QUANTIFICATION OF THE EFFECT OF WARM UP AND STRETCHING ON THE OXYGEN METABOLISM USING AN IMPROVED VERSION OF A fNIRS DEVICE

by

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ABSTRACT

QUANTIFICATION OF THE EFFECT OF WARM UP AND STRETCHING ON THE OXYGEN METABOLISM USING AN IMPROVED VERSION OF A fNIRS DEVICE

In the vastly improved field of exercise physiology, it is an imperative to exercise a a warm up and stretching routine before training. The reasoning is closely associated with the oxygen (O_2) metabolism in the blood. Warm up provides the necessary means to activate enzymatic reactions to accelerate oxy-hemoglobin (HbO_2) break up by increasing the body temperature and slightly decreasing the pH of blood. Stretching, meanwhile, acts as a regime to educate the muscle by extending the sarcomere to its full length and lining up the extracellular matrix (predominantly, the collagen fibers) in the line of action.

This M.Sc. thesis is involved with the analysis of the effect of warm up and stretching on the O_2 metabolism. The device used in the quantification process is a fNIRS equipment, named NIROXCOPE 301. Niroxcope 301, an improved version of Niroxcope 201[1], locally measures the deoxy-hemoglobin (Hb) and oxy-hemoglobin (HbO_2) change with respect to time and relative to a baseline determined at the calibration stage. The Arterial Occlusion Protocol was applied by using Niroxcope 301 in an effort to compare the gastrocnemius of the subjects ready for exercise (i.e. with warm up and stretching) with the unprepared gastrocnemius in terms of the pre-determined three parameters of post occlusion. Hb_{max} data, due to its great difference compared to that of the control groups resulted in p = 4.71 e-5 in ANOVA analysis. 90% index provided the most sterile data as it is normalized by a maximal value. It resulted in p = 0.0054. t_{rec} was a more controversial data due to its dual nature (more recuitment vs. better recovery of a single unit) and displayed a mixed pattern.

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Keywords: warm up, stretching, O_2 metabolism, functional Near-Infrared Spectrscopy (fNIRS), Niroxcope, ischemia

[1] Developed in the Biophotonics Lab, Boğaziçi University, Istanbul

ÖZET

ISINMA VE AÇMA-GERME HAREKETLERİNİN OKSİJEN METABOLİZMASI ÜZERİNDEKİ ETKİLERİNİN YENİLENMİŞ BİR iYKÖS CİHAZI TARAFINDAN ÖLÇÜMLENMESİ

Son yıllarda büyük ilerleme kaydeden egzersiz fizyolojisinin temel ilkelerinden biri idmandan önce ısınma ve açma-germe egzersizleri yapılmasıdır. Bunun nedeni kandaki oksijen metabolisması ile yakından ilgilidir. Isınma hareketleri, vücut ısısını arttırıp kandaki pH miktarını düşürerek, enzim reaksiyonlarını harekete geçirir ve oksi-hemoglobinin parçalanmasını hızlandırır. Açma-germe hareketleri ise, sarkomeri maksimal uzunluğuna getirerek ve ekstrasellüler matriksi (ağırlıklı olarak kolajen liflerini) hareket hattıyla aynı doğrultuya dizer.

Bu yüksek lisans tezi, ısınma ve açma-germe hareketlerinin oksijen metabolizması üzerindeki etkisini irdelemektedir. Ölçme işleminde kullanılan aygıt, NIROXCOPE 301 adlı bir iYKÖS cihazıdır. Niroxcope 201[2]'in gelişmiş sürümü olan Niroxcope 301, deoksi-hemoglobin (Hb) ve oksi-hemoglobin (HbO_2) parametrelerinin zamana karşı değişmini lokal olarak ve kalibrasyon aşamasında belirlenmiş bir baz değere göre ölçer. Atardamar Engeli Protokolü, gastrocnemius üzerine uygulanarak egzersize hazırlanmış bir kası, hazırlanmamış haliyle 3 parametreye göre karşılaştırır: Maksimal [Hb] seviyesi ANOVA (değişkenlerin analizi) ile p = 4.71 e-5, %90 indisi ise p = 0.0054 sonucunu vermiştir. Geri dönüm süresi ise çift yönlü etkenler sebebiyle istikrarsız sonuçlar üretmiştir.

Anahtar kelimeler: ısınma, açma-germe (esnetme) hareketleri, oksijen metabolizması, işlevsel Yakın-Kızılötesi Spektroskopi (iYKÖS), Niroxcope, iskemi

[2] Boğaziçi Üniversitesi Biyofotonik Laboratuvarı tarafından geliştirilmiştir.

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A	Light attenuation measured in optical distance
I_0	Incident light intensity
I	Received light intensity
$\alpha_{Hb\,/\,HbO2}$	Specific extinction coefficient of deoxy-hemoglobin / oxy-hemoglobin ($OD.cm^{-1}.\ \mu M^{-1}$)
$c_{Hb\,/\,HbO2}$	Concentration of deoxy-hemoglobin / oxy-hemoglobin (μM),
d	Distance between the points where light enters and leaves the tissue (cm)
B	Geometrical path length factor such that ($d \cdot B$) is the real path length taken by the light between the optodes
G	Factor which takes into account the scattering properties of the medium

LIST OF ABBREVIATIONS

NIR	Near-infrared
NIRS	Near-infrared spectroscopy
fNIRS	Functional near-infrared spectroscopy
ACh	Acetyl choline
ADP / ATP	Adenosine Di-Phosphate / Adenosine Tri-Phosphate
PNF	Proprioceptive Neuromuscular Facilitation
fMRI	Functional magnetic resonance imaging
PET	Positron emission tomography
HbO_2 / Hb	Oxy/Deoxy-hemoglobin
MbO_2 / Mb	Oxy/Deoxy-myoglobin
[HbO_2] / [Hb]	Concentration of oxy/deoxy-hemoglobin
CtOx	Cytochrome oxidase
$NIRS_{cws}$	Continuous-wave near-infrared spectroscopy
FIR	Far-infrared
VO_2	Volume of oxygen consumption
MVC	Maximum voluntary contraction
PMT	Photomultiplier tube
LED	Light emitting diodes
DAQ	Data acquisition
PD	Photodetector
GUI	Graphical user interface
BF	Blood flow
BMI	Body mass index
Oxy	Oxygenation
BV	Blood volume
SP	Systolic pressure
anova1	One-way analysis of variables

1. INTRODUCTION

1.1. Motivation and Objective

Understanding the physiological changes due to exercise has been a topic of interest for decades that led to the establishment of Sports Medicine departments in medical schools. However, it is not a common practice for these departments to collaborate with professional sports agents (sports clubs, trainers, professional athletes) on a regular basis. Living in an environment of sports and competition by working at the Efes Pilsen Sports Club as a basketball coach and getting acquainted with the ever improving area of medical instrumentation by studying Biomedical Engineering in Boğaziçi University, I am given the opportunity to assemble theory with application. Working with youngsters between the ages of 13-17, I have experienced difficulty in motivating my players to warm up and to stretch out properly even though it is a procedure done 10 times a week, before each and every training session. This has led me to come up with a project to investigate the physiology of warm up and stretching in detail.

Near-infrared spectroscopy provides the necessary means to measure local differences in muscle O_2 consumption and delivery in a sensitive manner with its main advantage being its non-invasive nature. It is obvious that non-invasive methods have proven their advantage over invasive ones in the medical innovations of the current medical world. Niroxcope, the functional NIRS device, was the instrument used to measure local oxygenation change caused by warm up and stretching. As for all instrumentation, upgrading and renovation with a newer version is an imperative for Niroxcope.

The objective of my M.Sc. project is to measure the effect of warm up and stretching by the agent of local O_2 in the blood. The work includes developing an upgraded version of Niroxcope due to specific needs: finding an appropriate protocol to apply, comparing the results attained using the protocol before and after the warm up and stretching procedure and reporting the data in an understandable format.

1.2. Outline of the Thesis

By writing this thesis, I have provided thorough physiological, medical and theoretical information on warm up, stretching and NIRS modality. Then, two separate applications constitute the remainder: Development of a new version of the fNIRS device and fulfillment of a comparative study to investigate the effect of warm up and stretching on the oxygen metabolism.

Chapter 2 provides the background of theory for exercise physiology, especially in warm up and stretching as well as the role of O_2 in energy production mechanisms in the body, and near-infrared spectroscopy (NIRS).

Chapter 3 focuses on the instrumentation, namely the Niroxcope, and its operating principle. It describes the current version (Niroxcope 201) and the enhancements made in the updated version (Niroxcope 301) in comparison with the current version.

Chapter 4 includes the experimental stage of the project. It introduces firstly the subjects that took place in the application along with the protocol used (Arterial Occlusion Protocol). Then, the parameters to be considered (Parameters of Postocclusion) are determined to be reported in a comparative manner (before vs. after warm up and stretching). Statistical correlation between the cases is investigated.

Finally, Chapter 5 provides a discussion of the work done, focusing especially on the experimental procedure, and provides recommendations for future work in instrumentation as well as the measurement methods and parameters.

2. THEORETICAL BACKGROUND

2.1. Exercise Physiology

Exercise represents one of the highest levels of extreme stresses to which the body can be exposed. For example, in a person who has an extremely high fever approaching the level of lethality, the body metabolism increases to approximately 100% above normal; by comparison, the metabolism of the body during a marathon race increases to 2000% above normal. This section of the document describes the basic physiology of exercise [1]. Exercise physiology, as a term, is "the identification of physiological mechanisms underlying physical activity, the comprehensive delivery of treatment services concerned with the analysis, improvement, and maintenance of health and fitness, rehabilitation of heart disease and other chronic diseases and/or disabilities, and the professional guidance and counsel of athletes and others interested in athletics, sports training, and human adaptability to acute and chronic exercise." (by ASEP – American Society of Exercise Physiologists)

2.1.1. Histology of Skeletal Muscle

The term exercise is closely associated with the skeletal muscle system as the skeletal muscles provide the means for human motion. Figure 2.1 shows the organization of a typical muscle (i.e. the biceps) from a gross anatomical view, down to the microscopic anatomy of the individual units of muscle contraction. The organization of the muscle: contractile protein filaments are bundled into myofibrils; bundles of myofibrils are contained in each individual muscle fibre (the cell); muscle fibres are bundled to form fascicles and the fascicles are bundled to form the muscle belly. The light and dark striations seen with the light microscope are caused by the precise orderly arrangement of actin and myosin myofilaments within each myofibril and the orderly arrangement of the myofibrils within the muscle cell (fibre).

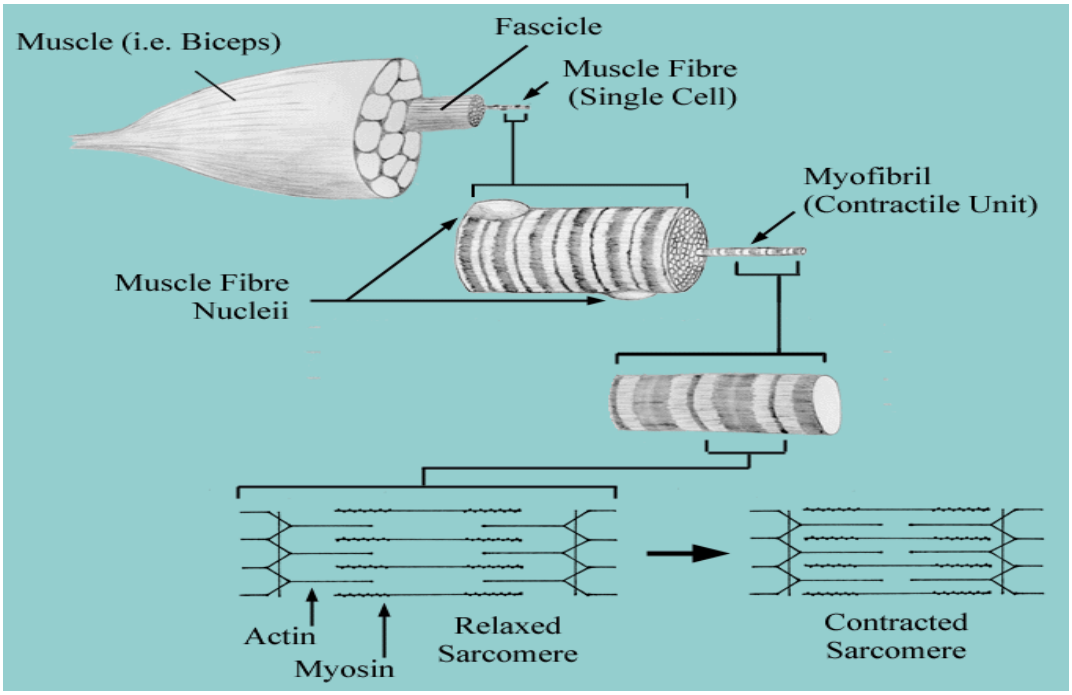


Figure 2.1 – The schematic organization of the skeletal muscle (biceps). The skeletal muscle cell is named as the muscle fibre (Developmental Biology Online).

The basic contractile unit of skeletal muscle is called the sarcomere. Each line or set of lines is given a letter name, which is the accepted way to refer to the specifics of these striations. When there is a stimulus for contraction, the actin filaments are pulled along the myosin filaments (an active process requiring ATP). This is represented by the Sliding Filament Theory. In that case, as in Figure 2.2, the Z discs are pulled closer together, causing the sarcomere to shorten. Although the difference in length produced by one sarcomere contracting is very small, when many sarcomeres arranged in series within musle fibers contract, there is considerable shortening of the muscle, causing generation of movement.

The diagram in Figure 2.2 compares the striations seen on the muscle fibre to the arrangement of the actin and myosin filaments which account for these striations. The proper name for each band of striations is shown for both the myofibril and the sarcomere. The I band crosses two sarcomeres, so it is only labelled on the myofibril section (Half of the I band is in each of two sarcomeres).

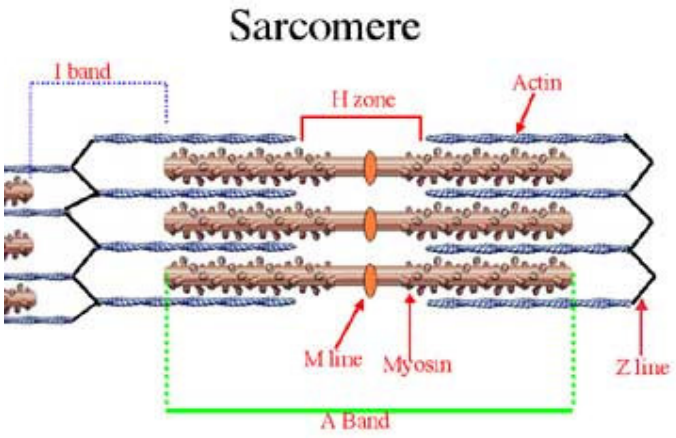


Figure 2.2 – The components of the sarcomere, the basic contractile unit.

2.1.2. The Extracellular Matrix

Although certain aspects of muscular adaptation to longterm stimulation are well-known [2], several facets remain unexplored. Changes in fiber-type, metabolic activity, muscle mass, contractile strength, and speed have been documented in numerous reports e.g., but little attention has been paid to alterations within the extracellular matrix. This is an important aspect of the adaptation process since the mechanical properties of muscle tissue are heavily influenced by the interstitium which binds individual muscle cells into a cohesive unit. Collagen types I and III are the major forms of interstitial collagen found in skeletal muscle and have been shown to strongly influence tissue extensibility.

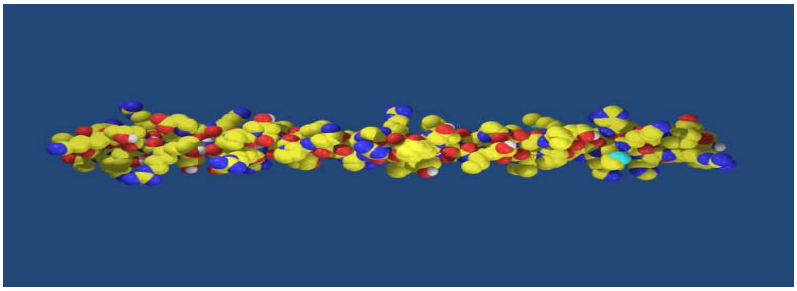


Figure 2.3 - α-chains in triple helices are found in collagen type III fibers.

Type III collagen is also the main component of connective tissue in non-capillary blood vessels. Fibril-forming collagens of types I, III, and V are made up of linear alpha-chains of about 100 kDa in molecular weight, which form closely coiled triple-stranded helical molecules. α-chains and triple helices have linear dimensions on the order of 100 nm (Figure 2.3). A network of elastic fibers in the extracellular matrix provides muscle tissue with the resilience required to recoil after a transient stretch. These flexible fibers comprise an amorphous core of elastin surrounded by microfibrils, of which fibrillin is the primary element. The amount and relative proportion of these interstitial components has been shown to change with exercise training [3].

2.1.3. Contraction of the Skeletal Muscle

As Figure 2.4 shows, the contraction of the skeletal muscle is a process of high requirements and subsequent steps to[1]:

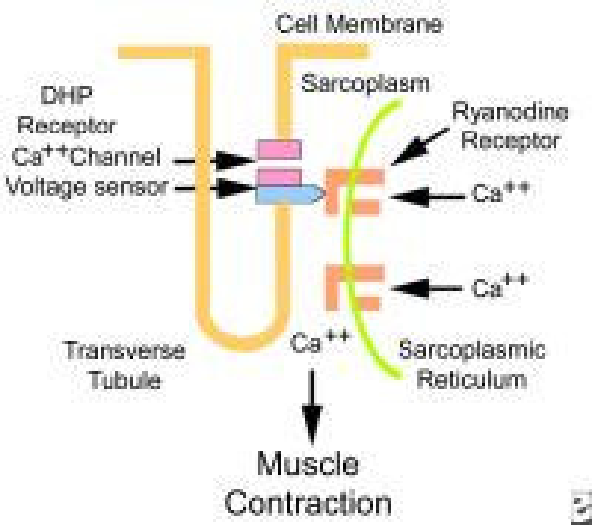


Figure 2.4 – The schematic diagram of skeletal muscle contraction.

- Nerve impulse (action potential) reaches the axon terminal, which triggers calcium influx into the axon terminal.

