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4 Guideline on non-clinical and clinical development of 5 similar biological medicinal products containing 6 recombinant human insulin and insulin analogues

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This guideline replaces 'Guidance on similar medicinal products containing recombinant human soluble insulin' (EMEA/CHMP/BMWP/32775/2005).

Comments should be provided using this template. The completed comments form should be sent to BMWP.secretariat@ema.europa.eu.

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Guideline on non-clinical and clinical development of similar biological medicinal products containing recombinant human insulin and insulin analogues

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Executive summary

This guideline lays down the non-clinical and clinical requirements for recombinant insulin-containing products, including human insulin and insulin analogues (both referred to as insulin), claiming to be similar to another one already authorised (the reference medicinal product).

The non-clinical section addresses the requirements of *in vitro* pharmacodynamic studies and cases when there is a need for additional *in vivo* toxicological assessment. The clinical section addresses the requirements for pharmacokinetic, pharmacodynamic and safety studies as well as the risk management plan.

1. Introduction

The Marketing Authorisation (MA) application dossier of a recombinant insulin claimed to be similar to a reference medicinal product already authorised shall provide the demonstration of biosimilarity of the product applied for to this reference medicinal product.

Human insulin is a non-glycosylated, disulphide-bonded heterodimer of 51 amino acids. Insulin analogues differ from human insulin by the substitution of amino acids or other chemical changes such as addition of a fatty acid chain within the molecule. Insulin preparations differ mainly by their kinetic/pharmacodynamic profiles. They are usually classified as rapid- (faster acting than soluble human insulin), short- (e.g. soluble human insulin), intermediate- (e.g. human isophane insulin = NPH insulin), and long-acting preparations (insulins with action profiles significantly longer than NPH insulin), and are used alone or as free mixtures or premixed preparations of rapid/short-acting insulin and intermediate/long-acting (biphasic) insulin in various proportions.

Suitable physico-chemical and biological methods are available to comprehensively characterise the primary, secondary and tertiary structures of the recombinant insulin molecule, as well as its receptor affinity and biological activity *in vitro* and *in vivo*. Attention should be given to product related substances/impurities and process related impurities, and in particular to desamido forms and other forms that may derive from the expression vector or arise from the conversion steps removing the C-peptide and regenerating the three-dimensional structure.

Currently available insulins are administered subcutaneously or intravenously. The effects of insulin are mediated predominantly via stimulation of the insulin receptor but insulin is also a weak natural ligand of the insulin-like growth factor-1 (IGF-1) receptor.

Antibodies to insulin occur frequently, mainly as cross-reacting antibodies. These are usually without relevant consequences for efficacy or safety. The potential for development of product/impurity-specific antibodies needs to be evaluated. Possible patient-related risk factors of immune response are unknown.

2. Scope

The guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues (EMEA/CHMP/BMWP/42832/2005) lays down the general requirements for demonstration of the similar nature of two biological products in terms of safety and efficacy.

This product-class specific guideline presents the current view of the CHMP on the non-clinical and

clinical requirements for demonstration of biosimilarity of two recombinant insulin-containing medicinal products. This guideline should be read in conjunction with the requirements laid down in the EU Pharmaceutical legislation and with relevant CHMP guidelines (see section 3 Legal Basis and relevant guidelines).

3. Legal basis and relevant guidelines

- Directive 2001/83/EC, as amended, in particular in Directive 2001/83/EC Art 10(4) and Part II of the Annex I of Directive 2001/83/EC, as amended
- Guideline on similar biological medicinal products (CHMP/437/04)
- Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues (EMEA/CHMP/BMWP/42832/2005)
- Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: Quality issues (EMEA/CHMP/BWP/49348/2005) to be replaced by EMA/CHMP/BWP/247713/2012
- ICH guideline S 6 (R1) Preclinical safety evaluation of biotechnology-derived pharmaceuticals (EMA/CHMP/ICH/731268/1998)
- Guideline on the clinical investigation of the pharmacokinetics of therapeutic proteins (EMEA/CHMP/ 89249/2004)
- Guideline on the investigation of bioequivalence (CPMP/EWP/QWP/1401/98)
- Guideline on Immunogenicity Assessment of Biotechnology-derived Therapeutic Proteins (EMEA/CHMP/BMWP/14327/2006)
- Guideline on good pharmacovigilance practices (EMA/500020/2012)
- Guideline on good pharmacovigilance practices, Module V – Risk management systems (EMA/838713/2011)

4. Non-clinical studies

Before initiating clinical development, non-clinical studies should be performed. These studies should be comparative in nature and should be designed to have appropriate sensitivity to detect relevant differences in the response to the similar biological medicinal product and the reference medicinal product and should not just assess the response *per se*. The approach taken will need to be fully justified in the non-clinical overview.

