Anti-inflammatory Therapy with High Dose Insulin in Brain Dead Organ Donors

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Submitted July, 2012

A thesis submitted to McGill University in partial fulfillment of the requirements of the Master degree in Science.

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Acknowledgments

For the most part I would like to express my deepest gratitude to my wife Hawazen Sharif for all her help in making this research project and thesis a reality. This thesis would not have been possible without her never ending encouragement and moral support.

I also want to thank God for blessing me with three wonderful children; Zain, Nour and Amnah. Their presence with the occasional and much needed distractions brought together from their enthusiastic play and laughter; made me accomplished so much. I am grateful to my family, mom and dad back home in Saudi Arabia for their continuous support and prayers.

Reaching the goals of my research would not have been possible without members of the hospital staff and the Hepatobiliary Transplant Surgery Division at the McGill University Health Center. I am grateful and thankful to Dr. M. Hassanain, Dr. P. Chaudhury, Dr. S. Paraskevas, and Dr. T. Schricker for assisting me to complete this work.

I specially thank Ms. A. Salman, the clinical co-ordinator; who was involved in all logistical aspects of the project. As well, I would like to show my gratitude to Dr. T. Nouh and Dr. M. Shaheen, who helped me in blood samples collection, spinning and serum storage of some patients.
I want to acknowledge Quebec Transplant, all the ICU departments and medical records. Their assistance during the study is highly appreciated.

I would like to acknowledge the collaboration of Dr. L. Wykes and Mr. E. Nitschmann, from the McGill School of Dietetics and Human Nutrition, who helped me in measuring serum cytokines using the Luminex 200 machine. As well, I want to recognize Mr. X. Tan an epidemiologist from the Biostatistics Core Facility, McGill University health Center, who helped me with the statistical analysis.

I am grateful to the King Abdul Aziz University, College of Medicine, Department of Surgery for sponsoring me and funding my clinical and research activities.

Finally, I am heartily thankful to my supervisor, Dr. P. Metrakos, whose encouragement and guidance over the past 2 and half years from the initial to the final level enabled me to develop a clear understanding of the subject. Without his support this work would not have surfaced.

Lastly, I offer my regards and blessings to all of those who supported me in any respect during the completion of the project.

Murad Aljiffry
List of Abbreviations

<table>
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<tr>
<th>Abbreviation</th>
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<tbody>
<tr>
<td>ACTH</td>
<td>Adrenocorticotropic Hormone</td>
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<td>ANCOVA</td>
<td>Analysis of Covariance</td>
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<tr>
<td>BMI</td>
<td>Body Mass Index</td>
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<tr>
<td>CO</td>
<td>Cardiac Output</td>
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<tr>
<td>CVA</td>
<td>Cerebrovascular Accident</td>
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<td>CVP</td>
<td>Central Venous Pressure</td>
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<tr>
<td>D20W®</td>
<td>Dextrose 20%</td>
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<tr>
<td>DCD</td>
<td>Donation after Cardiac Death</td>
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<td>DI</td>
<td>Diabetes Insipidus</td>
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<td>DRI</td>
<td>Donor Risk Index</td>
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<tr>
<td>ELISA</td>
<td>Enzyme-Linked Immunosorbent Assays</td>
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<tr>
<td>GH</td>
<td>Growth Hormone</td>
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<tr>
<td>HLA</td>
<td>Human Leukocyte Antigen</td>
</tr>
<tr>
<td>HNC</td>
<td>Hyperinsulinemic Normoglycemic Clamp</td>
</tr>
<tr>
<td>HR</td>
<td>Hormonal Resuscitation</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Intracellular Cell Adhesion Molecule</td>
</tr>
<tr>
<td>ICH</td>
<td>International Conference on Harmonization</td>
</tr>
<tr>
<td>ICP</td>
<td>Intracranial Pressure</td>
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<tr>
<td>ICU</td>
<td>Intensive Care Unite</td>
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<tr>
<td>IL</td>
<td>Interleukin</td>
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<tr>
<td>IR</td>
<td>Ischemia-Reperfusion</td>
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IV: Intravenous
MAP: Mean Arterial Pressure
MCP-1: Monocyte Chemotactic Protein-1
MD: Medical Doctor
MHC: Major Histocompatibility Complex
MUHC: McGill University Health Center
NS 0.9%: Normal Saline
PHN: Pince Hyperinsulinémique Normoglycémique
QT: Quebec Transplant
REB: Research Ethics Board
SAS: Statistics Software
SD: Standard Deviation
ST: Sample Time
SVR: Systemic Vascular Resistance
T3: Triiodothyronine
T4: Thyroxine
TGF: Tumor Growth Factor
TNF-α: Tumor Necrosis Factor-α
TRH: Thyrotropin-Releasing Hormone
TSH: Thyroid-Stimulating Hormone
UNOS: United Network for Organ Sharing
VCAM-1: Vascular Cell Adhesion Molecule-1
β-cell: Beta-cell
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Abstract

Brain death is considered a major stress on the body that is associated with a massive inflammatory response or what is known as the “cytokine storm”, which is characterized by the exaggerated release of pro-inflammatory cytokines. This heightened inflammatory response in brain dead organ donors leads to major disturbances in glucose homeostasis resulting in insulin resistance and systemic hyperglycemia. Acute hyperglycemia is intimately related to the inflammatory response and marks an increased risk of morbidity and mortality. Severe inflammation in brain dead donors can also lead to increased graft immunogenicity before transplantation and increased risk of graft dysfunction following transplantation. In addition, to the maintenance of normoglycemia Insulin therapy has expressed anti-inflammatory effects in clinical and experimental studies.

The rational of this project was to investigate the anti-inflammatory properties of high dose insulin therapy on brain dead organ donors and if this therapy is successful in maintaining normoglycemia in these donors. The anti-inflammatory effect was measured by comparing the change in the levels of serum cytokines in these donors. Insulin therapy was delivered using the hyperinsulinemic normoglycemic clamp (HNC) technique. The study was carried out in the context of a prospective pilot trial registered at clinicaltrial.gov (NCT01304290).

Fifteen brain dead organ donors were recruited including 6 donors were given the HNC protocol “experimental group” and 9 donors received routine management “control group”. The insulin therapy was provided for a minimum of 6 hours and continued until
the organ retrieval procedure. The donors were assigned to either experimental or control groups based on the location of the donation procedure. Blood samples were taken from all patients at various time points. The samples were analyzed to identify the levels of several predetermined inflammatory cytokines. Comparison of the changes in these levels with therapy in both groups was performed.

High dose insulin therapy in the form of HNC was successful in maintaining normoglycemia in the brain dead organ donors, without severe hypoglycemia. Furthermore, the anti-inflammatory effect was clearly demonstrated in the experimental group as expressed by the decreased levels of several pro-inflammatory cytokines as compared to the control group following treatment. Future studies with a focus on the effect of such therapy on the transplanted organs and patients are warranted.
Résumé

L’état de mort cérébrale est considéré comme un stress majeur pour l’organisme qui est associé à une réaction inflammatoire massive, que l’on appelle la « tempête de cytokine », caractérisée par la libération excessive de cytokines pro-inflammatoires. Cette réaction inflammatoire aiguë chez les donneurs d’organe en état de mort cérébrale est à l’origine de perturbations majeures de l’homéostasie du glucose qui provoquent l’insulinorésistance et l’hyperglycémie systémique. L’hyperglycémie aiguë est étroitement liée à la réaction inflammatoire et se traduit par un risque accru de morbidité et de mortalité. Une inflammation sévère chez les donneurs en état de mort cérébrale peut également augmenter l’immunogénicité du greffon avant la transplantation et le risque de dysfonctionnement de ce dernier à l’issue de la transplantation. Outre qu’elle permet de maintenir la normoglycémie, l’insulinothérapie a des effets anti-inflammatoires selon des études cliniques ou expérimentales.

L’objectif du présent projet était d’étudier les propriétés anti-inflammatoires de l’insuline administrée à fortes doses à des donneurs en état de mort cérébrale et d’établir si cette thérapie permet de maintenir la normoglycémie chez ces donneurs. L’effet anti-inflammatoire a été mesuré en comparant les fluctuations des niveaux de cytokines sériques chez ces donneurs. L’insulinothérapie a été administrée à l’aide de la pince hyperinsulinémique-normoglycémique (PHN). L’étude a été effectuée dans le contexte d’un essai pilote prospectif inscrit sur le site clinicaltrial.gov (NCT01304290).
Quinze donneurs en état de mort cérébrale y ont pris part, dont six ont fait partie du « groupe expérimental » qui a suivi le protocole PHN et neuf du « groupe de contrôle ». L’administration d’insuline a duré au moins six heures et s’est poursuivie jusqu’au moment du prélèvement d’organe. La répartition des donneurs dans le groupe expérimental ou le groupe de contrôle était fondée sur le lieu de la procédure de don. Des échantillons de sang ont été prélevés chez tous les patients à différents moments. Ces échantillons ont été analysés afin de mesurer les niveaux de plusieurs cytokines inflammatoires prédéterminées. Des comparaisons ont été établies entre les fluctuations de ces niveaux et l’administration de la thérapie chez les patients des deux groupes.

L’insulinothérapie à forte dose à l’aide de la PHN a permis de maintenir la normoglycémie chez les donneurs d’organe en état de mort cérébrale sans provoquer d’hypoglycémie sévère. Qui plus est, à l’issue du traitement, l’effet anti-inflammatoire a été clairement démontré dans le groupe expérimental, comme en témoignent les niveaux réduits de plusieurs cytokines pro-inflammatoires, comparativement au groupe de contrôle. Des études ultérieures s’imposent qui porteraient essentiellement sur l’effet de cette thérapie sur les organes transplantés et les patients.
Introduction and literature review

Organ donation and transplantation

Transplantation is the standard of care for patients with end-stage organ failure. Solid organ transplantation improves survival and the quality of life of the recipients. Improvements in donor management, surgical techniques, and perioperative care, together with more effective immunosuppression therapy, have contributed to an increasing success rate of transplantation in recent years. However, further improvement in donor management is required to increase the organ yield per donor.

Allografts are retrieved from deceased (cadaveric) donors or living donors (Figure 1). The two categories of cadaveric donors include heart beating donors (meet the criteria for brain death) or non-heart beating donors (do not meet the criteria for brain death) also referred to as donation after cardiac death (DCD) donors. Living donors can be related or unrelated. Most transplant grafts in North America are retrieved from cadaveric heart beating donors. The ideal organs are those from younger donors with no co-morbidities. However, as the North American population ages and the prevalence of obesity increases the number of donors with co-morbidities with less favorable grafts is increasing.
The increasing gap between supply and demand

As transplantation has become increasingly successful it has become the choice of treatment for more and more patients, thus the transplant wait lists have been growing during the last decade. Although initially the number at transplants performed were also rising they have now leveled off but the number of patients waiting is still increasing. Issues that need to be addressed to increase the number of organs available for transplantation include: a) increasing public awareness so that consent to organ donation increases, b) better donor identification and management so potential grafts are maintained in a viable state, and c) regularly updating the established criteria that predict post transplant viability and function of donor organs. Several strategies have been adopted to increase the organ pool without compromising the outcome of recipients.

Expanding the donor pool

Living donor transplantation can contribute significantly to the decreasing supply of organs available for transplantation; many kidney transplant programs derive up to 50% of their grafts from live donation. An added benefit from of live donor kidney transplant is that the outcomes are substantially better than cadaveric kidneys. Live donor liver, lung, and pancreas transplant on the other hand has not made a large contribution to the donor pool. Another strategy is to utilize expanded criteria donors (ECD). ECD include organs from older donors, donors with significant comorbidities, and specific organ related issues such as hepatic steatosis. These donors usually yield lower quality organs.
with a higher risk of graft failure after transplantation than standard criteria donors \(^1,11,12\). However, by decreasing the cold ischemia time and match these ECD organs to appropriate recipients many of these organs have been found to have reasonable outcomes \(^1\). Although the definition for ECD is not well established for all organs; it is best characterized kidney donors. The United Network for Organ Sharing has established a definition of expanded criteria kidney donors \(^13\). This includes all donors greater than or equal to 60 years of age, plus those donors between 50 and 59 years with at least two of the following three: serum creatinine greater than 1.5 mg/dL, cerebrovascular accident as cause of death, and a history of hypertension \(^13\). Currently, cross-species transplantation (xenotransplantation) and tissue engineering using stem cells are among the strategies still at the experimental stage as solutions to organs shortage \(^14,15\).

### Donor characteristics

Certain donor-specific factors could influence the quality of the organs retrieved thus affecting graft and / or recipient survival \(^16\). These factors include the category of donor (living, heart beating and non-heart beating donors), cause of death, donor age, and donor comorbidities. A good example of assessing the impact of donor characteristics on transplant outcomes is the effect of expanded criteria donors on kidney outcomes and the description of the donor risk index (DRI) in liver transplantation \(^13,17\). The DRI is calculated by using several donor characteristics. These include donor demographics (e.g. age, race), donor comorbidities, cause of death, if donor was a DCD and the surgical management of the graft (whole versus partial) \(^18\). This index can give an insight on liver
transplant outcomes independent of recipient characteristics. However, none of these scores has been tested for correlation with the impact of inflammatory stress induced by brain death on the organ and its future quality.

A factor that may be very important but is not routinely assessed is the intensity of the inflammatory response that is generated by the brain dead state. A landmark study described the correlation between the plasma level of interleukin (IL-6) in brain dead donors and recipients six month hospital-free survival after solid organ transplantation. This prospective cohort study took place in two large intensive care units of tertiary care university hospitals in the United States. A total of 30 consecutive brain dead organ donors and 78 recipients were recruited. The investigators demonstrated that higher plasma levels of IL-6 in donors were significantly associated with a lower six month hospital-free survival in recipients (hazard ratio 1.77; 95% confidence interval, 1.17–2.69, p<0.007).

