

# Hormone circuits Lecture notes

## Uri Alon (Spring 2021)

### Lecture 4

## Beta-cell tissue size control has fragilities that lead to type-2 diabetes: Dynamical compensation and mutant resistance in tissues

We continue to use the glucose-insulin system as a model to understand fundamental principles of endocrine organs, the official name for hormone secreting glands. Endocrine organs communicate with distant organs via hormones that flow in the bloodstream. We will see that endocrine organs face at least three universal challenges. They must:

- (i) Signal precisely to distant organs whose parameters are unknown. This is the problem of *robust homeostasis*.
- (ii) Maintain a proper organ size, despite the fact that cells tend to grow exponentially. This is the problem of *organ size control*.
- (iii) Avoid mutant cells that can grow and take over the tissue. This is the problem of *mutant resistance*.

In this lecture we will see that principles arise to allow organs to work robustly, keep the right functional size and resist mutants. In fact, a unifying and quite beautiful circuit design addresses all three problems at once!

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

We ended the last lecture with a mystery. The insulin-glucose feedback loop can provide rapid responses to a meal on the timescale of hours. However, it is sensitive to changes in physiological parameters like insulin sensitivity,  $s$ . The minimal model predicts that baseline glucose and its dynamics depend on  $s$ : insulin resistance (low  $s$ ) causes in the model a rise from 5mM glucose baseline, and a longer response time. This is in contrast to the observation that most people with insulin resistance have normal glucose steady-state concentration and glucose responses. The minimal model is not **robust** to parameters like  $s$ .

Therefore, 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 by making more insulin. It does this by increasing the number and mass of beta cells. This is beta cell *hyperplasia* - more cells, and

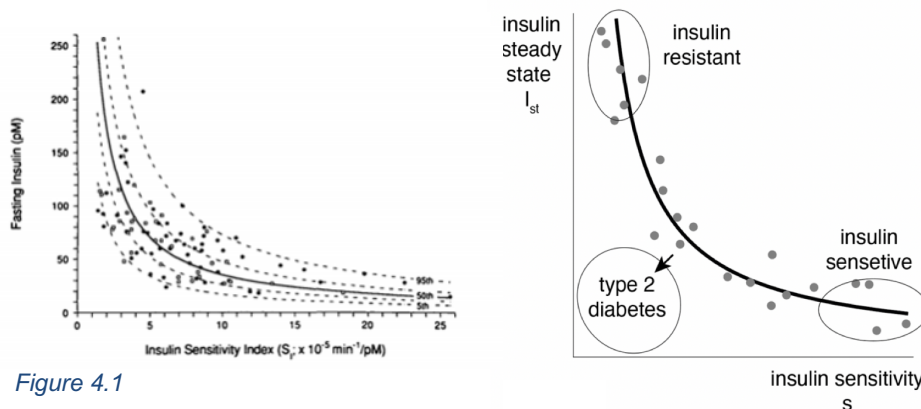


Figure 4.1

*hypertrophy* - bigger cells. Thus, the total mass of beta cells rises. More beta cell mass means more insulin. Remarkably, **the increase in insulin exactly matches the decrease in  $s$** . For example, people with obesity are insulin resistant and have more and larger beta cells than lean individuals. They thus secrete more insulin, compensating for insulin resistance.

This compensation is seen in the **hyperbolic relation** that exists between healthy people: an inverse relationship between  $s$  and steady-state insulin that keeps the product of the two constant:  $sI_{st} = \text{const}$  (Kahn *et al.*, 1993). People thus compensate for low insulin sensitivity with more insulin (Fig 4.1). People with diabetes have values that lie below this hyperbola - they appear to lie on their own hyperbola shifted to the left. The origin of this hyperbolic relationship has long been mysterious, but we will soon understand it.

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

To explain how such compensation can come about, we need to expand the minimal model. We need to add equations for the way that beta-

cell total mass,  $B$ , can change.

Here we enter the realm of the **dynamics of cell populations**. Cell dynamics are quite unlike the dynamics for the concentrations of proteins inside cells or molecules in the blood. For example, for glucose we used equations that, at their core, have production and removal terms,  $dG/dt = m - \alpha G$ , and safely converge to a stable fixed point,  $G_{st} = m/\alpha$  (Fig 4.2).