- Calcium influx causes synaptic vesicles to release ACh (acetyl choline) via exocytosis.
- ACh diffuses across synaptic cleft and binds to the ACh receptor on the sarcolemma (the skeletal muscle cell membrane).
- When ACh binds the receptor, it opens chemically regulated ion channels, which are sodium channels through the receptor molecule. Sodium, which is in high concentration outside cells and in low concentration inside cells, rushes into the cell through the channels.
- The cell, whose resting membrane potential along the inside of the membrane is negative when compared with the outside of the membrane, becomes positively charged along the inside of the membrane when sodium (a positive ion) rushes in. This change from a negative charge to a positive charge along the inner membrane is termed depolarization.
- The depolarization of one region of the sarcolemma initiates an action potential, which is a propagating wave of depolarization that travels along the sarcolemma. Regions of membrane that become depolarized rapidly restore their proper ionic concentrations along their inner and outer surfaces in a process termed repolarization.
- The action potential also propagates along the membrane lining the Transverse tubules entering the cell.
- This action potential traveling along the T tubules causes the sarcoplasmic reticulum to release calcium into thesarcoplasm.
- Calcium binds with troponin, causing it to pull on tropomyosin to change its orientation, exposing myosin-binding sites on actin.
- An ATPase, which also functions as a myosin cross-bridging protein, splits ATP into adenosine diphosphate (ADP) and phosphate (P) in the previous

contraction cycle. This energizes the myosin head. The energized myosin head, or cross-bridge, attaches to a myosin-binding site on actin.

- Power stroke occurs. The attachment of the energized cross-bridge triggers a pivoting motion of the myosin head. During the power stroke, ADP and P are released from the myosin cross-bridge. The power stroke causes thin actin myofilaments to slide past thick myosin myofilament toward the center of the A bands.

- ATP attaches to the myosin head again, allowing it to detach from actin.

- ATP is broken down to ADP and P, which cocks the myosin head again, preparing it for the next power stroke if needed.

- Repeated detachment and reattachment of the cross-bridges results in shortening without much increase in tension during the shortening phase (isotonic contraction) or results in increased tension without shortening (isometric contraction).

- Release of the enzyme acetylcholinesterase in the neuromuscular junction destroys ACh and stops the generation of a muscle action potential. Calcium is taken back up in the sarcoplasmic reticulum, and myosin cross-bridges separate. ATP is required to separate myosin-actin cross-bridges. The muscle fiber resumes its resting state.

2.1.4. The Role of O_2 in Exercise Physiology

The primary function of skeletal muscle is to contract and generate mechanical force to provide support to the skeleton and to produce movement of joints. This mechanical activity consumes large amounts of energy and therefore requires delivery of considerable amounts of oxygen and substrates, as well as the efficient removal of metabolic waste products. Both delivery and removal functions are performed by the circulation.

There are 3 different muscle metabolic systems that supply the energy required for various activities:

- Phosphagen system (for 10-15 second bursts of energy)
- Glycogen lactic acid system (for another 30-40 seconds of energy)
- Aerobic system (provides large amounts of energy that is only limited by the body's ability to supply oxygen and other important nutrients)

The aerobic system in the body is used for sports that require an extensive expenditure of energy, such as a marathon race. Therefore, endurance sports require aerobic energy. A large amount of ATP must be provided to muscles to sustain the muscle power needed to perform such events without an excessive production of lactic acid. This can only be accomplished when oxygen in the body is used to break down the pyruvic acid (that was produced anaerobically) into carbon dioxide, water, and energy by way of a very complex series of reactions known as the citric acid (Krebs) cycle. This cycle supports muscle usage for as long as the nutrients in the body last. The breakdown of pyruvic acid requires oxygen and slows or eliminates the accumulation of lactic acid. Figure 2.5 summarizes this process [1].

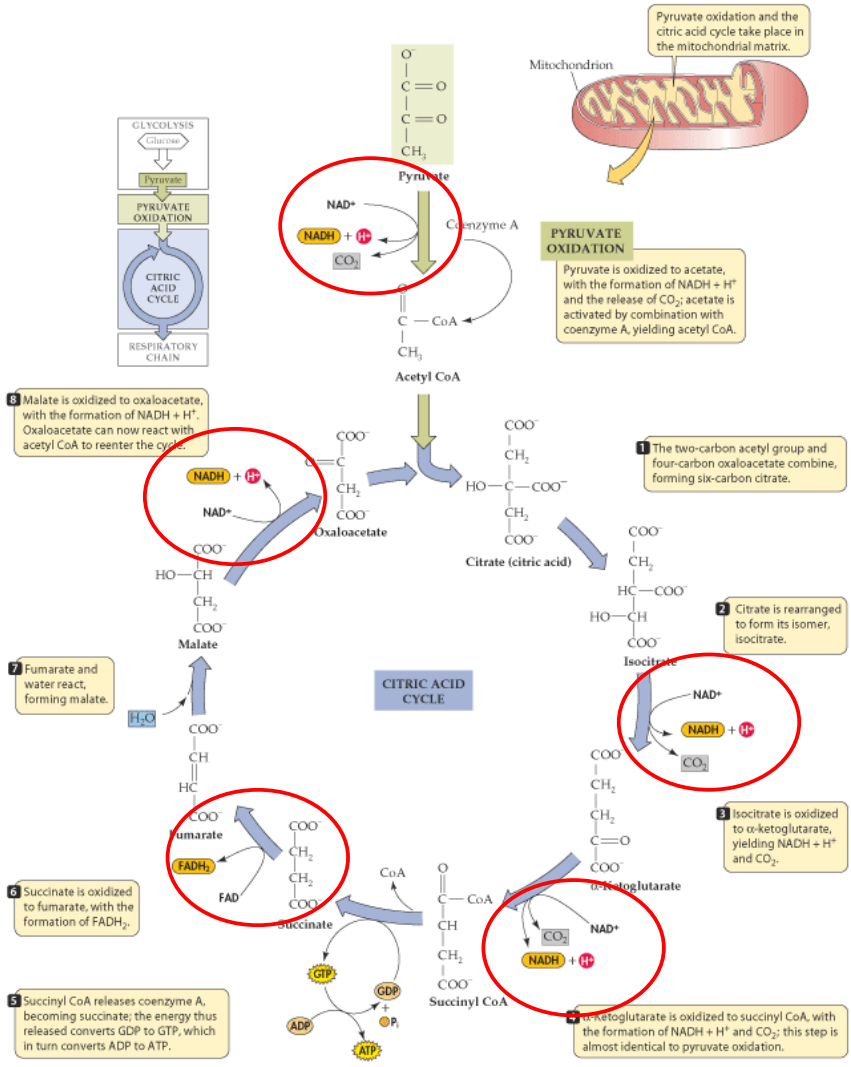


Figure 2.5 – The Krebs Cycle. Oxygen is required for the electron transport chain to function, which recycles NADH back to NAD+ and FADH2 back to FADH (marked in red).

In a more totalistic approach, the aerobic system is totally dependent on the oxygen supply to the muscle, and, naturally, the cardiac output. In the resting human, up to about 20% of cardiac output goes to skeletal muscle. This occurs not because blood flow is exceptionally high in resting muscle, but because skeletal muscle makes up about 40% of body mass. In the resting, non-contracting state, muscle blood flow is about 3 ml/min per 100g. However, when muscles contract during exercise, blood flow can increase more than 20-fold. Such increase in blood flow is termed active hyperemia. For muscle contraction, occuring during whole body exercise (e.g., running), more than 80% of cardiac output can

be directed to the contracting muscles. Therefore, skeletal muscle has a very large flow reserve (or capacity) relative to its blood flow at rest [4].

2.1.5. Physiology of Warm Up

Warm up is usually performed before participating in (technical) sports or exercising. It generally consists of a gradual increase in intensity in physical activity. It is important that warm ups should be specific to the exercise that will follow, which means that exercises should prepare the muscles to be used and to activate the energy systems that are required for that particular activity. Warm up can be active or passive. An active warm up involves exercise to raise core and muscle temperature, whereas a passive warm up relies on using external means to raise body temperature. Both methods increase muscle temperature but with the major difference being an active warm up has been shown to improve maximum oxygen uptake.

Changes derived from a warm-up that is specifically designed for endurance events have been documented. These produce a higher percentage of energy production from aerobic processes because of the facilitation of aerobic mechanisms that occurs after passive warm-up or rest. Ingjer and Stromme (1979) verified these benefits and listed the changes:

- Release of adrenaline
  - Heart rate increases to enable the O_2 in the blood to travel with greater speed.
  - Dilation of capillaries allow O_2 in the blood to travel in higher volume.
- Increase of temperature in the muscle
  - Muscle viscosity is reduced, resulting in an improvement in mechanical efficiency.
  - The enzyme activity is facilitated to encourage the breakdown of oxyhemoglobin (HbO_2) for the delivery of oxygen to the working muscle

  - The release of oxygen from myoglobin is increased.
- Increase of muscle metabolism
  - The activation energy for vital cellular metabolic chemical reactions is lowered which speeds up the breakdown of glycogen.
  - Injuries related to the muscles, tendons, ligaments, and other connective tissues may be reduced.
  - The cardiovascular response to sudden, strenuous exercise is improved
  - Lactic acid levels are lowered during following exercise.
- Increase in nerve conduction speed
  - Nervous impulses travel more rapidly and the sensitivity of nerve receptors is augmented.

2.1.6. Physiology of Stretching

Stretching is a regime of educating the muscle physiologically as well as mentally. Stretching is the activity of gradually applying tensile force to lengthen, strengthen, and lubricate muscles, often performed in anticipation of physical exertion and to increase the range of motion within a joint. Stretching is an especially important accompany to activities that emphasize controlled muscular strength and flexibility, such as ballet, acrobatics or martial arts [4].

There are different methods of stretching. Those are:

- Passive stretching is the one in which one assumes a position and holds it with some other part of the body, or with the assistance of a partner or some other apparatus.

- Active stretching is achieved by an individual contracting his own muscles without any help from an external device or a body.

- Isometric stretching is a type of static stretching (meaning it does not involve motion) which involves the resistance of muscle groups through isometric contractions (tensing) of the stretched muscles. The use of isometric stretching is one of the fastest ways to develop increased static-passive exibility and is much more effective than either passive stretching or active stretching alone. Isometric stretches also help to develop strength in the "tensed" muscles (which helps to develop static-active exibility), and seems to decrease the amount of pain usually associated with stretching. An example of using the wall to provide resistance would be the well known "push-the-wall" calf-stretch (Figure 2.6) where the subject is actively attempting to move the wall although he cannot actually do it [5].

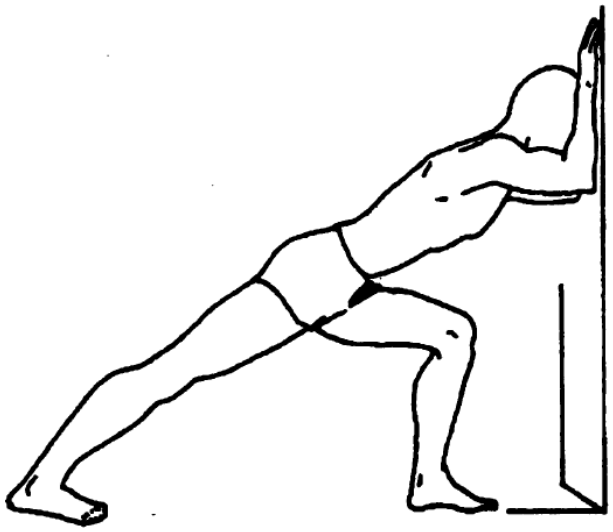


Figure 2.6 – "Push-the-wall" calf stretch is an example of isometric stretching where the length of the muscle does not change.

- Proprioceptive Neuromuscular Facilitation (PNF) stretching is relatively a new method that suggests alternating stretching and contraction patterns. The subject moves the muscle into a stretched position and then, with the aid of an external agent, isometrically contracts the muscle for 5-10 seconds [6].

As Figure 2.7 implies, the stretching of a muscle fibre begins with the sarcomere, the basic unit of contraction in the muscle fibre. As the sarcomere contracts, the area of overlap between the thick and thin myofilaments increases. As it stretches, this area of overlap decreases, allowing the muscle fibre to elongate. Once the muscle fibre is at its maximum resting length (all the sarcomeres are fully stretched), additional stretching places force on the surrounding connective tissue. As the tension increases, the collagen fibers in the connective tissue align themselves along the same line of force as the tension. Hence when you stretch, the muscle fibre is pulled out to its full length sarcomere by sarcomere, and then the connective tissue takes up the remaining slack. When this occurs, it helps to realign any disorganized fibres in the direction of the tension. This realignment is what helps to rehabilitate scarred tissue back to health. When a muscle is stretched, some of its fibres lengthen, but other fibres may remain at rest. The current length of the entire muscle depends upon the number of stretched fibres similar to the way that the total strength of a contracting muscle depends on the number of recruited fibers contracting [1].

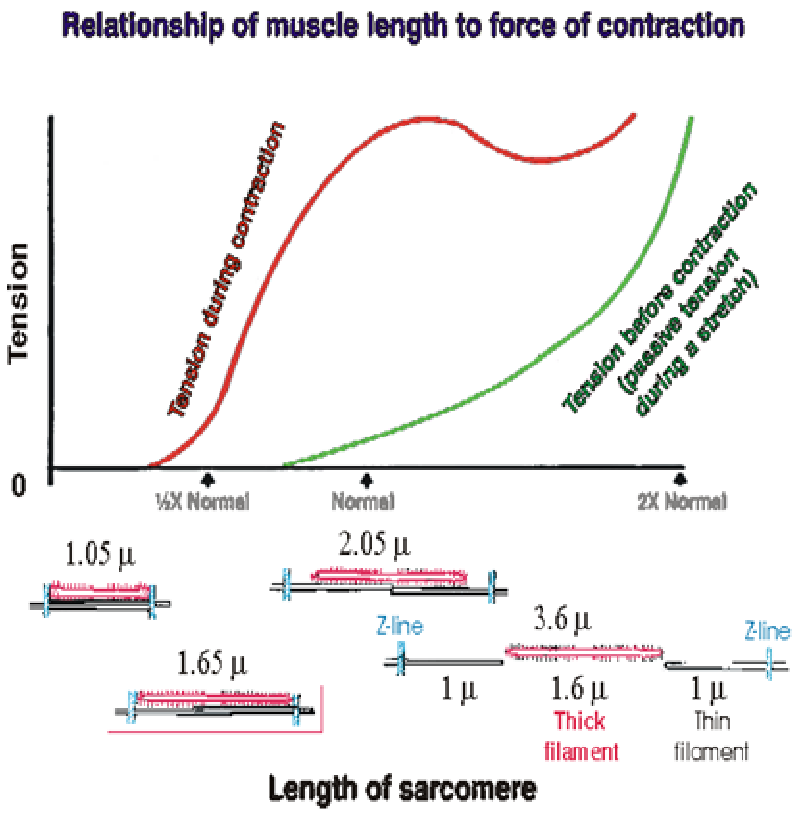


Figure 2.7 – Force vs. Length characteristic curve for a skeletal muscle. The total tension depends on two factors: The tensile power of the sarcomere (red) and the passive stretch (green) of the connective tissue (esp. the collagen fibers).

2.2. Functional Near-Infrared Spectroscopy (fNIRS)

Monitoring oxygen consumption has been a topic of interest in the 20th century, especially for researchers of muscle and brain, two tissues whose activity is predominantly determined by global and local oxygen consumption. Also, it is obvious that non-invasive methods have proven their advantage over invasive ones in the medical innovations of the current medical world. Revitalizing both concepts, non-invasive oxygen consumption monitoring has greatly enhanced our understanding of systemic metabolism and oxygen delivery kinetics. Near-infrared spectroscopy (NIRS) has been shown to be an extremely sensitive modality for studying local differences in muscle O_2 consumption and delivery. In addition to their high sensitivity, optically based monitoring designs are both wearable and are capable of providing real-time information about the condition of the patient. These

features afford such wearable, optical designs with a uniquely flexible applicability to the fields of both clinical health monitoring and enhanced fitness training [7]. All other methods (e.g. fMRI, PET) that serve the same goal of oxygen monitoring are either very expensive, non-portable, non-user friendly or invasive. In other words, the presence of functional NIRS (fNIRS) provides the best solution for the need of a non-invasive, portable, real-time rapid tissue oxygenation monitoring device to investigate local brain and muscle oxidative metabolism.

2.2.1. NIRS – The Theory

The diagnostic potential of optical methods has been widely known since Jöbsis (1977) first demonstrated that transmittance measurements of near-infrared (NIR) radiation could be used to monitor the degree of oxygenation of certain metabolites [8]. Human tissues contain a variety of substances whose absorption spectra at NIR wavelengths are well defined, and which are present in sufficient quantities to contribute significant attenuation to measurements of transmitted light. The concentration of some absorbers, such as water, melanin, and bilirubin, remain virtually constant with time. However, some absorbing compounds, such as oxygenated hemoglobin (HbO_2), deoxy-hemoglobin (Hb), and oxidized cytochrome oxidase (CtOx), have concentrations in tissue which are strongly linked to tissue oxygenation and metabolism. Increasingly dominant absorption by water at longer wavelengths limits spectroscopic studies to less than about 1000 nm. The lower limit on wavelength is dictated by the overwhelming absorption of Hb below about 650 nm. However, within the 650-1000 nm window, it is possible with sensitive instrumentation to detect light which has traversed up to 8 cm of tissue [9].

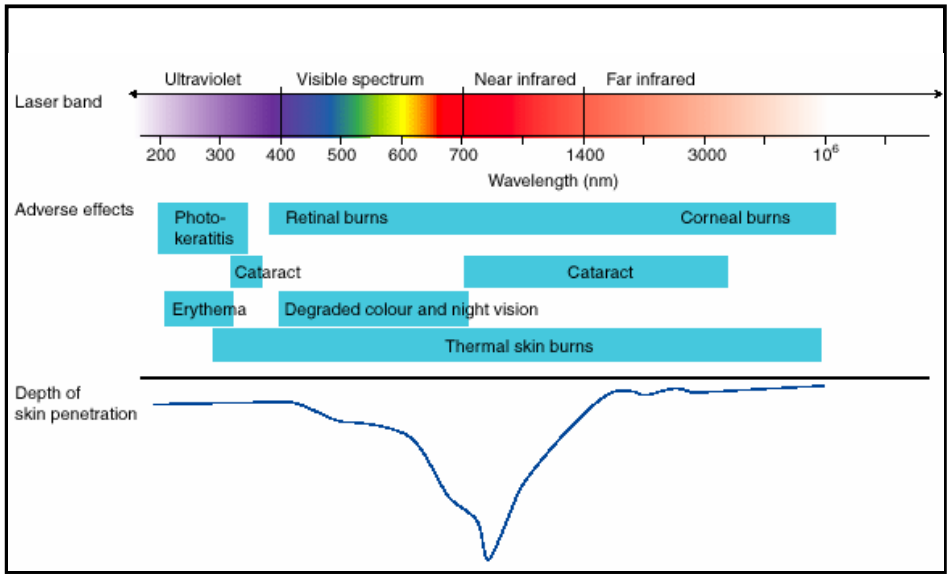


Figure 2.8 - The wavelength dependency of light in terms of skin penetration.

