SUPPLEMENTARY INTRODUCTION

The most relevant experiments at the University of Cape Town demonstrated that brain-dead animals were unable to aerobically metabolize radiolabeled metabolites (^{14}C-U-glucose, ^{14}C-U-1-palmitate, and ^{14}C-1-pyruvate) administered intravenously (3, reviewed in [2]). For example, following the injection of ^{14}C-1-palmitate to a live baboon, the exhaled $^{14}CO_2$ peak level occurred at two hours, but, following injection into a brain-dead baboon, a two-hour $^{14}CO_2$ peak was not observed, but was replaced by a low, flat curve. If T_3 was administered to the brain-dead baboon, however, the exhaled $^{14}CO_2$ was similar to that observed in the live animal.

The inability of the brain-dead animal to metabolize cellular fuels aerobically indicates that the mitochondrial aerobic pathways are progressively inhibited and can no longer meet the ATP requirement to support cellular homeostasis. Pyruvate does not enter into mitochondria, but accumulates as lactate (26). As time post-brain death progresses, the inhibition of the Krebs cycle becomes greater, and mitochondrial failure leads to depletion of high energy phosphates, tissue lactic acidosis (27), loss of cellular homeostasis, inability to maintain ion compartmentalization, and deactivation of sodium/potassium cellular pumps. Calcium uptake no longer occurs and calcium is released from the sarcoplasmic reticulum into the cytosol, finally resulting in cell death.

The experimental studies indicated that these metabolic changes were associated with a decline in myocardial function. The administration of T_3/T_4 (+/- other hormones)

reversed the inhibition of the aerobic pathways, and improved the metabolic and hemodynamic status of the brain-dead animal.

Hormonal therapy that included T_3 was first administered to a series of brain-dead potential organ donors at Groote Schuur Hospital in 1984 (4,5). The initial regimen involved the hourly administration of T_3 (2μg), cortisol (100mg), and insulin (20units).

Twenty-six conventionally-treated donors showed a progressive hemodynamic deterioration, requiring significantly increased inotropic support to maintain hemodynamic stability, and significantly increased bicarbonate administration to maintain a normal acid-base balance (5). Of this group, 5 (19%) of the donors were considered unsuitable as cardiac donors due to progressive cardiovascular deterioration or sudden ventricular fibrillation. Hormonal therapy was administered to 21 donors, resulting in a significant improvement in hemodynamic status, reduced inotropic support, and reduced requirement for bicarbonate (because of reduced accumulation of lactate). In these donors, all organs were acceptable for transplantation, which was followed by excellent organ function in the recipients (5).

SUPPLEMENTARY METHODS

Data retrieval

Data were provided from January 1, 2000, to December 31, 2009. All parameters of interest were retrospectively retrieved from the UNOS worksheet “Deceased Donor Registration Worksheet” in which all donor demographics, causes of death, and

procured/transplanted organs are included (28). Other parameters, e.g., inotropic support and hemodynamic stability, were not well-documented, and therefore did not undergo analysis.

In Study 2, two metrics were calculated – (i) the average number of organs procured from a donor, and (ii) the percentage of donors from which a specific individual organ, e.g., heart, was procured. The mean number of organs procured per individual donor given a hormonal treatment modality was calculated as the total number of organs procured divided by the total number of donors in that treatment modality group. The percentage of donors given a hormonal treatment modality from which a specific organ, e.g., heart, was procured was calculated as the number of specific organs procured divided by the number of donors in that modality group. The number of organs obtained in each subgroup was summarized as mean±standard deviation.