Pathophysiological changes after brain death

The pathophysiological changes seen in the brain dead donor are multi-factorial and are related to loss of brainstem function and the associated systemic inflammatory response. It all starts by an increase in the intracranial pressure (ICP), which usually precedes brain death. This pressure increase induces hemodynamic instability, endocrine abnormalities, hypothermia, electrolyte disturbances, and a robust inflammatory response.
Hormonal changes following brain death

The hormonal changes in the brain dead donor are variable in timing and severity. In animal models an induced increase in ICP to establish brain death will result in a progressive loss of both posterior and anterior pituitary functions. This ultimately leads to a rapid deterioration in cardiac function and a shift from aerobic to anaerobic metabolism. Brain death in humans has an inconsistent profile of hormonal disturbances; most commonly the posterior pituitary function is lost, leading to a decrease in vasopressin release and the development of diabetes insipidus (DI) in up to 80% of patients. If not recognized and adequately treated, this will result in polyuria, hypovolaemia and hypotension, together with hypernatraemia and a hyperosmolar state. In contrast, the changes in anterior pituitary function after brain death are variable. The anterior pituitary is regulated by the hypothalamus-pituitary axis, which may be partially or completely affected, depending on the degree of ischemia to the area. This will lead to decreased or occasionally normal circulating levels of different hormones such as adrenocorticotropic hormone (ACTH) and thyroid stimulating hormone (TSH).

Under normal circumstances, the hypothalamus stimulates the pituitary gland to secrete TSH, by releasing the Thyrotropin-releasing hormone (TRH) in response to low levels of circulating thyroid hormones. TSH prompts the thyroid gland to release Thyroxine (T4) and Triiodothyronine (T3). In addition, it promotes the peripheral conversion of T4 to T3 (active form). If the hypothalamus-pituitary-thyroid axis integrity is lost with brain
death, TSH release and T4 conversion drops, which results in a decline of T3 levels. This can lead to a progressive loss of cardiac contractility, increased anaerobic metabolism and accumulation of lactic acid\textsuperscript{24,26}. In a study by Sazontseva et al. involving 22 brain dead organ donors, TSH, T4 and T3 levels were decreased in 85%, 55% and 90% of patients respectively\textsuperscript{27}. It is believed that thyroid hormonal changes in brain death usually mimics the ‘euthyroid sick syndrome’ or severe nonthyroidal illness that is seen with acute major stress and in the critically ill patients. This is characterized by low serum levels of T3 and T4 with low to normal TSH serum levels\textsuperscript{28}.

The fate of the other anterior pituitary hormones, including ACTH, prolactin, growth hormone, and gonadotropin, is not clearly defined and reports have been inconsistent\textsuperscript{28}. For instance, Gramm et al. studied 32 potential organ donors aiming to determine the serum concentrations of hypothalamic-pituitary hormones after brain death. Although DI developed in 78% of the donors and in 62% T3 levels were below normal, ACTH levels were stable and the levels of TSH and growth hormone (GH) showed some increase from baseline values after 30-40 hours\textsuperscript{26}. However, in another study involving 31 brain dead donors, the investigators found that 50% of patients were ACTH deficient as defined by a serum cortisol below 400 nmol/L\textsuperscript{29}.

- **Hyperglycemia in brain dead donors**

In general, the majority of critically ill patients and those with major stress (e.g. brain death) will have high blood glucose values\textsuperscript{30}. Following brain death pancreatic function
appears to be preserved in potential donors. However, insulin production slightly drops and to a larger extent a peripheral state of insulin resistance develops, leading to a decrease in intracellular glucose levels. Mostly, this is related to the catecholamine surge and the autonomic storm that accompanies severe cerebral vascular accidents. In addition, brain death could disrupt the balance of the hypothalamic-pituitary axis system. This might lead to either up- or down-regulation of several hormones involved in controlling blood glucose. The release of epinephrine, glucagon and cortisol in response to stress promotes glucose production and suppresses insulin. The net effect is a disturbance in glucose homeostasis that eventually leads to energy deficit and a shift to anaerobic state resulting in systemic hyperglycemia and acidosis. Hyperglycemia is a risk factor for higher morbidity and mortality in patients with critical illness. In addition, a linear correlation has been shown between hyperglycemia and the systemic inflammatory response. Multiple studies have revealed that pro-inflammatory cytokine levels such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) increases with acute hyperglycemia. Moreover, there are certain cytokines, for example TNF-α that can induce insulin resistance, which in turn potentiate the hyperglycemia.

- **Systemic inflammatory response after brain death**

Brain death induces the highest known stress on the body that is associated with a massive inflammatory response or what is known as a “cytokine storm”. Severe trauma and critical illness are commonly associated with systemic inflammation. During the
inflammatory response endothelial and epithelial cells as well as neutrophils, macrophages and lymphocytes are stimulated to release many pro-inflammatory mediators. An exaggerated response can overwhelm local defense mechanisms and results in defense system failure\(^\text{37}\). In the case of brain death this contributes to donor hemodynamic instability and eventually may lead to organ hypoperfusion and damage\(^\text{38-40}\).

The mechanism by which the inflammatory response is induced following brain death is rather complex and related to multiple factors\(^\text{7,19}\). First, brain damage following ischemia leads to the release of inflammatory mediators from the cells of the central nervous system, particularly the astrocytes and microglia cells\(^\text{41-43}\). Second, the marked sympathetic stimulation (catecholamine surge) associated with stress could induce inflammation by creating a generalized ischemia-reperfusion (IR) injury due to the intense vasoconstriction and raised systemic vascular resistance leading to central redistribution of blood and visceral ischemia\(^\text{24,44}\). Third, there are obvious metabolic changes after brain death, such as a shift from aerobic to anaerobic metabolism, which might alter the inflammatory response, since metabolism is intimately related to inflammation\(^\text{45,46}\). Fourth, the patient’s preexisting condition (e.g. intracranial hemorrhage, trauma to other organs, hemodynamic instability, hypoxia, etc.) that precedes brain death also contribute to the initiation of the inflammatory response\(^\text{47-49}\). Moreover, failure to adequately restore the cardiovascular and ventilatory states following brain death could potentiate the condition, because hypotension and oxygen deprivation in organs intensifies the inflammatory response\(^\text{50}\).
The systemic inflammation following brain death is characterized by an increase in the serum levels of inflammatory cytokines and an up-regulation of their receptors in the somatic organs \(^{40, 51, 52}\). Inflammatory cytokines are low molecular weight cell-signaling molecules that are secreted by numerous cells in the body. Their primary action is regulating the inflammatory response through intercellular communications, either as pro- or anti-inflammatory mediators \(^{42, 53}\). Important pro-inflammatory cytokines include TNF-\(\alpha\) and interleukins such as IL-6 and IL-1\(\beta\), whereas IL-10 is believed to have anti-inflammatory effects \(^{19, 54}\). Adhesion molecules are also implicated in this inflammatory response like E-selectin, intracellular cell adhesion molecule (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) \(^{55}\). Cell adhesion molecules are proteins located on the surface of leukocytes and endothelial cells \(^{56}\). They mediate inflammation by inducing leukocyte tethering to the endothelial vascular wall promoting their migration into tissues and by facilitating the release of oxygen free radicals \(^{57, 58}\).

Several studies have shown increased levels of adhesion molecules and pro-inflammatory cytokines in kidneys from brain dead donors \(^{59, 60}\). In addition, it has been demonstrated that transient cerebral ischemia will up-regulate the transcriptional levels of TNF-\(\alpha\) and IL-6 \(^{61}\). Furthermore, livers from brain dead donors were found to have a significantly increased transcription rate of IL-6, TNF-\(\alpha\), monocyte chemotactic protein-1 (MCP-1) and tumor growth factor (TGF) \(^{40, 62}\). Overall, inflammatory markers are highly expressed in organs from brain dead donors compared to in living controls \(^{19, 40}\).
The impact of Inflammation on organs from brain dead donors

There are several reasons for lower organ yield and lower graft survival from brain dead donors when compared to living donation. The systemic inflammatory response could be an important contributor to this difference. The heightened inflammation in brain dead donors is well known to reflect negatively on the transplanted grafts.

- **Graft immunogenicity**

The inflammatory response has been shown to result in increased graft immunogenicity before transplantation. Inflammatory cytokines have different effects on the immune system, both agonistic and antagonistic. After brain death they seem to orchestrate an inflammatory reaction with rapid attraction of leukocytes in organs, through the activation of adhesion molecules and the release of chemokines, such as ICAM-1 and MCP-1. This will lead to increased inflammatory cellular infiltrates in all organs. Adherent leukocytes release pro-inflammatory mediators resulting in the up-regulation of major histocompatibility complex (MHC) class I and II in graft cells. MHC antigens could increase graft immunogenicity via enhancing the T-cell recognition process. An immunohistochemical study compared pre-transplant donor biopsies from living kidney donors with those from deceased kidney donors. The authors found increased expression of Human Leukocyte Antigen (HLA-DR) in the proximal tubules and higher levels of endothelial E-selectin in biopsies from deceased donors compared to those from living donors. Furthermore, these findings were significantly associated with early acute
rejection following transplantation\textsuperscript{55}. In another study the expression of adhesion molecules, VCAM-1 and ICAM-1 detected by immunohistochemistry in donor kidneys, is associated with inferior graft function after transplantation\textsuperscript{66}.

- **Graft dysfunction**

Increased levels of inflammatory markers have been related to malfunction of donor solid organs after transplantation. This organ dysfunction has been shown to decrease the recipient’s survival post transplantation\textsuperscript{4}. In a prospective study, a cytokine pattern consisting of elevated levels of IL-6 and TNF-\(\alpha\) in the heart shortly after transplantation corresponds to impaired hemodynamics\textsuperscript{63}. The hemodynamic impairments found in this study included a reduced stroke volume, elevated mean pulmonary artery pressure, and elevated left and right ventricular filling pressures that resulted in tachycardia and a reduced left ventricular performance. These impairments were found to be independent of cellular rejection and may indicate an unfavorable graft prognosis. In another study along the same theme, the expression of TNF-\(\alpha\) in the donor heart predicted right ventricular failure after transplantation\textsuperscript{67}. In a recent study, high levels of pro-inflammatory cytokines in donors correlated with severe IR injury post liver transplantation, which was accompanied by increased incidence of acute rejection\textsuperscript{62}. The systemic inflammatory response associated with brain death has also been shown to promote pulmonary infiltration with neutrophils and enhancing lung inflammation prior to transplantation\textsuperscript{47}. In addition, high levels of IL-8 in the bronchoalveolar fluid of donor lungs correlate with early graft failure\textsuperscript{68}. 
In summary, brain death is associated with the release of pro-inflammatory cytokines leading to a heightened inflammatory response in all organs considered for transplantation. This ultimately could result in immunologically activated organs before procurement, causing histological damage, decreased function, and lower graft survival compared with organs from living donors \(^{65, 69, 70}\).

**Donor management (anti-inflammatory therapy)**

Despite all the efforts to expand the donor pool by broadening the criteria of suitable donors, not all potential organs are recoverable from these donors \(^{71, 72}\). One reason for this can be the damage that is caused by the brain death induced systemic inflammatory response \(^{73-75}\). Therefore, managing donors in a way that modifies the inflammatory response prior to procurement is an attractive concept. It would be important to initiate the therapy as early as possible to maximize the benefits. However in clinical settings, starting any intervention prior to the declaration of brain death poses medical and ethical challenges. This holds true unless the treatment is also beneficial for patients with severe brain injury who are not declared brain dead. In addition, the intervention to ameliorate inflammation should not be potentially harmful to any of the transplantable organs. Unfortunately, there are not many anti-inflammatory therapeutic options that are proven to be safe and effective to suppress donor inflammation \(^3\). This is due to the paucity of experimental studies on donors given the logistical and ethical difficulties associated with these types of interventions.
• **Hormonal therapy**

Standardizing donor management through the establishment of protocols and guidelines has increased the number of organs retrieved from each donor\(^7^4\). However, the anti-inflammatory component (such as the use of steroids) is inconsistent or sometimes lacking in most of these guidelines\(^2^2,^7^6\). The United Network for Organ Sharing\(^7^7\) developed a critical pathway for organ donor management in the USA in 1999\(^7^7\). The pathway recommends definite treatment and monitoring goals in the management of donors, such as hemodynamic control and electrolytes replacement guidelines. Following its introduction, the rate of organs transplanted from brain dead donors has increased by 11.3%\(^7^4\). Consequently, the hormonal (in part anti-inflammatory) elements were implemented in the brain dead donor care. The so called triple hormonal therapy or ‘hormonal resuscitation’ (HR) consists of methylprednisolone bolus and infusions of vasopressin and either T3 or L-thyroxine\(^2\). In a retrospective study analyzing UNOS data, 10,292 consecutive brain dead donors were included, of whom only 701 received the three-drug HR. Significantly more organs were transplanted from donors who received HR compared to those who did not\(^2\). A subsequent study by the same group also using UNOS data to analyze heart transplant recipients showed an improved short-term graft function and survival with the use of HR in donors\(^7^8\). In addition, the authors found that steroids given alone or as part of combined hormonal therapy significantly reduced prolonged graft dysfunction. Although combined hormonal therapy has been shown to improve organ procurement rate and possibly improve cardiac graft survival in these retrospective analyses of UNOS data, it is however, still non-uniformly applied\(^3\). The
inconsistent delivery and heterogeneity of the hormonal regimens, as well as the potential
differences in other aspect of donor and recipient care between centers limited the
conclusions that can be made from these studies. Hence, more data is required to reach
sound conclusions.

- **Triple hormonal therapy**

On reviewing the triple hormonal therapy (methylprednisolone, vasopressin and thyroid
hormones) used in donor management, methylprednisolone is the only
immunomodulatory agent identified that is able to suppress the associated inflammatory
response. Vasopressin is routinely used to treat DI, a common complication in brain dead
donors. In addition it is useful in maintaining hemodynamic stability in the donor. On the other hand, thyroid hormone supplementation in the donor is rather selective. It is
recommended when the cardiac performance is impaired, defined as donor left
ventricular ejection fraction of less than 40% despite standard donor management.