Cells, however, **live on a knife's edge**. Their basic biology contains an inherent instability, due to exponential growth. Cells divide (proliferate) and grow at rate  $p$ , and are removed at rate  $r$  (Fig 4.3). The removal rate includes active cell death (apoptosis), and also 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, proliferation is intrinsically autocatalytic, a rate constant times the total mass of the cells: *proliferation* =  $p B$ . This is unlike the glucose equation above,  $dG/dt = m - \alpha G$ , in which the production term  $m$  is not multiplied by  $G$ . 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 proliferation and removal rates

$$(3) \quad dB/dt = pB - rB = (p - r)B = \mu B.$$

The net **growth rate** of cells,  $\mu = p - r$ , is equal to the difference between proliferation and removal parameters. If proliferation exceeds removal, growth rate  $\mu$  is positive and total cell mass rises exponentially,  $B \sim e^{\mu t}$  (Fig 4.4). Such explosive growth occurs in cancer. If removal exceeds proliferation,  $\mu$  is negative, and cell

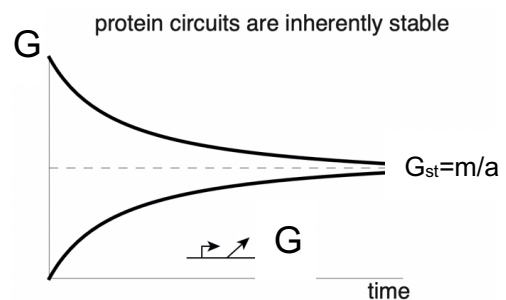


Figure 4.2

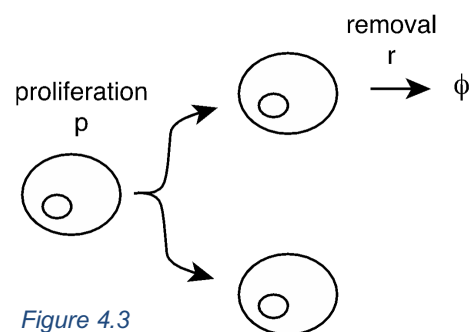


Figure 4.3

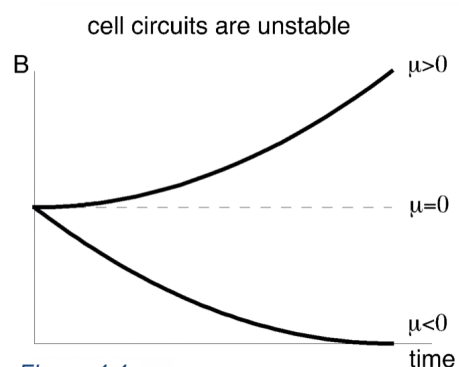


Figure 4.4

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**.

Organ size control is an amazing 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. If the production and removal rates were not precisely equal, we would exponentially explode or collapse.

To keep cell numbers constant, we need additional feedback control, because we must **balance proliferation and removal** in order to reach zero 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.

Such feedback control occurs for beta cells, as was pointed out by Brian Topp and Dianne Finegood (Topp *et al.*, 2000). The feedback signal is blood glucose: glucose controls the proliferation and removal rates, so that  $\mu = \mu(G)$ . As measured directly on rodent islets, the removal rate of beta cells is high at low glucose, and falls sharply around 5mM glucose (Fig 4.5). Removal rate rises again at high glucose, a phenomenon called **glucotoxicity**, which we will return to soon. For now, let's focus on the region around 5mM. Proliferation (which includes both cell division and growth of mass per cell) rises with glucose. Therefore, the curves describing the rates for proliferation and removal cross near  $G_0 = 5mM$  (Fig 4.6). Therefore,  $G_0 = 5mM$  is the fixed point that we seek with zero growth rate.

This way of plotting proliferation and removal rates is called a **rate plot**, an important tool for understanding tissue-level circuits. The crossing points of the curves are the steady-states, because cell production equals cell removal. At steady state, total cell mass doesn't change.

Another way of plotting this is to use the net growth rate  $\mu$ , defined as the difference between proliferation and removal. Growth rate reaches zero at  $\mu(G_0) = 0$  (Fig 4.7).

We can add the beta-cell mass changes to make a revised model, the BIG model (Beta-cell-Insulin-Glucose model, Fig 4.8). It is simply the minimal model with a **new equation for the total beta-cell mass B**:

- (4)  $dG/dt = m - s I G$
- (5)  $dl/dt = q B f(G) - \gamma I$
- (6)  $dB/dt = B \mu(G), \quad \mu(G_0) = 0$

