Glucolipotoxicity of the pancreatic beta-cell: Myth or reality?

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Abstract

The glucolipotoxicity hypothesis postulates that chronically elevated levels of glucose and fatty acids adversely affect pancreatic beta-cell function and thereby contribute to the deterioration of insulin secretion in type 2 diabetes. Whereas ample experimental evidence in in vitro systems support the glucolipotoxicity hypothesis, the contribution of this phenomenon to beta-cell dysfunction in human type 2 diabetes has been questioned. The reasons for this controversy include differences between in vitro systems and in vivo situations; time-dependent effects of fatty acids on insulin secretion (acutely stimulatory and chronically inhibitory); and the ill-defined use of the suffix “-toxicity”. In vitro, prolonged exposure of insulin-secreting cells or isolated islets to concomitantly elevated levels of fatty acids and glucose impair insulin secretion, inhibit insulin gene expression and, under certain circumstances, induce beta-cell death by apoptosis. Recent studies in our laboratory have shown that cyclical and alternate infusions of glucose and Intralipid in rats impair insulin gene expression, providing evidence that inhibition of the insulin gene under glucolipotoxic conditions is an early defect that might indeed contribute to beta-cell failure in type 2 diabetes, although this hypothesis remains to be tested in humans.

Keywords

Glucolipotoxicity; Insulin; Beta-cell; Islet; Diabetes

INTRODUCTION

Large-scale prospective trials in humans such as the UKPDS study [1], have shown that beta-cell function deteriorates during the years following the diagnosis of type 2 diabetes. This has led to the concept that the metabolic perturbations associated with type 2 diabetes are themselves deleterious to beta-cell function and, thereby, contribute to its failure. Amongst these, chronic hyperglycemia [2] and hyperlipidemia [3], have been postulated to harm the beta-cell, two phenomena commonly referred to as glucotoxicity and lipotoxicity, respectively. The term glucolipotoxicity was subsequently coined in recognition of the fact that glucotoxicity and lipotoxicity are interrelated and have some common mechanisms of action [4]. Somewhat surprisingly, despite several years of intensive investigations, the

mechanisms of glucolipotoxicity in the pancreatic beta-cell are still only partially understood and often a matter of debate. In addition, the contribution of this phenomenon to beta-cell failure in type 2 diabetes is still uncertain, in part because of the scarcity of studies examining this issue in humans. An additional confounder is the lack of clear consensus on the definition of the term glucolipotoxicity. For the purpose of this discussion, we will define glucolipotoxicity as the deleterious effects of elevated glucose and fatty acids on pancreatic beta-cell function and mass. In this review we will present the evidence in favor of the concept, address some of the questions that have been raised regarding its clinical validity, and hypothesize on the role glucolipotoxicity may play in various stages of beta-cell failure in human type 2 diabetes.

GLUCOLIPOTOXICITY IN THE BETA-CELL: WHAT IS THE EVIDENCE?

In vitro studies

A number of in vitro studies, using insulin-secreting cells and isolated islets, have attempted to identify the mechanisms of glucolipotoxicity. Prolonged exposure of isolated islets or insulin-secreting cells to simultaneously elevated levels of fatty acids and glucose leads to inhibition of glucose-induced insulin secretion, impairment of insulin gene expression, and induction of cell death by apoptosis (reviewed in [5]). Here we will focus on the work performed in our laboratory concerning the inhibition of the insulin gene by fatty-acids, as an example of in vitro studies which were recently extended to in vivo models. Thus, our own work has mainly focused on the mechanisms by which fatty acids, in the presence of elevated glucose, impair insulin gene expression in isolated islets. These studies have shown that the mechanisms whereby fatty acids affect insulin secretion are distinct from those by which they impair insulin gene expression. First, whereas both palmitate and oleate inhibit insulin secretion, only palmitate affects the insulin gene [6]. Since only palmitate, but not oleate, can serve as a substrate for de novo ceramide synthesis, we examined whether ceramide generation mediates palmitate inhibition of the insulin gene. We observed that a 72-h culture of isolated islets in the presence of palmitate is associated with an increase in ceramide content that can be largely blocked by inhibitors of de novo ceramide synthesis, and that inhibition of ceramide generation prevents the decrease in insulin mRNA levels upon exposure to palmitate [7]. Second, palmitate inhibition of insulin mRNA levels is not due to changes in mRNA stability, but to direct inhibition of glucose-induced insulin promoter activity in primary islets [7]. Third, we have observed that palmitate inhibition of insulin gene transcription is due to decreased binding activity of PDX-1 and MafA [8]. PDX-1 appears to be affected in its ability to translocate to the nucleus, whereas MafA is affected at the level of its expression [8]. The mechanisms whereby ceramide generation from palmitate impairs PDX-1 subcellular localization and MafA expression are unknown. Recently, we have examined the contribution of ERK and PKB in palmitate inhibition of insulin gene expression in isolated islets. We have observed that palmitate further augments the activation of ERK1/2 and PKB phosphorylation in response to glucose in isolated rat islets and MIN6 cells. (G. Fontés, V.P., unpublished results). Pharmacological inhibition of ERK1/2, but not that of PKB, partially prevents palmitate inhibition of insulin gene expression. In the same study we have also examined the contribution of PAS kinase, which has been shown to mediate glucose-regulation of insulin gene expression [9]. We observed