Methylprednisolone is a synthetic steroid with a predominant glucocorticoid function. It
is usually administered intravenously in brain dead donors at the time of organ retrieval
as an anti-inflammatory agent in a dose of 15 mg/kg. In a prospective randomized
study, steroid therapy in brain dead donors resulted in a significant reduction of liver
inflammation as demonstrated by decreased levels of several inflammatory cytokines
such as IL-6, TNF-α and MCP-1. A retrospective series compared 118 consecutive
brain dead donors who received methylprednisolone with 38 controls. The steroid group
had a significant improvement in donor oxygenation and a higher lung retrieval rate \(^8^2\). In another study, the use of methylprednisolone was associated with a reduction in progressive extravascular lung water accumulation \(^8^3\). A similar pattern of decreased inflammation in different organs (e.g. heart and kidney) with steroid use after brain death has been demonstrated elsewhere \(^8^4, 8^5\). On the other hand, a placebo-controlled blinded randomized clinical trial showed that steroid pretreatment of organ donors did not improve outcomes after liver transplantation \(^8^6\). Moreover, it should be noted that hyperglycemia is common in brain dead donors and is exacerbated by steroid administration, which warrants careful monitoring and treatment. Overall, there is multitude of evidence suggesting that methylprednisolone use in donors is associated with increased organ retrieval, reduced inflammation with the absence of major side effects \(^8^1, 8^7\).

- **Insulin therapy**

Insulin concentrations slightly decline and insulin resistance develops after brain death \(^7, 3^1\). In general, patients under major stress frequently encounter a catabolic response characterized by a series of hormonal and metabolic changes that can culminate in hyperglycemia \(^3^2, 3^3\). In addition, the release of pro-inflammatory cytokines exacerbates the condition even further by elevating the blood glucose levels \(^8^8\). For instance, TNF-\(\alpha\) can reduce the uptake of glucose into peripheral tissues \(^8^9\). Furthermore, the enhanced metabolic effects can cause a breakdown of skeletal muscle proteins and thereby provide gluconeogenic precursors in the liver. Lipolysis in adipose tissue is increased and glucose
utilization becomes impaired by this process leading to further hyperglycemia and the release for free fatty acids. Collectively, these effects contribute to an insulin resistance state in the patient and cause blood glucose levels to rise.

Acute hyperglycemia resulting from an inflammatory response marks an increased risk of morbidity and mortality in critically ill patients. In a brain dead donor, poor glucose control could lead to the development of severe osmotic diuresis and profound hypovolemia, thereby adversely affecting the donor renal function. Therefore, insulin supplementation with standard ICU protocols is often required to maintain adequate glucose control in donors. In fact, strict glucose control by insulin therapy has been shown to improve the outcome in medical and surgical ICU patients. A randomized clinical trial conducted by Van den Berghe et al. reported reductions in hospital mortalities by 30% among critically ill patients receiving tight-glucose control therapy. Several professional organizations including the American Association of Clinical Endocrinologists and the American Diabetes Association recommend intensive insulin therapy to maintain glucose control in critically ill patients. Certainly, in patients with brain injury, strict glucose control has shown to decrease the rise in the intracranial pressure, reduce the need for inotropic support, and the risk of seizures, as well as improves long-term rehabilitation. However, widespread adoption of intensive insulin therapy and tight glucose control has been hindered by concerns about the risk of severe hypoglycemia and difficulty in achieving normoglycemia in critically ill patients. A meta-analysis which evaluated the benefits and risks of intensive insulin therapy in critically ill patients concluded that a benefit with this therapy was found only in surgical...
ICU patients and caused an increased the risk of hypoglycemia among all critically ill patients 99.

In addition to the maintenance of normoglycemia, insulin therapy has shown to have anti-inflammatory effects both in experimental and clinical studies 100-103. Theoretically, insulin therapy reduces trauma related insulin resistance, increases tissue glycogen stores, provides an anti-inflammatory effect and improves the immune system defense against infection 104, 105. In the past, studies on the use of insulin therapy focused on its metabolic properties such as increasing cardiac, hepatic and muscle glycogen content and inhibiting peripheral lipolysis. Recently, evidence suggests that insulin protects the organ by counteracting the inflammatory response following injury 100, 104, 106. While acute hyperglycemia induces the release of pro-inflammatory cytokines TNF-α, IL-1β and IL-18, insulin significantly lowers these cytokines 34. Enhanced production of IL-2 and IL-4 needed for combating the IL-1/TNF-α pro-inflammatory pathway was observed in an animal study that looked at the effect of insulin therapy on systemic inflammation 107.

We are not aware of any human studies evaluating the effect of insulin treatment in donors after brain death. However, Barklin et al. tested insulin therapy on a brain dead animal model using pigs, which resulted in an anti-inflammatory effect 103. Brain death was induced by the inflation of a balloon in the epidural space of the animal thereby increasing the intracranial pressure till it exceeded the mean arterial pressure. In this trial, eight female landrace brain dead pigs were placed on fixed dose insulin therapy and compared to eight others which did not receive insulin. The main finding was that insulin
showed anti-inflammatory effects after brain death, expressed by lower IL-6 concentration in the hearts and kidneys of the treated animals as compared to the control animals.

The following is a summary of the potential benefits from the direct effect of insulin on target organs that could be reached using insulin therapy prior to organ procurement:

1. **Heart**

The use of high dose insulin therapy has been shown to be beneficial in patients undergoing cardiac surgery\textsuperscript{100,108}. In a randomized controlled study, patients having elective coronary bypass surgery were given either a fixed dose continuous insulin therapy or the standard insulin sliding scale. The investigators found that high dose insulin therapy attenuates systemic inflammatory response in coronary artery bypass grafting patients\textsuperscript{100}. In addition, patients receiving the insulin therapy experienced earlier metabolic recovery of the heart, better myocardial protection and earlier functional recovery compared to the control group\textsuperscript{108}. Moreover, patients that received the high dose insulin therapy had no perioperative myocardial infarction.

2. **Liver**

There is a direct link between liver function after transplantation and its glycogen stores. Animal trials have demonstrated a linear relationship between nutrition status, liver
glycogen content, and liver function after transplantation\textsuperscript{109-112}. In addition, portal vein infusion with dextrose during transplantation modified the enzymatic profile of the transplanted livers\textsuperscript{113}. In human studies, high dose insulin therapy given to patients undergoing major liver resection resulted in an improved liver glycogen content, suppressed apoptosis and reduced postoperative negative outcomes\textsuperscript{106,114}. Furthermore, insulin has been proven to provide a protective effect in patients needing blood inflow restriction to the liver (Pringles maneuver) during liver resection surgery by suppressing the exaggerated release of IL-6\textsuperscript{115}.

3. Kidney

Insulin infusion has been shown to have multiple beneficial effects on kidney function. Insulin therapy can cause vasodilatation of the renal circulation leading to increased plasma flow and plasma renin activity\textsuperscript{116,117}. Another important property that is also mentioned in the literature concerning insulin infusion to the kidney is the reduction of oxidative stress and possibly IR injury in the early phase of kidney transplantation.

Monge and colleges randomized 43 kidney transplantation patients to receive either a combined glucose and insulin infusion (study) or glucose only infusion (control). After the first post-transplantation day, antioxidant plasma capacity was found to be stable in the study group and decreased in the control group\textsuperscript{118}. This shows that insulin infusion can help in maintaining antioxidant defenses post-transplantation and could decrease IR induced injury.
4. Pancreas

The concept of beta-cell (β-cell) rest evolved in the field of islet cell transplantation. Studies have shown improvements in β-cell insulin content and function following a period of feedback inhibition to insulin secretion. The use of intensive insulin administration appears to exert a protective effect, prolonging β-cell survival and hence improving graft survival. Experimentally, this phenomenon appears to be related to a link between the β-cell functional state, antigenecity and susceptibility to cytokine injury. Therefore, in theory, high dose insulin therapy in brain donors could have a protective effect on the potential pancreatic graft.

Insulin therapy protocols

High dose insulin therapy makes it possible to deliver a constant intravenous dose of insulin. Available insulin infusion protocols include the Portland protocol (automated continuous intravenous insulin) and the hyperinsulinemic normoglycemic clamp (HNC) technique. Fixed dose insulin administration has been shown to be safe and successful in maintaining glucose at reasonable levels in multiple studies. In the HNC technique, patients receive a fixed calculated IV insulin infusion based on body weight and IV dextrose is used concomitantly to maintain normoglycemia. Administration of IV insulin for glucose maintenance runs the risk of causing hypoglycemia as well as hypokalemia since potassium is transported intracellularly with
glucose and insulin. Therefore, it is important to check serum glucose and potassium concentrations regularly when providing fixed dose insulin therapy 127.

In summary, insulin has inherent anti-inflammatory and anabolic effects, and it is essential to maintain adequate glucose control in brain dead donors. Given this, we believe insulin therapy will have multiple protective effects on future transplanted organs with the absence of major side effects to the donor and the organs.
Objectives

The overall objective of this work is to increase the quality and quantity of donor organs for transplantation. This pilot study specifically tested the feasibility of high dose insulin therapy in brain dead donors. The specific objectives included:

1. To determine if high dose insulin therapy can maintain normoglycemia without the occurrence of hypo or hyperglycemia in brain dead donors.

2. To determine if high dose insulin therapy can reduce the inflammatory state found in brain dead donors.

**Hypothesis**

1. Hyperinsulinemic normoglycemic clamp (HNC) is an effective method of maintaining normoglycemia without the occurrence of severe hypo or hyperglycemia in brain dead donors.

2. High dose insulin therapy can attenuate markers of inflammation in brain dead donors.

Normoglycemia was defined as glucose level between 4-7 mmol/l, hypoglycemia as glucose level equal to or below 2.5 mmol/l, and hyperglycemia as glucose level higher than 7 mmol/L. The anti-inflammatory effect was examined by measuring the changes of
inflammatory cytokine levels in donors in relation to the insulin therapy. High dose insulin therapy was delivered using the HNC technique.
Methodology

Study Design and Participants

This is an open-label prospective-controlled pilot study. The study was approved by the McGill University Health Center (MUHC) and the Quebec Transplant’s (QT) Research Ethics Boards (REBs). QT is the official organization mandated by the Quebec Health and Social Services Ministry to recruit and manage donor organ donation. The study is registered at clinicaltrial.gov (NCT01304290).

All eligible brain dead organ donors identified within the province of Quebec between January, 2010 and June, 2011 were considered for the study. If the logistically the donor could be treated then the donor’s family was approached for consent. Recruitment was based on the following:

- Inclusion Criteria

The donors were required to meet the following criteria:

1. Donor had to be 18 years of age or over.
2. Brain dead donors only.
3. Donor’s family consented to the study under QT and MUHC REB regulations.
Exclusion Criteria

The donors were excluded if any of the following was identified:

1. Inability to provide research consent.
2. Time interval between the start of the study and organs retrieval in the operating room (aortic cross-clamping) was expected to be less than six hours.
3. No solid organs were to be retrieved for transplantation (i.e. tissue donor).
4. If the donor procedure was cancelled for any reason (this did not apply if the organs were retrieved but eventually not transplanted).
5. Donor was diagnosed with Type 1 Diabetes.
6. Donor received high dose steroid therapy recently (3 months).

Sample size

There are no previous studies that evaluated the anti-inflammatory effect of high dose insulin therapy on brain dead human organ donors. Therefore, we carried out a pilot project to examine the feasibility and safety of high dose insulin therapy in brain dead donors and to generate preliminary data that could be used to do sample size calculations in future studies. A total of 15 donors were included; nine untreated controls and six that received high dose insulin therapy (experimental).
Treatment group assignment

Assignment of donors to the experimental or control groups was determined by the location of the treating hospital in which the donor was located and where the organ retrieval procedure would eventually take place. Since there was no research associate available outside the MUHC to oversee the administration of the insulin protocol but the donor coordinators from QT could collection blood specimens for study, donors outside the MUHC were considered controls. Donors who consented to the study within the MUHC were considered as experimental subjects and received the insulin therapy via the HNC technique.

Informed Consent

Prior to intervention under this protocol, QT personnel obtained a signed and dated informed consent from the donor’s family, specifically the next of kin as per the province rules and regulations. The study investigators, clinical coordinators and physicians involved in the donor care did not participate in the consenting process. The consent for this project was obtained in two steps. As per QT standard operating procedures, the QT coordinator obtained a general consent for organ donation. Included in that organ donation consent form is the option to agree or refuse participation in research projects. If the family consented to the research provision, another consent for the insulin project was obtained. During the second consent, the background of the study and the potential benefits and risks associated with participation were explained in detail. The families
were provided with a copy of the study information sheet and were given the opportunity to ask any questions or seek clarification.

1. Donors at MUHC: If the second consent was obtained, the donor entered the experimental arm of the study. If the family refused the second consent but agreed to the first consent, they were approached for approval to obtain blood samples and collect data only. The donor was then considered a control and treated as per QT standard donor protocol.

2. Donors outside the MUHC: After the consents were obtained, the donor was managed according to standard protocol. Only data and blood samples were collected.

Two copies of the signed and dated consents were made. One was given to the donor’s next of kin and the second one was stored in the study binder. The original copy was stored with Quebec Transplant. The investigators ensured that the study was conducted in full conformity with the current revision of the ICH guidelines of good clinical practice, the institutional review board and Quebec Transplant REB.
Research protocol

- Intervention

1. Control group

In this group blood glucose levels were controlled using a standard insulin sliding scale by QT personnel. Blood glucose levels were checked every 30 min to one hour with an Accu-Chek® glucose monitor (Roche Diagnostics, Switzerland). IV insulin (Humulin® R regular insulin, Eli Lilly and Company, Indianapolis, IN) was administered in a bolus fashion or through a continuous infusion adjusted according to a standard sliding scale (Table 1). In most sliding scales insulin is given only when blood glucose is above 6 mmol/L (110 mg/dL), to maintain a level between 6-10 mmol/L (110-180 mg/dL). Other aspects of donor management were carried out according to QT and the treating institution’s standard of care in all participants. Management of the recipient organs was as per standard receiving institution’s protocol.

2. Experimental group (HNC)

Donors in this group received high dose insulin therapy with the HNC technique. Initially, before the HNC was started; a baseline blood glucose level was measured and the following was performed accordingly:
1. If blood glucose was <4 mmol/L, a 20mL bolus of Dextrose 20% was given.
   Dextrose 20% infusion was started at 40 mL/hour followed by the insulin infusion.
   Blood glucose was checked after 10 min and adjusted according to the provided protocol (Table 2).