Pharmacodynamic studies

In vitro studies

In order to assess any differences in properties between the biosimilar and the reference medicinal product, comparative *in vitro* bioassays for receptor binding, as well as tests for subsequent biological activity should be performed. Partly, such data may already be available from bioassays that were used to measure potency in the evaluation of physico-chemical characteristics. It is important that assays used for comparability testing are demonstrated to have appropriate sensitivity to detect any relevant differences and that experiments are based on a sufficient number of replicates, dilutions or time points per curve to characterise the whole concentration-response or time-response relationship accurately. Biosimilar and reference product should be compared head-to-head in the same

experiment. All assays should include appropriate controls to demonstrate the validity and suitability of the method.

Comparative receptor binding, including on-off kinetics should be shown for human insulin as well as human IGF-1 receptors.

Biological activity should be compared at three levels: receptor autophosphorylation, metabolic activity and mitogenic activity. For receptor autophosphorylation care should be taken that the dynamic range of the detection method used in the assay is not too limited which would reduce ability to detect relevant differences in levels of receptor autophosphorylation. For metabolic endpoints various assays are available, including glycogen formation, lipogenesis, inhibition of stimulated lipolysis as well as glucose transport, which can be studied in a variety of cells. There is no need to do them all; any of such assays may suffice as long as the data provide a clear view on how insulin receptor agonistic properties of biosimilar and reference product compare. Functional activity of the IGF-1 receptor can be evaluated by testing mitogenic potential in cells expressing this receptor. For all endpoints (receptor autophosphorylation, metabolic effects and mitogenicity), different experimental approaches exist. Thus, the Applicant should select suitable assays and justify the choice in light of the above mentioned criteria.

In vivo studies

Comparative study(ies) of pharmacodynamic effects would not be anticipated to be sensitive enough to detect differences not identified by *in vitro* assays, and are normally not required as part of the comparability exercise.

Toxicological studies

Generally, separate repeated dose toxicity studies are not required. In specific cases, e.g. when novel excipients are introduced, the need for additional toxicology studies should be considered following a risk-based approach (see also Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues).

Studies regarding safety pharmacology and reproduction toxicology are not required for non-clinical testing of a biosimilar containing insulin or insulin analogues. Studies on local tolerance are not required unless excipients are introduced for which there is no or little experience with the intended route of administration. If other *in vivo* studies are performed, local tolerance may be evaluated as part of these studies. Although measuring mitogenic activity (*in vitro*) is expected for comparison of functional activity of biosimilar and reference product, there is no need to perform carcinogenicity studies.

5. Clinical studies

Pharmacology studies

In addition to similar physicochemical and functional characteristics, demonstration of similar pharmacokinetic (PK) and pharmacodynamic (PD) profiles is considered the mainstay of proof of similar efficacy of the biosimilar and the reference insulin. For this purpose, cross-over, preferably double-blind insulin clamp studies using single subcutaneous doses of the test and reference agents are considered most suitable. The wash-out phase between study periods should take into account the duration of action of the investigated insulin preparation to avoid carry-over effects. The time-concentration and time-action profiles should preferably be studied simultaneously (in the same clamp study). Separate pharmacology studies for intravenous use, if applicable, are not required.

Study population

The study population should be homogenous and insulin-sensitive to best detect potential product-related differences and may consist of normal-weight healthy volunteers or patients with type 1 diabetes.

Besides their better availability, healthy volunteers usually exhibit lower intra-individual variability compared to patients with type 1 diabetes mellitus (T1DM) but have the disadvantage of presence of endogenous insulin which cannot be distinguished from exogenously administered insulin by the available assays, except for some insulin analogues. Ethnic differences in endogenous insulin levels have been reported, e.g., individuals of African, South Asian or Hispanic descent have reduced glucose clearance, which should be considered when planning comparative clamp studies. Methods for suppressing endogenous insulin or adjusting measured insulin serum concentrations for estimated endogenous insulin may be considered (see below).

