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# Changes in T cell metabolism in post-cardiac arrest patients

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BOSTON UNIVERSITY  
SCHOOL OF MEDICINE

Thesis

**CHANGES IN T CELL METABOLISM IN POST-CARDIAC ARREST PATIENTS**

by

**MEREDITH ALDEN HURLEY**

B.S., George Washington University, 2011

Submitted in partial fulfillment of the  
requirements for the degree of  
Master of Science

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Approved by

First Reader

---

Jean L. Spencer, Ph.D.  
Instructor of Biochemistry

Second Reader

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Michael W. Donnino, M.D.  
Associate Professor of Medicine  
Harvard University, School of Medicine

# CHANGES IN T CELL METABOLISM IN POST-CARDIAC ARREST PATIENTS

MEREDITH ALDEN HURLEY

## ABSTRACT

**Objective:** The survival rates for cardiac arrest patients to hospital discharge are very low. Post-arrest patients have an immune response and usually a period of immunosuppression. When CD3+ T cells activate, they switch from primarily relying on aerobic metabolism to primarily relying on anaerobic metabolism. The goal of this study is to characterize the immune system of post-cardiac arrest patients. The specific objectives are (1) to determine the time period after the occurrence of a cardiac arrest that a patient acquires an infection, (2) to identify the most common types of infections in post-arrest patients, (3) to compare in vitro the cellular oxygen consumption of immune cells post-cardiac arrest with healthy controls, and (4) to compare cell proliferation and ATP production of immune cells post-cardiac arrest with healthy controls.

**Methods:** We conducted a retrospective chart review of 170 cardiac arrest patients (Beth Israel Deaconess Medical Center) who had return of spontaneous circulation. We measured oxygen consumption rates of peripheral blood mononuclear cells (PBMCs) in cardiac arrest patients and healthy controls. We also measured cell proliferation and ATP production of CD3+ T cells in cardiac arrest patients and healthy controls.

**Results:** Of the 170 cardiac arrest patients we reviewed, 42% had at least one incidence of infection. The length of time from cardiac arrest to first positive culture was 4 days, with pneumonia and urinary tract infections the most common diagnoses. The PBMCs of cardiac arrest patients showed a significant decrease in oxygen consumption post arrest compared with healthy controls. When thiamine was added to the PBMC samples of cardiac arrest patients, there was a significant increase in oxygen consumption from baseline. There was no significant difference in cell proliferation or ATP production of CD3+ T cells between the two groups of post-cardiac arrest patients and healthy controls.

**Conclusion:** Many patients suffer from infections post-cardiac arrest, and future research is needed on this subject. Our data support the hypothesis that post-arrest patients have a period of hyperimmune response followed by a period of immunosuppression.

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## LIST OF ABBREVIATIONS

APACHE II	Acute Physiology and Chronic Health Evaluation II
APC	Antigen-Presenting Cell
ATP	Adenosine Triphosphate
CoA	Coenzyme A
CPR	Cardiopulmonary Resuscitation
CTL	Cytotoxic T Cell
FAD	Flavin Adenine Dinucleotide
FADH	Flavin Adenine Dinucleotide Reduced
FCCP	Carbonyl Cyanide 4-(Trifluoromethoxy)phenylhydrazone
GCS	Glasgow Coma Scale
GLUT1	Glucose Transporter 1
MACS	Magnetic-Activated Cell Sorting
MHC	Major Histone Complex
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide
n	Sample Size
NAD <sup>+</sup>	Nicotinamide Adenine Dinucleotide Oxidized
NADH	Nicotinamide Adenine Dinucleotide Reduced
PBMC	Peripheral Blood Mononuclear Cell
PDH	Pyruvate Dehydrogenase
PHA	Phytohemagglutinin
ROS	Reactive Oxygen Species

SE .....	Standard Error
SFI .....	Shen-Fu Injection
TCA.....	Tricarboxylic Acid Cycle
TCR .....	T Cell Receptor
Treg .....	T Regulatory Cell

## INTRODUCTION

In 2013 there were 359,400 out-of-hospital cardiac arrests with a 9.5% survival-to-discharge rate as well as 209,000 in-hospital cardiac arrests with a 23.9% survival-to-discharge rate for adults (Cardiac Arrest Statistics, 2014). The in-hospital mortality rate for patients who regained spontaneous circulation post-cardiac arrest was between 67% and 71%. This statistic has not changed appreciably over the last fifty years (Neumar et al., 2008).

Cardiac arrest causes whole-body ischemia and reperfusion and results in the most severe state of shock (Zhang et al., 2014). During a cardiac arrest, the delivery of oxygen and nutrients to the body is quickly stopped (Zhang et al., 2014). This results in post-cardiac arrest syndrome (Gu et al., 2014). After a cardiac arrest, there is an increase in cytokine release which leads to cytokine production dysregulation, including cytokines that activate the immune system (Zhang et al., 2014). This situation is due to hemodynamic and cardiovascular function disorders which commonly occur after return of spontaneous circulation (Zhang et al., 2014). Immunologic pathways are activated, and there is systemic inflammation as a result of the oxygen debt created by the cardiac arrest (Zhang et al., 2014). The generalized activation of the immune systems leads to multiple organ dysfunction (Gu et al., 2013). Adrie et al. (2002) studied post-cardiac arrest patients and showed that days after the hyperimmune response post-cardiac arrest, patients experience a period of immunosuppression, and their

condition rapidly declines. Adrie and coworkers attributed the period of immunosuppression to the increase of T cell activity immediately following the arrest. Many cardiac arrest patients do not survive to hospital discharge because of sepsis and multiorgan failure (Gu et al., 2014).

It is important to note that the immune response has been shown to be the same in patients therapeutically cooled to 33 °C and 36 °C, suggesting that therapeutic hypothermia does not affect the immune response (Beurskens et al., 2014).

## **T Cells**

T cells participate in the body's cellular adaptive immune response by directing or regulating immune response or by directly attacking another cell (Andersen et al, 2006; Immune System T Cells, 2008). T cells have receptors on their surface that recognize antigens on infected cells (Immune System T Cells, 2008).

Immature T lymphocytes are produced in bone marrow and move to the thymus to mature. In the thymus, the stromal cells, cytokines, and chemokines produce functional T cells from the immature T cells. CD4+ and CD8+ are first found on the surface of the T cells in the thymus (Luckheeram et al, 2012).

Mature T cells leave the thymus and go to secondary lymphoid organs including lymph nodes and spleen for further differentiation (Luckheeram et al, 2012).

Before activation, these naïve T cells undergo aerobic metabolism by oxidative

phosphorylation to produce a surplus of ATP by catabolism of glucose, amino acids, and lipids (Delmastro-Greenwood and Piganelli, 2013). Naïve T cells need a surplus of adenosine triphosphate (ATP) so they can quickly activate when needed (Delmastro-Greenwood and Piganelli, 2013). Naïve T cell metabolism is regulated by trophic signals rather than the available nutrients, and the quiescent state is actively maintained by cytokine signaling (Verbist et al., 2012). In this state, naïve T cells undergo homeostatic proliferation and have low rates of metabolism (Cao et al., 2014).

During the innate immune response, the infection causes antigen-presenting cells (APCs) to mature (Alberts, 2002). The foreign antigen is detected, and APCs drain to the secondary lymphoid organs (Delmastro-Greenwood and Piganelli, 2013). APCs then process the antigen and present it to the lymphocytes, which activate in the secondary lymphoid organs and further differentiate into subsets of effector T cells like helper Th1 cells (Alberts, 2002; Delmastro-Greenwood and Piganelli, 2013).

