

## Systems Medicine Chapter 2

### Dynamical compensation, mutant resistance, and type-2 diabetes

We now build on our work in chapter 1 on the glucose-insulin system. We will understand how it breaks down in type-2 diabetes and identify principles that apply more broadly to other hormone circuits.

The insulin circuit is one of a hundred or so endocrine systems in the body. Endocrine organs communicate with distant organs via hormones that flow in the bloodstream. We will see that endocrine organs face three universal challenges. They must:

- (i) Work precisely even though they communicate with distant organs that have unknown parameters that change over time. This is the problem of *robustness to parameter variations*.
- (ii) Maintain a proper organ size, even though cell populations tend to grow or shrink exponentially. This is the problem of *organ size control*.
- (iii) Avoid harmful mutant cells that can overgrow and take over the organ. This is the problem of *mutant resistance*.

We will discover a unifying and beautiful circuit design that addresses all three problems at once! This chapter also introduces the fundamental physiological laws that will accompany us through the book. Before we start, let's take a nice deep sigh of relief.

#### **The minimal model cannot explain the robustness of glucose levels to variations in insulin sensitivity.**

We ended the last chapter with a mystery. The insulin-glucose feedback loop of the minimal model explained the rise and fall of glucose after a meal but failed to explain how glucose levels are maintained when physiological parameters, like insulin sensitivity  $s$ , change.

The minimal model predicts that insulin resistance (low  $s$ ) raises the glucose baseline above 5mM and lengthens the response time in the glucose tolerance test. However, most people with insulin resistance, including people with obesity, maintain a normal 5mM glucose steady-state concentration, and exhibit normal glucose responses. The minimal model is thus not **robust** to parameters like  $s$ . It is also not robust to differences in blood volume, which dilute out insulin, or to the beta-cell maximal insulin production rate,  $q$ . In fact, the minimal model is not robust to *any* of its parameters.

Robustness must involve additional processes beyond the minimal model's glucose-insulin feedback loop. Indeed, the way that the body compensates for decreased insulin sensitivity is by making more insulin. Each beta cell upregulates its insulin production capacity to the maximal possible. Then, there is an increase in the number and mass of beta cells. This is called beta cell *hyperplasia*—more cells—and *hypertrophy*—bigger cells. The two processes together increase the total mass of beta cells. More beta cell mass means more insulin production. For example, people with obesity are insulin

resistant and have more total beta cell mass than lean individuals. This extra secretion compensates for insulin resistance.

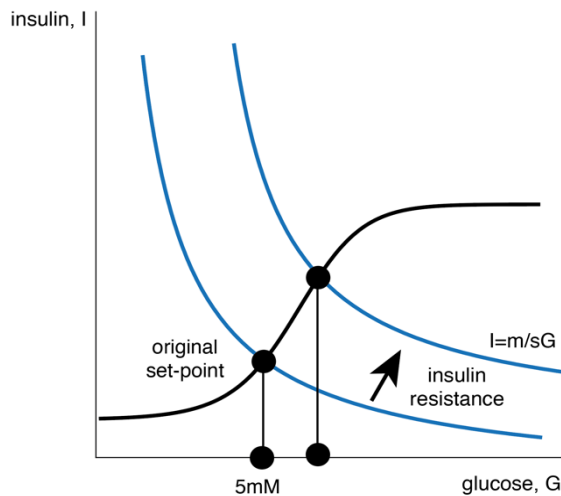


Figure 2.1 Insulin resistance raises the glucose set point.

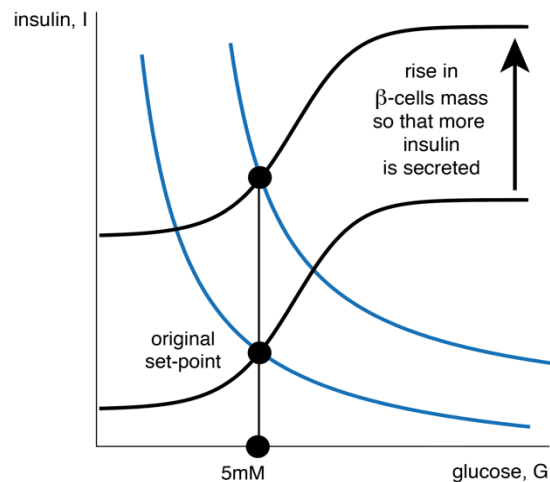


Figure 2.2 Beta cell mass rises to restore the glucose set point.

It's like factories making cars. To make more cars, one can increase production from each factory – but only up to a limit. Beyond that, more factories are needed.

Let's use the phase plot to understand the effect of beta cell mass changes (Fig. 2.1, 2.2). The original set point with 5mM glucose, occurs at the intersection of the two nullclines. Insulin resistance shifts the blue nullcline, and raises the fixed point to higher glucose (Fig 2.1). This is appropriate for short term (hours to days) physiological changes in insulin sensitivity, such as acute stress or inflammation, where elevated glucose is useful. However, long-term insulin resistance over weeks causes beta-cell mass to gradually increase. This raises the other nullcline, shifting glucose levels back towards their original level (Fig. 2.2). In this compensated state, insulin secretion is higher than in the original setpoint, due to the enlarged beta-cell functional mass.

For these shifts to produce precisely the right glucose level, 5mM, beta cells must stop expanding at exactly the right mass (Fig 2.2). Remarkably, they do. **The resulting increase in insulin exactly compensates for the decrease in  $s$ .** Although each unit of insulin is less effective, the amount of insulin produced is increased precisely enough to compensate.

This compensation is seen in the **hyperbolic relation**, in which healthy people show an inverse relationship between insulin sensitivity,  $s$ , and steady-state fasting insulin,  $I_{st}$ . This hyperbolic relationship,  $I_{st} \sim 1/s$ , maintains a constant product of the two variables:  $sI_{st} = const$  (Kahn et al. 1993) (Fig. 2.3). By contrast, for people with diabetes, the same product is lower (Fig. 2.3, right). The origin of this hyperbolic relationship has long been a mystery; we will soon understand it.

### A slow feedback loop on beta cell numbers provides compensation.

To explain how such precise compensation can come about, we need to extend the minimal model by adding an equation to describe how beta-cell total mass,  $B$ , can change.

Here we enter the realm of the **dynamics of cell populations**. These dynamics are unlike the dynamics of protein concentrations inside cells or molecules in the blood.

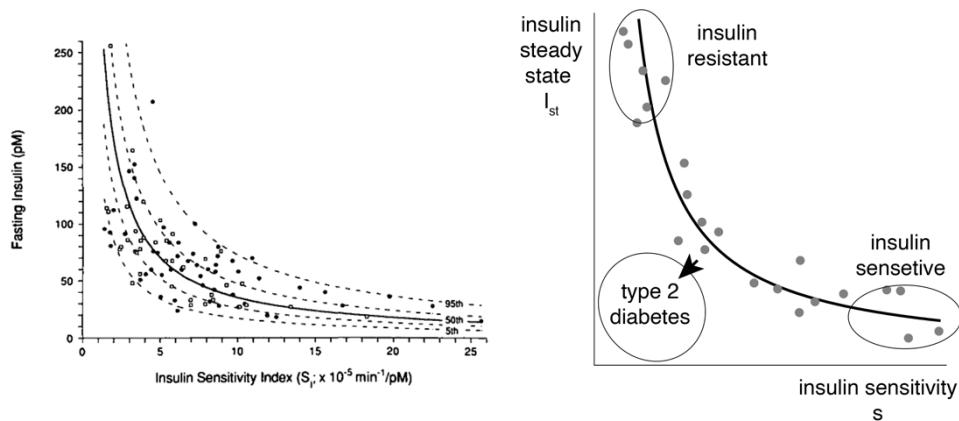


Figure 2.3 The hyperbolic relationship shows that fasting insulin is inversely proportional to insulin sensitivity in healthy people. Adapted from (Kahn et al., 1993)

For example, we used an equation for glucose that, at its core, has production and removal terms,  $dG/dt = m - \alpha G$ . Glucose safely converges to a stable fixed point,  $G_{st} = m/\alpha$  (Fig 2.4).

