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Oxidative stress, caloric intake and outcomes of critically ill patients

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SUMMARY

Background: The aim of this study was to investigate the patterns of oxidative stress in critically ill patients and the association with caloric intake and outcomes.
Methods: In this pre-planned sub-study of the PermiT (Permissive Underfeeding versus Target Enteral Feeding in Adult Critically Ill Patients Trial- ISRCTN68144998), we included patients expected to stay in the ICU for ≥14 days. Serum samples were collected on days 1, 3, 5, 7 and 14 of enrollment. We measured total anti-oxidant capacity (TAC), protein carbonyl concentration (a measure of protein oxidation) and 8-hydroxy-7,8-dihydro-2′-deoxyguanosine (8-OHdG) (a measure of DNA oxidation). We used principal component analysis (PCA) and hierarchical cluster analysis (HCA) to group patients according to oxidative stress.
Results: Principal component analysis identified 2 components that were responsible for 79% of the total variance, and cluster analysis grouped patients in three statistically distinct clusters. Majority of patients 78.6% (44/55) were included in cluster 1 with lowest TAC, protein carbonyl and 8-OHdG levels and cluster 2 which accounted for 16.1% (9/55) of patients had the highest levels of TAC and intermediate levels of protein carbonyl levels. Cluster 3 patients 5.4% (3/56) had the highest protein carbonyl levels. Incident renal replacement therapy was highest in cluster 2 (4/8, 50.0%), compared to cluster 1 (4/42, 9.5%) and cluster 3 (1/3, 33.3%, p 0.01). When adjusted to oxidative stress cluster membership, permissive underfeeding was not associated with 90-day mortality (adjusted odds ratio, aOR 1.37, 95% CI 0.36, 5.25, p 0.64) but was associated significantly with lower incident renal replacement therapy (aOR 0.02, 95% CI < 0.001, 0.57, p 0.02).
Conclusions: There are different distinct patterns of oxidative stress in critically ill patients. Incident renal replacement therapy was different among the three clusters. Our data suggest a protective effect of

Abbreviations: ICU, Intensive care unit; 8-OHdG, 8-hydroxy-7,8-dihydro- 2′-deoxyguanosine; TAC, Total anti-oxidant capacity; PCA, Principal component analysis; HCA, Hierarchical cluster analysis; ROS, Reactive oxygen species; RNS, Reactive nitrogen species; CR, Caloric restriction; LOS, Length of stay.

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permissive underfeeding on incident renal replacement therapy that may differ by clusters of oxidative stress.

1. Introduction

During aerobic metabolism, energy needed for cell biological functions is produced in the mitochondria. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated as by-products [1]. ROS and RNS hemeostasis plays an important role in regulation of cell survival. Moderate levels of ROS/RNS may function as signals to promote cell proliferation and survival, whereas severe increase of ROS/RNS can induce cell death [2]. In addition, ROS exert beneficial effects on immunological function such as destroying invading pathogens, but they can damage lipid, DNA, RNA and proteins. As a protective mechanism, the human body has several endogenous antioxidants, which counteract ROS [3]. The pro-oxidant/antioxidant balance determines the redox environment of the cell and is essential to maintain normal cell function [4–6]. Oxidative stress occurs commonly during critical illness and is caused by a higher production of ROS or a decrease in endogenous protective antioxidative capacity, which may lead to a serious alteration in cell structure and function [1,7,8].

Diet or one or more of its components could modulate the pro-oxidant/antioxidant balance [9]. Observational studies have suggested that negative calorie balance is associated with increasing morbidity and mortality. However, recent randomized controlled trials did not show mortality benefit with attempts to reach full caloric requirements compared to trophic or permissive underfeeding [10]. Studies have shown that caloric restriction prolongs life span in several species [11,12], promotes mammalian cell survival [13] and improves longevity biomarkers in humans [14]. These effects have been attributed to several mechanisms including reduction in oxidative stress [15], reduction in mitochondrial free radical generation [16] and up-regulation of plasma membrane anti-oxidant defense system [17]. The physiologically-stressed critically ill patients are likely to have augmented oxidative stress [18], however, the applicability of these findings to them is unknown. Augmenting antioxidant capacity by exogenous supplementation of antioxidants has been tested in several randomized controlled trials in critically ill patients [19–22], but were generally negative. No recent meta-analysis has demonstrated the advantages of large doses of antioxidants. Data regarding the effect of caloric intake on oxidative stress are scarce. The aim of this study was to investigate the patterns of oxidative stress in critically ill patients and the association with caloric intake and outcomes.

