




# Real-Time Energy Exposure Is Associated With Increased Oxidative Stress Among Feeding-Tolerant Critically Ill Patients: Results From the FEDOX Trial

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## Abstract

**Background:** Prospective randomized controlled trials (PRCTs) that found harm in patients receiving higher levels of energy exposure have been largely ignored, in part because of the lack of a known mechanism of harm. **Objective:** The current 7-day pilot study is a PRCT and post hoc analysis designed to explore the relationship between energy exposure and oxidative stress (as plasma total F2-isoprostanes) in mechanically ventilated intensive care unit patients with systemic inflammatory response syndrome. **Methods:** Thirty-five participants were randomized to receive either 100% or 40% of their estimated energy needs. Our intent-to-treat model found no differences in F2-isoprostanes between groups. A post hoc analysis revealed that on days when participants were in the highest tertile of daily kcal/kg, the real-time energy flow rate within 2 hours of the blood draw was predictive of increased oxidative stress. On these days, participants in the second or third vs the first tertile of real-time energy flow rate experienced a 41.8% ( $P = .006$ ) or 26.5% ( $P = .001$ ) increase in F2-isoprostane levels, respectively. This was confirmed through a within-group subanalysis restricted to participants with measurements on both sides of the median of real-time energy flow rate that found a 28.2% F2-isoprostane increase on days in the upper vs lower median of flow rate ( $P = .002$ ). **Conclusion:** The benefits of feeding may be more nuanced than previously suspected. Our findings imply a potential mechanism of harm in meeting the current recommendations for nutrition support in the critically ill that warrants further investigation. (*JPEN J Parenter Enteral Nutr.* 2020;44:1484–1491)

## Keywords

critical illness; enteral nutrition; F2-Isoprostanes; nutrition support; oxidative stress; prospective randomized controlled trial; systemic inflammatory response syndrome

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## Clinical Relevancy Statement

The processing of nutrient substrate at the electron transport chain during profound inflammation may lead to increased oxidative stress. We performed a 7-day randomized control feeding trial on 35 mechanically ventilated patients who had systemic inflammatory response syndrome and were receiving enteral nutrition. Post hoc analysis of this trial found that among patients receiving  $>15$  kcal/kg/d, the real-time energy rate of flow into the body from any source was associated with increased oxidative stress. Meeting resting energy expenditure in profoundly inflamed patients may carry negative consequences and requires further exploration in a larger cohort.

## Introduction

The provision of nutrition support is a foregone conclusion for most intensive care unit (ICU) patients, limited only by tolerance and feasibility. It is assumed that replenishing a patient's resting energy expenditure (REE) is beneficial or least benign, but findings from studies that achieved REE in their higher-fed groups imply it may not be so simple. Of the 7 prospective randomized controlled trials (PRCTs) that achieved estimated REE in their higher-fed groups,<sup>1-7</sup> 4 found harms in meeting REE<sup>1,2,5</sup> that ranged from increased nosocomial infection,<sup>2,5</sup> time on mechanical ventilation,<sup>2</sup> mortality,<sup>1</sup> and decreased likelihood of discharging early and alive from the ICU.<sup>2</sup> These harms have been largely ignored in the literature, as they run against the current profeeding dogma, and the studies tailored to explore potential mechanisms of harm in feeding the critically ill are lacking. The current study was designed to provide insight into one such mechanism.

Our group posited a mechanism of harm in feeding profoundly inflamed patients.<sup>8</sup> Briefly, we theorized that feeding inflamed patients would lead to increased oxidative stress from reactive oxygen species production at the mitochondrial electron transport chain (ETC). We based our theory on previous findings establishing that increased levels of nitric oxide had the potential to block specific complexes on the ETC, leading to increased superoxide radical production for the same amount of nutrition substrate.<sup>9,10</sup> This would then result in the production of peroxynitrite, which would further inhibit the ETC, further increasing superoxide production.<sup>11,12</sup> This would all lead to the depletion of antioxidants needed to restore the antioxidant enzyme systems that neutralize superoxide to water.<sup>13</sup> The result of this would be a buildup of hydrogen peroxide and oxidative stress in the mitochondria and local cellular environment that would lead to mitochondrial rupture and the eventual death of the cell.<sup>10</sup>

Based upon this theory, the objective of the current study was to explore the impact of higher vs lower energy exposure

on oxidative stress in critically ill, mechanically ventilated patients receiving enteral nutrition (EN).