During the first 24 hours of activation, T cells enlarge in size, and then every 4 to 6 hours afterward, they divide. Once they have divided, they differentiate into effector cells. Effector T cells produce cytokines and release cytotoxic granules (Delmastro-Greenwood and Piganelli, 2013). Early in activation, T cell metabolism changes from primarily relying on aerobic oxidative phosphorylation to primarily relying on a highly glycolytic metabolism (Cao et al., 2014). There is also an increase in oxidative phosphorylation, and some oxygen

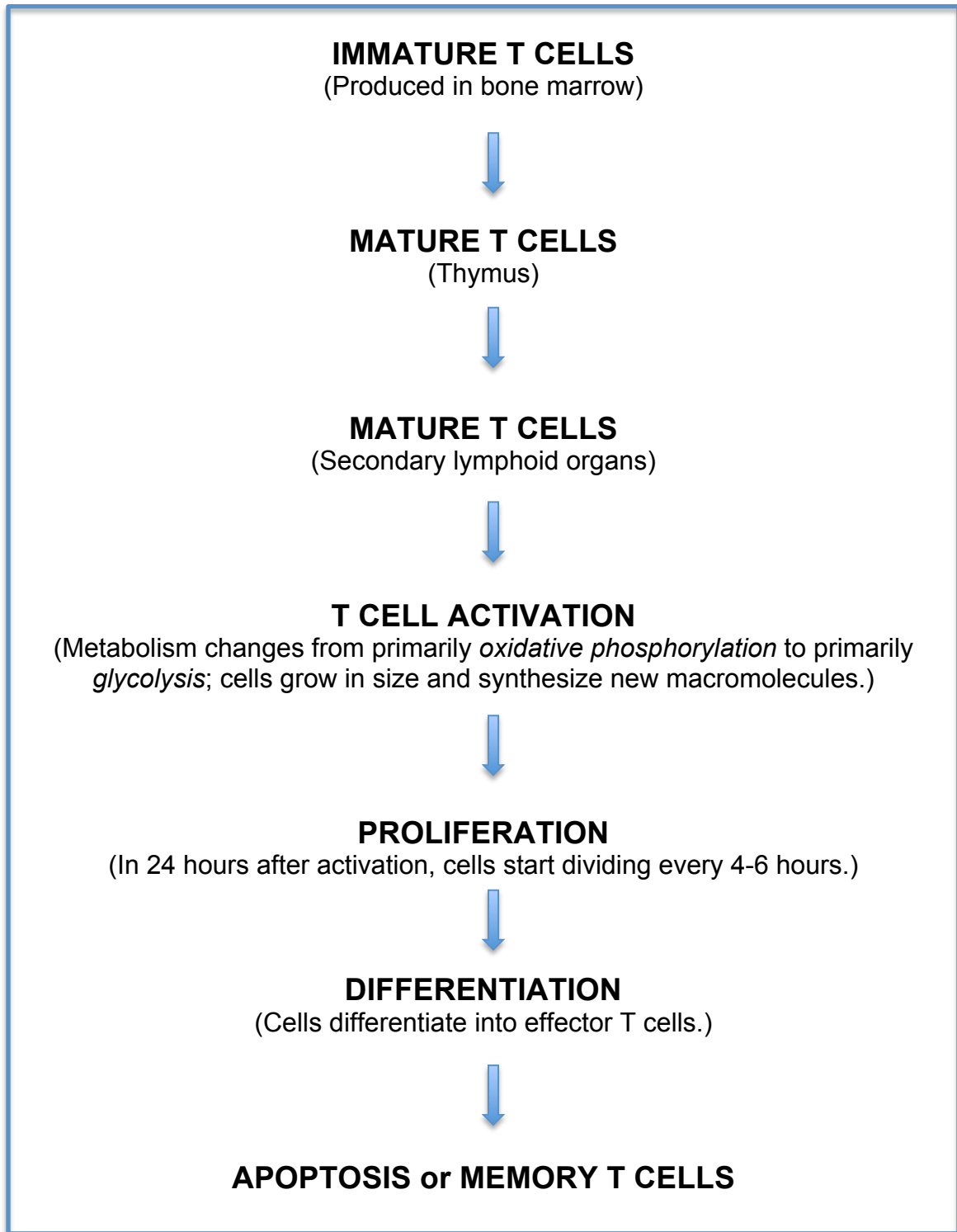
consumption still occurs (Cao et al., 2014; Delmastro-Greenwood and Piganelli, 2013). After activation, T cells migrate to the site of infection by going through the endothelial wall and away from their source of oxygen (Delmastro-Greenwood and Piganelli, 2013). Change in environment is not the only factor that controls the change in T cell metabolism once activated (Delmastro-Greenwood and Piganelli, 2013). Activated T cells use aerobic glycolysis (glycolysis in the presence of oxygen) because it is a hundred times faster in producing macromolecules and proliferating than oxidative phosphorylation. The ability to quickly produce macromolecules is crucial in the elimination of pathogens in the body (Delmastro-Greenwood and Piganelli, 2013). These metabolic changes are possible because there is an increase of expression of metabolic transporters, including glucose transporter 1 (GLUT1), which leads to an increase in glycolytic influx and glycolytic enzymes (Cao et al., 2014; Delmastro-Greenwood and Piganelli, 2013). The increase of GLUT1 transporters on the T cell surfaces has been shown to directly shift the metabolism from mostly oxidative phosphorylation to primarily glycolysis (Delmastro-Greenwood and Piganelli, 2013).

T cells utilize glycolysis when they are activated. After the pathogen has been cleared, the effector T cells have a diminished mitochondrial mass and can only survive by using glycolysis. These T cells will undergo apoptosis because they do not have adequate nutrition and cytokine stimulation. Some T cells, with greater mitochondrial mass as a result of biogenesis or asymmetrical cell

division, are able to switch back to oxidative phosphorylation after being activated and become memory cells (Delmastro-Greenwood and Piganelli, 2013). Memory T cells have similar metabolism to naïve T cells, except that they have a higher T cell receptor (TCR) affinity. Thus they can survive with less nutrients and cytokine stimulation, making them more advantageous (Delmastro-Greenwood and Piganelli, 2013) (Figure 1).

### **T Cell Differentiation**

T cells contain transmembrane proteins, like CD3 (part of the TCR), that transduce signals into the cell (Alberts, 2002; Cao et al., 2014). CD3 proteins transmit signals to the cell when major histone complex (MHC) binds to the protein. Helper T cells and cytotoxic T cells both contain a CD3 transmembrane protein. Helper T cells contain a CD4 transmembrane protein as part of the TCR that binds to MHC class II proteins. Cytotoxic T cells have a CD8 transmembrane protein included in their TCR that binds to MHC class I proteins (Alberts, 2002). When either MHC I or MHC II binds to the TCR, the binding acts through CD3 to trigger differentiation, metabolic reprogramming, proliferation, and growth (Cao et al., 2014).



**Figure 1. The life cycle of T cells.**

## **Cytotoxic T Cells**

Cytotoxic T cells (CTLs) destroy cells that have been infected by viruses and intracellular infections (Cao et al., 2014; Janeway et al., 2001; Masopust et al., 2007). CTLs identify parts of the virus or bacteria on the infected cell membrane (Immune System T Cell, 2008) and then kill the infected cell by inducing apoptosis either by the release of granules or by the expression of the Fas ligand (Henkart and Catalfamo, 2004; Janeway et al., 2001). CTLs release granules containing a variety of different substances that enter the targeted cell and induce apoptosis (Henkart and Catalfamo, 2014). The other way that CTLs kill infected cells is by using the Fas ligand located on their membrane. If the infected cell has CD95, or Fas receptor on its surface, the binding of CTL Fas ligand can induce apoptosis of the infected cell (Henkart and Catalfamo, 2014). CTLs can also secrete cytokines that directly damage the infected cell or inhibit replication of microbes (Henkart and Catalfamo, 2004). Activated CTLs have a highly glycolytic metabolism (Cao et al., 2014). When first activated, CTLs have a faster rate of growth and proliferation than helper T cells (discussed in the next section) (Cao et al., 2014). CTLs also have a higher rate of glycolysis compared with helper T cells following activation (Cao et al., 2014).

CTLs are divided into subpopulations dependent on their surface markers. There are four subpopulations: CD27, CD28, CCR7, and CD62L (Henkart and Catalfamo, 2004).

## Helper T Cells

Helper T cells fight bacterial and fungal infections as well as activate many different types of cells, including B lymphocytes and CTLs, and regulate the immune response (Cao et al., 2014; Luckheeram et al., 2012). There are two main types of helper T cells: Th helper cells and T regulatory cells (Tregs).

When activated, Th helper cells have a highly glycolytic metabolism and an increased rate of oxidative metabolism. Th helper cells have increased oxidative phosphorylation, higher mitochondrial mass, more activity, and greater maximal respiratory capacity compared with CTLs (Cao et al., 2014). Th helper cells are divided into subsets which include Th1, Th2, and Th17. The function of Th1 cells is to eliminate intracellular pathogens (Luckheeram et al., 2012). The immune response of Th2 cells is to attack extracellular parasites (Luckheeram et al., 2012), and the immune response of Th17 cells is against extracellular bacteria and fungi (Luckheeram et al., 2012).