Statistical analysis

Data analyses were performed using SAS V9.2.1 (SAS Inc., Cary, NC). For analyzing number of organs procured, Wilcoxon rank sum test (29) was used to compare each pair within each subgroup (i.e., donors with/without T_3/T_4 therapy) (Table 3) and van Elteren (30) test was used to compare the overall average number of organs procured between Group A (with T_3/T_4) and Group B (without T_3/T_4) adjusted for the other hormonal treatments. For analyzing percentage of organs procured, Pearson χ^2 tests were performed to compare each pair within each subgroup, e.g., A1 versus B1 (Table 2A), and the Cochran-Mantel-Haenszel test (31) was used to compare the overall

percentage of donors with a specific organ procured between Groups A and B adjusted for the other hormonal treatments. The significance levels for each pair comparison were adjusted for eight simultaneous multiple comparisons using the Bonferroni-Holm's step-down method (19).

To control possible confounding factors that may have impacted on the relationship between T_3/T_4 therapy and organ procurement/transplantation, multivariate logistic regression analyses were carried out for the number of organs procured and the success rate of organ transplantation. Odds ratios and their 95% confidence intervals were used to estimate the effect of treatment in addition to p values from Wald Chi-square tests.

Post-transplant graft and recipient survival were compared by the Kaplan-Meier method and log-rank test.

SUPPLEMENTARY DISCUSSION

The mechanism of action of the thyroid hormones has been previously discussed (22,23), and will not be considered in detail here. In summary, these hormones appear essential for re-activation of the Krebs cycle within the mitochondria, and this contributes to the hemodynamic stability of the organ donor. Brain-dead organ donors suffer from various degrees of deprivation of high energy phosphates, and, as time passes, there is a progressive accumulation of tissue and plasma lactate, which, despite bicarbonate replacement, leads to progressive acidosis. These donors

progressively become refractory to catecholamines and are eventually lost from the donor pool due to hemodynamic instability. The pathophysiology of brain death and the mechanisms by which hormonal therapy may improve donor organ function have recently been comprehensively reviewed (6).

The cause of the reduction associated with insulin remains uncertain, but may possibly be related to the observation that blood glucose levels and insulin resistance appear to be factors influencing mortality in critically ill patients admitted to intensive care units (32-36). The presence of an inflammatory state, with increased levels of tumor necrosis factor-α, interleukin-6, and C-reactive protein, correlates with a new inflammatory marker called resistin (named for resistance to insulin) which in humans derives from macrophages. Resistin was originally reported as an adipose tissue-specific hormone in critically ill patients (32). The systemic inflammatory response observed in brain-dead potential organ donors leads to a progressive loss of metabolic homeostasis. High requirements for insulin, leading to resistance to insulin, should be considered as a marker of marginal donor quality, from which fewer organs may be expected to be procured. However, there is at present inadequate data to support the conclusion that this point should be definitively considered in the selection of donors/organs for transplantation.

Our data indicate that Organ Procurement Organizations in all UNOS regions are utilizing T3/T4 therapy, though the incidence of use varies between approximately 25%

and 75% of potential donors (Supplementary Table 4). The criteria for use in the individual regions are not known.

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Table S1. Study 2 - Hormonal Treatment Subgroups

Group A (with T_3/T_4)		**Group B (without T_3/T_4)**	
Groups	Treatment	Groups	Treatment
A1	T_3/T_4+C+ADH+I	B1	C+ADH+I
A2	T_3/T_4+C+ADH	B2	C+ADH
A3	T_3/T_4+ADH+I	B3	ADH+I
A4	T_3/T_4+ADH	B4	ADH
A5	T_3/T_4+C+I	B5	C+I
A6	T_3/T_4+C	B6	C
A7	T_3/T_4+I	B7	I
A8	T_3/T_4	B8	None

Abbreviations used in all Tables:

T_3/T_4= triiodothyronine or levothyroxine

C = corticosteroids

ADH = anti-diuretic hormone (DDAVP or arginine vasopressin)

I = insulin

Table S2. Results of Multiple Regression Analysis*

Organ	Odds Ratio (OD)	OD 95% CI	p Value
Heart	1.28	1.21-1.35	<0.0001
Lungs	1.21	1.14-1.29	<0.0001
Kidneys	1.26	1.19-1.33	<0.0001
Liver	0.96	0.91-1.02	0.209
Pancreas	1.31	1.23-1.40	<0.0001
Intestine	1.23	1.18-1.28	0.560
All organs	1.23	1.18-1.28	<0.0001

*The multiple regression analysis adjusted for age, gender, ethnicity, cause of death, body-mass index (BMI), Organ Procurement Organization (OPO) region, and other hormonal therapy (i.e., non T_3/T_4).

