Cell Metabolism
Perspective

A New Biology of Diabetes Revealed by Leptin

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A variety of leptin actions require a re-examination of classic concepts of metabolic diseases. Here we present evidence for two physiologic pathways: a pathway that protects nonadipose tissues from overaccumulation of potentially toxic lipids and unrecognized paracrine interactions between α and β cells revealed by leptin's ability to suppress diabetic hyperglucagonemia. These observations strongly point to new therapeutic possibilities for both type 1 and type 2 diabetes.

Introduction

The discovery of leptin two decades ago has radically altered biologic perspectives of diabetes and metabolic diseases. Initially considered an antiobesity hormone, leptin was subsequently proposed to protect nonadipose tissues, such as liver, endocrine pancreas, and heart, from the lipotoxicity often associated with diet-induced obesity (DIO). Indeed, whenever leptin is congenitally absent or its action defective, a phenotype of lipotoxic disease of these organs appears.

Here, we review two areas in which leptin actions have forced revision or replacement of classic views of metabolic disease. First, we consider a physiologic antilipotoxic connection between leptin and nonadipose tissues; second, we examine physiologic and pharmacologic interactions between α and β cells uncovered through leptin's suppression of diabetic hyperglucagonemia; and third, we apply these new insights to improving treatment of diabetes.

Antilipotoxic Physiology for Leptin

Evolutionary Teleology of Leptin

The untenability of the belief that leptin was an antiobesity hormone left the adipokine without an accepted physiologic role. The fact that DIO is the only chronic upregulator of leptin expression and secretion (Considine et al., 1996; Mizuno et al., 1996) suggested that its physiologic role might be to prevent some adverse consequence of overnutrition (Lee et al., 2001). Indeed, the fact that leptin upregulates AMP kinase (Minokoshi et al., 2002; Steinberg et al., 2003), UCP1 (Scarpace et al., 1997), and enzymes of fatty acid (FA) oxidation (Suzuki et al., 2007) was consistent with a role in enhancing uncoupled FA oxidation of excess FAs (Shimabukuro et al., 1997). This role was supported dramatically by the fact that, in mice and rats with congenital lack of leptin action, ectopic lipid accumulation is much greater on a 6% fat diet than in normal rodents fed a 60% diet (Lee et al., 2001). Similarly, lipotoxicity is minimal in wild-type mice on a 60% fat diet, whereas it is a severe life-shortening clinical problem in leptin-deficient animals even on a 6% fat intake. This suggests that a physiologic role of leptin is to protect nonadipose tissues from lipotoxicity by minimizing ectopic lipid accumulation during periods of overnutrition and increasing caloric storage required to survive famine (Neel, 1962).

Evidence that Hyperleptinemia Prevents Lipotoxicity

To test the foregoing concept that leptin's physiologic role could be to protect nonadipose organs against lipotoxicity, we studied mice with heart-specific lipotoxicity resulting from transgenic expression of an acyl-CoA synthase gene controlled by the myosin heavy-chain promotor (Chiu et al., 2001). The phenotype consisted of severe dilated cardiomyopathy with 100% mortality within 100 days. Electron microscopy revealed cardiomyocytes filled with fat droplets (Lee et al., 2004). The dilated hearts were fibrotic. TUNEL staining provided evidence of ongoing lipoapoptosis (Chiu et al., 2001).

To test the premise that a physiologic role of diet-induced hyperleptinemia is to prevent lipotoxicity, we induced hyperleptinemia in the acyl-CoA synthase-MHC transgenic mice by injecting them with adenovirus containing either the leptin cDNA or a β-galactosidase cDNA as a control. In the hyperleptinemic mice, lipotoxic cardiomyopathy was completely prevented and the cardiomyocytes were devoid of fat droplets. Instead, they contained myriads of small mitochondria (Lee et al., 2004). All hyperleptinemic mice survived beyond 90 days. Thus, hyperleptinemia completely blocked lipotoxicity in the heart.