## Methods

### *Study Design and Patient Recruitment*

We performed a 7-day, single-center PRCT on critically ill, mechanically ventilated patients in an urban neurosurgical and medical ICU. We recruited patients who were  $\geq 18$  years old, were mechanically ventilated, and met the criteria for systemic inflammatory response syndrome (SIRS). Specifically, patients were eligible if their white blood cell count fell within the SIRS range for 2 consecutive days and their heart rate, respiratory rate, or temperature fell within the SIRS range at the time of screening. Patients were excluded from the study if they were pregnant, were severely immunocompromised, were expected to be extubated in  $<72$  hours, or did not require EN for their care.

### *Intervention*

Upon consent, patients were randomized to receive either 40% of their estimated caloric needs (40%ECN) or 100% of their estimated caloric needs (100%ECN). The lower-fed group was set at 40% to provide a reduction in energy exposure while preventing fasting-induced oxidative stress. Computer-generated randomization was performed off site by someone blinded to the patients' profiles. Energy needs were estimated to be 25–30 kcal/kg/d per the nationally recommended guidelines for feeding critically ill patients.<sup>14</sup> Patients were fasted 7 hours and then switched to Jevity 1.5 (Nestlé Nutrition, 1500 kcal, 63.8 g protein/L) as their main source of EN. Participants in the 40%ECN group were targeted to receive 10–12 kcal/kg/d. Participants in the 100%ECN group were targeted to receive 25–30 kcal/kg/d. EN flow rates were ramped up slowly, increasing 20 mL/h every 4 hours until the goal rates were achieved. Feeding interruptions were compensated as feasible by rate increases to avoid caloric deficits and returned to the original rate once the correction was achieved. The intervention occurred for 7 days or until ICU discharge or death.

### *Outcome Variables*

Our primary outcome was variable oxidative stress, assessed as total plasma F2-isoprostanes. This was quantified through liquid chromatography tandem mass spectrometry as described by Larose et al,<sup>15</sup> using a Q-trap mass spectrometer by the staff of UIC College of Pharmacy Mass Spectrometry, Metabolomics, and Proteomics Facility. Secondary outcome variables included ICU mortality, ICU and hospital length of stay, time on mechanical ventilation, and infections, but the current study was not specifically powered for any of these secondary outcomes.

## Data Collection

EN energy intake was monitored closely, as was energy intake from parenteral nutrition, intravenous drips, medications, and oral intake. Basic demographic variables were collected from the electronic medical record and confirmed with medical staff as necessary. Such variables included age, sex, admission diagnosis, ICU day, weight, height, comorbidities, vasopressor usage, insulin, glucose, complete blood count, electrolyte status, temperature, heart rate, respiratory rate, Acute Physiology and Chronic Health Evaluation Score II (APACHE II),<sup>16</sup> Sequential Organ Failure Assessment (SOFA) score,<sup>17,18</sup> blood gases, nutrition status, weight, and height.

Baseline blood was collected following the 7-hour fast and daily thereafter for the duration of the study. Blood samples were collected in EDTA tubes and placed inside a refrigerated centrifuge within 10 minutes of collection. Samples were centrifuged at 5000 rpm for 10 minutes. Plasma was then separated and placed in a  $-70^{\circ}\text{C}$  freezer until processing.

## Statistical Analyses

*Intent-to-treat analysis.* Basic descriptive statistics were run for demographic and baseline variables. All variables were assessed for normality and transformed as needed. Baseline variables were compared between groups using *t*-tests and Wilcoxon tests for parametric and nonparametric data, respectively.

