Systems medicine Lecture notes
Uri Alon (Spring 2019)

https://youtu.be/m6LYiNRCpxg

Lecture 2 - 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 tissues. Tissues are made of cells that signal to each other. Distant tissues communicate via hormones that flow in the blood stream. We will see that at the tissue level there are universal challenges. Tissues must:

(i) Maintain a proper size, despite the fact that cells tend to grow exponentially
(ii) Signal precisely to distant tissues whose parameters are unknown.
(iii) Avoid mutant cells that can grow and take over the tissue.

We will see that principles arise to allow organs to work robustly, keep the right functional size and resist mutants. In fact, a unifying circuit design can address all three problems at once.

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

We saw in the last lecture that the insulin-glucose feedback loop on its own 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) means a rise form 5mM glucose baseline, and longer response times. This is in contrast to the observation that most people with insulin resistance have normal glucose. The minimal model is not **robust** to parameters like s.

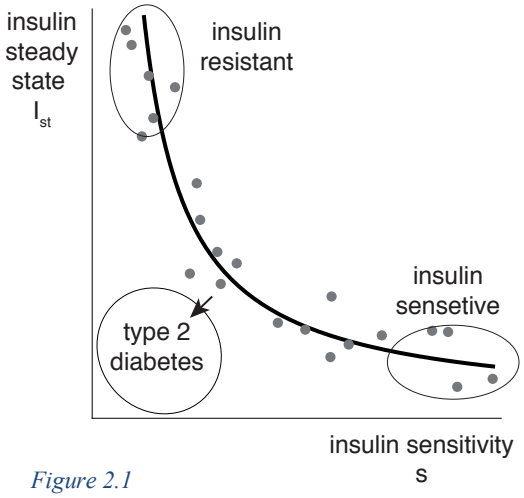

Figure 2.1

Therefore, robustness must involve additional processes beyond the minimal model's glucose-insulin loop. Indeed, the way that the body compensates for decreased insulin sensitivity s is by increasing the number of beta cells. More beta cells means more insulin, and this exactly matches the decrease in *s*. For example, people with obesity that are insulin resistant have more and larger beta cells than lean individuals (beta cell *hyperplasia* and *hypertrophy*). They thus secrete more insulin, compensating for insulin resistance.

This compensation is seen in a hyperbolic relation that exists between healthy people: an inverse relationship between s and steady-state insulin that keeps the product of the

two constant: $sI_{st} = const$ ((Kahn *et al.*, 1993)). People thus compensate for low insulin sensitivity with more insulin (Fig 2.1) People with diabetes have values that lie below this hyperbola. The origin of this hyperbolic relationship has long been mysterious, but we will soon understand it.

2.3 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 numbers, 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 form 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 2.2).

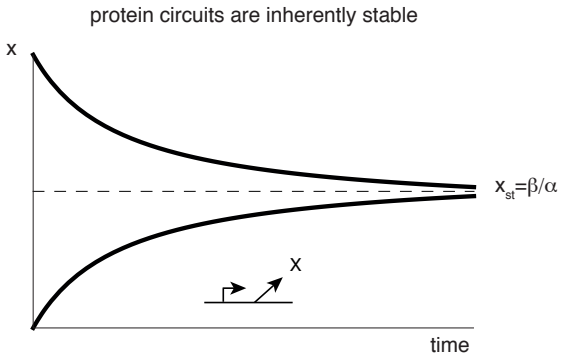

Figure 2.2

Cells, however, live on a knife's edge. Their basic biology contain an inherent instability, due to exponential growth. Cells divide (proliferate) at rate p, and are removed at rate d (Fig 2.3), which we will call death. Death 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 are made by cells, proliferation is intrinsically autocatalytic, a rate constant times the concentration of cells: *proliferation*=p B. This is unlike the glucose equation above, in which the production term m is not multiplied by G. Removal as usual is the number of cells times the rate at which cells are remover, d: removal= d B. As a result the balance between proliferation rate pB and death rate dB

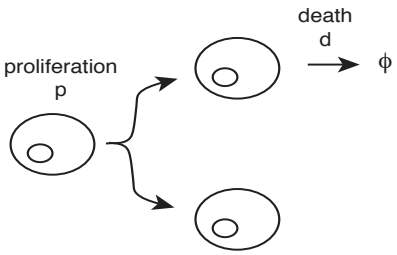

Figure 2.3

(2.3) $\frac{dB}{dt} = pB - dB = (p-d)B = \mu B.$

leads to a growth rate of cells $\mu = p - d$ equal to the difference between proliferation and death . If proliferation exceeds death, growth rate μ is positive and cell numbers rise exponentially, B~$e^{\mu t}$ (Fig 2.4). 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 in cell numbers occurs in degenerative diseases. This is the problem of **tissue size control.**

