

# Expert Views in Diabetes

Issue 2

Steno



Professor Peter C Butler is Director of the Larry L Hillblom Islet Research Center, Los Angeles, USA, which brings together a group of leading scientists in the area of islet research, to understand the cause of islet cell destruction leading to diabetes and to understand how islet cells can be replaced.

Professor Butler is also Professor of Medicine at the David Geffen School of Medicine, University of California, USA. From 1 January 2007, Professor Butler will become the Editor-in-Chief of *Diabetes*. Professor Butler graduated from the University of Birmingham,

UK, in 1980, and he also completed an Internship at the University in 1981. He was a Resident at the Universities of Edinburgh and Birmingham, UK, until 1987, when he joined the Mayo Medical College in Rochester, Minnesota, USA, as a Postdoctoral Researcher. Between 1990 and 1996, Professor Butler held the positions of Assistant Professor of Medicine at East Carolina University, USA, and Associate Professor and Associate Director of Medicine at the Mayo Clinic. From 1996 to 1999, he was the Chair of Diabetic Medicine, and Director of The Wellcome Trust Clinical Research Centre, both at the University of Edinburgh. From 1999 until 2004, when he assumed his current positions, Professor Butler was the Chief of the Division of Endocrinology and Diabetes, and Professor of Medicine at The Keck School of Medicine, University of Southern California, USA.

## Type 2 diabetes – a matter of $\beta$ -cell life or death

*Professor Peter C Butler, Director Larry L Hillblom Islet Research Center*

There are now 20 million people with diabetes in the United States. There is no question that lifestyle – especially lack of exercise and eating too much – can, to a large degree, predict whether genetic predisposition will result in type 2 diabetes, but 80% of people who are morbidly obese never develop diabetes.<sup>1</sup> However, the genetic basis for diabetes is something that patients cannot be blamed for. Impaired development of  $\beta$ -cells, or losing  $\beta$ -cells due to an underlying genetic defect, is, perhaps, something about which we are a little less prejudiced compared with a sedentary lifestyle. In contrast with type 2 diabetes, there is an element of sympathy for ‘unfortunate’ people with type 1 diabetes who have developed a disease – often in childhood – where they have lost their  $\beta$ -cells through ‘bad luck’. Indeed, a

current theme in diabetes research is that there is actually more in common between these diseases than there are differences. Against this background, I will try to address the ambitious title of this commentary – namely that type 2 diabetes is a matter of  $\beta$ -cell life or death.

### *Origins of the islets of Langerhans*

In developmental biology, the pancreas develops from two parts of the foregut, driven by Pdx1 and other transcription factors leading to the differentiation of endocrine cells. Though much is becoming known in this field, huge gaps remain in our understanding of  $\beta$ -cells. The mature pancreas is composed largely of acinar cells (99%) that synthesize the pancreatic juices and about one million islets of Langerhans that

constitute the endocrine cells. Between birth and adulthood, substantial growth in  $\beta$ -cell populations occurs.

An ongoing, as yet unpublished, study has been undertaken to evaluate the events that occur in the pancreas during childhood and adolescence. The exocrine pancreas grows faster than the endocrine pancreas. From birth to age 20 years, the pancreas becomes larger in a linear fashion. While the exocrine pancreas is growing, most of the growth of  $\beta$ -cells occurs by the age of 5 years. The  $\beta$ -cells are rather like the brain cells in that there are large populations to begin with and then the rate of growth and development of additional  $\beta$ -cells declines. Some kind of signal indicates that there are enough  $\beta$ -cells and further replication and