After an immune response, Tregs, a type of CD4<sup>+</sup> helper T cell, suppress the immune response through negative regulation. This is done to protect against immunopathology (Luckheeram et al., 2012). Unlike Th helper cells, activated Tregs have a primarily aerobic metabolism with high rates of fatty acid oxidation and low rates of glucose uptake (Delmastro-Greenwood and Piganelli, 2013). Also, unlike Th helper cells, lymphocytes differentiate into Tregs in the thymus (Luckheeram et al., 2012).

## **Aerobic Metabolism**

In humans, oxidative phosphorylation is the main way to produce ATP in aerobic cellular respiration (Delmastro-Greenwood and Piganelli, 2013). During aerobic respiration, cells oxidize glucose, fatty acids, and amino acids into acetyl coenzyme A (acetyl-CoA) (Delmastro-Greenwood and Piganelli, 2013). During aerobic metabolism, glucose turns into pyruvate. Pyruvate is converted into acetyl-CoA by a reaction catalyzed by pyruvate dehydrogenase (PDH) and its cofactor, thiamine (Koukourakis et al., 2005; Manzetti et al., 2014). Acetyl-CoA enters the tricarboxylic acid cycle (TCA), and intermediates nicotinamide adenine dinucleotide (NAD<sup>+</sup>) and flavin adenine dinucleotide (FAD) are reduced. The intermediate products of the TCA are nicotinamide adenine dinucleotide reduced (NADH) and flavin adenine dinucleotide reduced (FADH<sub>2</sub>). NADH and FADH<sub>2</sub> enter the electron transport chain where they produce adenosine triphosphate (ATP) using oxidative phosphorylation. Oxygen is required for this process (Delmastro-Greenwood and Piganelli, 2013).

The electron transport chain also creates reactive oxygen species (ROS). At high levels, ROS cause oxidative stress and damage molecules in the body (Delmastro-Greenwood and Piganelli, 2013).

## **Anaerobic Metabolism**

The metabolism of activated T cells changes from aerobic to anaerobic. Lymphocytes switch from primarily relying on oxidative phosphorylation to relying

on glycolysis. Glycolysis is a non-oxidative branch of glucose catabolism and is therefore anaerobic (Verbist et al., 2012). In anaerobic metabolism, glucose is broken down into pyruvate as it is in aerobic metabolism. However, instead of becoming acetyl-CoA, pyruvate is turned into lactate, NAD<sup>+</sup>, and ATP (Figure 2) (Delmastro-Greenwood and Piganelli, 2013). While anaerobic metabolism is less efficient in producing ATP per glucose molecule, it is thought that glycolysis, which produces ATP at a faster rate than oxidative phosphorylation, is needed for the energy demands of proliferating cells (Delmastro-Greenwood and Piganelli, 2013; Maciolek et al., 2014; Verbist et al., 2012). Glycolysis also produces important intermediates, like ribose-5-phosphate and glycine, for activated cells. In addition, glycolysis may be an adaptive advantage over oxidative phosphorylation, since high rates of oxidative phosphorylation lead to high levels of ROS which damage cells (Verbist et al., 2012).

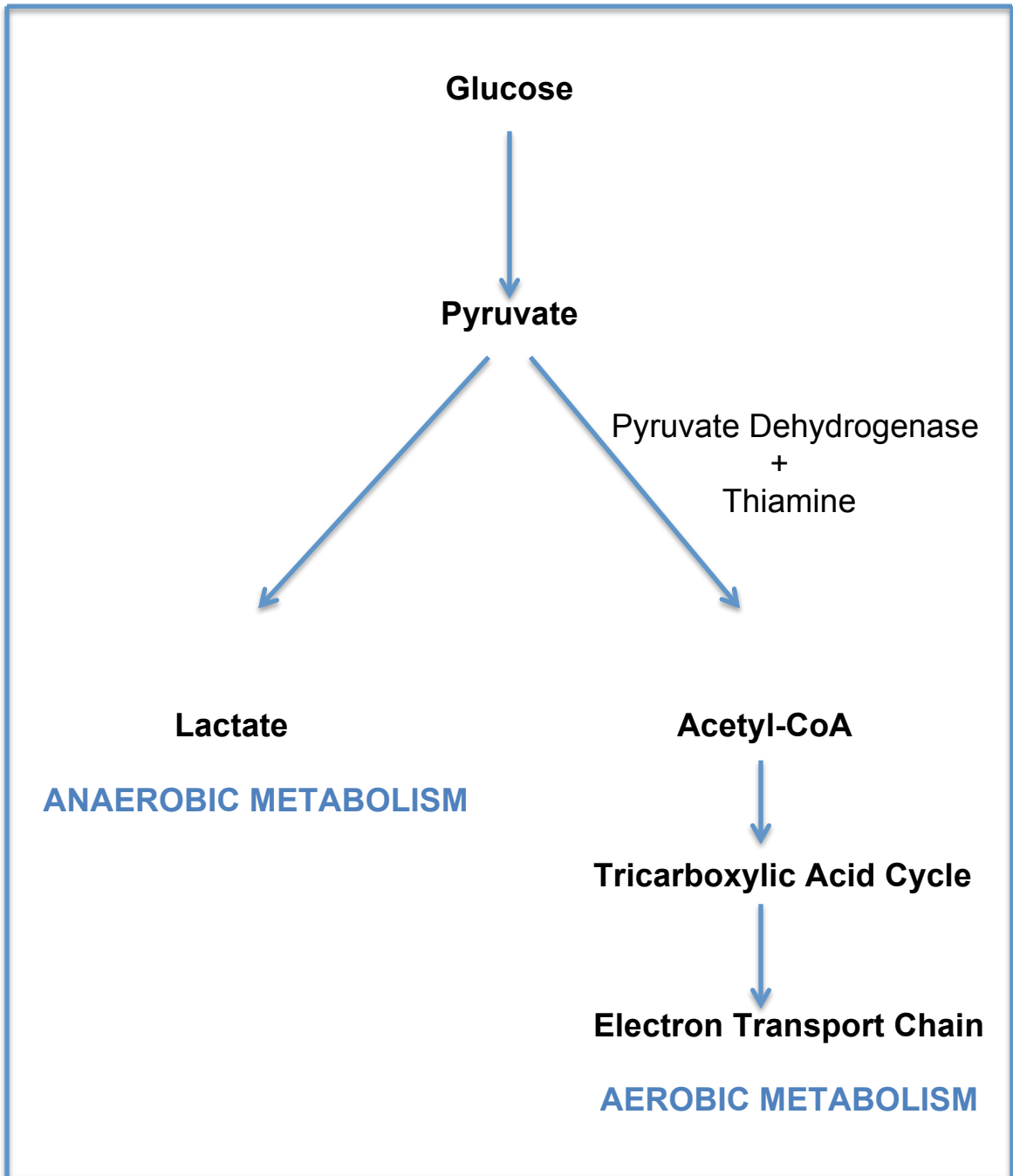


Figure 2. Differences in anaerobic and aerobic metabolism.

## **Current Research**

There are a few current explanations for immunosuppression after a cardiac arrest. Gu et al. (2013) proposed that patients experience a period of immunosuppression after a cardiac arrest because of an imbalance in the ratio of Th1 to Th2 T cells. They believed that the imbalance is due to the oxygen debt created during the cardiac arrest, which causes endothelial activation of cytokine releases. Gu and coworkers proved that there is an imbalance in the ratio of Th1 and Th2 cells after a cardiac arrest by using a porcine model and measuring the transcription factors responsible for Th1 and Th2 cell differentiation. They suggested that this imbalance contributes to the immune dysfunction post-cardiac arrest (Gu et al., 2013).

Another study by Gu et al. (2014) proposed that reperfusion after a cardiac arrest causes lymphocyte apoptosis. Their study examined the spleens in post-cardiac arrest porcine models and found lymphocyte apoptosis. The researchers attributed this finding to the post-cardiac arrest inflammatory response, which causes the lymphocyte apoptosis pathway to activate. They proposed that with fewer lymphocytes, the body has a decreased immune function (Gu et al., 2014).

Zhang et al. (2014) studied post-cardiac arrest hyperimmune response in a porcine model. They attributed the immune dysregulation from increased cytokine release as a result of global ischemia. The researchers proposed using Shen-Fu injection (SFI) to treat the cytokine dysregulation after a cardiac arrest.

They showed that SFI promotes immune system recovery by increasing anti-inflammatory cytokines and decreasing proinflammatory cytokines (Zhang et al., 2014).

