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Mechanism-based disease progression modeling of type 2 diabetes in Goto-Kakizaki rats

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Abstract

The dynamics of aging and type 2 diabetes (T2D) disease progression were investigated in normal [Wistar-Kyoto (WKY)] and diabetic [Goto-Kakizaki (GK)] rats and a mechanistic disease progression model was developed for glucose, insulin, and glycosylated hemoglobin (HbA1c) changes over time. The study included 30 WKY and 30 GK rats. Plasma glucose and insulin, blood glucose and HbA1c concentrations and hematological measurements were taken at ages 4, 8, 12, 16 and 20 weeks. A mathematical model described the development of insulin resistance (IR) and β -cell function with age/growth and diabetes progression. The model utilized transit compartments and an indirect response model to quantitate biomarker changes over time. Glucose, insulin and HbA1c concentrations in WKY rats increased to a steady-state at 8 weeks due to developmental changes. Glucose concentrations at 4 weeks in GK rats were almost twice those of controls, and increased to a steady-state after 8 weeks. Insulin concentrations at 4 weeks in GK rats were similar to controls, and then hyperinsulinemia occurred until 12–16 weeks of age indicating IR. Subsequently, insulin concentrations in GK rats declined to slightly below WKY controls due to β -cell failure. HbA1c showed a delayed increase relative to glucose. Modeling of HbA1c was complicated by age-related changes in hematology in rats. The diabetes model quantitatively described the glucose/insulin inter-regulation and HbA1c production and reflected the underlying pathogenic factors of T2D—IR and β -cell dysfunction. The model could be extended to incorporate other biomarkers and effects of various anti-diabetic drugs.

Keywords

Type 2 diabetes; Disease progression modeling; Insulin resistance; β -cell function

Introduction

Diabetes is “a group of diseases marked by high blood glucose resulting from defects in insulin production, insulin action, or both” [1]. Thus, the glucose–insulin system is central to the dynamics of maintaining glucose regulation. Circulating glucose arises from endogenous liver production and exogenous food intake. When necessary for maintaining circulating glucose concentrations, glucose can be derived by either breaking down glycogen stores in the liver or gluconeogenesis in the liver and kidney. Increased glucose stimulates pancreatic β -cells to secrete insulin and, in turn, the elevated insulin concentrations decrease glucose concentrations by stimulating peripheral tissue glucose uptake [2]. In addition, insulin stimulates glycogen synthesis in liver and muscle, and inhibits gluconeogenesis in the liver and kidney. Chronic hyperglycemia can result from the inability to dispose of ingested glucose, excessive gluconeogenesis or both. Disturbance of the glucose–insulin system results in hyperglycemia and subsequent diabetes.

About 90% of all diagnosed cases of diabetes are type 2 diabetes (T2D), a chronic and progressive metabolic disorder with uncontrolled glucose concentrations. The pathogenesis of T2D involves IR [3], in which most tissues do not use insulin properly. Therefore, β -cells secrete more insulin to compensate. Thus, hyperinsulinemia is usually observed in prediabetic patients. As long as β -cells can provide sufficient insulin, IR can last for decades without leading to disease. However, when β -cells do not function adequately to provide enough insulin, perhaps due to genetic factors, uncontrolled hyperglycemia occurs. Thus, the two key elements in the pathology of T2D are IR and β -cell dysfunction.

Classical pharmacodynamic modeling usually ignores the time course of disease progression. The assumption of consistency is not realistic for long-term studies, as T2D is a chronic progressive disease. Quantification of the disease progression with mathematical modeling can improve the understanding of T2D and facilitate the characterization of chronic drug effects. This approach is relevant to clinical pharmacology [4]. However, development of such models has been limited by the very slow time course of disease progression, high inter-individual variability, and the absence of placebo studies.

Animal models can be a useful substitute for humans in studies of disease progression. There are many T2D animal models available. Goto-Kakizaki (GK) rats are produced by repeated inbreeding of Wistar rats using glucose intolerance as the selection index. After 35 generations of breeding, diabetes in this animal model is stable. These rats exhibit a spontaneous polygenic T2D, which is similar to human T2D [5]. GK rats have been commercially available in the US since 2005 (www.taconic.com). GK rats show hyperglycemia, mild IR, impaired glucose-induced insulin secretion, and a decrease in β -cell mass as the animals age. In general, GK rats provide a satisfactory animal model for investigating T2D disease progression with or without treatment. Although the physiology of GK rats has been extensively studied, no one has quantitatively investigated the whole time course of disease progression in these rats.

In this report, we sought to carefully characterize disease progression in GK rats quantitatively using age-matched Wistar-Kyoto (WKY) rats as controls. A mechanistic disease progression model was developed, with plasma glucose and insulin as biomarkers.