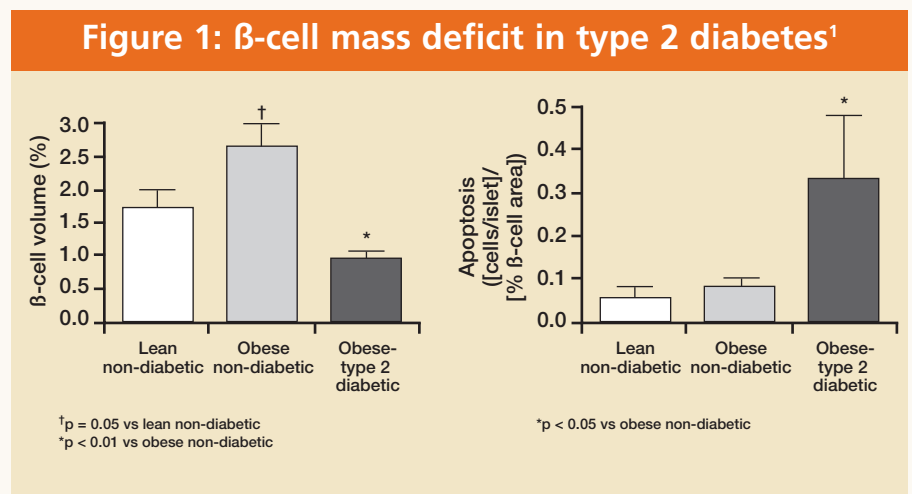
development are not needed. In contrast, in mice, continuing  $\beta$ -cell replication can be observed the longer a mouse lives.

Such data may lead to the assumption that growth in  $\beta$ -cell mass in early life will be an important determinant of an individual's subsequent risk for type 2 diabetes. Indeed, in type 2 diabetes, the focus is often on  $\beta$ -cell death, in the belief that this occurs in much the same way as bone development, where the amount of bone at age 20 years is important because individuals will lose bone thereafter at different rates. It is possible that a similar pattern occurs in the pancreas, and post-mortem studies are ongoing in patients who died suddenly, to determine if those with low  $\beta$ -cell mass at age 20 years had a family history of type 2 diabetes.

### **Clinical overview of the islets of Langerhans in type 2 diabetes**

Microscopic analysis of pancreatic islet tissue from individuals with type 2 diabetes shows very distinct abnormalities. In islet tissue from healthy non-diabetic individuals, there are approximately 2000–3000  $\beta$ -cells. In typical islets from subjects with type 2 diabetes, there are fewer  $\beta$ -cells and there is amyloid material within the islet. Unfortunately, the pancreas is a very inconvenient organ to study as tissue cannot be collected during life and the pancreas undergoes rapid autolysis after death. Thus, studies of pancreas pathology have been scarce.

A pathology study published in 2003<sup>1</sup> involving the analysis of  $\beta$ -cell mass in approximately 200 pancreases from obese versus lean non-diabetic individuals showed that obese individuals have approximately 50% greater  $\beta$ -cell mass (Figure 1). Thus it appears that obese individuals are,



ordinarily, able to adapt to insulin resistance by increasing  $\beta$ -cell mass. However, amongst those with a genetic predisposition to type 2 diabetes, obese diabetic individuals showed a deficit in  $\beta$ -cell mass of approximately 70%. Some of the confusion in this area comes from making the wrong comparisons – for example, obese individuals with type 2 diabetes compared with lean non-diabetic individuals, when we should be comparing subjects with comparable long-term insulin resistance. But, why do individuals with diabetes lose  $\beta$ -cells? Analysis of the frequency of apoptosis in these groups showed an increased frequency of  $\beta$ -cell apoptosis in the subjects with type 2 diabetes, implying that loss of  $\beta$ -cell mass may well be due to an increase in  $\beta$ -cell apoptosis.

Is a 70% deficit in  $\beta$ -cells enough to account for diabetes and hyperglycaemia? Studies in rats have shown that removal of 70% of the rat's pancreas does not cause it to develop diabetes.<sup>2</sup> Studies have suggested that humans can tolerate losing about 50% of their  $\beta$ -cell mass, although thereafter they will decline, and every further decrement of  $\beta$ -cell mass results in a marked increase in blood glucose. This is an interesting point from a clinical perspective if you are a  $\beta$ -cell regenerator and you want to try and restore  $\beta$ -cells – I suggest that you would not have to renew

them to a great extent in order to achieve quite a large improvement, if it were possible. These studies also show that a small loss of  $\beta$ -cell mass past a certain point results in marked deterioration. Furthermore, what about increasing  $\beta$ -cell apoptosis, and what is apoptosis anyway?