that palmitate prevents glucose induction of PAS kinase mRNA and protein expression, and that overexpression of a wild-type PAS kinase isoform totally prevents the inhibition of glucose-induced insulin gene expression by palmitate. These results suggest that PAS kinase, and to a lesser extent ERK1/2, are implicated in the mechanisms by which fatty acids affect the insulin gene.

In vivo studies

Most of the initial studies on glucolipotoxicity in rodent models were performed in the Zucker Diabetic Fatty (ZDF) rat, which harbors the *fa* mutation in the leptin receptor gene and displays marked obesity, insulin resistance, and diabetes (reviewed in [10]). While these experiments were instrumental in establishing the concept of glucolipotoxicity and identifying some of its basic mechanisms, the ZDF model suffers from limitations in that the effects of chronic hyperglycemia (glucotoxicity) are difficult to separate from those of chronic hyperlipidemia (glucolipotoxicity). The *fa* mutation results in defective leptin signaling and therefore perturbs intracellular fatty-acid metabolism, limiting the relevance of this model to human type 2 diabetes in which leptin receptor mutations are extremely rare [11]. In recent years, additional rodent models have been used and have provided important insights into the mechanisms of glucolipotoxicity in vivo. Mason et al. [12] have demonstrated that a 48-h perfusion of Intralipid or oleate impairs glucose-induced insulin secretion in normal rats. The influence of genetic predisposition on the insulin secretory response to excessive fatty acid levels is also illustrated by the recent observation that insulin secretion is impaired to a greater extent in heterozygous lean ZDF rats than in Wistar rats after Intralipid infusion [13]. To examine whether fatty acid-inhibition of insulin gene expression previously observed in isolated islets [7, 8] was also operative in vivo, we have recently performed a study in which normal Wistar rats were infused alternatively with glucose for 4 h and Intralipid + heparin for 4h, for a total of 72 h. In islets isolated at the end of the perfusion from animals infused alternatively with glucose and saline, insulin mRNA levels, PDX-1 nuclear localization, and PDX-1 binding to the endogenous insulin gene promoter were increased. In contrast, in islets from animals infused with glucose and Intralipid, insulin mRNA levels were reduced, PDX-1 localization was shifted towards the cytosol, and occupancy of the endogenous insulin promoter by PDX-1 was markedly diminished [14]. These results demonstrate that fatty acid inhibition of the insulin gene also occurs in vivo. Therefore, in vitro studies have provided important information regarding the molecular and cellular basis of glucolipotoxicity. They have shown that the various functional effects of chronically elevated fatty acids are mediated by distinct mechanisms, and some of the in vitro observations were reproduced in vivo in rodent models.

WHY HAS GLUCOLIPOTOXICITY BEEN CHALLENGED?

As mentioned above, the notion that glucolipotoxicity contributes to beta-cell failure in humans has been challenged, for several reasons.

An imprecise definition

There is no clear agreement on the definition of the term glucolipotoxicity. The root “toxicity” implies some form of cytotoxicity, which is not always observed. In fact, in our

experiments we have studied the functional effects of fatty acids in the absence of any measurable cell death [7]. Since the mechanisms underlying the various functional effects of fatty acids are distinct (as detailed in the above paragraph), it is not surprising that studies evaluating different endpoints (e.g. reduced insulin secretion, impaired insulin gene expression, apoptosis) have come to different – and seemingly discrepant – conclusions.

