sub-maximal exercise, whereas all other amino acids are taken up by the liver.^{44,50} Thus, the metabolism of BCAA is initiated in skeletal muscle, and the oxidation process is enhanced by exercise. Numerous studies have investigated the effects of BCAA supplementation on skeletal muscle soreness, where some studies found that BCAA may be useful for muscle recovery following acute sessions of exercise, both in trained and untrained individuals.^{25,26,47,51,52} However, nutritional supplementation and its effects on muscle soreness has not been examined in people with diabetes.

Approach of Studies

To study DOMS in people with diabetes, it is necessary to establish whether people with diabetes experience similar levels of soreness as healthy individuals or not? This question arises because it is known that people with diabetes have neuropathies, and with neuropathies they tend to have hypo or hyper-sensitivity.⁵³ Also, people with diabetes have high levels of free radicals and chronic inflammation, which could prolong muscle healing, and make it harder for these people to properly workout.^{53,54} Therefore, in chapter 2 of this dissertation, the problem of DOMS was examined in people with and without diabetes to see if muscle soreness after exercising was greater or less between them. We looked at DOMS in diabetic and healthy individuals at different areas of the body, and used a thermal imaging technique in addition to other biomarkers to quantify muscle soreness. As an outcome of the first study, in chapter 3 we expanded on this new thermal imaging technique and clarified how it can be used as a reliable biomarker to measure DOMS. An objective measure to determine and quantify muscle soreness is critical, because subjectively, two people could portray the same level of soreness whereas in reality they are experiencing different pain symptoms after performing a similar exercise. Once this new measuring technique was validated, in chapter 4 we used it in addition to other biomarkers to confirm if DOMS could be reduced with a nutritional supplement in people with diabetes. In this investigation our supplement of choice was BCAA, because there is abundant evidence suggesting that protein supplements which are high in BCAA can attenuate muscle damage, and reduce muscle soreness.^{25,26,55}

Investigation Purposes

The purpose of this investigation was three fold:

First, to identify whether people with diabetes become as sore, or sorer than healthy individuals after an intense exercise session, as there are hardly any studies that look at DOMS in people with diabetes.

Second, to look at biomarkers of muscle soreness, and establish a new objective means of quantifying muscle soreness. In this case, it was measuring skin temperature using thermal imaging, as there are no published studies done on DOMS and changes in skin temperatures.

Third, to verify whether nutritional supplementation could reduce muscle soreness and increase healing in people with diabetes. In this investigation, our focus was on BCAA, as no study to date has assessed the effectiveness of a BCAA supplement on DOMS in a diabetic population.

CHAPTER TWO

THE USE OF THERMAL INFRA-RED IMAGING TO ASSESS THE EFFICACY OF A THERAPEUTIC EXERCISE PROGRAM IN INDIVIDUALS WITH DIABETES

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Abstract

Background: Exercise is of great value for individuals with diabetes in helping to control their HbA1c levels, and increasing their insulin sensitivity. Delayed onset muscle soreness (DOMS) is a common problem in healthy individuals, and in people who have diabetes. People with diabetes are also faced with metabolic and endothelial impairments, which could make DOMS even worse. But because they usually have neuropathies, they may not feel this soreness appropriately, leading to premature return to exercise and causing further injuries.

Research Design: 118 subjects participated in this study, and were divided into 4 groups. Two groups (healthy, and diabetic) performed a series of abdominal exercises, and the other 2 groups (healthy, and diabetic) performed a series of arm exercises to induce DOMS. Skin temperature above the muscle was assessed using a thermal infra-red camera, and perceived soreness of the exercised muscle was assessed using a 100mm visual analog scale (VAS). Serum myoglobin concentrations were also measured.

Results: the results showed that there was a significant increase in skin temperature 24 hours post exercise for all 4 exercised groups ($P < 0.05$), where the combined average increase in skin temperature for all 4 groups was approximately 0.65 °C from baseline. Also, 24 hours post-exercise, all 4 groups were significantly sorer than they were at baseline ($P < 0.05$). Serum myoglobin levels were also significantly higher on day 3 when compared to day 1 ($P < 0.05$).

Conclusion: Infra Red Thermal Imaging may be a valuable technique of seeing which muscles are sore hours or even days after the exercise is over. Thus, thermal imaging would be an efficient and painless way of looking at DOMS in both healthy

individuals, and individuals who have diabetes, even if they are facing neurological problems.

Keywords: Diabetes, Muscle Soreness, DOMS, Thermal Imaging

Introduction

Exercise is recommended as part of the treatment and prevention of diabetes.^{1,2} The combination of both aerobic and resistance exercises have been found to be more effective in controlling blood glucose, and Hemoglobin A1c levels (HbA1c) when compared to only one type of exercise.^{1,3} It is imperative to include resistance training as a primary step in the exercise schedule of a diabetic patient, since some face the challenge of participating in aerobic exercise due to their low fitness levels. Also, muscle building exercises are critical for the diabetic population, as an increase in the skeletal muscle mass has been associated with better insulin sensitivity in people who have diabetes.⁴ But beginning with a resistive exercise routine after being physically inactive for prolonged periods of time, could cause delayed onset muscle soreness (DOMS).⁵⁻⁷ This would have the effect of reducing the individual's willingness to continue exercising.

While much is known about DOMS in young people, little is known about DOMS in people with diabetes. However, there may be some comparisons that can be made from studies of DOMS and ageing.⁸ Studies have shown that there is reduced proteolytic activity and an elevated production in free radicals in older individuals.^{8,9} This elevation prolongs healing time after excessive exercise.⁸ With metabolic impairments, endothelial

dysfunction, and higher levels of free radicals in people with diabetes ^{10,11} DOMS could be more severe and recovery periods longer in this population.

DOMS generally presents itself as initial soreness that starts within 24 hours of heavy exercise, where its associated symptoms can range from slight muscle tenderness to severe debilitating pain. ¹²⁻¹⁴ The severity of the symptoms depends on several factors including the fitness of the individual, their age, genetics, training, and the intensity of the activity. ¹⁵ The peak discomfort from muscle damage ranges between 24 to 72 hours post-exercise, but the symptoms may continue as much as 7 days post-exercise until they begin to diminish. ^{7,16} For this reason, DOMS is one of the most common recurrent forms of sports injury that can affect an individual's performance, and become intimidating for many. ^{5,17}

For the last 3 decades, the DOMS phenomenon has gained a considerable amount of interest amongst researchers and specialists in exercise physiology, sports, and rehabilitation. ¹⁸ There has been a variety of published studies investigating this painful occurrence in regards to its underlying mechanisms, treatment interventions, and preventive strategies. ^{5-7,10,17,19-24} However, it is evident from the literature that DOMS is not an easy pathology to quantify, as there is a wide amount of variability between the measurement tools and methods used to quantify this condition. ¹⁸ It is obvious that no agreement has been made on one best evaluation measure for DOMS, which makes it difficult to verify whether a specific intervention really helps in decreasing the symptoms associated with this type of soreness. Even though needle biopsies of the muscle, and blood levels of myofibre proteins or myoglobin might be considered a gold standard to some, ¹⁸ large variations in some of these blood proteins have been documented. ^{18,25}

Thus, it can be seen that muscle soreness is somewhat ambiguous, because many studies depend on measuring soreness using a visual analog scale (VAS).^{22,26-29} But with people who have diabetes, they often have neuropathies, and in this case they might not be able to feel the muscle pain as well as normal individuals, therefore giving false VAS readings.

Muscle soreness has a variety of underlying mechanisms, but a main mechanism of soreness is related to blood flow and inflammation in the muscle.^{5,6} Thus, when there is damage to the muscle, the blood flow should increase due to the inflammatory process, which should result in the increase of the skin temperature above it. There is ample evidence for this phenomenon. For example, a common technique for detecting some types of tumors is by using thermography.³⁰⁻³³ In many cases the tumor has higher blood flows than the rest of the examined area, which warms up a spot under the skin and shows up on a thermal image.³⁰⁻³³ Consequently, a muscle that is sore should also show up on a thermal image due to this same phenomenon. The advantage of using thermal imaging is that, unlike subjective pain measurements which can be altered by the individuals sensation of pain, and the associated neuropathies from diabetes, thermal imaging would actually provide very useful information relative to the damage in the muscle.

However, individuals with diabetes may have altered feeling of DOMS, or may not feel this type of muscle soreness due to their neuropathies. Therefore, a non invasive technique that could detect DOMS in its early stages would be very beneficial for this population, as it could prevent them from premature return to exercise. This may increase training and decrease their risks of injuries. It is surprising then that little has been done

on DOMS and changes in skin temperatures in this population. Therefore in the current study, we investigated changes in muscle soreness by looking at increases in skin temperatures using thermal imaging at 2 areas of the body. The first area was closer to the core of the body, and the second area was more peripheral, to see if we can detect a difference between the two regions. For the core area, the abdominal muscles were studied, as core muscles are usually warmer, and for the peripheral region, we examined the biceps muscle, because muscle and skin temperature in the peripheries are several degrees cooler than the core. Yet there have been no published studies looking at changes in skin temperature in individuals on days after they have done heavy exercise, to see if muscle soreness can be predicted by an increase in skin temperature.

Subjects & Methods

There were 118 subjects that participated in this study. Subjects were divided into 2 basic groups, 1 group of 80 subjects that participated in an abdominal exercise, and another group of 38 subjects that participated in biceps exercise. These 2 groups were also subdivided into healthy subjects (no diabetes), and subjects with diabetes. All the subjects were physically inactive for at least 3 weeks. Subjects were excluded if they were pregnant, had hepatic diseases, were diagnosed with Rhabdomyolysis, were diagnosed with an impaired circulatory disease (such as Raynaud's), had any recent upper limb or abdominal injuries, had severe neuropathies in their upper limbs, were hypertensive (blood pressure over 145/90 mmHg), or hypotensive (blood pressure lower than 90/50 mmHg), or were on high doses of alpha or beta agonist/antagonists, NSAID's, Cox 2 inhibitors, Calcium channel blockers, Pregabalins, or Pain reducers. Some diabetic

subjects had minimal cardiovascular disease but were within the blood pressure ranges stated above and had no major complications. The average ages, heights, weights, and demographics of the subjects are shown in table 1. All subjects were informed of all experimental procedures and protocols and signed a statement of informed consent as approved by either the institutions review board at Azusa Pacific University or Loma Linda University.

Table 1: General Characteristics of the Subjects (Means +/- SD)

		N	Age (Years)	Height (CM)	Weight (KG)	BMI	HbA1C
Abdominal Exercise	Healthy	40	28.75 (+/- 5.6)	171.25 (+/- 6.3)	88.15 (+/- 12.8)	30.10 (+/- 3.5)	7.7 (+/- 1.9)
	Diabetics	40	59.0 (+/- 9.9)	176.08 (+/-6.9)	95.95 (+/- 14.6)	31.43 (+/- 4.9)	
Arm Biceps Exercise	Healthy	22	28.45 (+/- 8.7)	167.91 (+/- 8.2)	73.09 (+/- 20.0)	25.52 (+/- 5.5)	7.1 (+/- 1.7)
	Diabetics	16	61.25 (+/- 12.6)	167.56 (+/- 14.8)	109.25 (+/- 39.6)	38.07 (+/- 10.2)	

The Exercise

The group doing the arm exercise used a dumbbell to exercise one arm. The resistance used was different for each subject, and was determined by testing each participant for their resistance maximum (RM). To do this, we used a strength measuring device with 4 strain gauge arranged as a Wheatstone bridge. This was interfaced with a computer through a BioPac (DAC-100) bioelectric amplifier module (BioPac Systems, Goleta, CA). The module was connected to a BioPac MP-100 analog to digital converter sampling at a frequency of 1,000 hertz per second, and at a resolution of 24 bits. The

device was fixed to a bench at a 45° angle, so that the subject would not recruit any muscle other than the biceps. After determining the RM for the biceps muscle of each participant in that group, we made them sustain the intended session of exercise with 35% of their RM calculated from the average of the 3 strength recordings done on the strain gauge device prior to the exercise.

The abdominal group accomplished a number of exercises as shown in the P90X video series which is designed for both men and women (Beachbody LLC, Los Angeles, California, USA.). The video includes several different exercise routines with various individuals doing each exercise differently, so the user could select low or high intensity variations of the exercise depending on their capability. For the purpose of this study, two workout videos were selected to emphasize the abdominal region. The first video, Core Synergistics lasted 44 minutes, followed by the Ab Ripper X video which lasted 16 minutes, giving a 60-minute total workout.

Skin Temperature

Skin temperature was measured using a Flir TC660 Thermal Camera (Stockholm, Sweden). The thermal image taken of the arm or abdominal regions were from approximately 1 meter away, and perpendicular to the skin (Fig. 1). From a series of tests done at our labs using the FLIR 660 IR Camera, this distance and angle were found to have the best correlation ($r = 0.93$) with thermocouple readings when compared to different distances from the skin (2, and 5 meters), and at different angles (15, 30, 45, and 60 degrees). The images were taken in a temperature controlled room which was maintained at approximately 23°C (+/- 0.5°C). The temperatures from the acquired image

were measured at 4 locations on the skin above the biceps or abdominal muscle using the “ThermoVision® ExaminIR™” software Version: 1.10.2 (Fig. 2). These 4 readings were analyzed individually and then averaged to give a full perspective of the temperature above the exercised muscle.

Soreness/Pain Visual Analog Scale

A visual analog scale (VAS) was used to assess subjective soreness of the arm or abdominal muscles before doing the exercise and after the exercise was completed. The scale had a 10 cm (100mm) long line marked “no pain” at one end, and “extremely sore” at the opposite end. Each participant was directed to make a vertical mark along the 10 cm line to indicate his or her response to soreness.



Figure 1: The setup of the Infra Red camera 1 meter away from the skin, and perpendicular to the area being examined.

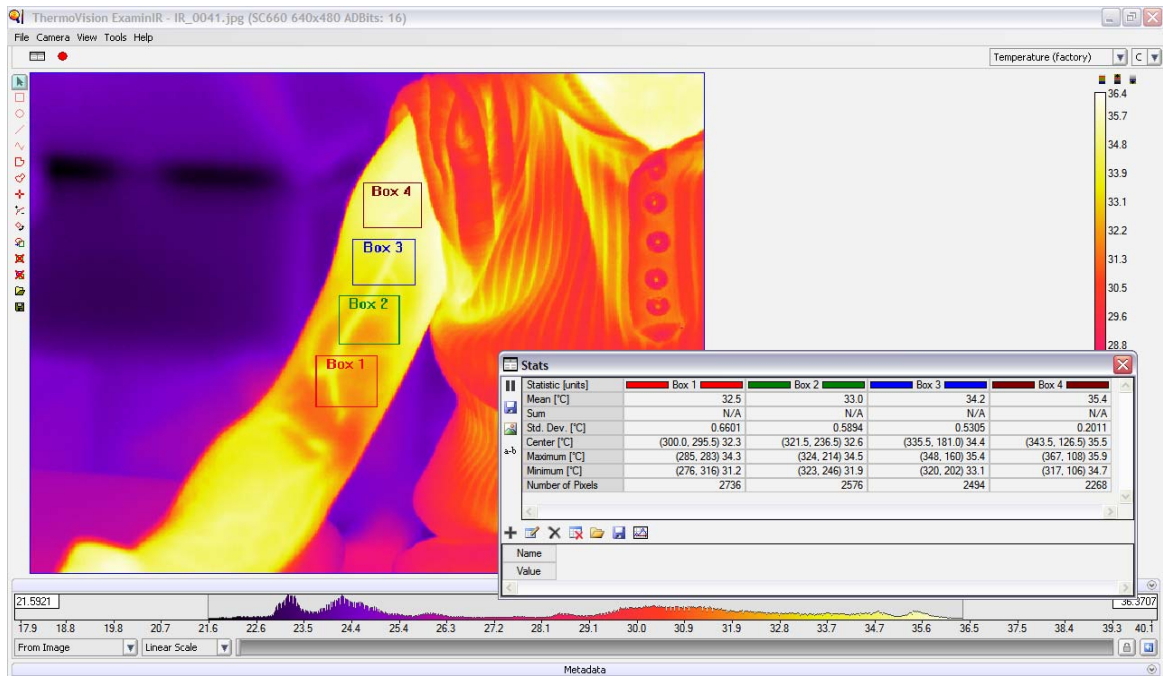


Figure 2: Software interface for the “ThermoVision® ExaminIR™” showing the 4 boxes of interest on an IR image of an exercised arm. Also shown are the statistical interpretations for each box from the software itself.

Blood Sampling & Measurement of Myoglobin Concentrations

Approximately 4 ml peripheral blood was collected from an antecubital vein.

Peripheral venous blood was drawn before (Pre), immediately after (Post), and 48 hours (2 days) after the exercise bout. The blood was spun down in a centrifuge at 4000 rpm for 10 min to separate the serum from the cells. The samples were stored at -80°C until the analyses of myoglobin was done.

Myoglobin was measured using a TOSOH “AIA®-360” Automated enzyme Immunoassay Analyzer (TOSOH Corp., Tokyo, Japan). The myoglobin Assay kits (Myo 025297, ST AIA-PACK Myoglobin) were used according to the manufactures

instructions. Controls were run before and after each analyses session, to verify that the measurements were accurate and within the manufacturers specified quality control ranges. The normal reference range for myoglobin using this method was 31.4 - 971 ng/ml. The intra-assay and inter-assay coefficient of variation was 2.76 %, and 4.45 % respectively.

Procedure

In this investigation, we started off with the biceps study and monitored the soreness and heat variations for the subjects over a period of more than 3 days. It was realized that the biggest change in temperatures was at 24 hours. We then did the abdominal study for only 3 days, to see if the core muscles have the same heating response as the peripheral muscles. Blood samples for the myoglobin readings were only carried out on the arm group. Myoglobin measurements were not carried out on the abdominal group due to costs.

After subjects attended to the fitness lab, baseline data, including a thermal image of the abdominal or arm muscles, and a soreness VAS reading were collected. Blood samples for the myoglobin analyses were also collected at this time, but only for the arm group. The targeted muscles for the subjects in the arm group were the elbow flexors. To provoke DOMS in these muscles, the subjects in this group carried out 4 sets of 25 repetitions of biceps concentration curls while seated on a chair, and with the elbows supported on their thighs. Subjects were advised to lower the weight and lift it at a steady state (approximately 3 seconds going down, and 3 seconds coming back up), to insure that both the eccentric and concentric components of the contraction were properly done.

