

## Pancreatic secretion, hepatic extraction, and plasma clearance of insulin from steady-state insulin and C-peptide measurements in critically ill patients

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**Abstract:** Hyperglycaemia is prevalent in critical illness and clinical practice is to reduce blood glucose by intravenous insulin infusion. We estimated pancreatic secretion, hepatic first-pass extraction ratio and plasma insulin clearance per minute from measured serum C-peptide and insulin in 9 critically ill adults in a pseudo steady-state situation, i.e. when nutrition and insulin infusion remained at a constant rate up to the test (min 20, median 240, max 510 min). To estimate pancreatic secretion, a population C-peptide kinetics model was used to convert C-peptide concentration to C-peptide secretion rate. Pancreatic secretion varied 18-fold from 5.2 to 93.5 mU·min<sup>-1</sup>, and the lowest secretion rates were in patients older than 70 years or patients with type 2 diabetes. Pancreatic secretion correlated positively, but not significantly with blood glucose. Blood glucose was not correlated with plasma insulin. A two-dimensional regression analysis of hepatic first-pass extraction and plasma insulin clearance showed that the smallest relative error between estimated plasma insulin and measured plasma insulin was obtained for an extraction ratio of 72% and plasma clearance of 0.34 min<sup>-1</sup>. Using these values, a negative correlation was found between post-hepatic insulin production and the rate of insulin infusion. These results indicate that 1) hepatic insulin extraction is increased in critical illness. This is also confirmed by the observation that steady-state plasma insulin concentrations in this study were relatively lower when compared to steady-state measurements in normal subjects; 2) blood glucose drives pancreatic secretion moderately; 3) there is substantial variation in pancreatic secretion between patients that cannot be explained from the blood glucose variation, but could be related to patient age and diabetic state; 4) there was also substantial variation in insulin sensitivity between patients, since similar levels of insulin sensitivity would have predicted a negative correlation between blood glucose and plasma insulin; 5) insulin infusions are used to compensate for inadequate pancreatic insulin secretion.

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### 1. INTRODUCTION

In the intensive care unit (ICU) more than half of the patients are affected by systemic inflammatory response syndrome (SIRS) to a degree sufficient to produce hyperglycaemia (Brun-Buisson, 2000; Jiménez-Ibáñez et al., 2012). There is both theoretical and experimental evidence to suggest that reduced insulin sensitivity is a part of the SIRS syndrome and that the hyperglycaemia seen in these patients is therefore a combination of increased mobilization of the intracellular glycogen reserves and a reduced ability to control the resulting glucose load, due to reduced insulin sensitivity (Dungan et al., 2009). In healthy individuals, insulin secretion increases if insulin sensitivity is reduced (Mari et al., 2005). The compensatory increase has been verified in obesity, in pregnancy and after glucocorticoid administration and similarly, a decrease in insulin secretion has been observed following exercise and weight loss (Ahrén and Pacini, 2004). Data from hyperglycaemic ICU patients indicate that the post-injury plasma insulin and C-peptide concentrations are elevated (Schmitz et al., 1984; Koch et al., 2010) and Duška and Anděl (2008) used a multiple regression model to demonstrate a relatively greater contribution by endogenous insulin secretion to plasma

insulin concentration than by exogenous insulin infusion. Since clinical practice attempts to reduce hyperglycaemia by exogenous insulin infusions or injections, the assessment of insulin secretion of the critically ill is important, in particular to avoid hypoglycaemic episodes (Ali et al., 2008).

