Sepsis uncouples serum C-peptide and insulin levels in critically ill patients with type 2 diabetes mellitus

Laurent Bitker*, Salvatore L Cutuli*, Luca Cioccari, Eduardo A Osawa, Lisa Toh, Nora Luethi, Helen Young, Leah Peck, Glenn M Eastwood, Johan Mårtensson and Rinaldo Bellomo

Sepsis triggers stress-induced hyperglycaemia,¹ and glycaemic instability is a marker of illness severity.²⁻⁵ Hyperglycaemia may impact survival by affecting the immune system's response to infection.¹,⁶⁻⁷ In contrast, pro-insulin connecting peptide (C-peptide), whose release by pancreatic β-cells is coupled with that of endogenous insulin, acts as an inflammation-modulating agent; it inhibits leukocyte adhesion, pro-inflammatory cytokine secretion, and reactive oxide species production.⁸⁻¹¹ Patients with type 2 diabetes mellitus (T2DM) are a specific population with abnormal pancreatic function, and an increased risk of both hyperglycaemia and infections.¹²⁻¹⁴ Evidence suggests that permissive moderate hyperglycaemia in such patients may be safe, and that in those with poor pre-admission glycaemic control, strict glycaemic control increases mortality.¹⁵,¹⁶ A large intensive care unit (ICU) trial showed that intensive insulin therapy increased mortality, compared with targeted mild hyperglycaemia.¹⁷,¹⁸ Taken together, these elements suggest that sepsis-induced hyperglycaemia may stimulate the endogenous pancreatic response, which may be downregulated by exogenous insulin therapy.¹⁹ Such pancreatic upregulation would increase circulating C-peptide levels, with potentially beneficial immune-modulating effects.

Hence, we hypothesised that, in patients with T2DM, critical illness and sepsis would increase C-peptide levels, and uncouple C-peptide levels from endogenous insulin levels. Moreover, we hypothesised that this C-peptide response would be inhibited by administering exogenous insulin. Accordingly, we measured pancreatic peptide levels in a cohort of septic and non-septic critically ill patients with T2DM, and compared these with pancreatic peptide levels in healthy subjects. We also evaluated the impact of insulin therapy on pancreatic peptide levels in the critically ill cohort.

Material and methods
This single-centre observational prospective study was approved by the Austin Health Human Research Ethics

ABSTRACT

Objective: To assess the effects of sepsis and exogenous insulin on C-peptide levels and C-peptide to insulin ratios in intensive care unit (ICU) patients with type 2 diabetes mellitus (T2DM).

Design, setting and participants: In this prospective, observational, single-centre study, we enrolled 31 ICU-admitted adults with T2DM. We measured serum C-peptide and insulin levels during the first 3 days of ICU stay and recorded characteristics of exogenous insulin therapy. Patients were compared on the basis of the presence of sepsis, and their exposure to exogenous insulin therapy. C-peptide levels were also measured in eight healthy subjects.

Main outcome measures: Serum insulin and C-peptide levels during the first 3 days in ICU.

Results: Median C-peptide levels were higher in the ICU population compared with healthy subjects (10.9 [IQR, 8.2–14.1] vs 4.8 [IQR, 4.6–5.1] nmol/L, P < 0.01). Sepsis was present in 25 ICU patients (81%). Among ICU patients unexposed to exogenous insulin, the 11 patients with sepsis had higher median C-peptide levels compared with the six non-septic patients (2.5 [IQR, 1.8–3.7] vs 1.7 [IQR, 0.8–2.2] nmol/L, P = 0.04), and a threefold higher C-peptide to insulin ratio (45 [IQR, 37–62] vs 13 [IQR, 11–17], P = 0.03). However, septic patients exposed to exogenous insulin had lower median C-peptide levels (1.2 [IQR, 0.7–2.3] nmol/L, P = 0.01) and C-peptide to insulin ratios (5 [IQR, 2–10], P < 0.01) compared with insulin-free septic patients. The C-peptide to insulin ratio was significantly associated with white cell count and severity of illness in insulin-free septic patients.