There was a 90 second resting period between each set and the other, and subjects either did the full set of 25 repetitions, or were instructed to stop the set if they failed to steadily control the descent of the weight and return their arm back to full flexion. As indicated previously, the resistance used was different for each subject, and was determined by testing each participant for their resistance maximum (RM).

For the abdominal group, after the baseline readings, the subjects participated in the 60-minute workout video mentioned above, to induce DOMS in their abdominal muscles. All subjects were asked to attempt the most difficult variation of each exercise, as they are able. Subjects were given four 3-minute breaks throughout the video to rest. Subjects were told that they are allowed to stop the exercise at any time if it became too strenuous.

Twenty-four hours post-exercise, thermal images of the arm or abdomen muscles were measured, and VAS readings were also taken at this time. Both these measures were also repeated at 48 hours post-exercise. Blood samples for the 2nd myoglobin reading were taken 30 minutes post-exercise, and the 3rd reading was taken 48 hours post-exercise.

Data Analysis

Data analysis involved means, and standard deviations. Measurements of all 3 variables (skin temperature, VAS, and myoglobin) were compared within each group using the repeated measures analysis of variance (ANOVA). Spearman's correlation was also used to identify any relationships between the variables. Statistical analysis was

performed using PASW Statistics Version 18.0.0 (SPSS, Inc., 2009, Chicago, IL, www.spss.com), and the level of significance was set at $P < 0.05$.

Results

Skin Temperature

The results of the skin temperature measurements for the 4 groups are shown in figure 3. As seen in figure 3, all the 4 groups had increased skin temperatures 24 hours post exercise. For the healthy group who performed the P90x abdominal exercise, their average skin temperature for the measured area increased by 0.83°C , from $32.50 \pm 0.37^{\circ}\text{C}$ to $33.33 \pm 0.39^{\circ}\text{C}$. This increase in skin temperature was significant ($P < 0.05$). The increase in skin temperatures from baseline to 24 hours post exercise for the diabetic group who performed the P90x abdominal exercise was also significant ($P < 0.05$), where it increased by 0.61°C , from $33.02 \pm 0.16^{\circ}\text{C}$ to $33.63 \pm 0.61^{\circ}\text{C}$.

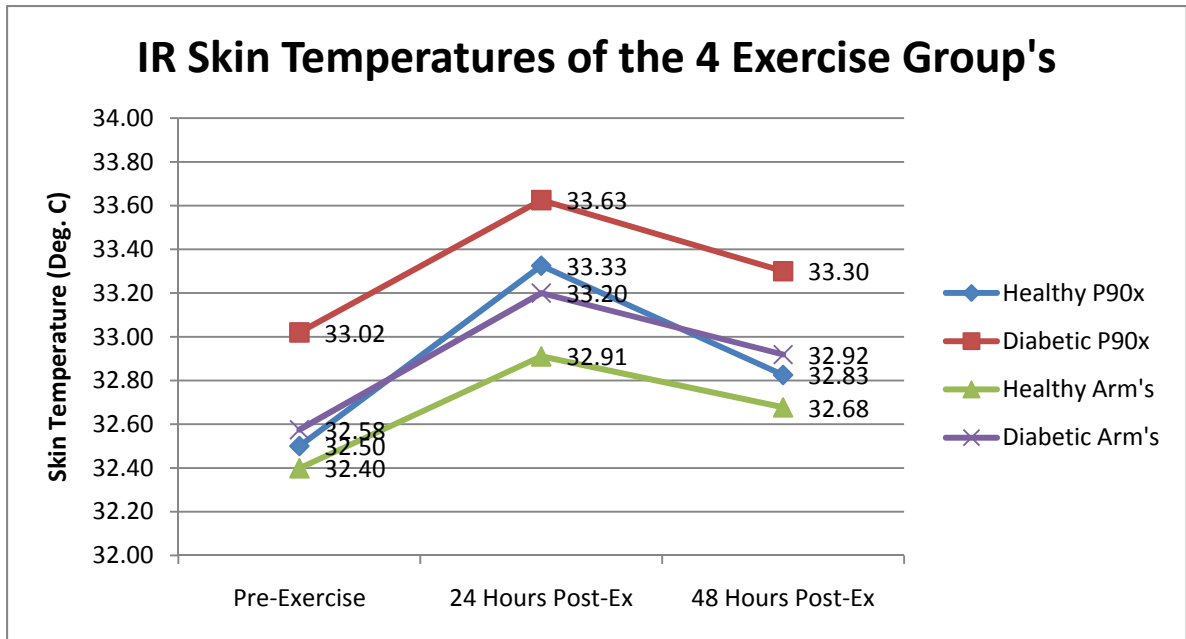


Figure 3: A representative graph of the differences in skin temperatures measured with the thermal camera in the healthy group who performed the P90x Abdominal exercise (diamonds), the diabetic group who performed the P90x Abdominal exercise (squares), the healthy group who performed the arm exercise (triangles), and the diabetic group who performed the arm exercise (X), over the 3 day time period.

For the groups that exercised their arm muscles, the healthy group had a 0.51°C increase in skin temperature between the first 2 days, from $32.4 \pm 0.87^{\circ}\text{C}$ to $32.91 \pm 0.96^{\circ}\text{C}$. This increase was significant ($P < 0.05$). Also, the diabetic group who exercised their arms had a significant increase in skin temperature ($P < 0.05$) from day 1 to day 2, where the increase was 0.62°C , from $32.58 \pm 0.85^{\circ}\text{C}$ to $33.20 \pm 0.74^{\circ}\text{C}$ (Fig. 4, A & B).

By the 3rd day (48 hours post exercise), skin temperatures were almost back to normal, where there was no significant difference between day 1 (pre exercise) and day 3 for all the groups ($P > 0.05$).

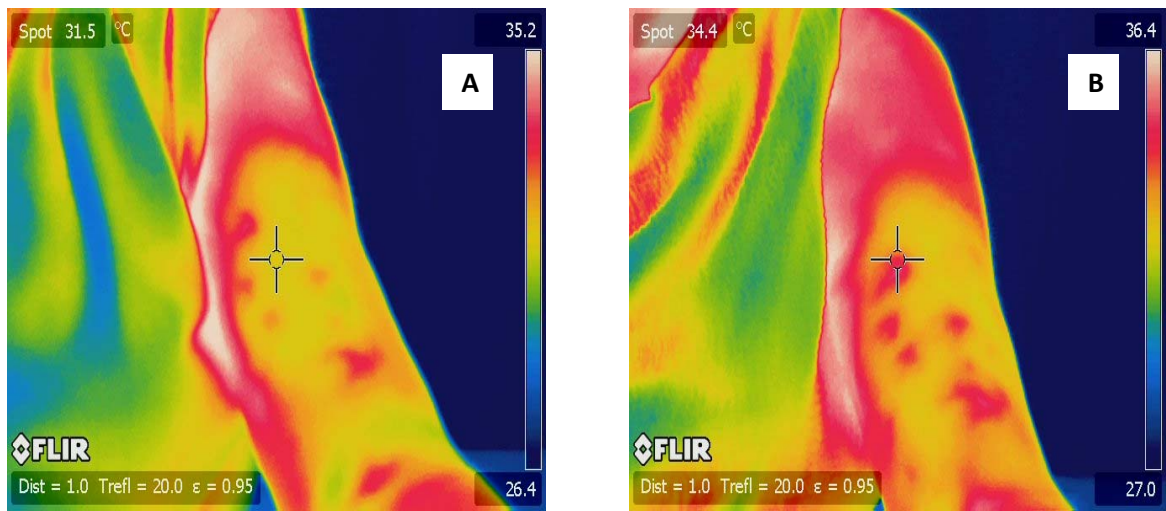


Figure 4: A) A thermal image of a diabetic individual's arm before exercising. B) A thermal image of the same individual's arm 24 hours post exercise (the temperature of the arm jumped from 31.5 °C to 34.4 °C)

Perceived Soreness (VAS)

The results of the perceived muscle soreness are shown in figure 5. As seen in figure 5, all 4 groups had a dramatic increase in muscle soreness 24 hours post exercise. The healthy group, who performed the P90x abdominal exercise, had an increase of approximately 41.5 millimeters (mm) on the pain scale, from 0 pain to 41.5 +/- 6.1 mm. This increase was highly significant ($P < 0.05$). The diabetic group who performed the same abdominal exercise had an even higher increase in muscle soreness than the healthy subjects. Their baseline muscle soreness was 7.3 +/- 1.7 mm, which had increased to 73.8 +/- 8.8 mm, 24 hours post exercise. That was a 66.5 mm increase which was also very significant ($P < 0.05$).

The biceps group also had large increases in soreness levels from pre exercise to 24 hours post exercise, where the healthy subjects had an 18.8 mm increase in muscle

soreness (from 4.1 +/- 6.9 mm to 22.9 +/- 15.1 mm), and the diabetic group had a 23.3 mm increase in muscle soreness (from 6.3 +/- 7.4 mm, to 29.5 +/- 11.6 mm). Both these increases were also significant ($P < 0.05$). It is also clear from figure 5 that the soreness levels for all the 4 groups were beginning to decrease but they didn't reach near baseline levels as was the case for skin temperature measurements.

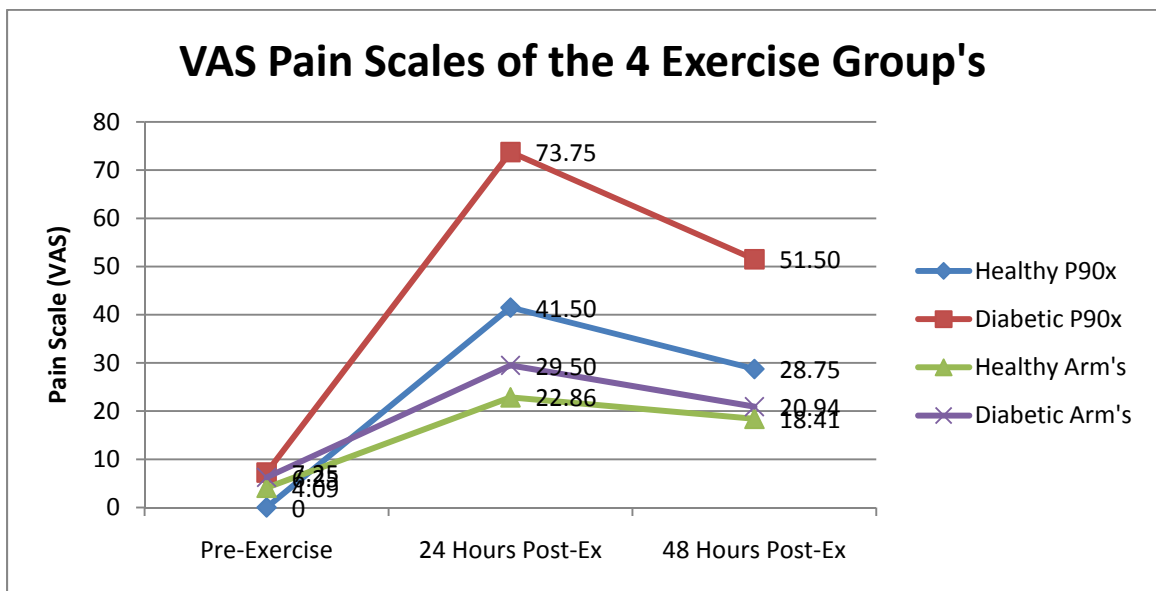


Figure 5: A representative graph of the differences in perceived muscle soreness measured with the VAS in the healthy group who performed the P90x Abdominal exercise (diamonds), the diabetic group who performed the P90x Abdominal exercise (squares), the healthy group who performed the arm exercise (triangles), and the diabetic group who performed the arm exercise (X), over the 3 day time period.

Myoglobin Concentrations

The results of the myoglobin readings for the 2 arm groups are shown in figure 6. As seen in figure 6, both groups had substantial increases in the myoglobin concentrations 48 hours post exercise. However, there was hardly any difference between

the 2 measurements (pre, and post) on day 1, for both the groups ($P > 0.1$). The healthy group had an increase of approximately 146 nanograms per milliliter (ng/mL) of blood on day 3, compared to day 1 (from 31.03 +/- 8.25 to 177 +/- 222). This increase was highly significant ($P < 0.05$). The diabetic group who performed the same arm exercise had an even larger increase in myoglobin concentrations, when compared to the healthy individuals. This group had an increase of almost 337 ng/mL of blood at 48 hours, when compared to baseline (from 50.9 +/- 19.2 to 388.35 +/- 411.9). This increase in the myoglobin level was also very significant ($P < 0.05$).

A correlation analysis was done between the myoglobin measurements and the other 2 variables (skin temperature and VAS). It was found that there was a strong correlation between the peak skin temperature measurements (day 2), and the peak myoglobin readings (day 3). This correlation was highly significant ($r = 0.41$, $P < 0.05$). There was also a relevant correlation between the myoglobin readings on day 3, and the VAS measurements on day 3. This correlation was also very significant ($r = 0.47$, $P < 0.05$).

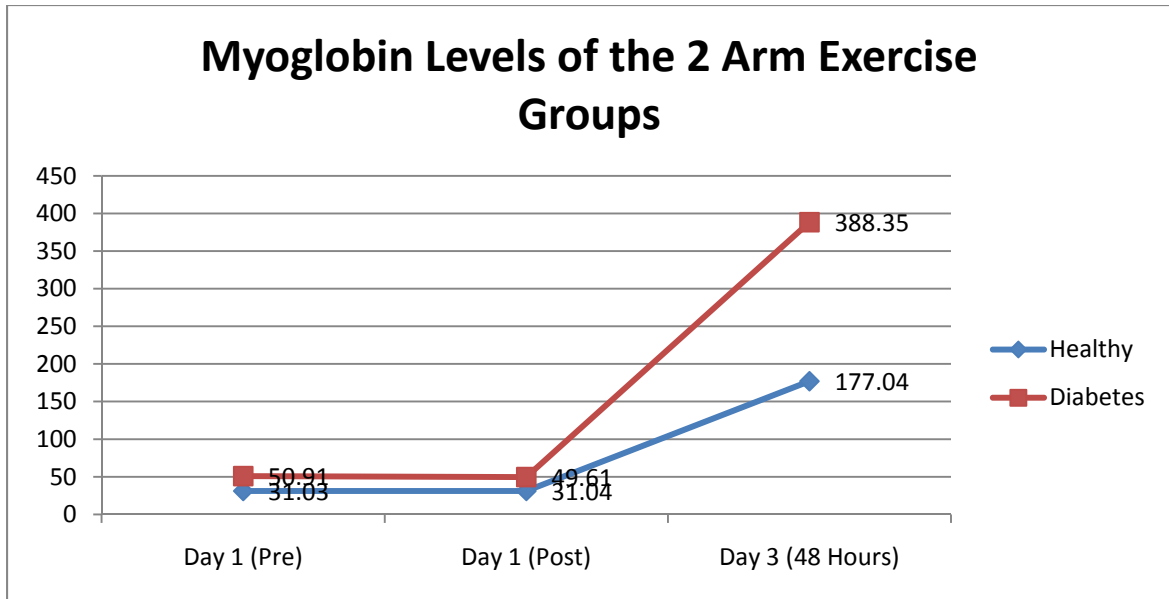


Figure 6: A representative graph of the differences in Myoglobin levels between the healthy group who performed the arm exercise (diamonds), and the diabetic group who performed the arm exercise (squares) at 3 different time periods (Day 1 pre & post, and day 3).

Discussion

In the present investigation, subjects participated in an intense exercise program involving a single bout of exercise to identify the usability of thermal infra red imaging for detecting DOMS. From this investigation, it was found that IR imaging could be a valid technique for determining DOMS within the first day of exercising.

Diabetes causes a chronic increase in whole body inflammation.³⁴ Due to this, and to the metabolic, and endothelial impairments associated with diabetes, exercise performance and training may become impaired in this population.^{35,36} Even though in our investigation, both the diabetes groups did not exercise as hard as the other 2 groups, they had more muscle soreness than the healthier groups. However, exercise is still the best means of increasing glycemic control in diabetics because it enhances the body's

insulin sensitivity.¹ Exercise has also been associated with a mean reduction in HbA1c of approximately 0.80%, especially muscle building exercises, as an increased overall muscle mass has been associated with better insulin sensitivity.^{4,37} This obviously gives the diabetic individual a better chance of coping with the disease, and preventing its symptoms from getting worse.^{2,38}

An interesting finding in this study was that the 2 groups who performed the abdominal exercise had higher skin temperatures, and increased soreness levels when compared to the groups who did the arm exercise. This could be due to the fact that the abdominal exercise was slightly longer in duration than the arm exercise, or because the abdominal muscles are larger in size than the biceps muscle. Another justification would be that the abdominal muscles are closer to the core, and are slightly warmer to begin with when compared to the peripheral arm muscles.

Pennes³⁹ provided a very detailed model of heat flow from muscle to skin in limbs. This model predicts that heat in deeper tissues such as muscles can be dissipated into blood and into the skin through conductive heat exchange. When muscles exercise, heat is developed in the muscle due to frictional forces of the muscle fibers and from the increased metabolism.⁴⁰ Increased blood flows to the muscle would also contribute to the increased heat in the muscle after exercising.

Skin is a shell tissue and its temperature is usually about 6 degrees less than that of the core.⁴¹⁻⁴³ The skin must be kept cooler than the core so that heat can move from the core to the skin and be removed by radiation, conduction and evaporation.^{42,44-46} Heat moves from the muscle both into the blood perfusing muscle and is dissipated throughout the body and also flows to the cooler skin area.³⁹⁻⁴¹ This allows core temperature to be

maintained at a regulated level.^{35,39,47} Muscle is also a shell tissue, where its temperature is usually 32 to 33 °C, which is slightly warmer than that of skin. However when blood flow is increased to the exercised muscle, it approaches the temperature of the core tissues which is approximately 37°C.^{33,39} Blood flow in skeletal muscle is very dynamic. However, when tissue is damaged during exercise, blood flow can increase dramatically. Thus, warm blood entering muscle when tissue repair is being accomplished as part of the inflammatory response, can deliver a great deal of heat into the tissue.^{6,48} The increased blood flow should go back to normal within an hour or two of the exercise termination, but when temperatures are still elevated at 24 hours post exercise, this shows that damage has happened to the exercised muscle and causes a hot spot under the skin.

