Dynamical compensation and mutant resistance in tissues

We now turn to circuits at a higher level of organization - the level of tissues and organs. These circuits are made of cells that signal to each other. Even cells in distant tissues can communicate via hormones that flow in the blood stream. We will see that at the tissue level there are new problems to worry about: parameters of distant tissues can vary, the dynamics of cells are inherently unstable, and mutations can occur as cells divide. Despite these problems, organ systems must still work precisely. We will see that new principles arise to allow organs to work robustly, keep the right functional size and resist mutants and disease.

The insulin-glucose feedback loop is a well-studied model system

As a model system, we will use the insulin control of blood glucose. Glucose is the main sugar used by our cells. When we eat a meal, sugar is absorbed and our blood glucose concentration rises. Within a few hours, glucose returns to its baseline concentration of Go=5mM (Fig 9.1). This 5mM baseline is kept remarkably constant - to within 10% - over time and between people. Tight control over blood glucose is important: if glucose drops too low, the brain doesn't have enough energy and we can pass out and even die. If glucose is too high, it damages blood vessels and other systems over the years, causing the symptoms of diabetes.

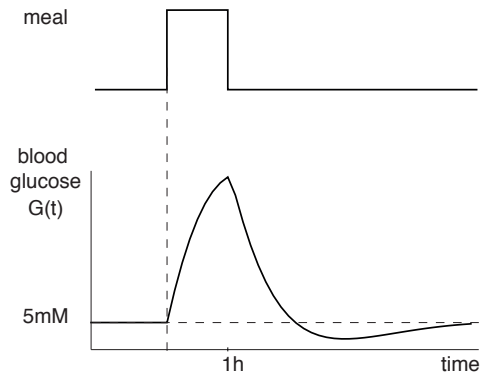

Figure 9.1

Not only is steady-state glucose kept constant, *the entire glucose dynamics G(t) after a meal is tightly controlled*. For example, in a clinical test for diabetes, called the glucose tolerance test, you are asked to drink 75g of glucose. Then, glucose levels are measured in the blood over the next two hours. Different healthy people show nearly the same glucose dynamics (Fig 9.1). Deviation from the expected dynamics (e.g. more than 11mM glucose after 2h) is a criterion to diagnose diabetes (Fig 9.2).

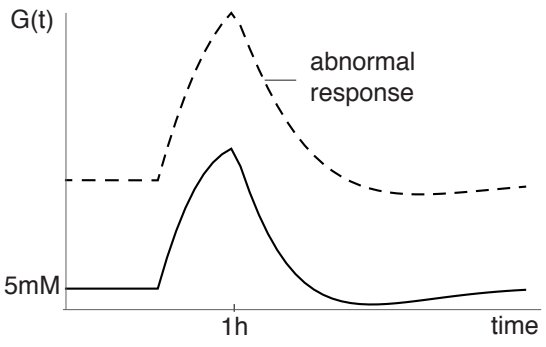

Figure 9.2

This exquisite control is carried out by a famous hormone circuit. Glucose is sensed by special cells in the pancreas called **beta cells**. Glucose causes beta cells to secrete the hormone **insulin**, a small protein that is carried by the blood to all tissues. Insulin is sensed by receptors in the cells of many tissues, and instructs the cells in the muscle, liver and fat to take up glucose from

the blood, reducing blood glucose concentration. This closes a negative feedback loop (Fig 9.3) whose timescale is hours. If there is high glucose, insulin levels rise, to bring glucose down again.

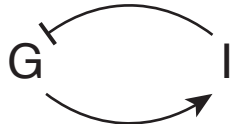

Figure 9.3

A classic model for this negative feedback loop, called the minimal model, was developed by Richard Bergman et al (1979), and is widely used to analyze clinical data. The level of glucose G is increased by a meal input m and is reduced by the action of insulin, I, that promotes removal of glucose from the blood. Thus, the removal rate of glucose rises with insulin:

(1) $dG/dt=m-Si\ I\ G$

The parameter Si, called **insulin sensitivity**, is the effect of a unit of insulin on the removal rate of glucose. Insulin, in turn, is produced by beta cells, that we denote X, at a rate that increases with glucose, f(G), and insulin is degraded at a rate γ, with a half-life on the order of 30 min:

(2) $dI/dt=q\ X\ f(G)-\gamma\ I$

Solving this model shows that a meal input causes a rise in glucose, eliciting a rise in insulin, causing glucose to drop back down (Fig 9.1) [1].