We conducted a clinical study to assess endogenous insulin secretion *in vivo* from arterial blood samples of critically ill patients with similar primary disease background and hyperglycaemia, who at the time of the study were treated with varying infusions of exogenous insulin or received no exogenous insulin. Because C-peptide is co-secreted with insulin on an equimolar basis from the pancreatic beta cell and is not extracted by the liver (Horwitz et al., 1975), any change in the secretion rate of insulin induces a change in the plasma concentration of C-peptide. Thus, plasma concentrations of C-peptide can be used to derive rates of insulin secretion under steady-state conditions provided that the mean clearance rates of C-peptide are constant. This assumption has been shown to be valid by Faber et al. (1978) over a wide range of C-peptide levels observed under normal physiologic conditions. Steady-state conditions can be approximated with the use of glucose clamping techniques, such as the hyperinsulinemic-euglycemic clamp originally proposed by DeFronzo et al. (1979). However, in the ICU

this type of experiment interferes heavily with the intensive care treatment of the patient and could be potentially harmful. Measurements were therefore attempted taken in a pseudo steady-state situation, i.e. in a situation where insulin infusion and nutrition by enteral or parenteral route, remained unchanged up to the taking of the blood samples. Insulin has a plasma half-life of 4-7 min (Faber et al. (1978)) and it was attempted to keep the insulin infusion rate and the nutrition at a constant rate for at least an hour up to the test. Using the two-compartment C-peptide kinetics model initially proposed by Eaton et al. (1980) and applying population parameters developed by Van Cauter et al. (1992) for Eaton's model, we tried to assess the relative roles of endogenous and exogenous insulin in critical illness.

## 2. METHODS

### 2.1 Patients

Nine ventilated adult ICU patients with traumatic brain injury, multiple trauma or subarachnoid haemorrhage were studied. Table 1 summarizes the basal characteristics of the studied patients. Exclusion criteria were insulin-dependent (type 1) diabetes, pregnancy, nursing and missing consent from relatives. Written informed consent for participating in the experiment was obtained from the patient's closest relatives. The Research Ethics Committee of North Jutland approved the study.

### 2.2 Study design

The study experiment was carried out while patients were routinely cared for, during the daytime hours with the help from a nurse assigned to take the blood samples for this study. All patients were unconscious and hyperglycaemic. Seven patients were treated with continuous intravenous insulin infusions. It was attempted to keep insulin infusion and nutrition constant for at least an hour (min 20, median 240, max 510 min) up to taking the blood samples. Only in 2 patients occurred a minor adjustment (from 6.5 to 7.5 U/h and from 5.0 to 5.5 U/h) within the hour that preceded the test. Because of the short half-life of insulin in plasma and due to the small size of the changes, these cases were not excluded from the following analysis. For the test, two samples for each patient, one for measuring the blood glucose and one for assaying insulin and C-peptide, were taken within one minute from the same arterial line. Plasma glucose was measured by blood gas analysis (ABL700 series, Radiometer Medical ApS, Brønshøj, Denmark). Arterial blood samples for measuring C-peptide and insulin concentrations from serum were centrifuged at 20°C and subsequently frozen at -80°C; the samples were analysed in batches by fluoroimmunoassay at Steno Diabetes Center, Gentofte. The intra- and interassay coefficients of variation in the C-peptide assay were 4.7 and 4.5%, respectively, according to the data sheet from Steno.

### 2.3 Data analysis

**Table 1 Basal characteristics**

Nr	Age	Sex	BMI (kg·m <sup>-2</sup> )	DM	SAPS II
1	72	F	23.6		34
2	46	M	33.6	Y	21
3	75	M	29.4		52
4	65	F	26.8		37
5	61	M	46.0	Y	60
6	44	F	23.8		21
7	68	M	26.8		52
8	40	F	32.1		n.a.
9	53	F	27.5		57

BMI, body mass index; DM, Diabetes mellitus (type 2); SAPS, Simplified Acute Physiology Score

Eaton et al. (1980) postulated a two-compartment model consisting of intravascular and extravascular exchanging pools to describe C-peptide kinetics. Mathematically, the model can be expressed in the form:

$$\frac{dC(t)}{dt} = -(k_1 + k_3)C(t) + k_2Y(t) + S(t) \quad (1)$$

$$\frac{dY(t)}{dt} = k_1C(t) - k_2Y(t) \quad (2)$$

where  $C(t)$  is the total amount of C-peptide in the plasma at time  $t$ ;  $Y(t)$  is the total amount of C-peptide in all extravascular sites;  $k_1$ ,  $k_2$  and  $k_3$  are fractional turnover rates; and  $S(t)$  is the endogenous rate of production of C-peptide.