To determine if hyperleptinemia can also block lipotoxicity in the endocrine pancreas, we isolated islets from normal rats and injected them into the portal vein of closely related diabetic recipients. The recipients had been made insulin deficient by previous treatment with streptozotocin (STZ) (100 mg/kg). The rationale was to induce steatosis in hepatocytes surrounding the islet transplants by exposing them to undiluted insulin from β cells. The high local insulin should upregulate the lipogenic transcription factor SREBP1c and its target genes in the hepatocytes, stimulating lipid production (Horton et al., 2002; Shimomura et al., 1999). Because islets secrete lipoprotein lipase (Marshall et al., 1999), triglycerides (TGs) would be hydrolyzed, exposing the islets to high FA levels, which should cause lipotoxicity. If lipotoxicity appeared, the rats would be made hyperleptinemic to test leptin's antilipotoxic activity in islets. This design might "cure" the chemically induced T1D in the recipient, but induce T2D in the donor islet transplants that cured the T1D.

Immunostaining of hepatocytes revealed SREBP1-positive hepatocytes surrounding each islet transplant (Lee et al., 2007). Oil-red O staining was also positive in hepatocytes

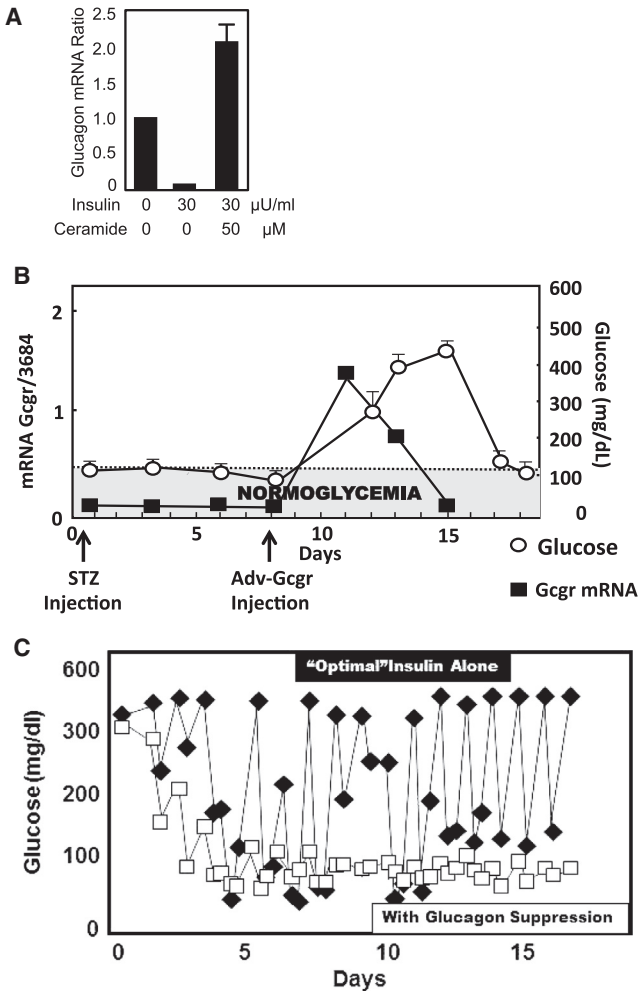

Figure 1. Evidence for New Pathophysiologic and Therapeutic Concepts in Diabetes

(A) In the absence of ceramide, 30 units of insulin cause marked downregulation of glucagon mRNA in cultured α cells. The presence of 50 μM ceramide completely prevents the downregulation by insulin.

(B) In glucagon receptor knockout (*GcgR*$^{-/-}$) mice, complete deficiency of insulin caused by high dose streptozotocin (STZ) administration does not cause hyperglycemia or any apparent clinical or metabolic abnormality (days 1–8). However, when adenovirus containing the glucagon receptor cDNA is injected, causing the glucagon receptor mRNA to appear in the liver, blood glucose rises near 500 mg/dl. When the transgenic glucagon receptor has disappeared from the liver (day 15), the hyperglycemia also subsequently disappears. These results show that the metabolic defects of diabetes are not caused directly by lack of insulin but rather require hyperglucagonemia caused by failure of paracrine insulin to suppress glucagon levels.

