

# A practical guide to the interpretation of PK/PD profiles of longer-acting analogue insulins. Part one: The principles of glucose clamp studies

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Glucose clamp studies are used to determine pharmacokinetics (PK) and pharmacodynamics (PD) of analogue insulins. With the development of longer-acting basal analogue insulins, including glargine 300 (Gla-300) and insulin degludec (IDeg), results from numerous glucose clamp studies are readily available. However, interpreting PK/PD profiles in a scientifically sound manner can be a challenging feat. This is the first in a series of publications that will suggest practical tips for interpreting and comparing results from glucose clamp studies. Variations in the glucose clamp methodology, duration of clamp studies and glucose clamp targets influence the study design and results significantly. Selection of study populations, including healthy patients or patients with Type 1 or 2 diabetes mellitus, has important implications. The dose of study insulin should reflect that of the general treatment population, and ideally steady-state conditions should be used. During the study the plasma insulin concentration and glucose infusion rate describe the pharmacokinetics and pharmacodynamics of the study insulin. With these practical tips in mind, results of glucose clamp studies can be interpreted in a scientifically correct manner. The next article in this series will discuss the interpretation of PK/PD profiles using two newly developed longer-acting basal analogue insulins: Gla-300 and IDeg.

**Keywords:** analogue insulins, glucose clamp, time–action profile, glucose infusion rate, pharmacokinetics

## Introduction

According to the guidelines for the treatment of diabetes published by the Society for Endocrinology, Metabolism and Diabetes of South Africa (SEMDSA), treatment with exogenous insulins is recommended for patients diagnosed with Type 2 diabetes mellitus who are unable to reach glycaemic control on oral antidiabetic agents.<sup>1</sup> Insulin forms the cornerstone in the treatment of patients with Type 1 diabetes mellitus due to an absolute deficiency of insulin.<sup>2</sup> The goal of treatment with exogenous insulins is to mimic physiological insulin secretion in patients with deficient endogenous insulin production to ensure maintenance of fasting blood glucose levels at 4–7 mmol/l.<sup>1</sup>

Although the insulin options available to the South African prescriber include analogue, human and premixed insulins, this article will focus on basal analogue insulins. Several basal analogue insulins are currently under development aiming at prolonged duration of action and more predictable absorption than the analogue insulins currently available.<sup>2–4</sup>

Regulatory approval of new biological products, including analogue insulins, requires complete description of pharmacokinetic (PK) and pharmacodynamic (PD) profiles of the new chemical entities in humans.<sup>5</sup> The glucose clamp study is regarded as the gold standard to establish PK and PD profiles of basal analogue insulins.<sup>6</sup>

A vast collection of data describing the time–action profiles of analogue insulins has been published recently.<sup>7–10</sup> However, while the correct interpretation of these profiles can guide the prescriber in selecting the most appropriate treatment for a patient, this proves to be a challenging feat. This series of publications will discuss practical tips for the accurate interpretation of pharmacokinetic and pharmacodynamic

profiles of analogue insulins obtained from glucose clamp studies.

## Pharmacokinetics and pharmacodynamics of analogue insulins

Pharmacokinetics describes the relationship between dose administered and observed plasma concentration, whereas the relationship between plasma concentration and the response elicited is described by the pharmacodynamics.<sup>11</sup> As such, pharmacokinetics is influenced by variables including dose, frequency of administration and route of administration. Pharmacodynamics may be affected by host factors such as receptor expression and sensitivity.<sup>11</sup>

The unique mode of protraction of longer-acting analogue insulins, including insulin glargine (Gla-300) and degludec (IDeg), results in extended duration of action. Gla-300 is soluble at acidic pH, and after injection into the subcutaneous tissue microprecipitates form a more compact soluble depot with smaller surface area in comparison with Gla-100, from which active monomers are steadily released.<sup>12</sup> In contrast, IDeg form multi-hexamers after administration in the subcutaneous tissue resulting in the formation of a soluble depot from which active monomers are steadily released.<sup>13</sup> The long half-life of these insulins translates into extended duration of action and therefore allows once-daily administration.<sup>2</sup> Half-life refers to the time required for the original plasma concentration of an administered drug to be reduced by half, which is determined by the rate at which drug is eliminated from the central compartment (plasma). For drugs with an intravenous bolus dose administration the original plasma concentration is established very rapidly so the plasma concentration profile over time is mainly determined by the bolus dose and factors that influence the rate at which

plasma concentration decreases, including the rate of metabolism and the rate of excretion.