Reformulating (1) and (2) in terms of concentrations with  $c(t) = C(t)/V_P$  and  $y(t) = Y(t)/V_Q$  yields:

$$\frac{dc(t)}{dt} = -(k_1 + k_3) \cdot c(t) + k_2 \frac{y(t) \cdot V_Q}{V_P} + \frac{S(t)}{V_P} \quad (3)$$

and

$$\frac{dy(t)}{dt} = k_1 \frac{c(t) \cdot V_P}{V_Q} - k_2 \cdot y(t) \quad (4)$$

where  $V_P$  and  $V_Q$  denote the distribution spaces in plasma and all extravascular sites, respectively. Assuming a steady-state situation where the derivatives  $dc(t)/dt$  and  $dy(t)/dt$  are zero, (3) and (4) become:

$$0 = -(k_1 + k_3) \cdot c_0 + k_2 \frac{y_0 \cdot V_Q}{V_P} + \frac{S_0}{V_P} \quad (5)$$

where  $c_0$ ,  $y_0$  and  $S_0$  are the steady-state values of  $c(t)$ ,  $y(t)$  and  $S(t)$ , respectively, and

$$0 = k_1 \frac{c_0 \cdot V_P}{V_Q} - k_2 \cdot y_0 \quad (6)$$

By rearranging (6)

$$k_2 \cdot y_0 = k_1 \frac{c_0 \cdot V_p}{V_Q},$$

subsequent substitution in (5) and solving for  $S_0$  we obtain

$$S_0 = k_3 \cdot c_0 \cdot V_p \quad (7)$$

the steady-state secretion rate of C-peptide.

C-peptide kinetics are usually determined in a separate experiment by analysing the C-peptide decay curve following a bolus injection of biosynthetic C-peptide. Van Cauter et al. (1992) developed equations to calculate population-based C-peptide kinetic parameters. We used these equations to calculate  $k_3$  and  $V_p$  of each patient (see Appendix A). Using (7) and the measured arterial C-peptide concentrations, we calculated the endogenous C-peptide production rates for the assumed steady-state situation. The rate of endogenous insulin production  $Q$  is the same as  $S$ , since C-peptide is co-secreted with insulin on an equimolar basis, and thus:

$$S(t) = Q(t) \quad (8)$$

The newly secreted insulin is delivered to the circulation in the portal vein and passes through the liver, where it undergoes first-pass extraction. We define  $h$  as the fractional hepatic extraction of the first-pass of endogenously released insulin and  $Q_h$  as the rate of hepatic first-pass extraction as follows:

$$Q_h(t) = Q(t) \cdot h \quad (9)$$

The rate of endogenous insulin that reaches the systemic circulation after first-pass extraction is denoted  $Q_{ph}$  and is related to the secretion rate in the following way:

$$Q_{ph}(t) = Q(t) \cdot (1-h) \quad (10)$$

The estimated change in plasma insulin concentration resulting from post-hepatic insulin delivery can be expressed as:

$$\frac{dI_{est(ph)}(t)}{dt} = -k \cdot I_{est(ph)}(t) + \frac{Q_{ph}(t)}{V_p} \quad (11)$$

where  $k$  is the fractional clearance of insulin from plasma by all peripheral tissues including removal by liver and kidneys.  $V_p$  is the plasma distribution volume of insulin, which is assumed equal to the C-peptide distribution volume calculated from Van Cauter et al.'s (1992) equations.