A fascinating thing about the tight regulation of glucose is that it occurs *despite large differences between people in insulin sensitivity, Si*. This parameter can be measured by injecting insulin and noting the reduction in blood glucose. People can vary by a factor of ten in insulin sensitivity, which is affected by exercise, pregnancy, infection, stress, obesity, genetics and other factors. Low insulin sensitivity is also called **insulin resistance.**

Insulin sensitivity varies between people because it is a physiological parameter that controls glucose allocation between bodily systems. For example, exercise increases insulin sensitivity and diverts more glucose to muscle tissues. Infection decreases sensitivity, causing more glucose to stay in the blood to be used by the immune system. In pregnancy, the fetus secretes hormones to decrease mom's insulin sensitivity and hence divert more glucose for the growth of the fetus - in pathological cases placing the mother at risk for diabetes.

Importantly, despite the large variation in insulin sensitivity, most people do not have diabetes, and show the normal glucose level of 5mM and the normal glucose dynamics in the glucose test. For example, people with obesity have very low Si (high insulin resistance), but more than 80%

[1] many effects are ignored for simplicity, such as production of glucose by the liver, insulin-independent uptake of glucose by the brain, the hormone glucagon which increases liver glucose production when glucose falls below 5mM, the effects of fat and amino acids in the diet, delay for insulin to reach peripheral tissues, and so on. These effects are not crucial to understand the principles in this chapter

of them have no diabetes, with 5mM glucose and normal glucose dynamics (Fig 9.4). Our goal is to understand how the system compensates for variations in an important parameter like Si.

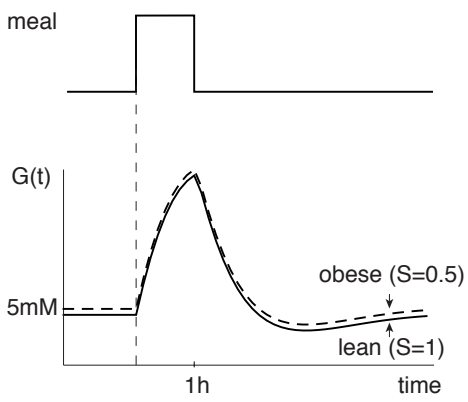

Figure 9.4

The minimal feedback model is not robust to changes in insulin sensitivity

So how does the insulin control-circuit compensate for variations in insulin sensitivity, namely variations in the intrinsic effectiveness of insulin on far-away tissues? The minimal model cannot account for this compensation. It shows a steady-state glucose level, and response dynamics, that depend on the parameter Si. Low levels of Si, for example, cause higher steady-state glucose, higher peak responses and longer response times (Fig 9.5) .

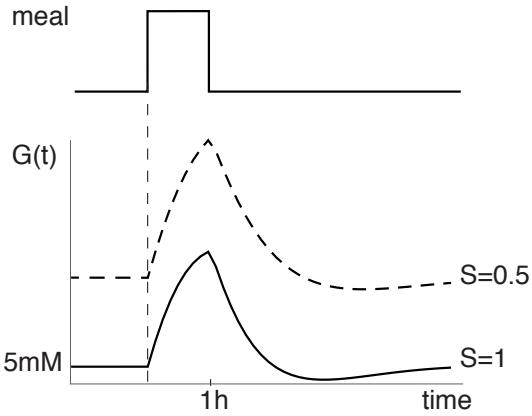

Figure 9.5

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Solved Example 1: Show that steady-state glucose depends on insulin sensitivity in the minimal model.

At steady-state, dG/dt=0 and dI/dt=0. Assuming a constant glucose input mo (say the basal production of glucose by the liver that occurs when we fast), we find Si Go Io=mo, and q X f(Go)=γ Io. Hence, Go=Si γ mo/p X f(Go). Lets use f(Go)=Go^2 as proposed by Topp(2000). This yields a steady state glucose level of Go=(Si γ mo/q X)^(1/3), which depends on Si. For example, 10-fold reduction in Si lead to ~2-fold increase in Go, with blood sugar going from 5mM to a pathological 10mM. The time it takes glucose to return to baseline is about ten times longer (excXX). The minimal model thus shows dynamics whose shape depends on the parameter Si. Such dependence on parameters is the typical behavior of most models that we can write down.

