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1 **Original Article**

2 **Exploration of muscle loss and metabolic state during prolonged critical illness:**
3 **implications for intervention?**

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17

18 Short title: Muscle loss and metabolic state during prolonged critical illness

19

20

21 **Abstract**

22 **Background:** Muscle wasting in the critically ill is up to 2% per day and delays
23 patient recovery and rehabilitation. It is linked to inflammation, organ failure and
24 severity of illness. The aims of this study were to understand the relationship between
25 muscle depth loss, and nutritional and inflammatory markers during prolonged critical
26 illness. Secondly, to identify when during critical illness catabolism might decrease,
27 such that targeted nutritional strategies may logically be initiated.

28 **Methods:** This study was conducted in adult intensive care units in two large
29 teaching hospitals. Patients anticipated to be ventilated for >48 hours were included.
30 Serum C-reactive protein (mg/L), urinary urea (mmol/24h), 3-methylhistidine
31 ($\mu\text{mol}/24\text{h}$) and nitrogen balance (g/24h) were measured on days 1, 3, 7 and 14 of the
32 study. Muscle depth (cm) on ultrasound were measured on the same days over the
33 bicep (bicep and brachialis muscle), forearm (flexor compartment of muscle) and thigh
34 (rectus femoris and vastus intermedius).

35 **Results:** Seventy-eight critically ill patients were included with mean age of 59
36 years (SD: 16) and median Intensive care unit (ICU) length of stay of 10 days (IQR: 6-
37 16). Starting muscle depth, 8.5cm (SD: 3.2) to end muscle depth, 6.8cm (SD: 2.2)
38 were on average significantly different over 14 days, with mean difference -1.67cm
39 (95%CI: -2.3 to -1cm), $p < 0.0001$. Protein breakdown and inflammation continued over
40 14 days of the study.

41 **Conclusion:** Our patients demonstrated a continuous muscle depth loss and
42 negative nitrogen balance over the 14 days of the study. Catabolism remained

43 dominant throughout the study period. No obvious ‘nutritional tipping point” to identify
44 anabolism or recovery could be identified in our cohort. Our ICU patient cohort is one
45 with a moderately prolonged stay. This group showed little consistency in data,
46 reflecting the individuality of both disease and response. The data are consistent with
47 a conclusion that a time based assumption of a tipping point does not exist. **Trial**
48 **Registration:** International Standard Randomised Controlled Trial Number:
49 ISRCTN79066838. Registration 25 July 2012.

50

51 **Keywords**

52 Critical illness, muscle breakdown, muscle depth loss, catabolism, nitrogen balance

53

54 **Introduction**

55 A problem common to virtually all intensive care unit (ICU) patients is the deterioration
56 in nutritional and functional status during and after their ICU stay, which has been
57 identified as a research priority (1; 2). In survivors of the acute respiratory distress
58 syndrome (ARDS) muscle weakness and fatigue determined long-term outcome, it
59 was shown that recovery time should be measured in months to years rather than
60 days to weeks (3). Muscle wasting and weakness were still evident one year after ICU
61 discharge in this cohort (3), whilst physical disability had been identified up to five
62 years later, with exercise capacity only reaching 76% of the predicted value (4). In a
63 similar cohort of ARDS survivors weight and fat mass increased 1 year after ICU
64 admission, whilst lean mass losses had not yet recovered (5).

65 Muscle wasting during critical illness is a multi-factorial process thought to be a
66 consequence of sepsis and inflammation, disuse atrophy, severity of illness (6;7) and
67 hypoxia *per se* (8). Muscle wasting may also be associated with increased cytokine
68 levels (9, 10) and glucocorticoid administration (11). Muscle protein breakdown rates
69 remained high over the first week of critical illness, whilst muscle protein synthesis
70 appeared to return to the level of a healthy fasted controls after seven days (7, 12).

71 Muscle mass declines early and rapidly, up to 2% per day during ICU stay (5, 7,13)
72 with significantly higher rates of wasting seen in patients with multi-organ failure than
73 those presenting in single organ failure (7). For the elderly, who are increasingly
74 represented in the intensive care population, these losses may be in addition to age-
75 related losses and will seriously compromise recovery to independence. Low skeletal
76 muscle mass and poor muscle quality on admission to the ICU has been shown to be

77 a risk factor for mortality (14; 15). Simply delivering more nutrients to critically ill
78 patients does not appear to ameliorate muscle wasting (7, 11).