Patients with T1DM recruited into clamp studies should have their serum C-peptide concentration measured to ensure absence of relevant remaining endogenous insulin secretion. In order to achieve comparable baseline conditions in all experiments, it is important to establish stable and comparable baseline blood glucose and insulin levels for some time (ideally one hour) prior to the study intervention, which may be more difficult in patients with T1DM compared to healthy subjects.

Clamp studies including either healthy subjects or patients with T1DM are considered appropriate for comparison of insulins with a short or intermediate duration of action, while patients with T1DM may be preferable for comparison of long-acting insulins.

Insulin sensitivity in women may vary during the menstrual cycle and it is unclear whether this may affect study results. Thus, inclusion of only men in the studies might be preferable.

Insulin clamp studies

There is general agreement that the euglycaemic hyperinsulinaemic clamp technique is the best available method for the measurement of insulin action. In these clamp experiments, the plasma insulin concentration is raised (e.g. by subcutaneous injection of insulin) and the blood-glucose level maintained ("clamped") at a pre-defined level by means of a variable infusion of glucose. Measurements of plasma insulin concentrations and glucose infusion rate (GIR) allow an estimation of the time-concentration and time-action profile, respectively, and, if investigated in the same clamp study, of the relation between exposure and glucose-lowering effect.

Different clamp methods and feedback algorithms for maintaining blood glucose levels exist. Clamp studies can be performed manually or by using an automated procedure. Both techniques require substantial experience. However, both methods have been reported to provide similar and reproducible results as long as there are no rapid changes in glucose requirements, which may not be recognised in time depending on the length of intervals between the blood glucose measurements during the manual clamp. A double-blind design is strongly recommended, especially for manual clamps which are more prone to bias by the examiner compared to automatic clamps. If this is not possible, other means should be applied to effectively reduce potential investigator-related bias.

Test conditions for a comparative clamp study need to be standardised as much as possible to reduce variability. Study subjects should undergo the clamp experiments after an overnight fast (usually 10 to 12 hours) and remain fasting throughout the tests to avoid a confounding effect on study results. In patients with diabetes, carry-over effects from the participants' last pre-study insulin injection should be minimised. Ideally, the clamp glucose target should be reached at least one hour before study insulin administration without any glucose infusion during this last hour. Standardisation of clamp technique and factors influencing insulin sensitivity such as time of day, physical activity and food

intake/diet, avoidance of alcohol, caffeinated drinks, smoking or medication other than the study medication and absence of intercurrent illness/infection or mental stress are important. Standardisation of habits may be relevant up to several days prior to the day of examination. In the test facility, the subjects should be allowed to adapt to the experimental situation to establish a comparable metabolic situation and should stay in a relaxed environment and avoid physical activity throughout the experiment. This highlights that even small details are important.

When healthy volunteers are enrolled in the clamp studies, their endogenous insulin production may interfere with PK and/or PD measurements. For evaluation of prandial insulins, the insulin bolus is expected to largely suppress endogenous insulin for the duration of the clamp. Endogenous insulin can usually be sufficiently suppressed by clamping blood glucose levels below the subject's fasting glucose (see below). Alternatively, a priming dose of rapid- or short-acting insulin, followed by a basal rate (e.g., 0.10 to 0.15 mU/min/kg) can be used but the co-administration of basal insulin infusion has been shown to alter the late glucodynamic profile of NPH insulin and possibly and even more relevantly of long-acting insulin preparations, overestimating the effect of the study insulins. Although somatostatin provides maximal suppression of endogenous insulin, glucagon and growth hormone during clamp studies, its use cannot be generally recommended due to tolerability issues. In addition, it should be noted that somatostatin reduces insulin clearance by about 20%, thus prolonging the duration of insulin action artificially. Serum C-peptide should be measured in parallel to insulin concentrations to estimate the extent and consistency of suppression of endogenous insulin throughout the experiment. In the absence of insulin suppression, C-peptide correction methods have been proposed but it is unclear whether they enhance sensitivity of the clamp studies or reduce variability of the clamp-derived measures. Regardless which method is used, it should be justified and consistent throughout the clamp studies to ensure comparable test conditions.