Power analysis and sample size was calculated using General Linear Multivariate Model Power and Sample Size (GLIMMPSE) software for mixed-level modeling. Based on findings for between-participant and within-participant variance in F2-isoprostanes from Ware et al<sup>19</sup> and Brown et al,<sup>20</sup> assuming an  $\alpha$  of 0.05 and a 20-pg/mL difference in F2-isoprostanes leading a slope increase of 3.57 in the 100%ECN group, it was estimated that 40 participants would provide a conservative power of 0.96.

Mixed-level modeling was used to explore the impact of randomization group on total plasma F2-isoprostane levels. Intercept as well as both linear and quadratic trends over time were explored as possible random effects in all mixed-level models.

*Post hoc analysis.* Exploration of daily energy exposure in the 100%ECN group revealed a large day-by-day, within-participant heterogeneity of energy exposure. This daily intra-individual variability eliminates the ability to detect the 7-day cumulative impact of energy exposure but still provides 7 days of energy data at many different levels of exposure. We therefore chose to model the average effect of energy exposure on any given day. To explore this, 2 key variables were created. The variable “daily kcal/kg” represented the total energy consumed from all sources (enteral,

parenteral, medication, drips, and oral) over 24 hours. A second variable termed “recent kcal/kg” was created and represented the total energy consumed per hour from all sources within 2 hours of the F2-isoprostane blood draw.

Overall tolerance of EN tends to be greater among healthier patients; thus, higher intake reflects lower severity of illness. Previous observational studies did not account for the improved EN tolerance commonly seen in less critically ill patients. Although we suspect the majority of low kcal/kg received reflects an increased illness severity (ie, due to nil per os status for tests, emesis, gastric hypoperfusion, or compassionate extubation prior to death), lower EN also occurs with the positive event of successful extubation. To prevent confounding by these different causes of energy deficit, we created a dichotomous variable, termed “beneficial extubation,” to capture whether these deficits were caused by improvements in health status (extubation due to improved blood gases) or events of critical illness (all other causes of deficit).

To account for daily EN tolerance and ameliorate biases caused by its relationship to illness severity, we modeled the association between recent kcal/kg and oxidative stress at different tertiles of daily kcal/kg through mixed-level modeling. All models were assessed for the potential confounders: beneficial extubation, age, gender, race, ICU day, study day, SIRS criteria, nutrition status, body mass index (BMI), admit diagnosis, APACHE II, baseline SOFA score, daily SOFA score, days overfed, propofol administration, vasopressor administration, and insulin administration. A variable was considered a confounder if it was statistically significant in the model ( $P < .05$ ) and altered the  $\beta$  estimate of recent kcal/kg by at least 10%.

Finally, recognizing that the above stratification on daily kcal/kg could create bias such that participants in the higher strata of recent kcal/kg may not have observations in the lowest stratum (ie, the reference group), we performed subanalyses on the relationship between recent kcal/kg and oxidative stress at each strata of daily kcal/kg, restricted to only those participants within that strata who had at least 1 measurement in each strata of recent kcal/kg. A median split was performed on recent kcal/kg to ensure adequate number of observations ( $\geq 5$ ) in each strata. This true within-group comparison was necessary to ensure our findings were not the result of different oxidative stress levels among participants in the lowest stratum vs participants in the higher strata.

With the exception of our power analysis, all statistical analysis was performed in Statistical Analysis Software (SAS 9.4).

## Results

Details and flow chart of the participant exclusion process as well as baseline demographic data are previously

**Table 1.** Baseline Values for Outcome Variables.

Variable	Overall N = 34	100%ECN n = 19	40%ECN n = 15	P-Value
F2-Isoprostanes	0.399 (0.13)	0.39 (0.15)	0.39 (0.12)	0.862

40%ECN, 40% of estimated caloric needs; 100% ECN, 100% of estimated caloric needs.