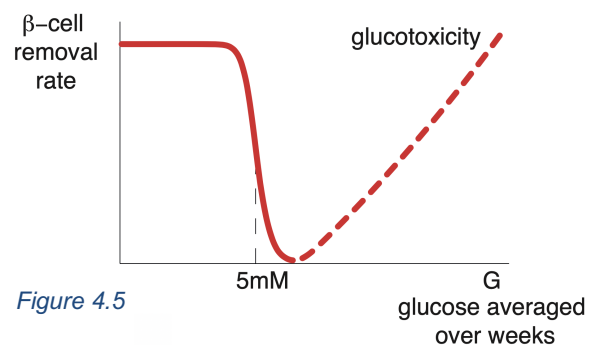


Figure 4.5

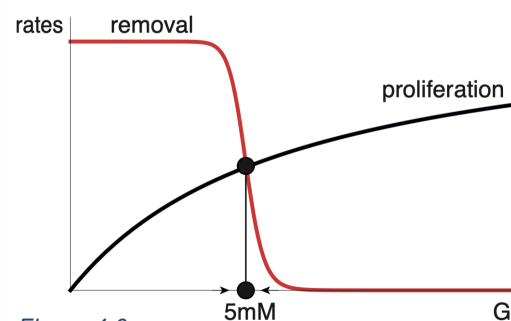


Figure 4.6

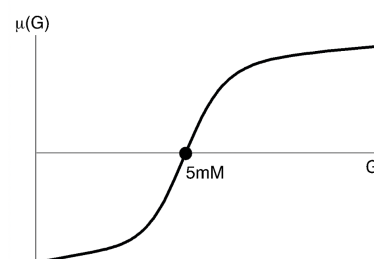


Figure 4.7

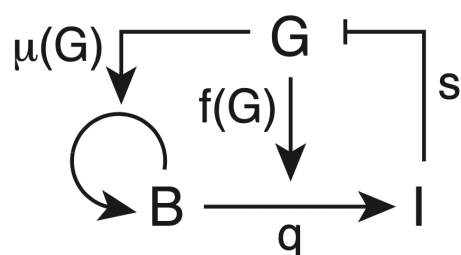


Figure 4.8

The point  $G_0 = 5mM$  is a **stable fixed-point** for both beta-cells and blood glucose. To see why the fixed point is stable, we need to see that perturbing glucose away from the point causes it to flow back. We can use our rate plot (Fig 4.9). If glucose is above 5mM, beta-cells proliferate more 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 than they are produced, leading to less insulin, pushing glucose levels back up. These stable dynamics are indicated by the arrowheads pointing into the fixed point in Fig 4.9.

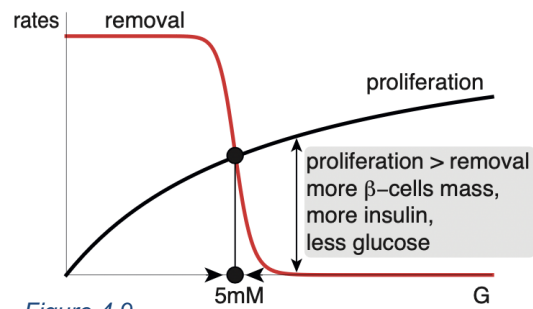


Figure 4.9

This cell-mass feedback loop operates on the timescale of weeks, which is the proliferation rate of beta cells. 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. This is powerful, because the only way to reach steady-state in Eq. 6 is when  $G = G_0$ .

This principle is, in essence, the same as integral feedback in bacterial chemotaxis (which we studied in the course Systems Biology. If you want to know more, see the 2018 videos on my website or the book “Introduction to Systems Biology”, Alon 2019).

The steep 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 proliferation rate (black curves) do not shift the 5mM fixed point by much (Fig 4.10). The steep removal curve is generated by the cooperativity of key enzymes that sense glucose inside beta cells (Karin *et al.*, 2016).

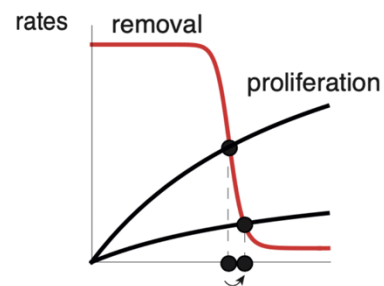
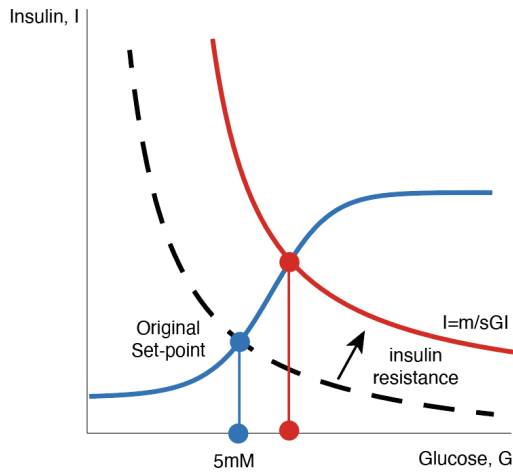
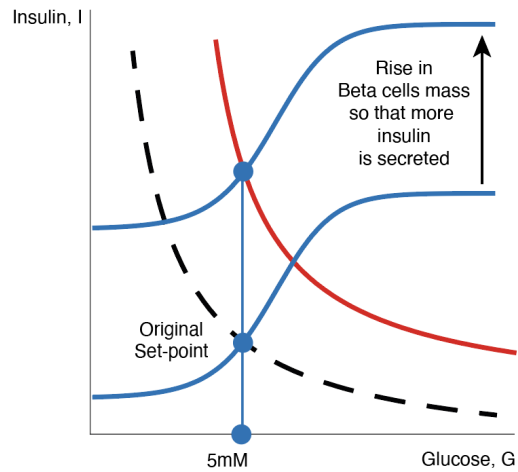