Cells, in contrast, **live on a knife's edge**. Their biology contains an inherent instability, due to exponential growth. Cells increase their biomass and divide (proliferate) at rate  $p$  and are removed at rate  $r$  (Fig 2.5). The removal rate includes active cell death (apoptosis) and other processes that take the cells out of the game like exhaustion, de-differentiation and senescence. Since all cells come from cells, and all biomass is made by biomass, production of biomass is intrinsically autocatalytic. It is a rate constant  $p$  times the total mass of the cells:  $production = p B$ . Removal of beta-cell mass  $B$  is, as usual,  $B$  times the rate at which cells are removed:  $removal = r B$ . As a result, the change in total cell mass  $B$  is the difference between production and removal rates:

$$(1) \quad dB/dt = pB - rB = (p - r)B = \mu B.$$

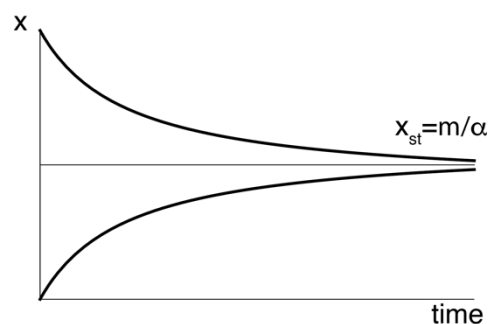


Figure 2.4 Circuits of molecular production and removal such as the glucose equation are inherently stable.

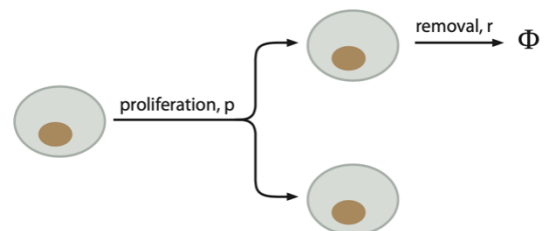


Figure 2.5 Cell circuits describe growth and removal of cell populations.

The key point is that the cell mass  $B$  appears in both growth and removal. It can therefore be taken outside the parentheses, leaving  $B$  times the **net growth rate** of cells,  $\mu = p - r$ , the difference between production and removal rates.

The problem is that if production exceeds removal, growth rate  $\mu$  is positive and total cell mass rises exponentially,  $B \sim e^{\mu t}$  (Fig 2.6). Such explosive growth occurs in early cancer. On the other hand, if removal exceeds production,  $\mu$  is negative, and cell numbers exponentially decay to zero, as in degenerative diseases. It is hard to keep total cell mass constant over time. This is known as the problem of **organ size control**.

Here we introduce the first of the three laws of physiology that are the foundation of this book

*Law 1: All cells come from cells.*

The problem of organ size control is a natural outcome of this law.

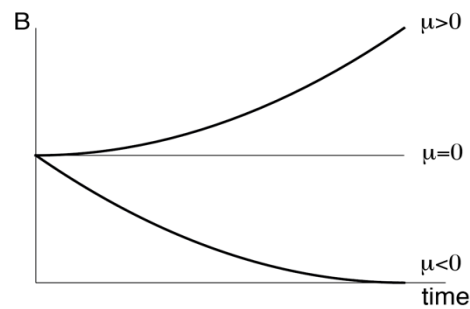
Organ size control is an amazing and universal problem. Our body constantly replaces its cells; about **a million cells are made and removed every second**. We make and remove about 100g of tissue every day (Sender and Milo 2021). If the production and removal rates were not precisely equal, we would exponentially explode or collapse.

To keep cell numbers constant, we need feedback control to **balance growth and removal** - to reach zero net growth rate,  $\mu = 0$ . Moreover, the feedback loop must keep the organ at a good functional size. Hence, the feedback mechanism must somehow register the biological activity of the cells and accordingly control their growth rate.

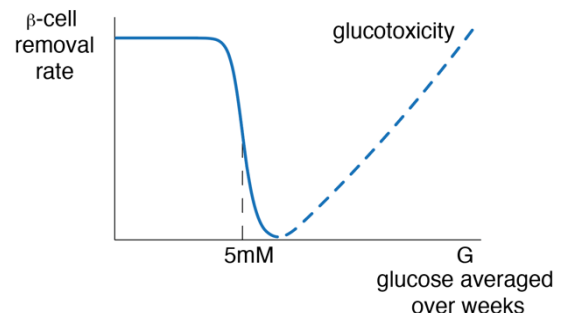
### Organ size control in beta cells is provided by feedback from glucose.

Organ size control of beta cells is achieved by means of glucose, as pointed out by Brian Topp and Dianne Finegood (Topp et al. 2000). The feedback signal is blood glucose, which controls both the growth and removal rates, such that  $\mu = \mu(G)$ . As measured in rodent islets, the removal rate of beta cells is high at low glucose, and falls sharply around 5mM glucose (Fig 2.7). Removal rate rises again at high glucose, a phenomenon called **glucotoxicity**, to which we will return soon.

For now, let's focus on the region around 5mM. Biomass growth (which includes both cell division and growth of mass per cell) rises with glucose. Therefore, the curves



*Figure 2.6: Cell circuits require stabilization because populations grow or decline exponentially unless net growth rate is*



*Figure 2.7: Beta cell removal rate drops at about 5mM glucose and rises again at high glucose levels.*

describing the rates for growth and removal cross near  $G_0 = 5mM$ , the fixed point that we seek with zero growth rate (Fig 2.8).

This way of plotting production and removal rates is called a **rate plot**, an important tool for understanding tissue-level circuits. The crossing point of the curves is the steady state, where cell production equals cell removal, and total cell mass does not change.

Another way of plotting this is to use the net growth rate  $\mu$ , defined as the difference between production and removal. Net growth rate crosses zero at  $\mu(G_0) = 0$  (Fig 2.9).

The fixed point  $G_0 = 5mM$  is **stable** for both beta-cells and blood glucose. It is stable because perturbing glucose away from the fixed point causes it to move back. We can see this on our rate plot (Fig 2.10). If glucose is above  $5mM$ , beta cells grow faster than they are removed. Total beta cell mass increases, leading to more insulin, pushing glucose back down towards  $5mM$ . Conversely, if glucose is below  $5mM$ , beta-cells are removed more rapidly than they grow, leading to less insulin, pushing glucose levels back up. These stable dynamics are indicated by the arrowheads pointing into the fixed point in Fig 2.10.

This cell mass feedback loop operates on the timescale of weeks, which is the growth rate of beta cell biomass. It is much slower than the insulin-glucose feedback loop that operates over minutes to hours. The **slow feedback loop** of cell mass dynamics keeps beta cells at a proper functional steady-state total mass and keeps glucose, averaged over weeks, at  $5mM$ .