(C) Comparison of glucose profiles of insulin-deficient T1D NOD mice treated with "optimal" insulin replacement (0.2 U/day, black squares) or with glucagon suppression with continuous infusion of metroleptin (20 μg/hr, open squares). Figures adapted from Lee et al., 2012 and Wang et al., 2010.

surrounding the islet transplants. We next compared the TG content of liver and the area of insulin-positive cells in the transplants in rats treated with adenovirus containing leptin or β-galactosidase cDNA. The administration of adenovirus containing leptin cDNA reduced liver TG content by 71% below the β-galactosidase controls. It was also accompanied by a 20-fold increase in the area of surviving insulin-positive β cells. If, as we postulate, the low survival of the untreated control β cells was due to lipotoxicity in the islet transplants, we could conclude that hyperleptinemia effectively prevented islet lipotoxicity and T2D in the transplanted β cells.

Leptin as a Physiologic Suppressor of SPT and Ceramide Synthesis

A more specific physiologic antilipotoxic role for leptin is suggested by the fact that hyperleptinemia downregulates the expression and activity of serine palmitoyl transferase (SPT) (Shimabukuro et al., 1998a). SPT catalyzes condensation of serine and palmitoyl CoA (Weiss and Stoffel, 1997) and is the rate-limiting enzyme of ceramide biosynthesis (Merrill, 2002). Ceramide has been implicated in both β cell lipoapoptosis (Shimabukuro et al., 1998b) and in insulin resistance (Chavez and Summers, 2012; Holland et al., 2007a, 2007b; Holland and Summers, 2008; Summers et al., 1998; Zhou et al., 1998). Evidence that patients with T2D have insulin-resistant hyperglucagonemia was first reported in 1975 (Raskin et al., 1975, 1976). Ceramide can cause resistance to insulin-mediated suppression of glucagon expression in cultured hamster α cells (Figure 1A). The combination of insulin resistance in α cells plus lipotoxic impairment of β cell function is a perfect pathophysiologic recipe for T2D. Based on these findings, we suggest that a physiologic role of leptin is to restrain ceramide formation in the endocrine pancreas. This leptin action would prevent or delay the ceramide-induced lipotoxic impairment of β and α cells that can cause T2D.

Although beyond the scope of this brief review, it is well established that certain metabolic actions of leptin on hepatocytes and adipocytes are mediated via the hypothalamus (Fujikawa et. al., 2013; Perry et. al., 2014).

Physiologic and Pharmacologic α-β Cell Interactions

Proving the Bihormonal Partnership in Glucose Homeostasis

Ever since its introduction in 1922, insulin has been accepted as the essential master regulator of metabolism, without which life is impossible. New evidence, much of it obtained by suppressing glucagon with leptin, now indicates that most metabolic actions of insulin are indirect and are mediated by glucagon. Moreover, in rodents a normal and healthy lifespan can exist in the total absence of insulin—provided glucagon action is suppressed.

These stunning observations were first uncovered through leptin suppression of glucagon in insulin-deficient rodents (Yu et al., 2008). However, the most incontrovertible evidence was obtained in glucagon receptor knockout (*GcgR*$^{-/-}$) mice (Gelling et al., 2003), provided by Maureen Charron of Albert Einstein School of Medicine. *GcgR*$^{-/-}$ mice with complete insulin deficiency induced by STZ remained normoglycemic and thrived for almost one year, despite the lack of detectable plasma levels of insulin and C-peptide. Because β cells of *GcgR*$^{-/-}$ mice are resistant to destruction by STZ (Omar et al., 2014), they received two or three times the STZ dose required for wild-type controls, which caused severe hyperglycemia, ketonemia, and death within 4 weeks (Lee et al., 2012).

To determine if transgenic expression of GcgR in the liver of the insulin-deficient *GcgR*$^{-/-}$ mice would make them as diabetic as the wild-type controls, we administered adenovirus containing either the GcgR or β-galactosidase cDNA. Two days after expressing GcgR mRNA in their liver, the *GcgR*$^{-/-}$ mice developed

Table 1. Comparison of Concentrations of Acutely Secreted Endogenous and Injected Exogenous Insulin Estimated Reaching Its Target Tissues

Tissue	Secreted Insulin	Injected Insulin
Islets	Rat: ~2,000–4,000 μU	~10 μU
Portal vein	Acute, human: ~100–500 μU	~10 μU
	Basal, dog: 21 μU	
Peripheral vein	Human: ~9–20 μU	~10 μU
	Dog: ~5–7 μU	