79 A recent research agenda review paper highlighted the need to distinguish between
80 the different phases of critical illness (catabolic vs. anabolic phase) to help identify
81 patients' "readiness for enhanced feeding" (16). Targeting nutritional strategies in the
82 very acute catabolic phase may be counter-productive as over-feeding may be harmful
83 (17). Targeting interventions once peak catabolism has started to wane could
84 therefore potentially support patients into a recovery phase without doing any harm.
85 The aims of this exploratory research were two-fold:

- 86 1. To explore the relationship between muscle depth loss (measured via
87 ultrasound) and nutritional and inflammatory markers during prolonged critical
88 illness using standard bedside equipment.
- 89 2. To identify when during prolonged critical illness catabolism might decrease
90 such that targeted nutritional or pharmacological strategies could be initiated.

91

92

93 **Materials and methods**

94 The study received a favourable ethical review from Camden & Islington Research
95 Ethics Committee (10/H0722/40), which included approval of the consent procedure
96 for patients lacking capacity. This study was conducted in four adult intensive care
97 units (mixed medical, surgical and trauma patients) in two large London teaching
98 hospitals from September 2010 to February 2013. All patients anticipated to be
99 ventilated for >48 hours were considered for inclusion into this study. Participants were

100 excluded if they were permanently wheelchair bound; were not able to provide
101 retrospective consent due to learning difficulties, psychiatric reasons or dementia;
102 were receiving long term steroid treatment, had severe Parkinson's disease, had
103 bilateral amputations or if they were pregnant.

104 **Descriptive measures:** Serum C-reactive protein (CRP), urinary urea and 3-
105 methylhistidine (3-MH), nitrogen balance and total muscle depth were measured
106 serially in patients.

107 Muscle depth change (cm) was measured using a Sonosite M Turbo™ ultrasound
108 machine with a 5 MHz linear array transducer (Sonosite Ltd, Hitchin, Hertfordshire,
109 UK). Muscle depth change was assessed over the bicep, forearm and thigh following
110 the protocol described by Reid *et al* (13). Reid provided training to the investigator
111 using the following protocol:

112 Participants were supine with measurements made on the right side of the body, on
113 the bicep, forearm and thigh to mark a halfway point on the limb from which the
114 ultrasound measurement would be made. Markings were made in indelible ink to
115 ensure that the same site would be measured throughout the patient's ICU stay. Three
116 ultrasound measurements were made per site using the built-in electronic calliper on
117 a frozen real-time cross-sectional image. The average of three measurements for each
118 site was used, up to a 0.2cm difference was accepted. The mean value from the bicep,
119 forearm and thigh was combined to provide a daily total muscle depth (cm). A
120 substantial amount of ultrasound gel was applied to ensure that the probe could rest
121 gently on the skin without compressing muscle or distorting underlying soft tissue.

122

123 **Bicep:**

124 The elbow was flexed to 90degrees and a point on the skin was marked between the
125 tip of the olecranon and the acromion with indelible ink. With the elbow extended and
126 the patient in a supine position, the forearm was supinated. The ultrasound probe was
127 applied at the pen mark on the upper arm to obtain a cross-sectional (axial) view which
128 included the humerus, biceps and brachialis muscle, subcutaneous tissue and skin.

129 **Forearm:**

130 The patient's arm was extended and forearm kept in supinated position. A point
131 between the antecubital skin crease and the ulnar styloid was marked with indelible
132 pen. The ultrasound probe was applied at the pen mark on the radial (lateral) side of
133 the forearm to obtain a cross-sectional (axial) view which included the radius. The
134 thickness of the flexor compartment was measured anteriorly between the superficial
135 fat-muscle interface and the interosseous membrane; radial or lateral side of the
136 forearm.

137 **Thigh:**

138 The patient was supine with knee extended. A halfway point on the thigh was identified
139 and marked. The thickness of the quadriceps muscle group anteriorly between the
140 superficial fat-muscle interface and the femur was measured (Vastus intermedius and
141 Rectus Femoris). The ultrasound probe was applied at the pen mark on the anterior
142 surface of the thigh to obtain a cross-sectional (axial) view which included the femur,
143 quadriceps muscles, subcutaneous tissue and skin.