Figure 4.10



Insulin resistance raises glucose set-point



Beta cell mass rises to restore glucose set-point

Figure 4.11

Figure 4.12

We can understand the effect of beta cell mass changes using the phase plot for insulin versus glucose (Fig. 4.11,4.12). The original set point with 5mM glucose, occurs at the intersection of the two nullclines. Insulin resistance moves one nullcline, and raises the setpoint to higher levels of glucose. This is appropriate for physiological changes in insulin sensitivity such as in exercise or inflammation. However long term changes cause beta cell mass to gradually increase. This raises the other nullcline. The beta cell rise stops precisely when the new setpoint has  $G=5\text{mM}$ . In this compensated state, insulin secretion is higher than in the original setpoint, due to enlarged beta cell effective mass.

### Dynamic compensation allows the circuit to buffer parameter variations

The slow feedback on beta cells can thus maintain a 5mM glucose steady-state despite variations in insulin sensitivity,  $s$ . Remarkably, this feedback model can also resolve the mystery 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 an input  $m(t)$  from 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 parameter  $s$ . Such independence of the entire dynamic curve on a parameter such as  $s$  is very unusual. Changing a key parameter in most models alters their dynamics.

Let's start simple, with calculating the steady-state of the BIG model. The glucose steady-state is  $G_{st}=5\text{mM}$  thanks to Eq 6 - the point where cell proliferation balances removal. Therefore, from Eq 4,  $I_{st} = m_{st}/sG_{st}$ . The lower  $s$ , the higher the insulin concentration. In fact, 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 3.1!

Finally, the beta-cell steady state can be found from equation 5, by setting  $dl/dt = 0$ , to find that  $B_{st} = \gamma \frac{I_{st}}{qf(G_{st})} = \gamma \frac{m_{st}}{qsG_{st}f(G_{st})}$ . This means that beta cell mass varies inversely with insulin sensitivity  $\sim 1/s$ . Beta-cell mass thus rises when  $s$  is small, as observed in people with insulin resistance. Therefore, the tissue-size control feedback over weeks makes **beta-cell mass expand and contract in order to precisely buffer out the effects of parameters changes** like insulin resistance. In fact, it keeps the 5mM steady-state despite variations in any of the minimal-model model parameters, including maximal insulin production per beta cell  $q$  and insulin removal rate  $\gamma$ .

The feedback does something even more dramatic: it makes the entire temporal response to a meal invariant to parameters like  $s$ . Robustness of a dynamical response to changes is sometimes called *rheostasis*, complementing the better-known concept of *homeostasis* which refers to maintaining a robust steady-state concentration (also called baseline concentration) of a key metabolite.

This is advanced material I did not discuss in class, but it is important to know. The ability of a model to compensate for variation in a parameter was defined by Omer Karin et al (Karin *et al.*, 2016) as **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 (technically, to be observable), for example, when you start away from steady-state. To establish dynamic compensation in our model requires rescaling of the variables in the equations, as done in the next solved example (feel free to skip this solved example right now if you don't want the details).

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### Solved Example 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 cells 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  $s$

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

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

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 start at steady-state at time  $t=0$ . Note that if the system starts not at 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 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 (exercises 3.5). There is no DC, however, to the insulin removal rate parameter,  $\gamma$ .

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). Let's look at the slow timescale first. Suppose that insulin sensitivity drops by a factor of two, representing insulin resistance (Fig 4.13). As a result, insulin is less effective and glucose levels rise. Due to the removal curve, beta-cells are removed less often, and their numbers rise over days to weeks (Fig 4.13 upper panels show the dynamics on the scale of weeks). More beta-cells means that more insulin is secreted, and average glucose gradually returns to baseline. In the new steady-state, there is twice the number of beta cells and twice as much insulin. Glucose returns to its 5mM baseline.