A second issue that has complicated interpretation of the data is that the term glucolipotoxicity implicitly refers to deleterious effects. This is somewhat paradoxical, since both glucose and fatty acids are, under physiological conditions and upon short-term exposure, stimulatory to beta-cell function. Therefore, in vivo a continuum must exist between the “positive”, stimulatory effects of these nutrients and their “negative”, harmful action on the beta-cell. In addition, it has been shown that after exposure to fatty acids, the glucose response is reduced but the response to fatty acids is enhanced [15]. This switch from glucose to fatty acids as the primary β -cell nutrient might be viewed in this context as an adaptation which should probably not be referred to as “glucolipotoxicity” but rather “glucolipoadaptation” [16], since it may contribute to β -cell compensation for insulin resistance and therefore be beneficial in maintaining normal glucose homeostasis. Clearly, depending on the experimental conditions used, different studies probably examine different stages of this spectrum and therefore yield different results.

Limitations of in vitro studies

The concentrations of fatty acids used in vitro vary amongst publications. The key determinant of fatty acid potency is the fraction that is unbound to bovine serum albumin, which depends on the molar ratio of fatty acids to albumin as well as the mode of preparation. Using a fluorescent probe that specifically measures the unbound fraction of fatty acids [17], we observed that when palmitate at a total concentration of 0.5 mM was pre-complexed to bovine serum albumin with a molar ratio of fatty acid:albumin of 5:1, the unbound concentration is in the 200 nM range (V.P., unpublished data), which represents approximately 3 times the unbound concentration measured in the plasma of lean individuals by the same method [18]. Thus, the concentration of fatty acids should be interpreted in the context of the concomitant albumin concentration. Finally, the concentrations of fatty acids in the vicinity of the β -cells in vivo are unknown and are determined by many different factors, including the activity of lipoprotein lipase, which accounts for some of the local delivery of fatty acids to the cells [19]. Therefore, the results of in vitro experiments using fatty acids should be interpreted with caution, particularly when marked cytotoxicity is observed. In this regard, it should be noted that while fatty-acid induced apoptosis can be shown in in vitro systems, to our knowledge there is no in vivo studies clearly demonstrating a direct effect of elevated circulating fatty acid levels on beta-cell death. The potential contribution of glucolipotoxicity to the decrease in beta-cell mass observed in type 2 diabetes [20] therefore remains to be clearly demonstrated.

Studies in humans

Studies examining the effects of prolonged fatty acids on insulin secretion in humans have led to conflicting results. Initial reports from Boden and colleagues indicated that a 48-h lipid infusion induces an appropriate insulin secretory response in healthy subjects [21] but

is defective in type 2 diabetic patients [22]. In contrast, Carpentier et al. [23] have shown that the increase in insulin secretion observed in response to an acute (90-min) lipid infusion in healthy subjects disappears when the infusion is prolonged to 48h. The loss of insulin secretion is specific to the response to glucose, as the response to arginine remains normal [24]. The same group further showed that obese, but not diabetic, subjects are susceptible to the inhibitory effect of lipids on glucose-induced insulin secretion [25]. Importantly, the increase in insulin secretion observed in non-diabetic subjects in response to a 24-h glucose infusion does not occur if lipids are infused simultaneously with glucose [26]. Finally, the group of Cusi and De Fronzo has carried out a series of studies in non-diabetic subjects with (FH+) and without family history of type 2 diabetes. They showed that a 4-day Intralipid infusion enhances insulin secretion (normalized for insulin sensitivity) in control subjects but inhibits glucose-induced insulin secretion in FH+ individuals [27], suggesting that perhaps part of the genetic predisposition to type 2 diabetes is related to the ability of the β cell to increase insulin secretion in response to elevated fatty acid levels. Importantly, reducing circulating fatty acid levels with Acipimox ameliorates insulin secretion in FH+ subjects [28]. Therefore, studies in humans indicate that prior to the occurrence of type 2 diabetes, the insulin secretory response to nutrient overload is impaired in subjects predisposed to developing the disease. These findings suggest that lipotoxicity is not restricted to the late stages of type 2 diabetes, but may actually play a role in early disease progression. The influence of glucolipotoxicity on beta-cell function after the onset of diabetes is less clear, probably in part because of the inherent difficulties in conducting such long-term studies in humans.

CONCLUSIONS

The physiological or pathological significance of the effects of fatty acids and glucose on pancreatic β -cell function is a matter of debate, and has been further confounded by the various interpretations given to the suffix “-toxicity”. Whereas one can reasonably assert that fatty acid-induced β -cell death is clearly a toxic manifestation, their effects on functional parameters such as insulin secretion or gene expression are more difficult to categorize as either beneficial or deleterious responses in a short time frame, although they are clearly deleterious in the long run. Based on the existing literature, one can hypothesize that excessive glucose and fatty acid levels have different effects at the various stages of beta-cell dysfunction during the course of type 2 diabetes. When insulin resistance develops, for instance as a result of obesity, the β cell mounts a compensatory response which involves coordinated increases in β -cell mass, insulin biosynthesis, and insulin secretion. Although the molecular signals that trigger functional adaptation of the β -cell are unknown, experimental evidence suggests that these may involve an increased response to fatty acids [29, 30]. The magnitude of the compensatory β -cell response is genetically determined [27] and, in turn, is a major determinant of the long-term ability of an individual to maintain glucose homeostasis in the face of insulin resistance. In genetically predisposed individuals, β -cell compensation eventually becomes insufficient and the β cell is no longer able to sustain a secretory response that matches the demand imposed by insulin resistance. At this stage, the effects of glucolipotoxicity probably contribute to the inexorable demise of the β cell. Due to the inherent difficulty in evaluating the effects of prolonged metabolic

