

**Title:** A Quantitative Look into the Effect of Glucose on Glucokinase Function in the Presence of an Activator

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**Objectives:** Hexokinase IV, or glucokinase (GK), is an allosteric enzyme responsible for phosphorylation of glucose to glucose-6-phosphate. Expressed in the liver, pancreas, gut and some CNS tissues, it acts as a glucose sensor, regulating glucose metabolism [1]. The activity level of GK is markedly low in low glucose conditions, but as glucose becomes more abundant, GK activity significantly increases, yielding increased glucose metabolism [2]. Mutations in GK have been linked to metabolic diseases, including maturity onset diabetes of the young type 2 (MODY2) and persistent hyperinsulinemic hypoglycemia of infancy [3], making it a target of interest in the treatment of diabetes. The activity of GK increases in the presence of a GK activator, which binds to the allosteric site, thus left-shifting GK's natural  $K_m$  for glucose [4]. The objective of this effort was to investigate how the dependence of GK activity on glucose affects the overall efficacy of a GK activator in a diabetic animal model.

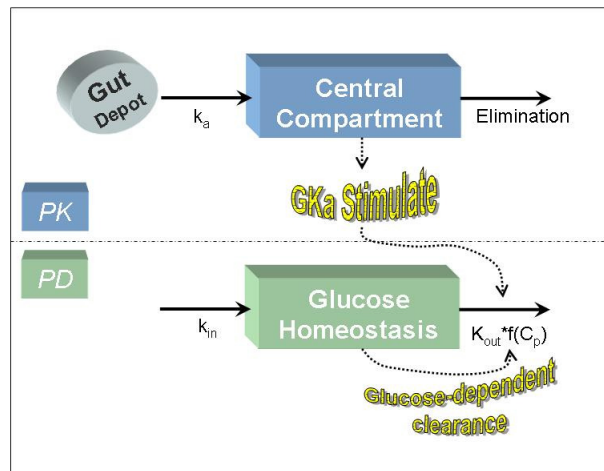
**Methods:** We first investigated the *in vitro* activity of GK in the presence of varying concentrations of glucose and glucose plus a GK activator using the wild-type GK mouse enzyme. Finding that the activator significantly left-shifts the  $K_m$  of GK for glucose, the activator was tested in an acute dose-response study using diet-induced obese (DIO) mice to assess the ability of the activator to reduce plasma glucose levels. Finally, we designed a mechanistically inspired PK/PD model to determine the exposure-response relationship between an activator and plasma glucose, integrating the *in vitro* biochemical data with the *in vivo* dose-response data. The resulting model is a modified indirect response model that assumes plasma glucose homeostasis in the absence of a GK activator (Figure 1). Upon activator exposure, the clearance rate of glucose from the plasma is accelerated in a glucose and activator concentration-dependent fashion. A Hill term is used to describe the nonlinear relationship between glucose and GK activity, governed by three parameters – maximum activity,  $K_m$ , and the Hill coefficient. The values of these parameters were obtained through the *in vitro* activity studies conducted. In the model,  $K_m$  is modulated as a function of the concentration of GK activator in plasma. The underlying assumption in this model is that the biochemical relationship between GK activity level and glucose concentration is the direct, predominant biological force controlling plasma glucose modulation caused by GK activator exposure. The model was calibrated to the dose-response data in DIO mice, yielding an estimation of maximum effect and  $EC_{50}$ . Mathematical analysis of the model was then conducted to identify limitations of the model to simulate glucose modulation under a variety of conditions. Simulations were then run within these limitations to explore predicted *in vivo* plasma glucose dynamics in the presence of an activator at various starting glucose levels.