This penetrating ability of infrared light into tissues has led to the development and increasingly widespread use of clinical near-infrared spectroscopy (NIRS), which offers a safe, non-invasive means of monitoring cerebral function at the bedside without the use of radioisotopes or other contrast agents as well as the metabolic rate in terms of exercise physiology of certain skeletal muscles.

Continuous wave NIRS ($NIRS_{cws}$) technology is based on the fact that light in the wavelength range 650-950 nm is weakly absorbed by tissues. Light in this range (called 'optical window') has a good penetration depth as seen in Figure 2.8. By transmitting and receiving light at least two wavelengths in the near-infrared range, it is possible to find concentration changes of deoxygenated ([Hb]) and oxygenated ($[HbO_2]$) hemoglobin with NIRS in tissue. Thus it gives dynamic balance information between O_2 supply and consumption. This method offers non-invasive and inexpensive method for the study of aerobic metabolism and hemodynamics and has become a widely used technology for the oxidative metabolism investigation in tissues, especially in brain and muscle [10].

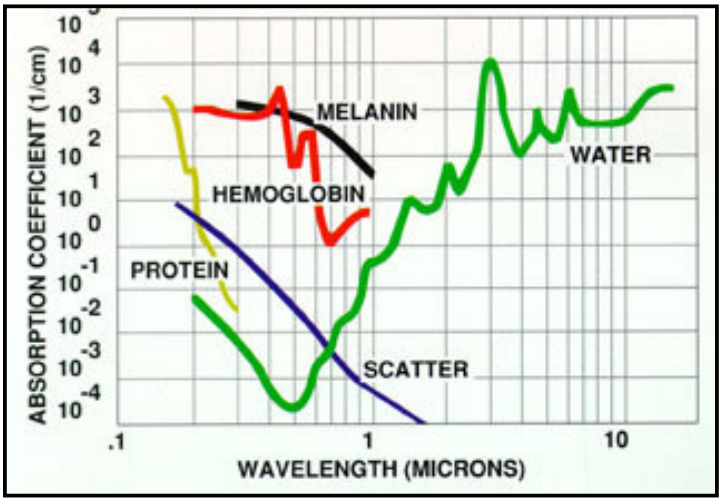


Figure 2.9 – Absorption coefficients of certain tissues.

In biological tissues, absorption is mainly caused by either water molecules in the far infrared (FIR) and higher wavelengths or macromolecules such as proteins and pigments in the visible or lower wavelengths. As Figure 2.9 suggests, the optical window is closely related to the range where the minimal absorption for water takes place due to the high content of water in all biological tissues. Whole blood is a strong absorber in the red-NIR regime, but because the volume fraction of blood is a few percent in tissues, the average absorption coefficient that affects light transport is moderate. However, when photons strike a blood vessel they encounter the full strong absorption of whole blood. Hence, local absorption properties govern light-tissue interactions, and average absorption properties govern light transport.

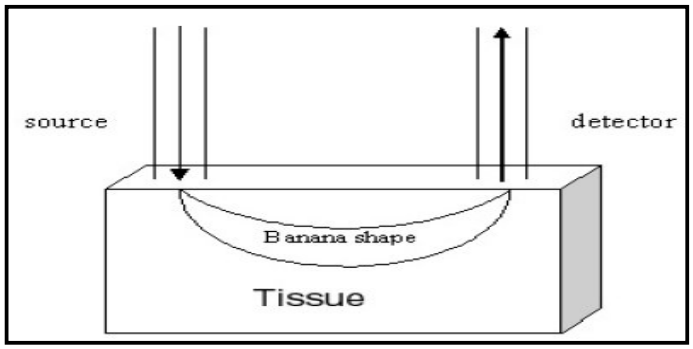


Figure 2.10 – The banana-shaped travel of light in tissues.

Light absorbed and scattered in the tissue reaches the light detector placed typically 1.5-4 cm apart from the source as in Figure 2.10. This distance is called the “optode distance”. Dominant light absorbing chromophores in near-infrared range are Hb and HbO_2, deoxgenated/oxygenated myoglobin (Mb and MbO_2) and cytochrome-c-oxidase. Concentration of cytochrome oxidase in living tissue is usually at least an order of magnitude below that of hemoglobin and its effect to NIRS attenuation is found to be about 2-5% [11]. Although absorption characteristics of hemoglobin and myoglobin overlap effect of myoglobin desaturation to optical signal is found to be less than 25% in studies by Chance(1992) and 20% in Mancini (1994). Hence, received light intensity change and NIRS signal is mainly due to hemoglobin. Since light passing through the large vessels is mostly absorbed, light that reaches the detector comes mainly from small blood vessels (arterioles, capillaries and venules) [10] providing a better circumstance for measurement in extremities [12].

2.2.2. The Use of fNIRS in Sports Medicine

A number of different approaches that utilize NIRS for enhancement of muscular and neural performance have been applied recently. Here is a comprehensive selection of varying perspectives on the topic:

- Quaresima et al. [13] investigated vastus lateralis and rectus femoris VO_2 at rest and during MVC using a 12-channel NIR_{cws} system. Although non-uniform over a certain area, VO_2 value was significantly higher than that of at rest as previously expected. However, at each of the 12 channels, no difference was observed between the mean values of VO_2 measured during a 5-sec MVC and those of 30-sec continuous MVC. The results provide a powerful tool for investigating the spatial and temporal featuresof muscle oxygenation.

- The monitoring of a single muscle fiber locations does not accurately represent the heterogenous oxidative metabolic responses to the same

exercise either within the same muscle or among different muscle groups. The ongoing development of comparative approaches, and especially multi-channel NIRS devices, offers the mentioned perspective. In the last decade, multi-channel NIR_{cws} systems have been developed to provide spatial maps of oxygenation changes every 5-8 sec during calf exercise [14]. Miura et al., found regional differences in the oxygenation of the gastrocnemius during exercise and recovery performing plantar flexion exercises for 120 seconds with the distal portion having greater oxygenation. This is consistent as the distal portion having a greater change in BF possibly because of the higher intramuscular pressure during exercise.

- Sport psychology field has a growing interest in investigating brain activation during exercise. Ide et al. [15] studied cerebral oxygenation and metabolism during sub-maximal cycling. Middle cerebral artery blood velocity with the cerebral oxygenation. This suggests that, during exercise, cerebral BF increases in excess the O_2 demand.

In conclusion, NIRS with its features is a potential tool of succesful application in sports medicine. It is highly likely that NIRS would present itself as an objective diagnostic imaging and quantization methodolgy en route to succesful therapeutic strategies and specific training programs.

3. NIROXCOPE

3.1. Instrumentation – The Operating Principle

The instrument to quantify the results obtained by NIRS is named NIROXCOPE developed at Boğaziçi University, Biophotonics Lab. (Figure 3.1) The device is composed of :

- a probe containing light sources and detectors on a flexible printed circuit board (PCB)
- a software to control the device and store the data on the computer for offline analysis
- transmitter/receiver circuits which control the LEDs, light sources with the software and LED currents.

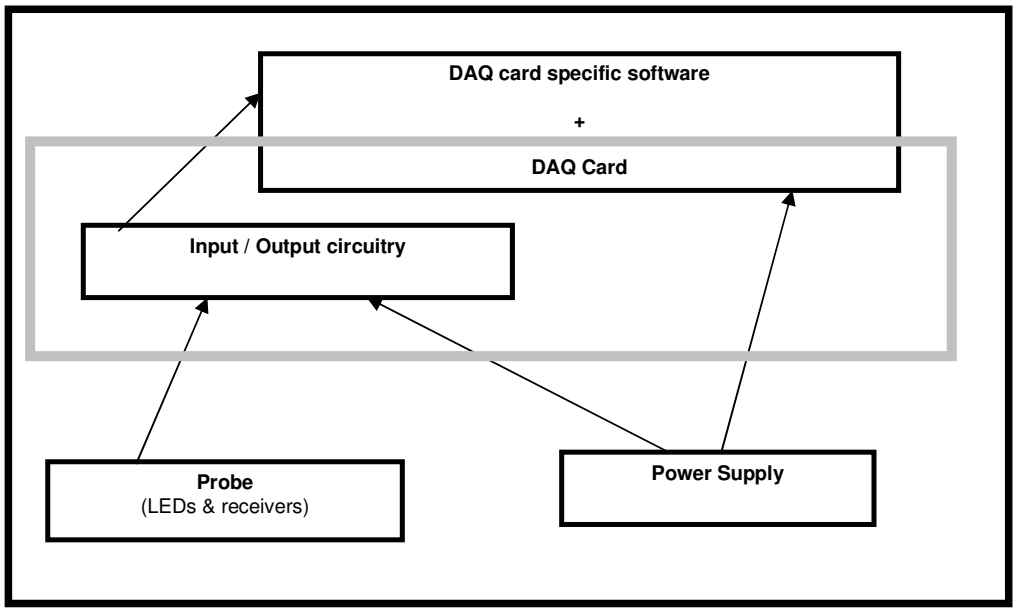


Figure 3.1 – The block diagram of Niroxcope. The data aqcuisition card and the input / output circuitry are embedded in a single box.

The probe is equipped with emitter and detectors for near-infrared light of 735 nm, 805 nm and 850 nm. The emitted light passes through the skin and, while scattering, reaches tissue where a portion is absorbed by Hb and then returns to the detectors. As Figure 3.2 suggests, with the 805 nm wavelength region –within the optical window (600 – 1000 nm), the region of equal absorption for oxygenated and deoxygenated Hb– as the isobestic point, deoxygenated Hb (Hb) causes an increase in absorption in the shorter wavelengths, while oxygenated Hb (HbO_2) causes an increase in absorption in the longer wavelengths. It thus follows that light from the emitter at 735 nm is more easily absorbed by deoxygenated Hb, while light at 850 nm is more easily taken up by oxygenated Hb. The amount of light that then returns to the detectors can be measured and in this way it is possible to measure approximately the oxygenation and deoxygenation states of Hb [16].

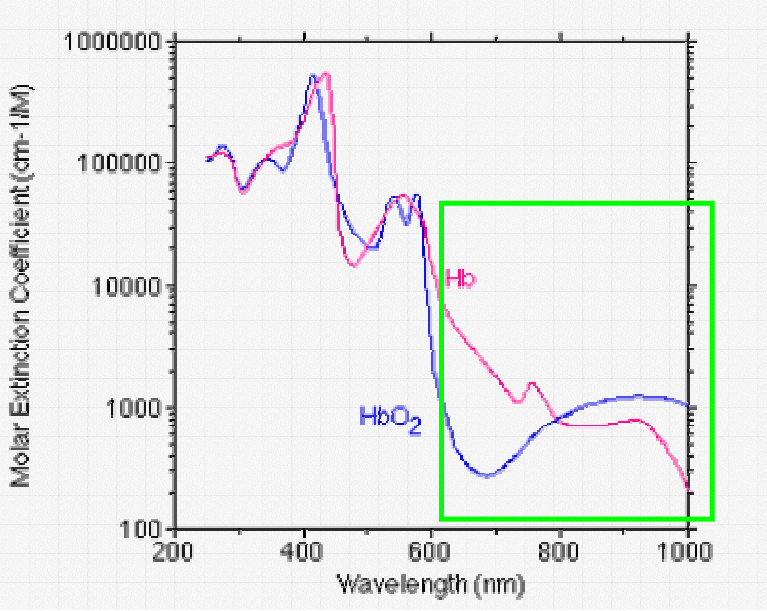


Figure 3.2 – Absorption spectra for Hb & HbO2 within the optical window (the green rectangle / 600 – 1000 nm). 805 nm is the isobestic point.

Changes in the concentrations [Hb] and [HbO_2] are calculated using the modified Beer-Lambert Law:

$$A = \log\left(\frac{I_o}{I}\right) = \alpha.c.d.B + G$$

where

A is the light attenuation measured in optical distance,

I_0 is the incident light intensity,

I is the received light intensity,

α is the specific extinction coefficient of the absorbing compound ($OD.cm^{-1}.\ \mu M^{-1}$),

c is the concentration of the absorbing compound in (μM),

d is the distance between the points where light enters and leaves the tissue (cm),

B is the geometrical path length factor such that ($d \cdot B$) is the real path length taken by the light between the optodes,

G is a factor which takes into account the scattering properties of the medium.

The assumption that *G* does not change during measurement period would require a modification of the general rule. For a medium containing [Hb] and [HbO_2] as dominant absorbing compounds, the equation becomes:

$$A = \left(\alpha_{Hb}.c_{Hb} + \alpha_{HbO_2}.c_{HbO_2}\right).d.B + G$$

Using this formula with the intensity changes for the three wavelengths, concentration changes of [HbO_2] and [Hb] are calculated as well as Oxy (oxygenation) signal which is the difference between concentration changes of [HbO_2] and [Hb] and BV (blood volume) which is their sum. It is siginificant to note that Oxy and BV are not absolute measurements, rather relative one in comparison to the base values determined at the calibration stage [17].

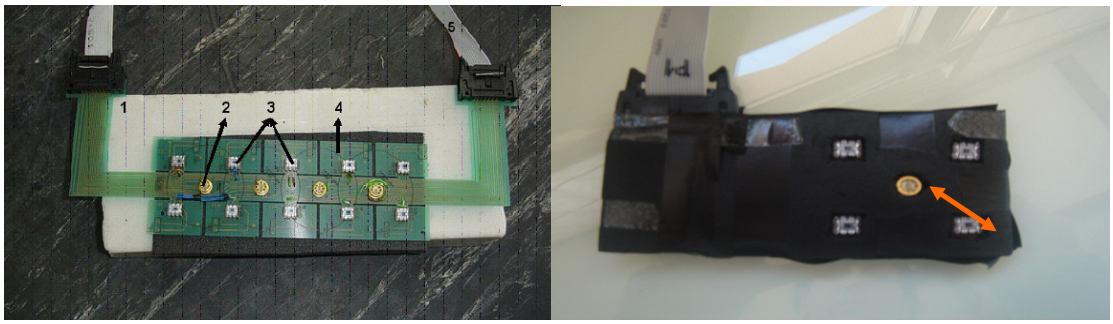
3.2. Components of Niroxcope 201

NIROXCOPE is a long term project initiated in 2002 by the Biophotonics Lab of the Institute of Biomedical Engineering in Boğaziçi University. The current version is named Niroxcope 201.

3.2.1. Probe

The probe houses light sources, photodetectors and special backing and band material as in Figure 3.3(a). It consists of four LEDs and ten detectors that require external control for their operation. Light sources used in this thesis are multi-wavelength light-emitting diodes (L4*730/4*805/4*850-40Q96-I, EPITEX, Japan). They emit in NIR spectrum at 730nm, 805nm and 850nm. Detector options ranged from multi-anode PMTs

(Photomultiplier Tube) to discretecommercial-grade photodiodes with external preamplifiers. A monolithic photodiode/preamplifier IC houses in a clear 8-pin DIP package (OPT101 from Burr-Brown) is used. This offers both the convenience and low-cost of a solid-state detector, combined with the electrical and optical isolation of an integrated preamplifier.



(a) **(b)**

Figure 3.3 – (a) A 4-LED [2] probe with 10 photodetectors [3] placed in a PCB [4] on a grey phantom [1]. (b) A single-quadrant probe to provide better localization with a LED to quadrant distance of 2.5 cm (shown in red).

The mean penetration depth of NIRS in living tissue is approximately one-half of the distance between the emitter and detector as verified directly and by Monte Carlo simulation (Chance et al., 1992). In NIROXCOPE 201, the distance between light sources (LEDs) and detectors is 2.5 cm as in Figure 3.3(b), which enables nearly 1.25 cm penetration depth in the tissue.

3.2.2. Software – The Control Unit

The control unit of Niroxcope 201 is named BRAININFO and includes both a computer and a DAQ card. Any computer which is compatible with Windows 2000 upper versions can be used. As for DAQ card, National Instrument NI-PCI-MIO-16E-4 card is used. This card provides up to 250 kS/s multichannel sampling rate. Four of the digital outputs and analog input channels of the DAQ card are used to control the system. DAQ card can be controlled either by LabVIEW or Visual C++.

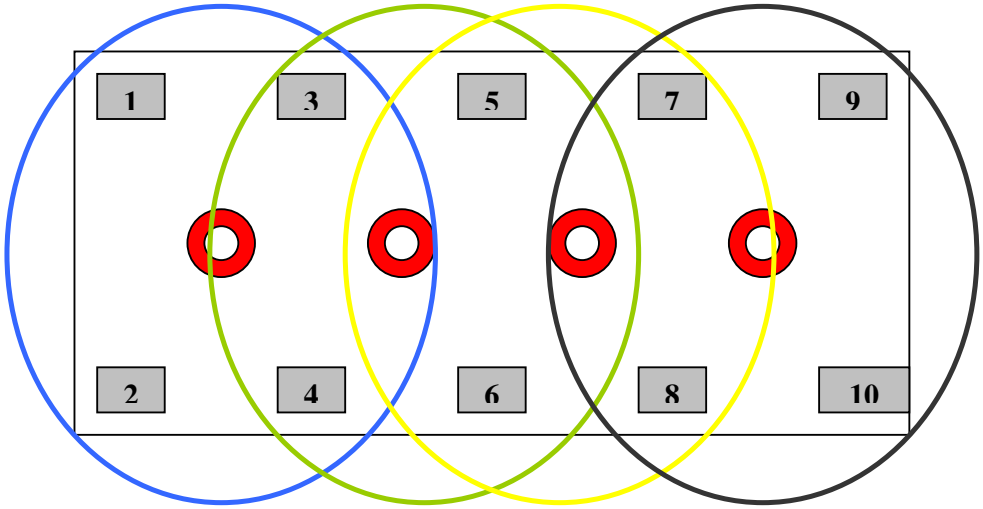


Figure 3.4 – The schematic diagram for the 4-LED probe. Each colored circle determines a quadrant (eg. Yellow circle is the 3rd quadrant – PD #5 to #8).

The programming routine that software follows is based on turning on and off LEDs and sampling the detected signals to the computer. Then, the program "listens" to all photodiode receptors in a specific order for each corresponding LED for a pre-determined number of cycles. All data for a single PD are averaged and the mean value recorded on a text file. This input procedure is repeated for each of the 3 wavelengths (735 nm, 805 nm and 850 nm).