The appropriateness of the application of glycosylated Hb (HbA1c), another firmly established clinical biomarker, to describe diabetes progression in rodents was also justified.

Methods

This study included 30 GK spontaneously diabetic and 30 WKY control male rats obtained from Taconic Farms (Germantown, NY). The research protocol adhered to the “Principles of Laboratory Animal Care” (NIH publication 85-23, revised in 1985) and was approved by the University at Buffalo Institutional Animal Care and Use Committee.

Animals were received at 21 ± 3 days of age. For analytical purposes, all animals were considered 22 days old at the time of arrival. Rats were maintained in a separate room in our animal facilities under stringent environmental conditions that included strict adherence to 12 h:12 h light:dark cycles. All animal handling was carried out between 1.5 and 3.5 h after lights on. All rats were housed in individual cages, and received a standard rat diet [10% energy from fat (Harlan TekLad 2016)] ad lib from the time of arrival in our facilities (shortly after weaning) until time of sacrifice.

Six animals from each group were sacrificed at ages: 4, 8, 12, 16, and 20 weeks. Animals were anesthetized with intraperitoneal injections of ketamine (80 mg/kg)/ valium (5 mg/kg), followed by aortic exsanguination using EDTA (4 mM final concentration) as anticoagulant. Plasma was prepared from exsanguinated blood by centrifugation ($2000 \times g$, 4°C, 15 min), aliquoted, and stored at -80°C .

Blood HbA1c

Blood HbA1c was measured by A1cNOW InView HbA1C test meters (Metrika, Sunnyvale, CA) in whole blood at the time of sacrifice.

Blood glucose

Blood glucose was measured at the time of sacrifice using a BD Logic glucose monitor (Nova Biomedical Corporation, Waltham, WA). However, at later ages, many GK rats had blood glucose values exceeding the range of the meter (600 mg/dl). Therefore, glucose concentrations were also determined in plasma by the enzymatic assay.

Plasma glucose

Plasma glucose was measured by the glucose oxidase method (Sigma GAGO-20, Sigma, St. Louis, MO). Manufacturer's instructions were modified such that the assay was carried out in a 1 ml assay volume, and a standard curve consisting of seven concentrations over a 16-fold range was prepared from the glucose standard and run with each experimental set. Experimental samples were run in triplicate.

Plasma insulin

Insulin was measured in plasma samples using a commercial RIA (RI-13K Rat Insulin RIA Kit, Millipore Corporation, St. Charles, MO). The assay was carried out according to manufacturer's directions with standards run in duplicate and experimental samples run in triplicate. Two experimental samples were selected as “quality control standards” to assess experimental variations between different runs. Such interassay variation was less than 10%.

Hematology

A Cell-Dyn 1700 (Abbott Laboratories, Abbott Park, IL) was used for the hematological tests: RBC count (10^6 per microliter), Hb concentration (grams per deciliter), mean

corpuscular hemoglobin (MCH) (picograms per cell). Hematological parameters in the blood with EDTA were analyzed within 4 h of blood collection. All procedures were based on the manufacturer's instructions.

Mechanism-based disease progression model

Feedback model of glucose–insulin dynamics

Figure 1 shows a general schematic for the entire disease progression model. Disease progression is initiated in the transit compartments based on pathophysiological elements in the development of diabetes. The glucose and insulin system was characterized with two linked turnover models, which described the dynamics of these biomarkers [6–9].

$$\frac{dG}{dt} = k_{inG} - k_{outG} \cdot G \cdot (1 + S_{Ins} [n] \cdot I), G(0) = G_0 \quad (1)$$

$$\frac{dI}{dt} = k_{inI} [m] \cdot (1 + S_G [r] \cdot (G - G_0)) - k_{outI} \cdot I, I(0) = I_0 \quad (2)$$

where G and I represent glucose and insulin plasma concentrations. Glucose is constantly produced with a zero-order rate constant k_{inG} and utilized with a first-order rate constant k_{outG} . Insulin is assumed to control glucose concentrations by stimulating its disposition with a linear efficiency constant (S_{Ins}). S_{Ins} represents the capability of insulin to promote glucose elimination, and is defined as insulin sensitivity. Insulin is also produced at a zero-order rate k_{inI} and degraded at a first-order rate k_{outI} . Change of glucose from its initial value (G_0) could stimulate insulin production with a linear efficiency constant S_G , which was defined as glucose sensitivity. The n , m and r are the numbers of transit compartments required to describe the changes of S_{Ins} , k_{inI} , and S_G . At time zero of the observation period, the system was assumed to be at its physiological steady-state yielding the following baseline equations:

$$G_0 = k_{inG} / k_{outG} \cdot (1 + S_{Ins0} \cdot I_0) \quad (3)$$

$$I_0 = k_{inI} / k_{outI} \quad (4)$$

where initial values G_0 and I_0 were fixed as the mean glucose and insulin concentrations of six rats at time zero for each strain.