2. If blood glucose was 4-6 mmol/L, insulin infusion was started together with
   Dextrose 20% infusion at 30 mL/hour. Blood glucose was checked after 10 min
   and adjusted according to the provided protocol (Table 2).

3. If blood glucose was 6-10 mmol/L, insulin infusion was started together with
   Dextrose 20% infusion at 20 mL/hour. Blood glucose was checked after 10 min
   and adjusted according to the provided protocol (Table 2).

4. If blood glucose was >10 mmol/L, only insulin infusion was started. Blood
   glucose was checked after 10 min and adjusted according to the provided protocol
   (Table 2).

Plasma insulin concentration was increased by a continuous intravenous insulin infusion
(Humulin® R regular insulin, Eli Lilly and Company, Indianapolis, IN) at a constant rate
of 0.3U/kg/hour (5 mU/kg/min). Insulin was prepared by mixing 250 U of Humulin® R
in 250mL of normal saline (NS 0.9%). A dextrose 20% (D20W®) infusion was titrated to
maintain a target blood glucose level between 4-6mmol/L (Table 2). The
Dextrose/Insulin therapy was maintained for a minimum of 6 hours and continued until
cross-clamping of the abdominal aorta (time of cardiac death) during the organ retrieval.
Blood glucose levels were measured every 10-15 min in the first hour with the Accu-
Chek® glucose monitor (Roche Diagnostics, Switzerland) to ensure euglycemia. After
one hour and once a steady state of glucose blood concentration was achieved; blood glucose was checked at 30-60 min intervals.

All exogenous sources of glucose were avoided and medications were mixed in normal saline (NS 0.9%). Caution was exercised and blood glucose was checked more frequently when transfusing blood products or replacing electrolytes. Serum potassium was measured every six hours and additional potassium supplementation was given during the clamp only if necessary (K+ <3 mmol/L). Other aspects of donor management were carried out according to QT and the treating institution’s standard of care in all participants.

- **Blood samples withdrawal and handling**

Blood samples were procured at three time points in all donors. The time point that the first sample was taken was considered the start of the study in both control and experimental donors and labeled as sample time zero (ST0). The second sample was taken six hours later, and labeled sample time one (ST1). The third blood sample was withdrawn at the time of aortic cross-clamping and organ perfusion and labeled as sample time two (ST2). At each time point, 20 mL of blood was collected in red-top test tubes from the central venous line after flushing and placed on ice (+4 °C) for transfer to the laboratory for processing. All the test tubes were labeled with the donor’s identification number and sample time and date. The bloods were then centrifuged for serum separation.
The serum was aliquoted into 0.5 mL aliquots and stored at -80 °C at the LD MacLean surgical laboratory, Royal Victoria Hospital for the duration of the study.

- Cytokine analysis

Concentrations of human IL-1β, IL-6, IL-8, IL-10, MCP-1, TNF-α and TGF-α (Table 3) were measured by suspension bead array immunoassay with the Luminex 200 X-map instrument (Luminex Corp, Austin, TX, USA). Analysis of the cytokines was carried out using a Milliplex human cytokine kit following manufacturer’s specifications (HCYTOMAG-60k, Millipore Corp, Bilerica, MA, USA). All samples were measured in duplicates and the kit had a sensitivity of 0.4 pg/mL.

Serial dilutions were made of a reconstituted human cytokine standard to produce a standard curve from 3.2 to 10,000 pg/ml. The standards were mixed 1:1 with 25µl of serum matrix and added to the microtiter plate. Then, serum samples were mixed 1:1 with 25µl of assay buffer and transferred to the appropriate wells of the plate. After sonication, 25µl of diluted antibody coated beads were added to all standard and sample wells. The plate was sealed and agitated on a plate shaker (Barnstead Int, Dubuque, IO, USA) for 16-24 hours at +4 °C. Fluid was aspirated and then the plate was washed two times with 200µl of wash buffer.

Following the wash, 25µl of detection antibody was added to all wells. The plate was again sealed and agitated at room temperature for one hour. Finally, 25µl of Streptavidin-Phycoerythrin was added and then incubated for an additional 30 minutes with agitation. The fluid was then removed and the plate was washed two more times with wash buffer.
The beads were re-suspended in sheath fluid and agitated for five minutes. The cytokines were analyzed on the Luminex instrument using MasterPlex CT 1.2 software (MiraiBio Inc, Alameda, CA, USA). Mean fluorescence intensity was obtained from a minimum of 50 beads per sample. Concentrations were calculated from the standard curve generated by the MasterPlex QT 4.0 analysis software (MiraiBio Inc, Alameda, CA, USA).

**Data collection and statistics**

The study coordinator and Dr. Aljiffry collected and stored all data prospectively. Data collection and accuracy were double checked by an independent clinical research assistant. All demographics and relevant clinical variables including the cause of death, blood group type (A, B, AB, O), hemodynamic parameters (mean blood pressure and central venous pressure), and number/type of organs retrieved and transplanted were collected. In addition, all glucose levels were recorded, insulin doses given in units, dextrose infusions given in grams, steroid use and the use of any inotropic support. Categorical (e.g. gender) and continuous variables (e.g. age) were expressed as summary statistics (n, percentage, median, range, mean, standard deviation) by groups and for all subjects. All comparisons between groups were carried out using a two-sided test at an alpha level of 10% unless otherwise specified. Fisher’s exact test (for categorical variables) and Wilcoxon Rank Sum test (for continuous non-normally distributed variables) was performed to assess differences between the groups.

The change of cytokines concentration (Table 3) with respect to time was compared in both groups. This change is defined as: $\Delta = \text{Difference in level (change)}$ for
cytokine X = (level of X at ST1 (or ST2) – level of X at ST0). Wilcoxon Rank-Sign and Wilcoxon Rank-Sum tests were conducted to test whether there is a significant difference in these outcomes (∆ change) within each group and between the groups respectively. As an exploratory analysis, we conducted Analysis of Covariance (ANCOVA) to estimate the effect of treatment on outcomes at ST1 (cytokine levels at ST1) controlling for the effect of baseline cytokine values (cytokine levels at ST0) and steroid use. We also conducted ANCOVA to estimate the effect of treatment on outcomes at ST2 (cytokine levels at ST2) controlling for the effect of baseline cytokine values (cytokine levels at ST0), steroid use, and time interval from ST0 to ST2. A p-value less than or equal to 0.1 was considered as a significant difference and evidence against the null hypothesis (no difference between the groups). All analyses were done using SAS version 9.2 statistics software.

All study records will be kept confidential in a password secured computer that belongs to Dr. P. Metrakos in the surgical research laboratory for 25 years and then will be discarded. The information will only be available to the study investigators and to assigned research personnel.
Results

Donor demographics

Fifteen of the 20 donors assessed for enrollment were assigned to the protocol including six donors in the insulin therapy group (experimental group) and nine donors in the control group (Figure 2). We excluded one donor in the experimental group because of a protocol violation where the donor had to be taken to the operating room due to hemodynamic instability after 2.5 hours of HNC, which is less than the minimum accepted time by the protocol. Four families declined participation.

There were no significant differences in the baseline donor characteristics between the two groups such as gender, age and body mass index (BMI) (Table 4, 5). The most common blood groups among all donors were blood group A and O constituting 14 out of the 15 donors included in the study. The cause of death was divided into CVA (intracranial bleeding) and others (trauma or anoxia). CVA was the most common cause of brain death affecting nine donors (60%), four (66.7%) in the experimental group and five (55.6%) in the control group. Steroid use was similar (p=1) in both groups; two out of six donors (33%) in the experimental group and four out of nine in the controls (44%).

The hemodynamic parameters that were measured include, mean arterial blood pressure (MAP) and central venous pressure (CVP), both before enrollment in the study and after six hours of therapy. The CVP did not differ significantly between the two groups before and after treatment. Similarly, the MAP was not different before the treatment between
the groups. Although, the MAP was lower (p=0.05) in the experimental group (median 72 mmHg) after treatment compared to the control group (median 81 mmHg), but it was still within the normal range (65 – 110 mmHg). The inotropic support was equal in all donors, in which vasopressin was the only vasopressor (inotrope) used during the study period with a dose of 1- 2.4 U/hour. The number of organs retrieved from donors ranged from two to six and those transplanted ranged from zero to six. The organ productivity rate from the donors (defined as organs retrieved/organs transplanted) was slightly higher in the experimental group (median 83%) as compared to the control group (median 75%) but not statistically significant (p=0.75).

**Feasibility**

Performing the HNC in the critical care unit went relatively smoothly. Nursing teams attended relevant educational sessions prior to the study and an on-call research MD remained available for questions and support. Having donors in the intensive care units created a comfort zone while applying the HNC for all involved staff due to the monitoring capability and the one-to-one nursing care. No complains regarding applying the HNC were reported for ICU nursing staff and MDs. QT coordinators were cooperative with the patient recruitment phase, and the only concern raised was the difficulty in obtaining two consent forms from the donor’s family. The ICU MDs were enthusiastic about the project and were very content and satisfied with the glucose control that was achieved.
Insulin and glucose balance

Although donors in both groups started with similar glucose levels, experimental donors had a statistically significant lower blood glucose levels while receiving insulin therapy (p<0.001) (Table 5). The experimental group reached targeted blood glucose levels (normoglycemia) (median 4.8 mmol/L) more often compared to the control group (median 9.0 mmol/L). There were no periods of severe hypoglycemia (glucose level \( \leq 2.5 \) mmol/L) in any donor during the study.

The insulin and dextrose doses were the same in both groups before enrollment into the study. As expected the experimental group received significantly more insulin (median 223U) and dextrose (median 191 g) following treatment compared to the control donors (Table 5).

Inflammatory cytokines

- Sample times

The levels of several inflammatory cytokines (Table 3) were analyzed at three time points from all donors (ST0, ST1 and ST2) (Table 5). a) ST0; baseline levels at the beginning of the study from all donors. b) ST1; levels at about six hours following therapy in the experimental donors and following enrollment in the control donors. This time interval was equal in both groups; median six hours and 20 min in the experimental group and median six hours in the controls. c) ST2; levels at the end of the study when donors went
for the organ retrieval procedure. This time interval was variable between donors; median 14 hours and 30 min in the experimental group and median 11 hours and 20 min in the controls. However, the difference was not statistically significant (p=0.72). In addition, at the ST2 interval, two samples (one donor from each group) were not available for cytokine analysis, due to blood clotting and inability to perform serum separation. Hence, the comparisons between cytokine levels at this time point (ST2) were performed for 13 donors only (five experimental and eight controls).

**Comparisons within each group**

1. **Experimental group**

   The levels of the pro-inflammatory mediators such as IL-6, IL-8, TNF-α, TGF-α and MCP-1 were lower after six hours of treatment (ST1) compared to their baseline values (Table 6), (Figure 3-8). This decrease was statistically significant for IL-6 (p=0.03), MCP-1 (p=0.03) and TGF-α (p=0.09). The same trend was seen at the second time interval (ST2), where there was also a statistically significant decrease for IL-6 (p=0.06), MCP-1 (p=0.06) and TGF-α (p=0.06) (Table 7), (Figure 9-14). IL-1β levels were undetectable in the majority of donors at all time points. We observed an opposite trend in circulating levels of the anti-inflammatory factor IL-10 in both post intervention time points (ST1 and ST2).
2. Control group

The levels of the pro-inflammatory markers did not demonstrate a consistent change after six hours (ST1) compared to their baseline values. Some cytokines had higher levels and others showed a minor decrease, however, no change was significant (Table 6), (Figure 3-8). The same trend was seen at the second time interval (ST2) (Table 7), (Figure 9-14). IL-1β levels were similarly undetectable in the majority of donors at all time points. The levels of IL-10 (anti-inflammatory cytokine) did not change at the ST1 time interval. However, they were surprisingly higher at the ST2 time point compared to their baseline value and this was statistically significant (p=0.054).

- Comparisons between the groups

Comparing the difference (Δ) or change in the levels of the inflammatory cytokines post intervention (time points ST1 and ST2) with respect to their levels at baseline between the two groups showed the following:

1. At the first time point (ST1)

The difference (Δ) of the levels of pro-inflammatory cytokines showed a greater decrease in the experimental group compared to the controls (Table 8). However, this difference was statistically significant for MCP-1 only (p=0.03). The Δ in IL-10 levels showed a statistically significant higher level of the anti-inflammatory mediator following therapy
in the experimental group (median 43.7 pg/mL) compared to the control group (median 3.9 pg/mL) (p=0.08).

2. **At the second time point (ST2)**

The difference (Δ) of the levels of IL-6, IL-8, and MCP-1 had a similar trend as what was observed at the ST1 time point (Table 9). There was a greater decrease in the levels of these pro-inflammatory cytokines in the experimental group at this time point compared to the controls, although the differences did not reach statistical significance (p=0.11, p=0.11 and p=0.31 respectively). The difference (Δ) of TNF-α and TGF-α levels was comparable in both groups at this time point (p=0.77 and p=0.46 respectively). For IL-10 the difference (Δ) showed an increase in the level of this marker in both groups with no statistical significance (p=0.31).

- **Exploratory analysis**

The effect of treatment (HNC) on cytokine levels at ST1 and ST2 in the whole cohort was tested using ANCOVA after controlling for baseline (ST0) cytokine values, steroid use and time duration from ST0 to ST2 (Table 10). The treatment had an overall anti-inflammatory effect on donors at both post intervention time points (ST1 and ST2). This was expressed by a lower level of most pro-inflammatory cytokines with treatment compared to controls, however, without a statistical significance. After controlling for the
insulin therapy effect, steroid use demonstrated similar anti-inflammatory properties on donors, again without statistical significance.
Discussion

Difficulties in performing interventional studies on organ donors

Improving the quality of transplanted organs requires that new and novel therapies to be assessed on brain dead donors in clinical studies. However, performing research on brain dead donors or patients with severe brain injuries is filled with many obstacles. The key problems related to this difficulty include the inability to gain consent for research from an incapacitated patient, whether the therapy could have a possible risk that could harm the future organs, whether the potential recipient should be consented to the study, and the uncontrolled time factor when applying therapeutic modalities to donors.