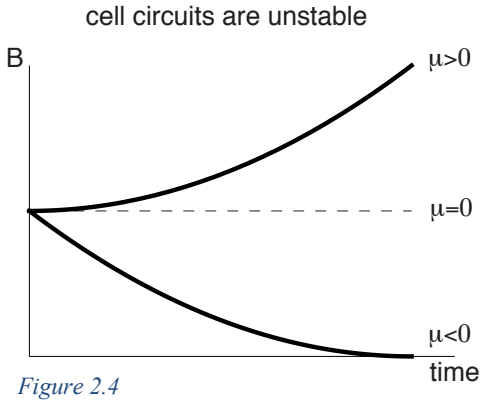

Figure 2.4

Tissue size control is an amazing problem: our body is constantly in turnover as 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 need to balance proliferation and death in order to reach zero growth rate, $\mu = 0$. Moreover, the feedback loop must keep the tissue 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 pointed out by Brian Topp and Dianne Finegood ((Topp *et al.*, 2000)). The feedback signal is blood glucose: glucose controls the cells proliferation and death rates, so that $\mu = \mu(G)$. The death rate of beta cells is high at low glucose, and falls sharply around 5mM glucose (Fig 2.5). Death rate rises again at high glucose, a phenomenon called **glucotoxicity**, which we will return to soon.

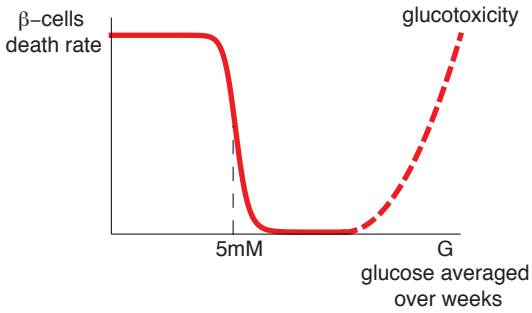

Figure 2.5

For now, let's focus on the region around 5mM. Proliferation rises with glucose, so that

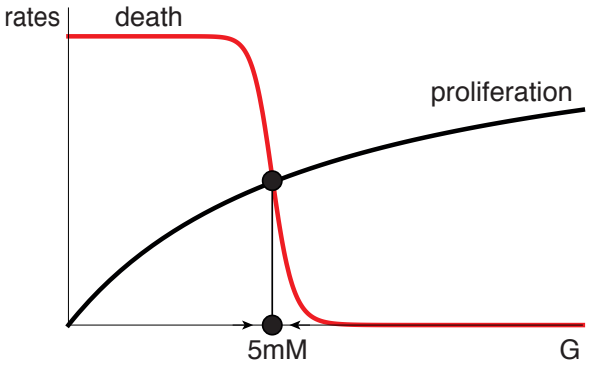

Figure 2.6

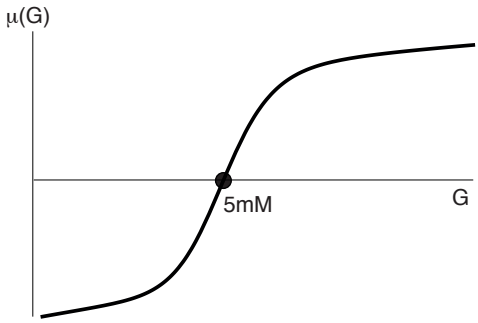

Figure 2.7

the curves describing the rates for proliferation and death cross near $G_0 = 5mM$ (Fig 2.6). Therefore, $G_0 = 5mM$ is the fixed point that we seek with zero growth rate, $\mu(G_0) = 0$ (Fig 2.7).

Our revised model, the BIG model (Beta-cell-Insulin-Glucose model, Fig 2.8), includes a new equation for the beta cells B

$$2.4 \quad \frac{dG}{dt} = m - s\,I\,G$$

$$2.5 \quad \frac{dI}{dt} = qXf(G) - \gamma I$$

$$2.6 \quad \frac{dB}{dt} = B\,\mu(G) \qquad \mu(G_0) = 0$$

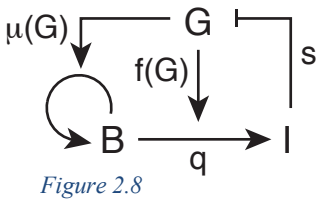

Figure 2.8

The point $G_0 = 5mM$ 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 they divide, leading to less insulin, pushing glucose levels back up.