Maturation in WKY rats

Insulin resistance (IR) IR is defined as $1/S_{Ins}$. Age-related development of IR in WKY rats was described by a function of S_{Ins} using a series of transit compartments with an inhibition factor k_{dis1} , which reflects the effect of aging on development of IR. Each transit compartment was connected by k_t , the turnover rate constant for each compartment. The equations and initial conditions describing the first and last event compartments are:

$$\frac{dS_{Ins-WKY}[1]}{dt} = k_t \cdot S_{Ins0} \cdot (1 - k_{dis1}) - k_t \cdot S_{Ins-WKY}[1], S_{Ins-WKY}[1](0) = S_{Ins0} \quad (5)$$

$$\frac{dS_{Ins-WKY}[n]}{dt} = k_t \cdot S_{Ins-WKY}[n-1] - k_t \cdot S_{Ins-WKY}[n], S_{Ins-WKY}[2 \cdot n](0) = S_{Ins0} \quad (6)$$

Transduction models with different numbers were tested with other model components to find the appropriate number which best captures the IR development. Transit steps may have no direct physiological correlation individually. The series as a whole represents the propagation of a signal with two main features. First, a temporal delay can be seen between the presence of the driving force and the observed effect. Unlike an explicit time delay, which can produce an acute change and be computationally confounding, transit compartments result in a smoothing effect. The magnitude of the effect is drawn out and extended over a longer period of time than the original stimulus [10, 11]. The hypothetical compartments with linear transduction produce smooth changes of disease components.

Maturation of β -cells Similarly, a series of transit compartments with a stimulation factor were used to describe the increase of β -cell function when WKY rats are growing. In our model, the increase of β -cell function was represented by an essential insulin secretion rate k_{inI} . The first and final transit compartments are defined by:

$$\frac{dk_{inI_WKY}[1]}{dt} = k_t \cdot k_{inI0_WKY} \cdot (1 + k_{dis2}) - k_t \cdot k_{inI_WKY}[1], k_{inI_WKY}[1](0) = k_{inI0_WKY} \quad (7)$$

$$\frac{dk_{inI_WKY}[m]}{dt} = k_t \cdot k_{inI_WKY}[m-1] - k_t \cdot k_{inI_WKY}[m], k_{inI_WKY}[2 \cdot m](0) = k_{inI0_WKY} \quad (8)$$

According to Eq. 4, k_{inI0_WKY} was calculated as $k_{outI} \times I_{0_WKY}$.

The β -cells are assumed to respond homogeneously when secreting insulin in response to circulating glucose. The β -cell function indicates the response of the collective β -cell mass to glucose in relation to β -cell mass and glucose-stimulated insulin release while k_{inI} represents the β -cell function at basal state. In WKY rats, since it is assumed glucose sensitivity does not change over time, β -cell function only depends on k_{inI} .

Disease progression in GK rats

The key elements of T2D progression are IR and β -cell dysfunction. The two processes were also modeled using transit compartments with inhibition factors, as in the case of WKY rats.

IR—Development of IR in GK rats was handled by a transduction model with inhibition factor k_{dis3} as:

$$\frac{dS_{Ins_GK}[1]}{dt} = k_t \cdot S_{Ins0} \cdot (1 - k_{dis3}) - k_t \cdot S_{Ins_GK}[1], S_{Ins_GK}[1](0) = S_{Ins0} \quad (9)$$

$$\frac{dS_{Ins_GK}[n]}{dt} = k_t \cdot S_{Ins_GK}[n-1] - k_t \cdot S_{Ins_GK}[n], S_{Ins_GK}[2 \cdot n](0) = S_{Ins0} \quad (10)$$

β -cell dysfunction—In GK rats, the deficiency of β -cell function is represented by both decline of β -cell mass and glucose-stimulated insulin secretion. Therefore, efforts were made to model these two components. Basal β -cell secretion (k_{inI}) and glucose sensitivity (S_G) were down-regulated by diabetes, and described with a transduction model with the inhibition factor k_{dis4} . The gradual decrease of glucose sensitivity is modeled using the same strategy:

$$\frac{dS_G[1]}{dt} = k_t \cdot S_{G0} \cdot (1 - k_{dis4}) - k_t \cdot S_G[1], S_G[1](0) = S_{G0} \quad (11)$$

$$\frac{dS_G[r]}{dt} = k_t \cdot S_G[r-1] - k_t \cdot S_G[r], S_G[2 \cdot r](0) = S_{G0} \quad (12)$$