Table S3. Mechanisms, Circumstances, and Causes of Death of the 63,593 Brain-Dead Potential Donors in this Study

Causes	With T_3/T_4 n (%)	Without T_3/T_4 n (%)	Total n (%)
Mechanisms of Deaths			
Drowning	231 (49.04)	240 (50.96)	471 (0.74)
Seizure	259 (51.39)	245 (48.61)	504 (0.79)
Drug Intoxication	841 (51.06)	806 (48.94)	1647 (2.59)
Asphyxiation	749 (51.73)	699 (48.27)	1448 (2.28)
Cardiovascular	2377 (45.33)	2867 (54.67)	5244 (8.25)
Electrical	13(50.00)	13 (50.00)	26 (0.04)
Gunshot Wound	3596 (57.25)	2685 (42.75)	6281 (9.88)
Stab	47 (52.81)	42 (47.19)	89 (0.14)
Blunt Injury	8685 (52.49)	7860 (47.51)	16545 (26.02)
SID	26 (31.33)	57 (68.67)	83 (0.13)
Intracranial Hemorrhage/Stroke	12789 (44.59)	15892 (55.41)	28681 (45.10)
Natural Causes	391 (49.43)	400 (50.57)	791 (1.24)
Other	958 (53.73)	825 (46.27)	1783 (2.80)
Total	**30,962 (48.69)**	**32,631 (51.31)**	**63,593(100.00)**
Circumstances of Deaths			
MVA	6686 (52.83)	5970 (47.17)	12656 (19.90)
Suicide	2665 (56.09)	2086 (43.91)	4751 (7.47)
Homicide	2233 (56.86)	1694 (43.14)	3927 (6.18)
Child Abuse	343 (45.07)	418 (54.93)	761 (1.20)
Non-MVA	2773 (51.37)	2625 (48.63)	5398 (8.49)
Natural Causes	8500 (44.25)	10708 (55.75)	19208 (30.20)
Other	7762 (45.95)	9130 (54.05)	16892 (26.56)
Total	**30,962 (48.69)**	**32,631 (51.31)**	**63,593(100.00)**
Causes of Deaths			
Anoxia	4713 (49.41)	4825 (50.59)	9538 (15.00)
Cerebrovascular/Stroke	12229 (44.47)	15269 (55.53)	27498 (43.24)
Head Trauma	13207 (53.58)	11440 (46.42)	24647 (38.76)
CNS Tumor	245 (51.36)	232 (48.64)	477 (0.75)
Other	568 (39.64)	865 (60.36)	1433 (2.25)
Total	**30,962 (48.69)**	**32,631 (51.31)**	**63,593(100.00)**

Table S1. Use of T3/T4 Therapy in Management of Brain-Dead Potential Organ Donors by UNOS Region

UNOS REGION	MANAGEMENT			T3/T4 USE RATE (%)
	Without T3/T4	With T3/T4	Total	
1	1387	458	1845	24.82
2	2398	4857	7255	66.95
3	8083	2807	10890	25.78
4	3587	2602	6189	42.04
5	4699	4597	9296	49.45
6	622	1712	2334	73.35
7	3506	1745	5251	33.23
8	1910	2180	4090	53.30
9	1850	1648	3498	47.11
10	2259	3540	5799	61.05
11	2330	4816	7146	67.39
Total	**32631**	**30962**	**63593**	**48.69**

Figure S1. Flow chart indicating the numbers of donors in which detailed hormonal therapy were available.