Conclusion: C-peptide levels were elevated in critically ill patients with T2DM. In this population, sepsis increased C-peptide levels and uncoupled serum C-peptide and insulin levels. Exogenous insulin decreased both C-peptide levels and C-peptide to insulin ratios.
Table 1. Characteristics of patients included in the study*

<table>
<thead>
<tr>
<th>Variables</th>
<th>Patients with sepsis (n = 11)</th>
<th>Patients without sepsis (n = 6)</th>
<th>Patients with sepsis and insulin exposure (n = 14)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>69 (65–77)</td>
<td>60 (59–72)</td>
<td>66.5 (53.2–77.5)</td>
<td>0.56</td>
</tr>
<tr>
<td>Sex (male)</td>
<td>5 (45%)</td>
<td>2 (33%)</td>
<td>10 (71%)</td>
<td>0.23</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>109 (80–121)</td>
<td>99 (80–101)</td>
<td>90 (77–99)</td>
<td>0.34</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>34.2 (26.2–47.8)</td>
<td>35.0 (33.1–36.9)</td>
<td>30.7 (26.8–37.9)</td>
<td>0.64</td>
</tr>
<tr>
<td><strong>Admission characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medical admission</td>
<td>10 (91%)</td>
<td>4 (67%)</td>
<td>14 (100%)</td>
<td>0.03</td>
</tr>
<tr>
<td>Surgical admission</td>
<td>1 (9%)</td>
<td>2 (33%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><strong>Sepsis description</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.86</td>
</tr>
<tr>
<td>Primary source of infection</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory</td>
<td>6 (55%)</td>
<td>—</td>
<td>9 (64%)</td>
<td></td>
</tr>
<tr>
<td>Biliary</td>
<td>1 (9%)</td>
<td>—</td>
<td>1 (7%)</td>
<td></td>
</tr>
<tr>
<td>Soft tissue</td>
<td>0</td>
<td>—</td>
<td>3 (21%)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>3 (27%)</td>
<td>—</td>
<td>1 (7%)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>1 (9%)</td>
<td>—</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Positive bacterial culture</td>
<td>8 (73%)</td>
<td>—</td>
<td>8 (57%)</td>
<td>0.68</td>
</tr>
<tr>
<td>Bacteraemia</td>
<td>4 (36%)</td>
<td>—</td>
<td>4 (29%)</td>
<td>0.99</td>
</tr>
<tr>
<td>Septic shock†</td>
<td>3 (27%)</td>
<td>—</td>
<td>4 (29%)</td>
<td>0.47</td>
</tr>
<tr>
<td><strong>Severity of disease</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>APACHE III score</td>
<td>69.0 (65.0–77.5)</td>
<td>59.0 (41.5–66.8)</td>
<td>68.5 (47–79)</td>
<td>0.47</td>
</tr>
<tr>
<td>SOFA score at inclusion</td>
<td>8 (6–9)</td>
<td>6 (5–8)</td>
<td>9 (6 to 11)</td>
<td>0.30‡</td>
</tr>
<tr>
<td><strong>Organ support§ and outcome</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mechanical ventilation</td>
<td>7 (64%)</td>
<td>4 (67%)</td>
<td>12 (86%)</td>
<td>0.48</td>
</tr>
<tr>
<td>Vasopressor requirement</td>
<td>6 (55%)</td>
<td>3 (50%)</td>
<td>12 (86%)</td>
<td>0.15</td>
</tr>
<tr>
<td>Renal replacement therapy</td>
<td>1 (9%)</td>
<td>0</td>
<td>2 (14%)</td>
<td>0.99</td>
</tr>
<tr>
<td>Enteral nutrition</td>
<td>0</td>
<td>0</td>
<td>3 (21%)</td>
<td>0.28</td>
</tr>
<tr>
<td>ICU mortality</td>
<td>1 (9%)</td>
<td>0</td>
<td>2 (14%)</td>
<td>0.99</td>
</tr>
<tr>
<td><strong>Biochemistry at inclusion</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemoglobin (g/L)</td>
<td>110 (94–123)</td>
<td>113 (103–118)</td>
<td>107 (94–138)</td>
<td>0.86</td>
</tr>
<tr>
<td>White cell count (x 10⁹/L)</td>
<td>11.4 (8.8–15.3)</td>
<td>10.6 (10.1–11.2)</td>
<td>11.4 (9.4–15.3)</td>
<td>0.71</td>
</tr>
<tr>
<td>Serum creatinine (µmol/L)</td>
<td>179 (162–324)</td>
<td>88 (71 to 92)</td>
<td>148 (78–298)</td>
<td>0.12</td>
</tr>
<tr>
<td>pH</td>
<td>7.36 (7.29–7.42)</td>
<td>7.44 (7.4–7.49)</td>
<td>7.39 (7.33–7.43)</td>
<td>0.38</td>
</tr>
<tr>
<td>Bicarbonate (mmol/L)</td>
<td>22 (19–25)</td>
<td>26 (24–28)</td>
<td>24 (21–25)</td>
<td>0.06</td>
</tr>
<tr>
<td>Base excess (mmol/L)</td>
<td>–3 (−6 to −1)</td>
<td>3 (1–4)</td>
<td>−1 (−2 to 1)</td>
<td>0.06</td>
</tr>
<tr>
<td>Lactate (mmol/L)</td>
<td>1.6 (1.2–2.2)</td>
<td>1.1 (0.9–2.5)</td>
<td>1.6 (1.3–2.4)</td>
<td>0.42</td>
</tr>
</tbody>
</table>