Similar to (11), the estimated change in plasma insulin concentration resulting from exogenous insulin infusion alone can be expressed as:

$$\frac{dI_{est(ex)}(t)}{dt} = -k \cdot I_{est(ex)}(t) + \frac{Q_{ex}(t)}{V_p} \quad (12)$$

where  $Q_{ex}(t)$  is the exogenous insulin infusion. The kinetics of endogenous and exogenous insulin is identical and, thus  $k$  has the same value in (11) and (12). The total change in

**Table 2 Plasma insulin, plasma glucose, and serum C-peptide**

Nr	$Q_{0ex}$ (mU·min <sup>-1</sup> ·kg <sup>-1</sup> )	BM (kg)	BG (mmol·l <sup>-1</sup> )	$I_{0a}$ (mU·l <sup>-1</sup> )	$c_0$ (pmol·l <sup>-1</sup> )
1	0.71	59	7.3	24.8	212
2	1.09	115	6.5	57.7	118
3	1.88	80	7.5	114.1	736
4	0.69	72	7.0	42.0	1149
5	1.63	133	8.0	112.7	249
6	1.43	64	8.0	62.9	1118
7	0	85	8.0	25.8	2750
8	1.32	95	7.3	92.3	940
9	0	75	10.0	14.1	1937

$Q_{0ex}$ , steady-state exogenous insulin infusion rate; BM, body mass; BG, blood glucose;  $I_{0a}$ , measured arterial plasma insulin;  $c_0$ , measured arterial C-peptide

plasma insulin resulting from post-hepatic and exogenous insulin can be estimated from:

$$\frac{dI_{est}(t)}{dt} = -k \cdot I_{est}(t) + \frac{Q_{ph}(t) + Q_{ex}(t)}{V_p} \quad (13)$$

where  $I_{est}(t) = I_{est(ph)}(t) + I_{est(ex)}(t)$ . Inserting (10) in (13) under steady-state conditions where the derivative  $dI_{est}(t)/dt$  is zero, the estimated insulin concentration becomes:

$$I_{0est} = \frac{Q_0 \cdot (1-h) + Q_{0ex}}{k \cdot V_p} \quad (14)$$

where  $I_{0est}$ ,  $Q_0$  and  $Q_{0ex}$  are steady-state values.  $I_{0est}$  is dependent on values of  $k$  and  $h$ , and  $k$  defines the steady-state relation between post-hepatic insulin and exogenous insulin infusion.

Using (14), we estimated joint parameter values for  $k$  and  $h$  as parameters in a two-dimensional regression analysis that minimized the root mean squared error between calculated  $I_{0est}$  values and measured arterial plasma insulin concentrations  $I_{0a}$ :

$$\min \sqrt{\frac{1}{N} \sum (I_{0a} - I_{0est})^2} \quad (15)$$

We also analysed cross-correlations by calculating Pearson's correlation coefficients between the following variables: measured arterial concentrations of plasma blood glucose (BG), serum C-peptide ( $c_0$ ) and plasma insulin ( $I_{0a}$ ), exogenous insulin infusion ( $Q_{0ex}$ ), calculated endogenous insulin secretion ( $Q_0$ ) and the steady-state post-hepatic insulin delivery ( $Q_{0ph}$ ) using a significance value of  $p < 0.05$ .

### 3. RESULTS

The results from the experiment are shown in Table 2 and Table 3. The mean BG was 7.7 mmol/l. The mean  $I_{0a}$  was  $60.7 \pm 38.1$  mU·l<sup>-1</sup> for all nine patients, and  $72.3 \pm 41.1$  mU·l<sup>-1</sup> only for the seven patients with continuous insulin infusion, respectively. There was no correlation between BG and  $I_{0a}$ .

**Table 3 Results of the regression analysis**

Nr	$Q_0$ (mU·min <sup>-1</sup> )	$Q_{0ph}$ (mU·min <sup>-1</sup> )	$I_{0est(ph)}$ (mU·l <sup>-1</sup> )	$I_{0est(ex)}$ (mU·l <sup>-1</sup> )	$I_{0est}$ (mU·l <sup>-1</sup> )
1	5.97	1.97	1.6	39.7	41.3
2	5.23	1.73	0.7	61.1	61.8
3	23.20	7.66	4.5	105.4	109.9
4	34.86	11.50	7.5	39.0	46.6
5	11.14	3.68	1.3	91.5	92.8
6	34.86	11.50	8.5	80.5	89.0
7	93.54	30.87	17.1	0.0	17.1
8	35.86	11.83	5.9	73.9	79.8
9	61.37	20.25	12.7	0.0	12.7