### **Specific Aims and Objectives**

The goal of this study is to characterize the immune system of post-cardiac arrest patients. Specifically, the objectives are:

1. To determine the time period after the occurrence of a cardiac arrest that a patient acquires an infection, and to characterize the most common types of infections.
2. To compare in vitro the cellular oxygen consumption of immune cells post-cardiac arrest with healthy controls, and to determine if there is a change in cellular metabolism post-cardiac arrest.
3. To measure in vitro the cell proliferation and ATP production of T cells post-cardiac arrest.

Characterization of post-cardiac arrest immunosuppression will provide us with insight into how to alter T cell metabolism and potentially manipulate its function. This knowledge will help us develop the capability to dampen the hyperimmune response immediately following a cardiac arrest and to decrease the severity of the immunosuppression period.

## **METHODS**

This study was done in three parts. For the first part, we performed a retrospective chart review of cardiac arrest patients to determine relevant statistics including (a) the time period after a cardiac arrest when patients acquired their first infection and (b) the types of infections that were most common. In the second part, we compared the oxygen consumption rates of peripheral blood mononuclear cells (PBMCs) from cardiac arrest patients and healthy controls. We also examined the effect of thiamine on respiration results for the cardiac arrest patients. Lastly, we tested the blood of cardiac arrest patients after the arrest and healthy controls to determine T cell proliferation and ATP production. We also evaluated these parameters by adding phytohemagglutinin (PHA) to the T cell samples.

### **Retrospective Chart Review**

We retrospectively reviewed 170 cardiac arrest patient charts (January 2008 to November 2014) from Beth Israel Deaconess Medical Center (Boston, MA). Patients had to meet the following criteria to be included in our study:

1. Patient suffered an in-hospital or out-of-hospital cardiac arrest.
2. Patient had return of spontaneous circulation post-cardiac arrest.
3. Patient had cardiopulmonary resuscitation (CPR) performed during his or her cardiac arrest.
4. Patient was at least 18 years old at the time of the arrest.

We recorded the following information by reviewing the patient's discharge summary note and microbiology culture results:

1. Patient's last name
2. Patient's medical record number
3. Patient's age at the time of the cardiac arrest
4. Arrest location: inside or outside a hospital
5. Date of the cardiac arrest
6. Patient's hospital disposition
7. Patient's discharge date
8. Patient's Acute Physiology and Chronic Health Evaluation II (APACHE II) score on admission. The APACHE II score (0-71) indicates the severity of a patient's disease (Knaus et al., 1985).
9. Patient's Glasgow Coma Scale (GCS) score on admission. The GCS score (3-15) indicates the patient's level of consciousness (Teasdale et al., 2014).
10. Presence of an infection prior to the arrest and the diagnosis of the infection
11. Date of a positive culture prior to the arrest
12. Results of the positive culture prior to the arrest
13. Diagnosis of infection prior to the arrest
14. Date of the first positive culture post-cardiac arrest
15. Results of first positive culture post-cardiac arrest

16. Date of the second positive culture post arrest
17. Results of second positive culture post-cardiac arrest
18. Diagnoses of infection upon discharge

From this information we calculated:

1. The length of time in days from the cardiac arrest to the first positive culture.
2. The length of time in days from the cardiac arrest to hospital discharge or death.

### **Prospective T Cell Oxygen Consumption**

Blood was drawn from five post-cardiac arrest patients 24 to 48 hours after their arrest. These patients met the following criteria:

1. Patient suffered an in-hospital or out-of-hospital cardiac arrest.
2. Patient had return of spontaneous circulation post-cardiac arrest.
3. Patient had CPR performed during his or her cardiac arrest.
4. Patient was at least 18 years old at the time of the arrest.

Blood was also drawn from five healthy individuals to be used as the control group. Control group individuals were at least 18 years old.

Oxygen consumption rates of PBMCs were determined using an XF<sup>e</sup>96 Analyzer (Seahorse Bioscience, North Billerica, MA). PBMCs were isolated from blood samples by spinning the blood down with a centrifuge. The plasma was removed, and a Ficoll-Paque solution (1.077 g/ml; GE Healthcare, Uppsala,

Sweden) was added for density gradient centrifugation. After being spun down for a second time, the middle layer containing the PBMCs was extracted and placed in a separate tube. PBMCs were then seeded (500,000 cells per well) into Seahorse 96-well XF cell culture microplates. Depending on the amount of blood provided by the patient or control, each sample was seeded into three to five wells. Phosphate-buffered saline or phosphate-buffered saline with thiamine (1  $\mu\text{g}/\text{mL}$ ) was added to some of the wells. The plates rested at room temperature for one hour and were incubated overnight. Cells were then washed with Seahorse XF Cell Mito Stress Test Assay Medium. The plates were placed in a 37 °C incubator without carbon dioxide for one hour.

Each plate was then loaded into the XF<sup>e</sup>96 Analyzer, and oxygen consumption rates were measured following the manufacturer's protocols. The XF Cell Mito Stress Test targeted components of the electron transport chain to determine different parameters of cellular oxygen consumption, including basal respiration rate and maximum respiration rate. Three different compounds—oligomycin, carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP), and a mixture of rotenone and antimycin A—were added sequentially to the samples in the XF<sup>e</sup>96 Analyzer. Oligomycin targeted complex V of the electron transport chain, ATP synthase, and caused a decrease in the oxygen consumption rate. FCCP targeted the inner mitochondrial membrane (mimicking an energy demand) and increased the oxygen consumption rate. Finally, rotenone targeted complex I in the electron transport chain and antimycin A targeted complex III in

the electron transport chain, causing the oxygen consumption rate to decrease to a level representing non-mitochondrial respiration.

Basal respiration was defined as the oxygen consumption rate of the cells under baseline conditions. The basal oxygen consumption rate was measured by the XF<sup>e</sup>96 Analyzer as the oxygen consumption rate before oligomycin addition minus the non-mitochondrial respiration (oxygen consumption rate after rotenone and antimycin A addition). The maximum respiration rate was defined as the highest rate of oxygen consumption obtainable by the cells. The maximum oxygen consumption rate was measured by the XF<sup>e</sup>96 Analyzer as the oxygen consumption rate after FCCP addition minus the non-mitochondrial respiration (XF Cell Mito Stress Test Kit User Guide.pdf, n.d.).

Thiamine was added to some of the samples to determine if it could increase the oxygen consumption rate by making the cells rely more on aerobic metabolism (see Figure 2).

### **Prospective T Cell Proliferation and ATP Production**

Blood was drawn from five post-cardiac arrest patients at 48 hours after their arrest. Patients had to meet the following inclusion criteria:

1. Patient suffered an in-hospital or out-of-hospital cardiac arrest.
2. Patient had return of spontaneous circulation post-cardiac arrest.
3. Patient had CPR performed during his or her cardiac arrest.
4. Patient was at least 18 years old at the time of the arrest.

Blood was drawn from eight healthy individuals for control samples. Control group individuals were at least 18 years old.

CD3<sup>+</sup> T cells were isolated from blood samples using a magnetic-activated cell sorting (MACS) separation protocol (Miltenyi Biotec, Auburn, CA). The final concentration of CD3<sup>+</sup> T cells varied from sample to sample. For cardiac arrest patients, concentrations of CD3<sup>+</sup> T cells ranged from (1.5 to 2.0) x 10<sup>6</sup> cells / 7 mL of blood. For healthy controls, concentrations of CD3<sup>+</sup> T cells ranged from (0.6 to 1.5) x 10<sup>7</sup> cells / 7 mL of blood.

T cell proliferation of both the cardiac arrest patients and the healthy individuals was measured using the Vybrant MTT Cell Proliferation Assay Kit (Molecular Probes, Eugene, OR). In this colorimetric assay, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), a water-soluble yellow dye, was reduced to an insoluble purple formazan by living cells. Cells were seeded into wells and either PHA (16 µg/mL) or nothing was added to each well. This was followed by the addition of 5 mg of MTT and 1 mL of phosphate-buffered saline to the wells. The microplate was incubated at 37 °C for four hours, and then 1 g of sodium dodecyl sulfate and 10 mL of 0.01 M hydrogen chloride were added to the wells. The microplate was incubated in a 37 °C humidified chamber for another four hours. The plate was then placed into the SpectraMax 190 UV-Vis Microplate Spectrophotometer (Molecular Devices, Sunnyvale, CA), and the absorbance was read at 570 nm.

