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CLINICAL REVIEW

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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Abstract

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

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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.[1] Insulin forms the cornerstone in the treatment of patients with Type 1 diabetes mellitus due to an absolute deficiency of insulin.[2] 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.[1]

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 that associated with the analogue insulins currently available.[2-4]

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.[5] The glucose clamp study is regarded as the gold standard to establish PK and PD profiles of basal analogue insulins.[6]

A vast collection of data describing the time-action profiles of analogues insulins have been published recently. [7-10] 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.[11] 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.[11]

The unique mode of protraction of longer acting analogue insulins, including insulin glargine (Gla-300) and degludec (IDeg), result in extended duration of action. Gla-300 is soluble

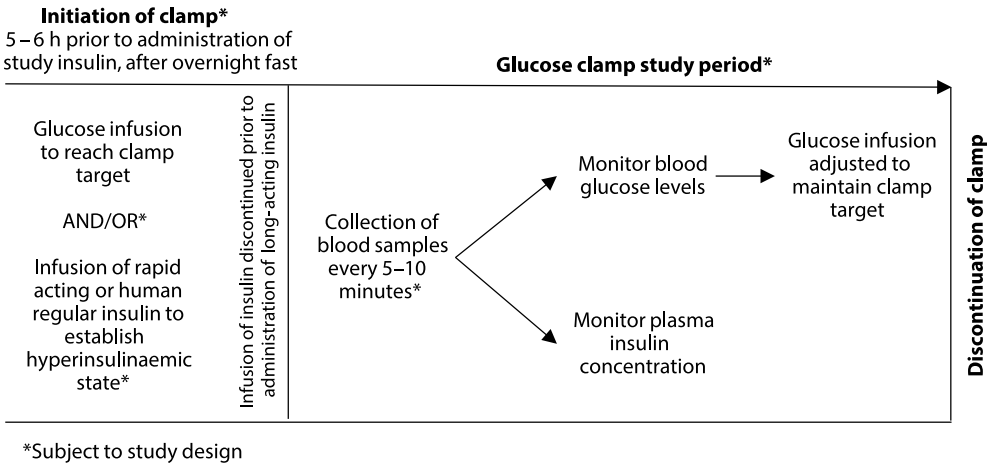

Figure 1: Euglycemic clamp methodology.

at acidic pH, and after injection into the subcutaneous tissue microprecipitates form a more compact soluble depot with smaller surface area in comparison to Gla-100, from which active monomers are steadily released.[12] 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.[13] The long half-life of these insulins translates into extended duration of action and therefore allows once daily administration.[2] 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.[6] Many of these factors are related 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 will also impact the clinical outcome observed with different analogue insulins.

Glucose clamp study procedure

Originally established to describe insulin secretion and resistance during the 1970's[14], 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.[6]

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 hyperinsulinemic 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).[14,15] As the euglycemic clamp model is most commonly employed to determine the time-action profile of long-acting analogues 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 hypokaleamia.[15] 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.[7,8]

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 I.

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

Parameters	Definition	Influencing factors	Clinical implication(s)
Area under the curve (AUC)[16]	Total quantity of drug the subject was exposed to	Dose	Total amount of glucose utilised during clamp due to exogenous insulin
		Frequency of administration	
		Elimination rate	
Elimination half-life ($t_{1/2}$)[16]	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[2,4]	Duration of pharmacological effect	Rate of absorption from subcutaneous depot	-
		Elimination rate	
Glucose infusion rate (GIR)[14]	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[3]	Time when blood glucose concentration reaches 8.3 mmol/L *	Dose	Roughly translating, the required frequency of dosing
		Rate of absorption	
		Elimination rate	

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

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.[17] 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.[17]

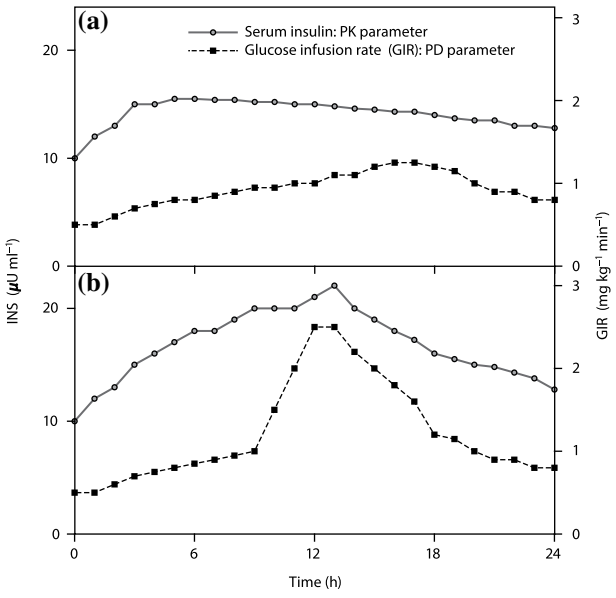

Figure 2: PK/PD profile of a longer acting basal analogue insulin where (A) indicate the ideal PK/PD profile and (B) indicate a non-ideal PK/PD profile. The PK/PD profile of a longer acting basal analogue insulin obtained from a euglycemic glucose clamp study should ideally have a flat, peakless serum insulin concentration curve suggesting reduced risk of hypoglycaemia.[6] A minimum duration of action of 24 h should be observed in order to allow for once daily administration.[2] 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.