The step drop of the removal curve at  $G_0 = 5mM$  is important for the precision of the glucose fixed-point. Due to the steepness of the removal curve, variations in growth rate (black curves) do not shift the  $5mM$  fixed point by much (Fig 2.11). The steep removal curve is generated by the cooperativity of enzymes that sense glucose inside beta cells (Karin et al. 2016b).

**The cell mass feedback circuit maintains homeostasis despite parameter variations.**

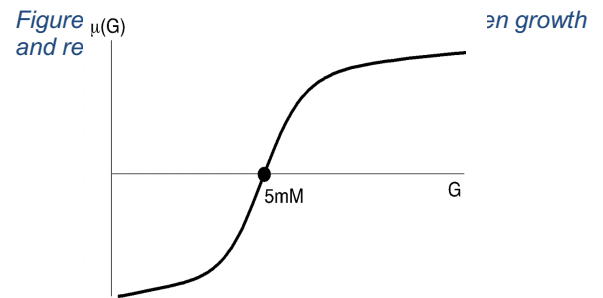
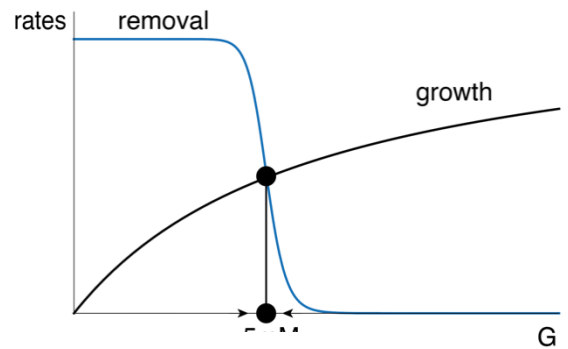


Figure 2.9: Beta cells reach a fixed point when their net growth rate equals zero.

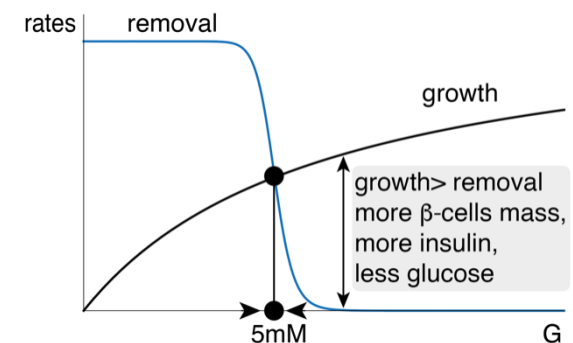


Figure 2.10: The  $5mM$  fixed point is stable because higher glucose makes beta cell mass grow over weeks producing more insulin and pushing glucose back down; a similar restoring force occurs when glucose is lower than  $5mM$ .

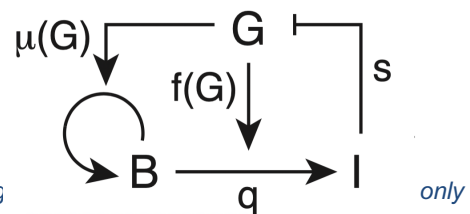
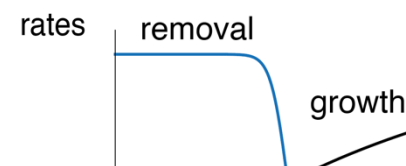


Fig 2.12: The BIG model circuit in which glucose controls beta cell total mass, contributing a timescale of weeks to the faster insulin-glucose feedback loop.

The slow feedback on beta cells can maintain a 5mM glucose steady state despite variations in insulin sensitivity,  $s$ . To explain things in a quantitative way, we need to see this mathematically, not only graphically. To do so, let's add beta cell mass changes to the minimal model. This leads to a revised model, the BIG model which stands for the Beta-cell-Insulin-Glucose model, Fig 2.12. It is simply the two equations of the minimal model of chapter 1 with a new equation, Eq 4, for the total beta-cell mass  $B$ :

$$(2) \quad dG/dt = m - s I G$$

$$(3) \quad dI/dt = qBf(G) - \gamma I$$

$$(4) \quad dB/dt = B \mu(G), \quad \mu(G_0) = 0$$

The only way to reach steady state in Eq 4 is either at  $B=0$ , meaning no beta cells at all, and therefore no insulin; or at zero net growth rate  $\mu(G) = 0$ , which occurs when  $G=G_0=5\text{mM}$  glucose. The latter is the stable solution that describes healthy people. This powerful locking of glucose is similar to controllers in engineering known as integral feedback loops. If you want to know more about integral feedback in biology, see the 2018 Systems Biology course videos on my website or the book "Introduction to Systems Biology" (2019).

It is easy to calculate the steady state of the BIG model, thanks to Eq. 4, that locks glucose steady state at  $G_{st} = G_0 = 5\text{mM}$ . We can use this to find the insulin steady state level by plugging  $G_{st}$  into the steady state of Eq. 2, by setting  $\frac{dG}{dt} = 0$ , to find  $I_{st} = m_{st}/sG_{st}$ . The lower  $s$ , the higher the insulin concentration. This means that the product of insulin steady-state level and insulin sensitivity is constant,

$$sI_{st} = \frac{m_{st}}{G_{st}} = \text{const.}$$

This explains the hyperbolic relation of Fig 2.3!

Finally, the beta-cell steady-state mass can be determined from equation 3, by setting  $dI/dt = 0$ , to find that

$$(5) \quad B_{st} = \gamma \frac{I_{st}}{qf(G_{st})} = \frac{\gamma m_{st}}{qsG_{st}f(G_{st})}.$$

Interesting: beta cell mass varies inversely with insulin sensitivity,  $B \sim 1/s$ . Beta-cell mass grows when  $s$  is small, as observed in people with insulin resistance. Beta cell mass shrinks when insulin sensitivity is high, as in starvation. In fact, beta cell mass varies with every parameter in the minimal model. Therefore, the organ-size control feedback makes **beta-cell mass expand or contract to precisely buffer out the effects of parameter changes**. It keeps the 5mM steady-state despite variations in any of the minimal-model model parameters, including maximal insulin production per beta cell,  $q$ , insulin removal rate,  $\gamma$ , and even the fasting supply of glucose by the liver,  $m_{st}$ .

### The same circuit appears in many hormone systems - a circuit motif

The same circuit logic appears in many hormone systems that perform homeostasis, namely tight control of an important factor in the body. The cells that secrete a hormone in response to a signal also grow in response to the same signal.

For example, the concentration of free calcium ions in the blood is regulated tightly around 1mM by a hormone called PTH, secreted by the parathyroid gland (Fig 2.13) (El-Samad, Goff, and Khammash 2002). The circuit has a negative feedback loop

similar to insulin-glucose, but with inverted signs: PTH causes increase of calcium, and calcium inhibits PTH secretion. The slow feedback loop occurs because parathyroid cell proliferation is regulated by calcium.

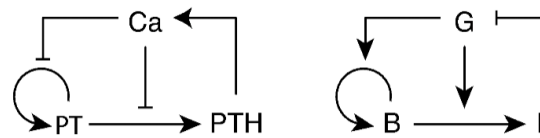


Figure 2.13: The secrete-and-grow circuit appears in the parathyroid hormone (PTH) system that controls blood calcium (Ca).