2. Methods

2.1. Study population

This is a pre-planned observational sub-study nested within the PermiT [10] (Permissive Underfeeding versus Target Enteral Feeding in Adult Critically Ill Patients- ISRCTN68144998) trial in which critically ill patients were randomized to permissive underfeeding (40–60% of calculated caloric requirements) or standard feeding (70–100%) for up to 14 days while maintaining similar protein intake in both groups. The trial found no difference in the primary endpoint of 90-day mortality between the permissive and standard feeding groups (relative risk 0.94, 95% confidence interval 0.76, 1.16, $p = 0.58$). In this sub-study which was separately funded by King Abdulaziz City for Science and Technology (KACST), Riyadh, Saudi Arabia (Grant Number - AT 32-25 KACST), we included patients who were enrolled into PermiT trial at King Abdulaziz Medical City, Riyadh, Saudi Arabia between September 2012 and September 2014 and were expected to stay for $\geq$14 days. A separate informed consent was obtained for participation in this sub-study. The study was approved by the Institutional Board Review of the Ministry of the National Guard Health Affairs, Riyadh, Saudi Arabia (Reference #: IRBC/308/14) and all experiments were performed in accordance with International Conference on Harmonization of Good Clinical Practice(ICH-GCP).

2.2. Nutrition

Caloric requirement was calculated using the Penn State equation for mechanically ventilated patients with BMI <30 kg/m^2 and the Ireton–Jones equation for mechanically ventilated patients with BMI $\geq$30 kg/m^2 and for spontaneously breathing patients [23]. Protein target was calculated at 1.2–1.5 g g/kg according to the clinical practice guidelines [24]. To achieve similar protein delivery to both feeding groups, additional protein (Beneprotein, Nestle Healthcare) was provided if needed.

2.3. Blood sampling and laboratory methods

Blood samples were collected on days 1, 3, 5, 7 and 14 of enrollment. Plasma was prepared from the blood samples by centrifugation at 4 °C at 1600 g for 20 min, then was stored at −80 °C prior to assay. We measured total antioxidant capacity (TAC, OxiSelectTMTAC Assay, Cell Biolabs Inc., San Diego, CA) for a global view of the antioxidant system. TAC was expressed as mM uric acid equivalents (UAE) in the plasma. This was derived from uric acid concentration used as standard in the kit. We also measured protein carbonyl concentration (OxiSelectTM Protein Carbonyl ELISA Kit, Cell Biolabs Inc., San Diego, CA), which is the most common product of protein oxidation in biological samples and was expressed as protein carbonyl concentration in nmol/mg protein. We also measured 8-hydroxy-7,8-dihydro-2′-deoxyguanosine(8-OHdG) by ELISA (OxiSelect™ Oxidative RNA Damage ELISA Kit (8-OHG Quantitation), San Diego, CA) as an indicator of oxidatively-damaged DNA with the concentration expressed as 8-OHdG ng/ml plasma. All tests were done in duplicates and the average result was reported.

2.4. Clinical data

We documented demographics and nutritional intervention data including caloric and protein intake over the 14-day intervention period. The following clinical outcomes were documented: mortality at ICU and hospital discharge, 90 days, 28 days, 180 days, mechanical ventilation duration, ICU and hospital lengths of stay (LOS), ICU-free days and incident renal replacement therapy during the ICU stay.

2.5. Statistical analysis

Statistical analysis was carried out using the Statistical Analysis Software (SAS, release 9.1, SAS Institute, Cary, NC). To identify distinct patterns of oxidative stress, we used principal component analysis (PCA), followed by hierarchical clustering analysis. PCA is an unsupervised data reduction technique that analyzes major sources of variation in multi-dimensional data without any prior assumptions, thus avoids introducing inherent bias. Because PCA is sensitive to the relative scaling of the original variables, we standardized biomarker data first by rescaling the three biomarkers to have a mean of zero and a standard deviation of one. We used principal component analysis to convert the possibly correlated biomarker variables into a set of values called principal components that are linearly uncorrelated and are responsible for most of data variance. Using the resulting principal components, we used hierarchical clustering analysis to group patients according to their similarities. This analysis was based on Ward's method and Euclidean distances, using the standardized matrix and a cluster tree was generated (Supplemental Fig. 1).

We compared baseline characteristics, interventions and outcomes among patients in the resulting clusters. Categorical data were presented as frequency with percent and were compared using the Chi-square test; continuous variables were presented as median with the first and third quartiles and compared using Mann–Whitney U test. We compared the biomarkers among the three clusters over time by repeated measures mixed linear models. We examined the association of randomization to permissive underfeeding or standard feeding on 90-day mortality and incident renal replacement therapy in each cluster. We further used logistic regression analyses to examine the association of permissive underfeeding compared to standard feeding on 90-day mortality and incident renal replacement therapy adjusting for membership into the three clusters of oxidative stress. We also examined the association of caloric intake (per 100 Kcal increase) and protein intake (per 10 g increase) on 90-day mortality adjusting for membership into the three clusters of oxidative stress.