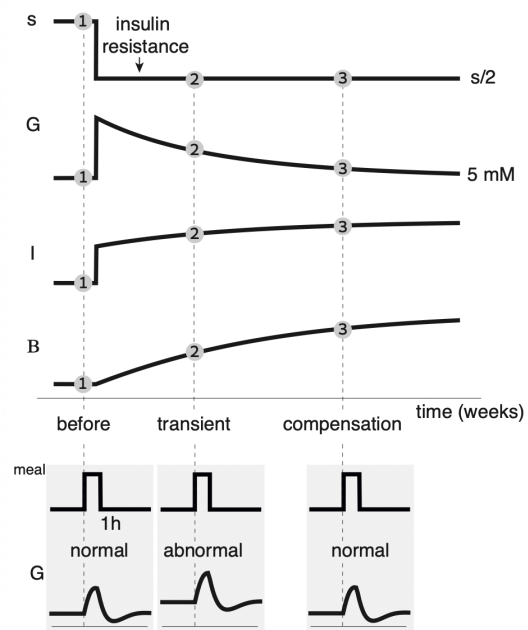


Figure 4.13

Let's now zoom in to the timescale of hours (Fig 4.13, lower panel). The response of glucose to a meal **long after the drop in  $s$  is exactly the same as before the change in  $s$**  (time-point 1 and timepoint 3). 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 numbers have not yet reached their new, compensatory, steady-state (time-point 2).

The dynamic compensation model predicts that people with different  $s$  should show the same glucose meal dynamics, but have insulin dynamics that scale with  $s$ . This is indeed seen in measurements that follow non-diabetic people with and without insulin resistance over a day with three standardized meals (lower panels in Fig 4.14) (Polonsky *et al.*, 1988). Insulin levels are higher in people with insulin-resistance (lower middle panel, Fig 4.14). But when normalized by the fasting insulin baseline,



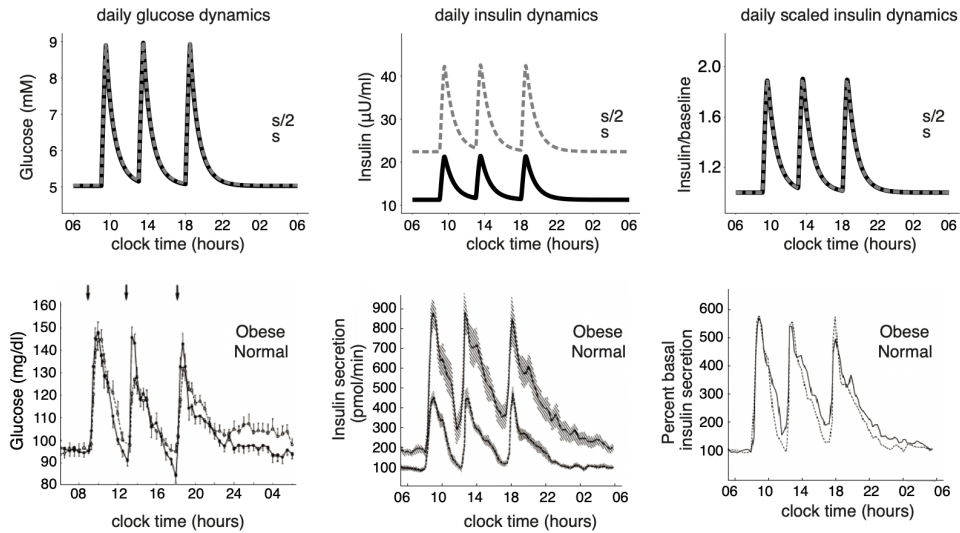


Figure 4.14 (adapted from Karin et al (2016) and Polonsky et al ((1988).

there is almost no difference between the two groups (lower right panel, Fig 4.14). The model (upper panels in Fig 4.14) captures these observations.

The DC property arises from the structure of the equations:  $s$  cancels out due to the linearity of the  $dB/dt$  equation with  $B$ , which is a natural consequence of cells arising from cells.  $s$  also cancels out due to the linearity in  $B$  of the insulin secretion term  $q B f(G)$ , a natural outcome of the fact that beta-cells secrete insulin.

These basic features needed for DC exist in most hormone systems that perform homeostasis, namely tight control of an important factor in the body. For example, free blood calcium concentration is regulated tightly around 1mM by a hormone called PTH, secreted by the parathyroid gland (Fig 4.15). 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.