APACHE = Acute Physiology, Age, and Chronic Health Evaluation. BMI = body mass index. ICU = intensive care unit. IQR = interquartile range. SOFA = Sequential Organ Failure Assessment. * Data are median (IQR) or number (%). † Septic shock was defined as the presence of sepsis, vasopressor requirement and lactate level > 2 mmol/L. ‡ No significant difference in individual organ failure SOFA scores. § In the 24 hours preceding Day 1.
Table 2. Glycaemic control at inclusion*

<table>
<thead>
<tr>
<th>Variables</th>
<th>Patients with sepsis (n = 11)</th>
<th>Patients without sepsis (n = 6)</th>
<th>Patients with sepsis and insulin exposure (n = 14)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-ICU glycaemic control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OHA dependency</td>
<td>10 (91%)</td>
<td>4 (67%)</td>
<td>12 (86%)</td>
<td>0.56</td>
</tr>
<tr>
<td>Insulin dependency</td>
<td>2 (18%)</td>
<td>3 (50%)</td>
<td>5 (36%)</td>
<td>0.32</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>6.6% (6.0–7.3%)</td>
<td>7.2% (6.1–7.7%)</td>
<td>7.7% (6.5–9.6%)</td>
<td>0.33</td>
</tr>
<tr>
<td>Pre-inclusion glycaemic control†</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exogenous insulin</td>
<td>0</td>
<td>0</td>
<td>14 (100%)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Exogenous insulin dose (U/kg)</td>
<td>—</td>
<td>—</td>
<td>0.4 (0.1 to 0.5)</td>
<td></td>
</tr>
<tr>
<td>Insulin analogues‡</td>
<td>1 (9%)</td>
<td>2 (33%)</td>
<td>4 (29%)</td>
<td>0.47</td>
</tr>
<tr>
<td>Secretagogues§</td>
<td>2 (18%)</td>
<td>1 (17%)</td>
<td>2 (14%)</td>
<td>0.99</td>
</tr>
</tbody>
</table>

HbA1c = glycated haemoglobin A1c. ICU = intensive care unit. OHA = oral hypoglycaemic agent. * Data are median (IQR) or number (%). † In the 24 hours before inclusion. ‡ Insulin aspart, lispro or glargine. § Sulfonylureas or dipeptidyl peptidase-4 inhibitors.

Committee (Melbourne, Australia; LNR/14/Austin/487), which waived the need for informed consent.