In the case of basal analogue insulins, this scenario is complicated by the slow release of insulin into the central compartment. Typically, this would lead to a ‘flattened’ concentration–time profile, e.g. if the rate of insulin entering the plasma compartment equals the rate at which insulin is eliminated from the same compartment, the concentration–time profile will be a horizontal line because the plasma insulin concentration does not change over time. This flattened profile enables less frequent dosing with the intermediate and long-acting basal analogue insulins. Factors that establish insulin plasma concentration include the dose, rate of release of insulin monomers and rate of absorption from the site of administration, in this case subcutaneous depots. Factors that influence the rate at which plasma insulin concentration decreases include the rate of metabolism and the rate of excretion.<sup>6</sup> Many of these factors are related to the physicochemical properties of the exogenous insulin, which can be manipulated to modify the pharmacokinetics of the drug. Since the plasma concentration directly relates to response, these modifications also have an impact on the clinical outcome observed with different analogue insulins.

**Glucose clamp study procedure**

Originally established to describe insulin secretion and resistance during the 1970s,<sup>14</sup> the glucose clamp methodology has been adapted to provide PK/PD profiles for long-acting basal analogue insulins. In brief, glucose clamp studies assess the intravenous glucose infusion rate required to maintain blood glucose levels at a predetermined target level, the clamp target, after administration of a long-acting basal analogue insulin.<sup>6</sup>

Variations of the glucose clamp study have been developed based on the glucose clamp target, i.e. the euglycemic and hyperglycaemic models. With the euglycemic clamp model a hyperinsulinaemic state is created through the infusion of insulin to reach a plasma insulin concentration of approximately 100 µU/ml. At the same time glucose is infused to reach the predefined plasma glucose concentration, which can either be the physiological plasma levels (euglycemic clamp model) or an elevated level (hyperglycaemic clamp model).<sup>14,15</sup> As the euglycemic clamp model is most commonly employed to determine the time–action profile of long-acting analogue

insulins, this model will be the focus of the article. The euglycemic glucose clamp methodology is illustrated in Figure 1.

After an overnight fast, a 20% dextrose solution and/or rapid-acting insulin is administered intravenously to reach clamp glucose and insulin target. Insulin is administered to suppress endogenous insulin and hepatic glucose production. Potassium phosphate may also be administered to prevent hypokalaemia.<sup>15</sup> Once the clamp target has been reached, infusion of rapid-acting insulin is gradually tapered off and discontinued prior to administration of the long-acting study insulin. The long-acting study insulin is administered at a predetermined dose. Blood samples are collected at regular intervals to determine blood glucose and plasma insulin concentrations. Based on the blood glucose levels determined, the glucose infusion rate is adjusted to maintain clamp target.<sup>7,8</sup>

**Interpretation of glucose clamp results**

Data obtained from glucose clamp studies are used to describe the duration of action of the study insulin and inter-individual variability observed among the study population. General outcome measures of glucose clamp studies are described, along with potential influencing factors, in Table 1.

The data obtained from glucose clamp studies are commonly represented in terms of the plasma insulin concentration (indicative of PK) and glucose infusion rate (indicative of PD) (Figure 2). The ideal long-acting analogue insulin will display a flat, peakless PD profile mimicking physiological endogenous insulin secretion.<sup>17</sup> Within-subject variability, i.e. variation in the response produced within the same individual after doses with the same insulin, may be determined from repeat clamp studies performed on the same study population. Within-subject variability is a clinically relevant measure and the ideal longer-acting insulin will have very low intra-individual variability. In clinical practice this translates into a predictable response after administration of the same dose of insulin on different days. This is particularly important to avoid unexpected hypoglycaemic episodes when the same dose is injected on different days.<sup>17</sup>

Several factors must be taken into consideration when interpreting the results of glucose clamp studies. A number of these factors are described below.