In GK rats, the feedback adaptation between loss of insulin sensitivity and β -cell secretion was modeled as interaction between S_{Ins} and k_{inf} . The feedback adaptation factor was assumed to be proportional to the rate of the decline of insulin sensitivity in the model. The steeper decline rate indicates a greater feedback effect.

$$FB[n] = - \frac{dS_{Ins-GK}[n]}{dt} \quad (13)$$

$$\frac{dk_{inf-GK}[1]}{dt} = k_t \cdot k_{inf0-GK} \cdot (1 - k_{dis4}) \cdot (1 + FB[n]) - k_t \cdot k_{inf-GK}[1], k_{inf-GK}[1](0) = k_{inf0-GK} \quad (14)$$

$$\frac{dk_{inf-GK}[m]}{dt} = k_t \cdot k_{inf-GK}[m-1] - k_t \cdot k_{inf-GK}[m], k_{inf-GK}[2 \cdot m](0) = k_{inf0-GK} \quad (15)$$

HbA1c dynamics—The values of erythrocyte indices such as RBC, Hb, and MCH changed significantly over time, and these changes need consideration before modeling HbA1c. The hematological baseline model for RBC and MCH proposed by Woo et al. [12] was adapted and applied to changes with age. RBC was modeled by a Gompertz equation:

$$RBC = RBC_0 \times e^{k_s(1-e^{-\alpha t})/\alpha} \quad (16)$$

where k_s is the growth constant, α is the retarding constant, and RBC_0 is the measured RBC count at the starting point.

MCH was modeled by a modified indirect response model as:

$$\frac{dMCH}{dt} = k_{syn}(t) - k_{out} \times MCH \quad (17)$$

$$\frac{dk_{syn}}{dt} = k_d \times \left(1 - \frac{k_{syn}}{k_{syn_min}}\right) \times k_{syn} \quad (18)$$

where $MCH(0) = MCH_0$, $k_{syn}(0) = k_{syn0}$. The change of MCH is controlled by a time-dependent change in the synthesis process [$k_{syn}(t)$], a constant first-order elimination process (k_{out}), and MCH_0 is the observed value at the beginning of the studies. It was assumed that the rate of production $k_{syn}(t)$ gradually decreases, with a first-order process k_d , and reaches its minimal value (k_{syn_min}).

The RBC and MCH were used to calculate the hemoglobin concentration as a product of RBC and MCH based on:

$$Hb(g/dl) = MCH(pg/cell) \times RBC(\times 10^6 \text{ cell}/\mu l) / 10 \quad (19)$$

HbA1c was described by a turnover model where the production depends on both blood glucose and hemoglobin concentrations:

$$\frac{dHbA1c}{dt} = k_{in_HA} \times Glu_{_Blood} \times Hb - k_{out_HA} \times HbA1c \quad (20)$$

where k_{in_HA} is the second-order HbA1c production rate constant and k_{out_HA} is the parameter for loss of HbA1c. The conversion ratio of glucose in blood and plasma is 1.05 (unpublished observations).

Data analysis

All samples were pooled for data analysis. All fittings were performed using the ADAPT II program [13] with the maximum likelihood method. The variance model was:

$$V_i = (\sigma_1 + \sigma_2 \cdot Y_i)^2 \quad (21)$$

where V_i is the variance of the i th data point, σ_1 and σ_2 are the variance model parameters, and Y_i represents the i th model predicted value.

Various proposed models for glucose–insulin dynamics with disease progression were fitted and compared. The final model was selected based on the goodness-of-fit criteria including visual inspection of the fitted curves, sum of squared residuals, Akaike information criterion, Schwartz criterion, and coefficients of variation (CV) of the estimated parameters. Only results of the final model fitting are presented in this paper.

Results

Dynamics of glucose, insulin and HbA1c

GK rats develop spontaneous T2D. Since these rats as well as WKY controls are produced by repeated inbreeding of Wistar rats, age-matched WKY rats were used as controls.

The plasma glucose and insulin changes over 20 weeks of age in WKY/GK rats are shown in Fig. 2. The data are expressed as means ($\pm$ SD). At all ages, glucose concentrations were significantly higher in GK rats ($P < 0.01$) even at the beginning of the study. Plasma glucose in GK rats increased until 12 weeks (>500 mg/dl), and then reached an apparent plateau. Calderari et al. [14] reported that 4 week old GK rats had glucose concentrations of 200 mg/dl, which is close to values in our 4 week GK rats. However, most studies report glucose concentrations approximately at 250 mg/dl [15–18], regardless of age. O'Rourke et al. [19] reported glucose concentrations of 374 mg/dl in 16 week old of GK rats, but the values reported in that study were substantially lower than we observed between 12 and 20 weeks of age.