published by McKeever et al.<sup>21</sup> Briefly, 485 patients met the inclusion criteria. Of these, 35 were successfully consented into the study. Participants were predominantly female (67%), African American (46%), and normal nourished (77%) with an average BMI of 33.7 (SD = 13.2), an average SOFA score of 8.8 (SD = 4.4), and an average APACHE II score of 21.0 (7.0) at baseline. Participants were not significantly different at baseline in these or any other demographic or clinical parameters. All participants met the study protocol subset of SIRS criteria at baseline. The primary outcome variable, F2-isoprostanes, was the same between groups at baseline (Table 1). The intervention successfully delivered more energy, protein, fat, and carbohydrates to the 100%ECN group vs the 40%ECN group (Table 2,  $P = .01$ ,  $P = .02$ ,  $P = .04$ ,  $P = .04$ , respectively). All were well within acceptable macronutrient distribution ranges for both groups. Despite this, daily events of critical illness (hypogastric perfusion, emesis, nil per os

**Table 3.** Tertile Distribution of Energy Variables.

	Daily kcal/kg (Over 24 Hours)	N of Observations	Recent kcal/kg (Within 2 Hours of Phlebotomy)	N of Observations
Tertile 1	<7.37	81	<0.25	83
Tertile 2	7.37–15.35	82	0.25–0.67	79
Tertile 3	>15.35	82	>0.67	84

status for tests, and extubation trials) led to considerable heterogeneity in energy exposure for the 100%ECN group (Figure 1).

### Intent-to-Treat Analysis

No significant differences were found between the 2 randomization groups for total F2-isoprostanes.

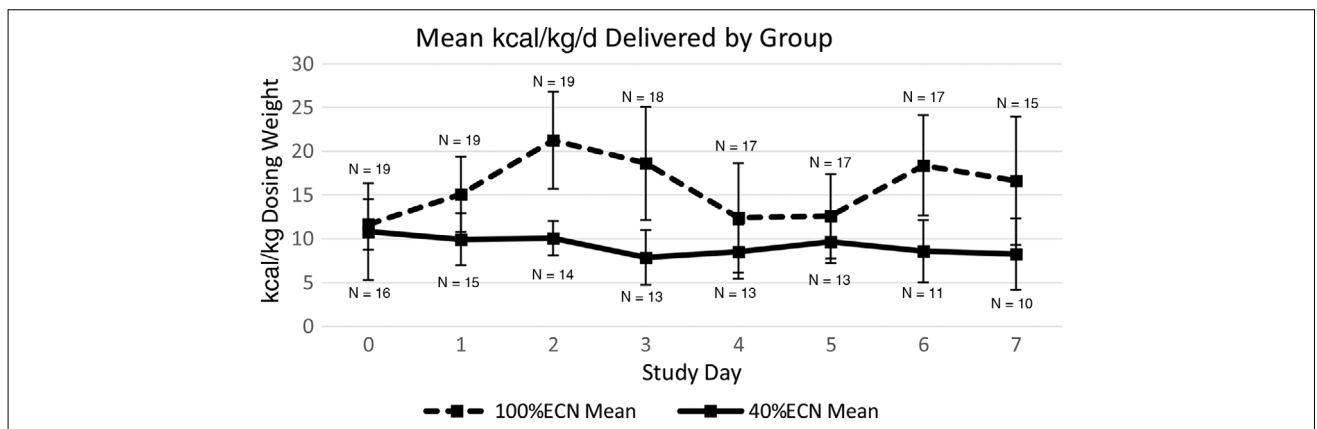
### Post hoc Analysis of Energy Exposure on Measures of Oxidative Stress

As described above to bypass the confounding effects that severity of illness impose on the relationship between feeding and oxidative stress, recent kcal/kg was explored at different tertiles of daily kcal/kg. Table 3 lists the tertile values for both variables.

**Table 2.** Nutrition Exposure Over the Study Period.

Variable	Overall N = 34	100%ECN n = 19	40%ECN n = 15	P-Value
Total kcal (SD)	948.96 (457.67)	1140.5 (504.6)	721.5 (316.9)	0.006
Mean kcal/kg (SD)	13.67 (6.24)	16.05 (6.04)	10.85.73 (5.39)	0.011
Mean protein g (SD)	39.10 (24.21)	46.62 (22.94)	30.18 (23.25)	0.021
Mean g/kg protein (SD)	0.5529 (0.2982)	0.6328 (0.2711)	0.4581 (0.3095)	0.084
Mean CHO g (SD)	121.79 (61.87)	144.8 (65.90)	94.45 (44.66)	0.036
Mean fat g (SD)	31.91 (20.21)	39.55 (22.14)	22.83 (13.25)	0.036

40%ECN, 40% of estimated caloric needs; 100% ECN, 100% of estimated caloric needs; CHO, Carbohydrate.