3. Results

3.1. Principal component analysis and cluster analysis

Principal component analysis identified 2 components that were responsible for 79% of the total variance (Supplemental Table 1). Supplemental Table 2 shows the correlation between the principal components and the individual standardized biomarkers levels. Principal component 1 correlated with TAC and 8-OHdG levels while principal component 2 correlated with protein carbonyl levels. Cluster analysis using these principal components grouped patients in three statistically distinct clusters (Supplemental Fig. 2). Majority of patients 78.6% (44/55) were included in cluster 1 which had the lowest TAC, protein carbonyl and 8-OHdG levels among the 3 clusters. Cluster 2 which accounted for 16.1% (9/55) of patients had the highest levels of TAC and intermediate levels of protein carbonyl levels. Cluster 3 which accounted for only 5.4% (3/56) of patients had the highest protein carbonyl levels (Supplemental Fig. 3).

3.2. Baseline characteristics of patients in the three clusters

Patients in the three clusters were similar in age, gender, admission category, APACHE II scores and mechanical ventilation (Table 1). Patients in cluster 1 had the lowest weight [cluster 1: 70.0 kg (58.5, 81.0), cluster 2: 87 (70, 114) and cluster 3: 80 (80, 104), p value = 0.04] and the lowest BMI [cluster 1: 26.0 (21.9, 30.6), cluster 2: 29.7 (26.4, 39.8) and cluster 3: 31.3 (27.7, 34.4), p value = 0.05], the lowest platelets count [cluster 1: 164.0 10^9/L (133.0, 242.5), cluster 2: 241 (184, 286) and cluster 3: 296 (270, 425), p value = 0.02] and the lowest transferrin levels [cluster 1: 1.3 g/L (0.9, 1.5), cluster 2: 1.5 (1.2, 1.8) and cluster 3: 2.1 (1.4, 2.2), p value = 0.03]. Cluster 2 had the highest serum creatinine [cluster 1: 81.5 μmol/L (63.5, 107.0), cluster 2: 124 (105, 154) and cluster 3: 80 (72, 118), p value = 0.03]. All patients in cluster 2 [9/9 (100%)] and cluster 3 [3/3 (100%)] were on vasopressors compared to 26/44 (59.1%) in cluster 1 (p value = 0.03).

3.3. Interventions during the study period for patients in the three clusters

Patients in cluster were equally assigned to permissive underfeeding and standard feeding whereas cluster 2 had more patients in the standard feeding and all 3 patients in cluster 3 were in the permissive underfeeding, although the differences did not reach statistical significance. Patients in the three clusters did not differ in the amount of caloric or protein intake, insulin therapy, type of formula or in the use of selected medications (Table 2).

3.4. Outcomes of patients in the three clusters

There were no significant differences in mortality among the three groups. Incident renal replacement therapy was highest in cluster 2 (4/8, 50.0%, compare to cluster 1 (4/42, 9.5%) and cluster 3 (1/3(33.3%), p = 0.01 (Table 3). ICU length of stay was shortest in cluster 1 [14 days (Q1, Q3: 8.5, 17.5)] compared to cluster 2 [28 (Q1, Q3: 19, 37)] and cluster 3 [36 (Q1, Q3: 5, 40), p value = 0.02], although the ICU-free days were not different among the clusters (Table 3).

When adjusted to oxidative stress cluster membership, permissive underfeeding was not associated with 90-day mortality (aOR 1.37, 95% CI 0.36, 5.25, p value = 0.64) but was associated significantly with lower incident renal replacement therapy (aOR 0.02, 95% CI < 0.001, 0.57, p value 0.02). Neither the caloric intake per 100 kcal was associated with 90-day mortality after adjusting for the cluster (aOR 0.90, 95% CI 0.74, 1.10), p value 0.28) nor was the protein intake per 10 g associated with 90-day mortality (aOR 0.90, 95% CI 0.65, 1.24, p value 0.53].

3.5. Changes in oxidative stress over time and with permissive and standard feeding

Over the intervention period, the levels of different oxidative stress biomarkers remained significantly different over time among the 3 clusters (Fig. 1).

Oxidative stress biomarkers were not different between permissive underfeeding and standard feeding over time and between the permissive underfeeding and standard feeding groups in cluster 1 and cluster 2 (Fig. 2). No comparisons were made for cluster 3 because of sample size.

4. Discussion

Using principal component analysis and cluster analysis, we identified different distinct clusters of oxidative stress in critically ill patients. These clusters differed in several baseline characteristics and outcomes. Incident renal replacement therapy was different among the three clusters. In addition, the data suggest a protective effect of permissive underfeeding on incident renal replacement therapy that may differ by clusters of oxidative stress.

Oxidative stress, which primarily Results from an imbalance between oxidants and antioxidants, is common in critical illnesses

Table 1
Baseline characteristics of enrolled patients according to the clusters of oxidative stress.