**Results:** Upon model calibration, we found a clear, dose-dependent, nearly direct response from plasma glucose due to exposure of a GK activator. One of the simulations is depicted in Figure 2, where the initial values of plasma glucose were set to correspond approximately with maximum glucose-lowering effect and homeostatic (baseline) plasma glucose values were left unchanged from the levels found in the dose-response study. Based on the rate of rebound to homeostasis, this simulation suggests that the ability of a GK activator to lower plasma glucose is dose-dependently decreased in moderately low glucose conditions.

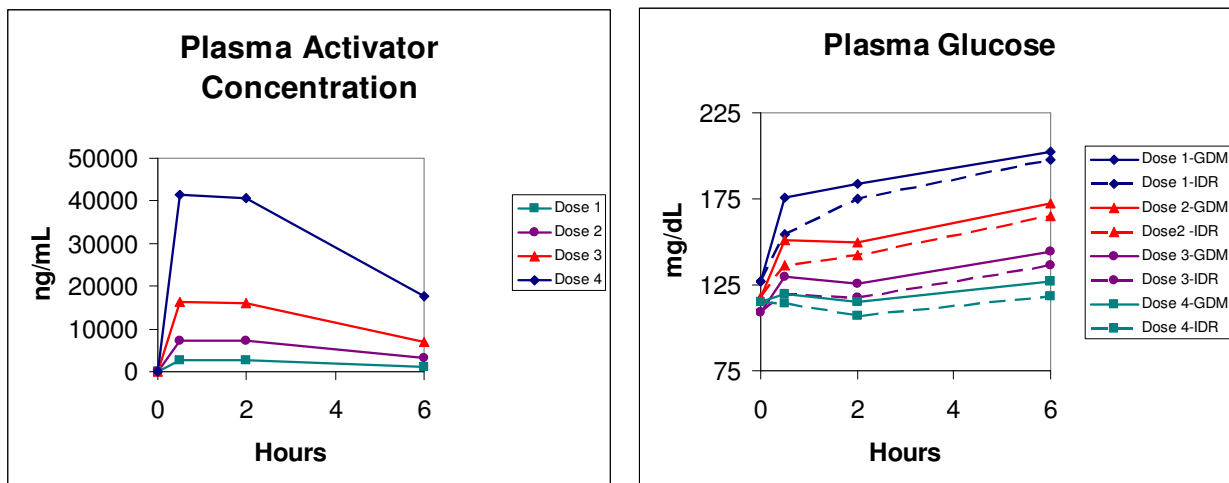
**Conclusions:** Through integration of biochemical understanding with *in vivo* data via PK/PD modeling, simulations suggest that a GK activator's ability to lower plasma glucose in the DIO mouse is decreased with decreasing glucose levels. Furthermore, this decrease appears to be dose-dependent, with the most significant decreases occurring in lower (clinically relevant) doses. This speaks to the possibility of designing a GK activator such that the risk of treatment-induced hypoglycemia is mitigated. This model-driven hypothesis, however, should be experimentally explored. A limitation of the model that the mathematical analysis admitted is that the homeostatic level of plasma glucose must remain unchanged from the level set during model calibration to the dose-response data in order to remain confident in simulations. This limitation could be eliminated with appropriate experimental studies and the addition of more mechanistic granularity in the model, in which case the space of experimental conditions in which we are confident to simulate in expands – an example of learning and confirming through a mechanistic approach.

**References:**

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- [3] Efanov AM, Barrett DG, Brenner MB, *et al.* A Novel Glucokinase Activator Modulates Pancreatic Islet and Hepatocyte Function. *Endocrinology* 146: 396-3701, 2005.
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**Figure 1:** PK/PD model describing the time-course of glucose response in DIO mice following a single oral dose of a GK activator.



**Figure 2:** PK/PD model simulations of GK activator PK and glucose time course in DIO mouse plasma. The initial plasma glucose was set to approximately 50% (maximum effect) of baseline. The mechanistically inspired model (GDM – solid lines) is compared to a standard indirect response model (IDR – dashed lines) that was fitted to the same dose-response data and does not account for the glucose dependence of GK activity.