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Therefore, compensation for changes in insulin sensitivity must involve additional processes beyond the minimal model's glucose-insulin loop. The way that the body compensates for decreased insulin sensitivity Si is by increasing the number of beta cells in order to increase insulin levels, to exactly match the decrease in Si. For example, people with obesity show much more beta cells than lean individuals. They thus secrete more insulin, compensating for their insulin resistance.

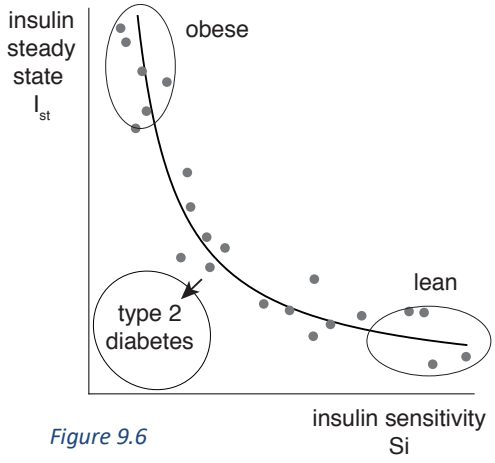

Figure 9.6

The compensation is clearly seen in a hyperbolic relation that healthy people show, which describes an inverse relationship between Si and steady-state insulin secretion that keeps the product Si Ist =const (Khan,1993). People thus compensate for low insulin sensitivity with more insulin (Fig 9.6) . People with diabetes lie below this hyperobla.

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 how beta-cell numbers, X, can change. Here we enter the realm of the **dynamics of cell populations**. Cell dynamics are quite unlike the dynamics we studied so far for the concentrations of proteins inside cells. For proteins circuits we used equations that, at their core, have production and removal terms, $dx/dt = \beta - \alpha x$, and safely to converge to a stable fixed point, $x_{st} = \beta/\alpha$ (Fig 9.7).

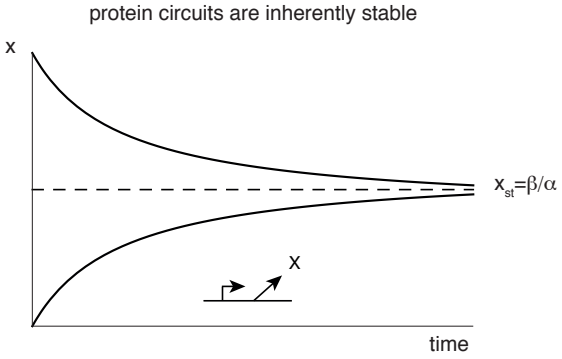

Figure 9.7

Cells, however, live on a knife's edge. Their basic equations contain an inherent instability. Cells can proliferate and die (Fig 9.8). Since all cells are made by cells, the proliferation rate is intrinsically autocatalytic, a rate constant times the concentration of cells, *proliferation=p X*. As a result the balance between proliferation p X and death d X leads to exponential growth of cells at rate $\mu = p - d$

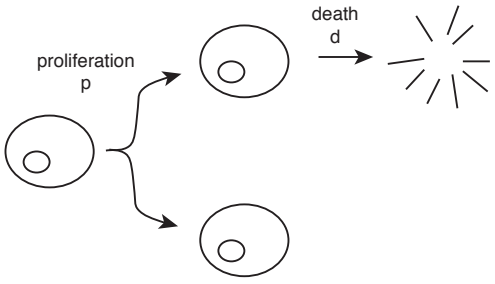

Figure 9.8

dX/dt=p X- d X= (p-d)X= mu X

If proliferation exceeds death, growth rate μ is positive and cell numbers explode exponentially, X~$e^{\mu t}$ (Fig 9.9). If death exceeds proliferation, μ is negative, and cell numbers exponentially decay to zero. Such an explosion in cells numbers occurs in cancer, and a decay occurs in degenerative diseases.