**Figure 1.** Statistics on kcal/kg dosing weight by group by study day; error bars represent 95% confidence intervals.

In the third tertile of daily kcal/kg, there were 81 observations spread over 26 participants. Conditional on tertile of daily kcal/kg and adjusting for ICU day, participant-days spent in the second or third vs the first tertile of recent kcal/kg were associated with a 41.8% ( $P = .006$ ) or 26.5% ( $P = .001$ ) increase in F2-isoprostane levels, respectively, among participant-days spent in the third tertile of daily kcal/kg (Table 4, Figure 2). On average, among participant-days spent in the highest tertile of daily kcal/kg, moving up 1 tertile in recent kcal/kg is associated with a 0.05-ng/mL increase in F2-isoprostanes ( $P = .009$ , Figure 2). This was confirmed through a within-group subanalysis exploring the impact of being in the higher vs lower median of recent kcal/kg on F2-isoprostanes restricted to only those participants who had measurements at both levels. There were 44 observations spread among 11 participants who had measurements on both sides of the median for recent

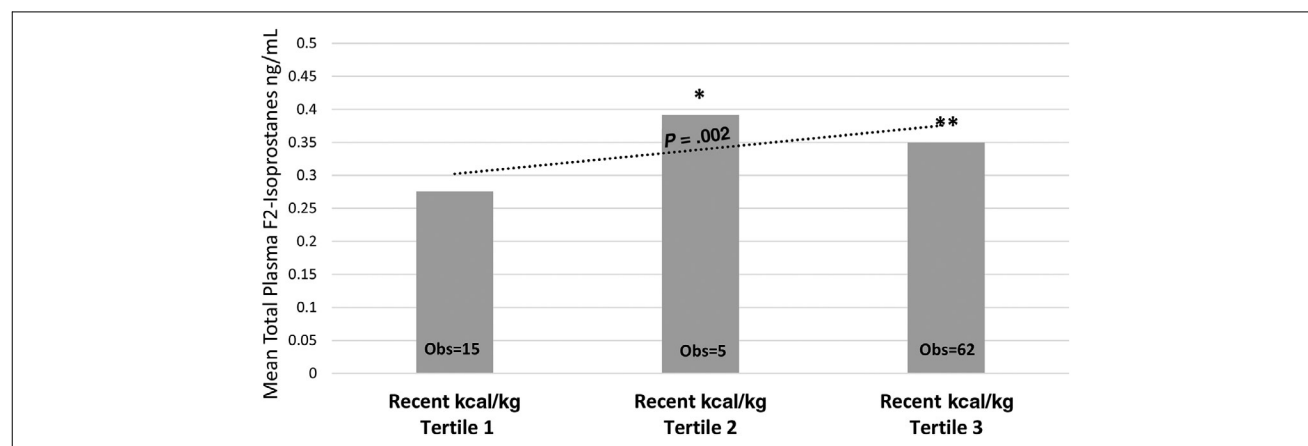
kcal/kg within this restricted set of participants. In this group, among participant-days spent in the highest tertile of daily kcal/kg and adjusting for ICU day, participant-days spent in the upper median of recent kcal/kg were 28.2% higher ( $P = .002$ , Figure 3) than participant-days spent in the lower median. None of the above associations were seen at lower levels of daily kcal/kg. Ten out of 35 participants were determined to have some energy deficit due to improved health (ie, they were extubated because of improved blood gases). Reason for energy deficit (improved health vs event of critical illness) was tested as a possible confounder but was not significant ( $P = .56$ ), nor did it appreciably affect the estimates for recent kcal/kg. ICU day was found to be a confounder in the relationship between recent kcal/kg and F2-isoprostanes. All presented models are adjusted for ICU day as well as its quadratic term when significant.