The islet insulin values were calculated from the maximal binding capacity of neutralizing antiserum perfused into normal rat pancreata to assess insulin's paracrine action on α cells (Maruyama et al., 1984). Values in the portal vein for acute glucose challenge in humans and basal concentration in dog are from Blackard and Nelson, 1970 and Sindelar et al., 1998, respectively. Values for human and dog peripheral vein are taken from Blackard and Nelson, 1970; Sindelar et al., 1998; and Moore et al., 2014. Values for plasma insulin injected at 0.11 nmol/kg/hr are from Moore et al., 2014. The results explain why no dose of injected insulin can mimic the stable glucoregulation maintained by the target-specific concentration gradients of secreted insulin.

severe hyperglycemia approaching 500 mg/dl (Figure 1B), accompanied by a robust increase in phosphorylated cAMP response element binding (CREB) protein and phosphoenolpyruvate carboxy kinase (PEPCK) protein in their liver. The transgenic GcgR mRNA disappeared spontaneously after 7 days, followed by disappearance of the hyperglycemia and accompanying metabolic abnormalities (Figure 1B).

These results indicate that the hyperglycemia and hypercatabolism of insulin deficiency are not directly due to lack of insulin, but rather are caused by glucagon excess resulting from deficiency of paracrine insulin (Lee et al., 2012). They support the idea, first proposed in 1975 (Unger, 1976), that β and α cells work as partners. The β cells sense glycemic change and deliver insulin via their paracrine connection to neighboring α cells to regulate glucagon release. This relationship precisely tailors the mix of the two opposing hormones to adjust hepatic glucose balance to meet the metabolic needs of the moment (Cherrington, 1999; Exton et al., 1970).

Why Insulin Monotherapy in T1D Cannot Normalize Glucose Homeostasis

In 1922 insulin therapy first converted uniformly fatal type 1 diabetes (T1D) into a condition compatible with years of near-normal life. But replacement of the deficient insulin did not restore the metabolic manifestations of the deficiency to normal or eliminate the late complications of the disease. Indeed, it is now clear that in T1D patients injected insulin can never duplicate the stable glucoregulatory homeostasis provided by secreted insulin in nondiabetic individuals. This is because injected insulin can never duplicate the concentration gradients produced by secreted insulin at target organs of nondiabetic subjects (Table 1).

The journey of secreted insulin begins with a paracrine mission to the juxtaposed α cells. The approximation of this paracrine insulin concentration presented in Table 1 is based on the maximal binding of the anti-insulin serum and the rate of its perfusion required to produce hyperglucagonemia (Maruyama et al., 1984). Portal vein and peripheral arterial insulin levels are based on direct measurements in human and dog (Blackard and Nelson, 1970; Moore et al., 2014), suggesting that glucagon suppression requires ~2,000–4,000 μU/ml per minute in the islet circulation. Even if this is an overestimate, the fact that insulin is virtually undiluted when it reaches α cells means that these islet cells receive, by far, the highest concentration of insulin anywhere in the body. Insulin reaches the liver in diluted form, estimated to be between ~100–500 μU/ml. More accurate measurements have been made by Moore (Moore et al., 2014) and Edgerton (Edgerton et al., 2006). Insulin then undergoes further dilution as it flows through the liver to the adipocytes and skeletal muscle, where it circulates at between 9 and 20 μU/ml (Edgerton et al., 2006). These dilutions are the consequence of the circulatory anatomy and cannot be duplicated by injected exogenous insulin.