$Q_0$ , endogenous insulin production rate;  $Q_{0ph}$ , post-hepatic insulin delivery rate;  $I_{0est(ph)}$ , estimated plasma insulin concentration resulting from post-hepatic insulin delivery;  $I_{0est(ex)}$ , estimated plasma insulin concentration resulting from exogenous insulin infusion;  $I_{0est}$  estimated plasma insulin concentration; all values estimated for an assumed steady-state

The mean intravenous insulin infusion rate (Patient 1—6 and 8) was 1.25 mU·min<sup>-1</sup>·kg<sup>-1</sup> and was also not correlated with  $BG$ .

The mean C-peptide concentration for all patients was 1023 ± 867 pmol·l<sup>-1</sup> and the highest arterial C-peptide concentrations were found in Patients 7 and 9 (the two patients without insulin infusion).

The regression analysis in the parameters  $k$  and  $h$  showed that the smallest root mean squared error (13.5 mU·l<sup>-1</sup> or 22%) between the estimated insulin concentration  $I_{0est}$  and the measured insulin concentration  $I_{0a}$  was obtained for  $k = 0.34$  min<sup>-1</sup> and  $h = 0.72$ . This means that only 28% of endogenously produced insulin reaches the systemic circulation after first-pass extraction and that the clearance of insulin from plasma is 34% every minute.

Table 3 shows the resulting model variables from using  $k$  and  $h$  with these estimated values. The endogenous insulin production  $Q_0$  varied 18-fold, from 5.23 to 93.54 mU·min<sup>-1</sup>.  $Q_0$  correlated moderately, but not significantly so, with  $BG$  ( $r = 0.62$ ,  $p = 0.08$ ). The difference in  $Q_0$  could not be explained by the variation in  $BG$ : A counterexample is for example the 8-fold difference in  $Q_0$  in the three patients who all had a measured  $BG$  of 8 mmol·l<sup>-1</sup> (Patient 5, 6 and 7). Relative to the other patients,  $Q_0$  was significantly lower in patients who were older than 70 years (Patient 1 and 3) or who had a diagnosis of type 2 diabetes (Patient 2 and 5).

A significant negative correlation was found between insulin infusion rate and post-hepatic insulin delivery, using (10) and the estimated value of  $h$  from the regression analysis to calculate  $Q_{0ph}$ .

#### 4. DISCUSSION

An advantage of our experimental design was that we studied a patient cohort under routine clinical care conditions and that the experimental setup did not disrupt clinical treatment. We studied only a small number of patients and the results should not be generalized; however, the results with regards to the large difference of endogenous insulin secretion, up to 18-

fold reduction in older patients and patients with type 2 diabetes, deserves clinical awareness.

We found no correlation between  $BG$  and  $I_{0a}$ . This indicates that there were substantial differences also in insulin sensitivity between patients, since similar levels of insulin sensitivity in all patients would have predicted a negative correlation between  $BG$  and  $I_{0a}$ , i.e. that patients with high  $I_{0a}$  would have low  $BG$  and patients with low  $I_{0a}$  would have high  $BG$ . Furthermore, the difference in insulin sensitivity between patients also predicts varying insulin infusion requirements. We did not find a correlation between  $BG$  and  $Q_{0ex}$  either. This absence is compatible with a clinical protocol, where the insulin infusion rate is increased until a clinically acceptable reduction in  $BG$  has been achieved.