Study cohort

We prospectively assessed all consecutive adult patients (aged ≥ 18 years) with previously diagnosed T2DM who were admitted to the ICU of a tertiary metropolitan teaching hospital (Austin Hospital, Victoria, Australia). We excluded patients with diabetic ketoacidosis or hyperglycaemic hyperosmolar syndrome, those who were pregnant, and those with a predicted ICU length of stay of < 48 hours.

Definitions of sepsis and exposure to exogenous insulin

Sepsis was diagnosed according to the Sepsis-3 criteria. Sepsis-associated organ dysfunction was assessed with the Sequential Organ Failure Assessment (SOFA) score, and the inflammatory response with the white cell count. We defined exposure to exogenous insulin as any insulin therapy (intravenous or subcutaneous, short- or long-acting, bolus or continuous infusion) administered during the 24 hours before the time of C-peptide and insulin measurement.

Definition of study groups

Patients were classified into three groups according to sepsis diagnosis and exposure to exogenous insulin during the 24 hours preceding study inclusion. The first two groups were unexposed to exogenous insulin before inclusion and comprised patients with sepsis (Sepsis + / Insulin –) and without sepsis (Sepsis – / Insulin –). The third group comprised patients with sepsis who were exposed to insulin therapy before study inclusion (Sepsis + / Insulin +).

Pancreatic peptide measurements

Serum insulin and C-peptide levels were measured at study inclusion (Day 1) and on the following 2 days (defining a 3-day observation period). We used a Cobas e602 analyser and Elecsys electrochemiluminescence immunoassays to measure serum insulin and C-peptide levels (Roche Diagnostics, Mannheim, Germany). Serum insulin concentrations were converted from mU/L to nmol/L using a multiplying factor of 7.175 × 10⁻³, as per the assay guidelines.

Although the C-peptide to insulin ratio in the portal circulation is 1:1, this ratio increases up to 10:1 in the systemic circulation of fasting healthy subjects, owing to rapid insulin hepatic clearance and slower C-peptide renal excretion. Hence, and in addition to the analysis of the absolute values of serum C-peptide and insulin levels, we calculated the C-peptide to insulin ratio. We also calculated the C-peptide to glycaemia ratio to assess the endogenous response to a given glycaemia level in non-fasting subjects.
as clinically indicated, and the values obtained closest to the time of C-peptide and insulin measurements are reported. Capillary glucose measurements were deemed unreliable. 25

Glycaemic control therapy during ICU admission

Glycaemic control followed a published protocol targeting moderate permissive hyperglycaemia (blood glucose 10–14 mmol/L). 15 Exogenous insulin was prescribed if blood glucose was > 14 mmol/L. We recorded details of exogenous insulin (cumulative dose and route of administration), oral hypoglycaemic agents (classified as secretagogues or non-secretagogues) and insulin analogues that were administered over the 24 hours preceding each daily measurement of serum C-peptide and insulin. 26,27

Collection of other patient data

We recorded patients’ demographic data, including pre-morbid diabetes-specific therapy, glycated haemoglobin A1c (HbA1c) level, and Acute Physiology, Age and Chronic Health Evaluation III (APACHE III) and SOFA scores. 21,28,29 Requirements for vasopressor therapy, mechanical ventilation, renal replacement therapy and nutrition (enteral or parenteral) were also reported.

Statistical analysis

We analysed data using the R software (version 3.3.1, The R Foundation, Vienna, Austria) with package lme4. 30,31 A P value below 0.05 was considered statistically significant. Continuous variables
ORIGINAL ARTICLES

Figure 2. C-peptide to insulin ratio versus severity of sepsis†

† The figure shows C-peptide levels versus white cell count (A) and number of organ failures (B) on Day 1 in patients unexposed to exogenous insulin (n = 17). The cumulative number of acute organ dysfunctions was assessed as the sum of positive organ-level SOFA score (cardiovascular, coagulation, liver, neurological, renal or respiratory score ≥ 1). The C-peptide to insulin ratio was significantly higher in patients with a white cell count > 15 × 10⁹/L (n = 3), compared with those for whom white cell count was ≤ 10 × 10⁹/L (n = 6), and significantly higher in those with four or more acute organ dysfunctions (n = 5) compared with those with 1 or 2 organ dysfunctions (n = 6). In each boxplot, the broad line represents the median value, and the lower and upper limits correspond to the first quartile (Q1) and third quartile (Q3), respectively. The whiskers correspond to the minimum and maximum values (Q1 – 1.5 × interquartile range, and Q3 + 1.5 × interquartile range), and the dots represent outliers (values above maximum or below minimum values). *** P < 0.05 compared with the reference level.

were expressed as median with interquartile range (IQR), and categorical variables as number with percentage.