Frequently used insulin doses in clamp studies are 0.2 to 0.3 U/kg bodyweight for rapid-/short-acting insulins, 0.3 to 0.4 U/ kg bodyweight for intermediate-acting insulins and 0.4 to 0.6 U/kg for long-acting insulins. Doses in the upper range usually produce a more reliable PD response, thereby reducing PD variability. The resulting levels of hyperinsulinaemia are expected to lie on the steep part of the dose-response curve of insulin and can thus be expected to be highly sensitive to detect potential differences in the time-action profiles of two insulins. Injection site and technique should be standardised to decrease variability.

In healthy subjects the blood glucose concentrations are usually clamped below (for example 0.3 mmol/L (5 mg/dL) or 10%) the subjects fasting glucose or at 4.4-5.6 mmol/L (80-100 mg/dL). In patients with T1DM, blood glucose concentrations are typically clamped at 5.6 mmol/L (100 mg/dL). Acceptable deviations of blood sugar levels from this value during the clamp should be pre-defined. Glucose levels below approximately 3.3 mmol/L (60 mg/dL) should be avoided because they result in the stimulation of counter-regulatory hormones (epinephrine, glucagon, cortisol, growth hormone) to increase blood glucose concentrations and lead to a rapid and pronounced worsening of insulin sensitivity, thus influencing the estimated time-action profile of the investigated insulin preparation.

The duration of the clamp studies needs to take into account the known duration of action of the investigated insulin preparation and its dose-dependency. The duration of action in glucose clamp studies may be defined as the time from insulin injection to GIR returning to baseline or to a predefined value (e.g. 0.5 mg/kg/min) or, in patients with diabetes, of blood glucose values exceeding a predefined threshold, e.g. 8.3 mmol/L (150 mg/dL). Typical clamp durations are 8 to 10 hours for rapid-acting and 10 to 12 hours for short-acting insulins. For intermediate and long-acting insulins, clamp durations of at least 24 hours are recommended.

A rationale for the selection of the clamp duration should always be provided taking into account the known effects of insulin dose and somatostatin use (if applicable) on duration of insulin action and ethnic differences in insulin clearance.

Endpoints/statistical analyses

Pharmacokinetics (PK)

In case of rapid- and short-acting insulins, $AUC_{(0-t)}$ (AUC until end of clamp) and C_{max} should be defined as primary endpoints and $AUC_{(0-\infty)}$, partial AUCs (such that are meaningful for the respective insulin), t_{max} and $t_{1/2}$ as secondary endpoints.

In case of intermediate-acting insulins, $AUC_{(0-\tau)}$ and C_{max} should be defined as primary endpoints and $AUC_{(0-t)}$, $AUC_{(0-\infty)}$, meaningful partial AUCs, t_{max} and $t_{1/2}$ as secondary endpoints.

Long-acting insulins typically exhibit a flat pharmacokinetic profile. Therefore, determination of C_{max} and t_{max} may not be possible and may be clinically meaningless. In this case, $AUC_{(0-\tau)}$ should be the primary endpoint and measures of partial AUCs, e.g. $AUC_{(0-\tau 50\%)}$ and $AUC_{(\tau 50\%-\tau)}$, the secondary endpoints. $T_{1/2}$ should be determined where possible.

For the primary endpoints AUC and C_{max} (where relevant), the 90% confidence interval of the ratio test/reference should be determined. In the absence of specific acceptance limits for biological medicinal products in general and for insulin specifically, the conventional acceptance range for bioequivalence, i.e. 80% to 125%, is recommended, unless otherwise justified. If high variability is anticipated, a replicate design study should be considered (e.g. 3-period cross-over design with replication of reference) to justify widening of the acceptance range (for details, reference is made to the Guideline on the investigation of bioequivalence, CPMP/EWP/QWP/1401/98 Rev.1). For the other parameters descriptive statistics would be appropriate.

Pharmacodynamics (PD)

The glucose-infusion rate (GIR) over time describes the time-action profile of an insulin preparation.