144 Reliability tests were performed between investigators prior to conducting this study,
145 intra-class correlation coefficients were 0.984, 95%CI (0.958-0.993) and 0.965, 95%CI

146 (0.882-0.990) respectively for intra- and inter-rater assessments, indicating good
147 reliability. Bland Altman data for intra-rater assessment: mean difference -0.05cm,
148 95% upper limit: 0.73cm, 95% lower limit: -0.83cm. Bland Altman data for inter-rater
149 assessment: mean difference 0.02cm, 95% upper limit: 1.04cm, 95% lower limit -
150 1.00cm.

151 C-reactive protein (mg/L) was measured from plasma samples collected on days 1, 3,
152 7 and 14 of the study. Urinary studies from study days 1, 3, 7 and 14 were analysed
153 in accredited clinical laboratories and included analysis of 3-methylhistidine
154 ($\mu\text{mol}/24\text{h}$), as a surrogate marker for skeletal muscle breakdown, and urinary urea
155 ($\text{mmol}/24\text{h}$). Urinary 3-methylhistidine was selected as it appears almost solely in
156 skeletal muscle protein and is not re-used.

157 Urinary 3-methylhistidine were analysed using an amino acid analyser with cation
158 exchange chromatography (JEOL UK Ltd., Hertfordshire, UK). Samples were
159 analysed using ninhydrin detection and one inferred standard to quantify amino acid
160 content. External quality assurance was provided by ERNDIM Quantitative Amino Acid
161 Scheme. Urine urea was measured by a kinetic urease using an Abbott Architect
162 assay with Abbott reagents (Abbott Diagnostics, Berkshire, UK). External quality
163 assurance was provided by UKNEQAS Urine Chemistries Scheme. No reference
164 ranges are provided for urinary urea or 3-MH excretion per 24h.

165 Nitrogen balance (g/day) was calculated using both the British Dietetic Association's
166 Parenteral and Enteral Nutrition Group (18) recommended equation and the Deacon
167 equation (19) for estimating nitrogen excretion, assuming nitrogen balance equals
168 nitrogen intake minus nitrogen excretion.

169

170 **Nitrogen balance equations**

171 The British Dietetic Association's Parenteral and Enteral Nutrition Group (PENG) nitrogen balance
172 equation (18):

173 urinary urea (mmol/24h) x 0.033 + obligatory losses (2-4g nitrogen/24h for hair, skin and faecal losses).
174 Add 0.6g nitrogen per 1 degree above 37.5°C.

175 The Deacon equation for nitrogen balance:

176 urea excretion (mmol/24h) x 0.028. Add 20% for other urinary losses and a further 2g/day for losses by
177 other routes (19).

178 Weight (kg), height (m) and body mass index (kg/m²) data were recorded in addition
179 to daily energy (kcal) and protein (grams) intakes. Recent weights and heights were
180 obtained from medical notes. If this was not available family members were asked or
181 weight was estimated by an experienced dietitian. If height data was not available
182 heights were estimated from obtaining ulnar length (measured from the acromion to
183 the ulnar styloid). Enteral feed tolerance was monitored by assessing gastric residual
184 volumes (ml) and incidence of vomiting. Patients were fed within 48h of ICU admission
185 according to the local ICU feeding protocol; energy requirements were calculated with
186 predictive equations (20; 21) and Propofol derived energy was included. No indirect
187 calorimetry was available. Protein requirements were estimated at 1.2-1.5g/kg/day
188 (21; 22).

189 **Statistical analysis:** Continuous data were tested for normality and presented
190 as mean (SD), mean (95% confidence intervals) or median (IQR). Categorical data
191 are presented as frequencies. Correlations were performed using the Spearman's
192 rank correlation test for non-parametric data. The difference between starting muscle
193 depth to end muscle depth was calculated by paired t-test. Descriptive statistical

194 analyses were performed using GraphPad Prism 6.02 for Windows (GraphPad
195 Software, La Jolla, CA). For missing data a 'last observation carried forward' approach
196 was followed. A comparison of data over the first vs. the second week of study was
197 also performed using the Wilcoxon matched-pairs signed rank test, GraphPad Prism
198 6.02 for Windows (GraphPad Software, La Jolla, CA). Significance was set at 0.05.

