

Clamping Down

Glucose clamps now offer more adaptable and reproducible procedures than ever before. Highlighting the latest changes, Clayton Dehn of ICON discusses a valuable tool on the market used for the development of new agents to treat Type 1 and Type 2 diabetes

The pharmaceutical industry is constantly looking for efficiencies in the drug development process in order to speed up the delivery of a molecule to market at a cost-effective price. Pharmacodynamic models can play a pivotal role in providing evidence of target engagement and putative efficacy, as well as aiding with dose selection in subsequent patient trials. In the diabetes therapeutic area the glucose clamp is traditionally applied to measure insulin sensitivity, beta cell sensitivity, or to characterise the time-action profile of an insulin product. The procedure is a powerful, well-established metabolic research method that can facilitate the development of novel agents for the treatment of both Type 1 and Type 2 diabetes.

The technique is based on manipulation of a variety of infusions to directly explore a range of metabolic processes. As such, there are many different types of clamp that may be further modified with accessory techniques. In addition to diversity, the great scientific strength of the glucose clamp is the reproducibility of its results; this is the fundamental element of the glucose clamp's significance as a research tool. This combination of application flexibility and extreme reproducibility make the glucose clamp an appealing tool to streamline the development of new agents to treat Type 1 and Type 2 diabetes.

PROCEDURE

The glucose clamp procedure involves two cannulas being placed in the hands: one is for sample collection; the other for delivery of venous infusions (see Figure 1). Arterialised venous blood is collected from the sampling cannula placed in the dorsum of the hand in retrograde position, with heat applied constantly throughout the procedure. Sampling from a closed-loop system prevents blood volume lost to waste as blood glucose is traditionally

measured at the bedside at five-minute intervals during the clamp.

Infusions are delivered through a cannula placed in a suitable vein contralateral to the sampling cannula. Based on the results of the bedside blood glucose values, the subject's blood glucose is manipulated towards a target with an infusion of dextrose that is typically variable. In some instances this dextrose infusion may be set at fixed rates as with graded glucose infusions, assuming one considers these to be very basic glucose clamps. Glucose clamp procedures may be rudimentarily categorised by the glycaemic target of the procedure as follows:

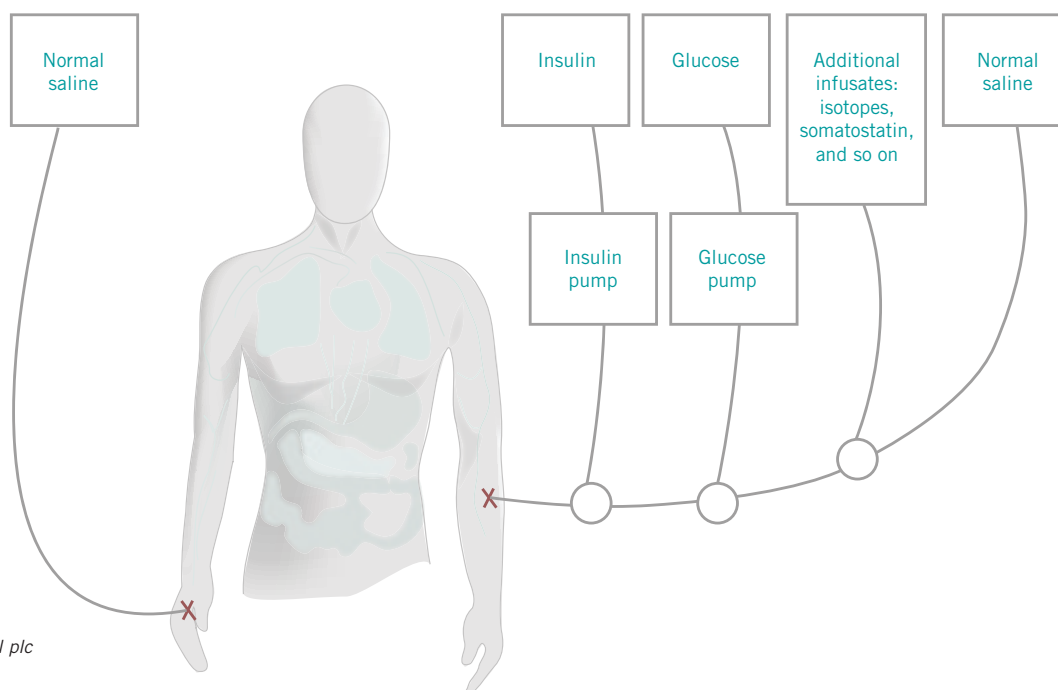
- Euglycaemic clamp: clamping subjects at the target blood glucose level typical of a fasting healthy person
- Hyperglycaemic clamp: clamping subjects at a target blood glucose level above what would be typical for a fasting healthy person
- Hypoglycaemic clamp: clamping subjects at a target blood glucose level below what would be typical of a fasting healthy person
- Isoglycaemic clamp: clamping subjects at the typical fasting blood glucose level for the population being studied. Isoglycaemic clamps in Type 2 diabetics would be at a target blood glucose level that would clinically be considered hyperglycaemic to fasting healthy populations. Isoglycaemic clamps in healthy subjects would also be euglycaemic