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

Clamp methodology

Glucose clamp studies can be performed manually or using an automated Biostator system. 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.[15] 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 - 36 hours[8,18,19] while euglycemic clamp studies for IDeg ranges from 24 – 42 hours.[7,20] 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 have been reported to be in excess of 30 hours and the duration of action of IDeg as 42 hours.[4]

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[9,31], patients diagnosed with type 1 diabetes mellitus[7,8,22] and type 2 diabetes mellitus[23,24] 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[25] 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.[15] 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.[9] 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 is fraught with complications. It has been demonstrated that time-action profiles for insulin glargine obtained in studies using healthy volunteers[9] did not reflect that of a study population of patients with Type 1 diabetes mellitus.[22,26] Furthermore extended duration of action of insulin in healthy volunteers may be observed due to continuous endogenous insulin secretion.[26]

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.[3,26] However, during the initial stages of type 1 diabetes mellitus β-cell function may not be entirely lost, resulting in production of endogenous insulin.[27] During infusion of glucose and exogenous insulin to establish the hyperinsulinemic 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.[25]

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.[28] 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.[3] 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.[3]

It has been suggested that the results of clamp studies where the study insulin was administered during the morning cannot be directly compared to that of clamp studies where insulin was administered at night.[29] As the majority of patients using basal analogues insulins administer these at night, the same dosing schedule should be followed in glucose clamp studies.[28] 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 time.[29]

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 long-acting insulin analogue.[7,8] The volume of glucose required at during the clamp period to maintain clamp target is indicated as the glucose infusion rate (GIR).[14] 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.[15,30]

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 allows for the quantification of insulin concentrations based on a detectable signal generated.[15] 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.[15] In order to ensure that the insulin concentration reported is accurate, the lower limit of quantification (LLoQ) of these assays must be reported.[25] 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[31], will not be accurately detected by antibody assays. Acetylated insulins have been demonstrated to bind to albumin, resulting in an extended duration of action[31], 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.[4]

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 from 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.

Conflict of interest

Prof Greeff and Dr JJ van Tonder have no conflicts of interest to declare. Dr K Naidu has previously received honoraria from Sanofi. Dr Mothilal is the Medical Director, Dr McMaster is the Medical Advisor and Dr A van Tonder is a Medical Science Liaison at Sanofi-Aventis South Africa (Pty) Ltd, a member of the SANOFI Group.