In a previous study by Ferrannini et al. (1983) a mean infusion rate of 1.0 mU·min<sup>-1</sup>·kg<sup>-1</sup> induced a mean plasma concentration of 90 ± 8 mU·l<sup>-1</sup> in healthy study participants. In comparison to this study, we measured a lower mean concentration of 72.3 ± 41.1 mU·l<sup>-1</sup> despite a higher mean insulin infusion of 1.25 mU·min<sup>-1</sup>·kg<sup>-1</sup>. We also found that the model-estimated values for  $k$  and  $h$  were higher than data published from studies with healthy humans. A previous review of data from the literature published by Arleth et al. (2000) reported a steady-state conversion factor of 98.1 kg·min·l<sup>-1</sup> between insulin infusion and insulin concentration. In comparison, by regression analysis we estimated a value  $k = 0.34$  that corresponds to a steady-state conversion factor of only 56.2 kg·min·l<sup>-1</sup> between insulin infusion and insulin concentration. Finally, the here estimated hepatic first-pass extraction ratio  $h = 0.72$  is higher than reported by Ferrannini et al. (1983), where the mean extraction ratio was 0.64 in healthy humans. Thus, both the experimental data (lower measured  $I_{0a}$  concentrations) and the model estimates ( $h$  and  $k$ ) indicate an overall increase of insulin clearance in our studied patient group.

The correlation between  $BG$  and  $Q_0$ , although not significant, indicates that  $BG$  moderately drives the endogenous insulin production. However, the substantial variation in  $Q_0$  between the patients cannot be explained by the variation in  $BG$ . For example, we found a significant reduction in  $Q_0$  between very old patients (>70 years) or with type 2 diabetes diagnosis compared with the other patients ( $p < 0.004$ ). This finding is interesting in a clinical situation when the exogenous insulin infusion requirement is determined.

The considerably high difference in  $Q_0$  in response to elevated blood glucose levels corresponds with the finding of the negative correlation between post-hepatic insulin delivery  $Q_{0ph}$  and exogenous insulin infusion  $Q_{0ex}$ . This means that insulin infusions are largely used to compensate for the reduced ability to produce adequate rates of endogenous insulin when blood glucose is elevated.

Two patients (Patients 3 and 6) were treated with norepinephrine, a commonly used vasopressor in critical care. Norepinephrine impairs insulin sensitivity and is a known inhibitor of insulin production in stress or during exercise. However, a recent study showed no significant changes of steady-state C-peptide and insulin levels when

norepinephrine was administered in clinically relevant pressor doses (Khoury and McGill (2011)). Further research about the influence of clinical vasopressor use on insulin secretion during critical illness is necessary.

## 5. CONCLUSION

The overall picture emerging from the present study is that insulin clearance is increased in critical illness. Large differences in insulin sensitivity levels and in insulin production rates are apparent and must be accounted for when decisions regarding exogenous insulin infusions are made in critical care.

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## APPENDIX A

The C-peptide model parameters  $k_3$  and  $V_p$  are calculated from the method proposed by Van Cauter et al. (1992), in which the amplitudes and time constants of a double-exponential decay are estimated as functions of the individual's age, sex, weight, body surface area, body mass index and diagnosis of type 2 diabetes. The formulas to calculate these parameters are shown in Table 4.

**Table 4 Identification of C-peptide kinetic parameters**

Steps	Normal (BMI < 30 kg·m <sup>-2</sup> )	Obese (BMI ≥ 30 kg·m <sup>-2</sup> )	Type 2 diabetes
1. Short half life $t_{1/2-short}$ (min)	4.95	4.55	4.52

2. $F$	0.76	0.78	0.78
3. Long half life $t_{1/2-long}$	$0.14 \cdot (\text{age [yr]}) + 29.2$		
4. Plasma volume $V_P$	Female: $1.11 \cdot BSA + 2.04$ Male: $1.92 \cdot BSA + 0.64$ With $BSA = \sqrt{\frac{\text{height[cm]} \cdot \text{weight[kg]}}{3600}}$		
5. C-peptide kinetic parameters	$k_2 = F \cdot (b-a) + a$ $k_3 = a \cdot b / k_2$ $k_1 = a + b - k_2 - k_3$ With $a = \ln 2 / t_{1/2-short}$ $b = \ln 2 / t_{1/2-long}$		

$BMI$ , body mass index;  $BSA$ , body surface area ( $m^2$ )