ROLE OF INFUSIONS

Glucose clamps may or may not include a venous infusion of exogenous insulin that interrupts the physiologically-

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Figure 1: Glucose clamp visualisation



Source: ICON plc

operating feedback loop between plasma glucose concentration and insulin secretion. This hyperinsulinaemia stimulates peripheral glucose uptake, suppresses endogenous insulin production, and suppresses hepatic glucose production. In this paradigm, the variable amount of dextrose infused to maintain the target blood glucose is indicative of the rate of disappearance of glucose from the periphery. In addition to the primary description of the glucose clamp target blood glucose, clamps may be further described with regard to venous exogenous insulinisation. Clamps that integrate a venous exogenous insulin infusion are typically labelled with the phrase 'insulin clamp' or 'hyperinsulinaemic'. These terms are absent from the title of clamps that do not include venous exogenous insulin infusions. For example:

- Hyperinsulinaemic-euglycaemic clamp: venous exogenous insulin is infused, as is a variable infusion of dextrose, with the aim of maintaining a target blood glucose level typical of a fasting healthy person
- Hyperglycaemic insulin clamp: venous exogenous insulin is infused, as is a variable infusion of dextrose with the aim of maintaining a target blood glucose level above what would be typical for a fasting healthy person
- Hyperglycaemic glucose clamp: venous exogenous insulin is not infused, but dextrose is infused to a target blood glucose level above what would be typical for a fasting healthy person

Additional infusions may be incorporated into glucose clamp procedures. The net effect of these additional infusions is an expansion of the glucose clamp platform application. For instance, the use of stable glucose isotope infusions allows for the quantification of residual hepatic glucose production. This could remain unsuppressed during hyperinsulinaemic-euglycaemic clamps due to hepatic insulin resistance in affected populations. This makes it possible to employ the glucose clamp technique in the study of insulin resistant populations such as Type 2 diabetics, impaired fasting glucose, impaired glucose tolerance or subjects with compensatory hyperinsulinaemia.

NEW USES IN DRUG DEVELOPMENT

The adaptable nature of the glucose clamp makes it a useful tool in metabolic drug development with relevance well beyond the traditional applications as a measure of insulin sensitivity, beta cell sensitivity, or time-action profiling of insulin products. Additional concomitant infusions and accessory techniques may also be included to achieve specific goals depending on the scope and focus of the clamp procedure. A selection of other augmented clamp procedures includes:

- Somatostatin infusion for maximal suppression of endogenous insulin, glucagon and growth hormone during hyperinsulinaemic-pancreatic clamp tests

“ The flexibility of clamp procedure design allows for controlled examination of an investigational product’s behaviour in almost any conceivable metabolic environment ”