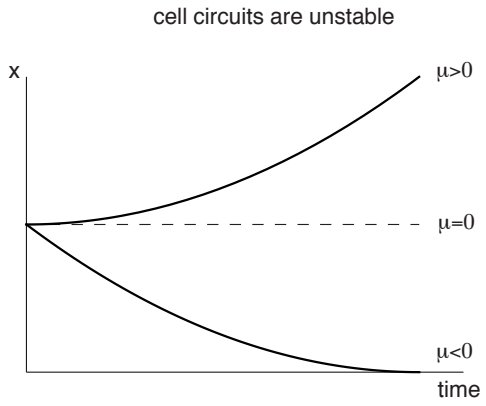

Figure 9.9

So to keep cell numbers constant we need additional feedback control, because we need a balance between proliferation and death in order to reach zero growth rate $\mu = 0$. The feedback needs to keep the tissue at a good functional size. Hence, the feedback mechanism must somehow register the biological activity of the cells and accordingly negatively control their growth rate.

Such feedback control occurs for beta cells, as pointed out by Brian Topp and Dianne Finegood (2000), with blood glucose as the feedback signal. In other words, glucose controls the cells growth rate, so that $\mu = \mu(G)$. The death rate of beta cells is high at low glucose, and falls sharply around 5mM glucose (Fig 9.10). Death 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. The curves describing the rates for proliferation and death cross near Go=5mM (Fig 9.11). Therefore, Go=5mM is the fixed point we seek with zero growth rate, $\mu(G_0) = 0$.(Fig 9.12)

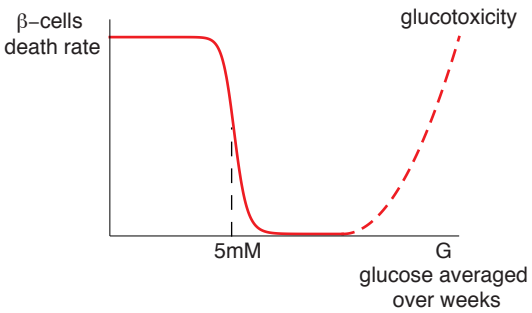

Figure 9.10

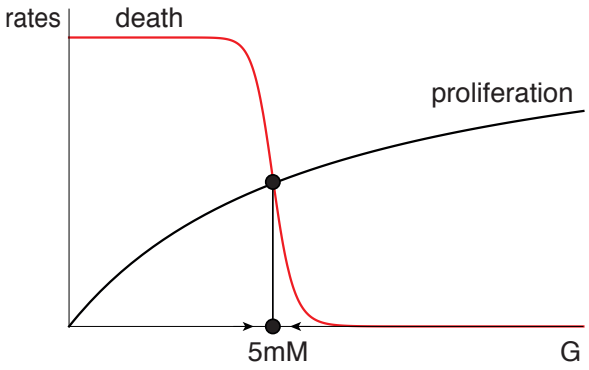

Figure 9.11

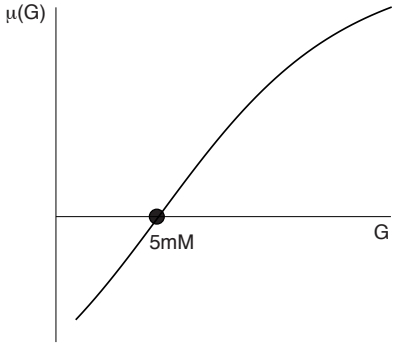

Figure 9.12

Our revised model, the BIG model (Beta cells-Insulin-Glucose model, Fig 9.13), includes a new equation for the beta cells X

(3) dG/dt=m-Si IG
(4) dI/dt=p X f(G)-γ I
(5) dX/dt=X mu(G) **mu(Go)=0**

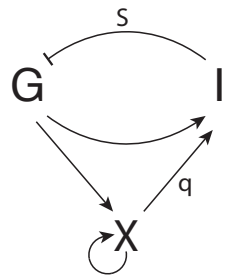

Figure 9.13

The point Go=5mM is a stable fixed point for both beta cells and blood glucose. If glucose is above 5mM, beta cells have proliferation>death, they increase in number, leading to more insulin, pushing glucose back down towards 5mM. If glucose is too low, beta cells die more than divide, leading to less insulin, pushing glucose back up.

This neat feedback loop operates on the timescale of days to weeks, which is the proliferation rate of beta cells. It is much slower than the insulin-glucose feedback that operates over minutes to hours. The slow feedback loop keeps beta cells at a good functional steady-state number and keeps glucose, averaged over weeks, at 5mM.