T cell ATP production was assessed using the CellTiter-Glo Luminescent Cell Viability Assay G7571 (Promega, Madison, WI). In this protocol, CellTiter-Glo Reagent was added to the cells, resulting in cell lysis and generation of a luminescent signal (luciferase reaction) proportional to the amount of ATP present in the cells. CD3<sup>+</sup> T cells were first seeded into plates. PHA (16 µg/mL) was added to some of the wells and incubated overnight. After incubation, plates were left at room temperature for thirty minutes. CellTiter-Glo Reagent was made from 10 mL of CellTiter-Glo Buffer and 0.01 mL of CellTiter-Glo Substrate. The CellTiter-Glo Reagent (100 µL ) was then added to the wells, and the plates were incubated at room temperature for 10 minutes. Afterward, luminescence was recorded using a SpectraMax M5 (Molecular Devices, Sunnyvale, CA).

PHA was utilized in both assays because it is a non-specific mitogenic lectin which binds to cell membranes and stimulates proliferation in T cells (Martin-Chouly et al., 2011).

## **Statistics**

For the chart review, we used a Mann-Whitney test to determine the statistical significance between the length of time from cardiac arrest to first positive culture for survivors and non-survivors. Statistical significance was defined as  $p < 0.05$ .

For the oxygen consumption rate, statistical significance was determined using a one-way ANOVA test. Statistical significance was defined as  $p < 0.05$ . Results are expressed as mean  $\pm$  standard error.

For proliferation and ATP production, statistical significance was determined between the healthy control group and the cardiac arrest group using a t-test for two independent means. A t-test for dependent means was used to determine the statistical significance when PHA was added. Statistical significance was defined as  $p < 0.05$ . Results are expressed as median values because the sample sizes were small.

## RESULTS

### Retrospective Chart Review

We reviewed the charts of 170 cardiac arrest patients (Tables 1 and 2). The average age of these patients was 64.3 years old. They had an average APACHE II score of 26.9 and an average GCS score of 4.7 on admission. A total of 105 cardiac arrests (62%) took place outside the hospital, while 65 (38%) took place in the hospital.

In 42% of the patients there was at least one incidence of infection post-cardiac arrest, and in 7% of the group there was evidence of multiple infections. Fifty-six patients had pneumonia, and 16 patients had urinary tract infections. Prior to their cardiac arrest, 25% of the patients had infections (Table 2).

On average, 4.2 days elapsed between the time of the cardiac arrest and the first incidence of a positive culture. Post-cardiac arrest patients who survived to hospital discharge and acquired an infection had a first positive culture 5.3 days after their arrest. Post-cardiac arrest patients who did not survive to hospital discharge and acquired an infection had a first positive culture 2.6 days after their arrest. The difference in length of time from cardiac arrest to first positive culture between survivors and non-survivors was statistically significant ( $p = 0.04$ ). The average length of time until hospital discharge or death was 8.5 days after the cardiac arrest (Table 1).

	Cardiac Arrest (n = 170)
Age in years	64.3
APACHE II on admission	26.9
GCS on admission	4.7
Days until discharge or death	8.5
Days elapsed to first positive culture	4.2
Days to first positive culture of survivors	5.3
Days to first positive culture of non-survivors	2.6

**Table 1. Summary of general descriptors from chart review of the cardiac arrest patients.** Tabulated results are from 170 cardiac arrest patient charts (January 2008 to November 2014) from Beth Israel Deaconess Medical Center (Boston, MA). APACHE II = Acute Physiology and Chronic Health Evaluation II score; GCS = Glasgow Coma Scale score.

	Number of Patients	Percentage of Total Patients
<b>Arrest Location</b>		
Out-of-hospital cardiac arrest	105	61.8%
In-hospital cardiac arrest	65	38.2%
Incidence of at least one infection	72	42.4%
<b>Discharge diagnosis</b>		
Pneumonia	56	32.9%
Urinary tract infection	16	9.4%
Other	10	5.9%
Multiple	12	7.1%
No diagnosis of infection	98	57.6%
Infections prior to arrest	42	24.7%

**Table 2. Summary of patient numbers from chart review of the cardiac arrest patients.** Tabulated results are from 170 cardiac arrest patient charts (January 2008 to November 2014) from Beth Israel Deaconess Medical Center (Boston, MA).

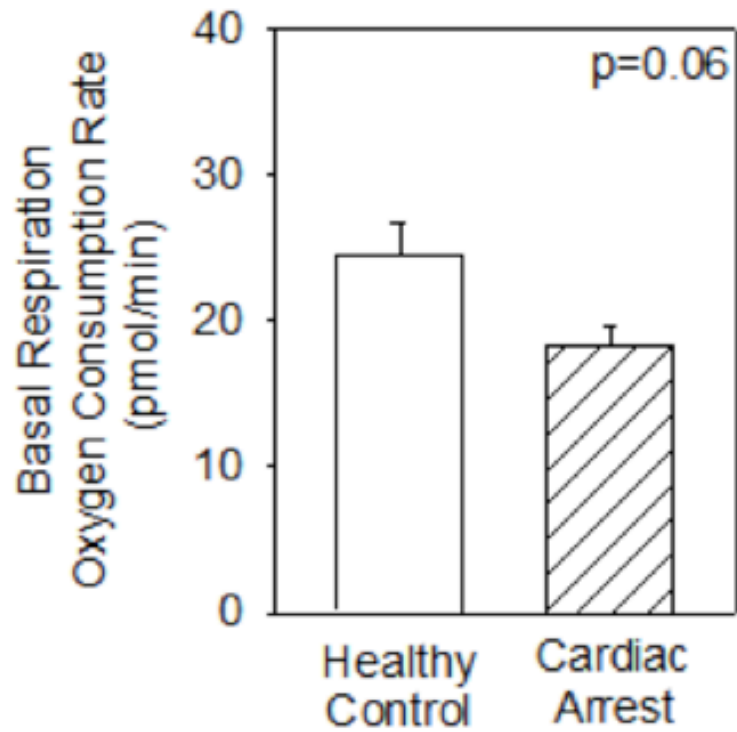
## **Prospective T Cell Oxygen Consumption**

We obtained the basal oxygen consumption rates for five healthy controls and five cardiac arrest patients (Figure 3). Healthy controls had a basal respiration rate (mean  $\pm$  SE) of  $24 \pm 2$  pmol/min. Cardiac arrest patients had a basal respiration rate of  $18 \pm 1$  pmol/min. There was no statistically significant difference between these two groups ( $p = 0.06$ ).

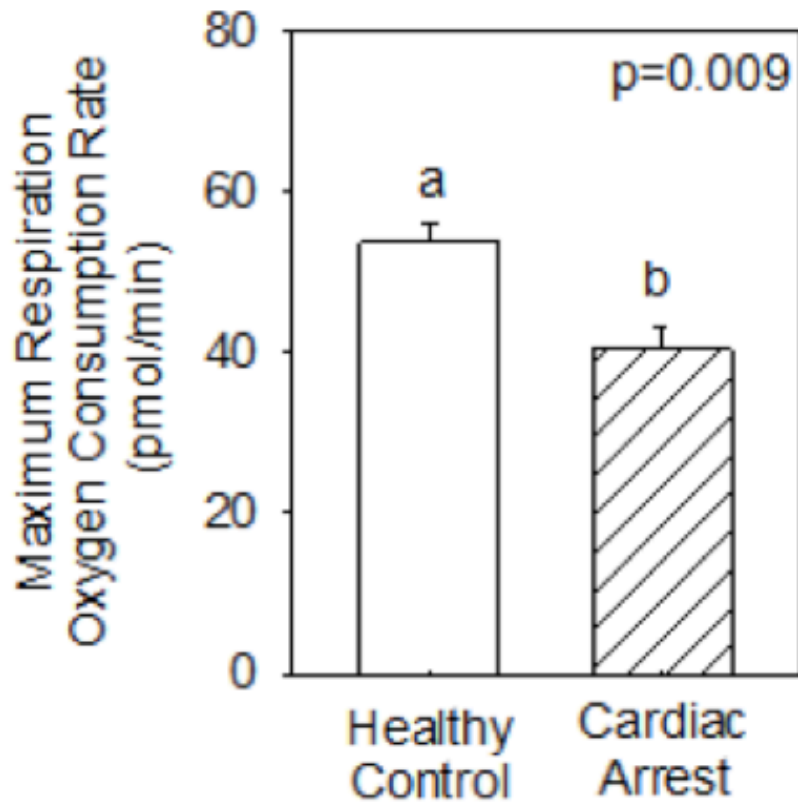
The maximum oxygen consumption rates were measured for both healthy controls and cardiac arrest patients (Figure 4). Healthy controls had a maximum respiration rate of  $54 \pm 2$  pmol/min. Cardiac arrest patients had a maximum respiration rate of  $40 \pm 3$  pmol/min. The difference between the maximum respiratory oxygen consumption rates of the cardiac arrest patients and the healthy controls was statistically significant ( $p = 0.009$ ).