- Supplementary potassium infusion in order to prevent insulin induced hypokalaemia allowing for supraphysiological infusions of insulin
- The addition of an enteral glucose challenge reactively compensated for by a corresponding reduction of venous dextrose infusion as an estimate of splanchnic glucose uptake during a clamp
- The incorporation of arginine stimulation as an insulinotrope during a clamp
- Infusion of incretin hormones such as GLP-1 as an insulinotrope during a clamp

The flexibility of clamp procedure design allows for controlled examination of an investigational product’s behaviour in almost any conceivable metabolic environment. The degree to which these conditions and results can be reproduced conveys the glucose clamp’s exploratory value. The intra-individual (within subject) coefficient of variation for the glucose clamp procedure is generally considered to be about 10 per cent. Other indices of insulin sensitivity of glucose metabolism are assessed with the physiologically operating, insulin-glucose feedback loop intact. Among these methods are frequently sampled intravenous glucose tolerance tests with minimal model analysis, insulin tolerance tests, and homeostasis models of glucose tolerance. These other models exhibit an intra-individual coefficient of variation two or three times higher than observed in the glucose clamp.

The relatively low inherent variability associated with well-executed glucose clamp procedures translates into enhanced assurance that changes observed are a treatment effect. This reduced variability also requires exposure to fewer subjects in order to arrive at statistically significant conclusions. Therefore, glucose clamp data can be used to support strategic drug development decisions at a very early stage and, by virtue of the procedure’s adaptable and reproducible nature, streamline the development of new agents to treat Type 1 and Type 2 diabetes.

Further Reading

1. Bergman RN, Finegood DT, and Ader M, Assessment of insulin sensitivity *in vivo*, *Endocr Rev* 6(1): pp45-86, 1985

2. Bokemark L, Froden A, Attvall S, Wikstrand J and Fagerberg B, The euglycemic hyperinsulinemic clamp examination: variability and reproducibility, *Scand J Clin Lab Invest* 60(1): pp27-36, 2000
3. Brehm, Atilla and Roden, Glucose Clamp Technique, in Roden M ed, *Clinical Diabetes Research Methods and Techniques*, pp42-76, 2007
4. DeFronzo RA, Tobin JD, Andres R, Glucose Clamp Technique: A Method for Quantifying Insulin Secretion and Resistance, *Am J Physiol: Endocrinol Metab Gastrointest Physiol* 6(3): E214-E223, 1979
5. Del Prato S, Ferrannini E and DeFronzo RA, Evaluation of insulin sensitivity in man, In Clarke WL, Larner W, Pohl SL eds, *Methods in Diabetes Research Clinical Methods*, pp35-76, 1986
6. Ferrannini E and Mari A, How to measure insulin sensitivity, *J Hypertens*, 16(7), pp895-906, 1998
7. Heinemann L, *Time-Action Profiles of Insulin Preparations*, 2004
8. Insel PA, Liljenquist JE, Tobin JD, Sherwin RS, Watkins P, Andres R, and Berman M, Insulin control of glucose metabolism in man: a new kinetic analysis, *J Clin Invest* 55(5): pp1,057-1,066, 1975
9. Matthew DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF and Turner RC, Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man, *Diabetologia* 28(7): pp412-419, 1985
10. Morris AD, Ueda S, Petrie JR, Connell JM, Elliott HL and Donnelly R, The euglycemic hyperinsulinaemic clamp: an evaluation of current methodology, *Clin Exp Pharmacol Physiol* 24(7): pp513-518, 1997
11. Soop M, Nygren J, Brismar K, Thorell A and Ljungqvist O, The hyperinsulinaemic-euglycemic glucose clamp: reproducibility and metabolic effects of prolonged insulin infusion in healthy subjects, *Clin Sci* 98(4): pp367-374, 2000

About the author



Clayton A Dehn is Director of Metabolic Research at ICON. Having completed an MS in Physiology at Texas Tech University Health and Science Center in 2003, Clayton began his professional career at a start-up biotech company developing investigational devices, techniques and

compounds for use in the fertility and assisted reproductive industry, before advancing to the role of Director of Clinical Research and Development. In 2006 Clayton refocused his research interests on metabolism; subsequently he has conducted thousands of clamps and brings substantial experience with tools of metabolic research to his current role.

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