Comparisons of baseline variables between the three study groups were performed using the Kruskal–Wallis test for continuous variables, and completed by a between-group post-hoc analysis using Dunn's test. Categorical variables were compared using Fisher's exact test. Comparisons between study groups of variables collected over the 3-day study period were performed using mixed-effects models, accounting for the repetition of measurements. When relevant, they were completed by a pairwise comparison of study groups, adjusted for multiple comparisons using the Tukey method.

Finally, we explored the associations of daily measurements of C-peptide, and of C-peptide to serum insulin ratio, using multivariable linear mixed-effects regression models (adjusted for the in-patient repetition of measurements), with an a priori set of relevant variables.

Results

Between 1 May 2016 and 2 April 2018, we assessed 31 critically ill patients with T2DM. Patients were enrolled within a median of 12 hours (IQR, 6–17 hours) of ICU admission. SEPSIS-3 criteria were met by 25 patients (81%). The characteristics of study patients are presented in Table 1, and their premorbid and baseline glycaemic control results are shown in Table 2.

Effect of critical illness on pancreatic peptide levels

Compared with healthy volunteers, serum glucose, C-peptide and insulin levels were significantly higher in critically ill patients with T2DM. However, their C-peptide to insulin ratios were similar (Online Appendix, available at cicm.org.au/Resources/Publications/Journal, Table 1).

Effect of sepsis on pancreatic peptide levels in insulin-free patients

In the absence of exogenous insulin exposure before inclusion, the 11 patients with sepsis had significantly higher C-peptide levels and C-peptide to insulin ratios at baseline than the six patients without sepsis (Figure 1). Baseline C-peptide to insulin ratios of septic patients unexposed to exogenous insulin were threefold higher than those for unexposed non-septic patients. Also, C-peptide to insulin ratios were significantly higher in patients with a high white cell count, and in those with four or more acute organ dysfunctions (Figure 2). Liver dysfunction did not affect the ratio of pancreatic peptides, but renal dysfunction did (Online Appendix, Figure 2).

During ICU admission, C-peptide levels differed significantly on all study days between the septic and non-septic groups (Online Appendix, Table 2), but serum insulin and serum creatinine did not. Daily C-peptide to insulin ratios remained significantly higher in insulin-free septic patients compared with insulin-free non-septic patients (Figure 3).

Effect of exogenous insulin exposure

The 14 patients with sepsis who were exposed to insulin therapy before inclusion had significantly higher blood glucose and serum insulin levels, and significantly lower C-peptide levels and C-peptide to insulin ratios, compared with insulin-free patients with sepsis (Figure 1). During the 3-day observation period, serum insulin levels remained significantly higher, while C-peptide levels and C-peptide to insulin ratios were significantly lower in insulin-exposed septic patients, compared with insulin-free septic patients (Online Appendix, Table 2).
Independent predictors of C-peptide levels and C-peptide to insulin ratios

On multivariable analysis, C-peptide levels were negatively associated with exogenous insulin dose and creatinine levels, and positively associated with elapsed time since inclusion (Table 3). Using the same explanatory variables, the C-peptide to insulin ratio was independently and negatively associated with glycaemia and administered dose of exogenous insulin, and positively associated with sepsis diagnosis (Table 3).

Discussion

In a prospective cohort of critically ill patients with T2DM, we assessed the concurrent effects of sepsis and exogenous insulin therapy on pancreatic peptides. First, we observed higher C-peptide and insulin levels in all critically ill patients with T2DM compared with healthy volunteers. Second, sepsis was associated with an increase in C-peptide levels and an uncoupling of C-peptide and serum insulin levels in insulin-free patients, in relation with the magnitude of the inflammatory response and the severity of illness. Third, exogenous insulin administration to septic patients was independently associated with a decrease in C-peptide levels.