Variables	Cluster 1 N = 44	Cluster 2 N = 9	Cluster 3 N = 3	P value
Baseline characteristics				
Age, yrs — median (Q1, Q3)	51.8 (25.5, 71.3)	62.8 (45.5, 69.7)	50.1 (26.9, 72.0)	0.68
Female sex, n (%)	10 (22.7)	4 (44.4)	1 (33.3)	0.39
Height, cm — median (Q1, Q3)	167.5 (160, 170)	165 (160, 170)	170 (160, 174)	0.77
Weight, kg — median (Q1, Q3)	70 (58.5, 81)	87 (70, 114)	80 (80, 104)	0.04
BMI, kg/m^2 — median (Q1, Q3)	26.0 (21.9, 30.6)	29.7 (26.4, 39.8)	31.3 (27.7, 34.4)	0.05
Diabetes, n (%)	22 (50.0)	7 (77.8)	2 (66.7)	0.29
Sepsis, n (%)	10 (22.7)	4 (44.4)	1 (33.3)	0.39
Admission category, n (%)				
Medical	24 (54.6)	6 (66.7)	2 (66.7)	0.62
Surgical	5 (11.4)	2 (22.2)	0 (0.0)	
Post-operative trauma	15 (34.1)	1 (11.1)	1 (33.3)	
Admission category, n (%)				
Medical	24 (54.6)	6 (66.7)	2 (66.7)	0.75
Non-medical	20 (45.5)	3 (33.3)	1 (33.3)	
Renal replacement therapy, n (%)	1 (2.3)	1 (11.1)	0 (0.0)	0.40
Mechanical ventilation, n (%)	41 (93.2)	9 (100.0)	2 (66.7)	0.15
Vasopressor, n (%)	26 (59.1)	9 (100.0)	3 (100.0)	0.03
APACHE II — median (Q1, Q3)	21 (12, 26)	21 (16, 25)	19.5 (13.0, 26.0)	0.73
SOFA Score Day 1 — median (Q1, Q3)	10 (8, 13)	11.5 (10.0, 13.0)	12 (6, 13)	0.43
PaO2: FiO2 ratio — median (Q1, Q3)	146 (83, 249)	74 (38, 130)	123 (54, 166)	0.14
SOFA Hypotension score	3.5 (1.0, 4.0)	4 (3, 4)	4 (3, 4)	0.49
GCS — median (Q1, Q3)	3 (3, 5)	3 (3, 7)	3 (3, 8)	0.90
Inclusion blood glucose, mmol/L — median (Q1, Q3)	9.7 (7.0, 13.2)	9.8 (7.3, 12.6)	13.6 (8.6, 15.3)	0.55
Hemoglobin, g/dl — median (Q1, Q3)	116.5 (96, 128)	98 (91, 105)	105 (84, 128)	0.10
INR — median (Q1, Q3)	1.1 (1.0, 1.3)	1.2 (1.0, 1.7)	1.0 (1.0, 1.2)	0.55
Platelets, 10^9/L — median (Q1, Q3)	164 (133, 243)	241 (184, 286)	296 (270, 425)	0.02
Bilirubin, μmol/L — median (Q1, Q3)	17.5 (10.7, 27.6)	11.8 (9.5, 26.7)	7.9 (6.1, 30.9)	0.49
Creatinine, μmol/L — median (Q1, Q3)	81.5 (63.5, 107.0)	124 (105, 154)	80 (72, 118)	0.03
C-reactive protein, mg/liter — median (Q1, Q3)	114.5 (60.7, 159.5)	137.5 (65.8, 237.0)	145.2 (13.6, 269.0)	0.93
Serum lipid profile, mmol/liter — median (Q1, Q3)				
Cholesterol	2.5 (1.8, 3.2)	2.5 (2.0, 2.8)	2.7 (1.2, 2.9)	0.87
Triglycerides	1.0 (0.8, 1.7)	1.6 (1.2, 2.2)	1.1 (1.0, 1.3)	0.18
HDL	0.6 (0.3, 0.9)	0.5 (0.4, 0.6)	1.0 (0.2, 1.1)	0.67
LDL	1.1 (0.5, 1.7)	0.7 (0.6, 1.5)	1.2 (0.4, 1.3)	0.91
Albumin, g/L — median (Q1, Q3)	28.0 (24.0, 31.5)	28 (28,29)	32 (30, 42)	0.18
Pre-albumin, g/L — median (Q1, Q3)	0.12 (0.09, 0.15)	0.10 (0.08, 0.11)	0.12 (0.04, 0.18)	0.49
Hemoglobin A1C — median (Q1, Q3)	0.06 (0.05, 0.07)	0.07 (0.05, 0.07)	0.08 (0.05, 0.09)	0.70
24- hours urinary nitrogen, mmol/— median (Q1, Q3)	245 (160, 344)	180.5 (42.5, 325.5)	188 (101, 702)	0.68
Transferrin, g/L — median (Q1, Q3)	1.3 (0.9, 1.5)	1.5 (1.2, 1.8)	2.1 (1.4, 2.2)	0.03
Minute ventilation, L/min — median (Q1, Q3)	8.9 (7.5, 10.9)	8.8 (8.6, 10.4)	10.4 (4.4, 13.6)	0.89
Maximum temperature, °C — median (Q1, Q3)	37.0 (36.6, 37.7)	37.2 (36.8, 38.3)	36.8 (36.7, 37.5)	0.71