Basal respiration rates were measured when thiamine was added to the PBMCs of cardiac arrest patients (Figure 5). The basal oxygen consumption rate for cardiac arrest patients with thiamine was  $26 \pm 2$  pmol/min. There was a statistically significant difference between the basal respiration rates of PBMCs with and without thiamine from cardiac arrest patients ( $p = 0.001$ ).

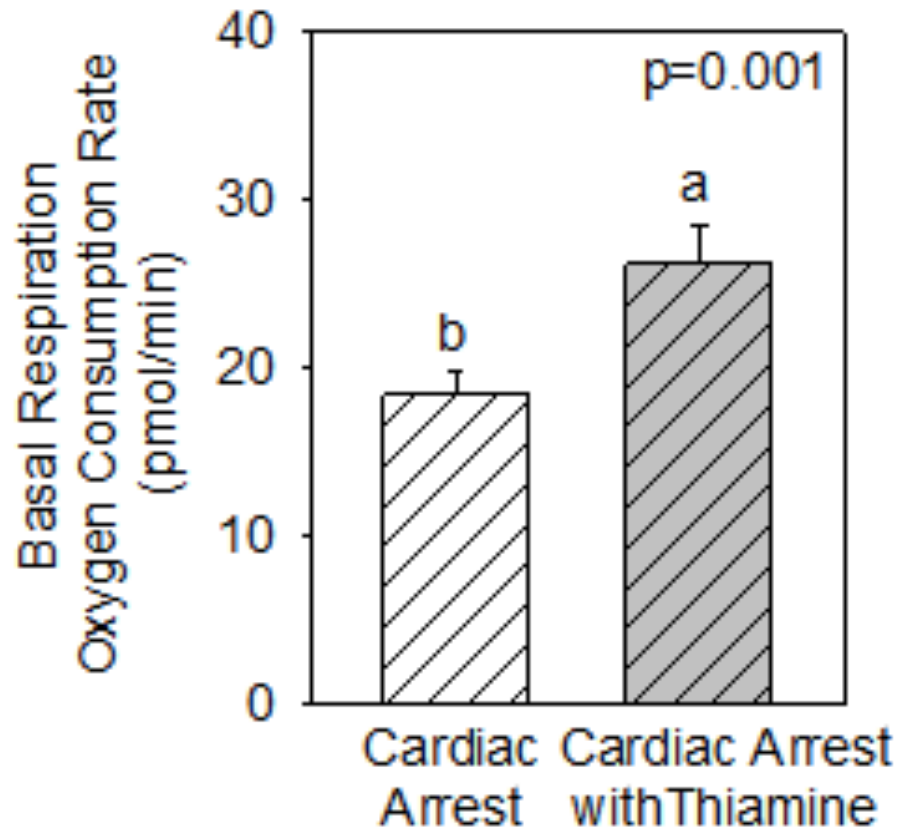
Maximum respiration rates of PBMCs with added thiamine were also measured (Figure 6). The maximum respiration rate for cardiac arrest patients with thiamine was  $56 \pm 4$  pmol/min. There was a statistically significant difference between the maximum oxygen consumption rates of PBMCs with and without thiamine from cardiac arrest patients ( $p = 0.01$ ).



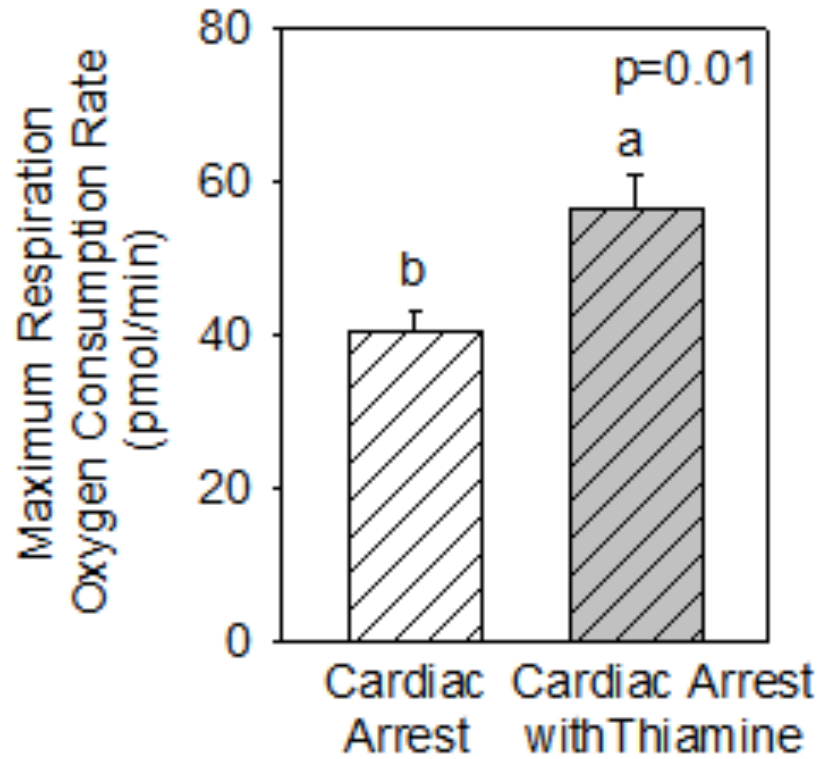
**Figure 3. Basal respiration rates in controls and cardiac arrest patients.** There is no statistical difference between the basal respiration oxygen consumption rates of PBMCs from healthy controls and cardiac arrest patients ( $p = 0.06$ ). Values are mean  $\pm$  SE;  $n = 5$ .



**Figure 4. Maximum respiration rates in controls and cardiac arrest patients.** The maximum respiration oxygen consumption rate of PBMCs is lower in cardiac arrest patients than in healthy controls ( $p = 0.009$ ). Values are mean  $\pm$  SE;  $n = 5$ .



**Figure 5. Effect of thiamine on basal respiration rates in cardiac arrest patients.** The basal respiration oxygen consumption rate of PBMCs from cardiac arrest patients is higher with added thiamine ( $p = 0.001$ ). Values are mean  $\pm$  SE;  $n = 5$ .



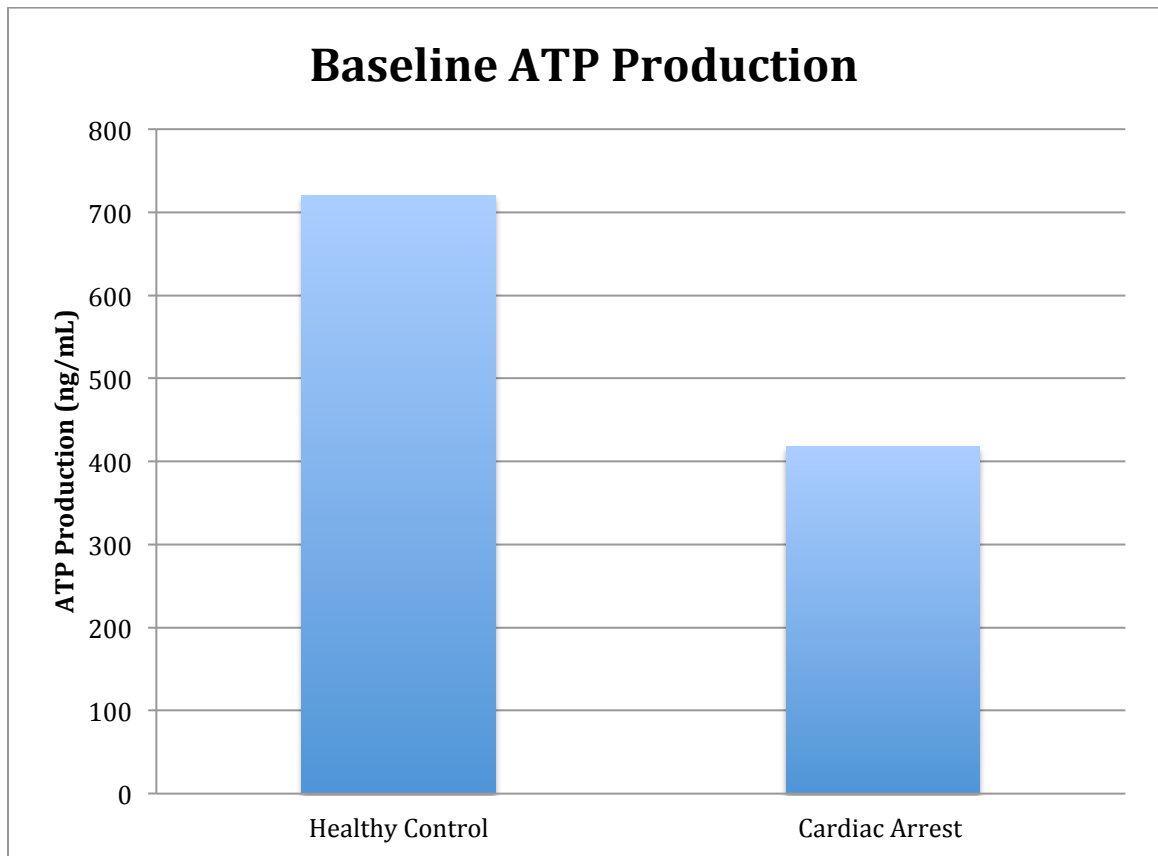
**Figure 6. Effect of thiamine on maximum respiration rates in cardiac arrest patients.** The maximum respiration oxygen consumption rate of PBMCs from cardiac arrest patients is higher with added thiamine ( $p = 0.01$ ). Values are mean  $\pm$  SE;  $n = 5$ .