Since critically ill patients are unable to give their own consent, proxy consent is often used. This implies having to approach the donor’s representatives for the consent, which inherently has its own problems. A survey found that individuals were more likely to donate their own organs than to consent to the donation of a relative’s organs. Moreover, the accuracy of the proxy in relaying the patient’s wishes may not always be accurate.

In the province of Quebec there are approximately 80-100 brain dead donors annually and about 20% of those are managed in the MUHC. A total of 15 donors were enrolled in this study, this reflects the difficulty in performing experimental research on donors. We
had four families that declined participation, and many others were not assessed for enrolment because of the inability to locate their families for consenting (Figure 2). Among families who refused participation, the main reason was the uncertainty if their relative’s wish was to participate in research. A solution to this is implementing the importance of donor research in the public awareness programs that focus on raising organ donation.

Another major issue is the time factor when applying therapeutic modalities to donors, which is related to the inflexibility in delaying the organ retrieval procedure. This raises the possibility of starting therapy in patients with severe brain injury before the declaration of brain death but on the other hand, it conflicts with protecting patients with critical illness \(^{131}\). Another problem that is related to conducting research on brain dead donors is whether the therapy could have a possible risk that could harm a future organ. This would raise the question, whether the potential recipient should be consented to the study. In this project recipients were not involved in the consenting process as per the MUHC and QT REB approvals. This was because insulin is not a new experimental drug and although it was used as a novel anti-inflammatory modality in brain dead human donors, insulin is an integral part in the management of glucose balance in donors \(^{22}\). In addition, insulin usage to control glucose balance has been proven to be safe and beneficial in critically ill patients and those with severe brain injury \(^{30,97}\). Furthermore, with the standard flushing process we trust that organs will not contain high doses of insulin that would affect the recipient after re-implantation, this phenomena was looked for especially in liver transplant recipients done at the MUHC. Thus, we believe that our
therapy had virtually no risk of harming the donor or the future organs since the process was monitored carefully and used with concomitant glucose infusion via the HNC technique.

**Donor characteristics**

A total of 15 donors were recruited, six experimental and nine controls. The donor population did not differ in terms of gender, age and body mass index (Table 4, 5). CVA was the most common cause of brain death in the whole cohort (60%), which is similar to recent trends where it has become a more common cause of brain death.\(^3\) The number of organs retrieved from donors ranged from two to six and those transplanted ranged from zero to six. Most of the organs that were discarded after procurement were due to possible organ dysfunction as assessed by biopsy, other physiologic variables, and surgical assessment of the organs.

**Variability of inflammatory status in donors**

The mechanism of the inflammatory response following brain death in humans is a complex process that is related to multiple factors (as discussed in the introduction). We found in this study that the serum levels of several inflammatory cytokines were variable from donor to donor in both groups at baseline (Table 11). For instance the median IL-6 level at baseline in the whole cohort was 193.4 pg/mL with a large range of (17.49 – 13252). This is contrary to what has been observed in brain death animal models, in
which the baseline markers were comparable. However, it should be noted that in animal models the exact time of insult (brain death) is known whereas in human donors the baseline sample can be many days after the donor have been ill. We believe that humans react to stress uniquely and certain characteristics could influence the magnitude of inflammation and related outcomes. Supporting this hypothesis is the description of expanded criteria donors and donor risk index for kidney and liver transplantation, respectively. It implies that there are certain donor characteristics that could influence transplant outcomes. In part this could be related to the age of the donor, cause of brain death, duration of illness prior to the declaration of brain death, management variation between institutions or simply due to the fact that every human being responds to stress differently.

**High dose insulin therapy**

In this study, high dose insulin therapy was delivered with the hyperinsulinemic normoglycemic clamp (HNC) technique. In this technique, patients received a fixed calculated intravenous insulin infusion based on body weight and intravenous dextrose was used concomitantly to maintain a normal glucose balance (Table 2). A constant insulin infusion of 0.3U/kg/hour (5 mU/kg/min) was delivered to the experimental donors. This dose was chosen because it has been shown to attenuate the inflammatory response in patients undergoing coronary artery bypass graft surgery. Dextrose 20% was used to maintain normoglycemia, which enabled the delivery of high glucose dose without the need to use large volume fluid replacements. This helped to
avoid hemodilution together with the inflammatory disturbances associated with large
volume crystalloid use $^{133-135}$.

The minimum time to observe an effect of high dose insulin therapy was set as six hours
in this study. No previous studies in human brain dead donors were available to
determine the optimal duration of therapy, but if HNC was to be useful in the donor
setting more than 6 hours it would not be feasible in most circumstances. However, in
the pig model of brain death, anti-inflammatory effects were demonstrated after six hours
of insulin therapy $^{103}$. Thus, we felt that six hours was the minimum time that would
demonstrate an effect related to insulin therapy.

The high dose insulin treatment was applied on MUHC brain dead organ donors while
donors form other institutions were treated as controls using the sliding scale.
Unfortunately, a proper randomization process was not possible. Due to the expanded
need for extra manpower and time to run this protocol at other hospitals within Quebec,
we only obtained approval to apply the HNC on brain dead donors within the MUHC,
and so performing the experiment in other centers was not possible. Furthermore, in order
to facilitate donor recruitment in a timely fashion, this approach of participant assignment
was used rather than randomizing MUHC donors only. Despite these constraints, it is
important to note that donor transfer to a certain hospital is a random process depending
on multiple logistic factors and it is not related to the health or condition of donors. In
addition, all hospitals that accept brain dead donors are similarly equipped with abilities
to manage these donors and perform the organ retrieval procedure. Therefore, we believe that this minimized the potential bias in the way treatment was assigned.

**Effects of insulin therapy**

The mechanism behind insulin’s beneficial effect in critical illness is debatable and it remains to be clarified if the effect is due to insulin per se, the avoidance of hyperglycemia or the combination of the two \(^{136}\). Insulin has proved to have anti-inflammatory properties. In addition, it has been shown to induce vasodilatation which could improve blood flow to organs. Furthermore, controlling hyperglycemia could be the reason behind the anti-inflammatory effect of insulin. We believe that insulin has an inert anti-inflammatory effect and controlling glucose levels adds an agonistic effect, as hyperglycemia is associated with exaggerated inflammatory response.

The following is a description of all the effects of high dose insulin therapy that were achieved:

- **Anti-inflammatory effect:**

  Severe inflammation marked by the elevated levels of pro-inflammatory cytokines such as IL-6 has been associated with poor prognosis in a variety of critical illnesses \(^{137, 138}\). In a study by Pathan et al, IL-6 was found to be a major mediator of myocardial depression in patients with sepsis \(^{139}\). Similarly, multiple studies strongly suggest that the “cytokine storm” seen following brain death negatively affects the function of the transplanted...
organs. For instance, significant activation of IL-6 and other inflammatory mediators in donor hearts has been associated with early cardiac allograft dysfunction after cardiac transplantation in the absence of cellular rejection \(^6\). Furthermore, high IL-6 levels in donors are associated with lower six months hospital free recipient survival \(^4\). This inflammatory response is thought to be caused by multiple mechanisms leading to poor allograft function and recipient outcome \(^7,19\).

While many have looked at the anti-inflammatory effect of insulin both in clinical studies and in experimental animal models, no study has examined this effect on human organ donors and on the inflammatory response after brain death. In a study on high dose insulin therapy and liver resection, the HNC technique was found to reduce postoperative liver dysfunction and complications. This was achieved through alteration of cytokine expression pattern (TNF-\(\alpha\), IL-8, MCP-1, IL-6, IL-10, and C-reactive protein) as well as the suppression of apoptosis \(^106\). The cardiac model of high dose insulin therapy has been explored widely by Albacker and colleges \(^100,108\). In their study in 2007, they found that high dose insulin therapy promoted early metabolic recovery of the heart via early extraction of lactate and higher oxygen extraction immediately postoperatively. The high dose insulin group also had a lower troponin I level four hours postoperatively, with greater improvement in cardiac indices. These changes led to better myocardial protection and functional recovery. In a later study by the same group, they found that high dose insulin therapy was able to reduce the early postoperative surge in inflammatory response after cardiopulmonary bypass by decreasing levels of IL6, IL8, and TNF-\(\alpha\).
This study was designed to assess the influence of HNC on the blood cytokine concentrations of human brain dead organ donors. Our main finding was that intervention with high dose insulin suppressed the inflammatory response in brain dead organ donors, which was evident when comparing the changes (Δ) in cytokine levels within each group (Figure 3-8). A significant decrease in the levels of several pro-inflammatory cytokines (particularly IL-6, MCP-1 and TGF-α) in the experimental group was achieved (Table 6, 7). This change was not observed in the control group (Table 6, 7). Levels of the anti-inflammatory mediator IL-10 also showed an increasing trend after six hours of therapy in the experimental group. Furthermore, comparing the changes (Δ) in the levels of the cytokines between the two groups showed a much greater decrease in the insulin therapy group (Table 8, 9). However, this was statistically significant only for the pro-inflammatory mediator MCP-1 after six hours of therapy. This could be explained by the small sample size in our study.

A prospective study examined the relationship between donor’s IL-6 level and outcomes in recipients. The study showed that a high IL-6 level (median >193 pg/mL) was associated with lower recipient six month hospital free survival. In our study the median IL-6 in the experimental group at baseline was 247.2 pg/mL (175 - 13252) and after six hours of insulin therapy the median IL-6 was 196.7 pg/mL (99 - 8604). Furthermore, the median IL-6 in the experimental group at the end of the study (time of organ retrieval) was 123.2 pg/mL (51.45 - 8604) (Table 11). Thus, high dose insulin therapy using the HNC was able to drop the IL-6 levels in these donors below the 193 pg/mL level that is associated with worse outcomes in recipients. On the other hand, the IL-6 levels in
control donors were below 193 pg/mL at all time points; at baseline 152.4 pg/mL (17.5 - 683.3), at six hours 115.1 pg/mL (34.83 - 1066), and at the end 169.2 pg/mL (42.4 - 3838). However, this shows a trend of increasing levels in control donors (Figure 9-14). Although statistically not significant, we could not pinpoint why the experimental donors had higher IL-6 levels at baseline compared to control donors, but we believe this is related to the variability in the inflammatory response between donors. Hence, we chose to compare the changes (Δ) in cytokine levels among donors rather than comparing certain values of these levels at different time points.

This pilot project clearly demonstrates the anti-inflammatory properties of insulin therapy in brain dead donors. The median percentage of change of IL-6 levels (relative to baseline) in the control group after six hours was approximately -13% and was about -32% in the experimental group. The standard deviation of the pooled percentage of changes was 35%. Using these parameters, we can calculate the sample size needed to perform a definite randomized controlled trial. The sample size calculation will be as follows:
Two-sided significance level (1-alpha): 95
Power (1-beta, % chance of detecting): 80
Ratio of sample size, control / experimental: 1
Difference in percentage of change: -19%
Standard deviation of percentage of change: 35%
Sample size – control group: 54
Sample size – experimental group: 54
Total sample size: 108

Note: We estimated the above parameters from the pilot data set. Specifically, the median percentage of change of IL-6 levels from baseline (ST0) to 6 hours (ST1) in the control group was estimated to be -0.13, and the median percentage of change of IL-6 levels from (ST0) to 6 hours (ST1) in the experimental group was estimated to be -0.32. The interquartile range (IQR) of the pooled (i.e., from both groups) percentages of change was 0.468, from which we estimated the pooled standard deviation as 0.35 (=0.468/1.349). Using these parameters we are able to estimate the sample size needed to reach a power of 80% of detecting the difference in percentage of 0.19 (0.32-0.13).

In summary, we have demonstrated that high dose insulin therapy can decrease the concentrations of inflammatory cytokines in brain dead donors. To our knowledge this is the first time that insulin was tested as anti-inflammatory therapy in human brain dead organ donors. This finding is consistent with studies on insulin in other clinical settings such as cardiac surgery and liver resection as well as in brain dead animal model 100, 103, 106, 114.
**Maintenance of glucose homeostasis**

Hyperglycaemia is a marker for higher morbidity and mortality in patients with critical illness and insulin is usually required to maintain adequate glucose control.  

Insulin supplementation in standard ICU protocols is often delivered with a sliding scale, in which a specific dose of insulin is administered in response to a certain glucose level (Table 1). Achieving a tight glucose control using insulin sliding scale has raised concerns regarding the risk of severe hypoglycemia with significant increase in complications and mortality as well as the difficulty in achieving normoglycemia in critically ill patients. We showed that, insulin therapy with the HNC technique counteracted the systemic hyperglycemia in brain dead donors by sustaining a normal serum glucose homeostasis (median 4.8 mmol/L) in experimental donors (Table 5). We did not encounter a period of severe hypoglycemia in any of the donors. Our findings support the hypothesis that intensive insulin treatment was able to maintain normoglycemia without causing hypoglycemia. This is explained by having the HNC in which dextrose is given along with the insulin infusion which would prevent hypoglycemia.

**Other effects**

Mean arterial blood pressure (MAP) is defined as Cardiac Output x Systemic Vascular Resistance (SVR) + Central Venous Pressure (CVP). It is recommended to keep the range between 65 and 110 mmHg to ensure adequate organ perfusion. We found that the
MAP was within normal range in both groups before enrollment in the study with no statistical difference (p=0.52). However, the MAP was lower (p=0.05) after treatment, despite being within the targeted range, in the experimental group (median 72 mmHg) and with a more narrow range (66–89 mmHg) compared to controls at 81 mmHg (71–129 mmHg), indicating a better control. Since the blood pressure management in all the donors was controlled (or driven) by volume resuscitation and the use of inotropic agents; we cannot attribute this finding as an effect of insulin therapy alone. However, it suggests that insulin might have contributed to this by lowering the SVR, due its vasodilatory effects that has been proven in multiple studies. Other hemodynamic parameters measured including CVP before and after treatment and inotropic support were similar in both groups.