In general, GIR-$AUC_{(0-t)}$ and GIR_{max} should be measured as primary endpoints for rapid- and short-acting insulins, GIR-$AUC_{(0-\tau)}$ and GIR_{max} for intermediate-acting insulins and GIR-$AUC_{(0-\tau)}$ for long-acting insulins. Other meaningful pharmacodynamic endpoints are time to onset of action and t_{GIRmax} for rapid-, short- and intermediate-acting insulins and partial GIR_{AUC} (such that are meaningful for the respective insulin).

If, based on comprehensive analytical characterisation and non-clinical *in vitro* tests using sensitive, orthogonal and state-of-the art methods, close similarity in physicochemical and functional characteristics can clearly be shown for the biosimilar and the reference insulin, all GIR-related parameters can be defined as secondary endpoints. Nevertheless, the PD results should always reasonably support the PK results.

Calculation of 95% confidence intervals will be required for all PD parameters. For primary GIR parameters, equivalence margins should be pre-defined and justified. In case a replicate design study is performed, intraindividual variability should also be documented for PD endpoints.

Quality of the insulin clamps

It is not easy to control the blood glucose concentrations during the clamp study. Depending on the measurement intervals and feedback algorithm, and due to the inherent measurement delay between sampling and resetting the glucose infusion and the subsequent delay of change in blood glucose levels in response to GIR changes, blood glucose values usually do not correspond to the exact target value but vary around it. In response to that, variations (“noise”) in GIR occur. The Applicant should provide

an estimate of the quality of the performance of the clamp study, e.g. by calculating mean values, root mean square deviation and coefficient of variation of the blood glucose concentrations. The results should be discussed and, where possible, compared with values reported in the literature. Listing of individual clamps should also be provided. The noise of the GIR measurements for the calculation of GIR_{max} and time-related parameters (such as t_{GIRmax}) can be reduced by fitting a mathematical model. The algorithm for GIR adjustment should be predefined and the appropriateness of the applied smoothing method demonstrated. In contrast, GIR-AUC is usually not strongly influenced by fluctuations and may be calculated from the unsmoothed GIR data.

Specifics of long-acting insulin preparations

Long-acting insulin preparations are intended to produce a time-concentration profile which, as far as possible, approximates physiological basal insulin secretion. Due to their flat pharmacokinetic profile, determination of C_{max} and t_{max} (for insulin and GIR) may not be possible and may be meaningless. Due to the slow decline in insulin action, together with the unavoidable variations of the GIR, especially in the "tail part" of the GIR curve, it may be difficult to determine the duration of action of a long-acting insulins, particularly in healthy subjects with interfering endogenous insulin. Therefore, patients with type 1 diabetes are generally considered more suitable to determine the time-action profile of long-acting insulins.

On the other hand, comparing the tail-end of the insulin/GIR profile of a long-acting insulin, administered e.g. once daily, may not be of great clinical relevance since the residual insulin and insulin action from the previous dose will usually be small compared to the effect of the following insulin dose. For this reason, $AUC_{(0-\tau)}$ rather than $AUC_{(0-t)}$ is recommended as primary PK endpoint (see above). It will be the responsibility of the applicant to justify the population used and the sensitivity of the test model/testing conditions to detect relevant differences, if present, in the PK and PD profiles between the test and the reference products.

Despite the above mentioned limitations and the increased intra-subject variability of long-acting compared to short-acting insulins, the hyperinsulinaemic euglycaemic clamp has been successfully used for the comparison of the PK and PD profiles of currently approved long-acting insulin preparations.

Requirements for different preparations containing the same active ingredient

In case a biosimilar manufacturer develops different preparations, e.g. short-acting, intermediate-acting and biphasic preparations containing the same active ingredient, PD data are not needed for all of these preparations. The following programme would be acceptable to show similar efficacy of such insulin preparations with their respective reference products:

1) Demonstration of similar PK and PD profiles for the soluble insulin preparations.

2) Demonstration of similar PK profiles of the other insulin preparations with their respective reference medicinal products. Any PD data collected during PK studies should be presented.

Clinical efficacy

There is no anticipated need for specific efficacy studies since endpoints used in such studies, usually HbA1c, are not considered sensitive enough for the purpose of showing biosimilarity of two insulins.