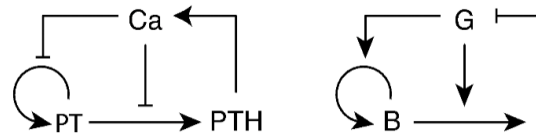


Figure 4.15

Other organ systems and even neuronal systems have similar circuits (Fig 4.16), in which the size of the gland or organ expands and contracts to buffer variation in effectivity parameters. For example, thyroid hormone, essential for regulating our temperature and metabolism, is secreted by the thyroid gland. The controlling signal is called TSH, which causes the thyroid gland to both secrete thyroid hormone and to proliferate. The systems shown in Fig 4.16 have essentially the same circuit as in the insulin-glucose system.

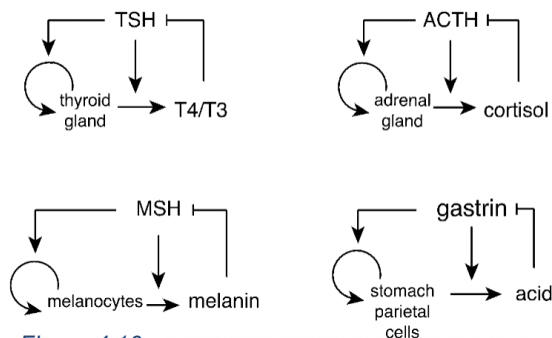


Figure 4.16



The feedback mechanism seems so robust. What about diseases such as diabetes? How and why do things break down?

Before full-fledged diabetes sets in, there is a stage called **prediabetes**. In prediabetes, blood glucose shifts to higher and higher steady state values above than 5mM: clinically, fasting glucose between 5.6mM and the diabetes threshold of 6.9 mM. It has no symptoms, and occurs in 1 of 3 Americans, though 80% don't know they have it.

Prediabetes occurs because of insulin resistance. When insulin resistance is strong, beta cells must grow in mass by a large factor to compensate for the reduced  $s$ . But there is, in biology, always **a limit to such compensation processes**. When beta cell mass hits its carrying capacity- its maximal value determines by the size of the islets- compensation stops working. As a result, glucose levels rise.

Another pathway to diabetes is rapid and continual rise in insulin resistance that is too fast for beta cells to grow and catch up. This may happen 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 muscle and fat cells. This may be one cause of **gestational diabetes**.

But the worst aspects of full-fledged diabetes are due to a dynamic instability that is built into the feedback loop, as we will see next.

### 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, and glucose levels go too high. It is linked with the phenomenon of glucotoxicity that we mentioned briefly above: at very high glucose levels, beta-cell removal rate rises (removal includes all processes that remove beta-cell function such cell death, de-differentiation and senescence) and eventually patients are not able to make enough insulin.

Glucotoxicity and cell death was quantified in an experiment by Efanova et al (1998) on rodent beta-cell islets. They incubated the islets 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 4.17).

Glucotoxicity is dangerous because it adds an **unstable fixed point**, the point at which proliferation rate crosses removal rate a second time (white circles in Fig 4.18). As long as glucose concentration does not exceed the unstable point, glucose safely returns to the stable 5mM point. However, if glucose (averaged over weeks) crosses the unstable fixed point, removal rate exceeds proliferation 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.

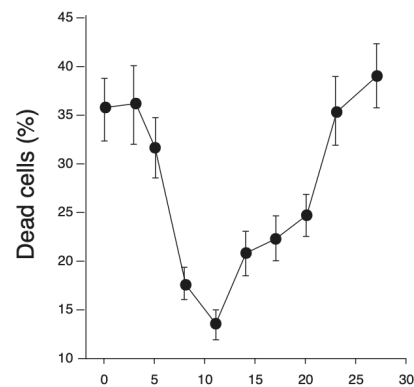


Figure 4.17 Glucose concentration, mM

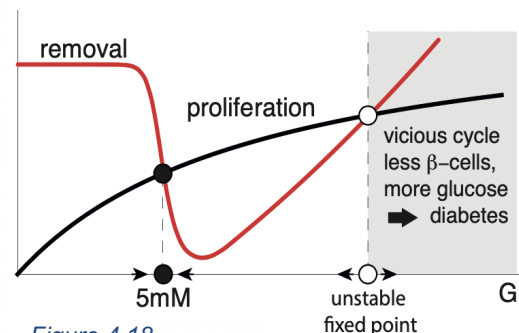


Figure 4.18

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 largely curable if addressed at early stages, by changing diet and exercising. This can bring average  $G$  back into the stable region even if the unstable fixed point was crossed.  $G$  then flows back to normal 5mM. The challenge is that it is difficult for many people to stick with such lifestyle changes.