199

200 **Results**

201 Eighty patients were recruited to the study, two died prior to testing, 78 were included
202 in the final analysis, **Fig 1**. 'Other' exclusions (**Fig 1**) refer to patients unable to provide
203 retrospective consent due to learning difficulties or dementia, patients enrolled into
204 other studies, patients receiving long-term steroids and previously wheelchair bound
205 patients.

206 **Fig 1. Patient recruitment on ICU .**

207 The 78 patients were representative of longer- staying patients in mixed medical and
208 surgical intensive care units (**Table 1**) with mean (SD) age of 59 (16) years,
209 predominantly male (69%), with a mean (SD) APACHE II score of 21.6 (7.7) and
210 median (IQR) ICU length of stay of 10 (6-16) days. Patients were recruited within 72h
211 of their ICU admission; 'Day 1' refers to day 1 of study, which occurred between days
212 1-3 of the patient's ICU admission. APACHE II score was determined according to day
213 of admission to the ICU, not day 1 of study.

214

215

216 **Table 1. Patient demographics (N=78).**

Demographic factors	ICU patients N=78
Age (years)	
Mean (SD)	59 (16)
Range	24-89
Sex Male n (%)	54 (69%)
BMI (kg/m ²)	
Median (IQR)	26 (22 -31)
APACHE II score	
Mean (SD)	22 (7.7)
ICU Length of stay (days)	
Median (IQR)	10 (6 -16)
Diagnostic categories - ICU admission:	
Pneumonia	19 (24%)
Cardiology/Cardiac surgery	14 (18%)
Neurology/neurosurgery	9 (11%)
Sepsis/Septic Shock	8 (10%)
Vascular surgery	6 (7.6%)
Major Trauma	6 (7.6%)
Traumatic Brain Injury	6 (7.6%)
Gastroenterology	3 (3.8%)
Gastrointestinal Surgery	3 (3.8%)
HIV	2 (2.5%)
Multi-organ failure	1 (1.3%)
Renal Failure	1 (1.3%)

217 ICU: Intensive Care Unit; SD: standard deviation; IQR: interquartile range; BMI: Body Mass Index; APACHE II: Acute
 218 physiological and chronic health evaluation II; HIV: Human immunodeficiency virus.

219

220 The flow of patients through the study is summarised in **Fig 2**, illustrating numbers
 221 achieved for each test day. Twenty-three patients (29%) died in ICU. Four patients
 222 (5%) died in hospital after ICU discharge.

223

224 **Fig 2. CONSORT diagram for patient discharges, deaths and patients not**

225 **measured.** USS: ultrasound scanning; CRRT: Continuous Renal Replacement Therapy. Blood tests: C - reactive protein

226 and serum albumin. Urine testing not performed: day 1 (6 misplaced samples, 14 patients anuric/CRRT), day 3: (7 misplaced

227 samples, 23 patients anuric/CRRT), day 7: (4 misplaced samples, 14 patients anuric/CRRT); day 14: (3 patients anuric/CRRT).

228

229 There was a significant reduction in muscle depth as measured by ultrasound, over 7

230 and 14 days (using all available data) presented in **Table 2**. This data includes the

231 total of three sites, the bicep (bicep and brachialis muscle), forearm (flexor
 232 compartment of muscle) and thigh (rectus femoris and vastus intermedius). **Table 3**
 233 shows the clinical characteristics of long stay patients (N=17) studied over 14 days on
 234 ICU.

