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# The Relationship Between Plasma Concentration and Plasma Disappearance Rate of Immunoreactive Insulin in Normal Subjects

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**Summary.** To investigate the mechanism of insulin degradation in normal subjects, a kinetic model of insulin disappearance was constructed: insulin was assumed to be extracted from plasma by two independent processes, one saturable and one non-saturable. On the basis of these assumptions, a linear (non-proportional) relationship between steady-state plasma insulin concentration and steady-state plasma disappearance rate was predicted over the concentration range studied. Constant infusion experiments were performed on eight healthy normal subjects, normoglycaemia and fasting plasma C-peptide concentrations being maintained during the experiments. Agreement was found between the predictions of the model and the experimental results, and it is concluded that insulin degradation in normal subjects may be described in terms of two processes: one that is saturated at physiological plasma insulin concentrations and one that is apparently non-saturable over a wide concentration range.

**Key words:** Insulin, insulin degradation, kinetics of insulin disappearance, constant infusion technique.

Plasma disappearance of immunoreactive insulin in normal man and animals has been studied extensively during the past decade. In a series of publications [1–4], proportionality between plasma concentration and degradation rate of insulin is assumed to exist without evidence over the range of plasma insulin concentrations studied (approximately 400–2300 pmol/l). Ooms et al. [5] and Franckson and Ooms [6] demonstrated from perfusion experiments on normoglycaemic dogs the existence of a saturable hepatic pathway and a virtually non-saturable renal pathway of insulin degradation. Sönksen et al. [7, 8] reported results from constant infusion experiments on normal subjects consistent with the existence of two such pathways. However, it has been pointed out that interpretation of these results is difficult due to the severe hypoglycaemia occurring in the experiments [3].

Therefore, the present study was designed to investigate the relationship between steady-state plasma insulin concentration and steady-state insulin disappearance rate in normoglycaemic normal subjects during constant (fasting) endogenous insulin release.

## Patients and Methods

### *Model*

1) One pathway of insulin degradation (possibly hepatic) is assumed to be saturable, such that insulin degradation rate ($V_h$, pmol · kg$^{-1}$ · min$^{-1}$) is a function f (C) of plasma insulin concentration (C, pmol/l) as follows:

$$f(0) = 0$$
$$\lim_{C \to \infty} f(C) = a$$

2) in another pathway of insulin degradation (possibly renal) insulin degradation rate ($V_r$, pmol · kg$^{-1}$ · min$^{-1}$) is assumed to be proportional to C over the range of plasma insulin concentrations studied: $V_r = b \cdot C$.

3) alternative pathways of insulin degradation are neglected. Total insulin degradation rate (V, pmol · kg$^{-1}$ · min$^{-1}$) is

$$V = V_h + V_r = f(C) + b \cdot C$$

and for "high" values of C,

$$V = a + b \cdot C$$

In the steady-state, total insulin degradation rate equals the sum of exogenous insulin infusion rate ($I_{ex}$, pmol · kg$^{-1}$ · min$^{-1}$) and the constant (but otherwise unknown) rate of endogenous insulin release ($I_{end}$, pmol · kg$^{-1}$ · min$^{-1}$)

$$V = I_{end} + I_{ex} = a + b \cdot C$$

$$C = -\frac{(a - I_{end})}{b} + \frac{1}{b} I_{ex}$$

(for "high" values of C)

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**Table 1.** Individual plasma insulin concentrations in eight healthy volunteers during intravenous insulin infusion

| Insulin infusion rate (pmol $kg^{-1}$ $min^{-1}$) | Time of blood sampling (min)[a] | Plasma insulin concentration (pmol/l) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Subject 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| 0 | −5 | 25 | 42 | 32 | 39 | 86 | 46 | 46 | 53 |
| | 0 | 18 | 40 | 30 | 34 | 93 | 35 | 46 | 49 |
| | | (22) | (41) | (31) | (36) | (90) | (40) | (46) | (51) |
| 7.18 | 25 | 215 | 258 | 251 | 388 | 474 | 402 | 438 | 582 |
| | 30 | 230 | 373 | 345 | 359 | 431 | 409 | 481 | 639 |
| | | (222) | (316) | (298) | (374) | (452) | (406) | (460) | (610) |
| 14.4 | 55 | 668 | 1077 | 862 | 1127 | 1235 | 1005 | 840 | 2082 |
| | 60 | 783 | 991 | 862 | 1091 | 1285 | 1012 | 912 | 1795 |
| | | (726) | (1034) | (862) | (1109) | (1260) | (1009) | (876) | (1938) |
| 21.5 | 85 | 1321 | 2578 | – | 1680 | 2262 | 1651 | 1436 | 3159 |
| | 90 | 1027 | 2491 | 1321 | 2082 | 2341 | 1580 | 1580 | 3087 |
| | | (1174) | (2534) | – | (1881) | (2302) | (1616) | (1508) | (3123) |
| 28.7 | 115 | 2319 | 3540 | 1860 | 2807 | 3145 | 2657 | 2513 | 4954 |
| | 120 | 2563 | 3138 | 1860 | 2886 | 3145 | 2728 | 2441 | 4452 |
| | | (2441) | (3339) | (1860) | (2846) | (3145) | (2692) | (2477) | (4703) |