Other organ systems have similar 'secrete and grow' circuits (Fig 2.14), in which the size of the organ expands or contracts to buffer variation in parameters. For example, thyroid hormone, essential for regulating metabolism, is secreted by the thyroid gland at the throat. The controlling signal is called TSH, which causes the thyroid gland cells to both secrete thyroid hormone and to proliferate. The thyroid is famous for overgrowing, sometimes to the size of a grapefruit, when more thyroid hormone is needed. This condition is called goiter.

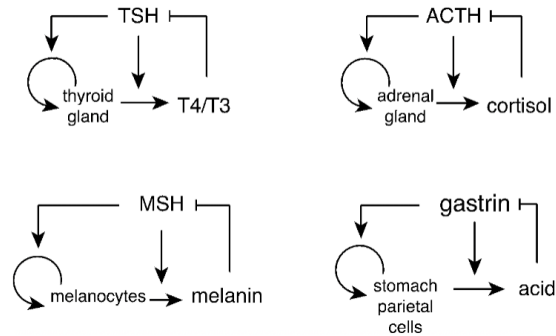


Figure 2.14: The secrete-and-grow circuit is a circuit motif because it appears in numerous hormonal systems.

Other systems with the same secrete-and-grow circuit shown in Fig 2.14 include acid secretion in the stomach by parietal cells under control of the hormone gastrin; secretion of cortisol by the adrenal gland under control of ACTH, and production of melanin by melanocytes in the skin under control of MSH. While the systems shown in Fig 2.14 differ in their molecular function, they share essentially the same circuit design as the insulin-glucose system. This is thus a **circuit motif**.

### The circuit also makes the dynamics robust

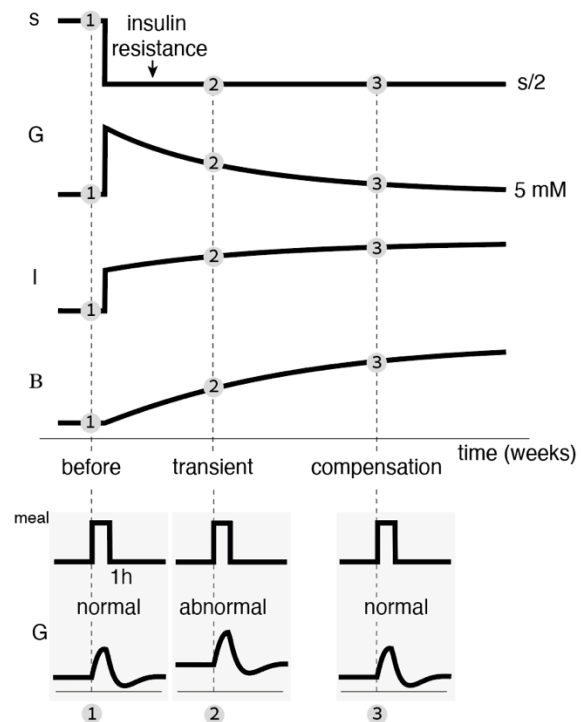
Remarkably, this circuit can also resolve the question of how glucose *dynamics* on the scale of hours are invariant to changes in insulin sensitivity. I mean that the BIG model shows how, in the glucose tolerance test, the response to a given input  $m(t)$ , such as drinking 75g of glucose, yields the same output curve  $G(t)$ , including the same amplitude and response time, for widely different values of the insulin sensitivity parameters.

This is unusual. Changing a key parameter in most models alters their dynamics. One might call such robustness of a dynamical response *rheostasis*, complementing the better-known concept of *homeostasis* which refers to maintaining a robust steady-state concentration of a metabolite.

This rheostatic ability was discovered by Omer Karin during his PhD with me (Karin et al. 2016a). We named it **dynamic compensation** (DC): Starting from steady-state, the output dynamics in response to an input is invariant with respect to the value of a parameter. To avoid trivial cases, the parameter must matter to the dynamics when the system is away from steady state (technically, to be observable). Solved exercise 1 at the end of the chapter shows how dynamic compensation occurs in the BIG model based on rescaling of the variables.

Let's see how dynamic compensation works. We will use the **separation of timescales** in this system: cell mass changes much slower (weeks) than hormones (hours). Suppose that insulin sensitivity drops by a factor of two, representing insulin resistance (Fig 2.15). As a result, insulin is less effective and glucose levels rise. Because glucose affects beta-cell growth rate, total beta cell mass rises over weeks (Fig 2.15 upper panels show the dynamics on the scale of weeks). More beta cells means that more insulin is secreted, gradually pushing glucose down to baseline. In the new steady state, there is twice the mass of beta cells and twice as much insulin. Glucose returns to its 5mM baseline.

Let's now zoom in to the timescale of hours (Fig 2.15, lower panels). **The response of glucose to a meal before the drop in  $s$  is identical to the response long after the drop** (time-point 1 and timepoint 3). In terms of glucose dynamics, the insulin resistance is invisible! The insulin response, however, is two times higher. Glucose dynamics in response to a meal are abnormal only during the transient period of days to weeks in which beta-cell mass has not yet reached its new, compensatory, steady-state (time-point 2).



*Figure 2.15: Dynamic compensation in the BIG model. A drop in insulin sensitivity causes glucose to rise within hours, but then beta cell mass rises over weeks and glucose returns to baseline. The glucose response to a meal (bottom panel) is abnormal during the transient period of weeks but returns to normal when beta cell mass adapts to its new level.*

Dynamic compensation thus allows people with different insulin sensitivity  $s$  to show the same glucose meal dynamics. Their insulin dynamics scale as  $1/s$ , namely more insulin when there is insulin resistance. This is indeed seen in experiments that follow non-diabetic people with and without insulin resistance over a day with three standardized meals (lower panels in Fig 2.16) (Polonsky, Given, and Van Cauter 1988). Their glucose levels rise and fall in the same way (lower left panel, Fig 2.16), but insulin levels are higher in people with insulin-resistance (lower middle panel, Fig 2.16). As the model predicts, when normalized by the fasting insulin baseline, there is almost no difference in insulin between the two groups (lower right panel, Fig 2.16). The BIG model (upper panels in Fig 2.16) captures these observations.



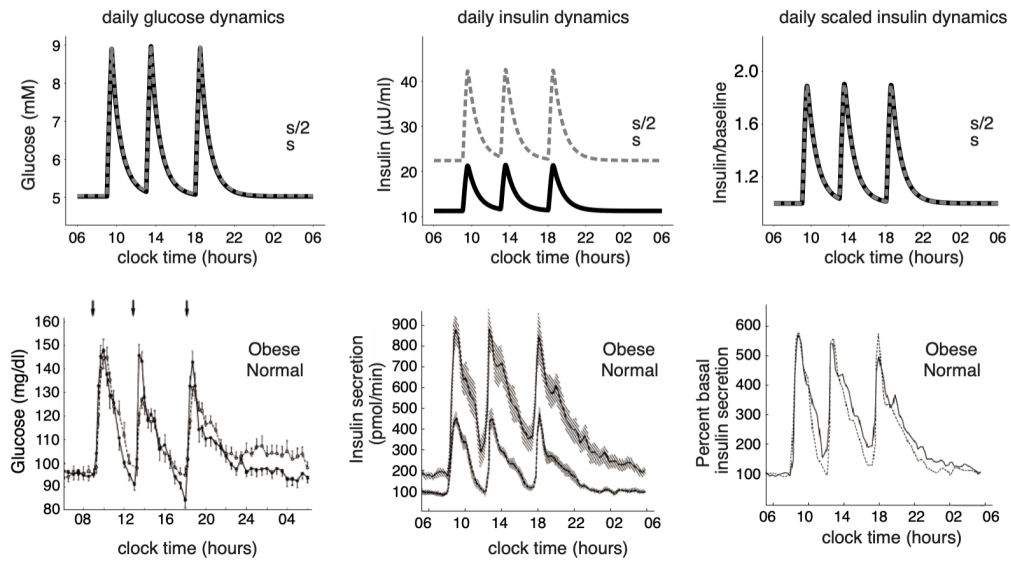


Figure 2.16 Nondiabetic obese people with insulin resistance show normal glucose responses to three meals. Their insulin is higher, but resembles normal insulin when both are scaled to their baselines. Top panel is the BIG model (Karin et al 2016), experimental data adapted from Polonsky et al 1988.