Thus, the elevated skin temperatures found 24 hours post exercise in all subjects is possibly the result of higher blood flows in muscle due to inflammation, and tissue damage repair.⁶ Therefore, if muscle blood flow remained elevated post exercise, the warmer core blood would keep the muscle warm and hence the overlying skin would stay warm. This occurrence has been seen with thermal imaging for breast tumors in women, where tumors under the skin have this same effect and therefore form the basis for breast thermal imaging to detect tumors.^{31,49} In fact, IR imaging has been used to detect and diagnose a number of other diseases,^{30,32,33,50-52} as changes in the human body's temperature has always been an indicator of some sort of dysfunction.⁵¹ Increased circulation associated with delayed onset muscle soreness could also be seen as increased tissue temperature resulting from a muscular dysfunction.

The elevated myoglobin concentrations on day 3 also verify this muscle soreness response after exercise. Myoglobin is a monomer protein, which has several functions

regarding oxygen storage and transport in the muscles. After strenuous exercise, myoglobin can be released in the bloodstream due to the breakdown of cell membranes in the exercised muscles.^{53,54} Muscle damage Biomarkers, such as myoglobin, may increase 30 minutes after exercising, and peak at 24 to 72 hours post exercise.⁵³ For that reason, we made a measurement of venous myoglobin at 48 hours post exercise, to identify the peak myoglobin response. Fortunately, as was seen in the correlation analyses, there was a strong relationship between the myoglobin concentrations on day 3, and the skin temperature measurements on the day before. This finding validates the usability of thermal imaging to detect DOMS in its initial stages. Even though skin temperatures and soreness levels were returning back to normal at 48 hours post exercise, the myoglobin concentrations were dramatically high. This exaggerated release of myoglobin from muscle could be due to the muscles response to the low grade inflammation and damage after the exercise.^{53,55}

There were a couple of limitations in this study. First, the sample sizes weren't equal between the groups that did the abdominal exercises, and the arm exercise groups. A main reason for this could be due to the fact that the arm exercise session, which was done prior to the abdominal exercise session, needed more commitment from the subjects, as it was extended to more than 3 days. Second, myoglobin was only measured for the arm exercise group. This was due to cost. Third, the exercise routines between the 2 groups were different. However, this was necessary because the abdominal muscles are larger than the arm muscles, and an exercise that challenges the abdominal muscles, without causing back pain was needed for the purpose of this study.

Despite the limitations of this study, we have provided evidence to suggest that thermal imaging could be of great value in identifying which muscles have been exercised and are sore, hours or even days after the exercise is over. Thermal imaging would then be a painless, and non invasive way of detecting DOMS in its early stages, which could minimize further injuries from over-exercising in both healthy individuals, and in people who have diabetes. This could be more beneficial for the diabetic individuals, as they don't always realize that they are sore, due to the neuropathies and impairments they have.

Acknowledgements

We wish to acknowledge a contract (WS1763368) from Pfizer Pharmaceuticals for support in this work. We would also like to thank the Saudi Arabian Ministry of Higher Education (MOHE) for their support.

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CHAPTER THREE

THE USE OF THERMAL INFRA-RED IMAGING TO DETECT DELAYED ONSET
MUSCLE SORENESS

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Keywords: (DOMS, Imaging, Thermal, Infra-Red, Muscle, Soreness, Thermography)

Short Abstract

The purpose of this investigation was to assess whether using an infra-red thermal camera is a valid tool for detecting and quantifying the muscle soreness after exercising.

Long Abstract

Delayed onset muscle soreness (DOMS), also known as exercise induced muscle damage (EIMD), is commonly experienced in individuals who have been physically inactive for prolonged periods of time, and begin with an unexpected bout of exercise¹⁻⁴, but can also occur in athletes who exercise beyond their normal limits of training⁵. The symptoms associated with this painful phenomenon can range from slight muscle tenderness, to severe debilitating pain^{1,3,5}. The intensity of these symptoms and the related discomfort increases within the first 24 hours following the termination of the exercise, and peaks between 24 to 72 hours post exercise^{1,3}. For this reason, DOMS is one of the most common recurrent forms of sports injury that can affect an individual's performance, and become intimidating for many^{1,4}.

For the last 3 decades, the DOMS phenomenon has gained a considerable amount of interest amongst researchers and specialists in exercise physiology, sports, and rehabilitation fields⁶. There has been a variety of published studies investigating this painful occurrence in regards to its underlying mechanisms, treatment interventions, and preventive strategies^{1-5,7-12}. However, it is evident from the literature that DOMS is not an easy pathology to quantify, as there is a wide amount of variability between the measurement tools and methods used to quantify this condition⁶. It is obvious that no agreement has been made on one best evaluation measure for DOMS, which makes it

difficult to verify whether a specific intervention really helps in decreasing the symptoms associated with this type of soreness or not. Thus, DOMS can be seen as somewhat ambiguous, because many studies depend on measuring soreness using a visual analog scale (VAS)^{10,13-15}, which is a subjective rather than an objective measure. Even though needle biopsies of the muscle, and blood levels of myofibre proteins might be considered a gold standard to some⁶, large variations in some of these blood proteins have been documented^{6,16}, in addition to the high risks sometimes associated with invasive techniques.

Therefore, in the current investigation, we tested a thermal infra-red (IR) imaging technique of the skin above the exercised muscle to detect the associated muscle soreness. Infra-red thermography has been used, and found to be successful in detecting different types of diseases and infections since the 1950's¹⁷. But surprisingly, near to nothing has been done on DOMS and changes in skin temperature. The main purpose of this investigation was to examine changes in DOMS using this safe and non-invasive technique.

Protocol

The Exercise

The muscle of interest for this experiment was the elbow flexors (Biceps Brachii). Muscle strength was measured for each participant to be able to give each individual an appropriate resistance. This was determined by testing each participant for their resistance maximum (RM). For testing the RM, we used a strain gauge device interfaced with a computer through a BioPac (DA-100C) bioelectric amplifier module (BioPac Systems, Goleta, CA) to measure muscle strength. The module was connected to an MP-

100 analog to digital converter sampling at a frequency of 1,000 hertz per second, and at a resolution of 24 bits (Fig. 7).

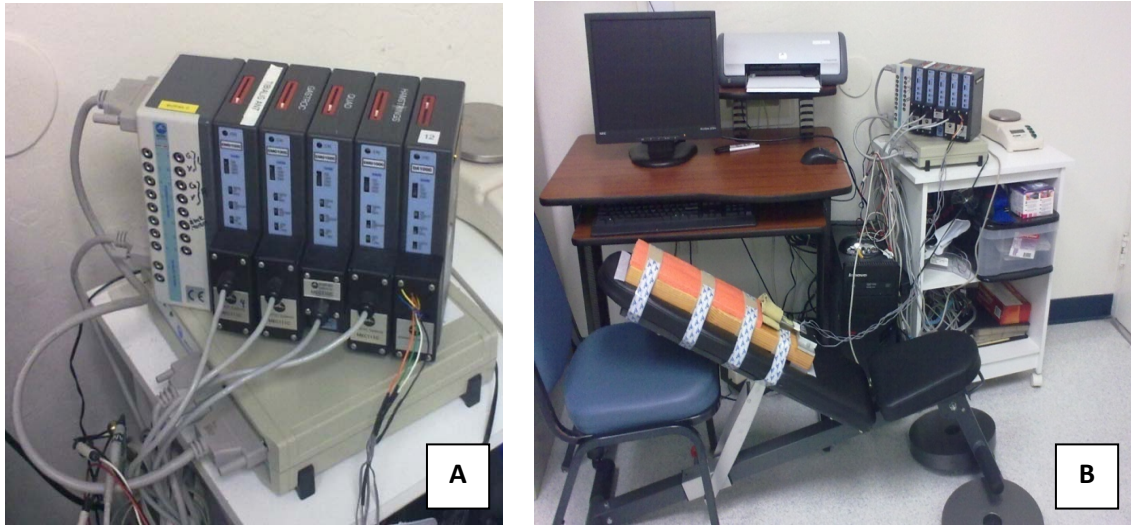


Figure 7. A) The BioPac Modules used for measuring the muscle strength. B) The strain gauge device fixed to a 45° angled bench and hooked to the BioPac system.

The strain gauge device was fixed to a bench at a 45° angle. The subjects were instructed to sit behind the device and rest their elbows on the padded area, so that the exertion force is through their wrists. This was the best way to insure that the subject will not recruit any muscle other than the biceps (Fig. 8).



Figure 8. A typical subject exerting force on the strain gauge device.

Strength was determined on 3 occasions with each contraction being 3 seconds in duration with approximately 45 seconds separating the contractions. The average of the 3 measurements was the RM. After determining the RM for the biceps muscle of each participant, the intended session of exercise was carried out with 35% of their RM. All the subjects underwent the same exercise using appropriate weighted dumbbells to induce the muscle soreness (DOMS). This was carried out by doing 4 sets of 25 repetitions of biceps concentration curls while seated on a chair, and with the elbows supported on their thighs (Fig. 9)

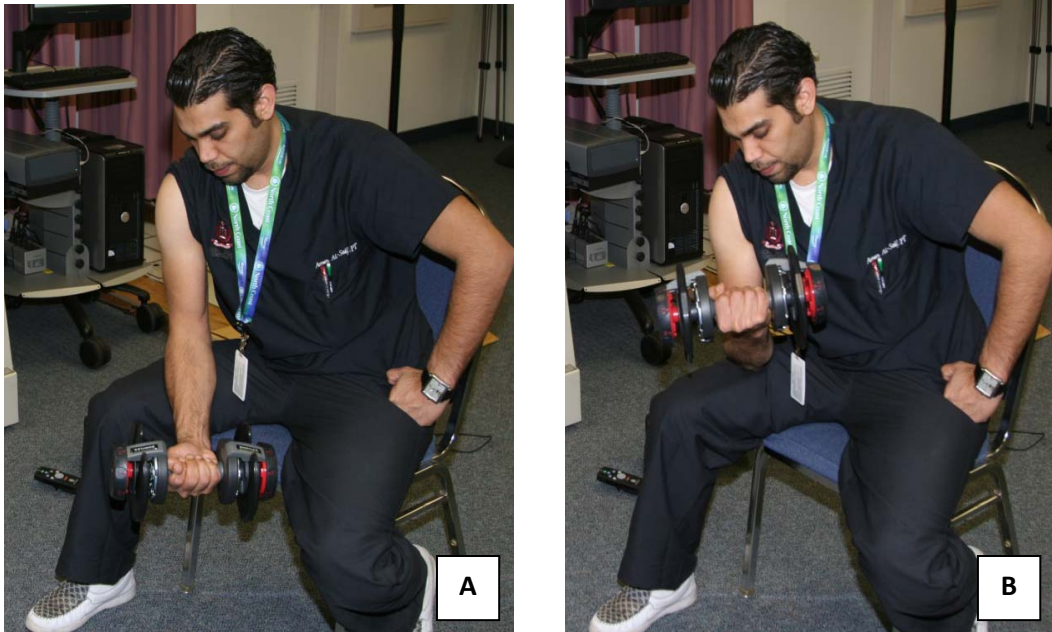


Figure 9. A subject undergoing the exercise protocol for inducing muscle soreness.

Each subject was given a 90 second resting period between each set. Subjects either did the full set of 25 repetitions, or were instructed to stop if they failed to steadily control the weight during the exercise.

Infra-Red Camera Preparation & Setup

The room where the infra-red imaging takes place was set at a constant temperature to minimize any external bias from differences in room temperature, which could lead to false thermal readings. For the purpose of this experiment we had a temperature controlled room which was maintained at approximately 23°C ($\pm 0.5^{\circ}\text{C}$). The camera was set at a distance of 1 meter away, and at a perpendicular angle to the skin being measured. (Fig. 10a)*

After the required distance was set up, the subjects were advised to stand still until the image has been taken. This shouldn't take more than a couple of seconds, but it is very critical to minimize movement to insure the accuracy of the taken image. It is preferable that the room has darker colored paint, rather than lighter colors, to minimize any infra-red interference. Lighting is also critical when dealing with infra-red images, because light source that emit infra-red waves like fluorescent or tungsten lighting could give false high readings. The best lighting option would be a room equipped with uniform LED lights, as LED lights hardly produce any infra-red interference. (Fig. 10b) *

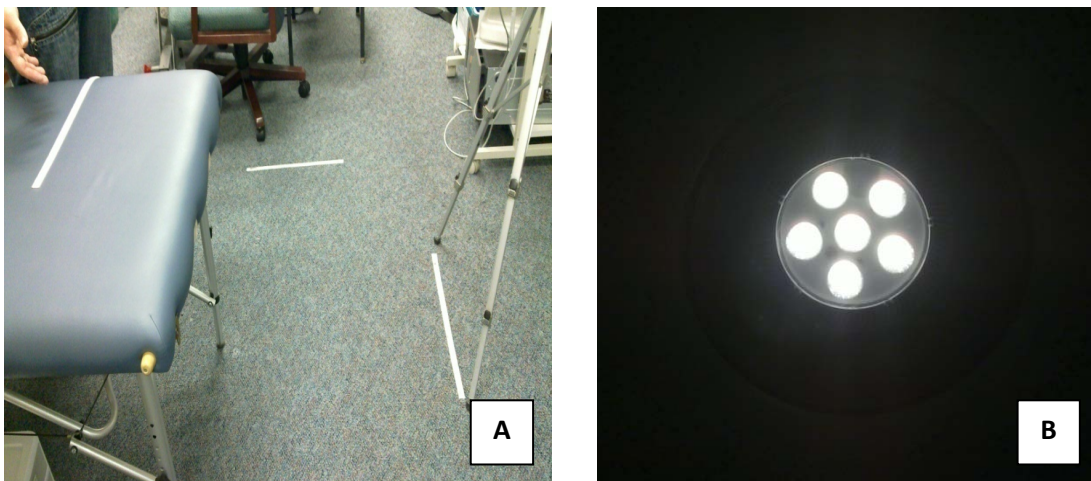


Figure 10. A) The Setup of the IR Camera 1 meter away from the subjects arm. B) The LED lights used in the lab where the images were taken.

* A series of tests were done at our labs using the FLIR 660 IR Camera (Fig. 11), where we compared images of the skin at different angles (0 (perpendicular), 15, 30, 45, and 60 degrees), and at different distances (1, 2, and 5 meters) from the skin, to accurately detect the temperature of the skin. All images were compared to calibrated thermocouples, and the best correlation between the images and the thermocouple readings was at a

perpendicular angle and at a distance of 1 meter away from the skin ($r = 0.93$). The different angles and distances caused a pixilation loss, and decreased the overall correlation between the images and the thermocouple readings.



Figure 11. The IR thermal camera used for this investigation (FLIR 660)

Image acquirement

For the purpose of this experiment, the image of the exercised muscle was taken before the exercise, and at 24, and 48 hours post exercise. Body heat from sources other than the target could disrupt the thermal image and give false readings. For this reason, no one should be standing beside or behind the intended target. In this investigation, pictures of both the exercised and non-exercised arm were taken for comparison purposes. We exercised one of the arms, as was mentioned previously, and the other arm was used as a control. (Fig. 12 and 13). Image numbers from the IR camera were recorded immediately on a separate spreadsheet, as it could be difficult to identify which image belongs to whom.

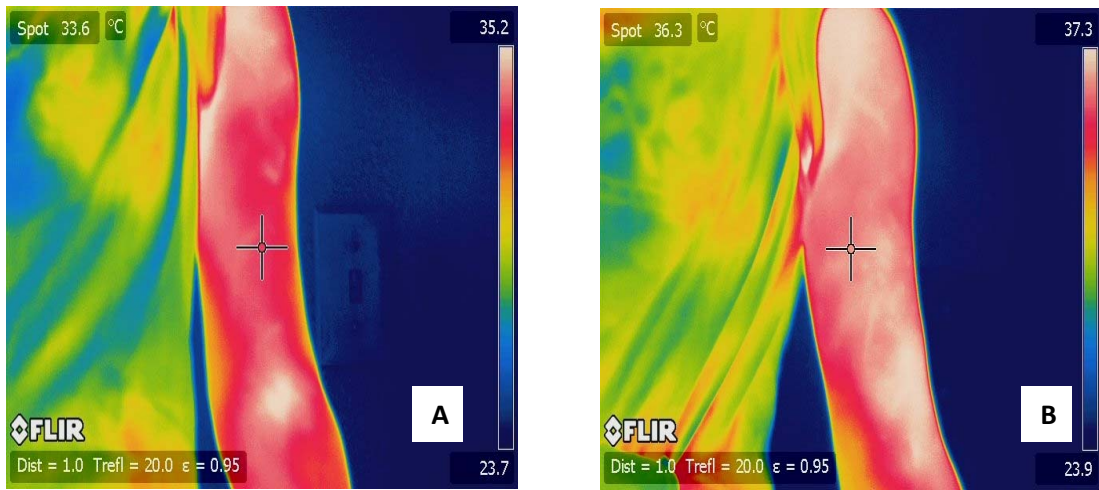


Figure 12. A) a typical IR image of a subject's **exercised** arm before the exercise. B) an IR image of the same subjects arm 24 hours after the exercise

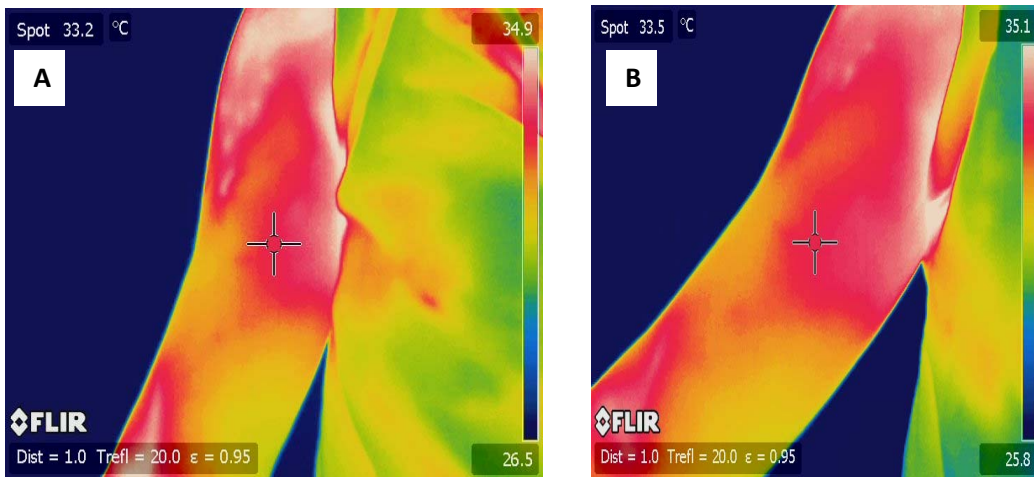


Figure 13. A) a typical IR image of a subject's **un-exercised** arm before the exercise. B) an IR image of the same subjects arm 24 hours after the exercise.