[a] Zero denotes the beginning of the first 30 min insulin infusion period. Mean values given in parentheses

### *Subjects*

The experiments were performed on eight healthy volunteers with body weights from 90 to 120% of average body weight [9], taking no medication and with no family history of diabetes mellitus. All subjects had fasting blood glucose concentrations between 2.9 and 3.9 mmol/l, a normal ECG and normal plasma concentrations of sodium, potassium and bicarbonate ions, phosphate, creatinine, alkaline phosphatase and aspartate aminotransferase.

All subjects (medical students, nurses and doctors) gave their informed consent, and the experimental design was approved by the National Health Service of Denmark.

### *Procedure*

The experiments began at 09.00 h after an overnight fast. No prior carbohydrate quantified diet was given. Crystalline porcine insulin (Insulin Leo Neutral, Nordisk Insulin Laboratorium) with 10 g/l human albumin was infused IV in one arm, using a programmable infusion pump [10]. The following insulin infusion rates were used: 0–30 min after the start of the experiment 1 mU $kg^{-1}$ $min^{-1}$ = 7.18 pmol $kg^{-1}$ $min^{-1}$; 30–60 min after the start of the experiment, 2 mU $kg^{-1}$ $min^{-1}$ = 14.4 pmol $kg^{-1}$ $min^{-1}$; 60–90 min after the start of the experiment, 3 mU $kg^{-1}$ $min^{-1}$ = 21.5 pmol $kg^{-1}$ $min^{-1}$; and 90–120 min after the start on the experiment, 4 mU $kg^{-1}$ $min^{-1}$ = 28.7 pmol $kg^{-1}$ $min^{-1}$. Normoglycaemia was assured by a blood glucose controlled infusion of 500 g/l = 2.5 mol/l glucose monohydrate solution. Blood was sampled from a superficial vein on the opposite arm. Blood glucose concentration and cardiac rate were determined every 10 min, blood for measurement of plasma insulin concentration was sampled 5 min before and 0, 25, 30, 55, 60, 85, 90, 115 and 120 min after the start of the experiment. Plasma C-peptide, plasma potassium ion and plasma phosphate concentrations were measured in samples taken 0, 30, 60, 90 and 120 min after start of the experiment. For the measurement of plasma insulin and plasma C-peptide concentrations, 10 ml of blood was drawn into glass tubes with 5000 units of aprotinin (Trasylol) and 500 units of heparin in a negligible volume and centrifuged immediately. Plasma was stored at −20 °C until analysis which was carried out within one month.

Plasma insulin was measured according to Heding [11]. The assay was demonstrated to be accurate over the range from 0 pmol/l to at least 3600 pmol/l. Plasma C-peptide concentration was measured according to Heding [12], using antiserum 1230 [13]. All samples from one subject were analysed in one series. The intra-assay standard deviation in the working range was 0.022 nmol/l. The detection limit of the assay was 0.05 nmol/l. Plasma C-peptide concentrations in 25 fasting normal subjects were 0.21–0.59 nmol/l. Fasting blood glucose concentrations were measured by a glucose oxidase method (photometry). During the experiments, blood glucose concentration was measured by Ames' Eyetone Reflectance Meter.

## Results

Normoglycaemia (range 2.3–5.2 mmol/l) was maintained throughout each experiment and plasma C-peptide concentrations (range 0.10–0.51 nmol/l) remained below the upper limit of the range for fasting normal subjects, indicating that endogenous insulin release during the experiments was not increased above fasting levels. Insulin infusion produced no symptoms or signs of hypoglycaemia in these eight subjects. In one subject (results not reported), transient sweating and tachycardia were observed, the insulin infusion was stopped and the glucose infusion rate was increased. Plasma potassium and plasma phosphate concentrations decreased slightly in all subjects ($\leqq$ 0.7 and $\leqq$ 0.51 mmol/l, respectively).

Table 1 shows the individual plasma insulin concentrations. Steady-state was assumed to exist at the end of each infusion period since the paired sign test demonstrated no difference between the results obtained at 25 (55, 85, 115) and at 30 (60, 90, 120) min.