235 **Table 2. Muscle depth change (total of three sites bicep, forearm, thigh).**

		N=43 over 7 days	N=17 over 14 days
Starting muscle depth (cm)	Mean (SD)	7.6 (3.7)	8.5 (3.2)
End muscle depth (cm)	Mean (SD)	6.5 (3.1) *	6.8 (2.2) *
Mean difference (cm)	(95%CI)	-1.1 (-1.5 to -0.7)	-1.67 (-2.3 to -1)
P value		<0.0001	<0.0001

236 * paired t-test

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249 **Table 3. Clinical characteristics of long stay patients studied for 14 days (N=17)**

	Age	Sex	Diagnosis	PMH	APACHE II score	MV (days)	ICU LOS (days)
1	72	F	Emergency AAA repair, MOF	DM II; High cholesterol, HTN	24	7	40
2	73	F	Laparotomy: ischemic small bowel, cardiac arrest. MOF	MI, PVD, IHD, Parkinson's, COPD	17	31	51
3	59	F	Poor grade SAH for coiling, chest sepsis, MOF	Nil, previously fit and well	26	18	26
4	52	M	Pancreatitis, chest sepsis, MOF	Pancreatitis, ETOH, GORD, cholesterol, Cholecystectomy'09, smoker 10/d	17	38	57
5	80	M	VF arrest post CABG, cardiogenic shock, MOF	CHD, MI, TURP, T2DM, ESRF on HD, AF, AAA repair'97, Cholesterol	38	19	32
6	53	M	Reduced GCS, seizures, MOF	Decompensated ALD, encephalitis, Pulmonary TB, ex-smoker, previous drug taking history?	23	17	22
7	57	F	Lithium toxicity	Psychotic depression, Bell's palsy	21	14	19
8	28	M	Myocarditis due to pneumonia	Nil	14	26	27
9	44	M	PEA arrest, chronic pancreatitis, MOF	Chronic pancreatitis, 28yr ETOH history	29	37	37
10	55	F	MSSA septicaemia	Large intra-abdominal cystic mass	9	9	17
11	85	F	Sepsis secondary to myeloma, Encephalitis	HTN, multiple myeloma - chemo	13	8	17
12	85	M	Emergency AAA repair	HTN, cholesterol, angina	16	25	25
13	24	F	Traumatic brain injury, MOF	Nil	24	17	21
14	40	M	Alcoholic Liver Disease, MOF	ALD	26	40	40
15	73	F	Chest infection	DM, HTN	14	22	24
16	77	M	Emergency AAA repair + open abdomen, MOF	HTN	27	104	108
17	32	M	Major Trauma	Nil	18	14	16

250 AAA: abdominal aortic aneurysm; AF: atrial fibrillation; ALD: alcoholic liver disease; CABG: coronary artery bypass graft; COPD:
 251 chronic obstructive pulmonary disease; DM: Diabetes Mellitus; ESRF: end stage renal failure; ETOH: ethanol misuse; HD:
 252 haemodialysis; HTN: hypertension; IHD: ischaemic heart disease; LOS: length of stay; MI: myocardial infarction; MOF: multi-
 253 organ failure; MSSA: Methicillin-sensitive Staphylococcus aureus; MV: mechanical ventilation; PVD: peripheral vascular disease;
 254 SAH: subarachnoid haemorrhage; TURP: transurethral resection of the prostate; VF: ventricular fibrillation

256 A strong association was observed between blood concentration of CRP and %
257 muscle depth loss at day 14 ($r = -0.66$, $p=0.017$). No correlation was observed between
258 % muscle depth loss and age: $r = -0.04$, $p=0.8$; APACHE II score: $r = -0.124$, $p=0.44$;
259 SOFA score: $r = -0.14$, $p=0.40$ and nitrogen loss: $r = -0.02$, $p=0.92$.

260 The biochemical data suggest that a catabolic state persists up to the end of the 14
261 days data collection with raised inflammatory markers, progressive protein breakdown
262 and continuing loss of muscle depth (**Figs 4-7**). **Fig 7a** shows the negative nitrogen
263 balance in the study cohort ($N=55$) and **Fig 7b** demonstrates this negative balance in
264 the long stay patients only ($N=14$). Protein losses were included in calculations in **Fig**
265 **7a and b**. Visual representation of median values for urinary urea, percentage muscle
266 depth loss, 3-MH and CRP (**Figs 3-6**) in long stay patients all suggest a persisting
267 catabolic state up to day 14, as does the continuing negative nitrogen balance (**Fig 7a**
268 **and b**), however of interest is the small change in trajectory of inflammatory and
269 catabolic markers in the second week of the study data. Markers appear to improve
270 from day 7 to 14. Median change (IQR) over 14 days is presented in a **Supporting**
271 **information, S1 - S3 Tables**.