This 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 that operates over minutes to hours. This **slow feedback loop** keeps beta cells at a proper functional steady-state number and keeps glucose, averaged over weeks, at 5mM. The only way to reach steady-state in Eq. 2.6 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 Alon 2006):
The steep drop of the death curve at G_0 is important for the precision 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 2.6). The steep death curve can be generated by the cooperativity of key enzymes that sense glucose inside beta cells ((Karin *et al.*, 2016)).

2.4 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 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 s. This independence of the entire dynamic curve on a parameter such as s is very unusual, because changing a key parameter in most models changes their dynamics.
Lets start simple, with calculating the steady state of the BIG model. The glucose steady state is G_0=5Mm thanks to Eq 2.6- the place where cell proliferation balances removal. Therefore, from Eq 2.4, $I_{st} = m_0/sG_0$. The lower s, the higher insulin. In fact, the product $sI_{st} = \frac{m_{st}}{G_0} = const$, which explains the hyperbolic relation of Fig 2.1. finally, the beta cell steady state can be found form equation 2.5, by setting $dI/dt = 0$, to find that $B_{st} = \gamma \frac{I_{st}}{f(G_o)} = \gamma \frac{m_0}{sG_0 f(G_o)}$. The number of beta cells also rises when s is small. Thus, the tissue-size control feedback over weeks makes beta cells expand and contract in order to precisely buffer out the effects of parameters changes like insulin resistance.

The feedback does something even more dramatic: it makes the entire response to a meal invariant to parameters like s. This is advanced material I did not discuss in class, but it is important to know: This 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, for example, when you start away from steady-state. To establish DC in our model requires rescaling of the variables in the equations, as done in the next solved example.

Solved Example2:
Show that the BIG 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 meal 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

$$(2.7)\ \frac{dG}{dt} = m - \tilde{I}G$$

Multiplying the insulin and beta-cell equations (Eq 2.5, 2.6) by s leads to scaled equations with no s

$$(2.8)\ \frac{d\tilde{I}}{dt} = q\,\tilde{B}f(G) - \gamma\tilde{I}$$

$$(2.9)\ \frac{d\tilde{B}}{dt} = \tilde{B}\mu(G)\ \text{ with } \mu(G_o) = 0$$

Now that none of the equations depends on s, we only need to show that the initial conditions of these scaled equations do not depend on s. If both the equations and initial conditions are independent on s, so is the entire dynamics. There are three initial condition values that we need to check, for G, $\tilde{I}$ and $\tilde{B}$. First, $G(t=0) = G_{st}$ is independent on s because $G_{st} = G_0$ is the only way for $\tilde{B}$ to be at steady-state in Eq 2.9. Therefore, from Eq 2.6, $\tilde{I}_{st} = m_0/G_0$ is independent of s, which we can use in Eq 2.7 to find that $B_{st} = \gamma\,\tilde{I}_{st}/f(G_o)$ 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. In fact, the product $sI_{st} = \frac{m_{st}}{G_0} = const$, which explains the hyperbolic relation of Fig 2.1. Also, $sB_{st} = const$, as beta cells rise 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 also to the total blood volume (exercises 2.5). There is no DC, however to the insulin removal rate parameter, γ.

==========

Let's see how dynamic compensation works. Suppose that insulin sensitivity drops by a factor of two, representing insulin resistance (Fig 2.9). As a result, insulin is less effective and glucose levels rise. Due to the death curve, beta cells die less, and their numbers rise over days to weeks (Fig 2.9 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.