There are a total of 10 photodiodes and 4 LEDs on the probe as seen in Figure 3.4. Due to the specific quadrant based multiplexing scheme, this corresponds to a maximum total of 16 photodetector outputs and each window in the software interface corresponds to one of these outputs as in Figure 3.5 with columns determining the quadrant (i.e. the LED) and rows determining the position within the quadrant (eg. 2nd row is the southwest photodetector).

To summarize, in a single cycle (i.e. a single row in the text files), 48 mean voltage values (3 wavelengths for 4 LEDs, 4 PDs associated for each LED) are recorded headed by

the time (in seconds) the cycle is completed. The procedure is carried out until the “Stop” button is pressed by the user. If the halting action happens within a cycle, the software delays it until the cycle ends.

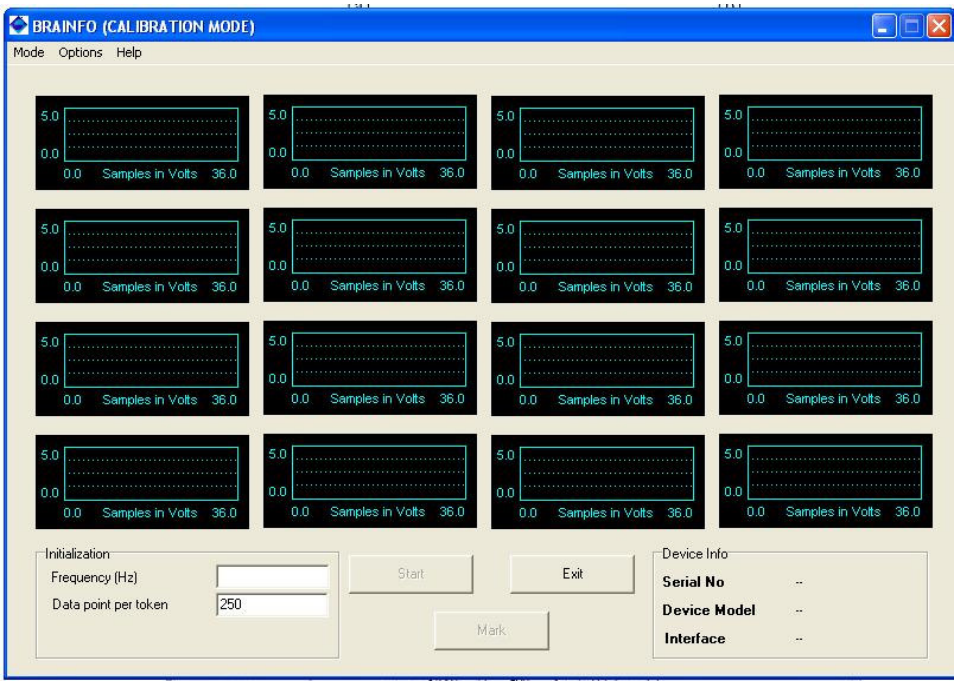


Figure 3.5 – The control unit software (BRAININFO) has 16 windows to keep track of photodetector inputs.

3.3. Niroxcope 301 vs. Niroxcope 201

As new needs arise, it is inevitable to update and modify any type of electronic equipment. This has been the case for Niroxcope as one of the integral parts of this thesis involved updating Niroxcope 201 to its new version Niroxcope 301. The modifications are included in Table 3.1:

Table 3.1

A comparison of Niroxcope 301 with Niroxcope 201.

	NIROXCOPE 201	**NIROXCOPE 301**
DAQ card	NIDAQ (PCMCIA)	UDAS 1001E-2G (USB)
Platform dependency	Requires MATLAB on PC	Windows 2000 / XP
User control		• Selective Mode • Input / Ouput rate control • GUI

3.3.1. DAQ Card

The data acquisition is the essential process which acts as the passageway between the software and the PCB components. The LEDs receive their turn on/off commands through the digital output ports of the DAQ card as the receivers on the PCB transfer their analog input to feed the software through it. The PCMCIA-based DAQ card by National Instruments of Niroxcope 201 was replaced by the UDAS DAQ card, named UDAS 1001E-2G (Figure 3.6) which uses a USB interface [18]. Having a USB interface primarily provides a much more user-friendly environment as USB is also the cornerstone of the "plug-and-play" technologies.

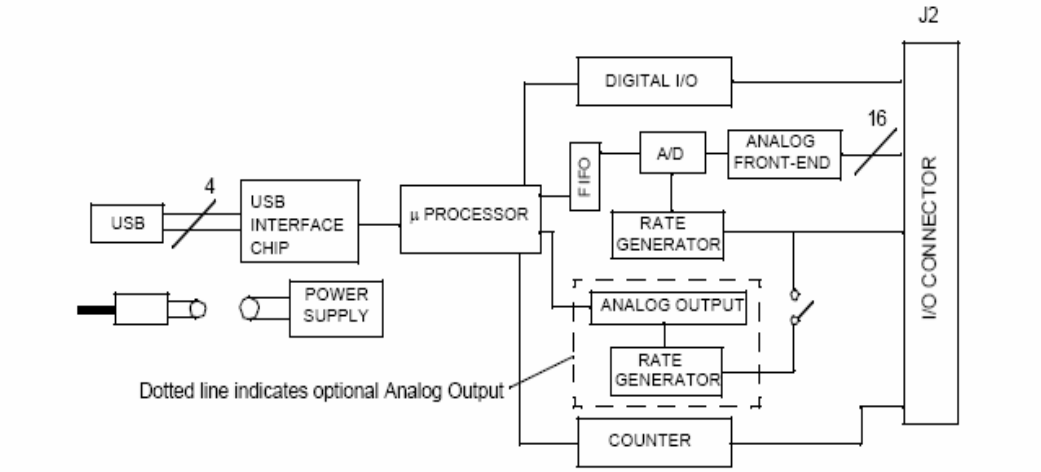


Figure 3.6 - UDAS 1001E Series Block Diagram.

The surplus that UDAS 1001E-2G provides can be classified as:

- The ability to exercise parallel processing as up to 127 systems can be connected to a single host

- The "UDAS Syscheck" function that is a useful tool for quickly verifying the hardware is to determine the proper matching of the components (Figure 3.7)

- When in auto zero mode, the inputs to the Analog input circuit are internally grounded, disabling the input multiplexers. This enables a known 0 (zero) volt signal to be measured, and the value read can be used to compensate for input offset errors.

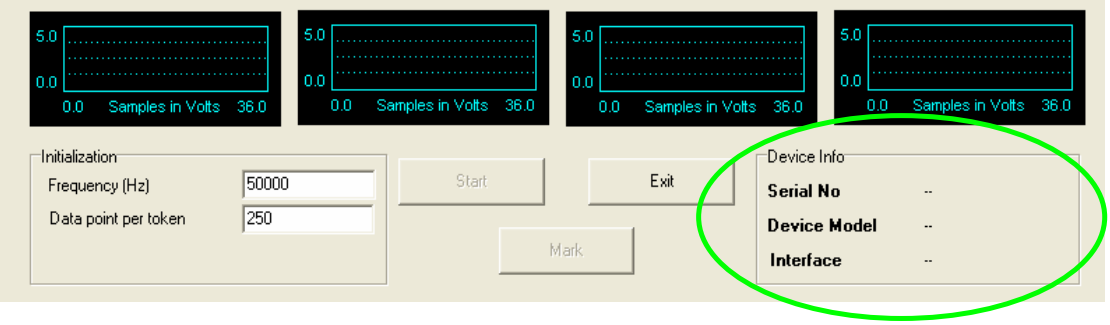


Figure 3.7 – Niroxcope 301 verifies the connection from the user interface.

- High-speed triggering to start or stop conversions that can be either an analog input or a digital input. External triggering (marked with green markers in the output plots) plays a very important role in synchronizing the system with an external unit such as an fMRI, EMG equipment as well as timed cognitive tests such as simulator activities to measure oxygenation in the brain. Niroxcope 301 is enabled to receive digital input triggering from the RS-232 parallel port as well as periodic internal triggers for the good of synchronization.

3.3.2. Platform dependency

Niroxcope 201 uses a chain of programs in the order of a C# program whose output is fed to an Excel macro only to be further evaluated and processed by a MATLAB code. The C# code deals with the DAQ card, the Excel macro performs data manipulations and the MATLAB code applies the Beer-Lambert law to produce the end results of blood volume and oxygenation. Niroxcope 301 combines these processes by only operating on a single Borland C program, which embeds the Excel macro and automatically triggers a MATLAB interpreter that fulfills the Beer-Lambert task without even notifying the user. The new compact form of software adds to the user-friendly approach as well as minimizing the user intervention and possible errors caused by it. More importantly, this approach of programming allows operating on computers without MATLAB installed on it. However, although much simpler for the end user, the performance of the current MATLAB interpreters is not yet at the desired level. Thus, the total time of computation of

Niroxcope 301 exceeds that of Niroxcope 201 for lengthy measurements. This is an issue of software design to be determined by the experimenting user as well as the coder.

3.3.3. User-controlled parameters

Even though it is aimed to minimize user intervention at the computation level, it is always a good practice to maximize the user controlled parameters in a software, increasing the user options to lead towards personalization and to increase the number of addressed individual needs. The modifications exerted on Niroxcope 301 over Niroxcope 201 in terms of the user-controlled parameters include:

- a graphical user interface (GUI) to select background and graph colors, axis determination and labelling, etc. to enable a more comfortable working environment for the user (Figure 3.8)

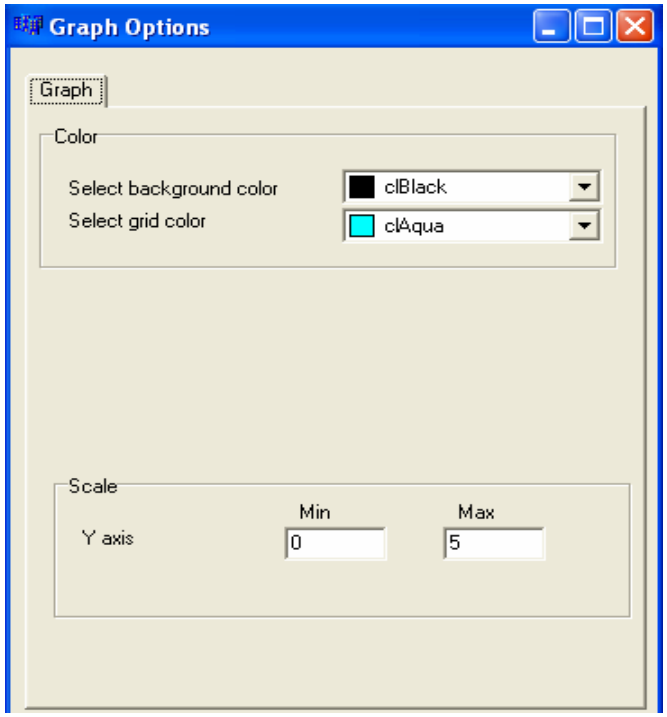


Figure 3.8 – The GUI of BRAININFO (selected from the "Options" menu tab in the interface).

- the ability to determine the input/output rates to control fast-flowing data online, to possibly relate data speed to the level of ischemia and exercise rate and to troubleshoot at the coding stage. (Figure 3.9)

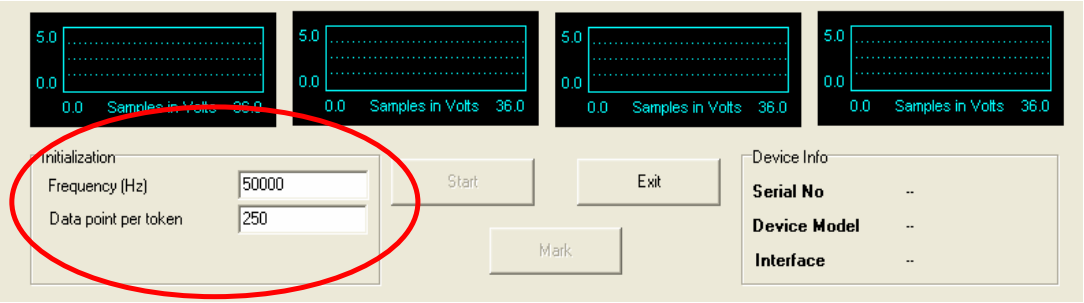


Figure 3.9 – The frequency (sampling rate) is a user-determined parameter in Niroxcope 301.

- a selective mode to choose a specific led and a wavelength of interest to simplify by getting rid of abundant data. (Figure 3.10) This enables the usage of the single-LED probe (Figure 3.3.(b)) without any loss time to ensure better localization on a single group of muscle.

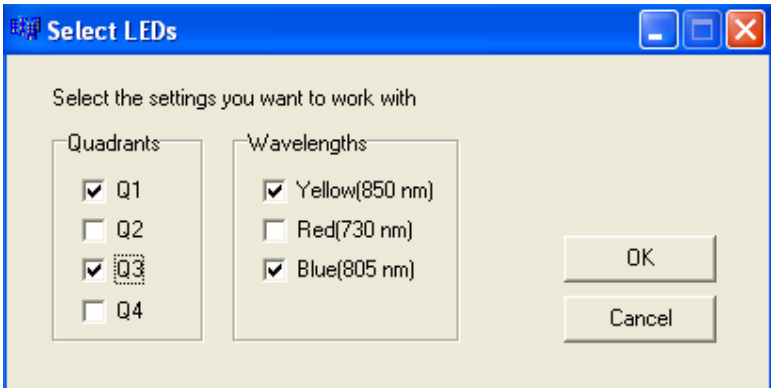


Figure 3.10 – The Selective Mode menu of BRAININFO. The current selection includes LED #1 and LED #3 with only 805 nm and 850 nm LEDs active (therefore, a measurement only for HbO2).

4. QUANTIFICATION OF WARM UP & STRETCHING USING NIROXCOPE 301

4.1. Subjects

Thirteen healthy, nonsmoking male volunteers (mean age 23.1, range 21–28) took part in the study, after giving informed written consent [see Appendix A]. The subjects, all sedantaire, were selected carefully to be of similar antropometric properties (mean height 181cm, range 176 – 188 cm and mean weight 81.1 kg, range 72 – 90 kg) with the mean BMI of 24.5 within the range of 20.6 to 28.1. All subjects were asked not to perform any extensive physical activity on the two consecutive days in which the test is performed. All the experiments were carried out at the ambient temperature of 21 °C. Table 4.1 shows the characteristics of the volunteers.

Table 4.1

Basic antropometric data of interest for the 13 volunteers (BMI: Body Mass Index, SP_{leg}: Systolic pressure of the leg).

	Age	Height (cm)	Weight (kg)	BMI	SP_{leg}
AA	21	184	86	25.4	145
CK	21	178	84	26.5	140
DC	22	176	81	26.1	165
EA	25	179	90	28.1	140
EB	28	183	83	24.8	155
EE	26	185	76	22.2	150
EY	26	174	76	25.1	175
HF	21	179	80	25.0	160
MB	26	183	78	23.3	170
MK	21	185	88	25.7	165
MM	22	184	76	22.4	170
RN	22	187	72	20.6	180
SR	26	188	86	24.3	180
MEAN	**23.6**	**181.9**	**81.2**	**24.6**	**161.2**
STD DEV	**2.6**	**4.3**	**5.5**	**2.0**	**14.2**

4.2. Methodology - The Arterial Occlusion Protocol

As Niroxcope 301 quantifies the oxygen usage relative to a baseline value locally, applying ischemia to the area of interest is a very common practise as it is with most of the fNIRS measurements [19]. Prevention of blood flow in and out of a certain area (presently the lower leg) suggests a constancy of blood volume (Δ[BV]) and, therefore, a better idea about the oxygenation (Δ[Oxy]).

Ischemia in the Arterial Occlusion Protocol was achieved by inflating a thigh cuff to a pressure of 30 mmHg above the value of the individual systolic pressure of the leg of each subject (measured by plethysmography in a pharmacy, within the range of 170 – 210 mmHg) [20]. Normally the systolic pressure (SP) of the leg exceeds the pressure of the aorta and brachial vessels due to compliance of the vessel wall [21]. The cuff was placed above the knee on the thigh (Figure 4.1) and was inflated in less than 1 min to the pressure needed for the arterial occlusion. A standard cuff was used for this purpose.

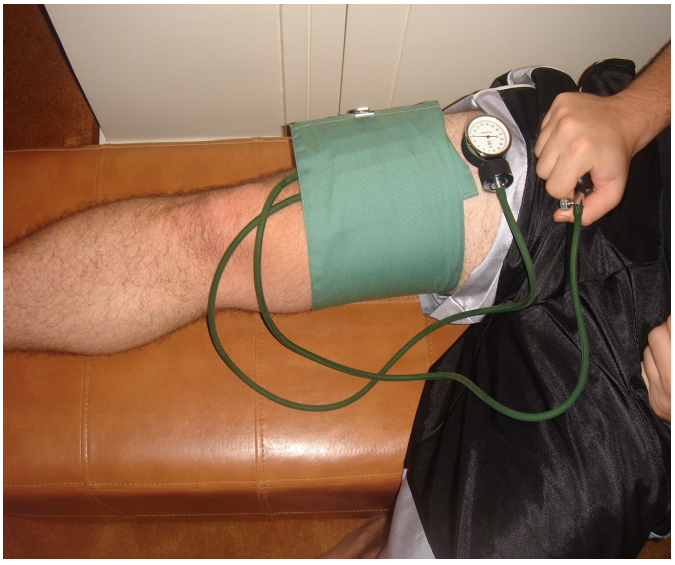


Figure 4.1 – A thigh cuff placed above the knee for lower leg ischemia.

The Arterial Occlusion Protocol takes approximately 11 minutes, consisting of four different periods all of which are separated with an internal trigger of Niroxcope 301:

- 30 seconds of introductory period (IP)
- 300 seconds of arterial occlusion (AO)
- 300 seconds of recovery (R)
- 15 - 30 seconds of finalization period (FP)

To observe the effect of warm up and stretching on the oxygen metabolism, a comparative study was held. The main routine included applying the Arterial Occlusion Protocol once before and once after a certain warm up and stretching routine and compare the resulting [Hb] and [HbO_2] plots [see Appendix B]. For an objective comparison, the following points were taken into consideration:

- The muscle studied was gastrocnemius, therefore, the probe was placed on the dorsal part of the lower leg as in Figure 4.2 and fixed with bandage. However, the leg is placed in a supine position (unlike in Figure 4.2) in order to reduce the effect of gravity in the ischemia procedure.