Potential benefit of using steroids in donors

A one-way analysis of covariance (ANCOVA) was conducted to estimate the effect of treatment on outcomes at ST1 and ST2 (cytokine levels at ST1 and ST2) in all donors, controlling for the effect of baseline cytokine values (cytokine levels at ST0) and steroid use (Table 10). The high dose insulin therapy had an overall anti-inflammatory effect on donors at both post intervention time points (ST1 and ST2). This was expressed by a lower level of most pro-inflammatory cytokines with treatment compared to no treatment. For instance, the IL-6 levels in donors who received the HNC were lower by 431.9 pg/mL after 6 hours of therapy compared to those who did not. Similarly, steroid use has been proven to have anti-inflammatory effects in brain dead donors in many studies.
Interestingly, after controlling for the insulin therapy effect, steroid use demonstrated similar anti-inflammatory properties on donors, in which donors who received steroids had lower IL-6 levels by 712.29 pg/mL compared to those who did not. However, both of these anti-inflammatory effects did not reach statistical significance, which is explained by the small sample size in this study. We believe that high dose insulin therapy combined with steroid administration will have an agonistic effect on suppressing the inflammatory response in brain dead donors. Further studies are needed to prove this theory and to determine if this immunomodulating effect will benefit organ function and ultimately future recipients.

**Analysis of inflammatory cytokines**

Luminex 200 X-map instrument was used to measure the concentrations of several inflammatory cytokines in this study (Table 3). The Luminex System is a flexible analyzer based on the principles of flow cytometry. It enables the simultaneous measurement of up to 100 analytes in a single microplate well, using very small sample volumes. The system delivers fast and cost-effective bioassay results and it has been used in a variety of studies\(^\text{143-145}\). This technology offers the benefits of the ELISA (enzyme-linked immunosorbent assays), but also enables the added value of higher throughput, increased flexibility, reduced sample volume, and low cost with the same workflow as ELISA. Moreover, the performance parameters of the Luminex 200 X-map assay has been validated by correlation with the "gold standard" ELISA\(^\text{146}\).
Limitations of the study

There are several limitations to our study:

First, this was a pilot study which was limited by a small sample size. However, because it is the first of its kind in humans, feasibility needed to be determined in a small number of patients.

Second, residual confounding could not be excluded despite detailed evaluation of donor characteristics. Residual confounding may occur because we were not able make more detailed measures of other aspects of donor management in both groups, such duration of illness and hospitalization before brain death and the variability of ICU departments and their staff.

Third, we have studied the effect of insulin on cytokines in donors only at one standard time point (ST1; six hours after treatment or enrollment). From a logistic point it was difficult to standardize the second time point (ST2; organ retrieval in the operating room), because this time was dependant on operating room and staff availability as well as the donor’s hemodynamic stability and recipient’s preparation. Although the median of ST2 was not statistically different between the two groups, this time was extremely variable between the donors in each group with a large range (Table 5). In addition, the cytokine analysis at the second time point was done on 13 patients (out of 15) with two patients missing (one from each group) due to blood sample clotting and inability to perform serum separation. However, even with these points taken into consideration a similar trend of change in cytokine was observed in both post intervention time points (ST1 and
ST2). It would have also been interesting to measure cytokine changes over a period of time because different cytokine levels have been hypothesized to peak at different times after brain death. In a future study it would be worthwhile to collect samples at multiple time points in order to study the kinetics of cytokine changes and understand the interplay of these cytokines in the inflammatory response especially following insulin treatment. Having multiple fixed time points following treatment will allow us to use area under the curve analysis which will capture both the dimensions of magnitude and time. Doing area under the curve analysis in the present study was not possible due to the fact that we have only two time points following intervention and the second time point (ST2) was not constant.

Fourth, as the decision to procure and transplant organs was primarily based on local QT criteria and the judgment of transplant physicians, our results of organ productivity rate may be difficult to generalize to other populations. Nonetheless, clinical teams were not involved in the HNC management, and therefore these data could not have been influenced by clinical management decisions.

Fifth, we did not analyze the association of this cytokine profile changes in donors with organs and recipient survival, as it was not the focus of this pilot study. Therefore, future studies are needed to examine whether this anti-inflammatory effect would in fact lead to improved function of organs transplanted from brain dead donors.
Conclusions

In conclusion, our study demonstrated that high dose insulin therapy in the form of HNC to be safe and feasible in human organ donors. In addition, insulin therapy was able to achieve a normal glucose balance. We also proved the concept that high dose insulin therapy has anti-inflammatory effects on brain dead organ donors. This was demonstrated by the decrease in inflammatory cytokine concentrations in the serum of these donors. Insulin is commonly used as a measure to control glucose levels in brain injured patients. Therefore a more intensified insulin therapy after the event of brain death is possible to achieve anti-inflammatory effects. Further studies are needed to elucidate whether this immune modulating effect will lead to improved function of transplanted organs from brain dead donors. That will eventually translate into improved graft and recipient survival. Using the data generated from this pilot project a sample size calculation will be possible to perform larger randomized controlled studies.
Table 1: Routine insulin sliding scale orders

<table>
<thead>
<tr>
<th>Blood glucose level</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤4.0 mmol/L</td>
<td>Stop insulin infusion and give 25 mL of D50%</td>
</tr>
<tr>
<td>≤6.0 mmol/L</td>
<td>Stop insulin infusion</td>
</tr>
<tr>
<td>&gt;6.0 and &lt;8.0 mmol/L</td>
<td>Maintain same rate</td>
</tr>
<tr>
<td>&gt;6.0 and &lt;10.0 mmol/L</td>
<td>↑ infusion by 1 U/h</td>
</tr>
<tr>
<td>&gt;10.0 mmol/L</td>
<td>↑ infusion by 2 U/h</td>
</tr>
</tbody>
</table>
### Table 2: Hyperinsulinemic Normoglycaemic Clamp (HNC) protocol

<table>
<thead>
<tr>
<th>Blood glucose level</th>
<th>Action</th>
<th>Additional instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 3.4 mmol/L</td>
<td>↑ D20W by 30 mL/h, and give 20 mL of D20W bolus</td>
<td>If persist call research MD</td>
</tr>
<tr>
<td>3.9 – 3.5 mmol/L</td>
<td>↑ D20W by 20 mL/h</td>
<td></td>
</tr>
<tr>
<td>4.0 – 6.0 mmol/L</td>
<td>Maintain same rate</td>
<td></td>
</tr>
<tr>
<td>6.1 – 7.5 mmol/L</td>
<td>↓ D20W by 10 mL/h</td>
<td></td>
</tr>
<tr>
<td>&gt; 7.5 mmol/L</td>
<td>↓ D20W by 20 mL/h</td>
<td>If persist call research MD</td>
</tr>
</tbody>
</table>
Table 3: Analyzed inflammatory cytokines

<table>
<thead>
<tr>
<th></th>
<th>Interleukins (IL)</th>
<th>Tumour Necrosis Factor (TNF)</th>
<th>Tumour Growth Factor (TGF)</th>
<th>Monocyte Chemotactic Protein (MCP)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IL-6, IL-10, IL-1β, IL-8</td>
<td>TNF-α</td>
<td>TGF-α</td>
<td>MCP-1</td>
</tr>
</tbody>
</table>
Table 4: General characteristics of donors

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control group (n = 9)</th>
<th>Experimental group (n = 6)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>60 (44–77)</td>
<td>58.5 (43–78)</td>
<td>0.77</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td>0.62</td>
</tr>
<tr>
<td>Males, (n %)</td>
<td>3 (33.3)</td>
<td>3 (50)</td>
<td></td>
</tr>
<tr>
<td>Females, (n %)</td>
<td>6 (66.7)</td>
<td>3 (50)</td>
<td></td>
</tr>
<tr>
<td>Blood group, n (%)</td>
<td></td>
<td></td>
<td>0.75</td>
</tr>
<tr>
<td>A</td>
<td>5 (55.6)</td>
<td>2 (33.3)</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>0 (0)</td>
<td>1 (16.7)</td>
<td></td>
</tr>
<tr>
<td>O</td>
<td>4 (44.4)</td>
<td>3 (50)</td>
<td></td>
</tr>
<tr>
<td>Cause of death (CVA, n %)</td>
<td>5 (55.6)</td>
<td>4 (66.7)</td>
<td>1.00</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24.1 (20.2–30.8)</td>
<td>24.4 (18.5–25.6)</td>
<td>0.95</td>
</tr>
<tr>
<td>Steroid use n (%)</td>
<td>4 (44.4)</td>
<td>2 (33.3)</td>
<td>1.00</td>
</tr>
<tr>
<td>MAP at baseline (mmHg)</td>
<td>80 (70–105)</td>
<td>86.5 (72–114)</td>
<td>0.52</td>
</tr>
<tr>
<td>MAP after treatment (mmHg)</td>
<td>81 (71–129)</td>
<td>72 (66–89)</td>
<td><strong>0.05</strong></td>
</tr>
<tr>
<td>CVP at baseline (mmHg)</td>
<td>9 (6–12)</td>
<td>8 (5–10)</td>
<td>0.47</td>
</tr>
<tr>
<td>CVP after treatment (mmHg)</td>
<td>8 (5–13)</td>
<td>8.5 (6–13)</td>
<td>0.84</td>
</tr>
<tr>
<td>Organs retrieved</td>
<td>4 (2–6)</td>
<td>3 (2–6)</td>
<td>0.32</td>
</tr>
<tr>
<td>Organs transplanted</td>
<td>3 (0–6)</td>
<td>2 (0–6)</td>
<td>0.33</td>
</tr>
<tr>
<td>Donor organ productivity (%)</td>
<td>75 (0–100)</td>
<td>83.3 (0–100)</td>
<td>0.75</td>
</tr>
</tbody>
</table>

Data are expressed as n (%) or median (range)
Table 5: Glucose levels, Insulin and Dextrose doses and sample times

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control group (n = 9)</th>
<th>Experimental group (n = 6)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood glucose at baseline (mmol/L)</td>
<td>8.6 (6.3–13.7)</td>
<td>9.8 (4.4–11.3)</td>
<td>0.32</td>
</tr>
<tr>
<td>Blood glucose after treatment (mmol/L)</td>
<td>9 (5.6–11.7)</td>
<td>4.8 (4–6.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total Dextrose given (g)</td>
<td>52.5 (0–103)</td>
<td>191.5 (12–360)</td>
<td>0.02</td>
</tr>
<tr>
<td>Total Insulin given (U)</td>
<td>1 (0–52)</td>
<td>223 (126–540.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sample time 1 (ST1) hours from baseline</td>
<td>6 (5.83–7)</td>
<td>6.3 (6–6.75)</td>
<td>0.22</td>
</tr>
<tr>
<td>Sample time 2 (ST2) hours from baseline*</td>
<td>11.3 (8–25.8)</td>
<td>14.5 (6.05–25.4)</td>
<td>0.72</td>
</tr>
</tbody>
</table>

Data are expressed as median (range)

* The number of donors was 5 experimental and 8 controls
Table 6: Sign-Rank test for change (Δ) of cytokine levels at ST1 from baseline (ST0) value within each group

<table>
<thead>
<tr>
<th>Inflammatory Cytokine (Time Points)</th>
<th>Control group (n = 9)</th>
<th>Experimental group (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 (ST1 vs. ST0)</td>
<td>Signed Rank = -0.5</td>
<td>Signed Rank = -10.5</td>
</tr>
<tr>
<td></td>
<td>P = 1.0000</td>
<td>P = 0.0313</td>
</tr>
<tr>
<td>IL-8 (ST1 vs. ST0)</td>
<td>Signed Rank = 4.5</td>
<td>Signed Rank = -1.5</td>
</tr>
<tr>
<td></td>
<td>P = 0.6523</td>
<td>P = 0.8438</td>
</tr>
<tr>
<td>MCP-1 (ST1 vs. ST0)</td>
<td>Signed Rank = 5.5</td>
<td>Signed Rank = -10.5</td>
</tr>
<tr>
<td></td>
<td>P = 0.5703</td>
<td>P = 0.0313</td>
</tr>
<tr>
<td>TNF-α (ST1 vs. ST0)</td>
<td>Signed Rank = 1.5</td>
<td>Signed Rank = -5.5</td>
</tr>
<tr>
<td></td>
<td>P = 0.9102</td>
<td>P = 0.3125</td>
</tr>
<tr>
<td>TGF-α (ST1 vs. ST0)</td>
<td>Signed Rank = 4.5</td>
<td>Signed Rank = -8.5</td>
</tr>
<tr>
<td></td>
<td>P = 0.6523</td>
<td>P = 0.0938</td>
</tr>
<tr>
<td>IL-10 (ST1 vs. ST0)</td>
<td>Signed Rank = 6.5</td>
<td>Signed Rank = 7.5</td>
</tr>
<tr>
<td></td>
<td>P = 0.4961</td>
<td>P = 0.1563</td>
</tr>
</tbody>
</table>
Table 7: Sign-Rank test for change ($\Delta$) of cytokine levels at ST2 from baseline (ST0) value within each group

<table>
<thead>
<tr>
<th>Inflammatory Cytokine (Time Points)</th>
<th>Control group (n = 8)</th>
<th>Experimental group (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 (ST2 vs. ST0)</td>
<td>Signed Rank = 0</td>
<td>Signed Rank = -7.5</td>
</tr>
<tr>
<td></td>
<td>$P = 1.0000$</td>
<td>$P = 0.0625$</td>
</tr>
<tr>
<td>IL-8 (ST2 vs. ST0)</td>
<td>Signed Rank = 2</td>
<td>Signed Rank = -3.5</td>
</tr>
<tr>
<td></td>
<td>$P = 0.8438$</td>
<td>$P = 0.4375$</td>
</tr>
<tr>
<td>MCP-1 (ST2 vs. ST0)</td>
<td>Signed Rank = -4</td>
<td>Signed Rank = -7.5</td>
</tr>
<tr>
<td></td>
<td>$P = 0.6406$</td>
<td>$P = 0.0625$</td>
</tr>
<tr>
<td>TNF-$\alpha$ (ST2 vs. ST0)</td>
<td>Signed Rank = -10</td>
<td>Signed Rank = -4.5</td>
</tr>
<tr>
<td></td>
<td>$P = 0.1953$</td>
<td>$P = 0.3125$</td>
</tr>
<tr>
<td>TGF-$\alpha$ (ST2 vs. ST0)</td>
<td>Signed Rank = -10</td>
<td>Signed Rank = -7.5</td>
</tr>
<tr>
<td></td>
<td>$P = 0.1953$</td>
<td>$P = 0.0625$</td>
</tr>
<tr>
<td>IL-10 (ST2 vs. ST0)</td>
<td>Signed Rank = 14</td>
<td>Signed Rank = 2.5</td>
</tr>
<tr>
<td></td>
<td>$P = 0.0547$</td>
<td>$P = 0.6250$</td>
</tr>
</tbody>
</table>
Table 8: Comparison of changes (Δ) in cytokine levels at ST1 from baseline (ST0) value between groups