The steepness of the death curve is important for the robustness of the fixed point. Due to the steepness of the death curve, variations in proliferation rate do not shift the 5mM fixed point by much (Fig 9.11). The steep death curve is thought to be generated by the cooperativity of key enzymes that sense glucose inside beta cells, glucokinase and AMPK (exercise XX).

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, Si. 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 test, the response to an input m of 75g glucose yields the same output G(t), including the same amplitude and response time, for widely different values of the insulin sensitivity parameter Si. This independence on Si is very unusual, because varying a key parameter in most models would change their dynamics.

This ability of a model to compensate for variation in a parameter was defined by Omer 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, for example, when you start away from steady-state. To prove DC in our model requires rescaling of the variables in the equations.

Solved Example2:

Show that the beta-cell-insulin-glucose model has dynamic compensation (DC).

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

$$(5)\ \frac{dG}{dt} = m - \tilde{I}G$$

Multiplying the insulin and beta-cell equations (Eq 3,4) by Si leads to scaled equations with no Si

$$(6)\ \frac{d\tilde{I}}{dt} = q\ \tilde{X} f(G) - \gamma \tilde{I}$$

$$(7)\ \frac{d\tilde{X}}{dt} = \tilde{X}\mu(G) \qquad \text{with } \mu(G_o) = 0$$

Furthermore, at steady-state, the initial condition of these scaled equations also does not depend on Si. There are three initial condition values we need to check, for G(t=0)=Gst, I~(t=0)=I~st and X~(t=0)=X~st. First, Gst is independent on Si because Gst=Go which is the only way for $\tilde{X}$ to be at steady state in Eq 7. Therefore, from Eq 5, $\tilde{I} = m_{st}/G_0$ is independent on Si, which we can use in Eq 6 to find that $\tilde{X}_{st} = \gamma\ \tilde{I}_{st}/f(G_o)$ is also independent of Si. Since the dynamic equations and initial conditions do not depend on Si, the output G(t) is invariant to Si, and we have DC.

Although G(t) is independent on SI, insulin and beta cell levels do depend on Si, as we can see by returning to original variables $X = \tilde{X}/S_i$ and $I = \tilde{I}/S_i$. The lower Si, the higher the steady-state insulin, keeping the product Si Ist=const=mst/Go, explaining the hyperbolic law of Fig 9.6. Also, Si Xst=const, as beta cells rise to precisely compensate decreases in Si.

Similar considerations show that the model has DC with respect to the parameter q, the rate of insulin secretion per beta cell, and also to the total blood volume (Exc XX). There is no DC, however to the insulin removal rate parameter, γ .

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Let's see how dynamic compensation works. Suppose insulin sensitivity drops by a factor of ten, representing insulin resistance (Fig 9.14). As a result, insulin is less effective and glucose levels rise. Due to the decreasing death curve, beta cells die less, and their numbers rise over days to weeks. More beta cells mean that more insulin is secreted, and average glucose returns to baseline (Fig 9.14 upper panels show the dynamics on the scale of weeks). In the new steady state, there are ten times more beta cells and ten times more insulin. Glucose returns to its 5mM baseline.

Let's now zoom in to the timescale of hours (Fig 9.14 lower panel). The response of glucose to a meal long after the drop in Si (timepoint 3) is exactly the same as before the change in Si (timepoint 1), but the insulin response is ten 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 (timepoint 2).