Image Processing & Analyses

The acquired IR images were processed using the “ThermoVision® ExaminIR™” software Version: 1.10.2. After selecting the required image for analysis, four regions of interest were identified on the acquired image of the arm using statistical boxes on the software interface. (Fig.14)

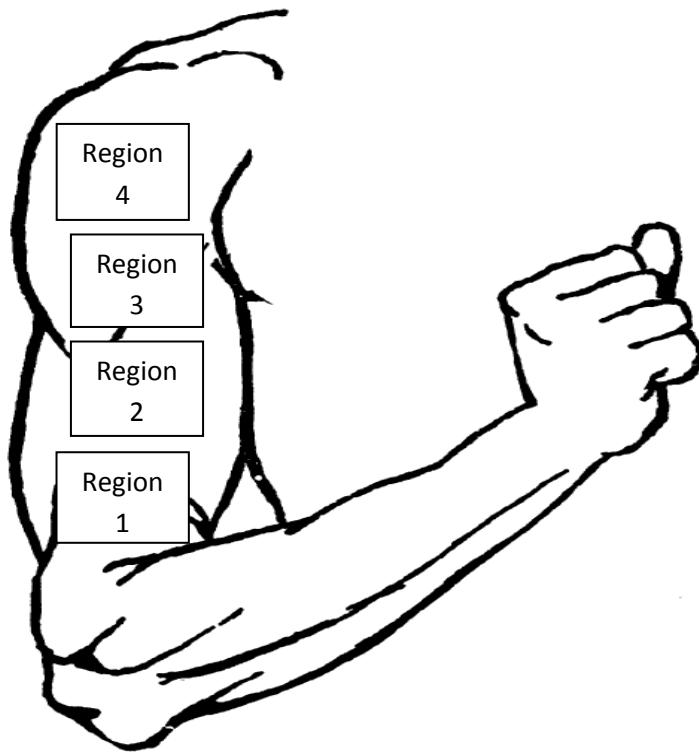


Figure 14. An illustration of the 4 regions of interest for analyzing the thermal image of the arm

When the required regions across the arm have been located, the software shows the Means and Standard Deviations of the temperatures for each of the selected regions.

We can then either cross compare each region individually or obtain an average temperature of the whole arm. (Fig. 15)

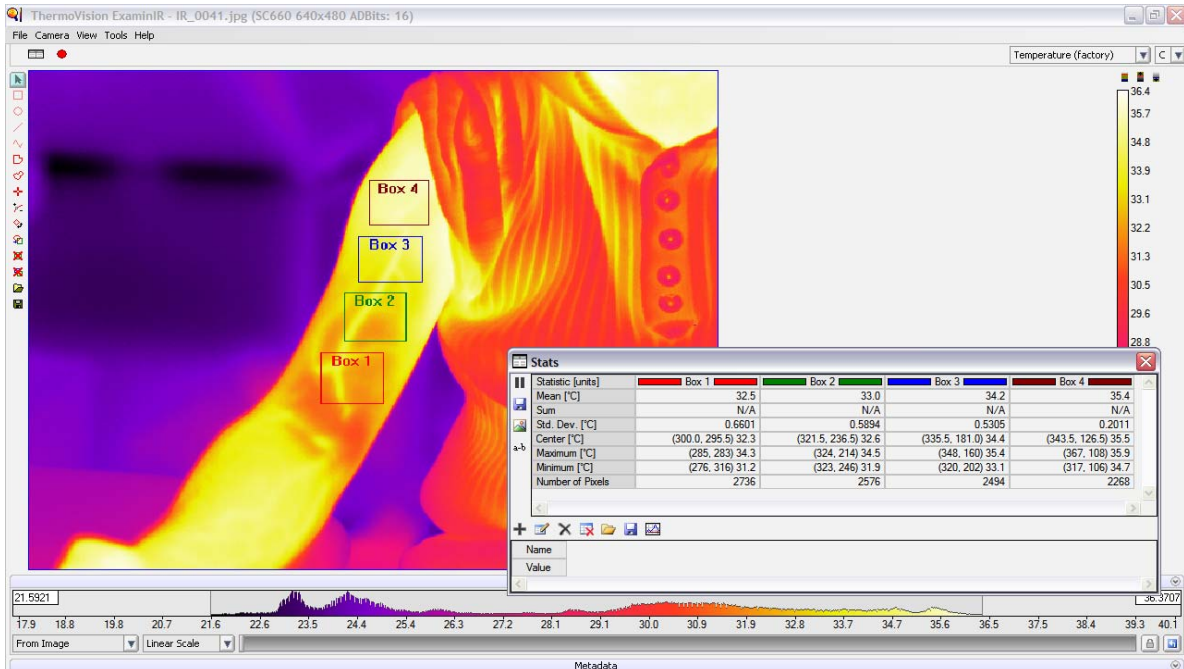


Figure 15. Software interface for the “ ThermoVision[®] ExaminIR[™] ” showing the 4 boxes of interest on an IR image of an exercised arm. Also shown are the statistical interpretations for each box.

Visual Analog Scale and Blood Analysis

Visual analog scales (VAS) were used to assess subjective soreness of the arm. The scale had a 10 cm (100mm) long line marked “no pain” at one end, and “extremely sore” at the opposite end. Each participant was directed to make a mark along the 10 cm line to indicate their response to soreness. The VAS’s were administered to the subjects before the exercise, 24 hours after the exercise, and at 48 hours. Peripheral blood was collected from the subjects to measure myoglobin concentration levels in the blood.

The blood was drawn from the subjects antecubital vein before the exercise, 30 minutes after the exercise was over, and at 48 hours. The blood was centrifuged at 4000 rpm for 10 min to separate the serum from the cells. The samples were then stored at -80°C until the analyses of myoglobin was done. Myoglobin was measured using a TOSOH “AIA®-360” Automated enzyme Immunoassay Analyzer (TOSOH Corp., Tokyo, Japan). The myoglobin Assay kits (Myo 025297, ST AIA-PACK Myoglobin) were used according to the manufactures instructions.

Representative Results

The results of IR thermal images taken during this investigation are clearly represented in Figure 16. Images taken at the 3 time periods (pre-exercise, 24 hours post-exercise, and 48 hours post-exercise) for the exercised arms of the 41 subjects, showed a noticeable increase in temperature on day 2 (24 hours post-exercise) when compared to pre-exercise temperatures, and temperatures taken at 48 hours. As shown in Figure 1, the average skin temperature was 32.80 +/- 1.03 °C for day 1 (pre-exercise), and 33.96 +/- 1.46 °C for day 2 (24 hours post-exercise), and 32.82 +/- 1.29 for day 3 (48 hours post-exercise). This difference in skin temperature from day 1 to day 2 was significant (ANOVA $p < 0.01$).

However, for the un-exercised arm, changes amongst the 3 time periods were not evident. Figure 16 shows that the average skin temperature was 33.08 +/- 0.83 °C for day 1 (pre-exercise), and 32.79 +/- 1.42 °C for day 2 (24 hours post-exercise), and 33.17 +/- 0.95 for day 3 (48 hours post-exercise). This difference in skin temperature over the 3 days was not significant (ANOVA $p = 0.38$).

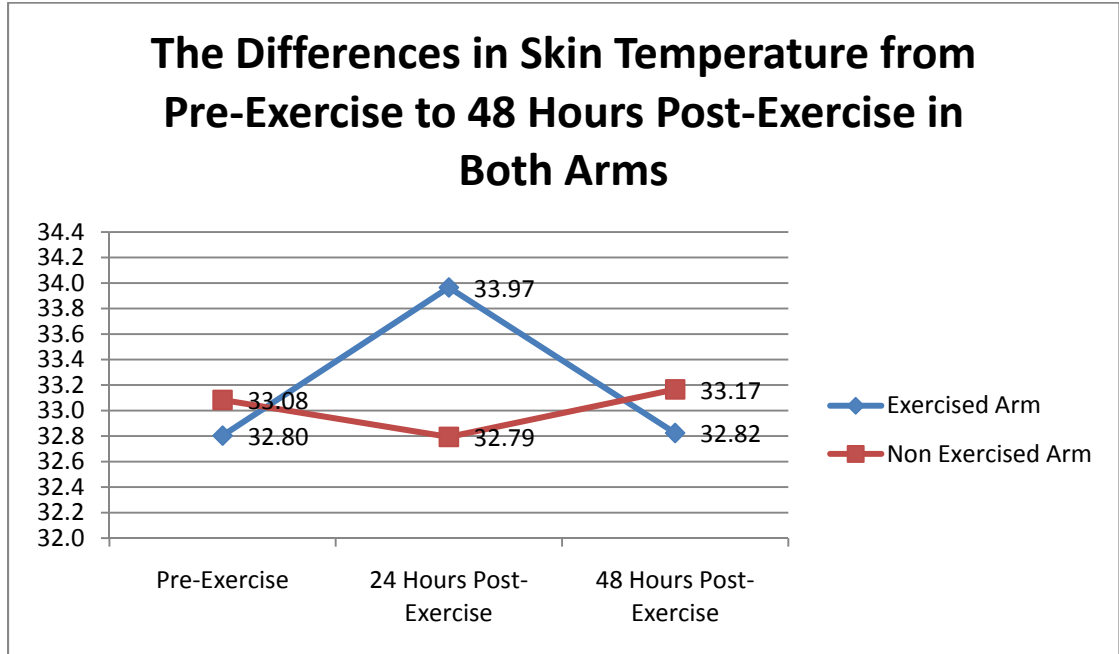


Figure 16. A representative graph of the differences in skin temperature in the exercised arms (Diamonds), and un-exercised arms (Squares) of the 41 subjects over the 3 day time period.

The results of the pain readings from the VAS are shown in Figure 17. As seen in Figure 17, the reported pain had a dramatic increase on days 2 and 3. Pain levels of the exercised muscle increased from 3.6 +/- 6.1 on day 1, to 36.3 +/- 22.8 on day 2, and 37.5 +/- 25.3 on day 3. This increase from day 1 was significant (ANOVA $p < 0.01$).

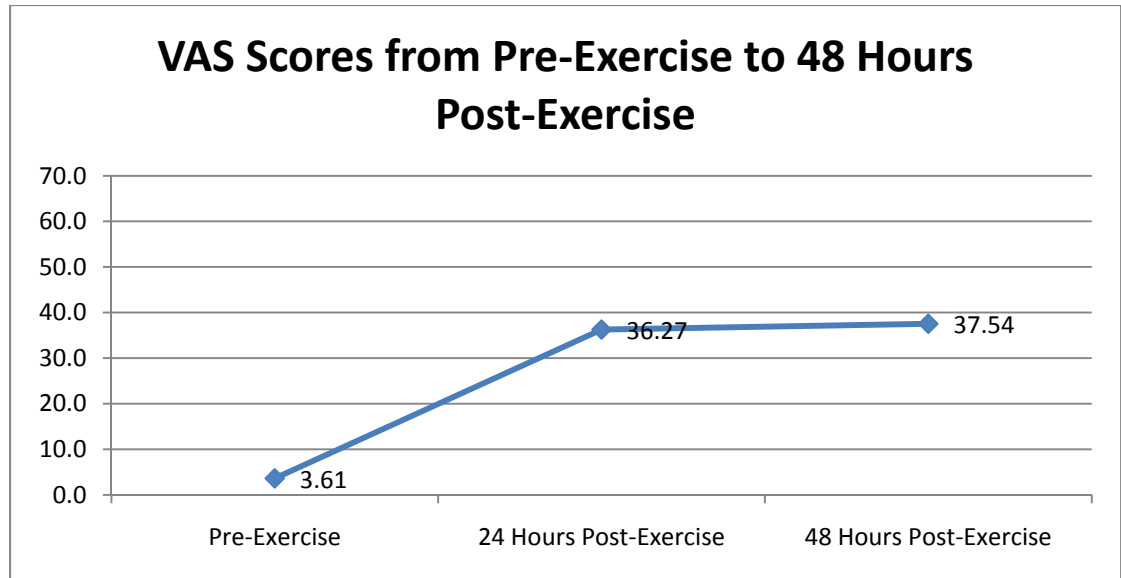


Figure 17. A representative graph of the differences in perceived muscle soreness measured with the VAS over the 3 day time period for all the 41 subjects.

The results of the myoglobin concentration levels are shown in Figure 18. As seen in this figure, there was hardly any change between the 2 myoglobin concentrations on day 1 (pre, & 30 minutes post exercise). But on day 3, the increase in myoglobin was very large. This increase on day 3 was approximately 147 nanograms per milliliter (ng/mL) of blood when compared to the first 2 concentrations on day 1. Myoglobin concentrations were 30.12 +/- 7.66 ng/mL at baseline, 31.66 +/- 11.89 ng/mL 30 minutes post exercise, and 178.96 +/- 249.51 ng/mL on day 3. This increase on day 3 was highly significant (ANOVA $p < 0.01$).

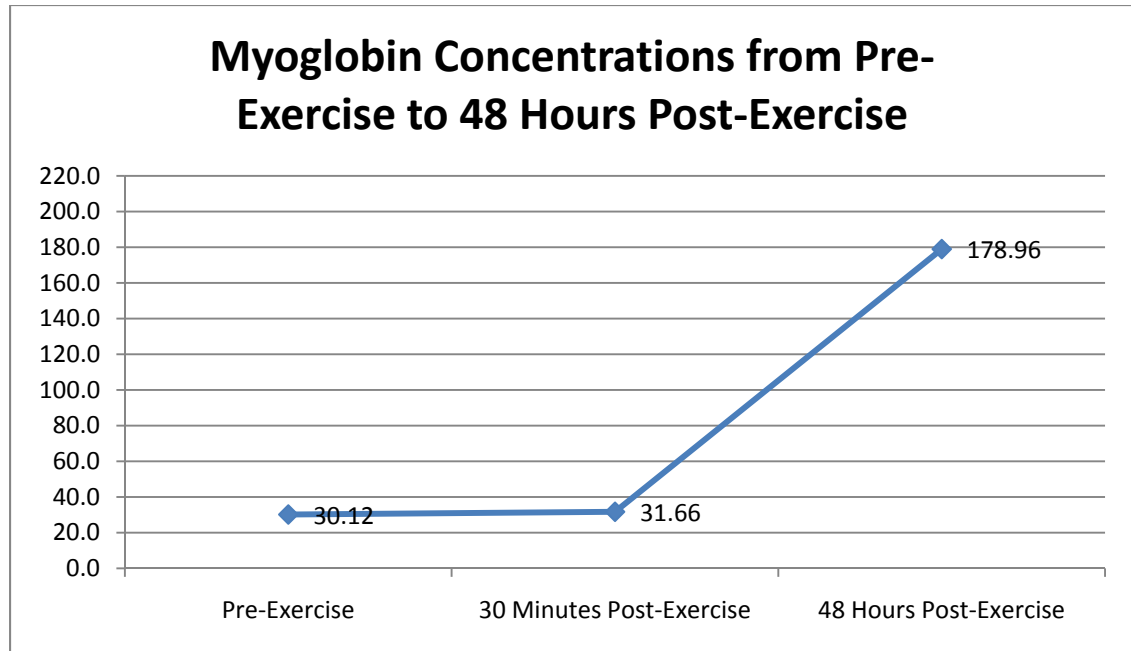


Figure 18. A representative graph of the differences in myoglobin concentrations for all the 41 subjects over the 3 time periods.

A correlation analysis was done between the skin temperatures obtained from the IR images, and the VAS soreness levels. It was found that there was a considerable correlation between the VAS readings on day 2, and the skin temperature measurement on day 2. This correlation was significant ($r = 0.312$, $p < 0.05$). However, there was no evident correlation between the VAS readings and the skin temperatures on day 3. This correlation was insignificant ($r = 0.047$, $p = 0.77$).

Discussion

The primary purpose of this investigation was to assess the usefulness of thermal IR imaging in detecting and measuring muscle soreness after strenuous exercise, and our results suggest that IR imaging could be a valid technique for detecting DOMS,

especially within the first 24 hours of exercising. This is not surprising, as Pennes¹⁸ provided a very detailed model of heat flow from muscle to skin in limbs. This model predicts that heat in deeper tissues such as muscles can be dissipated into blood and into the skin through conductive heat exchange. When muscles exercise, obviously, tremendous heat is developed in the muscle due to frictional forces of the muscle fibers and because of the increased metabolism. Increased blood flows to the muscle would also contribute to the increased heat in the muscle after exercising. Because muscle is a shell tissue, the temperature is usually 32 to 33 °C, however when blood flow is increased to the exercised muscle it approaches the temperature of the core tissues which is 37°C^{18,19}. This increased blood flow should go back to normal within a couple of hours after the exercise termination. But when temperatures are still elevated at 24 hours post exercise, this shows that damage has happened to the exercised muscle. This damage in the muscle causes additional heat transfer from the muscle to the overlying skin, which causes a detectable hot spot under the skin.

IR imaging has been used to detect and diagnose many diseases^{17,19-24}. Changes in the human body's temperatures have always been indicators of dysfunction, where increased heat is mainly associated with some sort of inflammation or infection¹⁷. Thus, the elevated skin temperatures found 24 hours post exercise in all subjects is possibly the result of higher blood flows in muscle due to inflammation, and tissue damage repair². Also, no noticeable increases in the skin temperature of the un-exercised arm occurred during the 3 days of the study. Therefore, if muscle blood flow remained elevated in the exercised arm, the warmer blood would keep the muscle warm and hence the overlying skin would stay warm. As a result, breast tumors in women and skin cancer can be easily

detected by IR imaging because of the increased blood flows to the affected lesion site^{20,21}.