### **$\beta$ -cell apoptosis**

Apoptosis is a cell transcription program that 'kicks in' and tells a cell to die. It is extremely important in developmental biology – there is a balance between replication making new cells, turning on transcriptional pathways to differentiate the cells and then removing the cells that we do not need. The disposal of cells is accomplished via pathway programs that indicate to the cell that it is no longer required. The three pathways that are defined are the intrinsic pathway, which is largely characterized through mitochondrial dysfunction, free radicals and so forth; the extrinsic pathway, which is activated classically through cytokines acting through surface receptors – which is how cytokines are believed to be responsible for  $\beta$ -cell death in type 1 diabetes – and endoplasmic reticulum (ER) stress. Glucose toxicity has also been implicated as an inducer of  $\beta$ -cell apoptosis, and free fatty acid (FFA) toxicity through lipolysis also has its advocates (Figure 2).

## Figure 2: Factors involved in $\beta$ -cell apoptosis

- Mitochondrial dysfunction
- Free radicals
- Cytokines
- Endoplasmic reticulum (ER) stress
- Glucose toxicity
- Free fatty acid (FFA) toxicity
- Islet-amyloid polypeptide (IAPP)

### *Islet-amyloid polypeptide*

Our own work is focused on islet-amyloid polypeptide (IAPP) which is by no means the only potential mechanism of increased  $\beta$ -cell apoptosis in type 2 diabetes. Amyloid – found in the islets of type 2 diabetic patients – is the biological equivalent of plastic: it is abnormal and should not be found there. In the nineteenth century, amyloid pectin was thought to be starch, but it is, in fact, proteinaceous, with a long, fibrillous structure. Amyloid is extra-cellular and, in neuro-degenerative diseases such as Alzheimer's disease, Parkinson's disease, prion disease and Jacob-Kreutzfeld disease, the amyloid is a local depositor of locally-expressed protein. It transpires that IAPP is co-expressed and co-secreted with insulin, so that when you eat your breakfast, you secrete both insulin and IAPP, also known as amylin, and this is secreted in the circulation, ordinarily. Indeed, IAPP and its role in  $\beta$ -cell death is controversial in type 2 diabetes. In contrast, in the neuro-degenerative diseases, there is fairly strong agreement that these local amyloid deposits are a cause of such diseases.

Looking at the sequence of IAPP, it is interesting to note that humans, monkeys, cats, mice and rats have identity in the carboxy- and the amino-terminal regions. However, there are also quite a few divergents in the sequence. The reason why IAPP forms fibres, or oligomers, is because of the region in the sequence that is hydrophobic – it is 'sticky' and allows the peptide to stick to itself. In this respect, humans, monkeys and cats have a very similar sequence and these are the species that develop diabetes. Therefore, if you study monkeys or wild cats, a proportion of them will have type 2 diabetes. In contrast, wild-type rats and mice do not develop diabetes, it is created in them by humans. This suggests that if you do not have hydrophobic motifs in this peptide, you will not develop type 2 diabetes. I think this is quite compelling evidence and must be something to do with the process. If we return to the amyloid situated outside of the cell, one of the questions that arises is, where did the amyloid form? Was it originated inside the cells and then deposited outside, or is it formed outside of the cells?

We suggested in 1994 that initially, a material seems to form intracellularly, and this apparently has some effect on local membranes. This area is ripe for transgenic technology, where it is possible to take a gene from the one group and place it in the other group to observe whether disease develops as a result. We studied transgenic mice and rats (the so-called HIP rat) to see what happens if we put the gene for human IAPP into a HIP rat at a high expression level. We found that the rat developed the extra-cellular islet amyloid, lost  $\beta$ -cell mass and exhibited a high rate of  $\beta$ -cell

apoptosis. In contrast with humans, we have been able to take samples of pancreas from these rodents over time. By doing this we can observe the disease evolving and potentially carry out intervention studies to see what happens in terms of preventing the progression. This is potentially a powerful tool. However, there is a conundrum – the studies showed  $\beta$ -cells surrounded by amyloid but still in existence. So, if you try to argue the case that amyloid is killing the cells, how come these cells are sitting there without any apparent problem? Studies have shown that if you make amyloid, taking IAPP in a test tube, it does not do a thing to  $\beta$ -cells; you get photographs of cells wearing amyloid; it does not hurt them. What is interesting is, if you take IAPP and you put it into the same cells when it is freshly dissolved, when you return the next day, the cells are dead. This hinted that there may be something that is not monomeric IAPP, and that an amyloid exists somewhere between these two, which is perhaps toxic. Research carried out by Charley Glabe's group at the University of California, Irvine,<sup>3</sup> showed that they had achieved a breakthrough. Glabe's group were finally able to synthesize a specific structure, the toxic oligomers, and what was fascinating was their identical structure whether you make them from IAPP or Alzheimer beta protein or synucleins. All these amyloidogenic proteins have the propensity to form a certain kind of toxic oligomer, which, it transpires, is membrane perforating. They enter membranes and serve as non-selective ion channels. This causes a leak in the membrane and appears to be the mechanism by which the cells are probably killed.