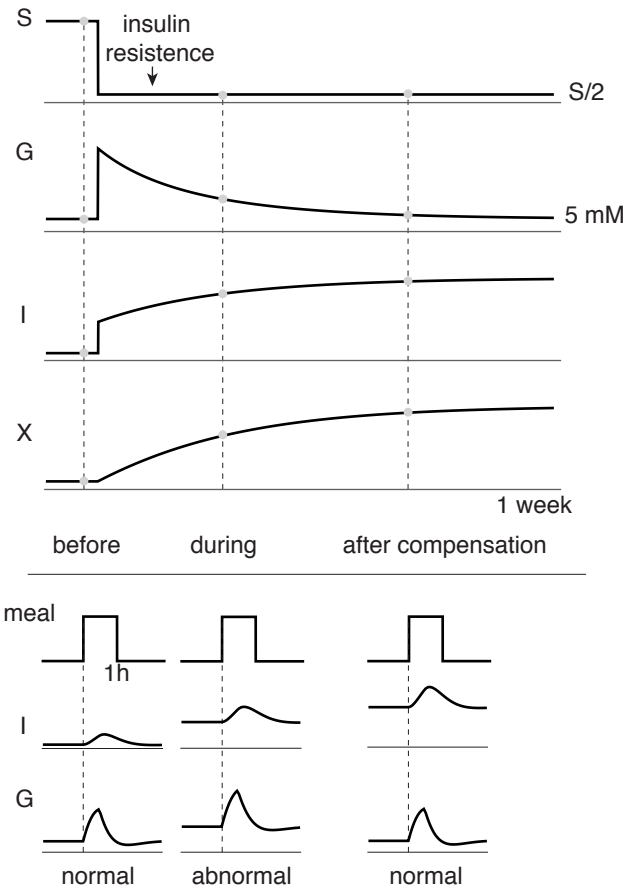

Figure 9.14

The DC model predicts that people with different Si should show the same glucose meal dynamics, but have insulin dynamics that scale with Si. This is indeed seen in measurements that follow non-diabetic people with and without insulin resistance over a day with three meals. Insulin levels are higher in people with insulin-resistance, but when normalized by the fasting insulin baseline, there is almost no difference between the two groups (Fig 9.15)

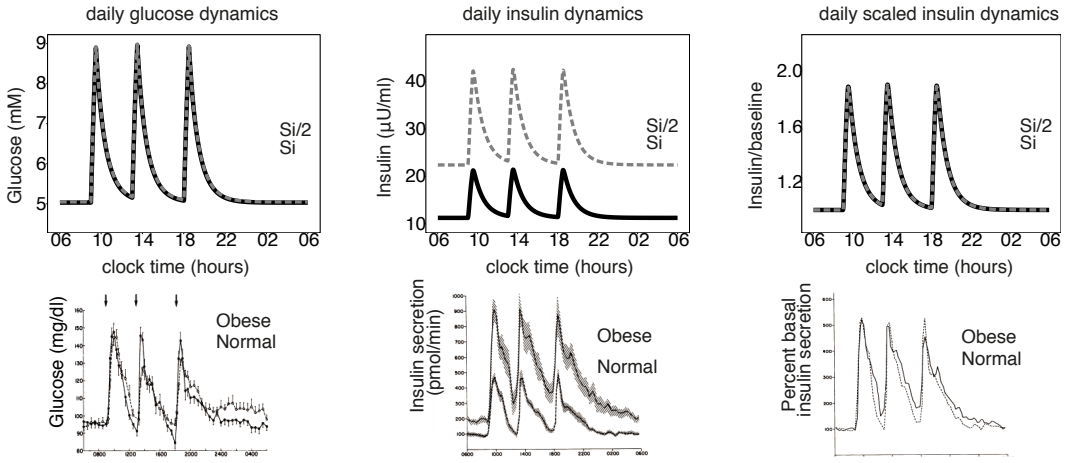

Figure 9.15

The DC property depends on the structure of the equations: Si cancels out due to the linearity of the dX/dt equation with X, which is a natural consequence of cells arising from cells. Si also cancels out due to the linearity in X of the of insulin secretion term q X f(G), a natural outcome of the fact that beta cells secrete insulin.

The basic features needed for DC exist in all hormone systems, in which glands made of cells secrete hormones that work on other tissues. Indeed, hallmarks of DC are found in several other hormone systems. For example, blood calcium concentration is controlled tightly around 10mM by a hormone called PTH, secreted by the parathyroid gland (Fig 9.16). 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. An additional slow feedback loop occurs because parathyroid cell proliferation is regulated by calcium.

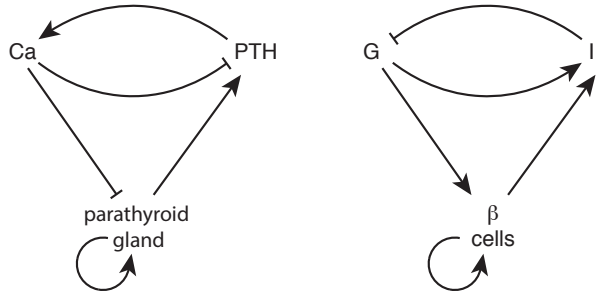

Figure 9.16

Other organ systems and even neuronal systems have similar hallmarks, giving a picture in which the size of the gland or organ expands and contracts to buffer variation in effectivity parameters. Moreover, as embryos and children grow, these slow feedback loops can help each gland grow precisely at a rate that keeps important variables such as glucose and calcium at their desired level.