BMI: body mass index; APACHE II: Acute Physiology and Chronic Health Evaluation II; INR: International normalized ratio; SOFA: Sequential Organ Failure Assessment; PaO_2:FIO_2 ratio: the ratio of partial pressure of oxygen to the fraction of inspired oxygen;GCS: Glasgow coam scale; HDL: High.density lipoproteins; LDL: Low density lipoproteins.
The denominators for all percentages is the N for each column. Continuous variables are represented as median (quartile 1 and quartile 3).

such as systemic inflammatory response syndrome, sepsis, traumatic brain injury, acute myocardial infarction and acute stroke [8,18,25,26]. Oxidative stress may lead to damage to lipids, proteins, and DNA and has been implicated in the development of multiorgan dysfunction [26]. In the present study, clusters 2 and 3 both had increased oxidative stress compared to cluster 1, had higher weights and BMI and had more patients on vasopressors compared to cluster 1. These findings are consistent with studies demonstrating that fat accumulation correlates with systemic oxidative stress in humans and mice [27]. The mortality did not differ according to the cluster of oxidative stress [28,29], although incident renal replacement therapy was different, suggesting the association between oxidative stress and acute kidney injury. The differences in requirement of renal replacement therapy among the clusters could not be explained based on differences in protein load.

Studies that evaluated oxidative stress in critically ill patients often used predefined criteria to define increased oxidative stress. However, such approach may be imprecise as it does not account for the variability in baseline levels among patients and it does not account for unexpected co-linarites between different biomarkers. For example, principal component 1 in our analysis correlated with TAC and 8-OHdG levels while principal component 2 correlated with protein carbonyl levels. The use of principal component analysis and cluster analysis has the advantage of grouping patients based on their own variables rather on any prior assumptions or definitions.

Caloric restriction, thought to promote health partly because of its effect on oxidative stress reduction has been investigated in different settings [11–14]. Caro et al. found that mice subjected to caloric restriction had lower oxidative DNA oxidation and protein oxidation, glycoxidation and lipoxidation compared with the control group [30]. Heilbronn et al. studied the effect of 6-month caloric restriction on longevity biomarkers, metabolic adaptation, and oxidative stress in overweight individuals and randomized 48 participants to 4 groups for 6 months: control (weight maintenance diet), caloric restriction (25% of baseline energy requirements), caloric restriction with exercise (12.5% energy requirements plus 12.5% increase in energy expenditure by structured exercise) and very low-calorie diet (890 kcal/d until 15% weight reduction, followed by a weight maintenance diet) [14]. Protein carbonyl

Table 2
Interventions by clusters.

Variables	Cluster 1 N = 44	Cluster 2 N = 9	Cluster 3 N = 3	P value
Feeding group, n (%)				
Permissive underfeeding	22 (50)	2 (22.2)	3 (100)	0.06
Standard feeding	22 (50)	7 (77.8)	0 (0.0)	
Calculated caloric requirement, kcal/day — median (Q1, Q3)	1750 (1579, 2086)	1870 (1770, 1968)	1916 (1825, 2046)	0.71
Study caloric target, kcal/day — median (Q1, Q3)	1381.5 (1054, 1863)	1800 (1680, 1968)	1150 (1102, 1222)	0.16
Daily caloric intake for the intervention duration –				
No. of kilocalories — median (Q1, Q3)	994.2 (795.4, 1242.1)	1108.1 (931.4, 1171.2)	781.2 (378.8, 990.6)	0.27
Percent of requirement — median (Q1, Q3)	56.6 (46.2, 73.2)	56.4 (54.6, 67.6)	40.8 (20.8, 48.4)	0.14
Caloric source for the intervention duration, kcal/day — median (Q1, Q3)				
Enteral	910.9 (646.4, 1135.1)	947.1 (733.9, 1136.1)	575.8 (68.8, 844.2)	0.18
Propofol	74.1 (24.2, 103.7)	47.9 (0.0, 161.0)	139.8 (82.3, 205.4)	0.31
Intravenous dextrose	3.9 (0.0, 45.6)	0.0 (0.0, 0.7)	6.6 (0.0, 227.8)	0.40
Total parenteral nutrition	0 (0.0)	0 (0.0)	0 (0.0)	–
Calculated protein requirement, g/day — median (Q1, Q3)	77.5 (68, 92.0)	84 (75, 91)	93 (73, 103)	0.46
Daily protein intake for the intervention duration				
No. of grams	52.8 (40.1, 68.4)	43.6 (37.3, 59.1)	41.1 (4.5, 66.4)	0.50
Percent of requirement	70.4 (55.7, 82.8)	65.0 (39.7, 83.1)	44.2 (6.2, 64.5)	0.23
Protein source- g/day — median (Q1, Q3)				
Main enteral formula	35.3 (35.0, 52.7)	34.9 (28.7, 56.5)	17.9 (2.5, 26.6)	0.09
Supplemental enteral protein	11.2 (0.0, 26.5)	0.3 (0.0, 6.1)	22.3 (2.0, 39.8)	0.32
Parenteral protein	0 (0, 0)	0 (0, 0)	0 (0, 0)	–
Duration of intervention, days — median (Q1, Q3)	10 (6, 14)	14 (7, 14)	14 (4, 14)	0.75
Co-interventions during study period				
Insulin				
Use – no. (%)	26 (59.1)	7 (77.8)	2 (66.7)	0.57
Dose – units/day —median (Q1, Q3)	2.7 (0.0, 24.7)	18.9 (2.5, 79.4)	6.8 (0.0, 55.1)	0.23
Blood glucose, mmol/L – median (Q1, Q3)	7.6 (6.5, 10.5)	10.3 (6.6, 11.3)	7.2 (6.6, 8.3)	0.75
Enteral formulae on day 1 – no. (%)				
Disease-non-specific[a]	22 (50.0)	2 (22.2)	1 (33.3)	0.29
Disease-specific[b]	22 (50.0)	7 (77.8)	2 (66.7)	
Medications given during the ICU stay – no. (%)				
Beta blockers	16 (36.4)	6 (66.7)	0 (0.0)	0.09
Aspirin	14 (31.8)	4 (44.4)	0 (0.0)	0.36
Angiotensin-converting enzyme inhibitors	4 (9.1)	2 (22.2)	1 (33.3)	0.30
Angiotensin II receptor blockers	0	0	0	0
Statins	16 (36.4)	4 (44.4)	1 (33.3)	0.89