Glucose was measured in whole blood at time of sacrifice and in plasma by an enzymatic assay. The conversion factor between whole blood and plasma in our study is 1.05 (95% confidence interval 1.01–1.08). This value is close to the conversion factor in humans, 1.11 [20].

The two strains had similar insulin concentrations at 4 weeks. An increase in plasma insulin between 4 and 8 weeks of age in both strains was observed, with GK generally higher than WKY values between 8 ($P < 0.01$) and 16 weeks. Other labs [21, 22] also observed

increased insulin concentrations in adult WKY rats. However, at 20 weeks of age, GK values appeared lower than WKY controls. The variation among animals within a group exhibited high standard deviations, possibly due to true animal variation rather than experimental error, since inter-assay variability was less than 10% for all experimental and QC samples. Samples were also analyzed by an ELISA method which resulted in an almost identical insulin profile (data not shown).

HbA1c was measured in whole blood at the time of sacrifice, and mean values for each strain are presented in Fig. 3. The HbA1c gradually increased with age in both strains until 12 weeks of age, where it reached an apparent plateau. The pattern of HbA1c closely mirrored that of plasma glucose except that the difference in HbA1c between WKY and GK at 4 weeks of age is less pronounced. The differences between WKY and GK rats at all ages were significant ($P < 0.01$). The high glucose concentrations observed in our study could be influenced by feeding, since our rats were maintained in a normal fed state rather than fasting before sacrifice. Another possible reason is the minimal handling of our rats, as we did not perform any procedures other than measuring body weights twice a week until blood and tissue collection at sacrifice.

Disease progression model

The structure of our proposed mechanism-based model is shown in Fig. 1. Figures 2 and 3 present the observed data and model fitting results. Parameter estimates are reported in Tables 1 and 2. This model represented the final selection after comparing several other model versions. For example, when compared with the model without the decrease of S_G for GK rats, the current model resulted in lower AIC value (1,264 vs. 1,510) and better overall fittings. Some models employ a biophase for insulin [23]; this was unnecessary in our study owing to the slow changes over time. Glucose, insulin and HbA1c concentrations in both strains were captured nicely with the current disease progression model.

Despite the structural complexity of the model, most parameters are identifiable with reasonable precision. All parameters could be estimated simultaneously. Although the model generated good fitting and parameter estimates with reasonable precision ($k_t = 2.57$ with CV % as 7.8), slight oscillations with glucose profiles in GK rats were observed. Therefore, k_t was fixed as 2 for computational purposes.

Baseline values for glucose and insulin were determined as the average of observed values from WKY and GK rats at 4 weeks of age. The model employed the physiologic values of glucose and insulin utilization values obtained from previous animal studies. Glucose output rate constant (k_{outG}) was fixed as 464 per week, which corresponds to the circulation half-life of glucose as 15 min, within the range of literature reports [23]. The glucose production rate constant was fixed as a secondary parameter according to Eq. 3 and initial glucose values were fixed as the observed mean concentrations at 4 weeks. At the start of experiments, glucose sensitivity in WKY and GK rats was assumed to be the same, while over time, pancreatic glucose sensitivity gradually decreased in GK rats with deterioration of β -cell function but stayed constant in WKY rats.

Similarly, the insulin output rate constant k_{outI} was fixed as 2,857 per week, corresponding to a circulation half-life of 2.5 min. The insulin concentrations at 4 weeks in both strains were similar and the initial conditions were fixed as the real observation values. The insulin production rate was allowed to change over time due to age/growth (WKY rats) or disease progression (GK rats). The initial production rate constants were fixed as secondary parameters according to Eq. 4. In WKY rats, β -cell function increases with maturation, and this process was modeled as time-dependent changes in k_{inI} using Eqs. 7 and 8. Five compartments were needed to capture the progress. However, β -cell function decreases in

GK rats with diabetes evolution, and the dysfunction process was modeled in k_{inI} and S_G using Eqs. 11–15. Based on the model estimation, β -cell function decreased by around 60.8% in GK rats, but increased 65.0% in WKY rats over these ages.

The meanings of k_{outG} and k_{outI} are not the same as some k_{out} values reported in long-term studies where these parameters may represent the time to reach a new steady-state for glucose after antidiabetic drug treatment. Insulin clearance and insulin-independent glucose disappearance do not contribute to age-related glucose intolerance [24]. Thus, it is reasonable to assume that k_{outI} and k_{outG} are constant over time.

An almost doubled basal hepatic glucose production was found in 2 month old GK rats compared to age-matched WKY rats [21]. In our model, k_{inG} in GK rats is also twice that of WKY rats, (k_{inG} as a secondary parameter). Moreover, glucose production rates at 2 and 12 months was found to be almost the same in both GK and WKY rats [21]. Thus, it appears valid to assume k_{inG} as constant over time in both rat strains.