This circuit seems so robust. What about diseases such as diabetes? How and why do things break down?

### Prediabetes is due to an upper limit to beta cell compensation

Before full-fledged diabetes sets in, there is a stage called **prediabetes** (Fig. 2.17). In prediabetes, blood glucose shifts to higher and higher steady-state values, rising above 5mM. Prediabetes is clinically defined by fasting glucose between 5.6 mM and the diabetes threshold of 6.9 mM. Prediabetes has no symptoms, and occurs in 1 of 3 Americans, though 80% don't know that they have it. It is dangerous because people with prediabetes transit to type-2 diabetes at a rate of about 10% per year.

Prediabetes is often associated with insulin resistance. When insulin resistance is strong, beta cells must grow in functional mass - its ability to secrete insulin - by a large factor to compensate. But there is, in biology, always a limit to such compensation processes. This is our second law,

*Law 2: biological processes saturate.*

In adulthood beta cells stop dividing. They can compensate by increasing their insulin secretion per unit biomass and the size of each beta cell. When functional beta-cell mass approaches its

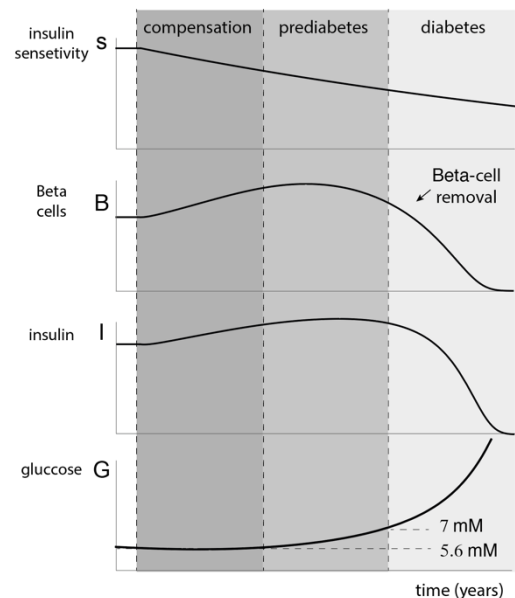


Figure 2.17: Prediabetes occurs when compensation for insulin resistance breaks down, due to a limit to beta cell mass growth, causing a rise in glucose. If glucose growth is unchecked, diabetes occurs and eventually beta cell mass and insulin secretion drops.

carrying capacity -- determined by the maximal insulin secretion per unit biomass time the maximal size of a beta cell -- compensation stops working. Beta cells hit a ceiling, and effectively the model returns to the minimal model of chapter 1 with a constant beta-cell mass. Recall that the minimal model has no robustness. Any further rise in insulin resistance causes glucose levels to rise above 5mM. The stronger the insulin resistance, the higher the glucose.

One insight from the model is that prediabetes can result not only from low  $s$ , but also from other parameter changes. As seen in Eq 5, beta cell mass goes as a specific combination of model parameters,  $B \sim \frac{m\gamma}{qs}$ . Thus, a decrease in beta-cell insulin production capacity  $q$  or insulin sensitivity  $s$ , or an increase in liver glucose production  $m$  or insulin removal rate  $\gamma$ , or a combination of these changes, can cause beta cells to hit their carrying capacity and compensation to saturate.

Having such a “parameter group” simplifies the understanding for the onset of disease. It also points to the way drugs or interventions work; for example, the diabetes drug metformin lowers liver production of glucose and thus lowers  $m$ , whereas exercise raises  $s$ . But both interventions act to prevent prediabetes.

Another pathway to diabetes is a rapid rise in insulin resistance that is too fast for beta cells to grow and catch up. This happens in some cases in pregnancy, when insulin resistance rises due to signals secreted from the placenta in order to direct glucose towards the fetus rather than mom's cells. This is one cause of **gestational diabetes**.

Eventually, if untreated, prediabetes leads to full-fledged type-2 diabetes. This disease shows a loss of beta cells and insulin, with a dramatic rise in glucose levels (Fig 2.17). At late stages, beta cells are gone, and the patient becomes dependent on insulin injections. We will next see that the transition to insulin-dependent type-2 diabetes is due to a dynamic instability that is built into the feedback loop.

### Type-2 Diabetes is linked with instability due to a U-shaped removal curve

Type-2 diabetes occurs when production of insulin does not meet the demand. Glucose levels go too high, damaging blood vessels and nerves.

The disease is linked with the phenomenon of glucotoxicity that we mentioned above: glucose at high levels kills beta cells. Patients lose their beta cells and are not able to make enough insulin.

Glucotoxicity was quantified in an experiment by (Efanova et al. 1998) on rodent beta-cell islets. Islets were incubated for 40h in different concentrations of glucose. The fraction of dead islet cells dropped sharply at 5mM glucose but then rose again above 10mM glucose (Fig 2.18).

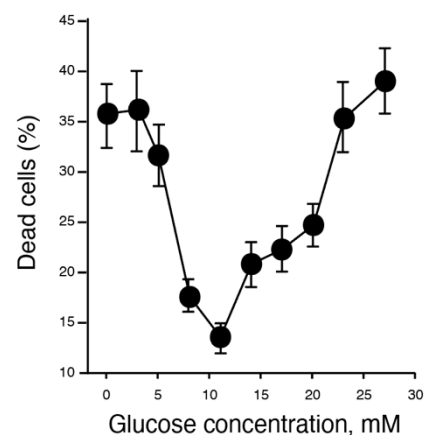


Figure 2.18: In glucotoxicity, beta cells are killed by high glucose levels as measured in rodent islets. Adapted from (Efanova et al., 1998).

The rate plot can help us see why glucotoxicity is so dangerous. It adds an **unstable fixed point**, the point at which proliferation rate crosses removal rate a second time (white circle in Fig 2.19). As long as glucose concentration lies below the unstable point, glucose safely returns to the stable 5mM point. However, if glucose (averaged

over weeks) crosses the unstable fixed point, beta cell removal rate exceeds growth rate. Beta cells die, there is less insulin and hence glucose rises even more. This is a vicious cycle, in which glucose disables or kills the cells that control it. It resembles end-stage type-2 diabetes.

This rate plot can explain several risk factors for type-2 diabetes. The first risk factor is a diet high in fat and sugars. Such a diet makes it more likely that glucose fluctuates to high levels, crossing into the unstable region. A lean diet can move the system back into the stable region.