Clinical safety

Generally, safety studies should be performed with specific focus on immunogenicity. Safety studies should include a reasonable number of patients with type 1 diabetes. If a mixed population is included, stratification for type of diabetes and pre-existing anti-insulin antibodies is necessary. It is

acknowledged that blinding of study participants is likely unfeasible but, at minimum, anti-drug antibodies should be determined in a blinded fashion. Since anti-drug antibodies, if any, usually develop early-on, a 6-month study investigating incidence and titres of antibodies to the test and reference medicinal products should be performed. However, there is no need to power the study to formally demonstrate non-inferiority regarding immunogenicity and, if considered desirable by the sponsor, it would be acceptable to calculate sample size based on an efficacy-oriented endpoint (such as HbA1c). The potential impact of anti-drug antibodies, if detected, on glycaemic control, insulin requirements and safety, especially local and systemic hypersensitivity reactions, should be investigated.

If a background insulin is given during the trial (e.g. an approved prandial or basal insulin in addition to the test insulin), this should not be changed during the evaluation period. In case a biosimilar manufacturer develops different preparations, e.g. short-acting, intermediate-acting and biphasic preparations containing the same active ingredient, only a single safety study is usually required using either all of these preparations, only the biphasic preparation or only the free combination of short- and intermediate-acting preparations and their respective reference products. However, if a formulation contains excipients for which no or very limited experience exists, a separate safety/immunogenicity study should be considered for this formulation.

In certain cases, a pre-licensing safety study including immunogenicity assessment may be waived. The following prerequisites apply: Firstly, biosimilarity between the biosimilar and the reference insulin can be convincingly concluded from the physicochemical and functional characterisation and comparison using sensitive, orthogonal and state-of-the-art analytical methods, and from the comparison of the pharmacokinetic and pharmacodynamic profiles. These data should provide sufficient reassurance that adverse drug reactions which are related to exaggerated pharmacological effects (e.g. hypoglycaemia) can be expected at similar frequencies. Secondly, the impurity profile and the nature of excipients of the biosimilar do not give rise to concerns. Appropriate scientific justification for waiving a safety/immunogenicity study should always be provided.

6. Pharmacovigilance plan

Within the authorisation procedure the applicant should present a risk management plan in accordance with current EU legislation and pharmacovigilance guidelines. The RMP of the biosimilar should always take into account identified and potential risks associated with the use of the reference product. In addition, it should be discussed in detail how these safety concerns will be addressed in post-marketing follow-up.

7. Extrapolation of indication

Demonstration of similar pharmacokinetic and, where needed, pharmacodynamic profiles of the biosimilar and the reference product and absence of safety issues with subcutaneous use will allow extrapolation of these data to intravenous use, if applicable, and to other indications and patient populations licensed for the reference product. If a rapid- or a short-acting biosimilar insulin is intended for use in pumps, additional stability data may be required.

8. Definitions

Pharmacokinetic parameters

$AUC_{(0-t)}$: Area under the plasma concentration curve from administration to end of clamp at time t;

366 $AUC_{(0-\infty)}$: Area under the plasma concentration curve extrapolated to infinite time;

367 $AUC_{(0-\tau)}$: AUC for the time of a dosing interval;

368 $AUC_{(0-\tau 50\%)}$: AUC during the first half of a dosing interval;

369 $AUC_{(\tau 50\%-\tau)}$: AUC during the second half of a dosing interval;

370 C_{max} : Maximum plasma concentration;

371 t_{max} : Time until C_{max} is reached;

372 $t_{1/2}$: Plasma concentration half-life;

373 Pharmacodynamic parameters

374 GIR-$AUC_{(0-t)}$: Area under the glucose infusion rate curve from administration to end of clamp at time t;

375 GIR-$AUC_{(0-\tau)}$: AUC for the time of a dosing interval;

376 GIR_{max} : Maximum glucose infusion rate;

377 t_{GIRmax} : Time until maximum glucose infusion rate is reached;

378 Time to onset of action: time after insulin injection at which first glucose infusion is required to
379 maintain euglycaemia or time after insulin injection at which GIR increase from baseline exceeds a
380 predefined cut-off (e.g. 10% or 20% increase in GIR from baseline).