Relationship to previous studies

Stress-induced hyperglycaemia has been thoroughly scrutinised in sepsis. In line with previous reports, we found that critical illness and sepsis triggered hyperglycaemia and hyperinsulinaemia secondary to development of acute insulin resistance. In this regard, we also found elevated C-peptide levels, comparable to those reported for critically ill patients with and without T2DM, and confirmed the impact of renal function on C-peptide levels. In addition, we found that exogenous insulin inhibited C-peptide release, in line with the findings of other researchers. To our knowledge, this is the first study to address the specific association between sepsis and high C-peptide levels in critically ill patients with T2DM.

The effects of C-peptide on the inflammatory response have previously been reported. In a mouse model of liposaccharide-induced endotoxemic shock, C-peptide infusion improved survival and alleviated acute lung injury. When combined with zinc, C-peptide administration reduced bacterial clearance, and improved survival in a mouse model of polymicrobial sepsis. In the present study, we observed higher circulating C-peptide levels, proportional to serum insulin levels, in exogenous insulin-free patients with high white cell count, suggesting an insulin-independent circulation of C-peptide triggered by inflammation. We observed higher C-peptide to insulin ratios in insulin-free patients with four or more acute organ dysfunctions. We hypothesise that the pancreatic response to critical illness triggers a release of C-peptide related to its immune role. Conversely, this could be related to modified hepatic or renal clearance of pancreatic peptides. However, after adjustment for renal function, sepsis remained associated with higher C-peptide to insulin ratio in our multivariable analysis. Also, we found no association between acute liver dysfunction and pancreatic peptide levels. Finally, catecholamines inhibit insulin secretion by pancreatic β-cells. However, this would not have affected the molar relationship between serum insulin and C-peptide.

Implications of study findings

Our findings show that sepsis increases C-peptide levels, and that this increase is greater than the increase in insulin...
levels. In line with the immune-modulating effects of C-peptide, we found that this dissociation of C-peptide and serum insulin levels was related to the number of circulating white cells and the cumulative number of organ failures. These findings may reflect increased C-peptide release by pancreatic \( \beta \)-cells during sepsis as a counter-regulatory, potentially protective, endocrine response to inflammation. Moreover, our findings highlight that exogenous insulin therapy inhibits such C-peptide release. Taken together, patients who required exogenous insulin may be more severely ill owing to more depressed endogenous pancreatic function. Finally, based on our results, future studies should test whether permissive hyperglycaemia and decreased exogenous insulin doses significantly affect the host immune response to sepsis.

**Strengths and limitations**

Our study has several strengths. It addresses the previously unexplored area of assessing pancreatic function in sepsis. The findings have biological implications relating to the potential immunological impact of exogenous insulin therapy in sepsis. To our knowledge, this is the first study to assess both C-peptide and serum insulin levels in a way that enables a detailed assessment of pancreatic peptides in patients with T2DM and sepsis, after accounting for the confounding effect of exogenous insulin. Moreover, we used the SEPSIS-3 consensus definitions to define sepsis, and obtained high data granularity in terms of glycaemic control therapies. The analysis was also strengthened by the use of mixed-effects multivariable models, accounting for the within-patient repetition of daily measurements. Finally, measurements in healthy volunteers allowed comparison of our findings with controls.

Our study also has several limitations. First, it is an observational study, thus any associations cannot be used to infer causality. However, our previously reported findings on the inhibitory effects of intensive insulin therapy on C-peptide levels are consistent with our results from the present study.