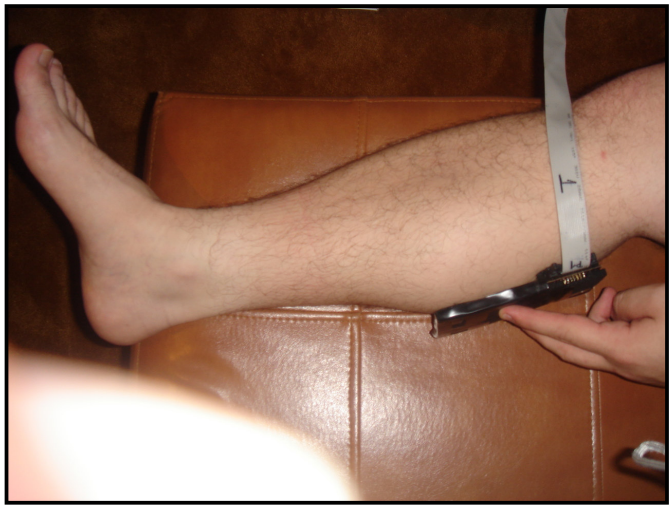


Figure 4.2 – The placement of the Niroxcope 301 probe on the gastrocnemius.

- The standardization of the warm-up and stretching of each subject was accomplished by assigning a jog (6.0 km/h) for 5 minutes on a treadmill followed by 12 times isometric contraction of the gastrocnemius (10

seconds per contraction) using an elastic band with a 10 second rest (Figure 4.3). Using a treadmill and an elastic band minimizes the intervention of the subject into the protocol achieving the same dose of exercise for all volunteers.

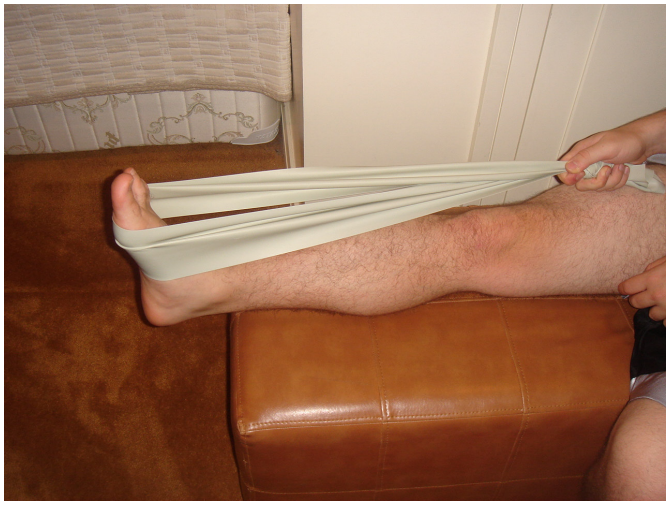


Figure 4.3 – Isometric stretching using an elastic band.

- The ischemia of 5 minutes has a cumulative effect if a sufficient amount of rest is not allowed. Therefore, the measurement before and after the warm up and stretching period were not carried out on the same day but rather on consecutive days. In order to maintain the same positioning of the probe on the second day, the exact location of the LED in the probe was marked on the leg of the subject after the first day.

- Also in the effort to reduce the differences caused by the effect of accumulation of the ischemia, the Arterial Occlusion Protocol was applied 30 minutes before the measurement phase on each of the two days of interest. This allows the gastrocnemius of the subject to be "in the same condition" as the real application takes place with the only difference being that on the second day, the subject performed the mentioned warm up and stretching routine.

- The single quadrant probe was used in the experiment to focus on a more specific area of interest. This provided four outputs per measurement. After applying wavelet denoising, the one (out of the four) with the least amount of noise was selected to be used in further evaluation.

In summary, the Arterial Occlusion Protocol was applied 4 times to each subject (I, II, III and IV) with I, II being on the first day and III, IV on the next day. I and III are only for calibrative purposes. II and IV are the actual measurements with II being before the warm up and stretching routine and IV being the protocol after that routine.

4.3. Parameters of Postocclusion

In a case of BV constancy, Niroxcope 301 effectively provides plots of [Hb] and [HbO_2] vs. time of the Arterial Occlusion Protocol as in Figure 4.4. The concentrations are not absolute values but they are are rather relative to a baseline determined at the calibration phase. The four phases of the protocol (IP, AO, R, FP respectively) are separated by red markers:

- There is no significant change of signal with rescpect to time in the IP and FP phases. These two phases act as buffers in which no ischemia nor recovery takes place. These phases are significant in determining the primary baseline before ischemia starts and final baseline, which is not necessarily the same as the primary one, after the recovery period ends. The subject is asked to sit still and refrain himself from any kind of energy consuming action in the IP and FP periods.

- It is significant to verify the constancy of volume in ischemia in the AO phase by observing the trend of [Hb] and [HbO_2] moving in opposite directions at a certain time (except for the initial part where total ischemia had not been established yet). As there is no blood supply to the region, [Hb] continuously

increases until it reaches a steady state or the ischemia is terminated (whichever comes first). [HbO_2] takes an initial positive value due to rushing of the blood into the lower leg from the pumping action of the cuff only to end as soon as arterial ischemia occurs. Later, it dips as oxygen in the region is continuously used up and HbO_2 is converted into Hb.

- In the R phase, the effect of the AO is progressively recovered in a damping oscillation trend. As soon as the ischemia is released, the accumulated Hb is replaced by HbO_2 from the rushing blood from the newly-opened arteries which causes a sharp fall in [Hb] and a sharp rise in [HbO_2]. Then, as time progresses, both graphs establish an equilibrium, not necessarily at the original baseline level which suggests that ischemia is creating a more permanent effect on the muscle which piles up unless the necessary resting time is provided.

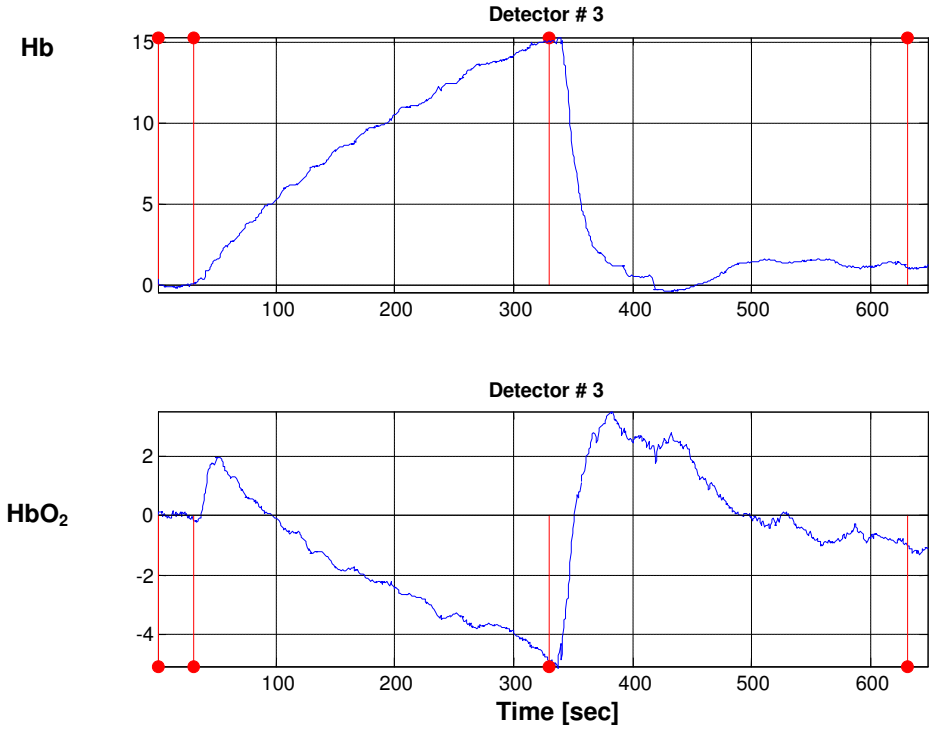


Figure 4.4 - [Hb] and [HbO2] vs. time plots of the Arterial Occlusion Protocol provided by Niroxcope 301, from Detector #3 of the probe for one of the subjects after the warm up and stretching routine.

After a thorough examination of the plots, different parameters were selected to be compared. It should be noted that all parameters worth of discussion are related to the [Hb] vs time plot. This is related to the lacking quality of the 850 nm signal which constitutes the [HbO_2] vs. time graph, only to be discussed in Chapter 5 of the document. Below are the parameters of postocclusion considered in this study:

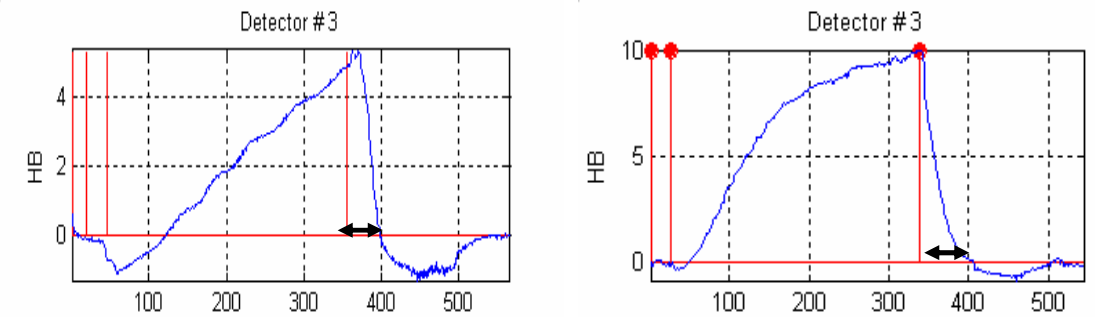


Figure 4.5 – A comparative study of the [Hb] vs. t: Arterial Occlusion Protocol before a warm up and stretching routine (left) vs. after the routine (right). Black arrows show t_{rec}.

- It is evident from the plots in Figure 4.5 that the experience of warm up and stretching allows a higher rate of conversion of [HbO_2] into [Hb] and, therefore, a faster increase in the [Hb] level. Time to reach the 90% (t_{90}) of the maximal [Hb] level is a common practise in quantifying the rate of change.[22] Adding a normalization parameter such as dividing t_{90} by the total time to reach the maximum level (t_{max}) gives a more accurate index of the rate of change of the [Hb] value in the AO phase of the Arterial Occlusion Protocol (i.e. during ischemia). This index is called the 90% index.

- The relative value of the maximal [Hb] level at the end of the AO phase itself is an issue of interest as it displays a significant difference in the two cases in which the protocol is applied (Figure 4.5). This value is referred to as Hb_{max}. Hb_{max} takes a greater value after the warm up and stretching routine, suggesting a greater number of motor units recruited.

- As the physiology of warm up and stretching is tightly knit around fatigue, the time of recovery (t_{rec}) is related to proving the cause. t_{rec} is the time between the release of the ischemia and the first time the [Hb] value reaches the initial 0 value after it as marked with black arrows in Figure 4.5. However, t_{rec} is not evidently biased to one of the cases, either before the warm up and stretching or after it. That is because, the recruitment of more motor units would take more time to recover to their original units (i.e. a higher t_{rec} for the protocol after warm up and stretching) whereas, the general tendency of a well-prepared muscle is to recover to the original state more quickly (i.e. a higher t_{rec} for the protocol without warm up and stretching).

4.4. Statistical Analysis

MATLAB 7.0 tools were utilized in the process of analyzing the data and the parameters of postocclusion. GINPUT is the MATLAB library function with which numerical data was extracted from the plots provided by Niroxcope 301. It enables the user to select points from the figure using the mouse for cursor positioning. Three points per plot were sufficient to obtain all parameters mentioned in Section 4.3 as marked with colored marks in Figure 4.6.

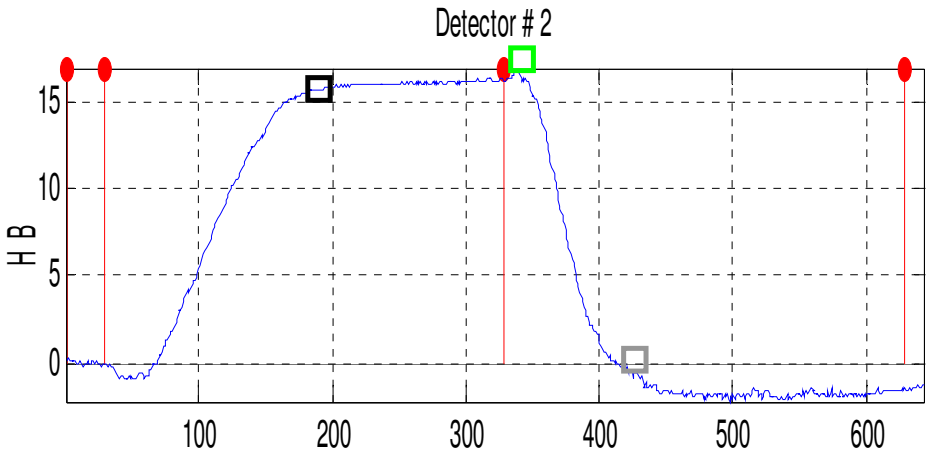


Figure 4.6 – Three input points to determine the parameters of postocclusion: 90% point (in black), maxima (in green) and recovery point (in grey).

For all parameters involved (i.e. Hb_{max}, t_{90}, t_{max}, %90 index, t_{rec}), the mean and the standard deviation values are calculated. Statistical significance between groups was tested by the One-Way Analysis of Variables (anova1 in MATLAB 7.0) Test. It should be noted that anova1 assumes the group of data be normalized. The statistically significant level of difference was considered to be at $p < 0.05$.

4.5. Results

Table 4.2 summarizes the results of the changes in [Hb] in a comparative manner:

Table 4.2

A comparative study: Parameters of Postocclusion (shaded) of the 13 subjects before and after the warm up and the stretching routine is applied.

	BEFORE					AFTER				
Subject	**Hb_{max}**	**t_{90}**	**t_{max}**	**%90 index**	**t_{rec}**	**Hb_{max}**	**t_{90}**	**t_{max}**	**%90 index**	**t_{rec}**
AA	**14.666**	249.6	338.5	**0.737**	**45.8**	**22.117**	217.6	340.9	**0.638**	**32.0**
CK	**15.089**	269.2	338.3	**0.796**	**46.4**	**23.406**	272.6	339.6	**0.803**	**38.2**
DC	**17.356**	303.6	336.5	**0.902**	**15.4**	**23.177**	168.3	335.1	**0.502**	**47.7**
EA	**16.162**	305.3	333.2	**0.916**	**21.9**	**16.430**	162.7	335.1	**0.486**	**76.0**
EB	**11.292**	288.6	339.3	**0.851**	**20.9**	**20.664**	211.7	342.5	**0.618**	**19.2**
EE	**15.028**	250.9	337.5	**0.743**	**65.2**	**16.681**	271.9	339.6	**0.801**	**26.0**
EY	**15.480**	298.6	336.1	**0.889**	**41.8**	**17.585**	238.1	336.9	**0.707**	**25.6**
HF	**22.028**	301.8	336.5	**0.897**	**14.6**	**16.786**	153.9	337.5	**0.456**	**72.0**
MB	**27.507**	270.5	413.0	**0.655**	**75.0**	**65.747**	281.3	363.9	**0.773**	**29.8**
MK	**30.107**	272.6	394.5	**0.691**	**62.1**	**70.529**	300.7	383.3	**0.785**	**50.0**
MM	**11.461**	291.4	412.4	**0.707**	**69.6**	**15.938**	256.3	399.3	**0.642**	**56.7**
RN	**12.943**	270.6	388.1	**0.697**	**47.0**	**16.639**	252.2	384.8	**0.655**	**42.1**
SR	**17.081**	292.8	405.1	**0.723**	**67.6**	**21.532**	255.0	383.5	**0.665**	**43.1**
MEAN	**17.400**	**282.0**	**362.2**	**0.785**	**45.6**	**26.710**	**234.0**	**355.5**	**0.656**	**43.0**
STD DEV	**5.783**	**19.3**	**33.9**	**0.094**	**21.7**	**18.613**	**47.9**	**23.8**	**0.119**	**17.5**

- Hb_{max} is a value relative to a baseline determined at the calibration phase which makes it very difficult to normalize due to the great variance between the subjects and the two separate measurements taken from each subject. Therefore, appyling an ANOVA test to the raw data is not feasible. However, as Table 4.3 states, the warm up and stretching clearly increases the maximal [Hb] by a mean multiplier of 1.455 (1.322 when outlier elimination takes place) accompanied by Figure 4.7. However, the anova1 (Figure 4.8) test can be applied to the data adjusted by outlier elimination, for which the p-value is determined to be 4.71 e-5, a value much smaller than the 5.00 e-2 treshold. (The criterion for outlier elimination is to take a value out of the scope of mean value ± std dev. In a quest to attribute a reasoning for the subjects that were left out, these subjects were determined to assume a higher fat layer, suggesting a deviation in the absorption by the photodetectors.) This results as more HbO_2 molecules are converted into Hb

in the total volume within the range of the Niroxcope probe suggesting a greater number of recruited motor units per volume. It perfectly coincides with the objective of the warm up to prepare the muscle for exercise by increasing body temperature and decreasing the pH to initiate the enzymatic reaction of muscle recriutment.

Table 4.3

The increase in the maximal [Hb] by the shaded Mult (multiplier) column is due to the warm up and stretching routine. Turquise shaded data are outliers and were replaced with the mean of the remaining data given in parantheses. The new mean and std dev are given in parantheses.