<table>
<thead>
<tr>
<th>Inflammatory Cytokine (Time Points)</th>
<th>Control group (n = 9)</th>
<th>Experimental group (n = 6)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 (pg/mL) (ST1 vs. ST0)</td>
<td>Median Δ -5.3 (-568 to 712.6)</td>
<td>Median Δ -82.3 (-6934 to -12.4)</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>Mean Δ 47.3 (±397)</td>
<td>Mean Δ -1964 (±3051)</td>
<td></td>
</tr>
<tr>
<td>IL-8 (pg/mL) (ST1 vs. ST0)</td>
<td>Median Δ 2.26 (-22.8 to 27.9)</td>
<td>Median Δ -14.9 (-97.8 to 174.9)</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>Mean Δ 1.97 (±15.2)</td>
<td>Mean Δ 7.6 (±91.2)</td>
<td></td>
</tr>
<tr>
<td>MCP-1 (pg/mL) (ST1 vs. ST0)</td>
<td>Median Δ 27.6 (-1929 to 4311)</td>
<td>Median Δ -618 (-1845 to -200)</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>Mean Δ 504.4 (±1757)</td>
<td>Mean Δ -748 (±587.4)</td>
<td></td>
</tr>
<tr>
<td>TNF-α (pg/mL) (ST1 vs. ST0)</td>
<td>Median Δ -0.24 (-5.4 to 9.2)</td>
<td>Median Δ -2.1 (-9.4 to 7.99)</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>Mean Δ 0.35 (±4.4)</td>
<td>Mean Δ -1.8 (±5.8)</td>
<td></td>
</tr>
<tr>
<td>TGF-α (pg/mL) (ST1 vs. ST0)</td>
<td>Median Δ 0.54 (-11 to 20.6)</td>
<td>Median Δ -4.71 (-8.3 to 2.09)</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>Mean Δ 1.74 (±9.3)</td>
<td>Mean Δ -4.04 (±3.97)</td>
<td></td>
</tr>
<tr>
<td>IL-10 (pg/mL) (ST1 vs. ST0)</td>
<td>Median Δ 3.9 (-89.8 to 46.37)</td>
<td>Median Δ 43.8 (-54.7 to 194.5)</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>Mean Δ 0.68 (±38.6)</td>
<td>Mean Δ 64.6 (±91.2)</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as median (range) and mean (± SD)
Table 9: Comparison of changes (Δ) in cytokine levels at ST2 from baseline (ST0) value between groups

<table>
<thead>
<tr>
<th>Inflammatory Cytokine (Time Points)</th>
<th>Control group (n = 8)</th>
<th>Experimental group (n = 5)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 (pg/mL) (ST2 vs. ST0)</td>
<td>Median Δ -1.5 (-629 to 3485)</td>
<td>Median Δ -128 (-8763 to -70.3)</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>Mean Δ 381.9 (±1303)</td>
<td>Mean Δ -2746 (±3896)</td>
<td></td>
</tr>
<tr>
<td>IL-8 (pg/mL) (ST2 vs. ST0)</td>
<td>Median Δ -2.7 (-38.6 to 91)</td>
<td>Median Δ -45.7 (-203 to 174.9)</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>Mean Δ 14 (±47.1)</td>
<td>Mean Δ -29.3 (±135)</td>
<td></td>
</tr>
<tr>
<td>MCP-1 (pg/mL) (ST2 vs. ST0)</td>
<td>Median Δ -240 (-3858 to 3799)</td>
<td>Median Δ -781 (-7835 to -450)</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>Mean Δ -470 (±2373)</td>
<td>Mean Δ -2182 (±3169)</td>
<td></td>
</tr>
<tr>
<td>TNF-α (pg/mL) (ST2 vs. ST0)</td>
<td>Median Δ -3.8 (-8.46 to 2.74)</td>
<td>Median Δ -1.54 (-18.4 to 8)</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>Mean Δ -2.6 (±4.4)</td>
<td>Mean Δ -4.3 (±9.8)</td>
<td></td>
</tr>
<tr>
<td>TGF-α (pg/mL) (ST2 vs. ST0)</td>
<td>Median Δ -8.3 (-24.3 to 14.6)</td>
<td>Median Δ -4.42 (-13 to -0.9)</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>Mean Δ -7.2 (±13.3)</td>
<td>Mean Δ -5.7 (±4.6)</td>
<td></td>
</tr>
<tr>
<td>IL-10 (pg/mL) (ST2 vs. ST0)</td>
<td>Median Δ 86.9 (-84.8 to 1165)</td>
<td>Median Δ 26.5 (-92.4 to 74.2)</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>Mean Δ 269.8 (±443.5)</td>
<td>Mean Δ 10.8 (±62)</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as median (range) and mean (±SD)
Table 10: The effects of insulin therapy and steroid on cytokine levels in donors
(using ANCOVA)

<table>
<thead>
<tr>
<th>Cytokine (pg/mL)</th>
<th>Effect of Insulin Therapy on Level at ST1</th>
<th>Effect of Steroid on Level at ST1</th>
<th>Effect of Insulin Therapy on Level at ST2</th>
<th>Effect of Steroid on Level at ST2</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>-431.9 P = 0.44</td>
<td>-712.2 P = 0.16</td>
<td>-710 P = 0.58</td>
<td>-1424 P = 0.18</td>
</tr>
<tr>
<td>IL-8</td>
<td>0.35 P = 0.99</td>
<td>-29.4 P = 0.38</td>
<td>-16.7 P = 0.78</td>
<td>-38 P = 0.50</td>
</tr>
<tr>
<td>MCP-1 (pg/mL)</td>
<td>-1260.7 P = 0.15</td>
<td>-325.9 P = 0.69</td>
<td>-1112.9 P = 0.46</td>
<td>-981 P = 0.51</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>-1.13 P = 0.70</td>
<td>-0.03 P = 0.99</td>
<td>0.88 P = 0.81</td>
<td>-5.8 P = 0.12</td>
</tr>
<tr>
<td>TGF-α (pg/mL)</td>
<td>-5.6 P = 0.17</td>
<td>4.2 P = 0.31</td>
<td>3.4 P = 0.57</td>
<td>4.2 P = 0.47</td>
</tr>
<tr>
<td>IL-10 (pg/mL)</td>
<td>70 P = 0.08</td>
<td>5.4 P = 0.88</td>
<td>-158.9 P = 0.47</td>
<td>282 P = 0.20</td>
</tr>
</tbody>
</table>

Data are expressed as cytokine concentration (pg/mL) relative to no treatment.
Table 11: Levels of inflammatory cytokines in both groups at different time points

<table>
<thead>
<tr>
<th>Inflammatory Cytokine</th>
<th>ST0 time point</th>
<th>ST1 time point</th>
<th>ST2 time point</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control:</td>
<td>Control:</td>
<td>Control:</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>152.4 (17.49 – 683.3)</td>
<td>115.1 (34.83 – 1066)</td>
<td>169.2 (42.4 – 3838)</td>
</tr>
<tr>
<td>Experimental:</td>
<td>247.2 (175 – 13252)</td>
<td>196.7 (99 – 8604)</td>
<td>123.2 (51.45 – 8604)</td>
</tr>
<tr>
<td>(p=0.19)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-8 (pg/mL)</td>
<td>Control:</td>
<td>Control:</td>
<td>Control:</td>
</tr>
<tr>
<td></td>
<td>45.22 (5.48 – 229.3)</td>
<td>50.58 (7.74 – 239.2)</td>
<td>49.83 (9.36 – 190.7)</td>
</tr>
<tr>
<td>Experimental:</td>
<td>119.4 (40.11 – 304.4)</td>
<td>99.2 (24.08 – 432.4)</td>
<td>77.74 (21.16 – 432.4)</td>
</tr>
<tr>
<td>(p=0.08)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCP-1 (pg/mL)</td>
<td>Control:</td>
<td>Control:</td>
<td>Control:</td>
</tr>
<tr>
<td></td>
<td>1612 (211.9 – 6617)</td>
<td>1188 (184.4 – 8475)</td>
<td>1451 (251.9 – 7678)</td>
</tr>
<tr>
<td>Experimental:</td>
<td>1393 (894.1 – 9017)</td>
<td>762.3 (694.2 – 7172)</td>
<td>496 (443.7 – 6458)</td>
</tr>
<tr>
<td>(p=0.35)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>Control:</td>
<td>Control:</td>
<td>Control:</td>
</tr>
<tr>
<td>(p=0.29)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGF-α (pg/mL)</td>
<td>Control:</td>
<td>Control:</td>
<td>Control:</td>
</tr>
<tr>
<td></td>
<td>16.58 (5.54 – 24.27)</td>
<td>14.7 (6.79 – 34.31)</td>
<td>6.09 (0 – 28.34)</td>
</tr>
<tr>
<td>Experimental:</td>
<td>15.65 (4.42 – 21.97)</td>
<td>8.69 (6.51 – 18.55)</td>
<td>7.53 (0 – 18.55)</td>
</tr>
<tr>
<td>(p=0.81)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-10 (pg/mL)</td>
<td>Control:</td>
<td>Control:</td>
<td>Control:</td>
</tr>
<tr>
<td></td>
<td>21.13 (3.51 – 167.1)</td>
<td>26.59 (2.26 – 108.3)</td>
<td>123 (7.81 – 1187)</td>
</tr>
<tr>
<td>Experimental:</td>
<td>30.01 (16.57 – 125.4)</td>
<td>70.77 (36.5 – 319.9)</td>
<td>68.78 (22.75 – 151.9)</td>
</tr>
<tr>
<td>(p=0.24)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as median (range) cytokine level (pg/mL)
Figure 1: Organ donor types

- Organ Donor
  - Deceased Donor
    - Heart beating (Brain dead)
  - Living Donor
    - Non Heart beating (DCD)
    - Living Related
    - Living Unrelated
Figure 2: Donors distribution

Patients assessed for enrollment (n=20)

Patients excluded (n=1)
  Refused participation (n=4)

Patients enrolled and assigned (n=15)

Control group (n=9)
Experimental group (n=6)
Figure 3: Box-Plot presentation of the Δ of IL-6 at ST1 in both groups

Box-Plot for Delta IL-6 (ST1 vs. ST0)
Figure 4: Box-Plot presentation of the $\Delta$ of IL-8 at ST1 in both groups
Figure 5: Box-Plot presentation of the $\Delta$ of MCP-1 at ST1 in both groups
Figure 6: Box-Plot presentation of the $\Delta$ of TNF-$\alpha$ at ST1 in both groups
Figure 7: Box-Plot presentation of the Δ of TGF-α at ST1 in both groups
Figure 8: Box-Plot presentation of the Δ of IL-10 at ST1 in both groups
Figure 9: Median concentrations of IL-6 in both groups

(ST0= baseline, ST1= 6h post intervention, ST2= end of study)
Figure 10: Median concentrations of IL-8 in both groups

(ST0= baseline, ST1= 6h post intervention, ST2= end of study)
Figure 11: Median concentrations of MCP-1 in both groups

(ST0 = baseline, ST1 = 6h post intervention, ST2 = end of study)
Figure 12: Median concentrations of TNF-α in both groups

(ST0= baseline, ST1= 6h post intervention, ST2= end of study)
Figure 13: Median concentrations of TGF-α in both groups

(ST0= baseline, ST1= 6h post intervention, ST2= end of study)
Figure 14: Median concentrations of IL-10 in both groups

(ST0= baseline, ST1= 6h post intervention, ST2= end of study)
References


130. Mossialos E, Costa-Font J, Rudisill C. Does organ donation legislation affect individuals' willingness to donate their own or their relative's organs? Evidence from European Union survey data. BMC Health Serv Res 2008;8:48.


Appendices

Appendix 1: Staff preparation

The study investigators conducted multiple teaching sessions and rounds for involved staff prior to recruitment. The goal of these teaching sessions was to give a brief background about the study, explain the benefits and potential risks of the proposed treatment, and describe the research protocol in detail. The targeted audiences were QT coordinators and physicians, and the MUHC intensive care units nursing staff as well as physicians (namely from Royal Victoria Hospital, Montreal General Hospital and Montreal Neurological Institute). When an eligible donor was identified, a study investigator was assigned as the research MD on a rotational basis. The QT coordinator discussed the inclusion and exclusion criteria with the research MD and determined whether the donor was a control or an experimental patient depending on the final site for the organ procurement procedure. Dr. Aljiffry had to be available to start and manage the HNC in the experimental donors. The study clinical coordinator supplied the test tubes, picked up and labelled the samples when they were ready.
Appendix 2: Team management

Team Management for SDR 09-054 (Anti-inflammatory Therapy with High Dose Insulin in Brain Dead Organ Donors)

- The Quebec Transplant coordinator will notify Dr. Aljiffry at (514) 625-9452 about the presence of an organ donor right after the diagnosis of brain death.

- Dr. Aljiffry will discuss inclusion and exclusion criteria with the transplant coordinator and will identify whether the donor is a control or a study depending on the final site for the organ procurement procedure.

If the donor is eligible for this study as a CONTROL:

- Dr. Aljiffry will page Ms. Ayat Salman at 514-406-0859 (Research Coordinator).

- Ms. Salman will provide the transplant coordinator with 6 red top vacutainers (2 for each blood sampling time points) and ice container (the blood samples will be done with coordination with the nurse taking care of the patient):
  - S0: At time of consenting for research (as soon as possible)
  - S1: 6 hours after the last blood sample
  - S2: right before cross-clamping in the operating room

- These blood samples will be kept on ice until Ms. Salman picks them up for further processing and no further manipulation will be done by Quebec Transplant’s Clinical Coordinator or nurses.