The feedback mechanism seems so robust. What about diseases such as diabetes? How and why do things break down? We will see that some forms of diabetes may be due to a dynamic instability that is built into the feedback loop.

Type-2 Diabetes is linked with instability due to a U-shaped death 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 high glucose levels, beta-cell death rate rises (by death here we include all processes that remove beta cell function such as beta-cell exhaustion, de-differentiation and senescence) and eventually patients are not able to make enough insulin.

Glucotoxicity is dangerous because it adds an unstable fixed point, the point at which proliferation rate crosses death rate a second time Fig 9.17. As long as glucose fluctuations do not exceed the unstable point, glucose safely returns to the stable 5mM point. However, when glucose (averaged over weeks) crosses the unstable fixed point, beta-cell death 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.

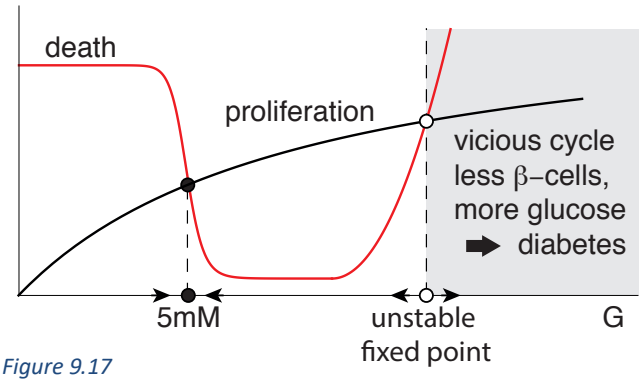

Figure 9.17

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 more lean diet can move the system back into the stable region. 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 9.18), 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 points appears to mildly increase in healthy people.

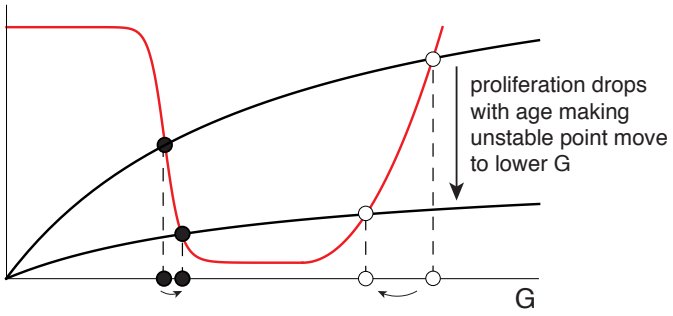

Figure 9.18

A final risk factor is genetics. It appears that the glucotoxicty curve is different between people. A shifted glucotoxicity curve can make the unstable fixed point come closer to 5mM (Fig 9.19).

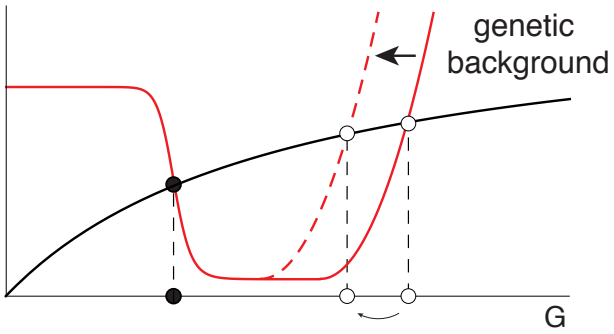

Figure 9.19

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 reactive oxygen species (ROS) generated by the accelerated glycolysis in beta cells presented with high glucose. ROS cause extensive cell damage. 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 systems that protect other cells types. Thus, it is intriguing to find a functional explanation for glucotoxicity.

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

Omer Karin et al (2017) provide an explanation for glucotoxicity by considering a fundamental fragility of tissue-level feedback circuits. The fragility is to takeover 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, given the huge number of cell divisions in a lifetime[2], 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 the input signal - glucose - by breaking glucose 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 glucokinase with a halfway-binding constant to glucose of K=40uM, but beta cells express a special isoform with K=8mM- perfect as a sensor for the 5mM range. Mutations that affect the K of glucokinase, reducing it, say , by a factor of five, cause the mutant cell to sense five times too much glucose (the mutant beta cells 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 G is actually 5G.