### **Endoplasmic reticulum stress**

How can we explain the increasing apoptosis we see in type 2 diabetes, which is shown as one or two cells disappearing over a period of weeks or months, but not a catastrophic disappearance? A current 'hot topic' is ER stress. IAPP and insulin are produced and mix in the ER – this is a remarkable organelle as the concentration of protein here is extremely high. However, protein does not precipitate in the ER. Recent data relating to  $\beta$ -cells in type 2 diabetes suggest that, for reasons which are still unclear, IAPP begins to oligomerize in the ER. This research shows that the chaperone proteins are failing – possibly because of genetic changes in the chaperone protein polymorphisms or problems in other components of the folding – and the oligomers that form these toxic oligomers are getting into the secretory vesicles. This causes the ER to leak calcium and, if calcium is leaked, this will very quickly induce the ER stress pathway of caspase-4 and -12 in humans and rodents, respectively. Interestingly, it appears that these oligomers are getting into the membranes of the mitochondria and, together with the release of local calcium, this will collapse the mitochondrial membrane potential which, in turn, will induce apoptosis. This is the underlying role of IAPP, at least in relation to inducing ER stress and cell death.

It appears that ER stress is the underlying factor in  $\beta$ -cell apoptosis in type 2 diabetes. However, it should be noted that studies also show that cytokines deplete ER calcium and can in turn cause ER stress and that fatty acids and the mitochondrial dysfunction pathway have also been shown to induce ER stress.

So it seems that ER stress will, in fact, be induced by almost anything that makes the  $\beta$ -cells 'unhappy'. Regarding the type 2 diabetes islet specifically, there is a deficit in  $\beta$ -cell mass in patients with type 2 diabetes – this is not the patient's fault, they have a genetic problem which, for some reason, results in the loss of  $\beta$ -cells because of increased apoptosis. There are multiple potential causes. One cause may relate not to this extra-cellular amyloid, which we think is irrelevant, but to the precursor of this; some small toxic oligomers that form in the ER, and are, in fact, causing loss of cells. This is highly analogous to what is happening in the brain of patients with Alzheimer's disease, Parkinson's disease and prion disease, and there is a considerable overlap in the underlying mechanism. ER stress will increase if the ER has to work harder, so  $\beta$ -cell rest – whether through basal insulin, insulin synthesis or lifestyle changes – would be a rational approach to try and unburden the ER.

### **Diabetes prevention and reversal: are there any fixes?**

Diabetes prevention is already possible for type 2 diabetes. In this commentary we suggest that the chosen strategy should attempt to prevent increased apoptosis. In type 2 diabetes, the implication is that anything which unburdens the ER is likely to help, as shown in the Buchanan study<sup>4</sup> with troglitazone in pregnant women, and studies of lifestyle improvements carried out in Scandinavia.<sup>5</sup> Studies with metformin and other new glitazone studies have all shown success, at least in delaying the onset of type 2 diabetes. I favour basal insulin treatment and, in

early-onset type 2 diabetes, a bedtime insulin shot will unburden the ER. I think that insulin is probably a very good anti-apoptotic drug and that this option should be addressed directly.

What about the reversal of diabetes? Can we make this decreased  $\beta$ -cell mass reappear? This would presumably require both decreasing whatever induces apoptosis and either allowing endogenous regeneration to repair the damage or perhaps fostering this. However, we do not yet have an idea of just what capacity there is for this in adult humans.

Finally, the alternatives that everybody is talking about include islet transplantation – which has not been as successful as was hoped for – and bone marrow transplantation. Studies carried out following the deaths of female recipients of male bone marrow transplants did not find any  $\beta$ -cells in the pancreases of these patients and this would suggest that, in humans at least, there does not seem to be any bone marrow cell conversion to  $\beta$ -cells. I would, therefore, suggest that you treat all these alternatives with a degree of scepticism.

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Steno