272

273 **Fig 3. Serial CRP (N=17) measurements in long stay patients: (i) Plots to explore**
274 **individual variability (ii) Box plots reported with median and IQR.** * CRP: C-reactive
275 protein. Medians and IQR are reported in the Supplementary Table (a), not on this figure due to wide variance.

276

277 **Fig 4. Serial urinary urea (N=14) measurements in long stay patients: (i) Plots to**
278 **explore individual variability (ii) Box plots reported with median and IQR.**

279 **Fig 5. Serial 3MH (N=14) measurements in long stay patients: (i) Plots to explore**
 280 **individual variability (ii) Box plots reported with median and IQR.** 3-MH: 3-methylhistidine.
 281 Medians and IQR are reported in the Supplementary Table (a), not on this figure due to wide variance.

282

283 **Fig 6. Serial % muscle depth change (N=17) measurements in long stay patients:**
 284 **(i) Plots to explore individual variability (ii) Box plots reported with median and**
 285 **IQR.**

286

287 **Fig 7. Nitrogen balance in (a) whole study cohort (N=55) and (b) long stay**
 288 **patients (N=14) over 14 days.**

289

290 In four of our patients that remained in ICU over 14 days the reduction in Day 14 CRP
 291 did not correspond with a change in surrogates for catabolism (urinary urea and 3-
 292 MH) with day 14 results still raised, **Table 4.**

293 **Table 4. Cases where CRP reduced at Day 14 whilst surrogate markers for**
 294 **catabolism (urinary urea and 3-MH) remained high.**

Patient	CRP1	CRP7	CRP14	UUrea1	UUrea7	UUrea14	3MH1	3MH7	3MH14
1	68.1	71.3	3	46	183	266	471	380	224
2	110	211.9	48.2	221.2	436.59	277.76	244	342	233
3	64.6	32.4	27	153	528.2	182	183	333	102
4	279.1	210.9	13.8	473.3	723	802.8	305	299	556

295

296

297 Energy balance (difference between estimated energy targets via predictive equations
298 and energy intake) and protein balance (difference between estimated protein targets
299 and protein intake) in patients remaining on the unit for ≥ 7 days (N=43) is shown in
300 **Fig 8**. Energy (and protein) targets were estimated as no indirect calorimetry was
301 available in this study.

302 **Fig 8. Predicted energy and protein balance of patients on the unit for ≥ 7 days**
303 **(N=43) over 14 days.**

304 Data were also compared between the first week and the second week of study, and
305 although they were not found to be significantly different between the two weeks,
306 graphical representation appears to suggest a modest difference, **Fig 9**.

307 **Fig 9. Data comparing the first versus the second week of study: urinary urea**
308 **(N=14), 3-MH (N=14), CRP (N=17) and muscle loss (N=17).**

309

310 **Discussion**

311 Our observational study focused on patients with a prolonged stay in ICU; the median
312 length of stay in our study is longer than national or international figures, because we
313 recruited only patients expected to be ventilated for >48 hours. These are patients with
314 greater original insults or more complications that result in longer lengths of stay. This
315 is illustrated by the day 14 data when 17 study patients still remained on ICU; 11 of
316 these remained ventilated, two were on non-invasive ventilation, and four were self-
317 ventilating. Although long stay patients may only represent a small group, they are an
318 important cohort as they are resource intensive and may have outcomes that are
319 worse than patients with an ICU stay of less than 10 days (23).

320 This is the first study, to our knowledge, that used clinical markers for inflammation,
321 nutrition and catabolism in conjunction with muscle depth change measured by
322 ultrasound to explore metabolic state during a period of critical illness. Results
323 demonstrate progressive muscle depth loss (**Table 2**), negative nitrogen balance (**Fig**
324 **7**), continued catabolism and marginal improvement of inflammation, in patients who
325 survive beyond 7 days (**Figs 3-6**). These results demonstrate an intense catabolic
326 state in line with others' work (24;25).