	Hb_{max}		
Subject	**Before**	**After**	**Mult**
AA	14.666	22.117	1.508
CK	15.089	23.406	1.551
DC	17.356	23.177	1.335
EA	16.162	16.430	1.017
EB	11.292	20.664	1.830
EE	15.028	16.681	1.110
EY	15.480	17.585	1.136
HF	23.428 (14.656)	16.786	0.762 (1.145)
MB	27.507 (14.656)	65.747 (19.177)	2.390 (1.308)
MK	30.107 (14.656)	70.529 (19.177)	2.343 (1.308)
MM	11.461	15.938	1.391
RN	12.943	16.639	1.286
SR	17.081	21.532	1.261
MEAN	**17.400 (14.656)**	**26.710 (19.177)**	**1.455 (1.322)**
STD DEV	**5.783 (1.846)**	**18.613 (2.721)**	**0.482 (0.216)**

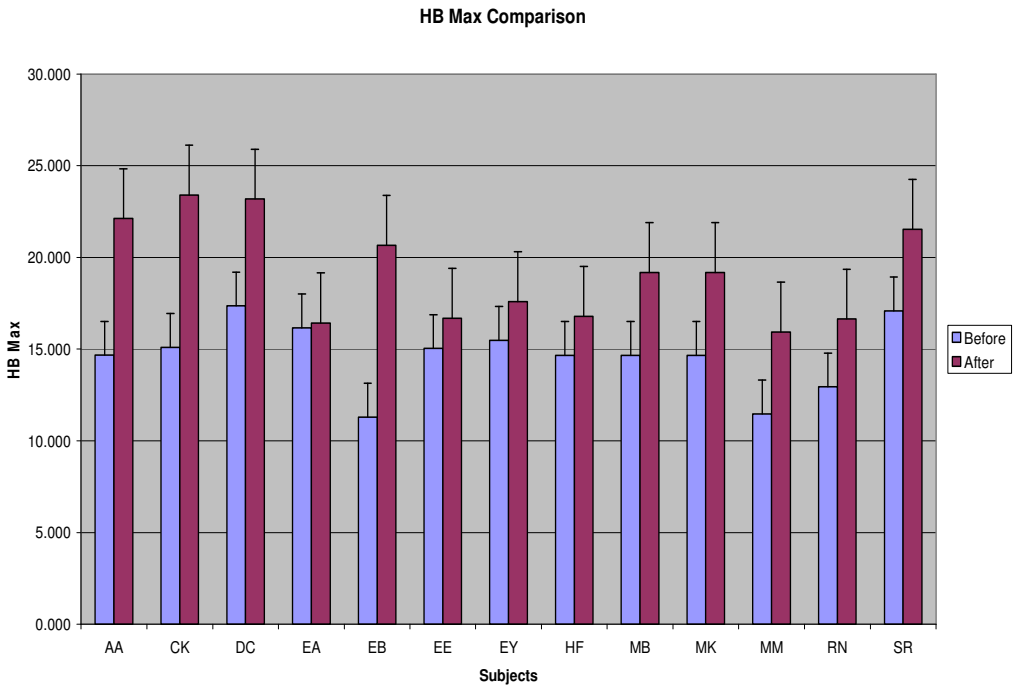


Figure 4.7 - The accompanying graph uses the adjusted values. Standard deviations are also present. The data provide a valid argument for augmented Hbmax values.

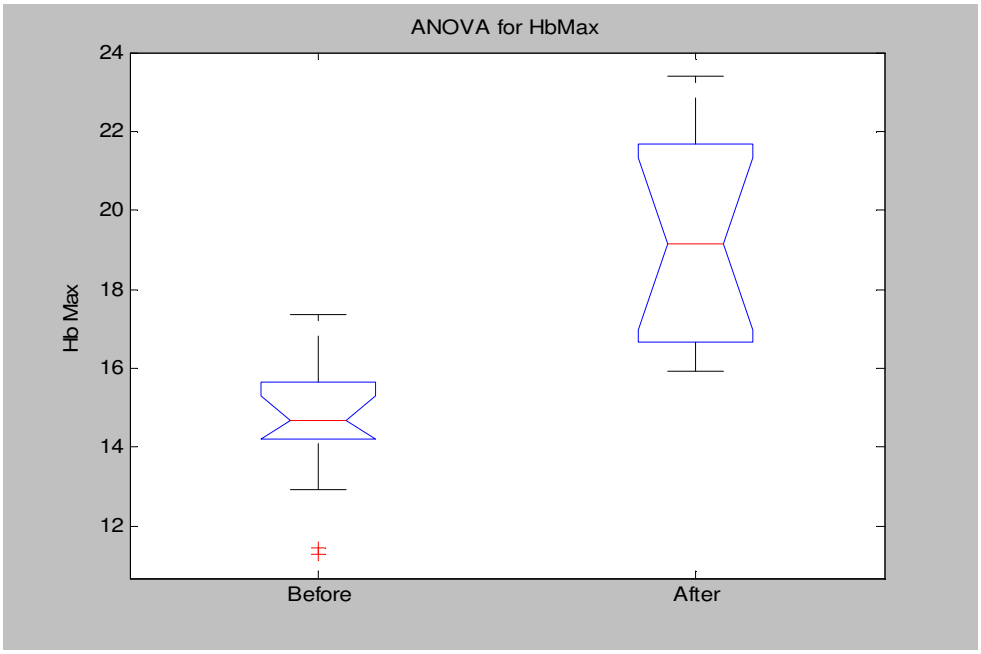


Figure 4.8 - ANOVA test results when the outliers from the Hb_{max} data are replaced by the mean of the remaining data (p=4.71e-5).

- 90% index is well suited for anova1 as t_{90} values were normalized by the t_{max} times which correspond to the end of ischemia. 90% index is an index of rate of change of HbO_2 into Hb. Figure 4.9 shows the bar graphs and indicates that the 90% index values for measurements before and after the warm up and stretching are statistically non-related with a p-value of 0.0054, a value much smaller than the treshold of 0.05. Considering the primary aim of warm up (increasing the maximal aerobic performance) as well as stretching (alignment of connective tissue in the direction of motion to allow maximum sarcomere length) and the fact that the two cases of the protocol are non-corellated, it is very sensible that the muscle is utilized much more efficiently by warming up and stretching in advance. This accounts for a quicker accomplishment of high utilization rate (i.e. a pleateau in the [Hb] vs. time plot), suggesting a more exponential format instead of the linear format of the plot of the model before the warm up and stretching. Curve fitting applications and corellation with biochemistry and biomechanics are to be discussed in Chapter 5 of the document.

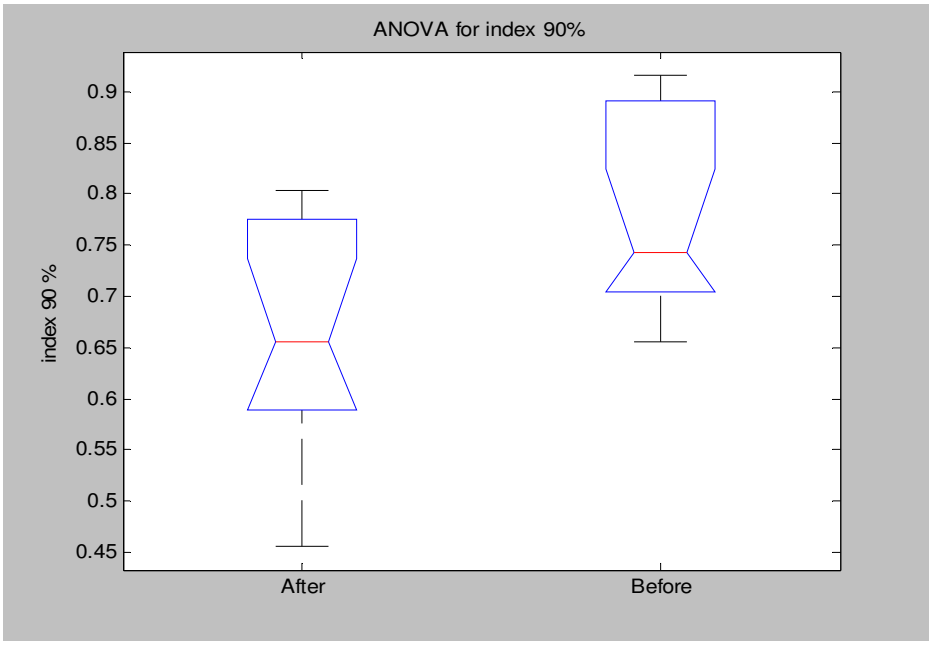


Figure 4.9 – The bar graphs for the cases of measurements before and after the warm up and stretching routine, providing an analyis of variables (ANOVA) with p = 0.0054.

- t_{rec} displays a mixed pattern as it stands in the middle of a trade off between two different phenomena happening concurrently:

    - The more the number muscle units recruited due to warm up, the longer it will take for a volume of the muscle to recover to their original states cumulatively. This makes t_{rec} larger for the case after the warm up and stretching routine.

    - It takes shorter for a single motor unit to recover in the case of stretching. This makes t_{rec} smaller for the case after the warm up and stretching routine.

It is not trivial to determine which of these two factor play a more dominant role. However, more hypertrophic subjects tend to have larger t_{rec} values with warm up than the others. Further discussion on the topic takes place in Chapter 5 of the document.

4.5.1. BMI vs. Parameters of Postocclusion

Although the subjects that volunteered in this experiment were selected to be of similar physical outlook and antropometric data, they present inevitable difference. Therefore, it would be a valuable analysis to evaluate the parameters of postocclusion that are in terms of the relative [Hb] values with respect to the BMI values, a parameter that combines major antropometric data. A higher BMI proposes a more hypertrophic figure (i.e. a more dense muscle, a higher number of motor units per volume). The same data outlier elimination was applied, only this time the subjects with eliminated BMI values have been ignored. Table 4.4 summarizes the data:

Table 4.4

The data table (of the 8 subjects after outlier elimination) that relates BMI with the parameters of postocclusion. The data is sorted according to the ascending order of BMI.

		BEFORE			AFTER		
Subject	**BMI**	**Hb_{max}**	**%90 index**	**t_{rec}**	**Hb_{max}**	**%90 index**	**t_{rec}**
RN	20.6	12.943	0.697	47.0	16.639	0.655	42.1
EE	22.2	15.028	0.743	65.2	16.681	0.801	26.0
MM	22.4	11.461	0.707	69.6	15.938	0.642	56.7
SR	24.3	17.081	0.723	67.6	21.532	0.665	43.1
EB	24.8	11.292	0.851	20.9	20.664	0.618	19.2
EY	25.1	15.480	0.889	41.8	17.585	0.707	25.6
DC	26.1	17.356	0.902	15.4	23.177	0.502	47.7
EA	28.1	16.162	0.916	21.9	16.430	0.486	76.0
MEAN	**24.2**	**14.601**	**0.803**	**43.7**	**18.581**	**0.634**	**42.1**
STD DEV	**2.4**	**2.411**	**0.095**	**22.4**	**2.782**	**0.103**	**18.7**

Figure 4.10 plots the maximal [Hb] value by the BMI of the subject. As expected, the increase in BMI, as an implication of a more hypertrophic figure, provides a higher Hb_{max} value both for the case before the warm up and sretching routine and after it due to the higher number of motor units in the range of the LED-light penetration of Niroxcope 301. The more interesting data is the rate of change (i.e. the slope in a linear curve fit) of the Hb_{max} values with respect to BMI. The slopes of the best-fit linear curves that belong to before warm up and stretching procedure and after it are very similar, with the exact values being 0.4815 and 0.4177 respectively. This suggests that the change in BMI has an almost identical effect on the O_2 metabolism of the muscle before and after pre-exercise routine.

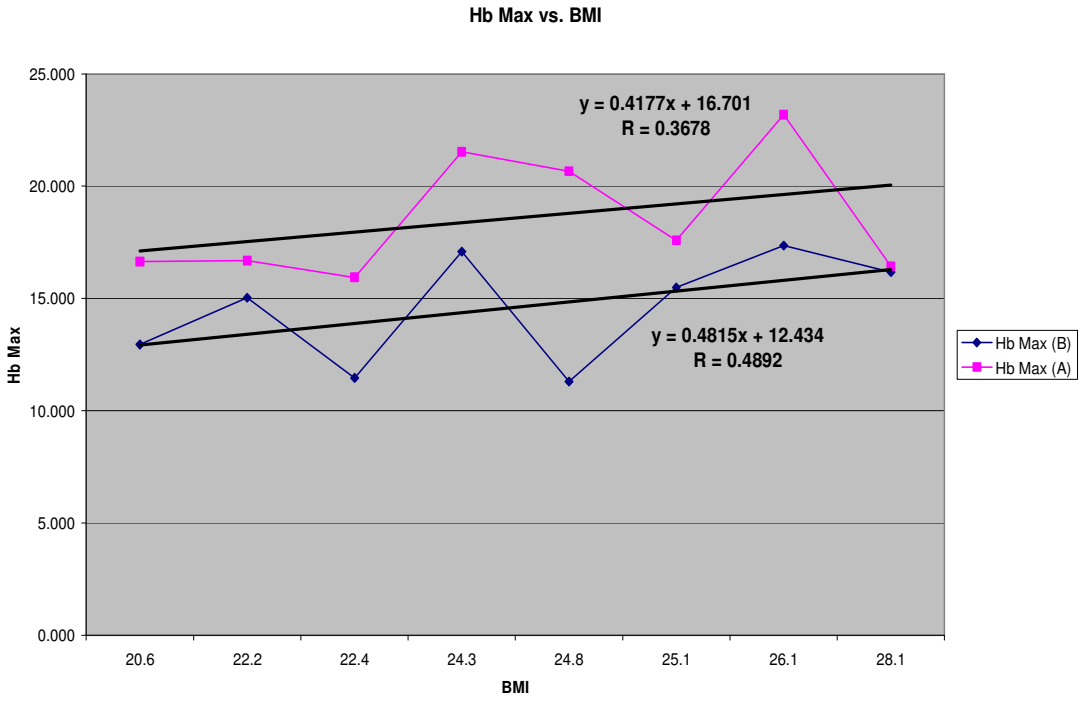


Figure 4.10 – The linear curve fitting application for the Hbmax values vs. BMI. The slopes of the resulting curves are similar. The R values are relatively off although the data has been outlier eliminated.

Figure 4.11 similarly plots the 90% index vs. BMI. The plot provides a better fit for the proposed equation than the Hb_{max} with higher R^2 values of 0.5150 and 0.8545. Although the magnitude of the slope for the two cases is once again close, the slopes have opposite signs: With warm up and stretching, the 90% index decreases with increasing BMI (slope = -0.0302) whereas it increases without it (slope = 0.0357). This means the difference of 90% indices is greater for a more hypertrophic body of higher BMI, suggesting a higher number of motor units involved. The comparative study in Chapter 4.5 has already proven a more efficient use of the muscle by warm up and stretching. The effect is enlarged with the presence of more available motor units in a body with a higher BMI.

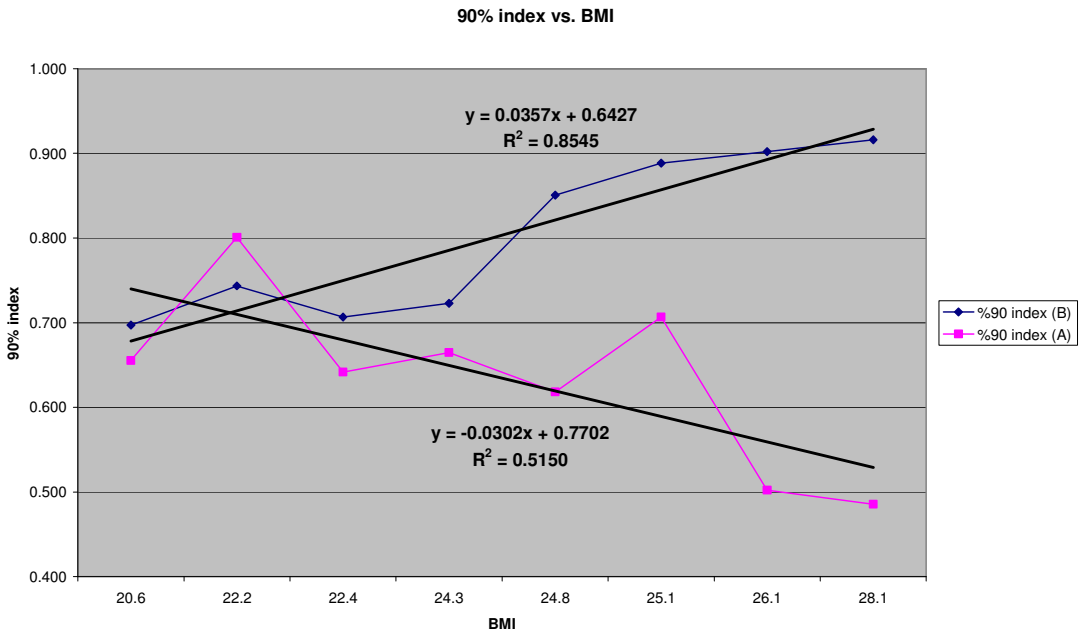


Figure 4.11 – 90% index vs. BMI plot for the 8 subjects that remained after outlier elimination. The trends (before vs. after warm up and stretching) move in different directions as BMI increases. R^2 values propose a better fit of the linear curve as they approach 1 (one).

t_{rec} vs. BMI plot in Figure 4.12 posseses a more complicated figure that is hard to fit a curve. That is presumably caused by the dual reasoning mentioned for the mixed pattern of the parameter. The BMI does not resolve in the quantification for a certain trend.

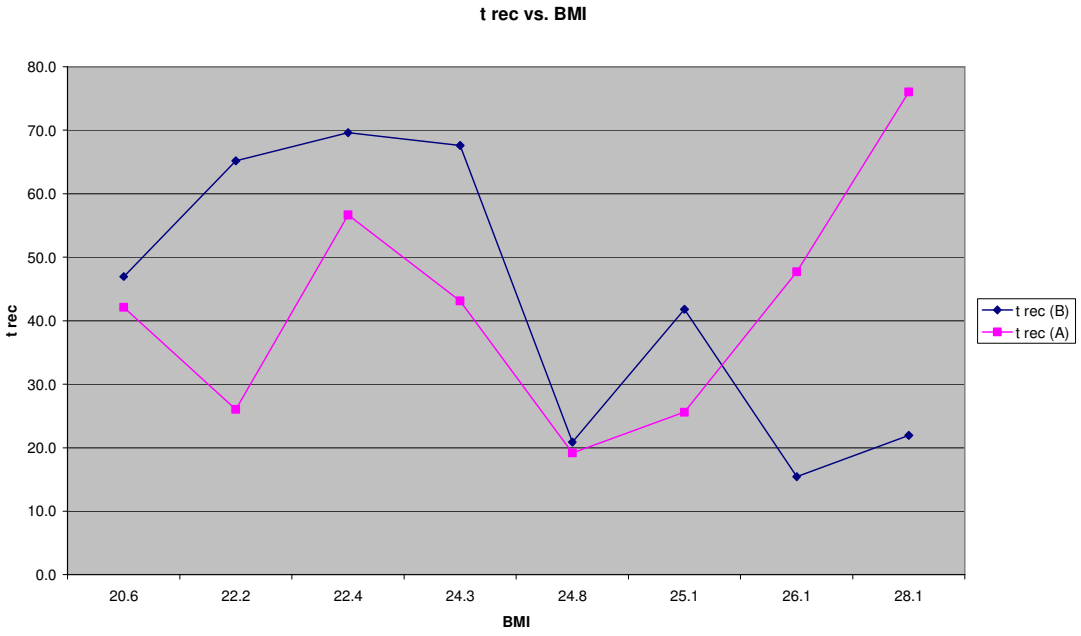


Figure 4.12 – t_{rec} vs BMI plot does not allow linear curve fitting as there is no evident trendline.

5. CONCLUSION

Conclusively, the summary of the results obtained from the experimental procedure and likely future work are to be discussed in this final chapter.