The elevated soreness levels (VAS) on day 2 and 3, and the increased myoglobin concentrations on day 3 are both indicators of DOMS. This shows that the subjects did get sore after the exercise session. As was seen in the results, there was a relationship between the increased skin temperatures on day 2, and the increased soreness levels on that same day. Myoglobin concentrations were still elevated on day 3, while skin temperatures were returning back to normal. This delayed release of myoglobin into the blood, could be due to the muscles slower response to inflammation and damage that occurs in the muscle fibers after exercise^{25,26}.

However, 3 to 5 days later, there can still be minor tissue repair and reconstruction, even though the blood flow maybe close to normal. That's why on the 3rd day we did not see a correlation between skin temperature and soreness levels, because the damage was already done. Therefore, we believe that this is predictive of soreness, because it shows that if you get sustained 24 hours increase in tissue blood flow, then you know that you've got damage to the tissue. This damage was verified by the VAS readings, and the myoglobin concentrations in the blood. Thus, the higher the skin temperature readings 24 hours post exercise, the sorer the subject would be later on.

Hence, IR thermal imaging would be of great value in detecting DOMS in its early stages. It would also be an interesting, and painless way of looking at muscles that have been exercised and are sore, hours after the exercise is over. In a sports setting, this early detection of DOMS could help in lowering the incidence of injuries from over-exercising sore muscles on days following the initial exercise.

Acknowledgements

We wish to acknowledge a contract (WS1763368) from Pfizer Pharmaceuticals for support in this work. We would also like to thank the Saudi Arabian Ministry of Higher Education (MOHE) for their support.

Equipment Used

Several devices were used throughout this study, and Table 2 below shows specific model information for these devices.

Table 2: Table of specific equipment.

Name of the Device	Company	Catalogue number	Comments (optional)
Infra-Red Thermal Camera	FLIR	FLIR SC660	
Thermal Infra-Red Analysis Software	ThermoVision ® ExaminIR™	Software Version 1.10.2	
Bioelectric Amplifier Module	BioPac	DA100C	The DA100C provides variable gain settings, and adjustable voltage references.
Analog to Digital Converter Module	BioPac	MP100	
Automated enzyme Immunoassay Analyzer	TOSOH	AIA -360	This device was used to analyze the blood samples, and obtain the myoglobin readings.

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CHAPTER FOUR

THE EFFECTS OF BRANCHED-CHAIN AMINO ACID SUPPLEMENTATION ON DELAYED ONSET MUSCLE SORENESS IN PEOPLE WITH DIABETES

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Abstract

For individuals with diabetes, exercise is important for maintaining proper glycemic control. However, exercise induces muscle soreness, making it problematic for these individuals to maintain exercise regimes. In younger non-diabetic individuals, studies have shown that nutritional supplementation including branched chain amino acids (BCAA), enhance muscle recovery. The purpose of this blinded study was to assess the effects of BCAA supplementation on delayed onset muscle soreness (DOMS) in diabetics compared to healthy controls. Forty-four subjects; 28 healthy, and 16 with diabetes, were randomly assigned to either the BCAA or placebo group. Measurements including muscle strength, electromyography response of the biceps muscle during maximum effort, perceived soreness, serum myoglobin concentration, elbow range of motion, and skin temperatures were collected before the biceps exercise session and for the five following days. The results showed that while serum myoglobin concentrations increased at 48 hours for both the healthy and diabetic subjects, it was significantly higher ($P = 0.013$) in the diabetic subjects who took the placebo supplement (pre-exercise = 54.5 ± 8 ng/mL, 48 hours = 767.1 ± 189 ng/mL) when compared to those who took the BCAA supplement (pre-exercise = 51.4 ± 7 ng/mL, 48 hours = 158.1 ± 62 ng/mL). Pain was 79.6% higher in the diabetic group who took the placebo when compared to the BCAA group ($P = 0.017$). In conclusion, BCAA supplementation reduced muscle damage and muscle soreness in diabetic subjects, whereas they had minimal effects on the healthy control subjects.

Keywords: BCAA, DOMS, Diabetes, Exercise

Introduction

The diabetes epidemic is on the rise in both developing and developed countries [1,2]. According to the World Health Organization (WHO) and recent estimates, the disease now affects approximately 345 million people worldwide and is expected to affect around 440 million by 2030, representing almost 8% of the global adult population [3,4]. Lifestyle characteristics such as obesity, a sedentary lifestyle, and lack of physical activity are regarded as the most important risk factors, both independently associated with diabetes and diabetes related co-morbidities [5,6].

Exercise is considered a major cornerstone of diabetes management. Studies have shown that exercise is recommended as part of the treatment and prevention of type 2 diabetes mellitus (T2DM) [7,5,8]. Even though aerobic exercise has beneficial effects for managing and treating T2DM, it has been found that resistance-type exercise is also effective in controlling blood glucose, and in reducing Hemoglobin A1c (HbA1c) levels [7,9-11]. It is best for individuals with diabetes to participate in a combination of both aerobic and resistance exercise to properly manage their blood glucose levels. The effects of combined exercise have shown more pronounced outcomes on glycemic control, muscle strength, and insulin sensitivity [7,9,12]. A study done by Church and colleagues [9], showed that a combination exercise group decreased their diabetes medication dependency, when compared to a control group who had increased their use of diabetes medications. It would be imperative then, to include resistance training as a primary step in the exercise schedule of a diabetic patient. This is particularly important for obese individuals, since aerobic exercise may be challenging for them.

Resistance training would be more beneficial for individuals with diabetes to begin with, as it contributes to the recruitment of previously inactive muscle fibers, which

would enhance the quality of the muscle, and lead to gains in muscle mass [13,7,14]. This would result in increased whole body insulin sensitivity, and improved glucose control [13,7,5].

Delayed onset muscle soreness (DOMS) however, is a phenomenon that occurs in skeletal muscle as a result of novel or unaccustomed exercise [15-17]. The severity of the damage and the extent of discomfort is exacerbated over time, and occurs minutes to days after the acute exercise bout [15,16]. The intensity of the symptoms and discomfort associated with DOMS usually peaks between 24 to 72 hours post exercise, and can last for up to 10 days, especially when the exercise bout encompasses an eccentric component [17-19]. Activities that are comprised of repeated eccentric contractions have been shown to result in damage to the ultrastructure of skeletal muscle[20,21]. This damage manifests itself as a temporary decrease in muscle function, restricted range of motion in the associated joint, increased muscle soreness, an increased swelling of the involved muscle group, and an increase in intramuscular proteins in blood such as creatine kinase, and myoglobin[22,23,20]. Consequently, DOMS is considered one of the most common recurrent forms of injury, which can lead to further injuries if a premature return to exercise is attempted [16]. For this reason, pain associated with DOMS can reduce an individual's willingness to exercise.

Not much is known about DOMS in people with diabetes. However, Studies have shown that older individuals have reduced proteolytic activity and an elevated production in free radicals [24,25]. This elevation in free radicals prolongs healing time after excessive exercise [24]. With higher levels of free radicals, metabolic impairments, and

endothelial dysfunction, in people with diabetes [26,27], DOMS has been found to be more severe in this population [26,28].

As a result of these observations, methods to alleviate the severity of DOMS, and to minimize the damage resulting from resistance exercises have been investigated. Treatment strategies including massage (depending on the time and type of massage) [29-31], water baths [32,33], and the administration of non-steroidal anti-inflammatory drugs (NSAID) [34-36] have shown varying results. Other treatment strategies such as cryotherapy, stretching, homeopathy, and ultrasound have shown minimal to no effect on the improvement of muscle soreness [15,37,38,16,39,40]. However, in regards to acute nutritional interventions, there is evidence that suggests ingesting protein supplements which are high in branched chain amino acids (BCAA) can attenuate muscle damage, reduce muscle soreness, and promote enhanced recovery of muscle function [41,22,23].

Resistance exercise causes decreases of some of the essential amino acids, including BCAA, during the recovery period after the exercise [42,43]. Resistance exercise then, alters the turnover of muscle protein by increasing the amino acid transport within the muscle [43]. This enhances both the rates of skeletal muscle protein synthesis, and protein breakdown during the recovery period following even a single bout of exercise [44,43]. Skeletal muscle however, remains in an overall catabolic state unless muscle protein synthesis surpasses muscle protein degradation, and for this to take place, adequate nutritional intervention is necessary during this recovery period [44,45]. Thus, when essential amino acids (EAA) are ingested in conjunction with an acute bout of resistance training, the hypertrophic response is enhanced resulting in a net increase in muscle protein synthesis, thereby improving performance [22,46].

Among these EAA, exceptional results have been observed with BCAA, and specifically leucine [46,47]. Studies have indicated that BCAA are mainly used by active skeletal muscles during submaximal exercise, whereas most other amino acids are metabolized in the liver [42,48].

Recent findings suggest that BCAA can be safely consumed in large doses with no adverse effects in healthy adults, when compared to other amino acids in protein [49,50]. Furthermore, the ingestion of acute doses of BCAA supplements containing all 3 BCAA (leucine, isoleucine, and valine) appears to be well tolerated by adults, because human cells have a tightly controlled enzymatic system for BCAA degradation [50,47].

Numerous studies have investigated the effects of BCAA supplementation on skeletal muscle soreness. Some found that BCAA may be useful for muscle recovery following acute sessions of exercise, both in trained and untrained individuals [22,51,45,52,23]. In contrast, other studies showed no effects of BCAA ingestion on DOMS [53,46]. However, no study to date has assessed the effectiveness of BCAA supplements on DOMS in a diabetic population. Thus, the purpose of this study was to examine the effectiveness of BCAA supplementation on reducing DOMS. We hypothesized that BCAA supplementation would be beneficial in alleviating DOMS by reducing muscle damage, and maintaining the functional performance of the exercised muscle.

Subjects and Methods

Forty four subjects participated in this study. There were 2 groups, one group of healthy individuals which consisted of 28 subjects, and another group of 16 diabetic

subjects. The participants in these 2 groups were randomly assigned into either the experimental group, who ingested the BCAA supplement, or the placebo group. All the subjects were physically inactive for at least 3 weeks prior to participating in the study. Subjects were excluded if they were pregnant, had hepatic diseases, were diagnosed with Rhabdomyolysis, or an impaired circulatory disease (such as Raynaud's), had any recent upper limb injuries, had severe neuropathies in their upper limbs, were hypertensive (blood pressure over 145/95 mmHg), or were on high doses of alpha or beta agonist/antagonists, Cox 2 inhibitors, calcium channel blockers, or pregabalins. Also, subjects were advised not to take any pain reducers, NSAID, or dietary supplements during the course of the study. The average age, height, weight, and demographics of the subjects are shown in Table 3. Subjects were informed of all experimental procedures and protocols and signed a statement of informed consent as approved by the Institutional Review Board (IRB) of Loma Linda University.

Table 3: Means (+/- SEM) of the general characteristics of the Subjects

Subject Type	Supplement	N	Age (Years)	Height (Cm)	Weight (Kg)	BMI	HbA1c (%)	Diabetes Duration (Years)
Healthy Individuals	BCAA	14	28.2 (+/- 2.0)	168.3 (+/- 2.1)	72.9 (+/- 4.7)	25.2 (+/- 1.3)		
	Placebo	14	28.07 (+/- 2.1)	168.0 (+/- 2.3)	72.7 (+/- 5.0)	25.5 (+/- 1.4)		
Diabetic Individuals	BCAA	8	60.6 (+/- 5.7)	166.7 (+/- 5.5)	98.7 (+/- 11.8)	34.8 (+/- 3.0)	7.5 (+/- 0.8)	7.7 (+/- 2.3)
	Placebo	8	61.9 (+/- 3.3)	168.5 (+/- 4.9)	118.9 (+/- 15.5)	41.3 (+/- 4.2)	6.7 (+/- 0.4)	5.5 (+/- 1.9)

Study Design

This study was a double blinded randomized controlled trial. The subjects and the examiners were blinded to the supplement taken, and the subjects were randomly allocated into either the experiment group, or the placebo group. All measurements were taken at 6 time periods: a baseline measurement taken pre-exercise (baseline), and post-exercise measurements at 30 minutes (day1), 24 hours (day2), 48 hours (day3), 72 hours (day4), and 96 hours (day5). The serum myoglobin measurements were taken at 5 occasions, where all measurements were taken at the same time periods except for day2 (at 24 hours).

The Supplement Protocol

The subjects in the experimental group took 3 doses of the BCAA supplement. The first dose was taken 30 minutes before the workout, the 2nd dose was taken immediately after completing the workout, and the 3rd dose was taken on the 2nd day (24 hours post exercise). The amount of BCAA supplement administered to the subjects in the experimental group on day 1 was a total dose of 1 gram of BCAA per kilogram of lean body mass (a bioelectric impedance analyzer was used to measure lean body mass as described below). This dose was divided in half and administered at the two specified occasions for the 1st day. The 3rd dose (on day 2) was equivalent to 0.8 grams per kilo of lean body weight, and was administered before any measurements were taken on that day. The ratio of isoleucine, leucine, and valine used in this study was 1:2.5:1 respectively. The subjects in the placebo group were administered 3 doses of a placebo supplement at the exact same times as above. The placebo supplement consisted of cellulose powder, which has no effects on skeletal muscle. Both the BCAA and the placebo supplement were mixed in a low calorie fruit flavored drink. The tastes of both the BCAA and placebo drinks were identical. Two teaspoons of artificial sweetener was added to both supplements to mask the slight bitterness of the supplements.

The Resistance Maximum (RM) and Muscle Strength (MS) Testing

A strength measuring device with 4 strain gauges placed on opposite sides of a steel bar (arranged as a Wheatstone bridge) was used to measure each individuals resistance maximum (RM) and muscle strength (MS). When the bar bends, the strain gauges get deformed and an electrical output is provided. This device was interfaced with

a computer through a BioPac (DAC-100) bioelectric amplifier module (BioPac Systems, Goleta, CA). This module was connected to a BioPac MP-150 analog to digital converter sampling at a frequency of 1,000 Hz per second, and at a resolution of 24 bits. The output signal was amplified 5,000 times. Data analysis and storage was done on Acknowledge 9.1 software from BioPac (BioPac Systems, Goleta, CA). This method has also been described elsewhere [28].

The device was fixed to a bench at a 45° angle, so that only the biceps would be recruited. Subjects sat behind this bench with their intended arm aligned to the device. The strap attached to the strain gauge device was placed on the subject's wrist, and an examiner instructed each subject when to exert their maximal force and when to relax (Fig. 19). Strength was determined on 3 occasions with each contraction being 3 seconds in duration and approximately 1 minute apart. The average of these 3 measurements was considered their maximum strength. The baseline (pre-exercise) MS measurement was also considered the subjects' RM. After determining the RM for the biceps muscle of each subject, the intended session of exercise was done with 35% of their RM.

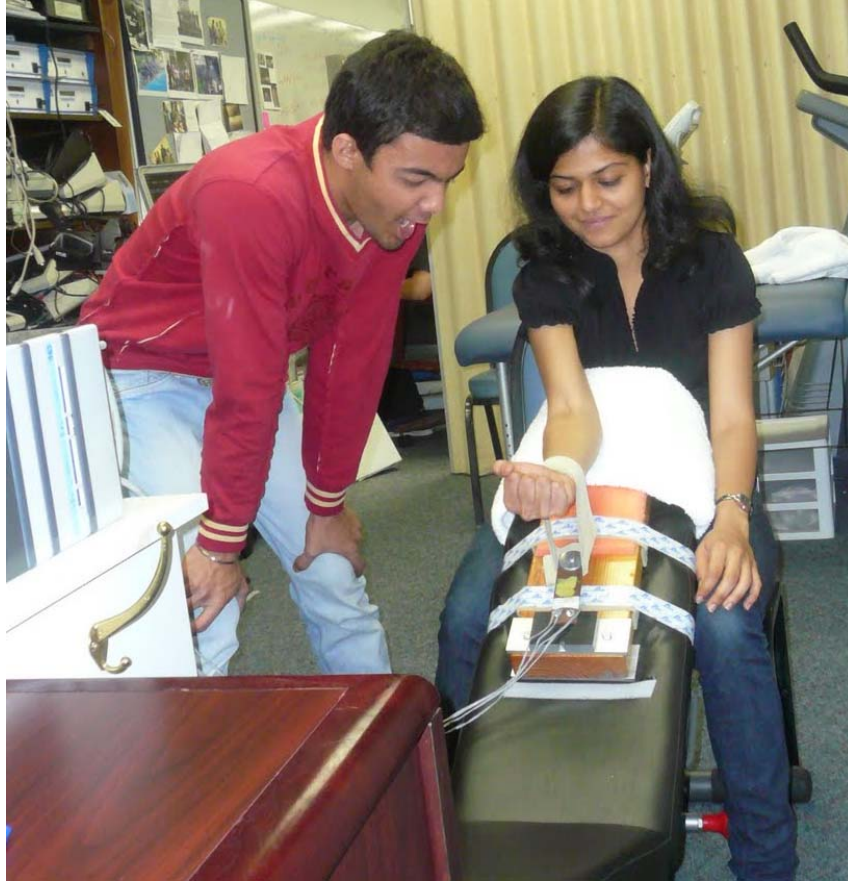


Figure 19: A typical subject setup for the repetition maximum (RM) and muscle strength (MS) measurements while the examiner is giving verbal cues. Also seen here is the strain gauge device with the wrist strap, securely attached to the 45° angled bench.

Electromyography (EMG) Assessment

Surface EMG was recorded from the exercised biceps muscle and sampled by 2 bipolar vinyl adhesive EMG electrodes with an active surface area of 0.5 cm². One electrode was placed on the mid-belly of the biceps brachii muscle and the other electrode was placed immediately distal to it. The position of both electrodes was immediately marked with permanent ink to ensure consistent placement on subsequent testing days. The ground electrode was attached to the forearm, and the position was also marked with permanent ink. The electrodes were connected to a BioPac (EMG-100B)

electromyogram amplifier module (BioPac Systems, Goleta, CA), which was interfaced with a computer. This module was connected to a BioPac MP-150 analog to digital converter. The raw EMG signal was collected at a frequency of 1,000 Hz per second, at a resolution of 24 bits, and amplified 5,000 times. Before recording, signals were visually inspected to ensure background noise and artifacts were minimized. The EMG measurements were standardized in that all measures were taken during the MS measurements. The EMG readings were recorded at the same time the subjects were instructed to contract their muscles for the MS measurement.