In fact, type-2 diabetes is often curable if addressed at early stages, by changing diet and exercising. This can bring average glucose  $G$  back into the stable region even if the unstable fixed point was crossed.  $G$  then flows back to normal 5mM. Unfortunately, it is difficult for many people to stick with such lifestyle changes.

The second major risk factor is aging. With age, the growth rate of cells drops in all tissues including beta cells. This means that the unstable fixed point moves to lower levels of  $G$  (Fig 2.20), making it easier to cross into the unstable region. Note that the stable fixed point also creeps up slightly. Indeed, with age the glucose set point mildly increases in healthy people.

A final risk factor is genetics. A shifted glucotoxicity curve can make the unstable fixed point come closer to 5mM (Fig 2.21).

Why does glucotoxicity occur? Much is known about *how* it occurs, which is different from *why* it occurs. Glucotoxicity is caused by programmed cell death that is regulated by the same processes that control beta cell growth and insulin secretion—glycolysis, ATP production and calcium influx. A contributing factor for cell death is reactive oxygen species (ROS) generated by the accelerated glycolysis in beta-cells presented with high glucose. Beta cells seem designed to die at high glucose—they are among the cells most sensitive to ROS, lacking the protective mechanisms found in other cell types.

Thus, it is intriguing to find a functional explanation for glucotoxicity—why is this dangerous effect not removed by natural selection?

Mainstream views are that glucotoxicity is a mistake or accident, exposed perhaps only recently due to our lifestyle and longevity. In this book, we take the point of view that

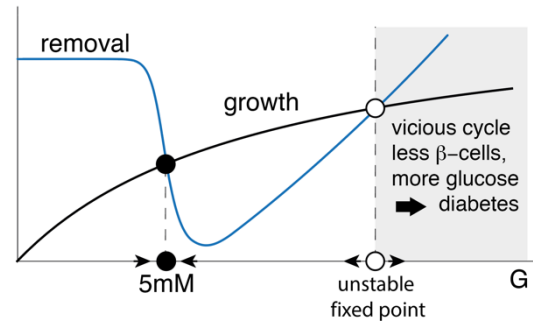


Figure 2.19: Glucotoxicity adds an unstable fixed point at high glucose levels, which leads to loss of beta cells when crossed.

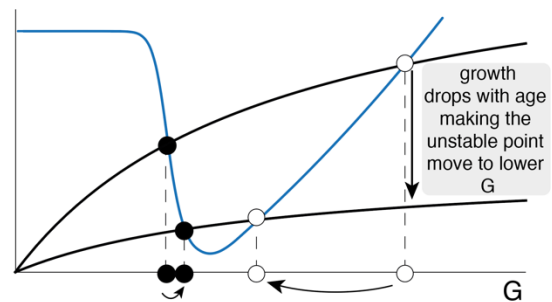


Figure 2.20: With age beta cell growth rate drops, bringing the unstable fixed point dangerously closer to the normal glucose set point.

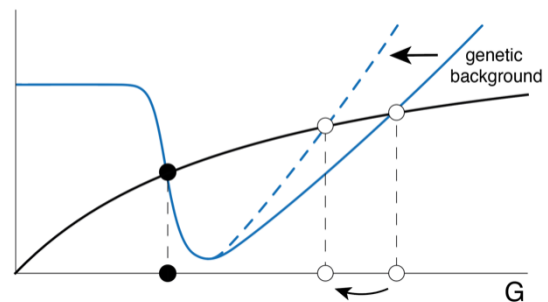


Figure 2.21: Factors which shift glucotoxicity also shift the unstable fixed point.

such processes have an important physiological role. They are crucial in the young reproductive organism. Their benefit outweighs the cost of diseases in the old.

### **The circuit is fragile to invasion by mutants that misread the signal**

In 2017, Karin et al (Karin and Alon 2017) provided an explanation for glucotoxicity by considering a fundamental fragility of the organ size-control circuit motif. The fragility is to **take-over by mutant cells that misread the input signal**. Mutant cells arise when dividing cells make errors in DNA replication, leading to mutations. Mutations also arise passively over time, even in non dividing cells. Rarely but surely, given the number of beta cells and the number of cell divisions in a lifetime<sup>1</sup>, a mutation will arise that affects the way that the cell reads the input signal. This is our third and final law,

*Law 3: cells mutate.*

Let's examine such a mutation in beta cells. Beta cells sense glucose by breaking it down in a process called glycolysis, leading to ATP production, which activates insulin release through a cascade of events.

The first step in glycolysis is to modify glucose chemically. This is done by the enzyme glucokinase. Most cell types express a glucokinase variant that binds even tiny (micromolar) amounts of glucose, with a halfway-binding constant to glucose of  $K = 40 \mu M$ . But beta cells express a special variant with  $K = 8mM$ . This half-way point is perfect for sensing the 5mM range of glucose in normal conditions.

A mutation that affects the binding constant  $K$  of glucokinase, reducing it, say, by a factor of five, causes the mutant beta cell to mis-sense glucose concentration as if it were five times higher than it really is. The mutant beta cell therefore does glycolysis as if there was much more glucose around. It's as if the mutant "thinks" that glucose concentration  $G$  is actually  $5G$ .

If our feedback design did not include glucotoxicity, such a mutant cell that interprets 5mM glucose as 25mM would have a higher proliferation rate (black curve) than removal rate (red curve). It would think 'Oh, we need more insulin!' and proliferate (Fig 2.22). The mutant cell therefore has a growth advantage over other beta cells, which sense 5mM correctly. The mutant cell will multiply exponentially, particularly if this mutation occurs during embryonic development or early childhood when beta cells proliferate rapidly. This will eventually produce a substantial population of mutant beta cells. This is dangerous because such a population of mutant cells produces a lot

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<sup>1</sup> Mutant cells that misread glucose are inevitable. Humans have about  $10^9$  beta cells. To generate these cells required at least  $10^9$  cell divisions starting from the fertilized egg. The mutation rate is about  $10^{-9}$ /base-pair/division. That means that every possible point mutation (single letter change in the genome) will be found in about 1 beta cell on average. Glucose mis-sensing can be caused by a large number of mutations, as exemplified by dominant activating glucokinase mutations (Christesen et al. 2008) so there should be multiple such mutant cells in everyone at birth. Human cells accumulate several tens of additional mutations per year even without dividing.

of insulin, attempting to push glucose down to a set-point level that they think is 5mM, but in reality is 1mM, causing lethally low glucose.

Mutant expansion has a second, devious property: as the mutant cell population starts to push glucose below 5mM, normal cells begin to be removed because their removal exceeds proliferation (they die to try to reduce insulin and increase glucose). The mutant's advantage is enhanced by killing off the normal cells.

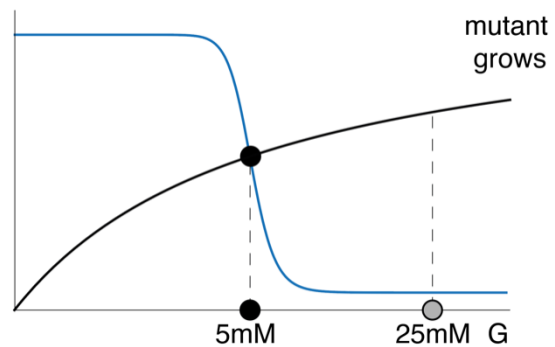


Figure 2.22: Without glucotoxicity, a mutant beta cell which misreads 5mM glucose as 25mM would grow.