A modified version of Niroxcope 201, named Niroxcope 301, has been developed with the main enhancement being the new control unit software. The software introduces collaboration with a new DAQ card that uses a USB interface enabling a "plug & play" technology. The hardware requirements and the corresponding input / output circuitry are set up in a single box with the help of the Biophotonics Lab, Boğaziçi University led by Murat Tümer. The new DAQ card, most importantly, allows and is programmed for communication with external devices (e.g. cognitive phantom tests, fMRI, EMG) in a possible effort to synchronize fNIRS data with data of different caliber.

The Arterial Occlusion Protocol was then applied by using Niroxcope 301 in an effort to compare the gastrocnemius of the subjects ready for exercise (i.e. with warm up and stretching) with the unprepared gastrocnemius in terms of the pre-determined three parameters of post occlusion (Hb_{max}, 90% index and t_{rec}). The results are as follows:

Hb_{max} data, due to its great variance, became meaningful only after outlier elimination, with the control groups providing $p = 4.71$ e-5 $<<$ 5.00 e-2 in ANOVA analysis.

90% index provided the most sterile data as it is normalized by a maximal value. It resulted in $p = 0.0054 << 0.05$.

t_{rec} was a more controversial data due to its dual nature (more recuitment vs. better recovery of a single unit) and displayed a mixed pattern.

5.1. Suggestions and Discussion for Future Work

The future work includes further improvement in Niroxcope 301 and on the experimental protocol:

- Choosing a right MATLAB interpreter (or even developing one) is very significant in terms of improved runtime of the single Borland C code of Niroxcope 301. This will be even more crucial for protocols that take longer to last as runtime has an exponential correlation with the duration of the protocol.

- The performance of the 850 nm LED can be improved. That is the likely reasoning of the lack of quality of the [HbO_2] vs. time plots. Attributing new parameters of postocclusion [APPENDIX C] for these graphs would add a new dimension to the present work.

- Curve fitting applications might follow this work as 90% index proposes a different characteristic for the rate of change of the [Hb] signal after the warm up and stretching compared to that before it.

- There exists a trade off in the trend for t_{rec} to point in opposite directions. This is likely to resolve with a different experiment that compares the effect of varying ischemia durations within the same comparative approach. Shorter ischemia means less number of motor units recruited although the effect of ischemia on a single motor unit remains the same (i.e. a relatively smaller t_{rec} after warm up and stretching).

APPENDIX A – Written Consent Form for Subjects

İZİN FORMU

Boğaziçi Üniversitesi Araştırma Katılım İzin Belgesi

Deneğin Ad ve Soyad Başharfleri:_____

Denek Adı:_____

Araştırma Başlığı : Isınma ve Açma-Germenin Egzersize Etkisinin NIRS ile Değerlendirilmesi

Araştırmanın Amacı

Gönüllü olmanız teklif edilen araştırma çalışması egzersiz öncesi ısınma ve açma-germe hareketlerinin performansa etkisinin değerlendirilmesini amaçlamaktadır. Yürütülmesi planlanan bu araştırmanın bir başka ilgi odağı da kasın oksijen tüketiminin, kasın antrenman seviyesi ile ilgili olarak ne şekilde değiştiğini görmektir. Araştırmamıza katılmanız durumunda, egzersize bağlı olarak kas hücrelerindeki oksijen değişme trendi, yakın kızılötesi ışık kullanılarak ölçülecektir. Bu teknik, düşük güçteki neredeyse gözle görülebilir düşük güçlü kırmızı ışık kaynağının kandan yansıma miktarını ölçer.

Bu çalışmada yaklaşık 12 gönüllü yer alacaktır. Bu çalışmaya fiziksel olarak sağlıklı olmanız, sigara içmemeniz ve sınır kabul edilen, günde 3 -5 fincan kahveden fazla kafein tüketmemeniz nedeniyle uygun görülmektesiniz.

Prosedür ve Süre

Tüm çalışmalar Boğaziçi Üniversitesi Biyomedikal Enstitüsü Biyofotonik Labaratuarı çalışanlarının gözetmenliğinde yapılacaktır. Bu çalışma, insan kas aktivitesi ve oksijen tüketiminin ısınma süreci öncesi ve sonrasında alt bacak iskemisine verdiği tepkiyle ölçülmesine ve ortaya çıkan parametrelerin incelenmesinde gastrocnemius verilerinin kullanılmasına odaklanmıştır.

Sizden bu çalışmayla ilgili olarak kısa sorulardan oluşan bir sorular bölümünü de doldurmanız istenecektir. Toplanacak olan fNIRS verilerinin değerlendirmemiz açısından sağlıklı olabilmesi için katılımcıların olabildiğince fiziksel olarak (boy, kilo...) benzer olmasına dikkat edilmektedir. Sorular bölümünde yer alan sorular, uygun katılımcıların seçilebilmesine yönelik hazırlanmıştır ve başka bir amaç için kullanılmayacaktır. Bütün bilgiler kesinlikle gizli tutulacaktır.

Uygun görüldüğünüz ve çalışmada yer almak istediğiniz takdirde, egzersiz sırasında plastik kaplı küçük ışık kaynakları ve dedektörler bulunan bir ışık kaynağı bulunan bir pet alt bacağınıza elastik bandajla tutturulacaktır. Düşük güçlü ışık kaynağı ilgili bölgede ışık saçacak ve dedektörlerce yansıyan ışık tespit edilecektir.

Prob denilen bu pedin bağlanması hiç bir rahatsızlık hissi vermez. Prob aracılığıyla kastaki oksijen ve kan akışı, ışığın probdaki dedektörlere dönmesiyle değerlendirilecektir.

Egzersiz süreci 3 aşamadan oluşacaktır. Başta 30 saniyelik, sonda ise 5 dakikalık (geriye dönüş - recovery) saniyelik istirahat durumu ölçümleri, arada da 5 dakikalık iskemi yapılacaktır. İskemi için uygun olan basınç her denek için ayrıca tespit edilecek olan sistolik basınç değerinin 30 mmHg

fazlası olarak belirlenmiştir. Toplam 11 dakikalık protokolün bir kez ısınma sürecinden öne bir kez de sonra uygulanması uygun görülmüştür. Fakat bu iki deneme, iskeminin biriken etkisinden dolayı farklı günlerde, bir gün ara ile yapılacaktır. Deneyler katılımcıların uygun olabileceği bir gün yapılacaktır. Ancak deneyden önce alkol ya da kafein tüketmemeniz beklenmektedir..

Riskler ve Oluşabilecek Rahatsızlıklar

1. Bu çalışmayla alakalı olarak riskler göz ardı edilebilecek kadar azdır. Kullanılacak yakın kızıl ötesi ışığın parlaklığı çok düşüktür ve güneşlenme sırasında maruz kalınan ışımaya denktir.Yakın kızıl ötesi ışının tüm etkileri bilinmemekle birlikte , şu ana kadar güneş ışınlarına maruz kalma dolayısıyla oluşan etkilerden daha farklı bir etki görülmemiştir. Bazı çok hassas cilt özelliklerine sahip bireylerde çok hafif güneş yanığı tarzında kızarıklıklar görülebilir.Uygulanacak prosedüre bağlı olarak hiç bir fiziksel rahatsızlık olmayacaktır.Ancak , herhangi bir sebeple rahatsızlık hissetmeniz halinde çalışmayı yürüten kişilerden birine durumu bildirip prosedürü hemen sonlandırabilirsiniz. Çalışmada kullanılan aletler tam olarak izolasyonludur, kontrolleri yapılmıştır ve elektriksel açıdan hastane güvenlik standartlarına tamamen uyduğundan sağlık açısından risk taşımaz.
2. Çalışmanın bazı aşamalarında alt bacak kaslarınızda iskemiye bağlı ağrı veya yanma hissedebilirsiniz. Eğer bu durumdan rahatsız olursanız istediğiniz zaman çalışmadan çekilebilirsiniz.
3. Prosedürün uygulanması sırasında istenmeyen durumların oluşması halinde proje görevlileri en uygun bakım ve tedavi sağlanıncaya kadar size eşlik edecektir.
4. Çalışmayı yürüten araştırmacılar sizin iyiliğiniz için gerekli gördükleri zaman prosedürü sonlandırma hakkına sahiptirler.
5. Çalışmanın bir parçası olarak, kişilik profiliniz, geçirmiş olduğunuz yaralanmalar ve tedavilerinizle ilgili sorular kısmını yanıtlamanız gerekmektedir.Bu bölümle ilgili tüm bilgileriniz gizli tutulacaktır.

Katılımcının Teyidi

- Yukarıda açıklanan tüm bilgileri okudum.
- Sorularımın hepsine cevap aldım.
- Yazılı iznimi gönüllü olarak veriyorum.

Katılımcının Adı Katılımcının İmzası Tarih

Onay Belgesini Kabul Eden Araştırmacının Adı ve İmzası Tarih

Araştırmacının Adı Araştırmacının İmzası Tarih

İzin Belgelerini Toplamaya Yetkili Kişiler

İsim	**Ünvan**
Yard. Doç.Dr.Ata AKIN	Proje Yürütücüsü
Emir ALKAŞ	Yüksek Lisans Öğrencisi
Ömer SEVER	Yüksek Lisans Öğrencisi
Ömer ŞAYLI	Doktora Öğrencisi

İsim_____ Tarih_____

KATILIMCI SORU BÖLÜMÜ
(LÜTFEN TÜM SORULARI ATLAMADAN CEVAPLADIĞINIZDAN EMİN OLUN)

Bölüm A

1.Kaç yaşındasınız ? ___
2.Boyunuzu ve kilonuzu yazın. ___ cm , ___ kg

Bölüm B

1. Ne sıklıkta egzersiz yaparsınız?
___ kere /ayda YADA ___ kere / haftada. Egzersizin türü: _____
2. Egzersizin haricinde , günlük yaşamınız orta yada ağır fiziksel aktivite içeriyor mu ?
Hayır
Evet (Lütfen açıklayınız) _____
_____

Bölüm C

1. Son 12 ay içinde ayağınızda , bacağınızda , kalçanızda ve/veya bel bölgenizde ağrı , travma benzeri rahatsızlık geçirdiniz mi? _____
Cevabınız 'Evet' ise lütfen zamanını , ne tür olduğunu ,ne kadar sürdüğünü ve ne şekilde tedavi olduğunuzu anlatın(ilaç, cerrahi…).
_____
_____
_____

2. Düzenli kullandığınız bir ilaç ve devam eden bir tedaviniz var mı?Varsa anlatınız.
_____
_____
_____

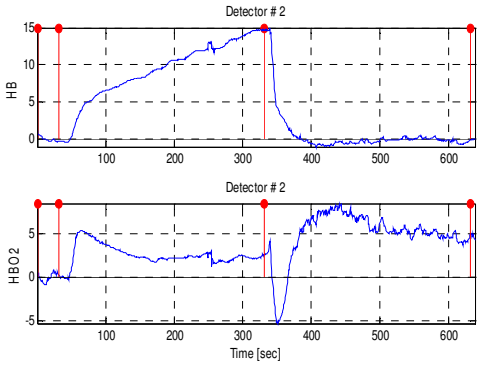
3.Metabolik veya sistemik bir hastalığınız var mı?(Diabet, tansiyon) Varsa anlatınız.
_____
_____
_____

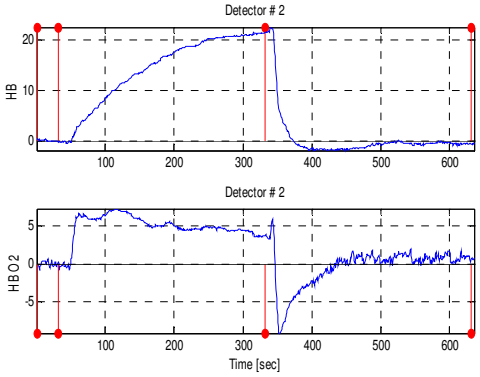
APPENDIX B – [Hb] and [HbO_2] vs Time plots of 13 subjects