perturbations on pancreatic beta-cell function in humans in an experimental setting, the precise contribution of glucolipotoxicity to the pathogenesis of type 2 diabetes remain to be established.

References

1. UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet*. 1998; 352:837–853. [PubMed: 9742976]
2. Unger RH, Grundy S. Hyperglycemia as an inducer as well as a consequence of impaired islet cell function and insulin resistance: implications for the management of diabetes. *Diabetologia*. 1985; 28:119–121. [PubMed: 3888754]
3. Unger RH. Lipotoxicity in the pathogenesis of obesity-dependent NIDDM. Genetic and clinical implications. *Diabetes*. 1995; 44:863–870. [PubMed: 7621989]
4. Prentki M, Joly E, El-Assaad W, Roduit R. Malonyl-CoA signaling, lipid partitioning, and glucolipotoxicity: role in beta-cell adaptation and failure in the etiology of diabetes. *Diabetes*. 2002; 51(Suppl 3):S405–413. [PubMed: 12475783]
5. Poitout V, Robertson RP. Glucolipotoxicity: Fuel Excess and {beta}-Cell Dysfunction. *Endocr Rev*. 2007
6. Moore PC, Ugas MA, Hagman DK, Parazzoli SD, Poitout V. Evidence against the involvement of oxidative stress in Fatty Acid inhibition of insulin secretion. *Diabetes*. 2004; 53:2610–2616. [PubMed: 15448091]
7. Kelpe CL, Moore PC, Parazzoli SD, Wicksteed B, Rhodes CJ, Poitout V. Palmitate inhibition of insulin gene expression is mediated at the transcriptional level via ceramide synthesis. *J Biol Chem*. 2003; 278:30015–30021. [PubMed: 12771145]
8. Hagman DK, Hays LB, Parazzoli SD, Poitout V. Palmitate inhibits insulin gene expression by altering PDX-1 nuclear localization and reducing MafA expression in isolated rat islets of Langerhans. *J Biol Chem*. 2005; 280:32413–32418. [PubMed: 15944145]
9. da Silva Xavier G, Rutter J, Rutter GA. Involvement of Per-Arnt-Sim (PAS) kinase in the stimulation of preproinsulin and pancreatic duodenum homeobox 1 gene expression by glucose. *Proc Natl Acad Sci U S A*. 2004; 101:8319–8324. [PubMed: 15148392]
10. Unger RH. Minireview: weapons of lean body mass destruction: the role of ectopic lipids in the metabolic syndrome. *Endocrinology*. 2003; 144:5159–5165. [PubMed: 12960011]
11. Clément K, Vaisse C, Lahlou N, Cabrol S, Pelloux V, Cassuto D, Gormelen M, Dina C, Chambaz J, Lacorte JM, Basdevant A, Bougneres P, Lebouc Y, Froguel P, Guy-Gtand B. A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature*. 1998; 392:398–401. [PubMed: 9537324]
12. Mason TM, Goh T, Tchepashvili V, Sandhu H, Gupta N, Lewis GF, Giacca A. Prolonged elevation of plasma free fatty acids desensitizes the insulin secretory response to glucose in vivo in rats. *Diabetes*. 1999; 48:524–530. [PubMed: 10078552]
13. Goh TT, Mason TM, Gupta N, So A, Lam TK, Lam L, Lewis GF, Mari A, Giacca A. Lipid-induced beta-cell dysfunction in vivo in models of progressive beta-cell failure. *Am J Physiol Endocrinol Metab*. 2007; 292:E549–560. [PubMed: 17003242]
14. Hagman DK, Latour MG, Chakrabarti SK, Fontes G, Amyot J, Tremblay C, Semache M, Lausier JA, Roskens V, Mirmira RG, Jetton TL, Poitout V. Cyclical and alternating infusions of glucose and intralipid in rats inhibit insulin gene expression and Pdx-1 binding in islets. *Diabetes*. 2008; 57:424–431. [PubMed: 17991758]
15. Busch AK, Cordery D, Denyer GS, Biden TJ. Expression profiling of palmitate- and oleate-regulated genes provides novel insights into the effects of chronic lipid exposure on pancreatic beta-cell function. *Diabetes*. 2002; 51:977–987. [PubMed: 11916915]
16. Prentki M, Nolan CJ. Islet beta cell failure in type 2 diabetes. *J Clin Invest*. 2006; 116:1802–1812. [PubMed: 16823478]