The number of transit compartments necessary to account for the disease progression was determined by trial and error, and varied between strains. Development of IR required six compartments in WKY rats and four in GK rats to account for the slow development. Insulin sensitivity in both strains changed over time, and the initial values were 1.358 ml/ng. Alteration of IR due to disease is more severe than aging, represented by parameters k_{dis3} and k_{dis1} (9% difference).

Although glucagon is also a regulator of glucose metabolism, both WKY and GK rats exhibit similar glucagon concentrations [25]. Thus, glucagon was not factored in the model.

Hematologic indices—Since hemoglobin does not remain constant in young rats, the hematologic changes over time were taken into account when modeling HbA1c. The data and model fittings are shown in Fig. 3. Both strains had similar patterns of MCH, RBC and Hb. As shown in Fig. 3, RBC counts and Hb increased with time, while MCH values decreased with time. With age, MCH decreased while RBC and Hb increased.

The model for RBC, MCH and Hb has been used to characterize the hematological changes with age in healthy rats [12]. A complex life-span model with a feedback regulatory loop model [26] was not considered, since the life-span of RBC was not affected by an acute disturbance such as occurs with rHuEPO treatment.

The production of HbA1c is governed by glucose and Hb in blood. The elimination of HbA1c depends on the life-span of erythrocytes, and it can be described as a first-order process. Since the life-span of Hb is already considered by Hb turnover, the elimination of HbA1c should not be expected to reflect loss of RBC. The Hb content per cell was not constant but gradually decreased over time, and this decrease was modeled by a time-dependent decrease in the production rate in the IDR model. It was assumed that such a decrease was governed by a first-order process, but as rats got older, the production rate reached a minimal value, and this value was maintained afterwards. The model fitted our data well and generated estimates with reasonable precision.

Discussion

The current study develops a mechanism-based model to describe maturation in WKY rats and disease progression in GK rats. The model integrates data for glucose, insulin, hematology, and HbA1c into a comprehensive system that contains the glucose–insulin feedback relationship and pathophysiologic elements of β -cell dysfunction and IR.

The simple feedback model combined with indirect response models can adequately describe the underlying physiology of the glucose–insulin system [27] and has been extensively applied [6, 7, 9, 28, 29]. The interaction was reflected as inhibition of glucose production and stimulation of glucose utilization by insulin. Mathematically, due to the feedback relationship, incorporation of both actions makes estimation of all parameters computationally difficult and sometimes not feasible. De Winter et al. [28] described insulin action as inhibition of glucose production in humans, arguing that the main regulation involves inhibition of hepatic glucose production and in the fasting state most glucose is taken up by insulin-independent tissues [30, 31]. However, the rats in our study had free access to food to avoid intentional physiological changes associated with starvation, and insulin should have dual effects on k_{inG} and k_{outG} which is not differentiable in the current experimental setting [32]. Furthermore, insulin is relatively ineffective in vitro in rodent liver [33, 34], and it has been suggested that insulin's hepatic action is secondary to its effects on peripheral tissues [35]. Jin and Jusko [7] tested insulin action with inhibition on k_{inG} or stimulation on k_{outG} when modeling glucose regulation by methylprednisolone in non-fasted rats. They found that stimulation of glucose utilization (k_{outG}) better captured the data. Most importantly, gene array analysis of livers in our rats showed that the excessive gluconeogenesis may only contribute to hyperglycemia at 4 weeks, and chronic hyperglycemia in later ages is likely due to impaired glucose disposal [36]. Thus, the feedback model with emphasis on glucose disposal was utilized.

The pathogenesis of T2D involves IR and β -cell dysfunction [3]. De Winter et al. [28] described disease progression in humans as a decrease in both β -cell function and insulin sensitivity, using empirical functions corresponding to HOMA outputs. However, the model was developed for anti-diabetic drug treatment and we did not obtain fasting glucose and insulin to calculate the HOMA index [37]. In another diabetes modeling effort, no real data and model fittings were included [38]. The model presented here incorporated similar ideas in terms of pathogenesis: growth of β -cell mass and increase of IR in WKY rats, and loss of β -cell function accompanying severe IR in GK rats. The model not only adequately predicted the time-course of biomarkers over the observation period, but also generated profiles of IR and β -cell function in agreement with physiology.

Dynamics in WKY rats

IR occurs in adulthood in healthy humans [39] and rodents [40]. When examining the isolated muscle from healthy rats, Goodman [41] found that insulin sensitivity progressively decreased from 3 weeks to around 16 weeks (no observations between 8 and 16 weeks), and then appeared to plateau until 96 weeks. According to our model, the development of IR in WKY rats (Fig. 4a) agrees well with Goodman's findings: IR gradually increased to a plateau at around 12 weeks, and remained constant afterwards.