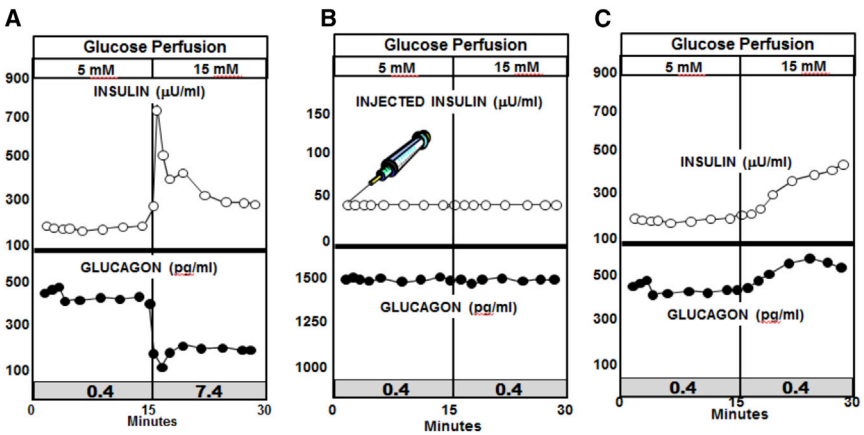

Figure 2. The α/β Cell Partnership in the Perfused Rat Pancreas

(A) Normally the insulin/glucagon response to glucose maintains tight control of glucose homeostasis in nondiabetic mammals. The bihormonal response is expressed as an insulin/glucagon ratio in the shaded zone at the base of each panel. Insulin and glucagon are released from juxtaposed cells. Insulin is a potent suppressor of glucagon. When blood glucose rises, insulin secretion increases sharply, particularly for 2–4 min. The spike of insulin is a powerful suppressor of glucagon from juxtaposed α cells. After the first few minutes, insulin levels fall, but are still well above baseline, and glucagon levels remain suppressed well below the baseline. This high insulin/glucagon ratio instructs the liver to stop producing glucose and take up ingested glucose entering the portal vein.

(B) In STZ-induced type 1 diabetes, there are almost no β cells within the islets. The only source of insulin is the subcutaneous injected dose. Thus, the level reaching the α cells is far below normal paracrine insulin levels, and it does not change in response to changes in glycemia. The frequent hypoglycemic episodes of well-controlled type 1 patients can be attributed to efforts to suppress glucagon-mediated hyperglycemia by increasing the insulin dose.

(C) In spontaneous type 2 diabetes, the insulin/glucagon ratio in perfused pancreata isolated from ZDF rats with T2D reveals that the first-phase insulin spike is missing, but that there is an abundance of paracrine insulin. And yet, glucagon secretion is not suppressed by the high glucose/high insulin. This suggests that the α cells, like many other insulin target tissues, are insulin resistant. Figure adapted from Raskin et al., 1975.

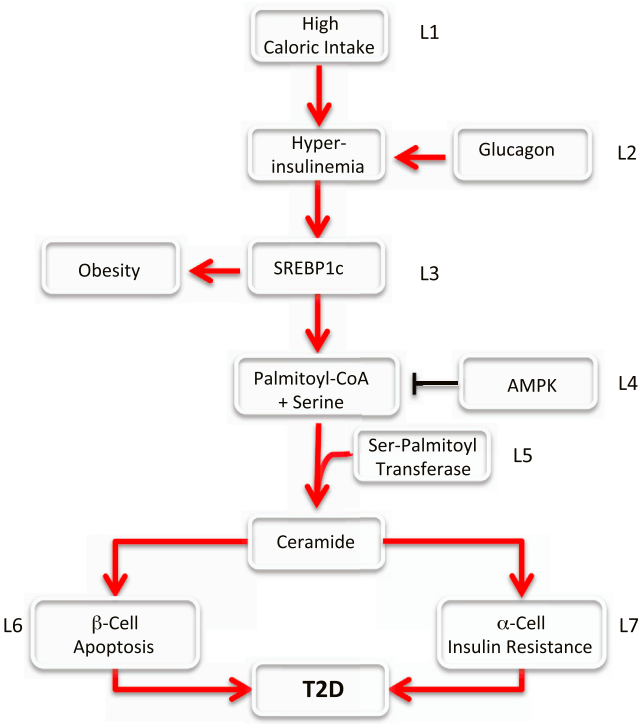

Figure 3. Proposed Pathway to T2D

Leptin, denoted by an "L," acts negatively and simultaneously on multiple points in the pathway. Hypothalamic leptin targets suppress caloric intake (L1). Leptin suppresses glucagon production by the α cells that enable diet-induced hyperinsulinemia (L2), inducing the SREBP1c-regulated program of lipogenesis (L3). Leptin activates AMPK, which will reduce palmitoyl-CoA levels (L4) and inhibits the serine palmitoyl transferase (SPT) control of de novo ceramide synthesis (L5). This will reduce β cell apoptosis (L6) and α cell insulin resistance (L7). All of these effects decrease the pathways leading to T2D. Unfortunately, obesity is usually accompanied by leptin resistance and fails to provide optimal protection against lipotoxicity.