[a] Disease-non-specific formula: Osmolite, Jevity, Promote, Ensure plus, Resource, Ensure, Resource plus, Jevity(1.2).
[b] Disease-specific formula: Glucerna, Nutric hepatic, Nepro, Pulmocare, Novasource Renal, Peptamen(1.0), Peptamen (1.2), Suplena, Oxepa.

Table 3
Outcome data by clusters.

Variables	Cluster 1 N = 44	Cluster 2 N = 9	Cluster 3 N = 3	P value
Death by 90 days — no. (%)	10 (22.7)	1 (11.1)	0 (0.0)	0.49
Death by 180 days, no. (%)	12 (27.3)	2 (22.2)	0 (0.0)	0.56
Death in the ICU — no. (%)	5 (11.4)	1 (11.1)	0 (0.0)	0.83
Death in the hospital, no. (%)	8 (18.2)	1 (11.1)	0 (0.0)	0.64
ICU length of stay, days — median (Q1, Q3)	14 (8.5, 17.5)	28 (19, 37)	36 (5, 40)	0.02
ICU-free days[a], days — median (Q1, Q3)	73 (46.0, 79.5)	62 (46.0, 71.0)	54 (50.0, 85.0)	0.49
Hospital length of stay — days— median (Q1, Q3)	38.5 (17.0, 79.0)	53 (28, 95)	43 (5, 97)	0.76
Duration of mechanical ventilation — days — median (Q1, Q3)	10 (6,15)	17 (11,30)	7 (3,21)	0.21
New renal replacement therapy, no. (%)	4/42 (9.5)	4/8 (50.0)	1/3 (33.3)	0.01
Renal replacement therapy free days, no. (%)	14 (14,14)	10 (4,14)	14 (12,14)	0.26

ICU: Intensive care unit.
[a] ICU-free days were calculated for the first 90 study days and were considered to be 0 for patients who died on or before day 90.

concentrations were not changed from baseline to month 6 in any group, whereas DNA damage was reduced from baseline in all intervention groups ($p < 0.005$) [14]. Krzystek-Korpacka et al. compared 114 overweight/obese children and adolescents who had different weight reduction programs (diet/exercise, bran-enriched diet/exercise, and diet/exercise plus metformin) with 53 lean controls [31]. Oxidation protein products decreased after the weight reduction program with the decrease being more pronounced in subjects on bran-enriched diet [31]. Meydani et al. randomized 46 overweight volunteers to caloric restriction of either 10% or 30% of basal caloric intake [32]. At 6 months, plasma glutathione peroxidase activity increased ($p = 0.04$) and plasma protein carbonyl levels decreased ($p = 0.02$) [32]. No significant change was observed in other plasma antioxidants such as superoxide dismutase and catalase [32]. The findings of these studies suggest that caloric restriction may modulate some but not all markers of antioxidants and oxidative stress. In the current study, we found no significant difference in oxidative stress whether the patients were permissively underfed or standard fed in the whole cohort or in clusters of patients with similar oxidative stress. This is in line with an animal study, which showed that short-term caloric restriction had no effect on oxidative damage, however, it lowered antioxidant