The second risk factor is ageing. With age, proliferation rate of cells drops in all tissues, including beta cells. This means that the unstable fixed point moves to lower levels of  $G$  (Fig 4.19), making it more likely to cross into the unstable region. Note that the stable fixed point also creeps up to slightly higher levels. Indeed, with age the glucose set point mildly increases in healthy people.

A final risk factor is genetics. It appears that the glucotoxicity curve is different between people. A shifted glucotoxicity curve can make the unstable fixed point come closer to 5mM (Fig 4.20).

Why does glucotoxicity occur? Much is known about *how* it occurs (which is different from *why* it occurs), because research has focused on this disease-related phenomenon. Glucotoxicity is caused by programmed cell death that is linked to the

same processes that controls cell division and insulin secretion (calcium influx). A contributing factor is reactive oxygen species (ROS) generated by the accelerated glycolysis in beta cells presented with high glucose. ROS causes extensive cell damage, and beta-cell removal. The sensitivity of beta cells to ROS does not seem to be an accidental mistake by evolution. Beta cells seem designed to die at high glucose- they are among the cells most sensitive to ROS, lacking protective mechanisms found in other cell types.

Thus, it is intriguing to find a functional explanation for glucotoxicity. Mainstream views are that glucotoxicity is a mistake, exposed perhaps only recently due to our lifestyle and longevity. In this course, we take the point of view that such processes have an important physiological role, preventing problems in the young. their benefit outweighs the cost of diseases in the old.

### Tissue-level feedback loops are fragile to invasion by mutants that misread the signal

Omer Karin et al (Karin and Alon, 2017) provided an explanation for glucotoxicity by considering a fundamental fragility of tissue-level feedback circuits. This 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. Rarely but surely,

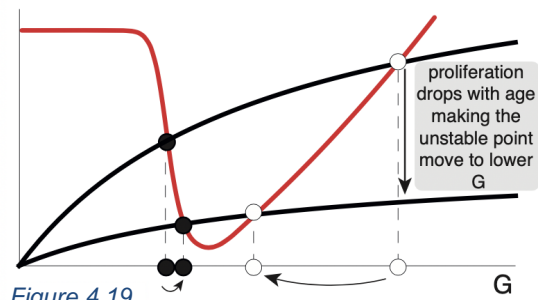


Figure 4.19

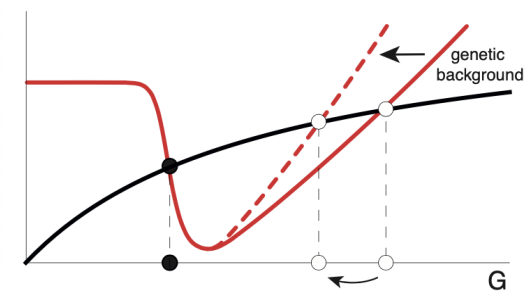


Figure 4.20

given the huge 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.

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 phosphorylation of glucose by the enzyme glucokinase. Most cell types express a glucokinase variant with a halfway-binding constant to glucose of  $K = 40 \mu M$ , but beta cells express a special variant with  $K = 8mM$ . This means that this glucose sensor has a half-way point of 8mM, perfect as a sensor for the 5mM range and to the glucose levels that exceed 5mM in normal conditions.

Mutations that affect the  $K$  of glucokinase, reducing it, say, by a factor of five, causes the mutant cell to mis-sense glucose concentration as if it were five times higher than it really is. The mutant beta cells therefore do glycolysis as if there was much more glucose around. It's as if the mutant distorts the glucose axis in the rate plots by a factor 5, "thinking" that glucose concentration  $G$  is actually  $5G$ .

If our feedback design did not include glucotoxicity, such a mutant 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 4.21). The mutant cell therefore has a growth advantage over other beta cells, which sense 5mM correctly. The mutant will multiply exponentially and eventually take over. This is dangerous because when the mutant cells take over, they push glucose down to a set-point level that they think is 5mM, but in reality, is 1mM, causing lethally low glucose.