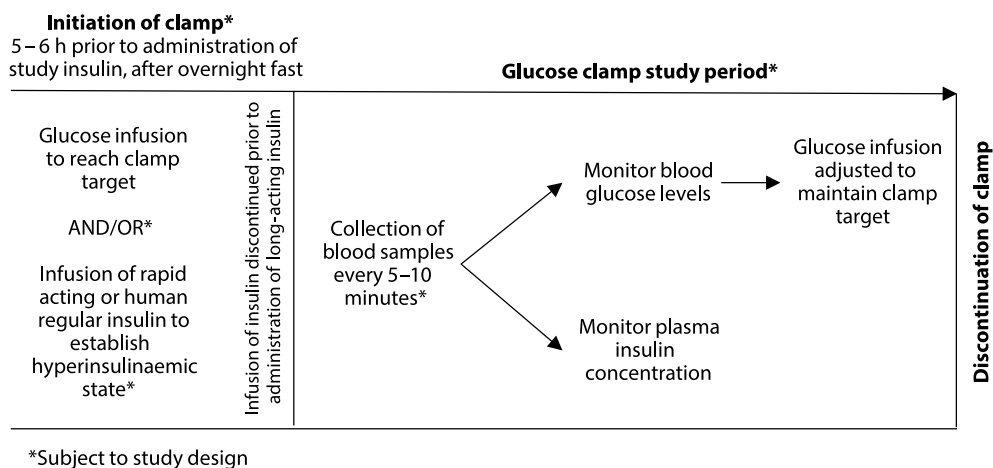
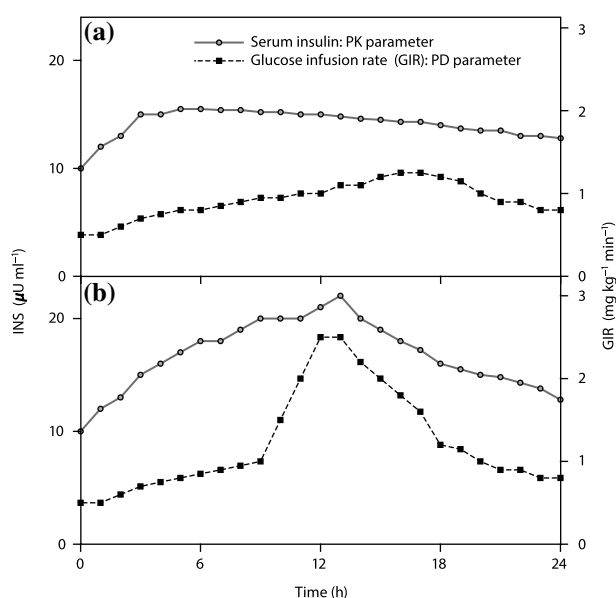


Figure 1. Euglycemic clamp methodology.

**Table 1:** General outcome measures of the euglycemic glucose clamp study

Parameters	Definition	Influencing factors	Clinical implication(s)
Area under the curve (AUC) <sup>16</sup>	Total quantity of drug the subject was exposed to	Dose Frequency of administration Elimination rate	Total amount of glucose utilised during clamp due to exogenous insulin
Elimination half-life (t <sub>1/2</sub> ) <sup>16</sup>	Time required for drug concentration to be reduced by half	Rate of metabolism Rate of excretion	Surrogate measurement of duration of action of the exogenous insulin
Duration of action <sup>2,4</sup>	Duration of pharmacological effect	Rate of absorption from subcutaneous depot Elimination rate	–
Glucose infusion rate (GIR) <sup>14</sup>	Rate at which i.v. glucose must be infused to maintain the clamp target	Glucose utilisation Endogenous insulin production Investigator bias	Glucose intake
End of action <sup>3</sup>	Time when blood glucose concentration reaches 8.3 mmol/l*	Dose Rate of absorption Elimination rate	Roughly translating, the required frequency of dosing

\*End of action may occur prior to end of study and is defined as GIR = 0 mmol/kg/min.