Soreness/Pain Measurements

The short form McGill pain questionnaire (SF-MPQ) has been identified as a reliable measure of pain [54,55], and was used to assess subjective soreness of the arm muscles on all days of the experiment. The benefit of this scale over the typical visual analog scale is that this scale is divided into 4 simple sections (a sensory pain rating index, an affective pain rating index, a present pain intensity rating, and an evaluative overall intensity of total pain), which gives a better overview of the type and intensity of the perceived pain. The subjects placed a check mark on the appropriate columns of each type of pain, to indicate their response to soreness.

Relaxed Elbow Range of Motion (RRoM)

Measurements of elbow resting angles were assessed using a universal goniometer with subjects positioned in a standardized manner. During all the measurements, the subjects were sitting in an armless chair so that they sat in an erect

position with the trunk supported and the feet on the floor. The arm, shoulder, and trunk were maintained in a neutral position throughout testing. The lines of the humerus and radius were used as standardization points. The lateral epicondyle of the humerus was considered the goniometric axis of movement, and was marked with a semi-permanent pen on all subjects. The reliability of this measurement technique has been demonstrated [56]. The range of motion measured for the purpose of this study was the elbow angle at rest, taken as the angle of the elbow while the arm hung loosely by the subjects side. Each measurement was repeated 3 times, and the average of all 3 measurements was the final measurement used. All measurements were obtained by the same experimenter, using the same goniometer, in order to minimize any measurement error.

Blood Sampling & Measurement of Myoglobin Concentrations

Approximately 4 mL peripheral blood was collected from an antecubital vein. Peripheral venous blood was drawn on all days of the experiment, except day 2. The blood was allowed to clot at room temperature for 10 minutes, before it was spun down in a refrigerated centrifuge at 4000 rpm for 10 min to separate the serum from the cells. The serum samples were then stored at -80°C until the analyses was done.

Serum myoglobin was determined using a TOSOH “AIA®-360” automated enzyme immunoassay analyzer (TOSOH Corp., Tokyo, Japan). The myoglobin Assay kits (Myo 025297, ST AIA-PACK Myoglobin) were used according to the manufactures instructions. Controls were run before and after each assay session, to verify that the measurements were accurate and within the manufacturers specified quality control ranges. The normal reference range for myoglobin using this method was 31.4 - 971

ng/mL. The intra-assay and inter-assay coefficient of variations were 2.76 %, and 4.45 % respectively.

Skin Temperature

Skin temperature was measured using a Flir TC660 Thermal Camera (Stockholm, Sweden). The thermal image taken of the exercised arm was taken from approximately 1 meter away, and perpendicular to the skin. From a series of tests done at our labs using the FLIR 660 IR Camera, this distance and angle were found to have the best correlation ($r = 0.93$) with thermocouple readings. The images were taken in a temperature controlled room which was maintained at approximately 23°C (+/- 0.5°C). The temperatures from the acquired image were measured at 4 locations on the skin above the biceps muscle using the “ThermoVision® ExaminIR™” software Version: 1.10.2. These 4 readings were analyzed individually and then averaged to give a full perspective of the temperature above the exercised muscle.

Lean Body Weight Determination

Lean body weight was determined by electrical impedance with an RJL Systems Quantum 2 Bioelectric Impedance Analyzer (Minneapolis, MN). The unit measured resistance and reactance with 1.0 ohms of resolution. Four electrodes were placed on the body, two source electrodes on the hand and foot and two recording electrodes on the hand and foot. The system placed a current of approximately 0.1 milliamps at 100,000 cycles per second frequency, through the source electrodes. The recording electrodes recorded the signal transmitted through the body, and use this to calculate body fat

content via the software provided by the manufacturer (RJL Systems). The Quantum 2 with the multiplexed cable, allows multi-zone and segmental measurements to be taken quickly and easily in 26 segments to calculate body water and body fat.

Food Dietary Analysis

Diet was assessed at baseline using the Brief Block Food Frequency Questionnaire (FFQ) (Block Dietary Data Systems, Berkeley, CA, www.nutritionquest.com) . This questionnaire has been shown to be a valid tool for assessing different types of nutritional components [57,58].

Procedure

First, demographical data was collected including: height, weight, age, body fat, body mass index (BMI), blood pressure, and medical history. Also, baseline data of all the previously indicated measurements were collected (MS, EMG, SF-MPQ, and RROM), including blood samples for the myoglobin measurement, and the administration of the brief FFQ. The initial dose of the supplement, whether BCAA or placebo, was also administered at this time. The targeted muscles for this experiment were the elbow flexors. The resistance exercise was performed using a dumbbell, and DOMS was only induced in the subjects' dominant arm. To provoke DOMS in these muscles, all subjects carried out 4 sets of 25 repetitions of biceps concentration curls while seated on a chair, and the elbows supported on their thighs. Subjects were advised to lower the weight and lift it at a steady rate (approximately 3 seconds going down, and 3 seconds coming back up), to ensure that the eccentric component of the muscle

contraction was properly done. There was a 90 second resting period between sets, and subjects either did the full set of 25 repetitions, or were instructed to stop the set if they failed to steadily control the descent of the weight and return their arm back to full flexion. As indicated previously, the resistance used was 35% of each subject's RM.

All the primary measurements were repeated after the exercise at 30 minutes, 24, 48, 72, and 96 hours except for the myoglobin measure which wasn't carried out at 24 hours. Also, the 2nd and 3rd dose of the supplements were taken immediately after the exercise and at 24 hours.

Data Analysis

Means, and standard errors (SEM) were calculated. Measurements of all variables (MS, EMG, RROM, SF-MPQ, Myoglobin, and Skin Temperatures) were compared over time between the experimental and placebo groups of each of the 2 main groups (healthy and diabetic) using a mixed factorial (2×6) analysis of variance (ANOVA). When a significant difference over time was found, a paired t-test was performed to determine any significant differences from baseline. Demographic data and the protein intake (from the FFQ analysis) were compared using independent-t tests. Statistical analysis was performed using PASW Statistics Version 18.0.0 (SPSS, Inc., 2009, Chicago, IL, www.spss.com), and the level of significance was set at $\alpha < 0.05$.

Results

Demographics and Protein Intake

There were no significant differences in demographic data between the two treatment groups of the healthy subjects group ($P > 0.05$). Also, no significant difference between the baseline protein intakes was observed. The same results were also seen for the diabetic subjects group, where no significant differences were found between the two treatment groups in regards to demographical data, and protein intake ($P > 0.05$).

Muscle Strength (MS)

There was no significant difference in MS between the two treatment groups in the healthy group ($P > 0.1$). However, there was a difference in MS over time, with a significant decrease in MS at day1 ($P < 0.01$), compared to baseline.

In the diabetic group, there was no significant difference in MS between the two treatment groups ($P > 0.1$). However, there was a significant decrease in MS at day1 ($P < 0.05$), compared to baseline. Figure 20 shows the MS response for all 4 groups.

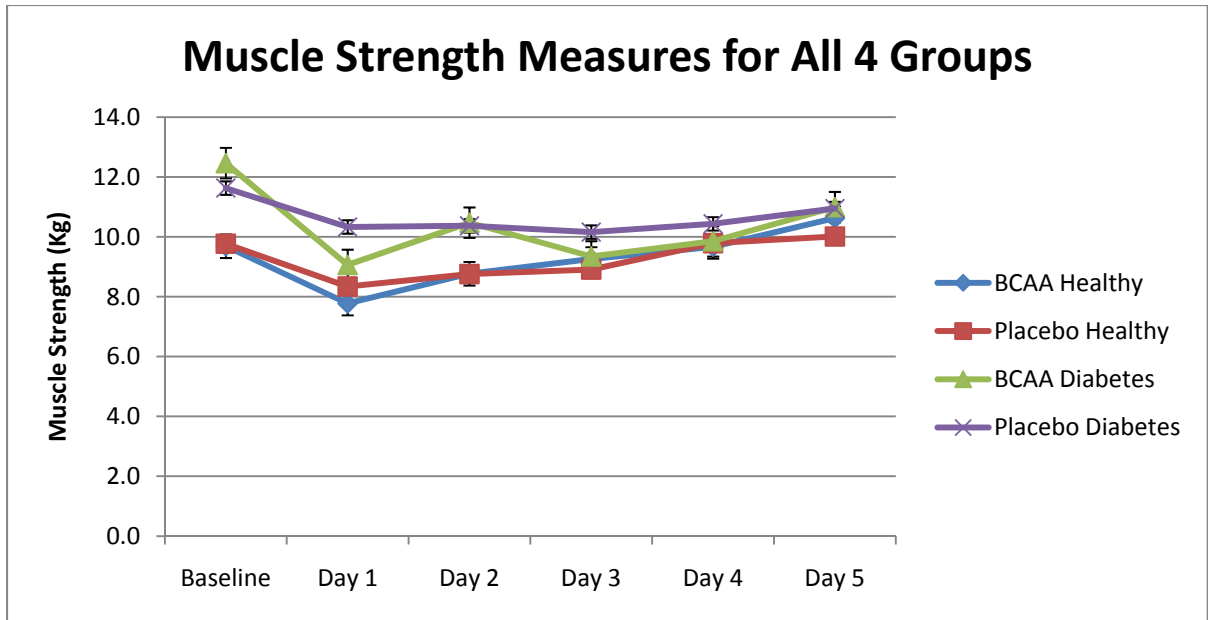


Figure 20: Graph of the muscle strength measures (Means +/- SEM) in the healthy group who ingested the BCAA supplement (diamonds), the healthy group who ingested the Placebo supplement (squares), the diabetic group who ingested the BCAA supplement (triangles), and the diabetic group who ingested the Placebo supplement (X), over the 6 time periods.

Electromyography (EMG)

There was no significant difference in EMG between the two treatment groups in the healthy group ($P > 0.1$). There was also no significant difference over time ($P > 0.05$).

In the diabetic group however, there was a significant difference in EMG between the two treatment groups ($P < 0.05$), where the placebo group had a significantly lower EMG amplitude during maximum effort when compared to the BCAA group. There was also a difference in EMG over time, with a significant decrease in EMG at day3, day4, and day5 ($P < 0.05$), compared to baseline. Figure 21 illustrates the EMG response during maximum effort for all 4 groups.

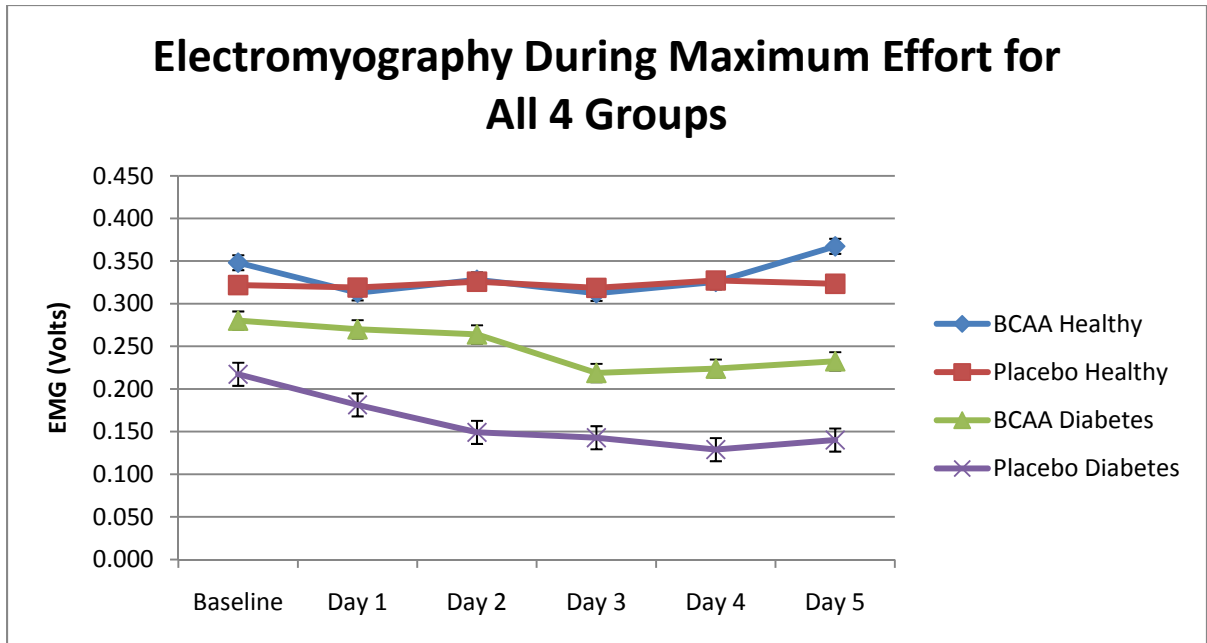


Figure 21: Graph of the electromyography (EMG) amplitude during maximum effort (Means +/- SEM) in the healthy group who ingested the BCAA supplement (diamonds), the healthy group who ingested the Placebo supplement (squares), the diabetic group who ingested the BCAA supplement (triangles), and the diabetic group who ingested the Placebo supplement (X), over the 6 time periods.

Soreness/Pain Response (SF-MPQ)

There was no significant difference in the pain scores from the SF-MPQ among the two treatment groups in the healthy group ($P > 0.1$). Pain scores differed overtime however, where there was a significant increase in pain at day1, day2, day3, and day4 ($P < 0.05$), compared to baseline.

In the diabetic group, there was a significant difference between the two treatment groups in regards to the level of perceived pain ($P < 0.05$), where the placebo group had higher pain than the BCAA group. A difference in pain over time was also found, where there was a significant increase in pain at day2, day3, and day4 ($P < 0.05$), compared to baseline in the placebo group. In the BCAA group however, the only significant time

difference from baseline was at day 3 ($P < 0.05$). Figure 22 shows the pain scores from the SF-MPQ for all 4 groups.

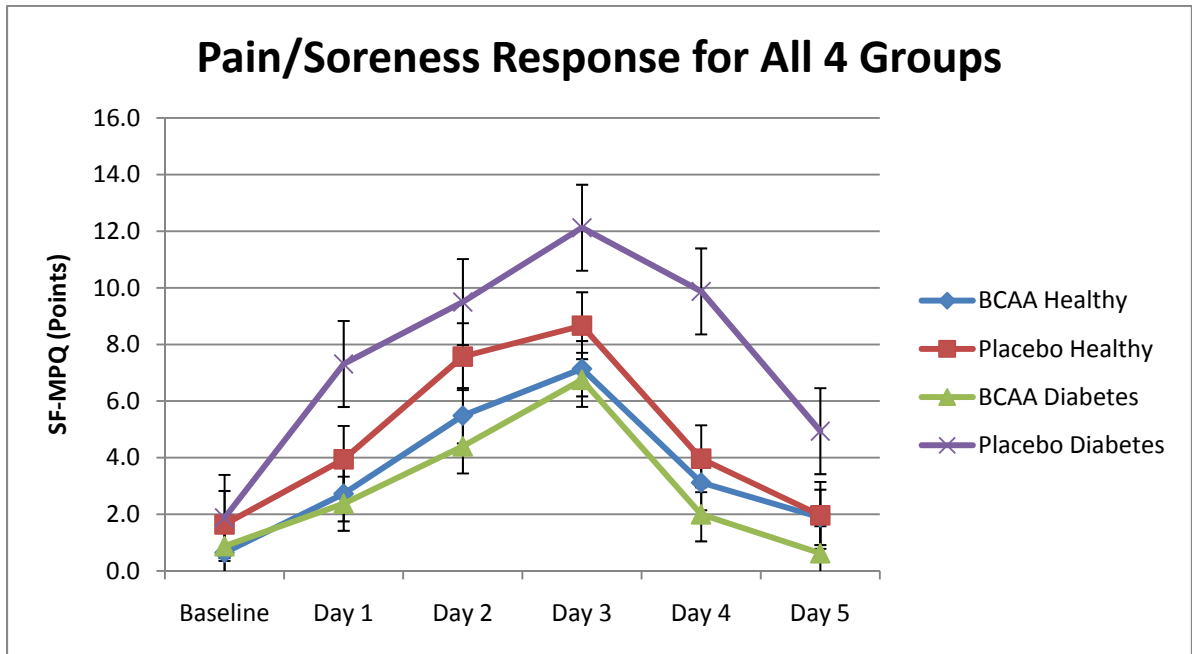


Figure 22: Graph of the soreness responses using the SF-MPQ (Means \pm SEM) in the healthy group who ingested the BCAA supplement (diamonds), the healthy group who ingested the Placebo supplement (squares), the diabetic group who ingested the BCAA supplement (triangles), and the diabetic group who ingested the Placebo supplement (X), over the 6 time periods.

Relaxed Range of Motion (RROM)

There was no significant difference in RROM between the two treatment groups in the healthy group ($P > 0.1$). However, there was a difference in RROM over time, with a significant decrease in RROM for all time points (day1, day2, day3, day4, day5) compared to baseline ($P < 0.05$).

Also in the diabetic group, where there was no significant difference in RROM between the two treatment groups ($P > 0.1$). However, there was a significant decrease in

RROM at day1, day2, day3, and day4 ($P < 0.05$), compared to baseline. Figure 23 shows the RROM measurements in all 4 groups.