**Table 4.** Mixed-Level Regression of Tertile of Recent kcal/kg (Study Days 0–7) on Mean log F2-Isoprostanes.

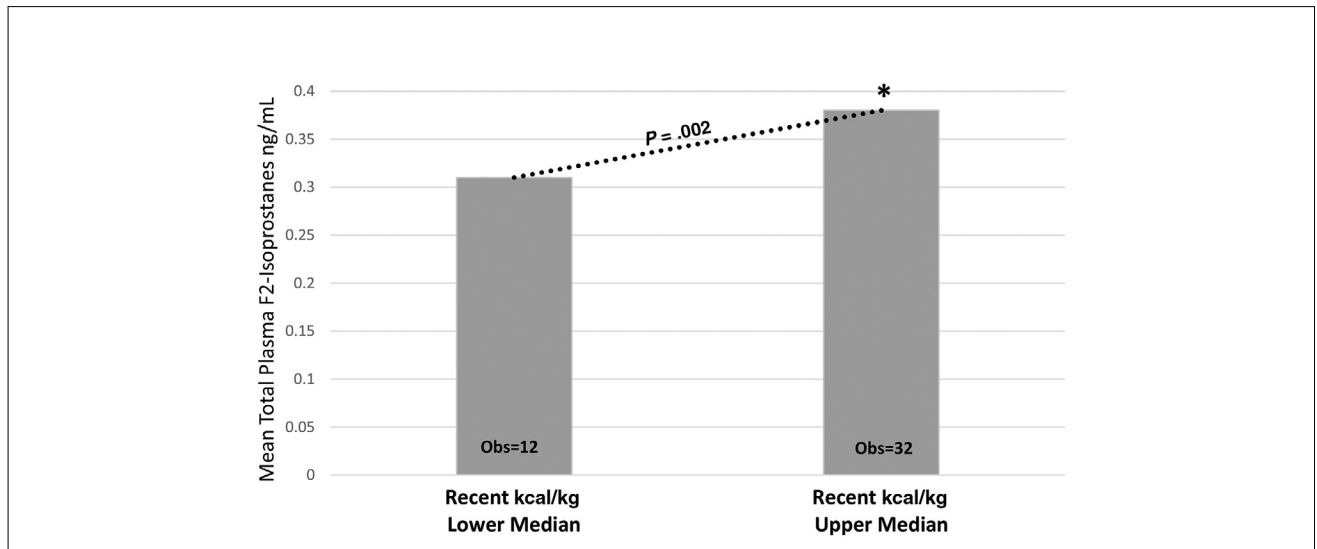
Parameter	Tertile Daily kcal/kg Ref = Tertile 1	Tertile Recent kcal/kg Ref = Tertile 1	Estimate	SE	P-Value
Intercept	—	—	−0.781	0.096	<0.0001
Daily kcal/kg tertile 2 vs 1	—	—	−0.014	0.060	0.817
Daily kcal/kg tertile 3 vs 1	—	—	−0.336	0.072	<.0001
Recent kcal/kg tertile 2 vs 1	—	—	−0.011	0.061	0.862
Recent kcal/kg tertile 3 vs 1	—	—	0.054	0.084	0.520
Interaction term for tertiles daily and recent kcal/kg	Second tertile	Second tertile	0.076	0.089	0.397
Interaction term for tertiles daily and recent kcal/kg	Second tertile	Third tertile	−0.212	0.114	0.063
Interaction term for tertiles daily and recent kcal/kg	Third tertile	Second tertile	0.360	0.138	0.010
Interaction term for tertiles daily and recent kcal/kg	Third tertile	Third tertile	0.182	0.107	0.090
Log ICU day			−0.086	0.041	0.034
Random-effects model Intercept $\sigma^2$	—	—	0.064	0.019	0.0003

−2 log L = 59.6

ICU, intensive care unit; SE, standard error.