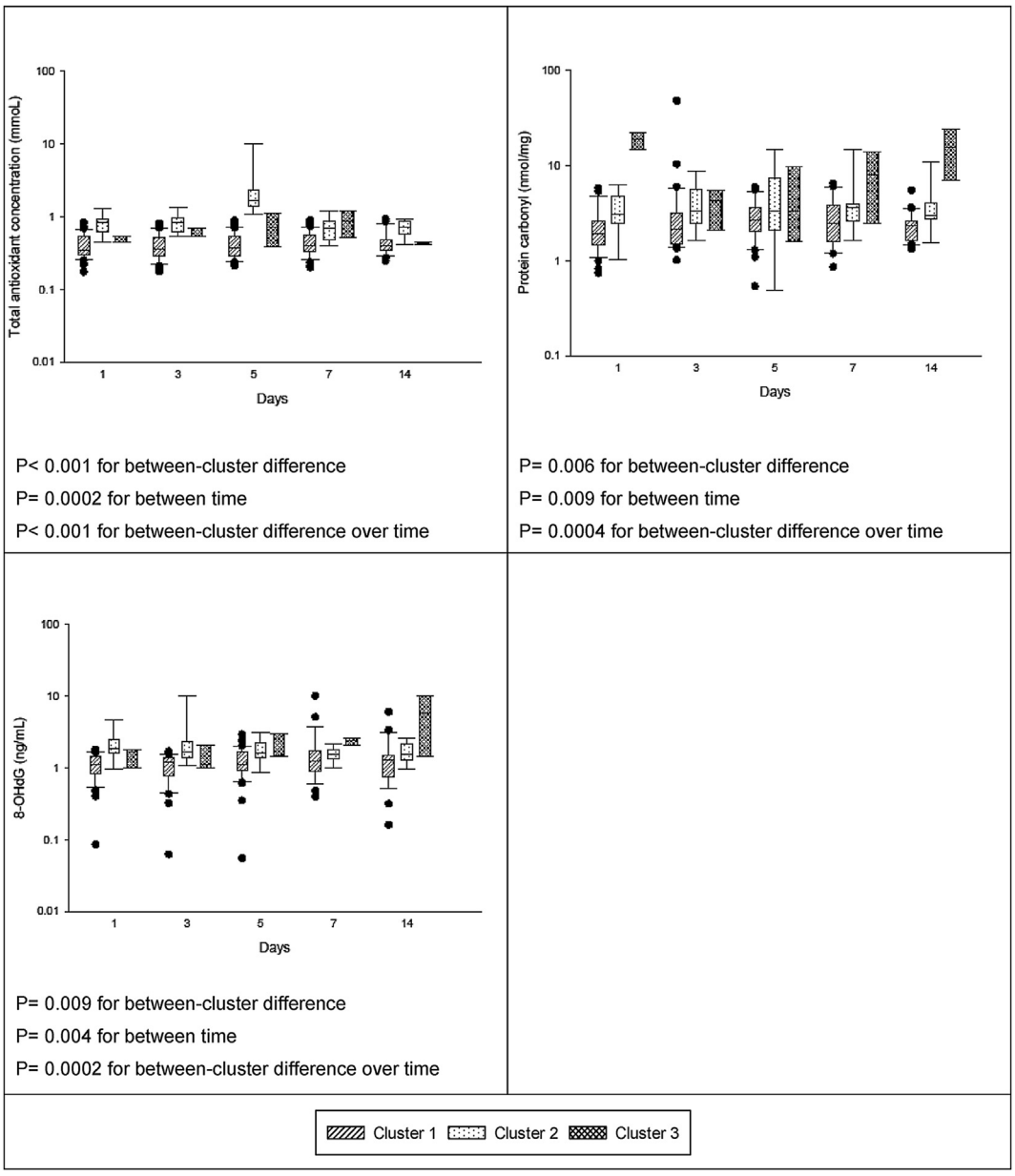

Fig. 1. Serial measurements of oxidative stress markers: total anti-oxidant capacity (TAC), protein carbonyl concentration and 8-hydroxy-7,8-dihydro- 2′-deoxyguanosine (8-OHdG) between the three clusters. P values for between-cluster differences and between-cluster differences over time are provided using mixed linear model.

activity [33]. A recent study have looked at the effect of very low caloric diet on body composition and metabolic state of obese individuals, and no sign of inflammation or oxidative stress was observed [34]. It is possible that caloric restriction may be effective in lowering oxidative stress, if caloric restriction occurred as precondition to the insult, which would not be the case in critically ill patients.

Protein intake is also thought to affect oxidative stress. In adult rats, long-term high casein-based protein intake did not increase markers of oxidative stress [35]. Reducing protein intake by 40% in Wistar rats for seven weeks was associated with 30–40% decreases in mitochondrial ROS production and in oxidative damage to nuclear and mitochondrial DNA [36]. We found no association of protein intake and mortality when adjusted for clusters of oxidative stress.

Interpretation of the findings should take in account the study strengths and limitations. The analysis of oxidative stress markers was carried out a priori as a part of sub-study of a randomized control trial. We measured oxidative stress markers in the blood and not at a cellular level. Study limitation includes the size of the clusters.

5. Conclusion

In conclusion, there are different distinct patterns of oxidative stress in critically ill patients. Incident renal replacement therapy, but not mortality, was different among the three clusters. Our data suggest a protective effect of permissive underfeeding on incident renal replacement therapy that may differ by clusters of oxidative stress.