**Figure 2.** PK/PD profile of a longer-acting basal analogue insulin where (A) indicates the ideal PK/PD profile and (B) indicates a non-ideal PK/PD profile. The PK/PD profile of a longer-acting basal analogue insulin obtained from a euglycemic glucose clamp study should ideally have a flat, peakless serum insulin concentration curve suggesting reduced risk of hypoglycaemia.<sup>6</sup> A minimum duration of action of 24 h should be observed in order to allow for once-daily administration.<sup>2</sup> It should be considered that the full duration of action of the study insulin may not be observed during the period of the glucose clamp study.

### Clamp methodology

Glucose clamp studies can be performed manually or using an automated Biostator system MTB (Medizintechnik, Amstetten, Germany). During a manual clamp study glucose levels are monitored at intervals of several minutes, whereas the use of the Biostator allows for the monitoring of glucose levels every minute.<sup>15</sup> A major advantage of the use of the Biostator is the exclusion of investigator bias in terms of adjustment of the glucose infusion rate.

### Duration of glucose clamp study

A standard duration of glucose clamp study has not been established. The duration of euglycemic clamp studies for Gla-300 ranges from 24 h to 36 h<sup>8,18,19</sup> while euglycemic clamp studies for IDeg ranges from 24 h to 42 h.<sup>7,20</sup> It must be considered that the duration of a glucose clamp study may be shorter than the duration of action of the study insulin. Indeed, the duration of action of Gla-300 has been reported to be in excess of 30 h and the duration of action of IDeg as 42 h.<sup>4</sup>

The differing clamp duration of these studies may confound comparison of the results and determination of the duration of action of a longer-acting insulin analogue. The maximum duration of action of a longer-acting insulin analogue that can be determined with a glucose clamp study is equal to the duration of the clamp. Therefore, only where the duration of action of the insulin is shorter than the duration of the clamp can an accurate duration of action be reported.

### Study population

Results from glucose clamp studies performed in healthy volunteers,<sup>9,30</sup> patients diagnosed with Type 1 diabetes mellitus<sup>7,8,21</sup> and Type 2 diabetes mellitus<sup>22,23</sup> have been published. However, for each population a number of potentially confounding factors must be considered when interpreting the results.

Where glucose clamp studies are performed in healthy volunteers or patients diagnosed with Type 2 diabetes mellitus, the effect of endogenous insulin secretion on the glucose infusion rate (GIR) must be accounted for. In order to compensate for secretion of endogenous insulin, the pre-study baseline insulin:C-peptide ratio is commonly used as reference<sup>24</sup> as C-peptide is secreted in equimolar amounts to insulin. Monitoring of serum C-peptide levels may be used to indicate the secretion of endogenous insulin. A reduction in serum C-peptide levels is indicative of a decline in endogenous insulin secretion.<sup>15</sup> Adjusting for endogenous insulin, using C-peptide concentrations, provides a more accurate assessment of the pharmacokinetics of an exogenous insulin and is particularly useful when assessing

biosimilarity between drugs as demonstrated by a recent study that evaluated the biosimilarity between different insulin glargine products.<sup>9</sup> While this approach may reduce the reported effect of endogenous insulin on glucose concentration, it does not entirely eliminate this confounding metabolic effect of the endogenous insulin.

Clamp studies performed in a healthy study population are fraught with complications. It has been demonstrated that time–action profiles for insulin glargine obtained in studies using healthy volunteers<sup>9</sup> did not reflect that of a study population of patients with Type 1 diabetes mellitus.<sup>22,25</sup> Furthermore, extended duration of action of insulin in healthy volunteers may be observed due to continuous endogenous insulin secretion.<sup>25</sup>

The use of a study population of patients diagnosed with Type 1 diabetes mellitus is recommended, as endogenous insulin secretion will not affect results obtained.<sup>3,25</sup> However, during the initial stages of Type 1 diabetes mellitus  $\beta$ -cell function may not be entirely lost, resulting in production of endogenous insulin.<sup>26</sup> During infusion of glucose and exogenous insulin to establish the hyperinsulinaemic euglycemic state prior to initiation of the clamp study, the duration of action of the exogenous insulin injected prior to the clamp and time of discontinuation prior to administration of study insulin must be considered to avoid masking the glucose-lowering effect of the study insulin.<sup>24</sup>