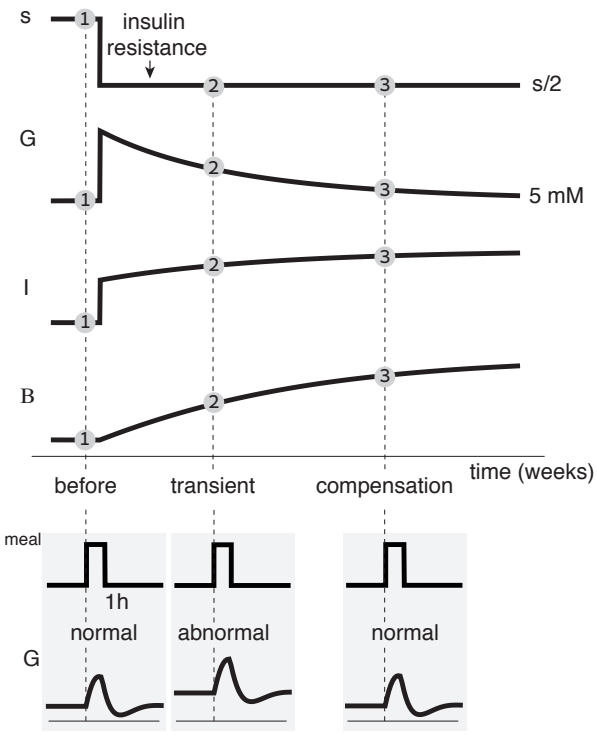

Figure 2.9

Let's now zoom in to the timescale of hours (Fig 2.9, lower panel). The response of glucose to a meal long after the drop in *s* (time-point 3) is exactly the same as before the change in *s* (time-point 1). 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 DC 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

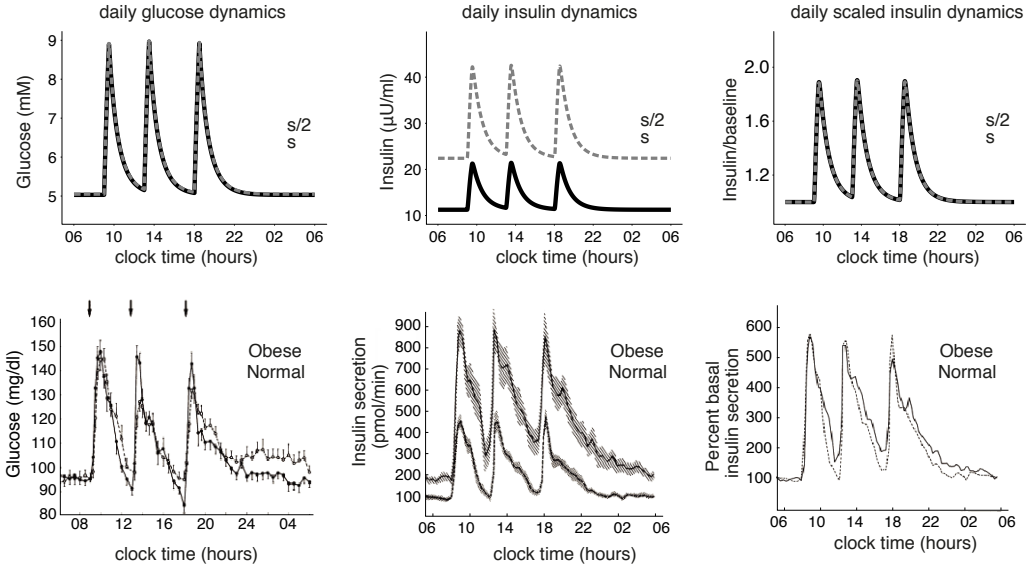

Figure 2.10 (adapted from Karin et al (2016) and Polonsky et al ((1988).

a day with three meals (lower panels in Fig 2.10) ((Polonsky *et al.*, 1988)). 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 2.10). The model (upper panels in Fig 2.10) 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 of 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, in which glands secrete hormones that work on distant tissues. For example, free blood calcium concentration is regulated tightly around 1mM by a hormone called PTH, secreted by the parathyroid gland (Fig 2.11). 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.

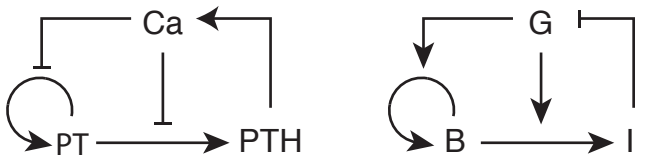

Figure 2.11

The slow feedback loop occurs because parathyroid cell proliferation is regulated by calcium.

Other organ systems and even neuronal systems have similar circuits (Fig 2.12), 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 steady-state level.