If the donor is eligible for this study as a STUDY:

- Dr. Aljiffry will page Ms. Ayat Salman at 514-406-0859 (Research Coordinator).

- Dr. Aljiffry will be starting the clamping protocol and providing the nurse taking care of the donor with the maintenance orders. Dr. Aljiffry will be available for any questions related to this protocol while the donor is receiving the therapy. Clamping will start right after S0 blood sample is taken.
• Ms. Salman will provide the transplant coordinator with 6 red top vacutainers (2 for each blood sampling time points) and one ice container (the blood samples will be done in coordination with the nurse taking care of the patient):
  
  o S0: At time of consenting for research (before the clamping protocol)
  o S1: 6 hours after the last blood sample
  o S2: Right before cross-clamping

• These blood samples will be kept on ice until Ms. Salman picks them up for further processing and no further manipulation will be done by Quebec Transplant’s Clinical Coordinator or nurses.
Appendix 3: Insulin orders (HNC) protocol

GLUCOSE/INSULIN CLAMP (HNC)
ORDER SHEET

- Start insulin infusion at 0.3U/kg/hour (mix 250 units of Humulin R in 250cc of normal saline)
- Blood glucose is checked after 5-10 min (Accu-Chek)
  - If blood glucose is low (< 4 mmol/l):
    - Give (20% Dextrose) 20cc bolus and continue with (Dextrose 20%) infusion at 40 cc/hr
    - Check blood glucose after 10 min and adjust according to the provided protocol
    - Check blood glucose every 60 min until OR
  - If blood glucose is within normal limits (4-6 mmol/l):
    - Start dextrose infusion (20% Dextrose) at 30 cc/hr
    - Check blood glucose after 10 min and adjust according to the provided protocol
    - Check blood glucose every 60 min until OR
  - If blood glucose is between 6-10 mmol/l:
    - Start Dextrose infusion (20% Dextrose) at 20 cc/hr
    - Check blood glucose after 10 min and adjust according to the provided protocol
    - Check blood glucose every 60 min until OR
  - If blood glucose is above 10 mmol/l:
    - Start insulin infusion only
    - Check blood glucose after 10 min and adjust according to the provided protocol
    - Check blood glucose every 60 min until OR

<table>
<thead>
<tr>
<th>Blood glucose level</th>
<th>Dextrose infusion</th>
<th>Additional instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 3.4 mmol/l</td>
<td>↑ D20W by 30 cc/h</td>
<td>Call research MD on-call</td>
</tr>
<tr>
<td>3.9 – 3.5 mmol/l</td>
<td>↑ D20W by 20 cc/h</td>
<td></td>
</tr>
<tr>
<td>4.0 – 6.0 mmol/l</td>
<td>Maintain same rate</td>
<td></td>
</tr>
<tr>
<td>6.1 – 7.5 mmol/l</td>
<td>↑ D20W by 10 cc/h</td>
<td></td>
</tr>
<tr>
<td>≥ 7.5 mmol/l</td>
<td>↑ D20W by 20 cc/h</td>
<td></td>
</tr>
</tbody>
</table>

MD Name and Signature __________________________

TIME POINTS:
Blood samples will be withdrawn from both groups (control and study) for study-related analysis:
- The first blood sample will be taken after consent: Sample time 0 (ST0)
- Second will be taken 6 hours post enrollment to the study: Sample time 1 (ST1)

Blood samples:
- 20cc required at each time point, need 2 red capped tubes
- Please contact the clinical coordinator once the blood sample is taken.

CONTACT: Clinical Coordinator, Ayat Salman, Pager: 514-406-0859 / Dr. Aljiffry at (514) 625-9452
Appendix 4: McGill donor management protocol

Donor Management Protocol
McGill University Health Centre

Initial Assessment

I. Laboratory Investigations

CBC
SMA-7 (Na, K, Cl, HCO3, BUN, Creatinine, Glucose)
Ca, Mg, PO4
Albumin, total protein
Liver Profile: AST, ALT, GGT, LDH, ALP (Alkaline Phosphatase), Bilirubin total,
Bilirubin direct
Coagulation profile: INR, PTT, PT
Amylase
CK, CKMB, troponin
Serology: Hepatitis B, Hepatitis C, CMV, HIV, HTLV1, HTLV2, F24, anti HBC,
Syphilis
PSA
ABG PRN
Blood Cultures X 2
Urine Gram Stain, stat
Sputum gram stain, stat
Urine analysis, urine sediment
Type and screen
Crossmatch X 4 units
EKG

II. Radiology

Chest X-Ray

III. Consults

Neurology: To confirm 2nd brain death assessment
Respiratory tech.: apnea test
Blood Pressure

1. Keep BP systolic > 100
   a. Levophed as required
   b. If Levophed requirement is greater than 15 ug/min then use
   c. Vasopressin 0.05-0.1 U/min. Can be used to lower Levophed
      requirements or as first line drug
   d. If Vasopressin not working, keep as an infusion and add or increase
      Levophed as required
   e. If levophed and vasopressin not working, use dopamine
   f. If refractory-consider assessing Cardiac Index

2. Hypertension
   a. A transient phenomenon: an indication of coning
   b. Manage with Labetolol boluses or nitroprusside drip as required

Temperature: Heat or Cool as required

Urine Output: Keep at 100 cc/hr

1. Low urine output:
   a. 50-99 cc/hr: correct CVP, correct BP
   b. <50 cc/hr: 40 mg IV Lasix Bolus, correct CVP and BP (in
      REVERSE ORDER)
   c. Start Lasix drip: 5mg – 10mg/ hour if required

2. High urine output: >300 cc/hr:
   a. ddAVP: 2 ug IV x 1
   b. if no response within 30 minutes (>200 cc in 30 mins) than 4
      ug ddAVP IV x 1
   c. Repeat q 2 hours as required

Electrolytes

1. Use D5W IV or per NG as required for Hypernatremia
2. Hyponatremia is a sign of over-hydration, ensure adequate urine output
   and monitor
3. Glucose: if >10, start insulin drip as required (target <10)
4. Correct Mg, Ca as required
Appendix 5: Consent form

DEPARTMENT OF SURGERY

Anti-Inflammatory Therapy with High Dose Insulin in Brain Dead Organ Donors

Protocol No. SDR-09-054

Principal Investigator: Dr. Peter Metrakos
    MUHC HPB and Transplant Surgery

Co-Investigators: Dr. M. Aljiffry, Dr. M. Hassanain, Dr. P. Chaudhury, Dr. T. Nouh, Dr. S. Paraskevas, Dr. R. Lattermann, Dr. T. Schricker, Ayat Salman

“Consent for this research study may be given by the following persons, as stipulated by the Civil Code of Quebec in articles 14 and 15, in order of priority, by the mandatory, tutor, curator, spouse (married, de facto, civil union), a close relative or a person who shows a special interest in the deceased.”

We would like to invite you to take part in a research study. Before deciding to participate you should understand its requirements, risks and benefits. This document provides information about the study, and it may contain words you do not fully understand. Please read it carefully, discuss it with friends and relatives if you wish and ask the study staff any questions you may have. They will discuss the study with you in detail. If you agree to take part in this study, you will be asked to sign this form as stipulated by the Civil Code of Quebec in articles 14 and 15 and a copy will be given to you to keep for your records.

The purpose of this study is to determine if the use of insulin (medication that reduces sugar levels in the blood) on organ donors, before removing the organs, can drop
the inflammation intensity in the body. You have the right to know about the procedures that will be performed during the study. This document will inform you of potential benefits and risks so you can decide with confidence whether or not you will take part in this study.

INTRODUCTION

You are being asked to agree to this study because have consented for organ donation and medical research purposes that are related to organ donation.

Brain death is defined as an irreversible form of unconsciousness characterized by a complete loss of brain function while the heart continues to beat. This event leads to multiple metabolic reactions that result in accumulation of harmful metabolites and intense inflammation. Eventually, this leads to progressive loss of function in all organs.

Pancreas production of insulin also drops after brain death causing a decrease in glucose entry into the cells, which will eventually lead to energy shortage and systemic hyperglycemia (high sugar content in the blood). The body’s immune system, also known as the body’s defense mechanism, will respond to this disruption by causing more inflammation. This heightened inflammatory response seen in the organ donors is negatively reflected on the transplanted organs and their future function after transplantation.

Insulin regulates blood sugar levels and suppresses inflammation when given at a higher does. Therefore, a high dose of insulin infusion is required to prevent the development of severe hyperglycaemia (high blood sugar levels) and the inflammation that follows.

High-dose insulin therapy makes it possible to deliver a constant level of insulin into the blood stream. The treatment involves giving a calculated dose of sugar in the form of dextrose to prevent low blood glucose level. This therapy is called dextrose/insulin clamp. It has been shown to be safe and successful in maintaining normal glucose levels.

These treatment options have no direct benefit to the organ donor. Data from scientific experiments indicate that insulin and/or glucose could have a local effect on those organs by protecting them against the stress of the surgery and preserving their function. Using this method, we are trying to provide the transplanted organs with what we think is a better treatment option during and after transplantation.

PURPOSE OF THE STUDY

As explained above, both brain death event and high blood sugar tends to induce a massive inflammation in patients diagnosed with brain death. Meanwhile, this inflammation greatly affects the quality and survival of the transplanted organs. The purpose of this study is to test if the use of a dextrose/insulin clamp on deceased donors will prevent high sugar in the blood and drop the inflammation related to that process.
This study will also look at the effect of this therapy on organ survival and function once transplanted.

**STUDY PROCEDURES**

All deceased donors are managed in the ICU before they go to the operating room. In the current standard of care, the donors are often given an intermittent average insulin dose depending on their blood sugar level, in what’s medically known as the insulin sliding scale.

In this study the donor will be given continuous infusion of high dose insulin in the form of a glucose/insulin clamp. The insulin infusion is combined by dextrose (sugar) infusion at rate required to maintain a normal blood glucose level.

Theoretically, this clamp has the advantage of achieving a steady blood glucose level rather than the fluctuation seen with the standard insulin sliding scale. Furthermore, the high insulin dose given will drop the inflammation seen in deceased subjects.

To see the effect of our treatment protocol (dextrose/insulin clamp), blood samples will be withdrawn from the donors to test for markers of inflammation and will be compared to blood tests from donors treated by the standard protocol (sliding scale). These blood samples are withdrawn before the start of the dextrose/insulin clamp, after 6 hours, and just before entering the operating room. The amount of extra blood taken for analysis is minimal and it will not be greater than 20 teaspoons.

**ANESTHETIC AND SURGICAL CARE**

Anesthetic and surgical treatment will be performed following the standards established by the Ministere de la Santé et des Services Sociaux du Québec. Your consent to participate in this study will not affect any surgical procedure or anesthetic care provided.

**RISKS AND DISCOMFORTS**

There are no expected risks and virtually no discomfort to the organ donor. Both insulin and dextrose are given to organ donors under close observation in the ICU. Similar therapy has been tested on living subjects with no reported harm or discomfort.
POTENTIAL BENEFITS

You should not expect any direct benefit from participating in this study. It’s hoped that the information collected from this study will benefit others in the field of organ transplantation in the future.

VOLUNTARY PARTICIPATION AND/OR WITHDRAWAL

Participation in this study is entirely voluntary. You are free to refuse your relative to participate or to withdraw his/her consent to participate in this study at any time without giving any reasons. Should you decide not to participate, there will be no consequences.

WHO HAS REVIEWED THIS STUDY

The Royal Victoria Hospital Ethics Review Board and Quebec Transplant Ethics Review Board have given their approval to this study. This study will be carried out according to Good Clinical Practice Guidelines as well as all local law(s) and regulation(s).

COMPENSATION

Every effort to prevent injury or pain that could result from this study will be taken by the investigator. In the event of injury suffered by participating in this study, that is highly unlikely, there will be no compensation.

CONFIDENTIALITY

A record of the progress will be kept in a confidential form at McGill University Health Centre as described below. Qualified representatives of the following organizations may inspect the medical/study records and retrieve information from those records for quality assurance and data analysis:

- Hospital Research Ethics Board
- McGill Faculty of Medicine Institutional Review Board
- Quebec Transplant
- Health Canada

The results of this study may be published in medical journals or reported at medical meetings; however, no information regarding your relative will be identified nor released or published. This information may include test results, reports of operation, x-rays or other body scan reports.
The organizations listed above have policies of strict confidentiality and will not release any information concerning your relative except to other investigators involved in this study. They will keep information about your relative confidential, to the extent permitted by applicable laws, in the following manner:

- His/her name will not be used in any reports about the study
- His/her date of birth (month and year) will only be used to confirm that his age meets the eligibility requirements of this study.
- He/she will be identified only by a study number
- Information will be kept behind locked doors and can be accessed only by the organizations listed above for 25 years

QUESTIONS AND CONTACT INFORMATION

For any questions related to the study, you can contact the clinical coordinator of this study, Ms. Ayat Salman, at (514) 934-1934 ext: 31917 or ayat.salman@muhc.mcgill.ca.

If you have questions concerning your rights as a research subject and wish to discuss them with someone not associated with the study, you may contact the RVH ombudsman at (514) 934-1934, local 35655.
Consent Form

Principal Investigator: Dr. Peter Metrakos
In association with Quebec Transplant

I have read and understood the information sheet for the above study. All of my questions have been asked and answered to my satisfaction.

I have been informed that my participation is voluntary and I am free to withdraw, at any time, without giving any reason, without any medical care or legal rights being affected.

I give permission for these organizations, as mentioned above, to have access to the donor’s medical records.

I, ________________________________, being the ________________________________
(Name of person giving consent) (Relationship to the deceased)

of ________________________________ do authorize the study team to include the above mentioned donor in this research study.
(Name of the deceased)

________________________________ __________________________________
- Physician’s Signature - - Date -

________________________________
- Physician Name - PLEASE PRINT -

________________________________
- Signature of person obtaining consent - - Date -

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- Name of person obtaining consent - PLEASE PRINT -