There is postnatal expansion of β -cell mass in the first 3 months after birth in rodents [42]. The increased insulin secretion observed in our young WKY rats could mainly result from maturation of β -cells. The model predicted that β -cell function represented by k_{inI} rapidly increases after 4 weeks and reached a steady-state at around 12 weeks (Fig. 4b). In addition, it was reported that increments in β -cell mass closely match that of total body weight, and suggested that the adaptation of β -cell mass and insulin secretion was essential in maintaining normal glycemia in physiological conditions [43]. The rapid increase of body weight in WKY rats also occurred in the first 3 months [44], and the model prediction offers support for the existence of this relationship.

Although β -cell hypertrophy in elderly rats appears to be determined by insulin sensitivity [21], no further augmented insulin secretion was observed after 12 weeks. The insulin

sensitivity did not decline much according to the model estimation and the study only covered 5 months of age.

Dynamics in GK rats

The primary disease defects in GK rats are ascribed to β -cell failure and IR. GK rats are not highly insulin resistant compared to other disease models [45], but β -cell dysfunction, probably due to genetic reasons, fails to meet the increased insulin demand and therefore leads to disease.

We observed non-fasting hyperglycemia at 4 weeks of age. Others reported mild fasting hyperglycemia, raised basal insulin secretion, β -cell insensitivity to glucose, and reduced glucose tolerance [25, 46]. A clamp study showed a glucose utilization rate in GK rats significantly lower than in WKY rats [21]. The development of IR in GK rats was handled in the same way as in WKY rats and increased sharply between 8 and 12 weeks (Fig. 4c). This increase of resistance could be mainly due to disease instead of growth, since there is no major alteration of age-related impairment in peripheral insulin action in GK rats [21]. The effect of disease was about 9% greater than that of aging. Considering that GK rats are mildly insulin resistant, the estimated value of the disease effect (k_{dis3}) is reasonable.

The defective β -cell mass and impaired glucose-stimulated insulin secretion has been consistently reported in GK rats. Portha et al. [45] found that not only did GK rats have significantly lower percent of β -cell mass in the pancreas, but the loss of β -cells is progressive. In GK rats, β -cell dysfunction develops as early as 3.5 weeks of age [47]. Therefore, at the start of our observations, some degree of β -cell dysfunction already existed. The decline of β -cell function was represented by both k_{inI} and S_G , allowing these parameters to change gradually over time with the same inhibition factor. In adult GK rats, total pancreatic β -cell mass and pancreatic insulin stores are decreased by 60% [48]. According to the model, at 16 weeks of age, k_{inI} in GK rats was around 70% less than in WKY rats (Fig. 4).

Although β -cell numbers decline over time in GK rats, β -cell failure only becomes evident after 16 weeks. The hyperinsulinemia between 8 and 12 weeks, also observed by O'Rourke et al. [19], was produced by the feedback compensation. This adaptation was assumed to be proportional to the rate of the decline of insulin sensitivity. Therefore, although k_{inI} kept declining, insulin production rates in GK rats actually increased until around 10 weeks of age, and then failed to provide a sufficient supply (Fig. 4e).

HbA1c is widely used to assess average glycemic control. The production of HbA1c is usually modeled as a pseudo first-order process, with proportionality to glucose concentrations [23, 28]. Our average estimate for k_{out} of HbA1c is in agreement with an erythrocyte lifespan of about 120 days in humans using the first-order model. However, although the fittings were good, this method generated an unrealistic k_{outHA} value, which was much larger than $1/TR$ ($TR = 60$ days, life span of RBC in rats). The reason may be that the assumption of the pseudo first-order process does not apply in the case of younger rats. Theoretically, Hb glycosylation is a second-order reaction between Hb and glucose. As the total Hb concentration in blood is large compared with the fraction glycosylated and remains constant in adults, a pseudo first-order process is reasonable. However, RBC reached a plateau at around 18 weeks of age in rats, and the effect of natural growth of rats on RBC/Hb has to be considered. Using the current method, one should not expect k_{outHA} to reflect the RBC lifespan, since the turnover of Hb is already taken care by other equations.

Although our model successfully described glucose, insulin, and HbA1c dynamics with age/disease simultaneously, it was limited by at least one factor. A known mechanism such as

hepatic IR was not included to avoid over-parameterization, assuming this plays a less important role in glucose–insulin homeostasis and maturation/disease progression.