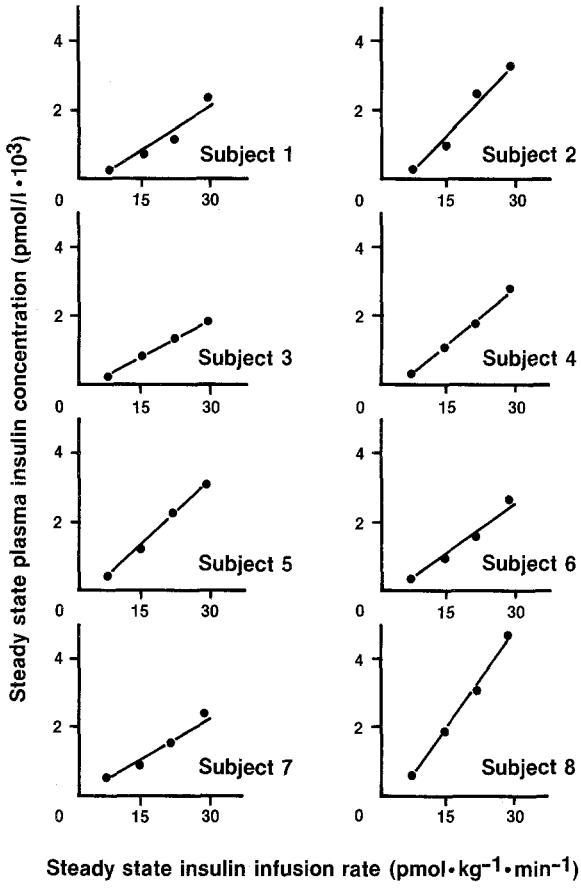


**Fig. 1.** The relationship between steady-state insulin infusion rate and steady-state plasma insulin concentration in eight healthy subjects

**Table 2.** The calculated parameters for the experimental model

| Subject | $a - I_{end}$ (pmol $kg^{-1}$ $min^{-1}$) | b ($1\ kg^{-1}\ min^{-1}$) |
|---|---|---|
| 1 | 4.9 | 0.012 |
| 2 | 5.1 | 0.0072 |
| 3 | 3.0 | 0.014 |
| 4 | 3.9 | 0.0091 |
| 5 | 3.6 | 0.0080 |
| 6 | 3.1 | 0.010 |
| 7 | 1.9 | 0.012 |
| 8 | 3.9 | 0.0054 |

In this model, it is assumed that the total insulin degradation rate (V) is the sum of the degradation rate of a saturable pathway (a, for 'high' plasma insulin concentrations) and the degradation rate of a virtually non-saturable pathway (b · C, b is a constant and C is the steady state plasma insulin concentration). Thus, in the steady state:

$$V = I_{end} + I_{ex} = a + b \cdot C \text{ and } C = -\frac{a - I_{end}}{b} + \frac{1}{b} I_{ex}$$

where $I_{end}$ denotes endogenous insulin release and $I_{ex}$ denotes the steady state exogenous insulin infusion rate

Figure 1 demonstrates the relationship between insulin infusion rate and steady-state plasma insulin concentration. In all experiments, a linear relationship was observed in the range 7.18–28.7 pmol · $kg^{-1}$ · $min^{-1}$. The straight lines were calculated by the method of least squares, weighting was performed with the inverse of the variances of concentration. The calculated parameters of the model ($a - I_{end}$ and b) are given in Table 2.

## Discussion

In the present study, no proportionality between plasma concentration and plasma disappearance rate of insulin was found. This result supports the findings of Sönksen et al. [7, 8] and throws doubt upon the validity of results based on the assumption of proportionality [1–4]. Our model is based on the assumption that insulin is extracted from plasma by two independent processes: a saturable pathway and a non-saturable pathway. The demonstration of a positive value for the maximal degradation rate of the saturable pathway and the finding of a linear relationship between steady-state plasma insulin concentration and insulin infusion rate seem to validate this assumption.

In conclusion, the plasma disappearance of unlabelled insulin in normal subjects may be described in terms of two pathways: one that is saturated at physiological plasma insulin concentrations and one that is apparently non-saturable over a wide concentration range. From the present study, no conclusions can be made concerning the sites of insulin degradation, but experiments on dogs indicate that the liver and the kidneys are responsible for saturable and non-saturable insulin degradation, respectively [5, 6]. We recognize that conclusions about the maximal degradation rate of the saturable pathway cannot be made with accuracy since the magnitude of endogenous insulin release, even if small, remains unknown.

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