### **Prospective T Cell Proliferation and ATP Production**

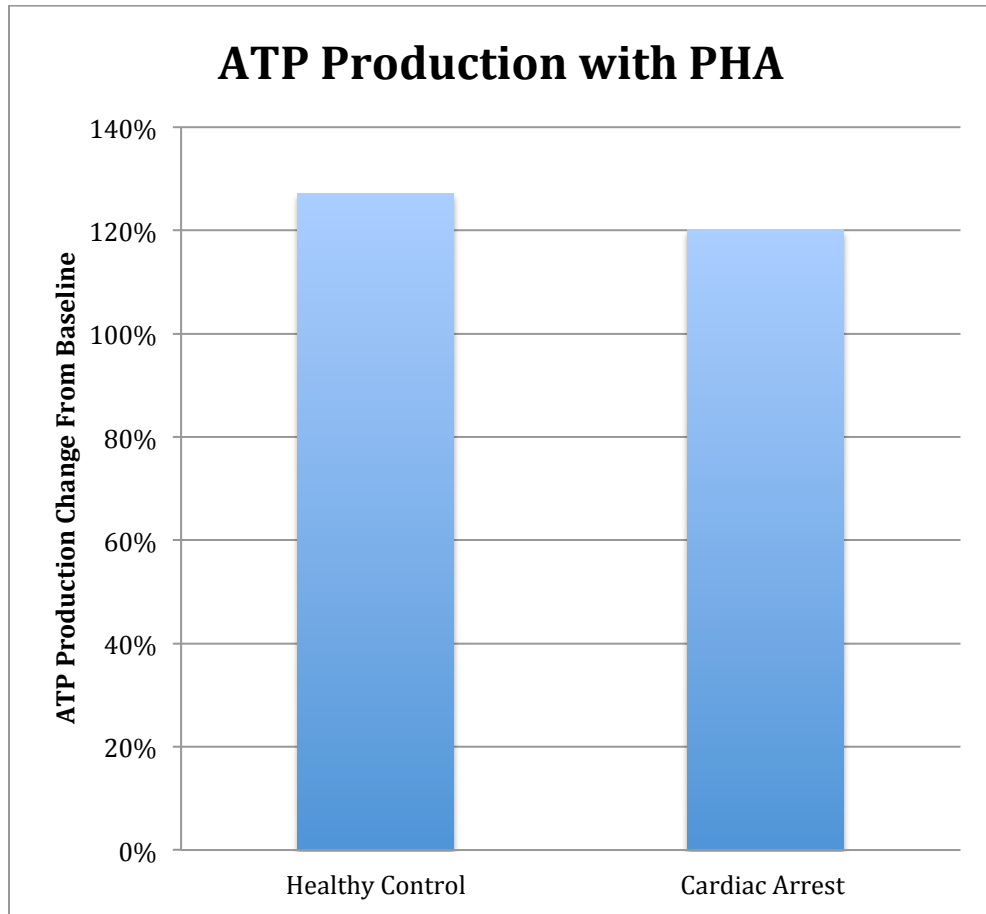
We measured ATP production in CD3+ T cells from healthy controls and cardiac arrest patients 48 hours after the arrest (Figure 7). The median baseline ATP production for eight healthy controls was 720 ng/mL. The median baseline ATP production for five cardiac arrest patients was 418 ng/mL. There was no statistically significant difference between these two groups ( $p = 0.44$ ).

We also measured ATP production in CD3+ T cells when PHA was added to the cells. The change from baseline ATP production was determined for both healthy controls and cardiac arrest patients (Figure 8). The median ATP production with PHA showed a 127% increase above baseline for CD3+ T cells from eight healthy controls. The CD3+ T cells from five cardiac arrest patients had a median ATP production with PHA that was 120% greater than baseline. No statistically significant difference was found between the two groups ( $p = 0.30$ ).

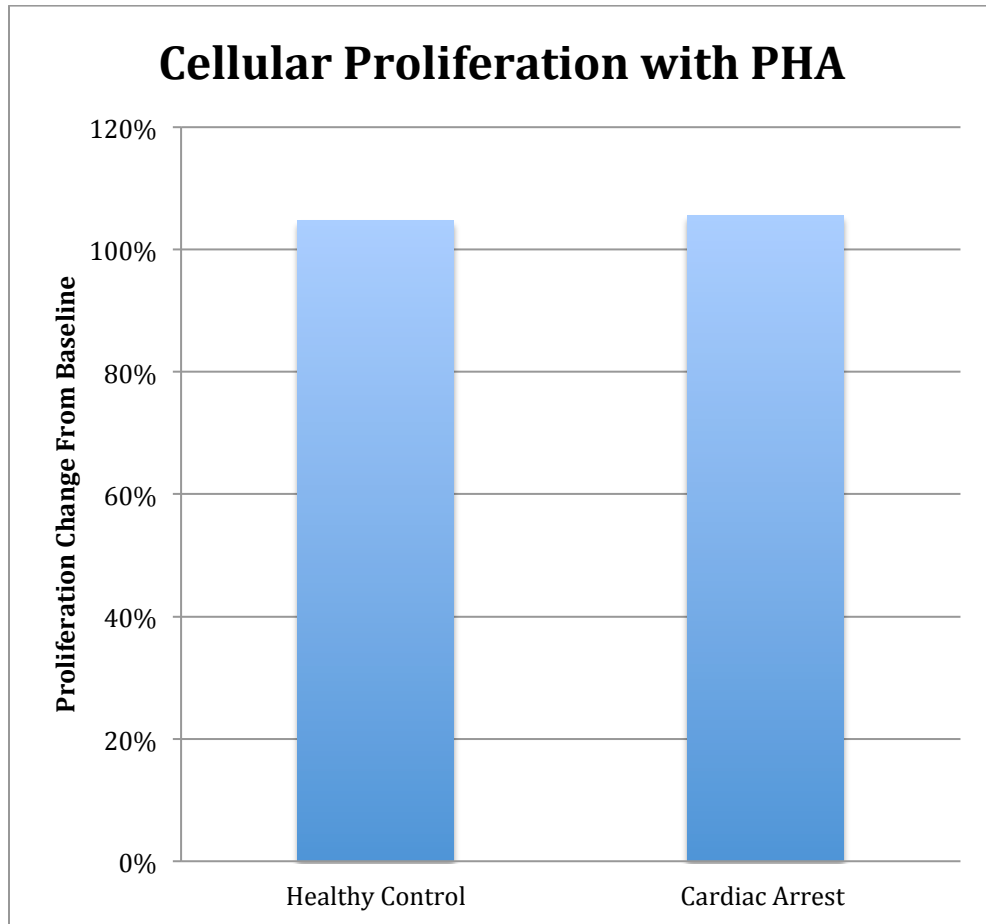
In addition to measuring ATP production, we measured the proliferation of CD3+ T cells with PHA in healthy controls and cardiac arrest patients. The change from baseline cell proliferation was calculated for both groups (Figure 9). In five healthy controls, the median increase in proliferation was 105% above baseline when PHA was added to the CD3+ T cells. For three cardiac arrest patients, a median proliferation increase of 106% above baseline was observed with the addition of PHA. There was no statistically significant difference between these two groups ( $p = 0.90$ ).