Thus, biology has a challenge not usually seen in engineering. Suppose you want to control temperature; you use a thermostat. You can count on the thermostat being precise. It will not start dividing and mutating. Biology, in contrast, needs special designs to prevent takeover by mutant cells.

### Biphasic (U-shaped) response curves can protect against mutant takeover

To resist such mutant cells, we must give them a growth disadvantage. This is what glucotoxicity does. The mutant cell misreads glucose as very high. As a result, its removal rate exceeds proliferation. The mutant kills itself (Fig 2.23). Mutants are removed.

Isn't that neat?

The downside of this strategy is that it creates an unstable fixed point, with its vicious cycle. There is thus a **tradeoff between resisting mutants and resisting disease**.

In our evolutionary past, nutrition and activity probably prevented average glucose from being very high for weeks. The unstable fixed point was rarely crossed. Our modern lifestyle makes it more likely for glucose to exceed the unstable point, exposing a fragility to disease.

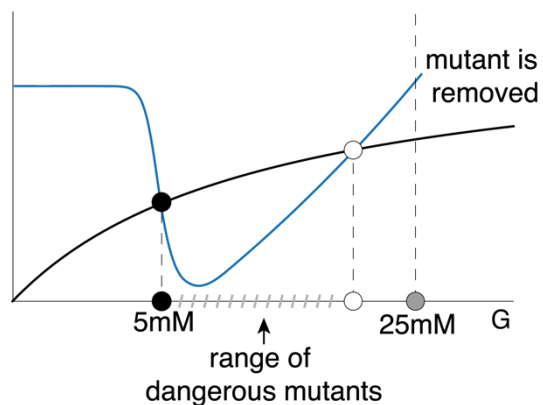


Figure 2.23: Glucotoxicity causes mutant beta cells that strongly hyper-sense glucose to kill themselves. This strategy has a loophole because mutant beta cells that only mildly hyper-sense glucose, in the range indicated by the hatched region, can still expand.

Glucotoxicity is a cell-autonomous strategy that eliminates mutants that strongly misread glucose. However, this strategy is still vulnerable to certain mutations of smaller effect - mutant cells that misread 5mM glucose as a slightly higher level that lies between the two fixed points (hatched region in Fig 2.23). Such mutant cells still have a growth advantage, because they are too weak to be killed by glucotoxicity, and have higher proliferation rate than removal rate.

Designs that can help against intermediate mutants are found in this system: beta cells are arranged in the pancreas in isolated islets clusters of ~1000 cells. A mutant might take over one islet, but not the entire organ. Relatively slow growth rates for beta cells

also help keep such mutants in check. Karin et al (Karin and Alon, 2017) estimate that only a small fraction of the islets are taken over by mutants in a lifetime. And, as we will see in chapter 4, there are additional safeguards against these mutants, whose failure provides a mechanism for why the immune-system attacks beta-cells in type-1 diabetes.

The glucotoxicity mutant-resistance mechanism can be generalized to other organs: to resist mutant takeover of a tissue-level feedback loop, the feedback signal must be toxic at both low and high levels. Such U-shaped phenomena are known as **biphasic responses**, because their curves have a rising and falling phase. Biphasic responses occur across physiology. Examples include neurotoxicity, in which both under-excited and over-excited neurons die, and immune-cell toxicity at very low and very high antigen levels. These toxicity phenomena are linked with diseases, for example Parkinson's disease in the case of neurons.

Not all endocrine systems have such biphasic responses, however, requiring other mutant resistance strategies as we will study in chapter 4.

### Summary

By modeling the glucose regulation system, we came upon new questions that reveal challenges shared by many organ-level circuits. First, organs have a fundamental instability due to exponential cell growth dynamics. They therefore require feedback to maintain steady-state and a proper size. This is the problem of *organ size control*.

The feedback loops use a signal related to the tissue function - blood glucose in the case of beta cells - to make organ size and function arrive at a proper stable fixed-point. This fixed point is maintained as the cells constantly turn over on the scale of days to months.

A second fact of life for hormone circuits is that they operate on distant target tissues by secreting hormones into the bloodstream. The challenge is that the target tissues have variation in their parameters, such as insulin resistance. Hormone circuits thus need to be robust to such distant parameters in order to maintain good steady-state values (*homeostasis*) and dynamic responses (*rheostasis*) of the metabolites they control. We saw how hormone circuits can achieve this robustness by means of dynamic compensation (DC). In dynamic compensation, tissue size grows and shrinks to precisely buffer the variation in parameters. As shown in the solved exercise below, DC arises due to a symmetry of the equations.

Finally, organ-level feedback loops need to be protected from the unavoidable production of mutant cells that misread the signal and can take over the tissue. This problem of *mutant resistance* leads to a third principle: biphasic responses found across physiological systems, in which the signal is toxic at both high and low levels. Biphasic responses protect against strong mis-sensing mutant cells by giving them a growth disadvantage. This comes at the cost of fragility to dynamic instability and disease.

Thus, all three constraints- organ size control, robustness and mutant-resistance- are addressed by a single integrated circuit design - the secrete-and-grow circuit. This circuit design is also found in numerous other hormone circuits.

## Exercises:

### Solved Exercise 1: Show that the BIG model has dynamic compensation (DC).

To establish DC, we need to show that when starting at steady-state, glucose output  $G(t)$  in response to a given input  $m(t)$  is the same regardless of the value of  $s$ . To do so, we will derive **scaled equations** that do not depend on  $s$ . To get rid of  $s$  in the equations, we rescale insulin to  $\tilde{I} = sI$ , and beta cell mass to  $\tilde{B} = sB$ . Hence  $s$  vanishes from the glucose equation

$$(7) \quad dG/dt = m - \tilde{I}G$$

Multiplying the insulin and beta-cell equations (Eq 5, 6) by  $s$  leads to scaled equations with no  $s$

$$(8) \quad \frac{d\tilde{I}}{dt} = q \tilde{B} f(G) - \gamma \tilde{I}$$

$$(9) \quad \frac{d\tilde{B}}{dt} = \tilde{B} \mu(G) \quad \text{with } \mu(G_0) = 0$$

Now that none of the equations depends on  $s$ , we only need to show that the initial conditions of these scaled equations also do not depend on  $s$ . If both the equations and initial conditions are independent of  $s$ , so is the entire dynamics.

There are three initial condition values that we need to check, for  $G$ ,  $\tilde{I}$  and  $\tilde{B}$ , which we assume begin at steady-state at time  $t=0$ . Note that if the system begins away from steady-state, there is no DC generally. The first initial condition,  $G(t=0) = G_{st}$  is independent on  $s$  because  $G_{st} = G_0$  is the only way for  $\tilde{B}$  to be at steady-state in Eq 9. This means that the second initial condition, from Eq 6,  $\tilde{I}_{st} = m_0/G_0$  is independent of  $s$ , which we can use in Eq 7 to find that the third initial condition  $B_{st} = \gamma \tilde{I}_{st}/G_0 f(G_0)$  is also independent of  $s$ . Because the dynamic equations and initial conditions do not depend on  $s$ , the output  $G(t)$  for any input  $m(t)$  is invariant to  $s$ , and we have DC.