17. Richieri GV, Anel A, Kleinfeld AM. Interactions of long-chain fatty acids and albumin: determination of free fatty acid levels using the fluorescent probe ADIFAB. *Biochemistry*. 1993; 32:7574–7580. [PubMed: 8338853]
18. Richieri GV, Kleinfeld AM. Unbound free fatty acid levels in human serum. *J Lipid Res*. 1995; 36:229–240. [PubMed: 7751810]
19. Cruz WS, Kwon G, Marshall CA, McDaniel ML, Semenkovich CF. Glucose and insulin stimulate heparin-releasable lipoprotein lipase activity in mouse islets and INS-1 cells. A potential link between insulin resistance and beta-cell dysfunction. *J Biol Chem*. 2001; 276:12162–12168. [PubMed: 11154699]
20. Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC. Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. *Diabetes*. 2003; 52:102–110. [PubMed: 12502499]
21. Boden G, Chen X, Rosner J, Barton M. Effects of a 48h fat infusion on insulin secretion and glucose utilization. *Diabetes*. 1995; 44:1239–1242. [PubMed: 7556964]
22. Boden G, Chen X. Effects of fatty acids and ketone bodies on basal insulin secretion in type 2 diabetes. *Diabetes*. 1999; 48:577–583. [PubMed: 10078559]
23. Carpentier A, Mittelman S, Lamarche B, Bergman R, Giacca A, Lewis G. Acute enhancement of insulin secretion by FFA in humans is lost with prolonged FFA elevation. *Am J Physiol*. 1999; 276.
24. Carpentier A, Giacca A, Lewis GF. Effect of increased plasma non-esterified fatty acids (NEFAs) on arginine-stimulated insulin secretion in obese humans. *Diabetologia*. 2001; 44:1989–1997. [PubMed: 11719829]
25. Carpentier A, Mittelman SD, Bergman RN, Giacca A, Lewis GF. Prolonged elevation of plasma free fatty acids impairs pancreatic beta-cell function in obese nondiabetic humans but not in individuals with type 2 diabetes. *Diabetes*. 2000; 49:399–408. [PubMed: 10868961]
26. Leung N, Sakaue T, Carpentier A, Uffelman K, Giacca A, Lewis GF. Prolonged increase of plasma non-esterified fatty acids fully abolishes the stimulatory effect of 24 hours of moderate hyperglycaemia on insulin sensitivity and pancreatic beta-cell function in obese men. *Diabetologia*. 2004; 47:204–213. [PubMed: 14712348]
27. Kashyap S, Belfort R, Gastaldelli A, Pratipanawatr T, Berria R, Pratipanawatr W, Bajaj M, Mandarino L, DeFronzo R, Cusi K. A sustained increase in plasma free fatty acids impairs insulin secretion in nondiabetic subjects genetically predisposed to develop type 2 diabetes. *Diabetes*. 2003; 52:2461–2474. [PubMed: 14514628]
28. Cusi K, Kashyap S, Gastaldelli A, Bajaj M, Cersosimo E. Effects on insulin secretion and insulin action of a 48-h reduction of plasma free fatty acids with acipimox in nondiabetic subjects genetically predisposed to type 2 diabetes. *Am J Physiol Endocrinol Metab*. 2007; 292:E1775–1781. [PubMed: 17299078]
29. Fex M, Nitert MD, Wierup N, Sundler F, Ling C, Mulder H. Enhanced mitochondrial metabolism may account for the adaptation to insulin resistance in islets from C57BL/6J mice fed a high-fat diet. *Diabetologia*. 2006; 50:74–83. [PubMed: 17093947]
30. Nolan CJ, Leahy JL, Delghingaro-Augusto V, Moibi J, Soni K, Peyot ML, Fortier M, Guay C, Lamontagne J, Barbeau A, Przybytkowski E, Joly E, Masiello P, Wang S, Mitchell GA, Prentki M. Beta cell compensation for insulin resistance in Zucker fatty rats: increased lipolysis and fatty acid signalling. *Diabetologia*. 2006; 49:2120–2130. [PubMed: 16868750]