**Figure 7. Baseline ATP production in controls and cardiac arrest patients.** There is no statistical difference between the ATP production in CD3+ T cells from healthy controls and cardiac arrest patients 48 hours after the arrest ( $p = 0.44$ ). Values are median;  $n = 5-8$ .



**Figure 8. Effect of PHA on ATP production in controls and cardiac arrest patients.** ATP production in CD3+ T cells increases above baseline production when PHA is added to healthy controls and cardiac arrest patients 48 hours after the arrest. There is no statistical difference between the changes in both groups ( $p = 0.30$ ). Values are median;  $n = 5-8$ .



**Figure 9. Effect of PHA on cell proliferation in controls and cardiac arrest patients.** Proliferation of CD3+ T cells increases above baseline proliferation when PHA is added to healthy controls and cardiac arrest patients 48 hours after the arrest. Baseline proliferation rate is 100%. There is no statistical difference between the changes in both groups ( $p = 0.90$ ). Values are median;  $n = 3-5$

## **DISCUSSION**

Many patients suffer from infections post-cardiac arrest. The purpose of this study was to characterize immunosuppression and T cell metabolism in post-cardiac arrest patients. The major findings of this study were as follows: (1) 42% of cardiac arrest patients who had return of spontaneous circulation acquired an infection, (2) infections occurred an average of 4 days after the cardiac arrest, with pneumonia and urinary tract infections the most common, (3) PBMCs of cardiac arrest patients had lower rates of maximum oxygen consumption compared with healthy controls, and (4) thiamine increased the basal and maximum oxygen consumption rates of PBMCs from cardiac arrest patients.

### **Retrospective Chart Review**

Of the 170 cardiac arrest patients we reviewed, 42% of them had at least one incidence of infection post arrest. This finding suggests that cardiac arrest patients suffer from a compromised immune system after an arrest. Moreover, this result emphasizes the importance of more intensive research in the area of post-cardiac arrest treatment.

Cardiac arrest patients had a first positive blood culture 4 days after their arrest. The most prominent diagnosis was pneumonia, followed by urinary tract infections. These results imply that the period of immunosuppression started sometime within 4 days of the cardiac arrest. Post-cardiac arrest patients who

survived to hospital discharge and acquired an infection had a first positive culture 3 days later than non-survivor cardiac arrest patients who acquired an infection. This finding suggests that patients with stronger immune systems, who can sustain a longer period of time before acquiring an infection, have a higher survival rate.

### **Prospective T Cell Oxygen Consumption**

We measured the PBMC oxygen consumption rates of cardiac arrest patients 24 to 48 hours after their cardiac arrest compared with healthy control patients. PBMCs include T cells as well as B cells and monocytes. Like T cells, B cells and monocytes switch to increased glycolysis utilization when they are activated (Delmastro-Greenwood and Piganelli, 2013; Dietl et al, 2010; Marsin et al., 2002). Compared with healthy controls, the PBMCs of cardiac arrest patients showed a significant decrease in the maximum respiration oxygen consumption rate. These findings suggest that after a cardiac arrest, PBMCs switch to a more anaerobic metabolism compared with their healthy counterparts. Since PBMCs change metabolism from primarily aerobic to primarily anaerobic when they are activated, this drop in maximum respiration may indicate that PBMCs are activated post-cardiac arrest. It is also possible that these findings reflect PMBC death post-cardiac arrest, a situation that leads to a decreased number of cells and lower oxygen consumption.

Thiamine significantly increased the basal and maximum oxygen consumption rates of PBMCs in cardiac arrest patients compared with their baseline values. This finding suggests that thiamine may be able to increase the aerobic metabolism of these cells and potentially decrease the use of the anaerobic pathway.

### **Prospective T Cell Proliferation and ATP Production**

We measured T cell proliferation and ATP production in healthy controls and cardiac arrest patients 48 hours after their arrest. The overall results were inconclusive. In both cardiac arrest patients and healthy controls, the CD3+ T cells had similar baseline ATP production, and when stimulated by PHA, they had similar increases in ATP production and in cellular proliferation. The lack of statistical significance between the cardiac arrest patients and the healthy controls may be due to the small sample size of each group.

### **Future Research**

More research needs to be done on this subject because a high percentage of cardiac arrest patients acquire an infection post-cardiac arrest with current treatments. A future study should include more patients and more time points after the cardiac arrest to draw blood for assessing oxygen consumption, ATP production, and cellular proliferation. It would also be helpful to isolate T cells from the PBMC mixture in order to more conclusively determine their

oxygen consumption rate post arrest. This would lead to a clearer picture of a hyperimmune response and immunosuppression timeline.

A future study should also continue to investigate the effects of thiamine on T cell metabolism in order to gain more conclusive results. There is a possibility that by giving cardiac arrest patients thiamine post arrest, we could change the T cell metabolism from primarily anaerobic to primarily aerobic. This switch in metabolism would decrease the severity of the hyperimmune response by decreasing the number of T cells activated immediately post-cardiac arrest. This action would in turn decrease immunosuppression of post-cardiac arrest patients and potentially lead to a decreased infection rate.

## **Conclusion**

Because many cardiac arrest patients suffer from immunosuppression post arrest, there is a need for ongoing research on this issue. Our study showed that there is a decrease in the oxygen consumption rate of PMBCs post-cardiac arrest. Our results also indicated that thiamine increases the oxygen consumption rate in PBMCs of cardiac arrest patients. More research is required to determine whether cardiac arrest patients and healthy controls have differences in CD3+ T cell proliferation and ATP production.

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## CURRICULUM VITAE

Meredith Alden Hurley  
Born 1988

### Education

**Boston University**, Boston, MA

Masters of Science in Medical Sciences: Class of 2015

**The George Washington University**, Washington, D.C.

Columbian College of Arts and Sciences: Class of 2011

Bachelor of Science in Biophysics; minor in Emergency Health Services

### Clinical Experience

**Boston Childrens Hospital**, Boston, MA

October 2014 – December 2014

*Emergency Department Clinical Assistant*

- 32 hours a week
- Assists pediatric nurses and physicians with procedures
- Take vital signs and preforms point-of-care testing

**Boston Medical Center bWell**, Boston, MA

January 2014 – May 2014

*Volunteer*

- Volunteer in the outpatient pediatric clinic three hours a week
- Provide healthy life style information to young patients and their parents and lead exercise activities like yoga and obstacle courses
- Help patients and their families navigate the pediatric unit

**Virginia Hospital Center**, Arlington, VA

May 2011 – August 2013

*Emergency Department Technician*

- Performed life saving interventions and CPR, started IVs, conducted EKGs, splinted limbs, took vital signs, assisted nurses and doctors, assessed patients and documented patient care
- Trained new emergency department technicians
- Performed in the top 5% of emergency department employees
- Part of the hospital interviewing team for prospective emergency department technicians

**George Washington University Emergency Medical Response Group**, Washington, D.C

January 2009 – May 2011

*NREMT-B*

- Volunteer EMT; 60 hours a month
- Promoted to crew chief October 2010, qualified to lead a crew of 4 EMTs
- Responsible for pre-hospital patient care and transport, including patient

- assessments, life-saving interventions and documenting patient care
- Mentor for new members, including training and team building
- 2010-2011 Compassion Award for providing comfort and support to patients in crisis
- 2009-2010 Assistant Coordinator of Administration Award recipient for commitment and dedication to the organization
- President's Volunteer Service Award for over 100 hours of volunteering

**Falmouth Hospital**, Falmouth, Massachusetts

July 2009 – August 2009

*Emergency Department Volunteer*

- Assisted patient check-in and triage
- Shadowed a nurse and assisted with patient care including helping prepare patients for tests and procedures

**Research Experience**

**Center for Resuscitation Sciences,**

Beth Israel Deaconess Medical Center, Boston, MA

August 2014 - Current

*Research Student*

- Researching post cardiac arrest patients' immune system by studying their T cell metabolism and proliferation
- Performing a literature review on current methods of treating post cardiac arrest immunosuppression
- Assisting with various research projects including a traumatic brain injury study

**Crystal Ball Experiment**, Mainz, Germany

May 2010 – June 2010

*Student Research Intern*

- Collided photons in a photomultiplier detector to research subatomic particles
- Worked with the A2 Collaboration at the Johannes Gutenberg University
- Wrote computer code to compress data in the form of signals to be used for analysis and more concise data storage

**Non-clinical Work Experience**

**Boston University**, Boston, MA

September 2014 – December 2014

*Tutor*

- Tutoring physiology to Boston University School of Dental Medicine students
- Tutoring graduate level biochemistry
- Helping students create effective study schedules and discuss test taking strategies