**Figure 2.** Mean total plasma F2-isoprostanes by tertile of recent kcal/kg (flow rate from any source within 2 hours of blood draw) among participant-days spent in the third tertile of daily kcal/kg. Obs, observations.



**Figure 3.** Subanalysis: Mean total plasma F2-isoprostanes by median of recent kcal/kg (flow rate from any source within 2 hours of blood draw) among participant-days spent in the third tertile of daily kcal/kg and restricted to participants with measurements on both sides of the median for recent kcal/kg. Obs, observations.

## Discussion

The primary finding of the current study was that real-time energy flow rate was associated with increased oxidative stress among mechanically ventilated SIRS patients capable of tolerating >15 kcal/kg/d. These findings, although post hoc in nature, are in the opposite direct of the known bias, in which patients who were more tolerant of feeding would be less critically ill and therefore expected to have lower oxidative stress. These results imply a mechanism of harm in meeting REE in critically ill patients that warrants further investigation.

It is interesting that the relationship between energy flow and oxidative stress was limited to the highest tertile of daily energy exposure. It is possible that this was due to the energy flow occurring in a context of higher levels of feeding that day. However, a more likely explanation concerns the difference between nutrition-induced oxidative stress and critical illness-induced oxidative stress. Patients in the lower 2 tertiles of energy exposure, by definition, had more “events of critical illness” that day, necessitating frequent and prolonged cessation of feeding. Patients in the highest tertile of daily kcal/kg were simply having a better day, necessitating fewer interruption to their feeding. Patients in the lower tertile likely had much higher critical illness-induced oxidative stress, which could have masked the nutrition-induced oxidative stress. Indeed, exploration of our data demonstrated significantly increased daily SOFA scores at lower levels of feeding and wider SDs for F2-isoprostanes in the lower 2 tertiles of daily kcal/kg. Despite the lack of findings in our EN-intolerant groups, our findings imply that feeding the EN-tolerant may be problematic from a redox perspective.

Mitochondrial ETCs, the dominant mechanisms for converting nutrient substrate to adenosine triphosphate (ATP), do not function normally in the context of profound inflammation. Mitochondrial dysfunction has been demonstrated to occur early in sepsis, impairing the machinery of ATP production. Garrabou et al<sup>10</sup> explored mitochondrial function in sepsis by comparing the peripheral blood mononuclear cells of septic patients with those of healthy volunteers. The septic patients’ mitochondrial ETC complexes I, III, and IV were inhibited, and oxygen utilization was 22%–42% lower compared with that of healthy volunteers. Septic patients in this study had signs of mitochondrial damage evidenced by a 3-fold increase in plasma mitochondrial proteins and depolarized mitochondria, an indicator of early apoptosis. Brealy et al<sup>11</sup> compared muscle biopsies on 28 critically ill patients with 9 biopsies of control patients undergoing elective hip surgery. They found decreased muscle ATP in patients who died compared with that in survivors and controls. They also found patients with increased norepinephrine requirements, an indicator of shock severity, had decreased mitochondrial ETC complex I activity. Collectively, these findings paint critical illness as a time when our tools for converting nutrient substrate to ATP are damaged and when feeding may result in oxidative stress and cell death.

Despite this, feeding trials in critically ill patients do not consistently favor any one outcome. Some find harm. Others find nothing. Still others find feeding to be beneficial, but the short list of prospective randomized feeding trials in critically ill patients is plagued with inconsistencies. The inappropriate conflation of study findings among trials that differ wildly in energy exposure and timing of intervention