References

1. Amod A, Motala A, Levitt N, et al. The 2012 SEMDSA Guideline for the Management of Type 2 Diabetes. JEMDSA. 2012;17(1):S1-S94.
2. Sorli C. New developments in insulin therapy for type 2 diabetes. Am J Med. 2014;127:S39-S48.
3. Swinnen SGHA, Holleman F, DeVries JH. The interpretation of glucose clamp studies of long-acting insulin analogues: from physiology to marketing and back. Diabetologia. 2008;51:1790–5.
4. Lamos EM, Younk LM, Davis SN. Concentrated insulins: the new basal insulins. Ther Clin Risk Manag. 2016;12:389-400.
5. Heinemann L, Khatami H, McKinnon R, et al. An overview of current regulatory requirements for approval of biosimilar insulins. Diabetes Technol Ther. 2015;17(7):510-26.
6. Arnolds S, Kuglin B, Kapitza C, et al. How pharmacokinetic and pharmacodynamics principles pave the way for optimal basal insulin therapy in type 2 diabetes. Int J Clin Pract. 2010;64(10):1415-24.
7. Heise T, Hövelmann U, Nosek L, et al. Comparison of the pharmacokinetic and pharmacodynamics profiles of insulin degludec and insulin glargine. Expert Opin Drug Metab Toxicol. 2015;11(8):1193-1201.
8. Becker RHA, Nowotny I, Teichert L, et al. Low within- and between-day variability in exposure to new insulin glargine 300 U/ml. Diabetes Obes Metab. 2015; 17:261-67.
9. Linnebjerg H, Lam ECQ, Seger ME, et al. Comparison of the pharmacokinetic and pharmacodynamics of LY2963016 insulin glargine and EU- and US-approved versions of Lantus insulin glargine in healthy subjects: three randomised euglycemic clamp studies. Diabetes Care. 2015; 38:2226-33.
10. Hompesch M, Jones-Leone A, Carr MC, et al. Albiglutide does not impair the counter-regulatory hormone response to hypoglycaemia: a randomised, double-blind, placebo-controlled, stepped glucose clamp study in subjects with type 2 diabetes mellitus. Diabetes Obes Metab. 2015; 17:82-90.
11. Tozer TN, Rowland M. Opening comments. In: Klingler A, ed. Introduction to pharmacokinetics and pharmacodynamics. The quantitative basis of drug therapy. Baltimore: Lippincott Williams & Wilkins. 2006.
12. Steinstraesser A, Schmidt R, Bergmann K, et al. Investigational new insulin glargine 300 U/ml has the same metabolism as insulin glargine 100 U/ml. Diabetes Obes Metab. 2014; 16(9):873–876.
13. Kurtzhals P, Heise T, Strauss HM, et al. Multi-hexamer formation is the underlying basis for the ultra-long glucose-lowering effect of insulin degludec. Diabetologia. 2011; 54:S426.
14. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am Physiol Soc. 1979; E214-23.
15. Heinemann L. Time-action profiles of insulin preparations. Kirchheim, Mainz. 2004.
16. Morello CM. Pharmacokinetics and pharmacodynamics of insulin analogues in special populations with type 2 diabetes mellitus. Int J Gen Med. 2011; 4:827-35.
17. Heise T, Peiber TR. Towards peakless, reproducible and long-acting insulins. An assessment of the basal analogues based on isoglycaemic clamp studies. Diabetes Obes Metab. 2007; 9:648-59.
18. Tillner J, Bergmann K, Teichert L. Euglycaemic clamp profile of new insulin glargine U300 formulation in patients with type 1 diabetes (T1DM) is different from glargine U100 (Abstract). Diabetes. 2013; 62:A234.
19. Becker RHA, Dahmen R, Bergmann K, et al. New insulin glargine 300 units.mL^{-1} provides a more even activity profile and prolonged glycaemic control at steady state compared with insulin glargine 100 units.mL^{-1}. Diabetes Care. 2014; 1-7.
20. Heise T, Hermanski L, Nosek L, et al. Insulin degludec: Less pharmacodynamic variability than insulin glargine under steady-state conditions (Abstract). Diabetologia. 2010; 53(Suppl 1):S387.
21. Heinemann L, Linkeschova R, Rave K, et al. Time-action profile of the long-acting insulin analogue insulin glargine (HOE901) in comparison with those of NPH insulin and placebo. Emerging Treatments and Technologies. Diabetes Care. 2000; 23:644-9.
22. Porcelatti F, Rossetti P, Busciantella NR, et al. Comparison of the pharmacokinetics and dynamics of the long-acting insulin analogues glargine and detemir at steady state in type 1 diabetes: a double-blind, randomized, crossover study. Diabetes Care. 2007; 30:2447-52.
23. Luzio S, Dunseath G, Peter R, et al. Comparison of the pharmacokinetics and pharmacodynamics of biphasic insulin aspart and insulin glargine in people with type 2 diabetes. Diabetologia. 2006; 49:1163-8.
24. Klein O, Lynge J, Endahl L, et al. Albumin-bound insulin analogues (insulin detemir and NN344): comparable time-action profiles but less variability than insulin glargine in type 2 diabetes. Diabetes Obes Metab. 2007; 9:290-9.
25. Home P. Pharmacokinetics and pharmacodynamics of biosimilar insulins: is clamp technology fit for purpose? Diabetes Care. 2015; 38:2234-2236.
26. Lepore M, Pampanelli S, Fanelli C, et al. Pharmacokinetics and pharmacodynamics of subcutaneous injection of long-acting human insulin analogue Glargine, NPH insulin and Ultralente human insulin and continuous subcutaneous infusion of insulin lispro. Diabetes. 2000. 49:2142-48.
27. Heinze E, Thon A. Honeymoon period in insulin-dependent diabetes mellitus. Paediatrician. 1983. 12(4):208-12.
28. Porcellati F, Lucidi P, Bolli GB, et al. How to accurately establish pharmacokinetics/pharmacodynamics of long-acting insulins in humans: relevance to biosimilar insulins. Diabetes Care. 2015; 38:2237-40.
29. Heise T, Zijlstra E, Nosek L, Heckermann S, Plum-Mörschel L, Forst T. Euglycaemic glucose clamp: what it can and cannot do, and how to do it. Diabetes Obes Metab. 2016; 18(10):962-972.
30. Bergman RN. Toward physiological understanding of glucose tolerance: Minimal-model approach. Diabetes. 1989; 38:1512-27.
31. Kurtzhals P, Havelund S, Jonassen I, et al. Albumin binding of insulins acylated with fatty acids: characterization of the ligand-protein interaction and correlation between binding affinity and timing of the insulin effect in vivo. Biochem J. 1995; 312(3):725-31.