327 Our data are however highly heterogeneous with substantial individual variation.
328 Inspection of our data does not appear to show an obvious "nutrition tipping point"
329 within the studied cohort where anabolism or recovery could be identified, granted
330 within a modest sample size. Additionally, the suggestion that CRP could potentially
331 be used as a surrogate to define recovery or anabolism as inflammation subsides, has
332 not been shown in our data. There appears to be a lack of relationship between CRP,
333 which was used as a surrogate for inflammatory state, and markers of catabolism and
334 muscle breakdown (24hr urinary urea and 3-MH). For some of the patients day 14
335 CRP results had effectively normalised yet day 14 urinary urea and 3MH were still
336 significantly raised, indicating ongoing catabolism. This points to the need for
337 individual monitoring using a number of markers to understand catabolism.

338 Although we initially aimed to identify a 'nutritional tipping point', which could be used
339 to predict whether patients remained catabolic or not using logistic regression, it was
340 difficult to define 'catabolism' accurately from the markers measured in this study. If
341 nitrogen balance, urinary urea and urinary 3-methylhistidine are used as surrogates
342 for catabolism, there is potential for missing data for patients on renal replacement
343 therapy.

344 Enteral feeding should commence early in the critically ill, within 24-48h of ICU
345 admission (21; 22), what is less clear is how much we should feed patients during the
346 first weeks of illness. Tailoring energy targets to the metabolic phase of critical illness
347 is widely discussed (16; 26), however determining how to identify the phases remains
348 challenging. It has been recognised that a 'dynamic marker' is required to help identify
349 which patients might be ready for increased feeding. This type of marker would ideally
350 be able to track changes in endocrine and metabolic markers (16). Yet there is no
351 agreed definition on the exact description of the phases, when they might occur or
352 how one would identify the transition point between them. ESPEN guidelines (21)
353 recently expanded the definition beyond the 'ebb' and 'flow phase', with an early acute
354 phase (where patients have metabolic instability and with increasing catabolism) and
355 a late acute phase (where metabolic disturbances have started to settle down whilst
356 significant muscle wasting is observed). Beyond 7 days has been defined as the 'post-
357 acute phase' in which patients either progress onto rehabilitation, or remain in a
358 'persistent inflammatory or catabolic state', which will require a prolonged period of
359 hospitalisation (21). Regardless of the definition used our impression is that
360 identification of these phases and the transition points between them will be very
361 challenging to determine, particularly at a population level. We believe that there is
362 need to move towards a more individualistic system, but how this can be achieved
363 remains uncertain. Whether metabolomic profiling could be used as future way to
364 distinguish metabolic phases (26) remains to be seen.

365 It is also not yet known whether catabolism could be reduced early, or at all during ICU
366 stay. Future strategies to reduce early catabolism might require as yet unidentified
367 pharmacological interventions or, more speculatively, nutritional interventions coupled
368 with physical activity or a combination thereof. Timing, dose and duration of nutritional

369 intervention are all important factors to consider in future studies. Nutritional
370 interventions combined with physical activity would likely provide the best opportunity
371 for recovery, since muscle anabolism is enhanced by the synergistic action of amino
372 acids and resistance exercise (27-29). How nutritional interventions and physical
373 activity relate to longer term physical and functional recovery of ICU patients also
374 needs to be explored.

375 Our patients lost on average 1.2% of muscle depth per day over 14 days, which
376 confirms previous studies that report muscle depth losses between 1-2% per day
377 (7;13). Reid *et al* (13) reported a median 1.6% muscle depth loss per day in 48 ICU
378 patients over 7 days, and Puthucheary *et al* (7) showed muscle losses, in patients with
379 similar demographics to the current study, of 1.7% per day over 10 days. This group
380 also showed that muscle breakdown remained elevated during the first week on ICU,
381 whilst synthesis returned to normal by the end of the first week (7). Patients in this
382 cohort were in negative energy and protein balance (**Fig 8**), a common finding in many
383 recent clinical trials in ICU (30-32). Negative energy and protein balances will
384 contribute to muscle depth loss and catabolism, however they are unlikely to be the
385 sole cause of ICU-related muscle wasting (13;33).

386 Muscle ultrasound offers a practical and feasible method to serially quantify muscle
387 mass or volume change in the ICU environment, in even the sickest patients.
388 Ultrasound can have large inter-observer variability (22), however reliable measures
389 are possible and have previously been reported (34). When using this technique it is
390 vital to ensure assessors are fully trained and intra and inter-rater reliability is tested.
391 We carried out extensive training and found good intra- and inter-rater reliability was
392 achieved by our assessors. A recent systematic review highlights the increased
393 potential value and excellent reported reliability of muscle ultrasound (34).