Although  $G(t)$  is independent on  $s$ , insulin and beta cells do depend on it, as we can see by returning to original variables  $B = \tilde{B}/s$  and  $I = \tilde{I}/s$ . The lower  $s$ , the higher the steady-state insulin, as well as beta-cell mass, which rises to precisely compensate for the decreases in  $s$ .

Similar considerations show that the model has DC with respect to the parameter  $q$ , the rate of insulin secretion per beta cell, and hence to the total blood volume. There is no DC, however, to the insulin removal rate parameter,  $\gamma$ .

Dynamic compensation arises from the structure of the equations: the parameter  $s$  cancels out due to the linearity of the  $d\tilde{B}/dt$  equation with  $\tilde{B}$ , which is a natural consequence of cells arising from cells.  $s$  also cancels out from the  $d\tilde{I}/dt$  equation because the insulin production term,  $q \tilde{B} f(G)$ , is also linear in  $\tilde{B}$ , a natural outcome of the fact that beta-cells secrete insulin.

**2. Brain uptake of glucose, BIG model:** The brain takes up glucose from the blood at an insulin-independent rate.

(a) Write a BIG model with a term describing this effect.

(a) Write a formula for the steady states of glucose, insulin and beta-cells,  $G_{st}$ ,  $I_{st}$  and  $B_{st}$ .

(b) Is the steady-state blood glucose level  $G_{st}$  affected by the brain's uptake rate? Compare this to the minimal model.

(d) Discuss why the BIG model design might be biologically useful when organs like the brain have varying fuel demands (50 words).

### 3. The BIG model – numerical simulation

Write a computer code to numerically solve the BIG model equations. Set all parameter values to 1,  $f(G)=G^2$  and beta-cell growth rate  $dB/dt=0.01$  (G-5). Note that due to the “0.01”, the rate of change of  $B(t)$  is much slower than the rate of change of  $G(t)$  and  $I(t)$ . This represents the slow rate of beta-cell turnover compared to the fast hormone reactions.

(a) Plot  $G(t)$ ,  $B(t)$  and  $I(t)$  when at time  $t=100$ , there is a drop of insulin sensitivity from  $s=1$  to  $s=0.2$ . The plot should show the transition of  $B(t)$  from one steady-state to a new one. (Hint: the initial steady state of  $B$  is determined by setting all the time derivatives in the BIG model to zero). Explain in 50 words.

(b) Plot  $G(t)$  and  $I(t)$  in response to a meal, in the situation of (a). Model a meal by a pulse of glucose input. Thus,  $m(t)$  goes from an initial value  $m_0 = 1$  to a higher value  $m_1 = 2$  for 1 time unit then back down to  $m_0$ . Let the meal begin at three different times, before, right after and long after the drop of insulin sensitivity:  $t_{meal}=90, 110$  and  $300$ . Plot a comparison of the response in the three meals in terms of how high and how quickly glucose rises and falls. Make sure the plots zoom in around the region of interest where glucose changes. Interpret using the concept of dynamical compensation (100 words).

### 4. A model for prediabetes:

In this exercise we add to the BIG model a carrying capacity to beta cells and study the consequences of their loss of ability to compensate for parameter changes. The BIG model with carrying capacity is:

$$\begin{aligned}\frac{dG}{dt} &= m - s I G \\ \frac{dI}{dt} &= q B f(G) - \gamma I \\ \frac{dB}{dt} &= B \left( p(G) \left( 1 - \frac{B}{C} \right) - r(G) \right)\end{aligned}$$

where  $p(G)$  is beta cell biomass growth rate,  $r(G)$  is biomass removal rate and the carrying capacity is  $C$ . The shapes of  $p(G)$  and  $r(G)$  are given schematically in figure 2.19.

(a) Since insulin has the fastest removal time, assume that  $I$  is at steady state and tracks the slower changes in  $B$  and  $G$ . Write the equation for  $I_{st}$  as a function of  $B$  and  $G$ .



- (b) Plug in this 1st solution to the other two equations. This reduces the model from three to two differential equations. Sketch the two nullclines for B and G. Note that the B nullcline has a shape of an inverted U.
- (c) How many fixed points are there? Interpret these fixed points in terms of healthy and diseased states.
- (d) Find steady state glucose as a function of insulin sensitivity  $s$ . What happens to the 5mM glucose set point when insulin resistance rises? What do changes in the other parameters do to the fixed points? Relate this to prediabetes.
- (e) When does the transition to late-stage type-2 diabetes occur, in which beta function is lost?

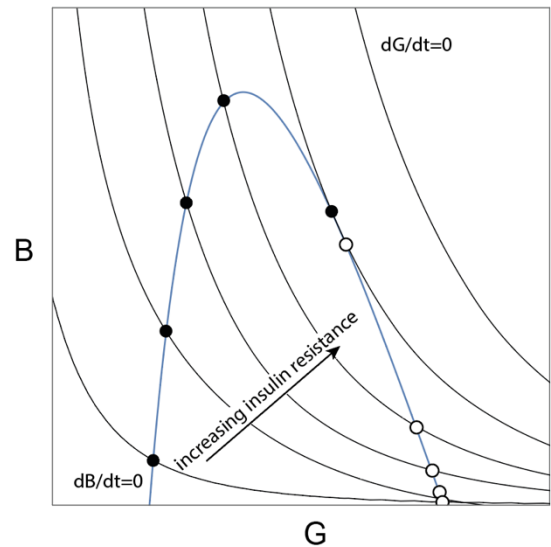


Figure 2.24 Phase plot of the slow variables  $B$  and  $G$  in a model with beta cell carrying capacity. The glucose steady state rises continuously with insulin resistance, until a critical value of insulin resistance where the stable and unstable fixed points collide and annihilate. Thereafter beta cell mass goes to zero and glucose rises.

*Turn and face the strange changes (ch-ch-changes)*

*insulin resistance will mean nothing to you!*

*Turn and face the strange changes*

*you can have your cake and eat it too!*

*Glands change in me,*

*and still here i stand*

*Turn and face the strange changes*

*if you want to be a better gland!*

*Turn and face the strange changes*

*glucose stays normal, now you understand,*

*Glands change in me,*

*and still here i stand*

*Based on Changes/Bowie*



## Further reading

### The BIG model

(Topp *et al.*, 2000) “A model of  $\beta$ -cell mass, insulin, and glucose kinetics: Pathways to diabetes”

### Dynamical compensation

(Karin *et al.*, 2016) “Dynamical compensation in physiological circuits”

(El-Samad *et al.* 2002) Calcium homeostasis and parturient hypocalcemia: an integral feedback perspective. *J Theor Biol.* 2002.

### Resistance to mis-sensing mutants

(Karin and Alon, 2017) “Biphasic response as a mechanism against mutant takeover in tissue homeostasis circuits”

### A general resource for models in physiology

(Keener and Sneyd, 2008) “Mathematical Physiology II: Systems Physiology”

Christesen, Henrik B. T., Nicholas D. Tribble, Anders Molven, Juveria Siddiqui, Tone Sandal, Klaus Brusgaard, Sian Ellard, et al. 2008. “Activating Glucokinase (GCK) Mutations as a Cause of Medically Responsive Congenital Hyperinsulinism: Prevalence in Children and Characterisation of a Novel GCK Mutation.” *European Journal of Endocrinology* 159 (1): 27–34. <https://doi.org/10.1530/EJE-08-0203>.

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<https://doi.org/10.1038/s41591-020-01182-9>.

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