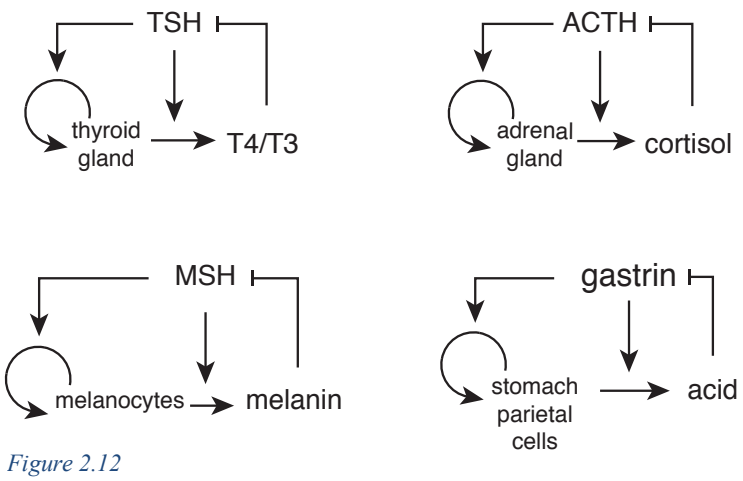

Figure 2.12

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.

11.5 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 very 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 (white circles in Fig 2.13). As long as glucose fluctuations do not exceed the unstable point, glucose safely returns to the stable 5mM point. However, if glucose (averaged over weeks) crosses the unstable fixed point, 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.

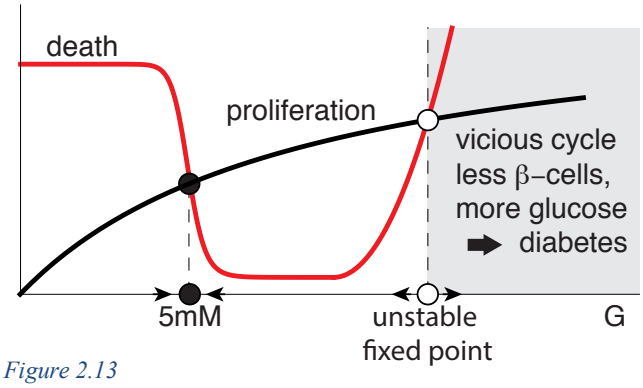

Figure 2.13

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.

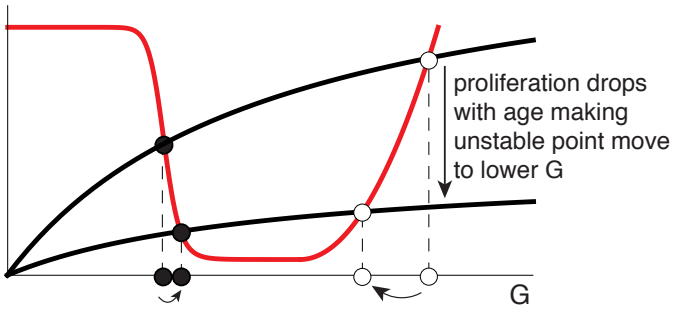

Figure 2.14

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 2.14), 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.

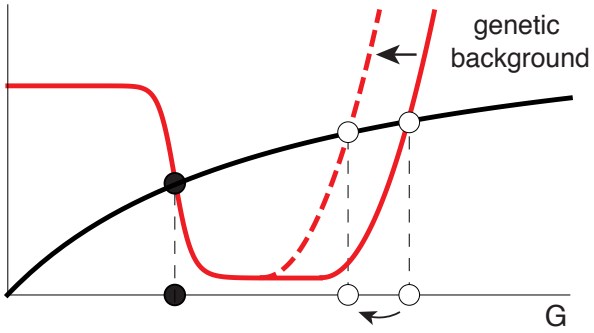

Figure 2.15

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 2.15).

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 cause extensive cell damage, and beta-cell death. 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 cells types. Thus, it is intriguing to find a functional explanation for glucotoxicity.

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

Omer Karin et al ((Karin and Alon, 2017)) provide an explanation for glucotoxicity by considering a fundamental fragility of tissue-level feedback circuits. This 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[1], 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 $K = 8mM$ - 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 have higher proliferation rate (black curve) than death rate (red curve). It would think 'Oh, we need more insulin!' and proliferate (Fig 2.16). 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 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.

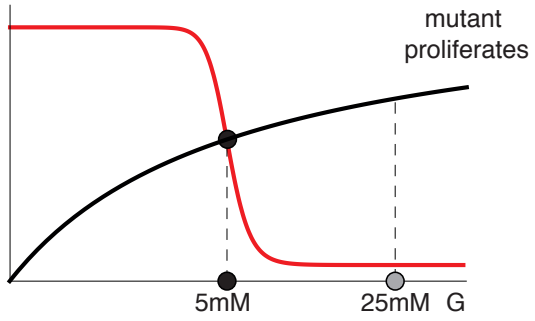

Figure 2.16

Mutant expansion is insidious because as the mutant population starts to push glucose slightly below 5mM normal cells begin to die (to reduce insulin and increase glucoe), enhancing the mutant's advantage.

