HIGHLIGHT

Beta-cell apoptosis in the pathogenesis of human type 2 diabetes mellitusOlga Leonardi¹, Gregory Mints¹ and Mehboob A Hussain^{1,2}*Departments of ¹Medicine and ²Pharmacology, New York University School of Medicine, New York, USA**(Correspondence should be addressed to M A Hussain, 550 First Avenue MSB 424, New York, New York 10016, USA; Email: hussam02@popmail.med.nyu.edu)*

Type 2 diabetes is accompanied by chronic insulin resistance and a progressive decline in β -cell function (1). Obesity is a major risk factor for the development of type 2 diabetes (2, 3) and is thought to confer increased risk for type 2 diabetes through obesity-associated insulin resistance (4). However, most people who are obese do not develop diabetes but compensate their relative insulin resistance by increasing insulin secretion (5). In rodent models of obesity without diabetes there is (as opposed to non-obese littermates) an adaptive increase in β -cell mass to meet metabolic demands (6). Although not many data are available, studies suggest that β -cell mass is also adaptively increased in non-diabetic obese humans (7, 8). β -cell mass is regulated by a balance of β -cell replication and apoptosis, as well as development of new islets from exocrine pancreatic ducts (neogenesis) (9, 10). Disruption of any of the pathways of β -cell formation or increased rates of β -cell death would result in decreased β -cell mass and thus reduced capacity to produce insulin. There is controversy whether β -cell mass is decreased in type 2 diabetes mellitus (8, 11–17). These discrepancies are in part due to the paucity of available data in humans. Furthermore, it is controversial whether β -cell apoptosis is truly increased in type 2 diabetes.

A recent (18) very carefully conducted study gives new and convincing data indicating that increased apoptosis rather than decreased neogenesis or replication may be the main mechanism leading to reduced β -cell mass in type 2 diabetics. Autopsy material from obese patients with diabetes or with impaired fasting glucose (IFG) or without diabetes as well as from lean patients was examined. The authors report that obesity in non-diabetic humans is accompanied by a 50% increase in relative β -cell volume as compared with lean non-diabetic humans. However, the non-diabetic obese humans died younger than the non-diabetic lean humans and the difference found on autopsy may be due to difference in age. Obese humans with IFG and type 2 diabetes had a respective 40 and 63% deficit in relative β -cell volume. The decreased β -cell volume in patients with type 2 diabetes was due to a reduced number of β -cells rather than a smaller volume of individual cells and occurred irrespectively

of whether they were treated with diet alone or oral medications or insulin. There was no difference in the mean fasting plasma glucose levels among these three treatment groups. Lean subjects with type 2 diabetes had a 41% deficit in relative β -cell volume compared with lean non-diabetic subjects. These findings are consistent with other carefully conducted recent studies in which β -cell mass is decreased in type 2 diabetes (8, 15, 17). Neogenesis, while increased in obesity, was comparable in all groups. Decreased β -cell replication was found with aging (18).

Because new islet formation, the predominant input into the β -cell mass, appears intact in type 2 diabetics (18), the mechanism for the decreased β -cell mass would have to be increased β -cell apoptosis. Indeed, a significantly increased frequency of apoptotic events were detected in lean type 2 diabetic vs non-diabetic cases. When normalized to the β -cell volume, the frequency of apoptosis was 3-fold higher in obese cases of type 2 diabetes and 10-fold higher in lean cases of type 2 diabetics as compared with their controls.

Thus, relative β -cell volume and therefore β -cell mass is decreased in both obese and lean humans with type 2 diabetes compared with non-diabetic age- and weight-matched controls. The fact that patients with IFG, a risk group for developing diabetes, had a 40% deficit in relative β -cell volume indicates that loss of β -cells is an early process in the pathogenesis of diabetes mellitus (18).

Once β -cell mass decreases below a critical level and insulin production no longer meets metabolic demands we have hyperglycemia. Does a 60% decrease in β -cell mass – as found in this study – translate into impaired glucose metabolism? Humans who have undergone 50% pancreatectomy have impaired glucose tolerance and insulin secretion in response to a hyperglycemic clamp (19–23). On this basis we may assume that a 60% reduction in β -cell mass in the face of insulin resistance may be sufficient to result in hyperglycemia.

What are the mechanisms for the increased apoptosis found in the islets of type 2 diabetics? The islet in type 2 diabetes is characterized by deposits of polypeptide (IAPP) (15, 24–29). This peptide causes apoptosis of β -cells (30, 31), particularly when it is in

the form of small IAPP oligomers (32). In addition, both glucotoxicity and lipotoxicity cause β -cell apoptosis. The molecular mechanisms behind gluco- and lipotoxic apoptosis in the human β -cell have recently been further elucidated.