394 As with all clinical studies undertaken in the intensive care environment there are a
395 number of limitations. As previously mentioned this study cohort consisted of a group
396 of patients with a moderately prolonged stay on ICU, which limits the wider applicability
397 of the findings. The attrition of patients from the study cohort due to discharge from
398 ICU to wards has meant a large amount of missing data at later time points,
399 complicating the interpretation of the data. We were unable to follow patients up on
400 the wards due to limited resources. We would recommend that follow up after
401 discharge from ICU should be included in future research. Similarly mortality in the
402 study cohort made data interpretation more difficult as some of the data presented is
403 from patients who ultimately died. Additionally, patients on continuous renal
404 replacement therapy were excluded from urine collection studies. Protein losses in the
405 critically ill could be underestimated from urinary urea (35), and muscle breakdown
406 may not be the sole contributor to 3-MH excretion as actin is present in other cells
407 (36). Additionally, as 3-MH is derived from actin and myosin, breakdown reflects that
408 of all muscle which may include skeletal, smooth and cardiac muscle. Using 3-MH as
409 a surrogate measure for muscle breakdown in conjunction with other outcome
410 measures to detect muscle or protein breakdown may reduce this limitation. Energy
411 expenditure was estimated using predictive equations rather than measured by
412 indirect calorimetry as calorimetry was not available. Energy and protein balances may
413 appear artificially low in our study as balances were calculated over a 24h period rather
414 than accounting for part days on the ICU. Finally, day 1 of study may not necessarily
415 be the first day of the patient's ICU admission (median ICU stay at day 7 of study was
416 8 days).

417

418 **Conclusion**

419 Strategies to limit muscle loss are much needed in this population to aid recovery, and
420 the timing of such strategies may be crucial to their success. Our patients
421 demonstrated a continuous muscle depth loss and negative nitrogen balance over the
422 14 days of the study. Catabolism remained dominant throughout the study period
423 whilst there appeared to be a marginal improvement in inflammation. No obvious
424 ‘nutritional tipping point’ to identify anabolism or recovery could be identified in our
425 cohort. Our ICU patient cohort is one with a moderately prolonged stay; a very
426 important group due to their high resource requirement. This group showed little
427 consistency in data, reflecting the individuality of both disease and response. Thus the
428 data are consistent with a conclusion that a time based assumption of a tipping point
429 does not exist. This supports an argument for individually characterising patient state
430 and personalising the approach; be that with a yet to be identified metabolic marker,
431 a novel method or a device.

432

433 **Key messages**

- 434 • In this ICU patient cohort with moderately prolonged stay, catabolism remained
435 elevated throughout 14 days follow up even though inflammation improved
436 marginally.
- 437 • CRP does not appear to be a good surrogate marker to assess anabolism and
438 recovery.
- 439 • Our data did not demonstrate an obvious time point where anabolism starts to
440 exceed catabolism or ‘nutritional tipping point’.

- 441 • There is a need to individually characterise patient state and personalise the
442 approach.

443

444 **Abbreviations**

445 APACHE II: Acute Physiology and Chronic Health Evaluation score II; ARDS: acute
446 respiratory distress syndrome; CRP: C - reactive protein; ICU: Intensive care unit; IQR:
447 Interquartile range; 3-MH: 3-methylhistidine; PENG: parenteral and enteral nutrition
448 group; SOFA: Sequential Organ Failure Assessment.

449 **Declarations**

450 **Ethics approval and consent to participate**

451 The study has ethical approval from Camden & Islington Research Ethics Committee
452 (10/H0722/40). In line with the Mental Capacity Act 2005 for persons who lack capacity
453 ‘assent’ was sought from a family member; retrospective consent was sought once
454 appropriate.

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577 **Supporting information**

578 **S1 Tables. All long stay patients: Median biomarker change (IQR) over 14 days**
579 **on ICU.**

580 **S2 Table. Survivors: Median biomarker change (IQR) over 14 days on ICU.**

581 **S3 Table. Non-survivors (ICU and hospital deaths): Median biomarker change (IQR)**
582 **over 14 days on ICU.**

583

584