#### **Dose of study insulin**

Glucose clamp studies aim to provide information as to the pharmacokinetic profile to be expected in the general treatment population and administration of study insulin should therefore reflect the doses and administration times of the general treatment population.<sup>27</sup> Glucose clamp studies have been performed using a single dose of study insulin. However, this does not reflect the use of long-acting analogue insulins in the treatment population. The time–action profiles of long-acting analogue insulins are thus best studied once steady-state conditions have been reached.<sup>3</sup> Steady-state is achieved when the absorption of a drug is equal to its elimination, after approximately four to five half-lives. It is recommended that glucose clamp studies for long-acting insulin analogues are performed at steady-state conditions in a study population of patients diagnosed with diabetes mellitus.<sup>3</sup>

It has been suggested that the results of clamp studies where the study insulin was administered during the morning cannot be directly compared with those of clamp studies where insulin was administered at night.<sup>28</sup> As the majority of patients using basal analogue insulins administer these at night, the same dosing schedule should be followed in glucose clamp studies.<sup>27</sup> However, a direct comparison of the results of glucose clamp studies of Gla-100 using morning and evening administration did not reveal a marked difference between the dosing times.<sup>28</sup>

#### **Glucose infusion rate**

Glucose clamp studies quantify the total volume of glucose required to maintain blood glucose levels at a predetermined clamp target after administration of a long-acting insulin analogue.<sup>7,8</sup> The volume of glucose required during the clamp period to maintain clamp target is indicated as the glucose infusion rate (GIR).<sup>14</sup> Samples are collected at regular intervals to

determine blood glucose levels and the GIR adapted accordingly to maintain blood glucose levels at the predetermined clamp target. GIR is therefore indicative of glucose utilisation and metabolism.<sup>15,29</sup>

#### **Plasma insulin concentration**

During the clamp study plasma insulin concentration is determined using immunoassays with radio-labelled or enzyme-linked antibodies. These antibodies recognise a peptide sequence in the insulin molecule, and this allows for the quantification of insulin concentrations based on a detectable signal generated.<sup>15</sup> However, antibodies to specific analogue insulins are not readily available and therefore endogenous and exogenous insulins may not be differentiated. Furthermore, these assays do not accurately quantify C-peptide, a product of the conversion of endogenous proinsulin to insulin, which may be used to distinguish endogenous and exogenous insulins.<sup>15</sup> In order to ensure that the insulin concentration reported is accurate, the lower limit of quantification (LLOQ) of these assays must be reported.<sup>24</sup> Levels of insulin lower than the LLOQ cannot be accurately determined using the currently available assays.

It must be considered that insulin bound to albumin, a phenomenon known as protein binding,<sup>30</sup> will not be accurately detected by antibody assays. Acetylated insulins have been demonstrated to bind to albumin, resulting in an extended duration of action,<sup>30</sup> but may result in an underestimation of plasma insulin concentration. Indeed, accurately determining the plasma concentrations of acetylated IDeg basal analogue insulin is not currently possible with immunoassays.<sup>4</sup>

Glucose clamp studies, when used appropriately, provide valuable insight into the time–action profiles of longer-acting insulin analogues, such as Gla-300 and IDeg. These studies are useful to determine the duration of action of an insulin analogue translating into frequency of administration, as well as inter- and intra-individual variability, translating into predictable response after repeated administration. Conclusions regarding the safety and efficacy of longer-acting insulin analogues, however, cannot be drawn from the results of glucose clamp studies. In order to establish the application of new analogue insulins in the clinical setting, phase three clinical trials with specific outcomes for diabetes are required.

#### **Conclusion**

Even though glucose clamp studies are recommended for the determination of the time–action profiles of long-acting analogue insulins, the advantages and confounding factors of glucose clamp methodology must be considered when interpreting and comparing results from different glucose clamp studies. However, using the guide as set out above, glucose clamp studies can be interpreted in a scientifically correct manner. The next article in this series will discuss the interpretation of PK/PD profiles of two newly developed longer-acting basal analogue insulins: Gla-300 and IDeg.

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