A series of studies (33–36) report on the roles of high glucose concentrations and different free fatty acids (FFAs) on β -cell proliferation, apoptosis and function in cultured human islets. The results showed that prolonged exposure of cultured human islets to high glucose levels increased β -cell apoptosis in a dose-dependent manner. In addition, chronic exposure of cultured human islets to the saturated fatty acid palmitic acid results in increased markers of β -cell apoptosis and decreased β -cell proliferation. However, the mono-unsaturated fatty acids palmitoleic acid and oleic acid did not affect DNA fragmentation and induced β -cell proliferation. Moreover when co-supplemented, each of the monounsaturated fatty acids prevented apoptosis, prevented impairment of β -cell proliferation and improved insulin secretion that was caused by palmitic acid and/or hyperglycemia.

Federici *et al.* (37) have demonstrated that cultures of human pancreatic islets exposed to elevated glucose levels showed overexpression of proapoptotic genes Bad, Bid and Bik, while the expression of anti-apoptotic gene Bcl-2 was unaffected, suggesting modulation of the balance towards apoptosis and β -cell death. β -cells produce and release interleukin (IL)-1 β in response to chronic exposure to hyperglycemia (33). This subsequently results in activation of an apoptotic pathway (NF- κ B activation, FAS up-regulation, DNA fragmentation) which is prevented by an IL-1 receptor antagonist. Moreover, IL-1 β -producing β -cells are reported in pancreatic sections of type 2 diabetic patients but not in normal control pancreata (33).

Chronic exposure to elevated levels of FFA leads to lipid overload of pancreatic cells, dysregulated insulin secretion (38, 39) and apoptotic cell death (36, 40–42). FFA-induced apoptosis and reduced β -cell proliferation capacity were observed in rodent (36, 41) and human pancreatic islets (40). Studies demonstrate that lipotoxicity is attributed to accumulation of saturated fatty acids, and not associated with exposure to unsaturated fatty acids (36, 43, 44).

Apart from the deleterious effects of saturated fatty acids on the pancreatic β -cell, elevated serum FFAs contribute to the pathogenesis of the metabolic syndrome and heart disease. While adipocytes store excess fatty acids in the form of triglyceride in lipid droplets, non-adipose tissues have a limited capacity for storage of lipids. In hyperlipidemic states, accumulation of excess lipid in non-adipose tissues leads to cell dysfunction and/or cell death. This lipotoxicity appears to be specific for saturated fatty acids in several tissues studied and is ameliorated by unsaturated fatty acids (36, 43–46).

How should we understand this difference in the effects of fatty acids? In their recent work Listenberger *et al.* (47) provide evidence that exogenous or endogenously generated unsaturated FFAs induce incorporation of saturated fatty acids into triglycerides, thus diverting saturated fatty acids from pathways that can lead to β -cell apoptosis. In their cell culture system (47), oleic acid does not affect uptake and accumulation of palmitic acid. However, the intracellular fate of the saturated palmitic acid is altered with co-supplemented oleic acid. The presence of oleic acid resulted in incorporation of palmitate to triglyceride stores, while absence of oleic acid showed no channeling of palmitate into triglyceride stores. Appropriate controls ensured that this effect was not simply due to increased total fatty acids in the media available to cells. Moreover, cells derived from mice lacking the enzyme for the final step of triglyceride synthesis (acyl CoA:diacylglycerol transferase 1 knockout mice (48)), fail to accumulate exogenously applied fatty acid and are more sensitive to fatty acid (saturated or not)-induced cell death as compared with controls. Thus it appears that the ability to synthesize triglyceride plays an important role in the protection from lipotoxicity. Triglyceride accumulation in response to increased cellular levels of unsaturated fatty acids may be a general metabolic phenomenon. Listenberger *et al.* (47) hypothesize that palmitate channeled toward triglyceride storage may be unavailable for pathways leading to cell death, such as the generation of reactive intermediates and ceramide.

While we have possible mechanisms to explain gluco- and lipotoxicity of β -cell apoptosis, it remains unclear what underlies the apoptotic loss of β -cells before we have frank hyperglycemia and/or hyperlipidemia. The occurrence of apoptosis in patients with IFG suggests an inherent defect in the β -cells of people who subsequently develop type 2 diabetes mellitus. A possibility for slowing the progression or even preventing type 2 diabetes may be to develop dietary and pharmacological strategies aiming at ameliorating increased β -cell apoptosis in people with a defined high risk for developing type 2 diabetes.

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