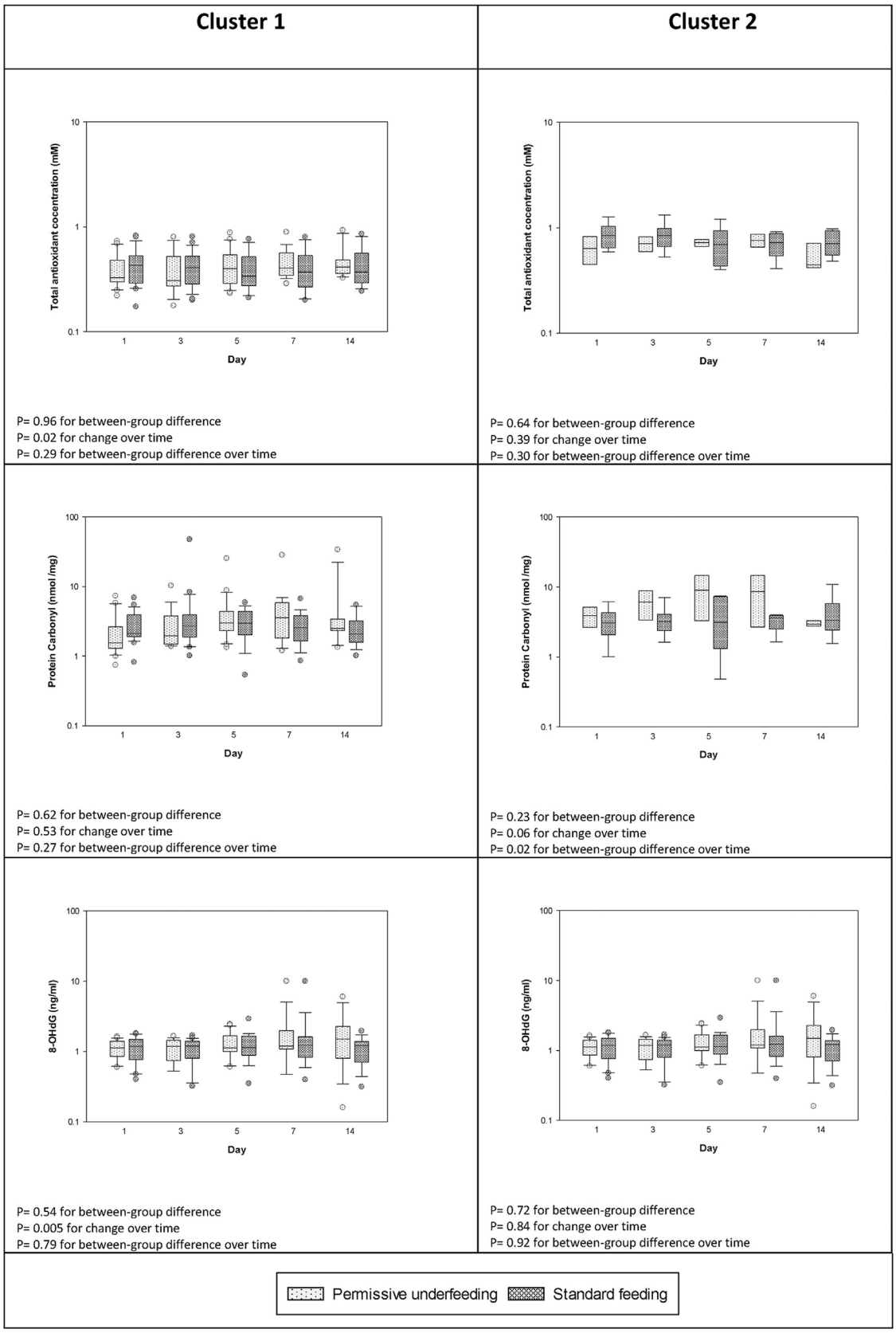

Fig. 2. Serial measurements of oxidative stress markers: total anti-oxidant capacity (TAC), protein carbonyl concentration and 8-hydroxy-7,8-dihydro- 2′-deoxyguanosine (8-OHdG) between permissive and standard feeding in Cluster 1(column 1) and Cluster 2 (column 2). P values for between-group differences and between-group differences over time are provided using mixed linear model.

Declaration

Ethics approval and consent to participate

The study was approved by the National Guard Health Affairs Institutional Review Board (IRB).

An informed consent was obtained from the subjects enrolled in PermiT trial to participate in this substudy.

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflict of interest (COI) statement

The authors declare that they have no conflict of interest.

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Authors' contributions

YA: Conception, acquisition of data, design, analytical plan, drafting of the manuscript and critical revision of the manuscript for important intellectual content, approval of the final version to be published.

DJ: Acquisition of data, and critical revision of the manuscript for important intellectual content, approval of the final version to be published.

AB: Acquisition of data, and critical revision of the manuscript for important intellectual content, approval of the final version to be published.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnesp.2018.11.011.

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