11.7 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, has a death curve that exceeds the proliferation curve, and kills itself (Fig 2.17). Mutants are removed.

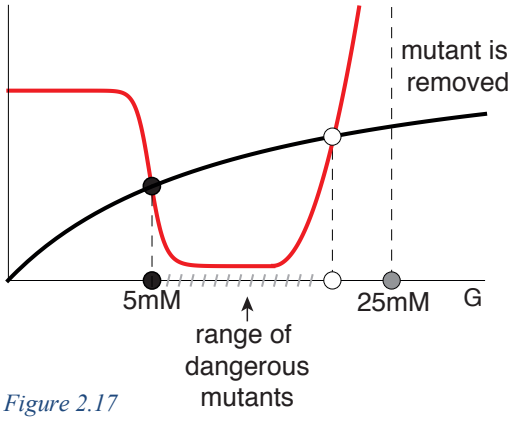

Figure 2.17

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, lifestyle and nutrition was probably such that average glucose rarely stayed very high for weeks, and thus the unstable fixed point was rarely crossed.

---

[1] A gram of tissue has about 10^9 cells. If they divide 1/month, there are about 10^{10} divisions in a year. Mutation rate is 10^{-9}/base-pair/division, so there will be about 10 cells expressing each possible point mutation. Depending on the tissue, cells are renewed on average every few days (colon epithelium) to a few months (most tissues- skin, lung) to never (most neurons).

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 2.17). Such mutants have a growth advantage, because they are too weak to be killed by glucotoxicity, but still have higher proliferation rate than removal rate.
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 in the pancreas in isolated clusters of ~1000 cells called islets of Langerhans, so that a mutant can take over just one islet and not the entire tissue. Slow growth rates for beta-cells also help keep such mutants in check. Karin et al ((Karin and Alon, 2017)) estimate that a small fraction of the islets are taken over by mutants in a lifetime.
This 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 U-shaped 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, for example Alzheimer's and Parkinson's in the case of neurons.

11.8 Summary

Tissues have robustness constraints beyond those of protein circuits inside cells. First, tissues have a fundamental instability due to exponential cell growth dynamics. They require feedback to maintain steady-state and a proper size. Such feedback loops use a signal related to the tissue function, 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.
Tissue-level circuits, such as hormone circuits, are also challenged by the fact that they 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 distant parameters by means of dynamic compensation (DC), which arises due to a symmetry of the equations. In dynamic compensation, tissue size grows and shrinks in order to precisely buffer the variation in parameters.
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 can 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.

Further reading

History of the minimal model
(Bergman, 2005) "Minimal model: Perspective from 2005"
The BIG model
(Topp *et al.*, 2000) "A model of β-cell mass, insulin, and glucose kinetics: Pathways to diabetes"
Dynamic 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.
Bergman, R. N. (2005) 'Minimal model: Perspective from 2005', in *Hormone Research*. doi: 10.1159/000089312.
Kahn, S. E. *et al.* (1993) 'Quantification of the relationship between insulin sensitivity and β- cell function in human subjects: Evidence for a hyperbolic function', *Diabetes*. doi: 10.2337/diabetes.42.11.1663.
Karin, O. *et al.* (2016) 'Dynamical compensation in physiological circuits', *Molecular Systems Biology*. doi: 10.15252/msb.20167216.
Karin, O. and Alon, U. (2017) 'Biphasic response as a mechanism against mutant takeover in tissue homeostasis circuits', *Molecular Systems Biology*. doi: 10.15252/msb.20177599.
Keener, J. and Sneyd, J. (no date) *I N T E R D I S C I P L I N A R Y A P P L I E D M A T H E Mathematical Physiology II: Systems Physiology*. Available at: https://link.springer.com/content/pdf/10.1007/978-0-387-79388-7.pdf (Accessed: 5 December 2018).
Polonsky, K.S., Given, B.D., and Van Cauter, E. (1988). Twenty-four-hour profiles and pulsatile patterns of insulin secretion in normal and obese subjects. J. Clin. Invest. *81*, 442–448.
Topp, B. *et al.* (2000) 'A model of β-cell mass, insulin, and glucose kinetics: Pathways to diabetes', *Journal of Theoretical Biology*. doi: 10.1006/jtbi.2000.2150.