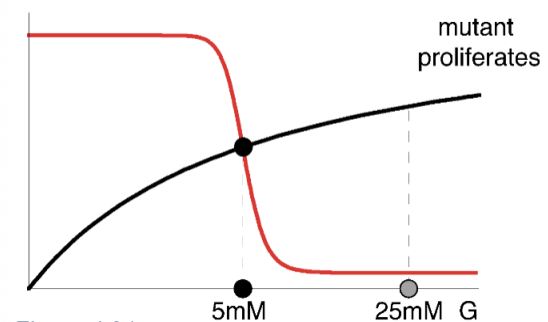


Figure 4.21

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

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

To resist such mutants, we must give them a growth disadvantage. This is what glucotoxicity does. The mutant cell misreads glucose as very high. As a result, its

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<sup>1</sup> A gram of tissue has about  $10^9$  cells, which is also the approximate number of beta cells in humans. If cells divide 1/month, there are about  $10^{10}$  divisions per year. Mutation rate is  $10^{-9}$ /base-pair/division. That means that every possible single-letter (base-pair) change in the genome (point mutation) will be found in about 10 cells. Mis-sensing can be caused by at least 100 different mutations, so every year at least 1000 mis-sensing mutants arise. Also just to generate the  $10^9$  cells during embryonic development and childhood growth there need to be at least  $10^9$  divisions starting from the fertilized egg, resulting in at least 100 mis-sensing mutants. Depending on the tissue, cells are renewed on average every few days (gut epithelium) to a few months (most tissues- skin, lung, blood cells) to never (most neurons, most muscle cells).

removal rate exceeds proliferation. The mutant kills itself (Fig 4.22). Mutants are removed.

Isn't that neat?

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

In our evolutionary past, activity and nutrition was probably such that average glucose rarely stayed very high for weeks, and thus the unstable fixed point was rarely crossed. Modern lifestyle makes it more likely for glucose to exceed the unstable point, exposing a fragility to disease.

The glucotoxicity strategy eliminates mutants that strongly misread glucose. However, this strategy is still vulnerable to certain mutants of smaller effect: e.g. mutants that misread 5mM glucose as a slightly higher level that lies between the two fixed points (hatched region in Fig 3.20). Such mutants have a growth advantage, because they are too weak to be killed by glucotoxicity, and have higher proliferation rate than removal rate.

Luckily, such intermediate-effect mutants are rarer than mutants that strongly activate or deactivate signaling. Designs that can help against intermediate mutants are found in this system: beta cells are arranged in the pancreas in isolated clusters of ~1000 cells called Islets of Langerhans. A mutant can take over one islet, but not the entire tissue. Relatively slow growth rates for beta-cells also help keep such mutants in check. Karin et al (Karin and Alon, 2017) estimate that often only a small fraction of the islets are taken over by mutants in a lifetime. And, as we will see in Part 2, 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 Alzheimer's and Parkinson's in the case of neurons.

## Summary

By modelling the system, we came upon new questions that reveal constraints shared by virtually all tissue-level circuits. First, tissues 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*. Such feedback loops use a signal related to the tissue function (blood glucose in the case of beta cells), to make both organ size and function stay 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 need to operate on distant target tissues by secreting hormones into the bloodstream. The challenge is that the target

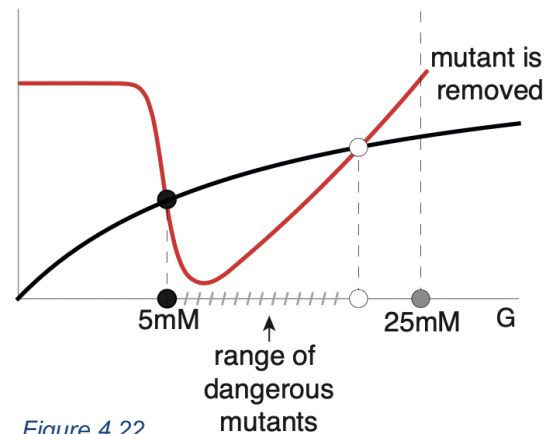


Figure 4.22

tissues have variation in their response 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 in order to precisely buffer the variation in parameters. DC arises due to a symmetry of the equations.

Finally, tissue-level feedback loops need to be protected from another consequence of cell growth- the unavoidable production of mutants 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 mutants 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, hemostasis and mutant resistance- are addressed by a single integrated circuit design. The circuit design of the glucose-insulin circuit is also found in numerous other hormone circuits.

## Further reading

### History of the minimal model

(Bergman, 2005) “Minimal model: Perspective from 2005”

### 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”

### 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, no date) “Mathematical Physiology II: Systems Physiology”

Bergman, R. N. *et al.* (1979) ‘Quantitative estimation of insulin sensitivity.’, *The American journal of physiology*. doi: 10.1172/JCI112886.

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Topp, B. *et al.* (2000) ‘A model of  $\beta$ -cell mass, insulin, and glucose kinetics: Pathways to diabetes’, *Journal of Theoretical Biology*. doi: 10.1006/jtbi.2000.2150.