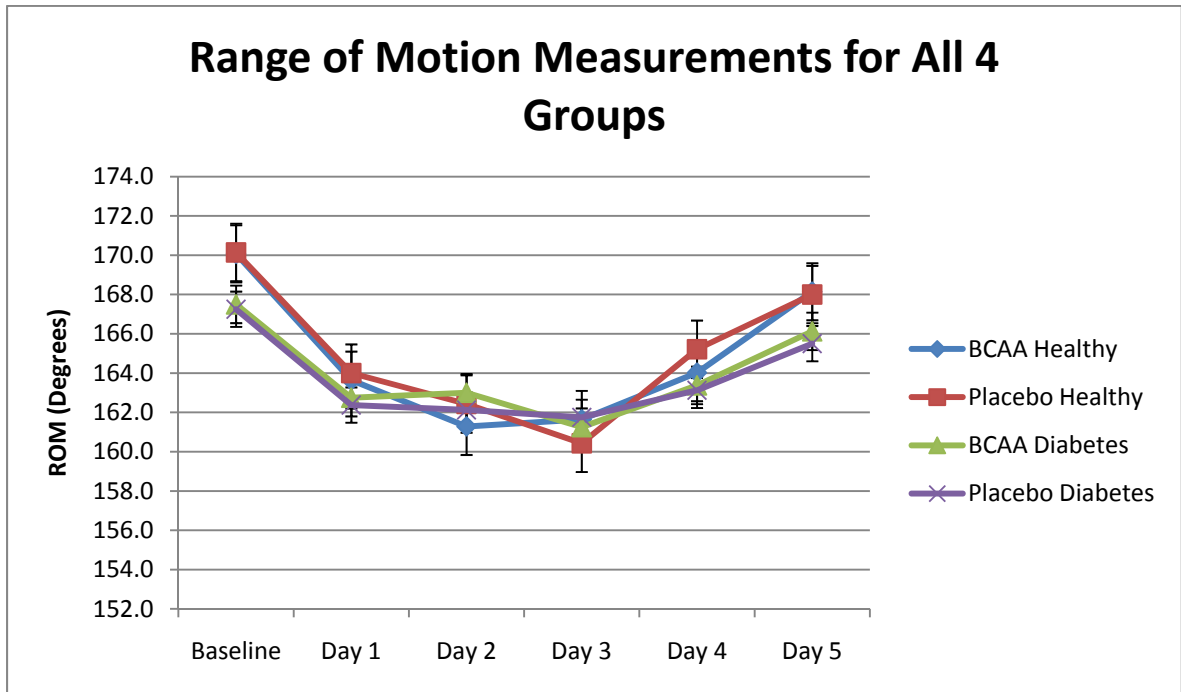


Figure 23: Graph of the relaxed range of motion (RROM) measurements (Means \pm SEM) in the healthy group who ingested the BCAA supplement (diamonds), the healthy group who ingested the Placebo supplement (squares), the diabetic group who ingested the BCAA supplement (triangles), and the diabetic group who ingested the Placebo supplement (X), over the 6 time periods.

Myoglobin Concentrations

There was no significant difference in myoglobin concentrations between the two treatment groups in the healthy group ($P > 0.1$). However, there was a difference in myoglobin concentrations over time, with a significant increase in myoglobin concentrations at day3, day4, and day5 ($P < 0.05$) compared to baseline.

In the diabetic group, there was a significant difference in myoglobin concentrations between the two treatment groups ($P < 0.05$), where the placebo group had

higher blood myoglobin concentrations than the BCAA group. A difference over time was also found, with a significant increase in myoglobin concentrations at day3, day4, and day5 ($P < 0.05$), compared to baseline in the placebo group. In the BCAA group however, the only significant time difference from baseline was at day 4 ($P < 0.05$).

Figure 24 shows the blood myoglobin concentrations for all 4 groups.

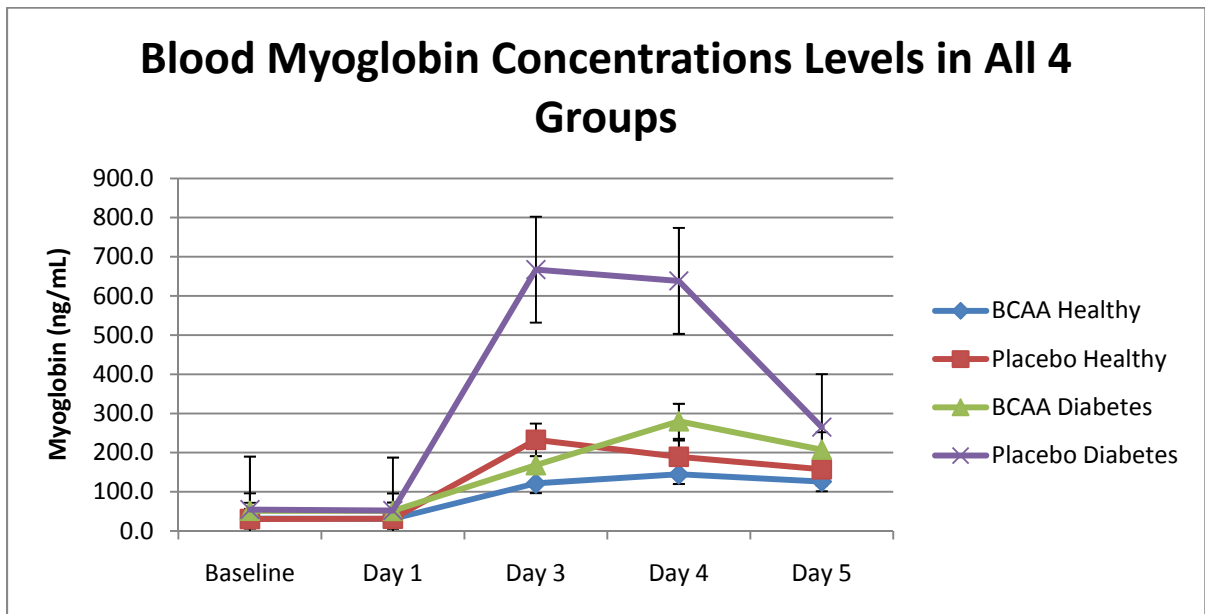


Figure 24: Graph of the blood myoglobin concentrations (Means +/- SEM) in the healthy group who ingested the BCAA supplement (diamonds), the healthy group who ingested the Placebo supplement (squares), the diabetic group who ingested the BCAA supplement (triangles), and the diabetic group who ingested the Placebo supplement (X), over the 5 time periods.

Skin Temperatures

There was no significant difference in skin temperatures between the two treatment groups in the healthy group ($P > 0.05$). However, there was a difference over time, with a significant increase in skin temperatures at day1 ($P < 0.05$), compared to baseline for both groups. Skin temperatures in the placebo group were still significantly

elevated at day 2 ($P < 0.05$) compared to baseline, whereas in the BCAA group, skin temperatures at day 2 were not significantly different from baseline ($P > 0.05$).

Also in the diabetic group, where there was no significant difference in skin temperatures between the treatment groups ($P > 0.05$). There was however, a time difference, where there was a significant increase in skin temperatures at day 1 ($P < 0.05$), compared to baseline for both groups. Also, similar to the diabetic group, skin temperatures for the placebo group were still significantly elevated at day 2 ($P < 0.05$) compared to baseline, whereas for the BCAA group there was no significant difference between day 2 and baseline measures ($P > 0.05$). Figure 25 shows the skin temperature measurements for all 4 groups.

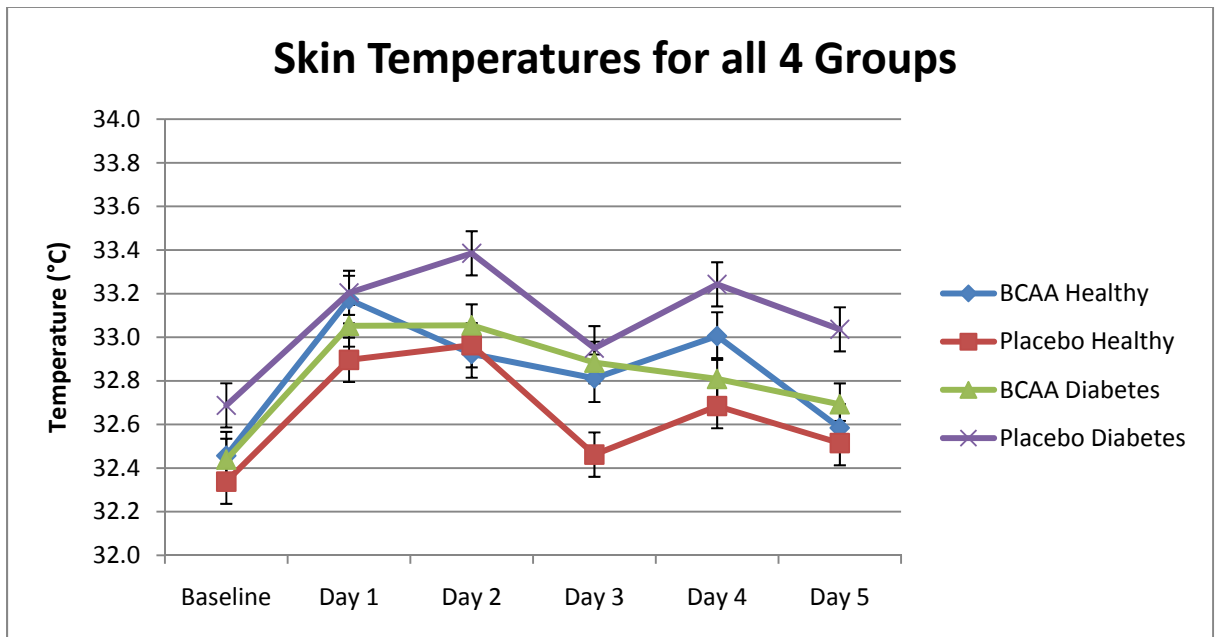


Figure 25: Graph of skin temperatures (Means \pm SEM) in the healthy group who ingested the BCAA supplement (diamonds), the healthy group who ingested the Placebo supplement (squares), the diabetic group who ingested the BCAA supplement (triangles), and the diabetic group who ingested the Placebo supplement (X), over the 6 time periods.

Discussion

Exercise is still considered one of the best means of increasing glycemic control in people with diabetes, particularly resistance exercise [7]. It has been shown that an overall increase in muscle mass is associated with better insulin sensitivity [13,9]. Resistance exercise however, especially the eccentric phase, has been attributable to micro-traumas and damage of protein structures within skeletal muscles [38,20,59]. This damage alters the protein turnover process within the muscle, which causes a decrease in performance and in the body's BCAA content (and some other essential amino acids) during the recovery period after exercise [44,43,42].

There has been a heightened interest lately in nutritional supplementation, in treating DOMS and its associated symptoms. In these studies, BCAA has been found to have promising effects in facilitating the restoration of muscle function, and attenuating muscle damage following exercise [22,45,60,51,52,23]. However, because people with diabetes have endothelial dysfunction, soreness levels are worse after exercise and recovery periods longer [26,28].

No study up to date has investigated BCAA effects on a diabetic population, which was the focus of this study. In this investigation, subjects participated in a moderate intensity resistance exercise to induce DOMS, and identify the effectiveness of BCAA supplementation in enhancing the recovery of DOMS, and its perceived symptoms. This study was also unique in the fact that we used a BCAA dosage dependent on lean body weight, rather than just the individual's weight. The reason for incorporating this method is that diabetic individuals, on average, have higher fat distributions when compared to non-diabetic individuals. Also, because BCAA is mainly

metabolized in skeletal muscle, we thought that the incorporation of the individual's whole body weight in the dosage calculation might not be necessary. From this investigation, we found that BCAA supplementation could be a useful method for treating DOMS and facilitating muscle recovery in a diabetic population.

BCAA when consumed before or after an acute exercise session has been found to further stimulate muscle protein synthesis, and reduce muscle protein breakdown, resulting in an enhanced muscle adaptation to the related damage [46,45,47]. These positive results were seen in the diabetic group, where some of the muscle damage markers were decreased when compared to the placebo group.

In regards to the EMG response, the diabetic BCAA group showed a better maintenance of their muscle activity output, while the diabetic placebo group had a larger decrease. This could be attributed to the fact that an increased dose of leucine has been found to help maintain the muscles force output during isometric contractions [22]. Perceived soreness, and myoglobin concentrations in the diabetic BCAA group were much lower than in the diabetic placebo group. This response has been observed in a number of studies that have incorporated the use of BCAA, or leucine supplementation on the markers of muscle damage and DOMS [52,60]. Although skin temperatures were not significantly different between the two diabetic groups, a notable finding was that skin temperatures were closer to baseline in the BCAA group at 24 hours, while temperatures were still elevated in the placebo group at that same time period. This indicates that there were higher blood flows in the exercised muscle of the placebo group, due to increased inflammation and tissue damage [59].

An interesting finding in our study was that the BCAA supplement had enhanced the recovery in the diabetic group, but had almost no effects on the healthy group, even though the dosage duration was short. A critical explanation for this occurrence could be ascribed to the fact that diabetic individuals have a high prevalence of gastrointestinal complications including dyspepsia, and abdominal pain [61,62]. Diabetes mellitus is known to provoke many complications such as retinopathy and nephropathy; gastrointestinal dysfunction is just one of these many complications [61,63]. Neuropathy and hyperglycemia have been found to cause abnormal gastrointestinal motility and disturbed digestion, which together have shown to cause impaired intestinal absorption [64-66]. This may result in the mal-absorption of essential amino acids from the proteins in the diet of a diabetic individual. Thus, an amino acid supplement would be much easier for the diabetic individual to absorb through an impaired gastrointestinal system.

On the other hand, we found no significant differences between the healthy BCAA group, and the healthy placebo group. A main reason for this finding could be related to the fact that the supplementation duration wasn't long enough. Studies have concluded that the duration of BCAA supplementation could have a large impact on its effects on DOMS [67,53]. For example, a study done by Sharp and colleagues [45] found that BCAA supplementation had significant effects on lowering DOMS, but their supplementation period was over the length of 4 weeks (3 weeks before the exercise, and 1 week after). These positive effects were seen even though the exercise routine they incorporated was intense, as it included muscles of the whole body (circuit training) rather than just the arm, or leg muscles. Also, another study done by Skillen and

colleagues [68], administered an amino acid supplement over a period of 2 weeks and found that it had beneficial effects on reducing muscle damage markers.

One of the limitations of this study is that we only assessed the subject's dietary intake at baseline, and failed to assess their intake over the course of the study. This obviously limits our ability to detect changes in intake, especially in regards to protein content. Another limitation would be the fact that we didn't incorporate a strict dietary program for the subjects during the time of the study. However, even though this would increase the studies internal validity, it might decrease its external validity. Suggestions for further studies would be to incorporate different vitamins into the BCAA supplement. Vitamin E would be a good example, as it is considered an antioxidant, which has been found to be effective in attenuating exercise induced muscle damage [15,69].

Despite the limitations of this study, we have provided suggestive evidence that BCAA supplementation in a diabetic population can be beneficial in enhancing muscle performance, and improving recovery from DOMS. BCAA supplementation would be a simple method for stabilizing the muscle's protein content after an acute bout of resistance exercise in people with diabetes. This could assist in minimizing further injuries from over-exercising in this population. However, further investigations are required to clarify and elucidate these findings.

Acknowledgments

No external financial support was used for this study. We would like to thank the Saudi Arabian Ministry of Higher Education (MOHE), and the Saudi Arabian Cultural Mission (SACM) for their cooperation and support. We would also like to thank the

Physical Therapy Department at the School of Allied Health Professions (SAHP) at Loma Linda University (LLU). We would also like to acknowledge everyone who assisted us in the clinical lab including: Paula Cavalcanti, Bhargav Dave, and Harold Moniz for their time and effort.

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CHAPTER FIVE

DISCUSSION

In the present research, we examined the intensity of DOMS in people who have diabetes, in 2 different body regions (Chapter 2). As an outcome of the first study, we then investigated a new technique to objectively quantify muscle soreness (Chapter 3). Finally, using this new technique and other biomarkers, we examined whether soreness could be reduced by ingesting a single nutritional supplement, which in this case was BCAA (Chapter 4).

Diabetes causes a chronic increase in whole body inflammation.⁵⁴ Because of this, and of the metabolic and endothelial impairments associated with diabetes, exercise performance and training may become impaired in this population.^{56,57} It has been shown that exercise which results in an overall increase in muscle mass is associated with better insulin sensitivity, and a mean reduction in hemoglobin A1c of approximately 0.80%.^{12,16} This obviously gives the individual with diabetes a better chance of coping with the disease and preventing its symptoms from getting worse.^{7,58} For these reasons, resistance exercise is considered one of the best means of increasing glycemic control in people with diabetes.¹⁰ Resistance exercise however, especially the eccentric phase, has been attributable to micro-traumas and damage of protein structures within skeletal muscles.^{23,34,59} This damage alters the protein turnover process within the muscle, which causes a decrease in BCAA and some other essential amino acids during the recovery period after

exercise.⁴⁴⁻⁴⁶ A decrease in performance occurs during this recovery period, but BCAA supplementation has been found to facilitate the restoration of muscle function.^{25,47,60}

While much is known about DOMS in young people, little is known about DOMS in people with diabetes. However, there may be some comparisons that can be made from studies of DOMS and ageing.⁶¹ These studies have shown that there is reduced proteolytic activity and an elevated production in free radicals in older individuals.^{61,62} This elevation prolongs healing time after excessive exercise.⁶¹ With metabolic impairments, endothelial dysfunction, and higher levels of free radicals in people with diabetes^{53,63} DOMS could be more severe and recovery periods longer in this population.

To understand DOMS however, we need a marker of DOMS, and in this dissertation we used common markers such as; myoglobin and soreness scales. But the most interesting finding was being able to use a non-invasive marker, which was objectively valid and correct.

In this investigation, it was found that infrared imaging could be a valid technique for determining DOMS within the first days of exercising. A purpose of this investigation was to assess the usefulness of thermal imaging in detecting and measuring muscle soreness after strenuous exercise. Our results suggest that thermal imaging could be a valid technique for detecting DOMS, especially within the first 24 hours of exercising. As well as being able to measure skin temperature, thermal cameras are fairly inexpensive.

Pennes⁶⁴ provided a very detailed model of heat flow from muscle to skin in limbs. This model predicts that heat in deeper tissues such as muscles can be dissipated into blood and into the skin through conductive heat exchange. When muscles exercise,

heat is developed in the muscle because of frictional forces of the muscle fibers and from the increased metabolism.⁶⁵ Increased blood flows to the muscle would also contribute to the increased heat in the muscle after exercising.

Skin is a shell tissue, and its temperature is usually about 6°C less than that of the core.⁶⁶⁻⁶⁸ The skin must be kept cooler than the core so that heat can move from the core to the skin and be removed by radiation, conduction, and evaporation.⁶⁸⁻⁷¹ Muscle is also a shell tissue, where its temperature is usually 32–33°C, which is slightly warmer than that of skin. However, when blood flow is increased to the exercised muscle it approaches the temperature of the core tissues, which is approximately 37°C.^{39,64} Blood flow in skeletal muscle is very dynamic, and when tissue is damaged during exercise, blood flow can increase dramatically. Thus, warm blood entering muscle when tissue repair is being accomplished as part of the inflammatory response can deliver a great deal of heat into the tissue.^{34,72} The increased blood flow should go back to normal within an hour or two of the exercise termination, but when temperatures are still elevated at 24 hours post-exercise, this shows that damage has happened to the exercised muscle and causes a hot spot under the skin.