Conclusions

Overall, augmenting the basic glucose/insulin feedback model by including growth and time course of changes in β -cell function and IR yields a reasonable disease progression model. Conflicting reports exist in the literature regarding insulin concentrations in GK rats. An increase, decrease, or no difference [19, 21, 49] compared to controls has been reported. This could be due to different ages at which the animals were studied, as indicated by model simulations (Fig. 5). Rationalization of the disease progression components and parameters requires direct measurement of these indices, and further validation of the model is necessary. Moreover, the use and modeling of HbA1c in rats should be considered in view of changes in erythrocyte indices over time. This model could be beneficial in designing pharmacological studies in GK rats. Because the model is derived from basic principles and physiology, it could be applied to other rat T2D models. After further model validation, perhaps a modified version might also be applied to human translational research, such as investigation of drug effects, as it permits a mechanistic framework to examine the effects of anti-hyperglycemic drugs.

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Fig. 1. Model schematic for inter-regulation of glucose, insulin and HbA1c during maturation (WKY) and T2D (GK) progression. *Parameters* and *symbols* are defined in the tables. *Lines with arrows* indicate conversion to or turn-over of the indicated responses. *Lines ending in closed circles* indicate an effect is exerted by the connected factors. *Open* and *solid boxes* differentiate stimulatory and inhibitory effects. *Dashed lines* depict pathways of maturation/disease progression

Fig. 2.
Time course of plasma glucose (*left*) and insulin (*right*) in WKY (*solid circles*) and GK (*open circles*) rats. All observations are reported as mean $\pm$ SD. Model fittings are shown as *lines*

Fig. 3. Time course of MCH (a), RBC (b), Hemoglobin (c) and HbA1c (d) in WKY (*solid circles*) and GK (*open circles*) rats. All observations are reported as mean $\pm$ SD. Model fittings are shown as *lines*

Fig. 4. Time courses of IR (**a**, **c**), insulin secretion rate constant (**b**, **d**), and/or insulin production rate (**e**), and S_{Glu} (**f**) in WKY (dashed line) and GK (solid line) rats according to model estimations

Fig. 5.
Simulated time courses of plasma glucose and insulin in WKY (*dashed line*) and GK (*solid line*) rats over 52 weeks of age

Table 1

Estimates of parameters for glucose and insulin dynamics

Parameter (units)	Definition	Estimate WKY/GK	CV%
Glucose dynamics			
k_{inG} (mg/dl/week)	Glucose production rate	316262/724518	Fixed ^c
k_{outG} (1/week)	Glucose output rate	464	Fixed ^a
S_{G0} (dl/mg)	Glucose sensitivity	0.0771	30.6
G_0 (mg/dl)	Initial glucose concentration	119/226	Fixed ^b
Insulin dynamics			
k_{outI} (1/week)	Insulin output rate	2857	Fixed ^a
k_{inI0} (ng/ml/week)	Initial insulin production rate	3400/4114	Fixed ^c
I_0 (ng/ml)	Initial insulin concentration	1.19/1.44	Fixed ^b
S_{I0} (ml/ng)	Initial insulin sensitivity	1.358	19.0
Disease progression dynamics			
k_t (1/week)	Transduction rate constant	2	Fixed ^d
k_{dis1}	Aging constant of S_I in WKY rats	0.885	13.4
k_{dis2}	Aging constant of k_{inI} in WKY rats	0.650	22.8
k_{dis3}	Disease progression constant of S_I in GK rats	0.975	42.8
k_{dis4}	Disease progression constant for β -cell function in GK rats	0.608	11.7

^aParameter fixed to values obtained from previous study^bParameter fixed as mean of experimental data^cSecondary parameter^dParameter fixed for computational purpose

Table 2

Estimates of parameters for hematology dynamics

Parameter (units)	Definition	Estimate WKY/GK	CV%
k_s (week ⁻¹)	Growth constant in Gompertz equation	0.134	13.0
α (week ⁻¹)	Retarding constant in Gompertz equation	0.287	15.7
k_d (week ⁻¹)	First-order rate constant for change in $k_{syn}(t)$	0.0210/0.0878	95.0/134
k_{syn_min} (pg/cell/week)	Minimal value of $k_{syn}(t)$	6.75/6.61	43.7/43.7
k_{syn0} (pg/cell/week)	Initial value of $k_{syn}(t)$	8.48/7.97	Fixed ^a
k_{out} (week ⁻¹)	Loss rate constant of MCH	0.571	69.8
k_{out_HA} (week ⁻¹)	Elimination rate constant of HbA1c	1.123	35.9
RBC_0 ($\times 10^6$ cells/ μ l)	Initial value of RBC count	4.55/5.39	Fixed ^b
MCH_0 (pg/cell)	Initial value of MCH	23.0/21.6	Fixed ^b
$HbA1c_0$ (%)	Initial value of HbA1c	4.18/4.62	Fixed ^b

^aSecondary parameter^bParameter fixed as mean of experimental data