has led to the conclusion that feeding is unequivocally benign. This approach is problematic because it assumes the relationship between feeding and outcome is linear. Relationships between nutrients and outcomes are rarely, if ever, linear. It is far more likely that the relationship between energy exposure and outcome is some variant of a U-shaped curve in which both low and high levels of energy exposure invoke harm, whereas exposure in the middle range is anywhere from benign to beneficial. Capturing this curve requires studies that straddle the borders where benign turns to harm. The Intensive Nutrition in Acute Lung Injury (INTACT) trial,<sup>1</sup> which found increased mortality in critically ill participants fed 25–30 kcal/kg vs standard care, explored the threshold for kcal/kg at which energy exposure significantly predicted mortality. They found that energy exposure began to significantly predict mortality as it approached 18 kcal/kg.<sup>22</sup> A retrospective analysis on participants who met the inclusion for INTACT study but were not consented into the trial performed a similar threshold analysis and found that energy exposure began significantly predicting mortality when it reached 16 kcal/kg.<sup>23</sup> Currently, these are the only thresholds in the literature for this question, but it is interesting that most of the studies that do not demonstrate harm at higher levels of feeding fail to straddle these thresholds. Most fall below the thresholds in both intervention and control groups,<sup>24–26</sup> whereas others hover above.<sup>3,5,27</sup> In trials that demonstrate clear harm with higher levels of energy exposure, such as the INTACT<sup>1</sup> and Early versus Late Parenteral Nutrition in Critically Ill Adults (EPaNIC)<sup>2</sup> studies, these thresholds are straddled. These studies may provide the first evidence toward a more nuanced understanding of nutrition support in the critically ill, and the current study clearly demonstrates nutrition-induced oxidative stress as a potential mechanism of harm to explain these findings.

We did not find a difference between feeding groups in our intent-to-treat model. We speculate this was due to the increased heterogeneity in our ability to provide our intervention to the 100%ECN group. Further, we speculate that, independent of severity of illness, which was similar between groups, the contribution of specific disease processes may confound the relationship between energy exposure and oxidative stress in ways that we could not capture. Measures of illness severity (APACHE II, SOFA, etc) measure disease state on a continuum of mortality and may not translate to other effects of critical illness. For example, aspects of critical illness that induce oxidative stress may not plot on the same continuum, thereby defying adjustment in statistical models. This necessitates a larger sample size so randomization will hold at finer levels of stratification, such as admit diagnosis and comorbidities. In our post hoc strategy, we further eliminated this confounding by conditioning on the ability to feed, which was the most direct adjustment possible for the intricate interac-

tions between illness severity, EN tolerance, and oxidative stress.

Our study had several strengths. Beyond being the first study to explore the relationship between feeding and oxidative stress in critically ill patients, its prospective design meant that data were collected accurately and in real time. To our knowledge, it is also the first study to explore both 24-hour energy exposure and real-time energy exposure to provide mechanistic insights that relate closely with actual energy delivery at the time of sample collection. Limitations of the study include the heterogeneity of energy exposure in the higher-fed group as well as our sample size. Although our repeated-measure analysis provided 245 data points on 34 participants, increasing our power to see an effect, this does nothing to improve generalizability. Furthermore, conditioning upon tertiles of daily energy exposure decreased our number of observations per level of conditioning to an acceptable but admittedly small size. For this reason, we recommend these findings should be interpreted with caution and viewed as hypothesis-generating. With this said, our within-group subanalyses were remarkably consistent with our nonrestricted findings despite the substantial decrease in power. This implies a true signal of nutrition-induced oxidative stress worthy of further exploration. Finally, although our findings imply feeding is associated with increased oxidative stress, we are unable to comment on whether this increased oxidative stress indeed invoked harm. A larger study will be needed to parse out this information.

The nonvolitional provision of nutrition support is a medical nutrition therapy with nuanced benefits and consequences. Our pretense that we understand these nuances is holding us back from truly mastering this valuable tool. Future PRCTs are needed to explore the impact of feeding on oxidative stress as well as other mechanisms of harm in critical illness.

## Statement of Authorship

L. McKeever contributed to the conception of the research; L. McKeever and C. A. Braunschweig equally contributed to the design of the research; L. McKeever, S. J. Peterson, S. Cienfuegos, J. Rizzie, and O. Lateef contributed to the acquisition of the data; L. McKeever, C. A. Braunschweig, and S. Freels contributed to the analysis and interpretation of the data; L. McKeever drafted the manuscript. All authors critically revised the manuscript, agree to be fully accountable for ensuring the integrity and accuracy of the work, and read and approved the final manuscript.

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