BEFORE

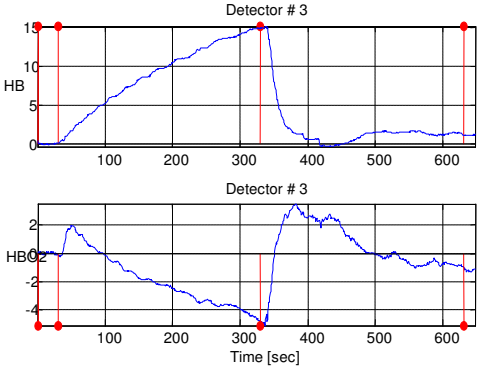
AFTER

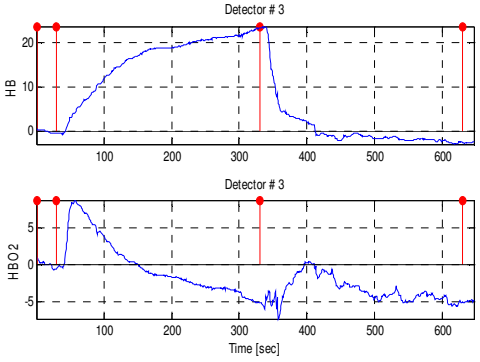
Subject : AA



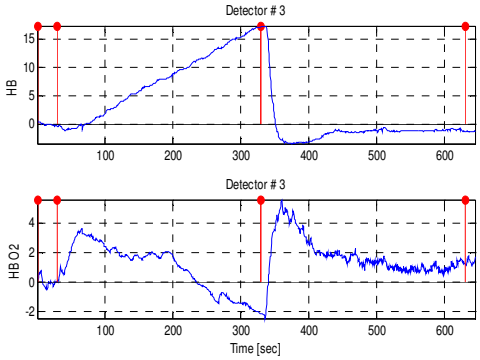


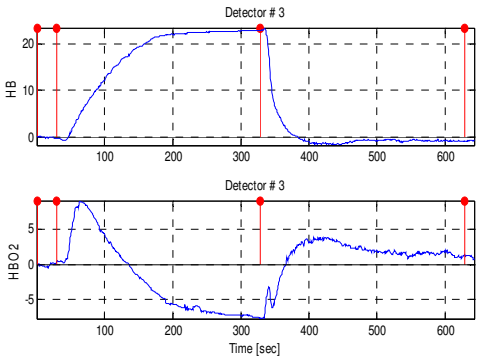
Subject : CK





Subject : DC

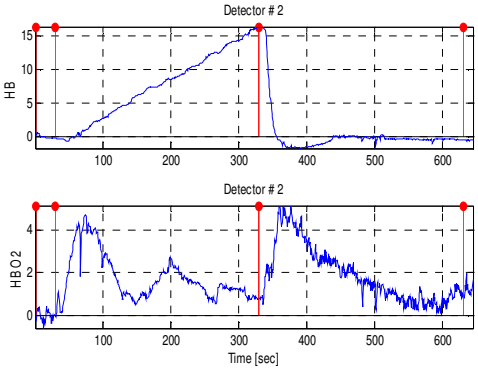


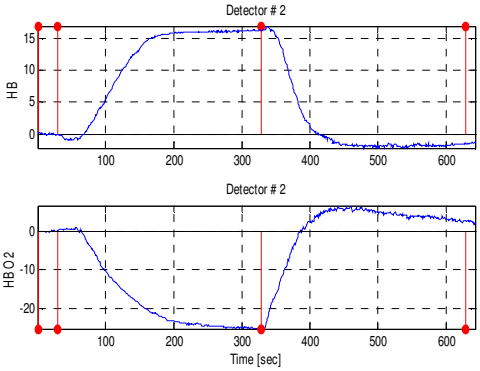


BEFORE

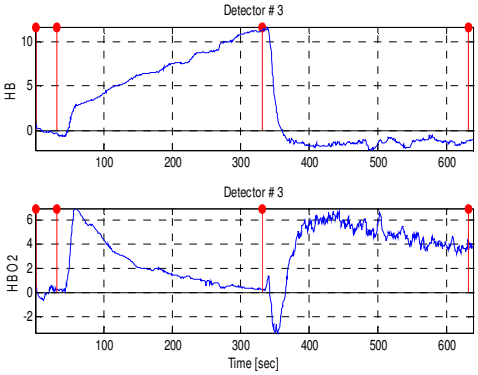
AFTER

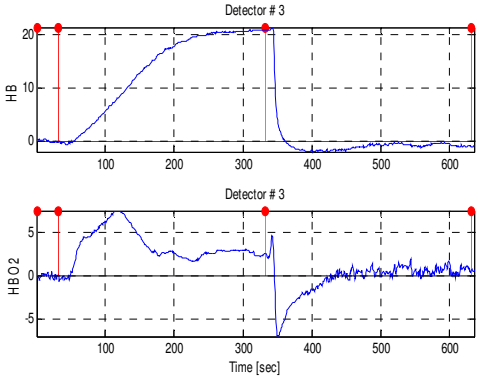
Subject : EA



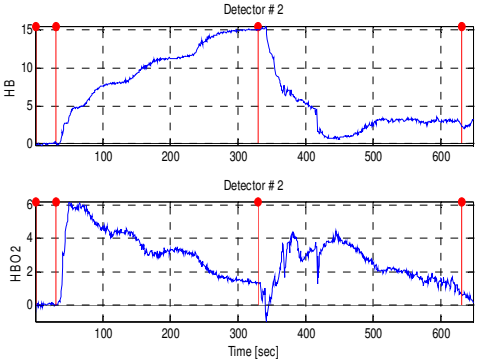


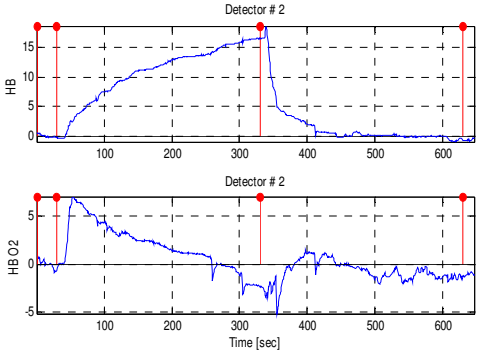
Subject : EB





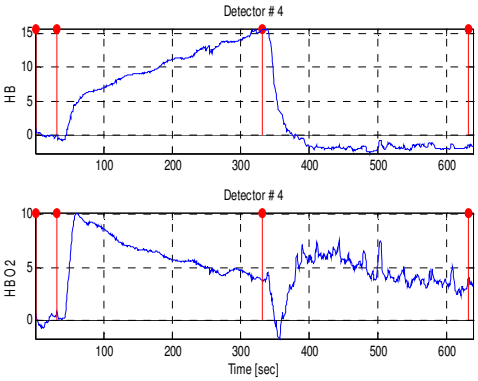
Subject : EE

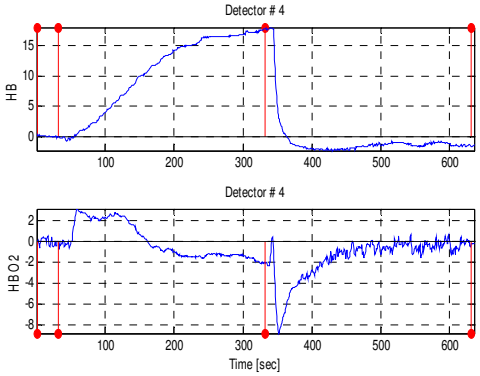




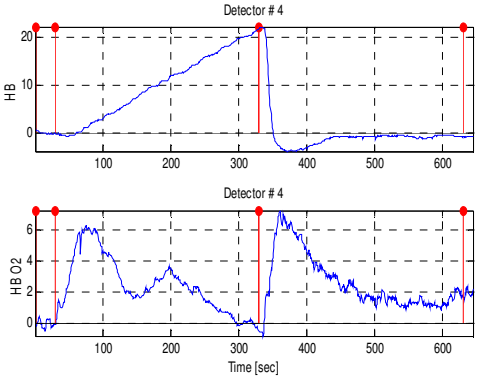
BEFORE **AFTER**

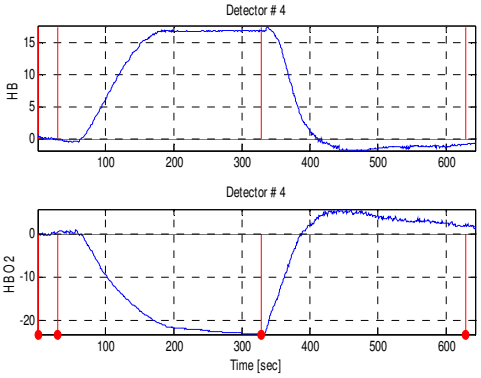
Subject : EY



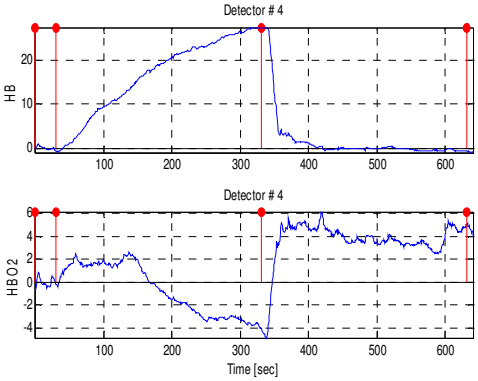


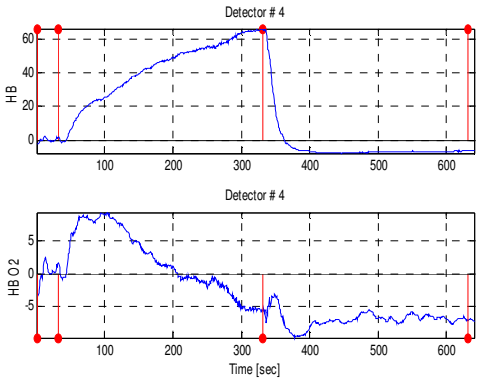
Subject : HF





Subject : MB

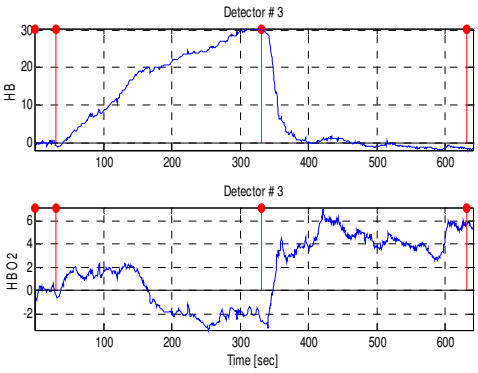


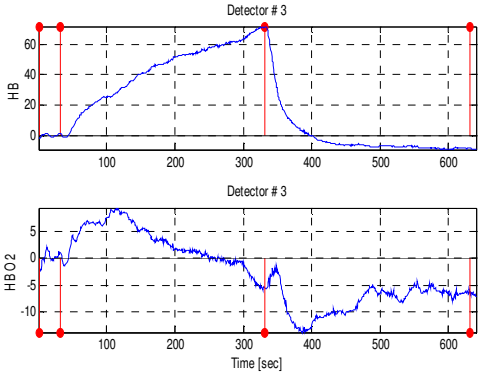


BEFORE

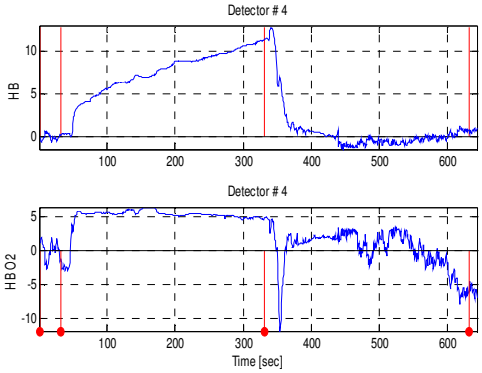
AFTER

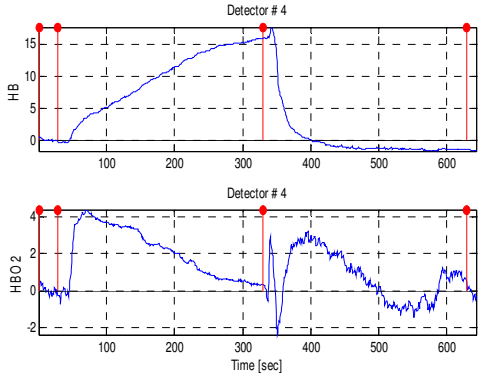
Subject : MK



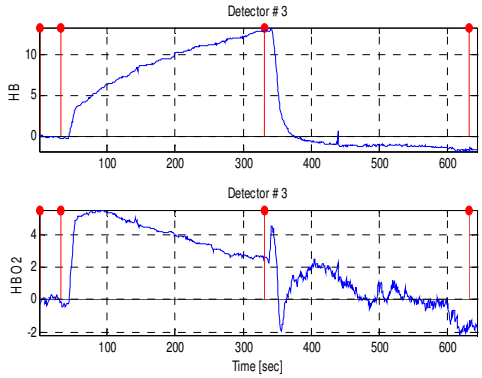


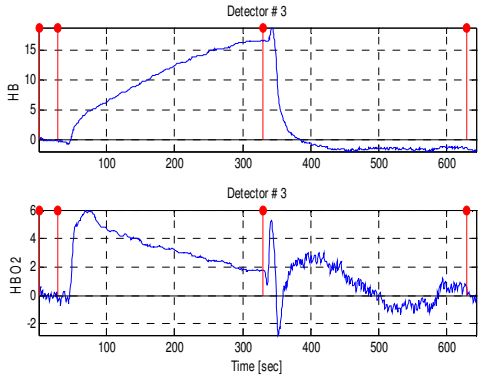
Subject : MM





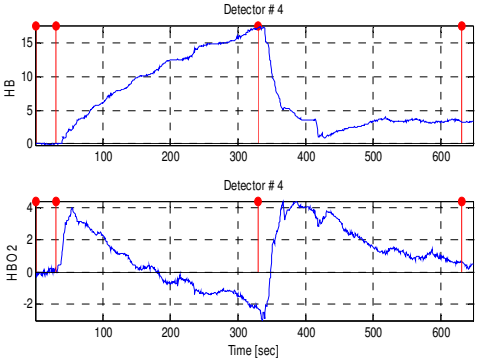
Subject : RN

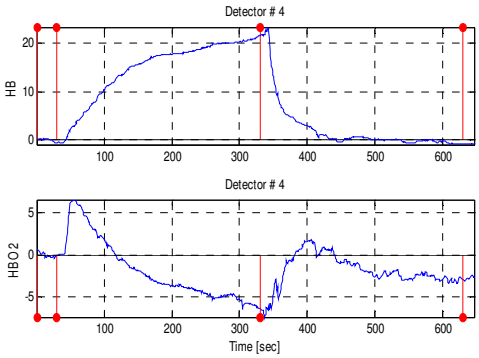




BEFORE **AFTER**

Subject : SR





APPENDIX C – Analysis of the [HbO_2] vs. Time plots

The protoype [HbO2] vs. time plot shows a different characteristic curve than the [Hb] vs. time plot making it more difficult to assign key values that determine the parameters of post occlusion. The Figure C.1 shows a typical plot with 4 input markers that suffice to obtain the parameters of post occlusion. The necessity of an extra marker arises from the initial local peak at the beginning of the AO phase due to the rushing of the blood to a distal part (i.e. the calf) when sudden ischemia is applied at a more proximal location (i.e. the thigh). The original trend of [HbO_2] under the condition of ischemia is a continuous decrease.

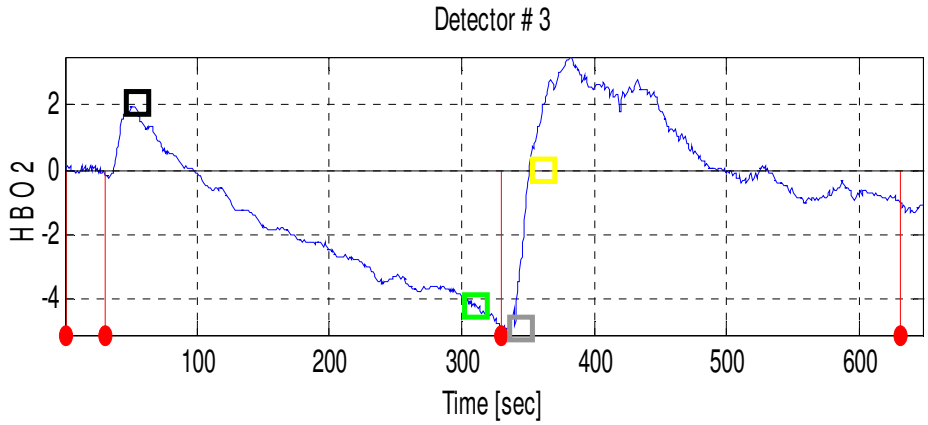


Figure C.1 – The input locations for determining the parameters of post occlusion for the [HbO_2] vs. time plot. The markers are named initial peak (black), 90% marker (green), minima (gray) and recovery (yellow).

The 90% marker is placed according to the declining curve that starts from the initial peak and ends in the minima (i.e. end of ischemia). In other words, the initial increase is taken into consideration because that amount of extra O_2 is used up in the process just like the already present amount before the extra rush. However, the recovery marker is placed on the original baseline to account for the recovery from the total effect of ischemia which includes the initial rush of blood, and therefore, the temporary increase in the [HbO_2] level.

Table C.1 summarizes the data of the parameters of post occlusion for the [HbO_2] vs. time using the same comparative approach for the case before and after warm up and stretching. Similarly, these parameters are $HbO_{2\,max}$, %90 index and t_{rec}.

Table C.1

A comparative study: Parameters of Postocclusion (shaded) for the [HbO_2] data of the 13 subjects before and after the warm up and the stretching regimen is applied.

	BEFORE					AFTER				
Subject	**$HbO_{2\,max}$**	**t_{90}**	**t_{max}**	**%90 index**	**t_{rec}**	**$HbO_{2\,max}$**	**t_{90}**	**t_{max}**	**%90 index**	**t_{rec}**
AA	**3.650**	255.7	320.0	**0.799**	**49.0**	**3.135**	317.7	344.1	**0.923**	**88.8**
CK	**6.985**	313.3	335.1	**0.935**	**15.5**	**14.401**	304.1	345.3	**0.881**	**50.4**
DC	**5.624**	280.3	330.8	**0.847**	**9.7**	**16.765**	210.1	327.0	**0.643**	**27.5**
EA	**3.759**	221.1	329.2	**0.672**	**34.1**	**26.224**	187.4	330.2	**0.568**	**53.4**
EB	**6.559**	257.1	337.7	**0.761**	**27.3**	**5.260**	174.5	336.1	**0.519**	**52.0**
EE	**4.948**	275.0	332.6	**0.827**	**24.4**	**7.844**	293.1	353.4	**0.829**	**28.5**
EY	**6.205**	266.1	336.9	**0.790**	**42.6**	**5.150**	244.6	343.3	**0.712**	**44.8**
HF	**6.938**	295.2	337.3	**0.875**	**16.2**	**23.846**	178.3	330.2	**0.540**	**55.0**
MB	**6.476**	288.2	335.6	**0.859**	**15.3**	**14.389**	288.6	334.1	**0.864**	**29.0**
MK	**5.289**	240.1	337.2	**0.712**	**16.1**	**11.301**	301.1	331.7	**0.908**	**41.1**
MM	**1.977**	300.4	341.9	**0.879**	**23.5**	**4.237**	304.4	334.6	**0.910**	**26.7**
RN	**2.852**	378.1	335.5	**1.127**	**48.6**	**4.266**	286.5	332.1	**0.863**	**37.3**
SR	**6.685**	318.2	336.7	**0.945**	**11.4**	**13.087**	299.4	340.4	**0.880**	**39.9**
MEAN	**5.227**	**283.8**	**334.3**	**0.848**	**25.7**	**11.531**	**260.8**	**337.1**	**0.772**	**44.2**
STD DEV	**1.671**	**39.9**	**5.4**	**0.115**	**13.8**	**7.549**	**54.1**	**7.6**	**0.154**	**16.9**

The discussion in Chapter 5 suggests a more erroneous measurement of the [HbO_2] compared to that of [Hb]. This is observed from the higher standard deviation values for relative concentration measurements (i.e. [Hb] and [HbO_2]) as reported in the 3 plots of Figure C.2, one for each parameter. On the other hand, the time-related parameters show hybrid character. The related data tables of mean values and standard deviations can be found in Table C.2.

Table C.2

A table for comparison of standard errors for each of the parameters of post occlusion.

		BEFORE			AFTER		
		Hb / $HbO_{2\ max}$	**90% index**	**t_{rec}**	**Hb / $HbO_{2\ max}$**	**90% index**	**t_{rec}**
HbO	**MEAN**	5.227	0.848	25.7	11.531	0.772	44.2
	STD DEV	1.671	0.115	13.8	7.549	0.154	16.9
	% STD DEV	**32.0**	**13.6**	**53.7**	**65.5**	**19.9**	**38.2**
Hb	**MEAN**	17.400	0.785	26.7	26.710	0.656	43.0
	STD DEV	5.783	0.094	18.6	18.613	0.119	17.5
	% STD DEV	**33.2**	**12.0**	**69.7**	**69.7**	**18.1**	**40.7**

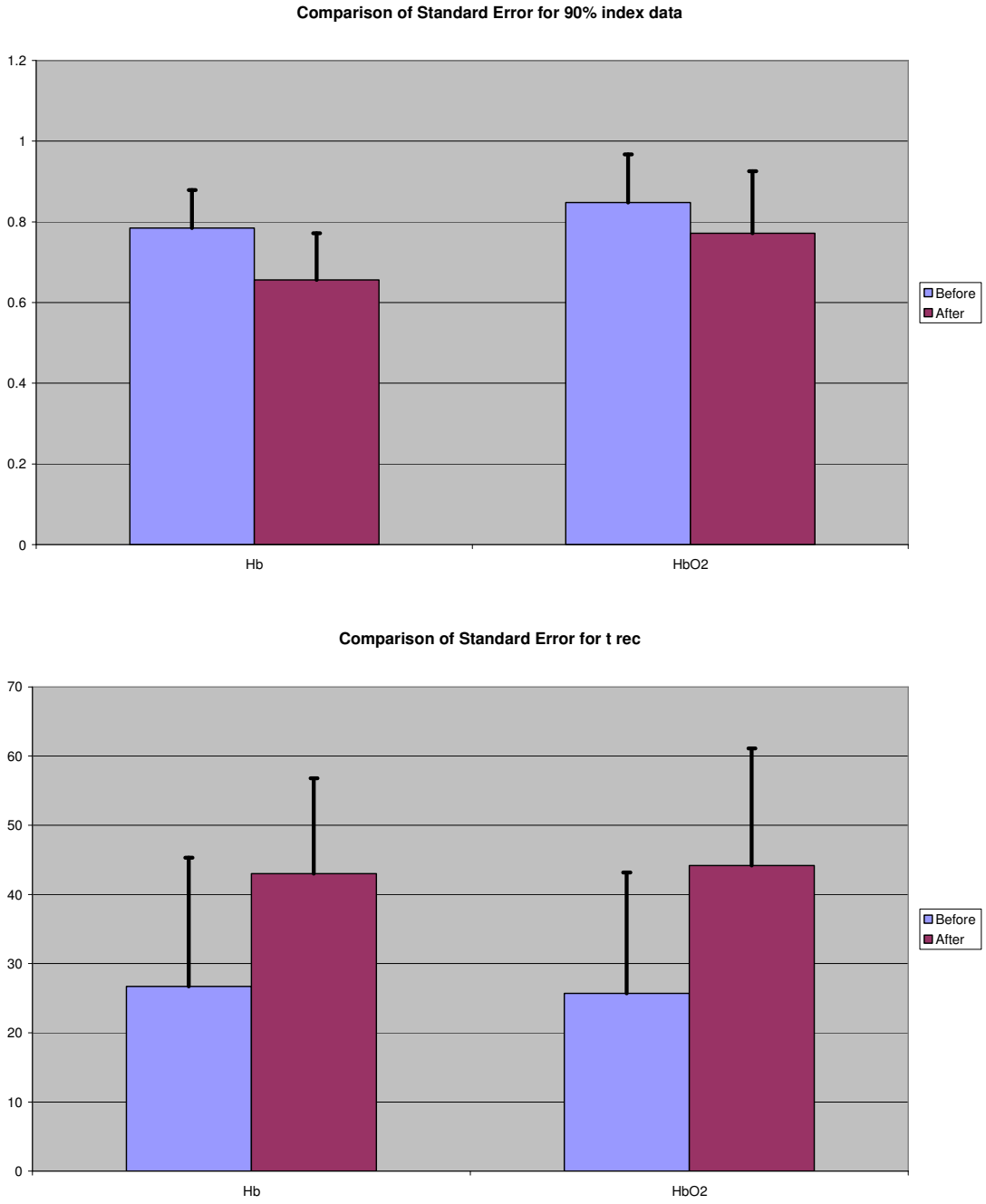


Figure C.2 – The error bars (dark black lines) show the standard error for the corresponding mean values obtained from the measurements of the 13 subjects (without outlier elimination). HbO_2 has a wider margin of error in the concentration measurements of maximal [Hb] and [HbO_2] whereas there is no obvious tendency for the time-related parameters (90% index and t_{rec}).

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