### Table 3. Variables associated with daily C-peptide levels and C-peptide to insulin ratio during ICU stay*

| Variables                          | Multivariable analysis | C-peptide level (nmol/L) | C-peptide to insulin ratio | P
<table>
<thead>
<tr>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>[\beta \text{ estimate} \pm \text{ SE}]</td>
<td>(\beta \text{ estimate} \pm \text{ SE})</td>
</tr>
</tbody>
</table>
| Age (per 10 year increase)         | –0.01 ± 0.02          | 0.63                     | –0.1 ± 0.3               | 0.59
| Pre-morbid insulin-treated T2DM    | 1                      | 1                        | 1                         | 1
| No                                 | 0.3 ± 0.5              | –3.7 ± 7.1               | 0.48                      |
| Yes                                | –0.1 ± 0.1             | 0.46                     | –1.6 ± 1.7               | 0.48
| HbA1c (per 1% increase)            | –0.01 ± 0.01           | 0.62                     | 0 ± 0.2                  | 0.85
| APACHE III score (per 1 point increase) | 0.5 ± 0.06          | 25.0 ± 9.3               | 0.56                      |
| Sepsis                             | 0.5 ± 0.6              | 25.0 ± 9.3               | 0.56                      |
| No                                 | 0.5 ± 0.06             | 25.0 ± 9.3               | 0.56                      |
| Yes                                | –0.1 ± 0.1             | 0.46                     | –1.6 ± 1.7               | 0.48
| Glycaemia (per 1 mmol/L increase)† | 0 ± 0.1                | 0.98                     | –1.9 ± 0.6               | < 0.05
| Serum insulin levels (per 1 mmol/L increase) | 1.1 ± 0.5            | 0.06                     | —                        | —
| Serum creatinine (per 100 mmol/L increase)† | 0.01 ± 0.00        | < 0.01                   | 0 ± 0.02                 | 0.91
| Exogenous insulin dose (per 1 U/kg increase)† | –1.3 ± 0.3           | < 0.01                   | –16.0 ± 3.5              | < 0.01
| Artificial nutrition modality‡     | 0.7 ± 0.4              | 5.5 ± 5.5                | 0.74                      |
| None                               | 1.9 ± 1.3              | 0 ± 13.4                 | 0.74                      |

**APACHE** = Acute Physiology, Age, and Chronic Health Evaluation. HbA1c = glycated haemoglobin A1c. ICU = intensive care unit. SE = standard error. T2DM = type 2 diabetes mellitus. * Analysis was performed on all available daily measurements (\(n = 86\)), and accounted for the within-patient repetition of daily measurements; \(P\) values were calculated using bootstrapping. † Reported on a daily basis, value closest to measurement. ‡ Administered over the 24 hours preceding measurement.
Moreover, the effects of C-peptide administration observed in experimental models of sepsis strongly support its role as an immune regulatory peptide.\textsuperscript{19,38} Second, our study was conducted in a single centre, thus limiting its external validity. However, our ICU has all the characteristics of a tertiary teaching centre and we recruited a broad spectrum of medical and post-operative patients. Third, no power calculation was performed before undertaking the study, and our study enrolled a limited number of patients, therefore cautious interpretation of statistical test results is needed. However, no data on the C-peptide to insulin ratio was available in this population at the time of study design, and all performed tests accounted for the small number of patients and the non-parametrical distribution of data. Fourth, C-peptide and serum insulin levels were measured in the systemic circulation, whereas pancreatic peptides are first released into the portal circulation. However, many of their effects are outside the portal system, making serum levels physiologically relevant. Also, serum insulin levels were obviously contaminated to an unknown extent in patients treated with exogenous insulin. Finally, we did not compare our results to a non-diabetic cohort with sepsis. However, this would have required a major change to our local protocol for glycaemic control in patients without diabetes. Nonetheless, studies of serum C-peptide and insulin levels in such patients would be highly valuable.

**Conclusion**

In critically ill patients with T2DM, sepsis is associated with increased C-peptide levels and an uncoupling of C-peptide and serum insulin levels. This uncoupling is enhanced in hyper-inflammatory states and with greater severity of illness. However, when exogenous insulin is administered, C-peptide levels decrease and the ratio is significantly lowered. Our findings suggest that a specific pancreatic response to sepsis may exist, and that it may be inhibited by exogenous insulin administration.

**Competing interests**

None declared.

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**References**

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