Thus, the elevated skin temperatures found 24 hours post-exercise in all subjects is possibly the result of higher blood flows in muscle due to inflammation and tissue damage repair.³⁴ Therefore, if muscle blood flow remained elevated post-exercise, the warmer core blood would keep the muscle warm, and hence the overlying skin would stay warm. This occurrence has been used to detect and diagnose several other diseases using thermal imaging.^{35-39,73-75} This is true because changes in the human body's

temperatures have always been indicators of dysfunction, where increased heat is mainly associated with inflammation or infection.³⁵

The elevated soreness levels, and the increased myoglobin concentrations are both indicators of DOMS. This verifies that the subjects did get sore after the exercise session. Myoglobin is a monomer protein, which has several functions regarding oxygen storage and transport in the muscles. After strenuous exercise, myoglobin can be released in the bloodstream because of the breakdown of cell membranes in the exercised muscles.^{76,77} Concentrations of muscle myoglobin markers in the blood, may increase 30 min after exercising and peak at 24-72 hours post-exercise.⁷⁶

Certainly being able to quantify DOMS is important, but preventing it is as important, especially in a diabetic population. Even though we would have liked to investigate several nutritional supplements and vitamins, but because of financial and time limitations, we chose to investigate only one supplement which was BCAA

There has been a heightened interest lately in nutritional supplementation, in treating DOMS and its associated symptoms. In these studies, BCAA has shown promising effects in attenuating muscle damage following exercise.^{25,26,47,51,52,60} However, because people with diabetes have endothelial dysfunction, soreness levels may be worse after exercise and recovery periods longer.⁵³ In addition, no study to date has investigated the effects of BCAA in people with diabetes, which was one of the focuses of this experiment.

BCAA, when consumed before or after an acute exercise, has been found to further stimulate muscle protein synthesis and reduce muscle protein breakdown, resulting in an enhanced muscle adaptation to the related damage.⁴⁷⁻⁴⁹ These positive

results were seen in our diabetic group, where some of the muscle damage markers including myoglobin concentrations, were much lower when compared to the placebo group.

On the other hand, we found no significant differences between the healthy groups, whether they ingested the BCAA or placebo supplement. A main reason for this finding could be related to the fact that the supplementation duration wasn't long enough. Studies have concluded that the duration of BCAA supplementation could have a large impact on its effects on DOMS.^{78,79} For example, studies that supplemented their subjects over a period of 2, and 4 weeks showed significant results on lowering DOMS, and reducing muscle damage markers.^{47,80}

An intriguing finding in our experiment was that the BCAA supplement had enhanced the recovery in the diabetic group, but had no effects in the healthy group. This difference was not likely due to a placebo effect, because all subjects were blinded to what supplement they were taking. A critical explanation for this occurrence could be attributed to the fact that diabetic individuals have a high prevalence of gastrointestinal complications including dyspepsia, and abdominal pain.^{81,82} Diabetes mellitus is known to provoke many complications such as retinopathy and nephropathy, and gastrointestinal dysfunction is just one of these many complications.^{81,83} Neuropathy and hyperglycemia have been found to cause abnormal gastrointestinal motility and disturbed digestion, which have shown to cause impaired intestinal absorption.⁴⁰⁻⁴² This may result in the mal-absorption of essential amino acids from the proteins in the diet of a diabetic individual. Thus, an amino acid supplement would be much easier for the diabetic individual to absorb through an impaired gastrointestinal system.

Suggestions for Future Research

Several suggestions that would enhance some of the findings in this dissertation would be to:

1. Look at different populations of people with diabetes, in regards to how long they have been diagnosed with diabetes, and whether this factor affects the intensity of DOMS in this population.
2. Look at diabetic individuals who have different levels of HbA1c control, and identify if people with higher HbA1c, because of increased neuropathic damage, feel more or less soreness compared to people with lower HbA1c.
3. Look at different durations of the supplementation, especially in the healthy control subjects because the BCAA supplement seemed to have minimal effects on that group. An extended duration might also benefit people with diabetes, where they might experience even less soreness with increased periods of supplementation.
4. Look at other types of nutritional supplements, such as vitamin A, C, D, E, and Co-Enzyme Q10, and what impact would these supplements have in regards to exercise performance, and reducing muscle soreness? Also, if different vitamins and supplements are coupled with BCAA, would this combination be even more beneficial in adding extra nutrients to people with diabetes, and assist in the muscle recovery process after exercise.

Conclusion

In this dissertation, we provided evidence that people with diabetes experienced higher levels of muscle soreness after an intense exercise session, when compared to

healthy controls. This muscle soreness was quantified non-invasively, as well as with invasive biomarkers. One interesting finding in this study was that we developed a new novel biomarker, thermal imaging, which provided an efficient and non-invasive technique of predicting and quantifying the extent of muscle soreness in healthy and diabetic individuals. This new biomarker was correlated with the pain scales, and with the myoglobin concentrations throughout this investigation. Thermal imaging would then be a painless and in-expensive way of detecting DOMS in its early stages,

By using this biomarker and other biomarkers it was also shown that at least one nutritional supplement, BCAA, reduced muscle soreness and enhanced recovery in people with diabetes. Therefore, BCAA supplementation would be a simple method for stabilizing the muscle's protein content after an acute bout of resistance exercise in people with diabetes.

From this investigation we found that thermal imaging would be an early predictor of muscle soreness. We also concluded that BCAA would be beneficial in decreasing DOMS, and in enhancing muscle recovery following exercise. Thus, an acute dose of BCAA supplementation would help in faster muscle recovery, and an early detection of DOMS would assist in minimizing the incidence of injuries from over-exercising sore muscles.

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APPENDIX A
SUBJECT DATA COLLECTION FORM

The Effects of Orally Ingesting Branched Chained Amino Acids on Delayed Onset Muscle Soreness in Diabetic Patients

Subjects Data Form:

Subjects ID #: _____ Gender: _____

Age: _____ Occupation: _____

Height: _____ Weight: _____

BMI: _____ Lean body weight: _____

Blood pressure: _____ Fasting blood glucose level: _____

Required Weight: _____ Required BCAA: _____

Reactance: _____ Resistance: _____

Activity Level: _____ Frame Size: _____

Upper limb neuropathy testing: _____

Medications or any other medical problems: _____

Comments: _____

Code:

APPENDIX B

SHORT FORM MCGILL PAIN QUESTIONNAIRE

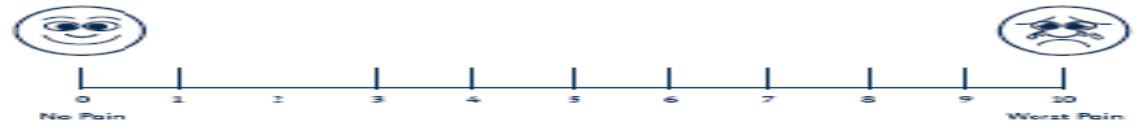
Study #:
Date:

Short-Form McGill Pain Questionnaire:

I. Pain Rating Index (PRI):
 The words below describe average pain. Place a check mark (✓) in the column that represents the degree to which you feel that type of pain. Please limit yourself to a description of the pain in your **Arm**:

		None	Mild	Moderate	Severe	
↑ a ↓	Throbbing	0	1	2	3	
	Shooting	0	1	2	3	
	Stabbing	0	1	2	3	
	Sharp	0	1	2	3	
	Cramping	0	1	2	3	
	Gnawing	0	1	2	3	
	Hot-Burning	0	1	2	3	
	Aching	0	1	2	3	
	Heavy	0	1	2	3	
	Tender	0	1	2	3	
	Splitting	0	1	2	3	
	↓ b ↓	Tiring-Exhausting	0	1	2	3
		Sickening	0	1	2	3
		Fearful	0	1	2	3
		Punishing-Cruel	0	1	2	3

II. Present Pain Intensity (PPI)–Visual Analog Scale (VAS). Tick along scale below for **Pain**



III. Evaluative overall intensity of total pain experience. Please limit yourself to a description of the pain in your **Arm**. Place a check mark (✓) in the appropriate column:

Evaluative		
0	No pain	
1	Mild	
2	Discomforting	
3	Distressing	
4	Horrible	
5	Excruciating	

IV. Scoring:

		Score
I-a	S-PRI (Sensory Pain Rating Index)	
I-b	A-PRI (Affective Pain Rating Index)	
I-a+b	T-PRI (Total Pain Rating Index)	
II	PPI-VAS (Present Pain Intensity-Visual Analog Scale)	
III	Evaluative overall intensity of total pain experience	

APPENDIX C
INFORMED CONSENT FORM



LOMA LINDA UNIVERSITY
School of Allied Health Professions

INFORMED CONSENT

TITLE: THE EFFECTS OF ORALLY INGESTING BRANCHED CHAINED AMINO ACIDS ON DELAYED ONSET MUSCLE SORENESS IN DIABETIC PATIENTS
SPONSOR: Loma Linda University, School of Allied Health Professions
PRINCIPAL INVESTIGATOR: Dr. Jerrold Petrofsky, PhD, Professor and Director of Research
Department of Physical Therapy, Loma Linda University, 909 558 7274

1. WHY IS THIS STUDY BEING DONE?

The purpose of the study is to test the effects of 3 types of basic protein products called "Amino Acids" on treating a type of muscle soreness that appears after exercising. This soreness usually reaches its maximum almost 3 days after exercising, and begins to subside after the 5th day. This type of soreness is called "Delayed Onset Muscle Soreness" (DOMS), and the amino acids that will be used are named "Branched Chained Amino Acids" (BCAA).

The rationale for this study is that BCAA's have been found to promote the muscle recovery process following the muscle fiber damage from exercising, which would in turn decrease the pain and soreness felt in the muscle.

You are invited to participate in this research study because you are either a healthy individual, or an individual with type 2 diabetes, between the ages of 20 to 90 years old. Your participation will involve taking part in the two trials of the study

Approximately 50 subjects will participate in this study, 25 healthy and 25 with type 2 diabetes. Your participation in this study may last up to 10 days, with 2 trials of 5 days that are 1 week apart

**Loma Linda University
Adventist Health Sciences Center
Institutional Review Board
Approved 11/19/10 Void after 9/7/2011
5100233 Chair R. J. Ruppberg**

Subject Initials _____

Date _____

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Consent Version Date: _____

A Seventh-day Adventist Institution

DEPARTMENT OF PHYSICAL THERAPY | Nichol Hall, Loma Linda, California 92350
(909) 558-4632 · (800) 422-4558 · fax (909) 558-0459 · www.llu.edu/llu/sahp/pt

2. HOW WILL I BE INVOLVED?

Participation in this study will involve the following:

- There will be 2 trials in the study, so for those who receive the BCAA supplement in the first trial, will get a supplement that has no effects (placebo) in the next trial, and vice versa.
- Depending on which group you are in, the supplement (either the BCAA or the placebo), will be taken by mouth, before and after the intended exercise is carried out. You will also be given the supplement on day 2, when you come in for the measurements.
- To determine which group you will start the study in, and which arm will be used first, a chance selection will be made (randomization). The chance of beginning in either one of the groups is 50%.
- The arm muscle (biceps) will be exercised for 4 sets until fatigue. The exercise will only be done on one arm per trial. The measurements of pain, pain with pressure, range of motion, muscle strength, muscle activity, ultrasound image, and blood analysis will be taken before the exercise, and after the exercise has been completed on day 1. These same measurements will also be taken on the 4 days following day 1 (once a day).
- A brief dietary food questionnaire, and a physical activity survey will also need to be filled out on day 1 (or can be taken home and brought back on day 2)
- The total experiment will take about 2.5 hours of your time per trial (1 hour for day one, and approximately 25 minutes on each of day 2, 3, 4, and 5), so for both trials it will take 5 hours
- You will not know which supplement you have taken until the end of the study.
- There will be a 1 week gap between the first trial and the second trial of the study.
- The amount of blood drawn from you on day 1 will be a total of 3.2 teaspoons for both samples (prior to and following the exercise). For the last 3 days the blood drawn will be 1.6 teaspoons per day. The total amount of blood drawn per trial will be 8 teaspoons, where 5 sticks will be used for the 5 days. For the whole study (both trials) the amount of blood drawn would be approximately 16 teaspoons.

Please Refer to Attachment 1 for a table which provides an overview of the procedures performed at each study visit.

Subject Initials _____

Date _____

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3. WHAT ARE THE REASONABLY FORESEEABLE RISKS OR DISCOMFORTS I MIGHT HAVE?

The committee at Loma Linda University that reviews human studies (Institutional Review Board) has determined that participating in this study exposes you to minimal risk. A mild to moderate discomfort (soreness) will most likely be felt in the studied muscle (the exercised arm). Slight pain or bruising from the blood collecting procedure may also be noticed during the collection process. In regards to the BCAA supplement, no events of over-dosage have been reported, and no known side effects have been documented, but it is contraindicated for those with hypersensitivity to BCAA components.

4. WILL THERE BE ANY BENEFIT TO ME OR OTHERS?

Although you are not likely to benefit directly from this study, the scientific information we learn from the study may benefit future individuals with this type of soreness, and a better understanding of whether Branched Chained Amino Acids alleviates this type of muscle soreness that is followed by exercise, would allow therapists and rehabilitation specialists to deal with this painful experience in better ways.

5. WHAT ARE MY RIGHTS AS A SUBJECT?

Participation in this study is voluntary. You may leave the study at any time. If at any time during a procedure you experience tiredness or discomfort beyond what you are willing to endure, just tell the person conducting the procedure you want to stop. Your decision whether or not to participate or withdraw at any time from the study will not affect your standing with those conducting the study and will not involve any penalty or loss of benefits to which you are otherwise entitled.

Likewise, those conducting the study may withdraw you from the study for any reason without your agreement or may stop the study entirely.

6. HOW WILL INFORMATION ABOUT ME BE KEPT CONFIDENTIAL?

Efforts will be made to keep your personal information confidential. We cannot guarantee absolute confidentiality, but all obtained data will be saved to a secure storage location. Your personal information may be disclosed if required by law. You will not be identified by name but by ID number in any publications describing the results of this study.

Subject Initials _____

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7. WHAT COSTS ARE INVOLVED?

There is no cost to you for participating in this study. The department of Physical Therapy will pay for the services, supplies, and procedures that will take place during the study. This includes the blood draws, blood analysis, and the equipment used for collecting the required data.

8. WILL I BE PAID TO PARTICIPATE IN THIS STUDY?

You will be entitled to a monetary compensation for participation, which will be given to you on the last day of the second trial. You will be given a \$50 gift card/voucher for completing this study. Premature withdrawal from the study will cause you to be excluded from receiving this compensation.

In order to receive such payments, you may be asked to provide your full name and home address.

9. WHO DO I CALL IF I AM INJURED AS A RESULT OF BEING IN THIS STUDY?

In the case of injury or illness resulting from this study, emergency medical treatment is available but will be provided at the usual charge. The Emergency Department at Loma Linda University Medical Center is located on the corner of Barton and Campus roads at 11234 Anderson Street, Loma Linda, California 92354, Phone #: 909-558-4000. No funds have been set aside to compensate you in the event of injury.

10. BIOLOGICAL STUDIES

Blood samples taken from you will be stored in specialized cooling systems until all the data is obtained and analyzed. Samples may be stored for up to 6 months, in case data is lost and needs to be analyzed again. After the study is over, all blood samples will be disposed of.

11. WHO DO I CALL IF I HAVE QUESTIONS?

If you wish to contact an impartial third party not associated with this study regarding any questions about your rights or to report a complaint you may have about the study, you may contact the Office of Patient Relations, Loma Linda University Medical Center, Loma Linda, CA 92354, phone (909) 558-4647, e-mail patientrelations@llu.edu for information and assistance.

Subject Initials _____
Date _____
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5100233 Chair R. R. R. R.*

12. SUBJECT'S STATEMENT OF CONSENT

- I have read the contents of the consent form and have listened to the verbal explanation given by the investigator.
- My questions concerning this study have been answered to my satisfaction.
- I have received a copy of the California Experimental Subject's Bill of Rights and have had these rights explained to me.
- Signing this consent document does not waive my rights nor does it release the investigators, institution or sponsors from their responsibilities.
- I may call Jerrold Petrofsky, PhD during routine office hours at (909) 558 4300 ex 82186 or leave a voice mail message at this number during non office hours if I have additional questions or concerns.
- I hereby give voluntary consent to participate in this study.

I understand I will be given a copy of this consent form after signing it.

Signature of Subject

Printed Name of Subject

Date

13. INVESTIGATOR'S STATEMENT

I attest that the requirements for informed consent for the medical research project described in this form have been satisfied – that the subject has been provided with a copy of the California Experimental Subject's Bill of Rights, that I have discussed the research project with the subject and explained to him or her in non-technical terms all of the information contained in this informed consent form, including any risks and adverse reactions that may reasonably be expected to occur. I further certify that I encouraged the subject to ask questions and that all questions asked were answered. I will provide the subject or the legally authorized representative with a signed and dated copy of this consent form.

Signature of Investigator

Printed Name of Investigator

Date

Subject Initials _____

Date _____

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The Effects of BCAA on DOMS in Diabetic Patients

Attachment 1: Study Flow Chart for trial 1 (trial 2 of the study will be exactly the same)

TRIAL DAY	Trial 1 Day 1 Pre-Ex	Trial 1 Day 1 Post-Ex	Trial 1 Day 2	Trial 1 Day 3	Trial 1 Day 4	Trial 1 Day 5
Medical history	X					
Vital signs	X					
Dietary Survey + Physical Activity Survey	X (or take Home)		(Bring with you)			
Muscle Strength	X	X	X	X	X	X
Muscle Activity	X	X	X	X	X	X
Ultrasound Image	X	X	X	X	X	X
Range of Motion	X	X	X	X	X	X
Blood Draw	X	X		X	X	X
Pain with Pressure	X	X	X	X	X	X
Questionnaires	X	X	X	X	X	X

Subject Initials _____

Date _____

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