In contrast to secreted insulin, subcutaneously injected exogenous insulin reaches every target tissue at an identical concentration determined by the dose of hormone (Table 1). An insulin dose sufficient to suppress α cells could require a dose ~100 times that required to suppress lipolysis in adipocytes. This would obviously predispose to dangerous glucopenia and contribute to the glycemic volatility that commonly plagues patients with T1D (Derr et al., 2003).

A bihormonal strategy, first proposed in 1975 (Unger, 1976) and tested in 1978 (Raskin and Unger, 1978), may solve the problem. Measurements of insulin and glucagon during the highs and lows of glycemic volatility reveal that during hypoglycemic dips insulin levels may average 16 ng/ml, almost 20× normal. This iatrogenic hyperinsulinemia (Wang et al., 2013) can be eliminated by reducing the insulin dose by as much as 90%, which can lower plasma insulin to below 1 ng/ml. The reduction of the insulin dose will cause a rise in glucagon and hyperglycemia at mealtime. This can be prevented by administering either a noninsulin suppressor of glucagon, such as somatostatin, leptin, liraglutide, GABA, or a glucagon receptor antagonist. It is expected that this strategy will provide stable normoglycemia and lower HgbA1c to below 6, with little or no risk of hypoglycemia (Figure 1C).

T1D and T2D Paracrinopathy

Although paracrinopathy underlies all forms of diabetes, the paracrinopathies of T1D and T2D could not be more different. The normal insulin/glucagon partnership is depicted in Figure 2A. The β cells serve as glucose sensor, reacting to the rise in glucose with a short burst of secreted insulin that sharply suppresses glucagon. The insulin/glucagon ratio rises to 7.4, a powerful signal to the liver to take up incoming glucose and store it as glycogen. In T1D (Figure 2B) there are no β cells, so that paracrine insulin is constantly deficient, despite an excess of insulin at downstream targets. This means that α cells will not be suppressed by an increase in hyperglycemia. Glucagon levels reaching the liver will always be inappropriately high, even after a glucose-containing meal. The high portal vein glucagon instructs the liver *not* to take up incoming glucose and *not* to convert it to glycogen. Rather, glucose will pass through the liver to enter the posthepatic circulation, accompanied by inappropriate glucagon stimulation of hepatic glycogenolysis and gluconeogenesis. Thus, the postprandial surges that plague even well-controlled T1D patients are the consequence, not only of ingested glucose, but also of endogenous glucose derived from inappropriately enhanced hepatic production.

By contrast, the paracrinopathy of T2D (Figure 2C) involves no dearth of paracrine insulin. In fact, in the first years of T2D, insulin levels may be supernormal. Nevertheless, glucagon levels are inappropriately elevated just as in T1D because the α cells, like other targets of insulin in T2D, are insulin resistant. α cell insulin resistance was first observed in 1976 in T2D patients, who required more than seven times as much insulin as nondiabetic subjects to lower plasma glucagon by 3 pg/ml (Raskin et al., 1975, 1976). This resistance may be the result of excess palmitate accumulation in islet cells as well as other target tissues of insulin (Piro et al., 2010). The palmitate may directly contribute to insulin resistance and/or it may condense with serine to form ceramide. In T2D, glucagon hypersecretion continues to maintain a subnormal insulin/glucagon ratio that causes inappropriate hepatic overproduction of glucose.

Leptin and the Pathway to T2D

Although leptin is not an islet hormone, it is a close collaborator with islet hormones, protecting them from T2D. Figure 3 depicts the postulated pathway from caloric excess to T2D. Virtually every pathogenic point on the pathway to lipotoxicity, from the high food intake to ceramide synthesis, is protectively influenced by leptin (indicated by "L" on the figure). While the metabolic syndrome is outside the scope of this review, it should be pointed out that lipotoxicity has been proposed to be a systemic disorder that affects all nonadipose organs and could therefore be its cause.

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