If our feedback design did not include glucotoxicity, such a mutant that interprets 5mM glucose as 25mM would think 'oh, we need more insulin!' and proliferate (Fig 9.20). The mutant cell therefore has a growth advantage over other beta cells, which sense 5mM correctly. The mutant will proliferate exponentially and eventually take over. This is dangerous because when the mutant takes over, it pushes glucose down to a set-point level that it thinks is 5mM, but in reality is 1mM - causing lethally low glucose.

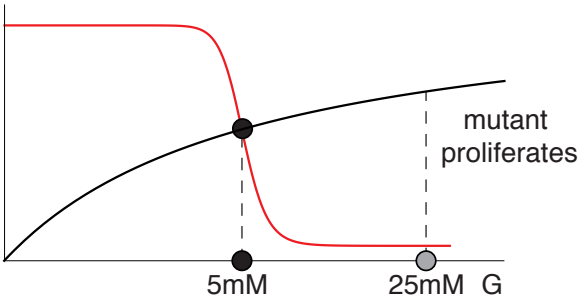

Figure 9.20

[2] Here is a conservative estimate. 1g of tissue has about 10^9 cells. If they divide 1/month, there are 10^12 divisions in a human lifespan. Mutation rate is 10^-9/letter/division, and so each position in the genome is mutated 10^3 times in this gram of tissue. There will be 1000 cells expressing each possible point mutation in each protein. Avoiding mutant growth may be one reason why adult cells divide much slower than they could (embryonic cells can divide every few hours). Depending on the tissue, cells are renewed on average every few days, weeks or months.

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 too high, and kills itself (Fig 9.21). Mutants are removed.

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.

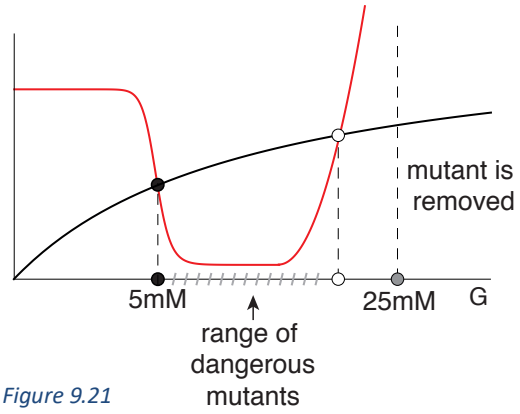

Figure 9.21

In our evolutionary past, lifestyle 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, but it 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 (Fig 9.21). Such mutants have a growth advantage, because they are too weak to be killed by glucotoxicity, but still have more proliferation than removal.

Luckily, such intermediate-effect mutants are much rarer than mutants that strongly activate or deactivate signaling. Designs that can help against intermediate mutants are found in beta cells: beta cells are arranged within the pancreas in isolated clusters of ~1000 cells called islands of Langerhans, so that a mutant can take over just one island and not the entire tissue. Slow growth rates for beta-cells also help keep such mutants in check. Exercise xx estimates that a small fraction of the islets are taken over by mutants in a lifetime (Ec XX)

The glucotoxicity mutant-resistance mechanism can be generalized: to resist mutant takeover of a tissue-level feedback loop, the feedback signal must be toxic at both low and high levels. Such phenomena are known as **biphasic responses**, and 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 such as Alzheimer's and Parkinson's in the case of neurons.

Summary

Tissues have robustness constraints that are not found when thinking about protein circuits inside cells. First, tissues have a fundamental instability due to exponential cell growth dynamics. They require feedback to maintain steady-state. Such feedback loops can use a signal related to the tissue function, to make both organ size and organ function stay at a stable fixed

point. This fixed point is maintained as the cells constantly turn over on the scale of days to weeks.

Tissue-level circuits, such as hormone circuits, are also challenged by the fact that they often need to operate on distant target tissues. These target tissues have variation in their interaction parameters, such as insulin resistance. Hormone circuits can show robustness to such parameters by means of dynamic compensation, which arises due to a symmetry of the equations to the parameter. In dynamic compensation, tissue size grows and shrinks in order to precisely buffer against such parameter variation.

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 constraint 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 mutants by giving them a growth disadvantage. This comes at the cost of fragility to dynamic instability and disease. Additional principles of tissue-level circuits no doubt await to be discovered.