

Leptin Effects on Pancreatic β -Cell Gene Expression and Function

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The hormone leptin is secreted from white adipocytes, and serum levels of leptin correlate with adipose tissue mass. Leptin was first described to act on the satiety center in the hypothalamus through specific receptors (leptin receptor [ObR]) to restrict food intake and enhance energy expenditure. Important peripheral actions of leptin involve inhibition of insulin biosynthesis and secretion in pancreatic β -cells. In turn, insulin stimulates leptin secretion from adipose tissue, establishing a hormonal regulatory feedback loop—the so-called “adipo-insular axis.” Multiple signal transduction pathways are involved in leptin signaling in pancreatic β -cells. We have identified the proinsulin gene and protein phosphatase 1 gene as leptin repressed genes and the gene for the suppressor of cytokine signaling 3 protein as a leptin-induced gene in pancreatic β -cells. The molecular effects of leptin culminate to restrict insulin secretion and biosynthesis to adapt glucose homeostasis to the amount of body fat. In most overweight individuals, however, physiological regulation of body weight by leptin seems to be disturbed, representing “leptin resistance.” This leptin resistance at the level of the pancreatic β -cell may contribute to dysregulation of the adipo-insular axis and promote the development of hyperinsulinemia and manifest type 2 diabetes in overweight patients. *Diabetes* 53 (Suppl. 1): S152–S158, 2004

The *ob* gene was identified through positional cloning in 1994, and it was demonstrated that mutations in the *ob* gene of *ob/ob* mice prevent synthesis of a functional leptin protein (1). The discovery that the protein encoded by the *ob* gene leptin is essential for body weight homeostasis (2) and associated metabolic regulation has inspired the whole field of metabolic physiology. Leptin was identified as one of the first hormonal substances synthesized and secreted from adipose tissue. This set the stage for the notion that adipose

tissue represents an endocrine organ, which regulates a host of physiological and pathophysiological conditions, such as satiety, energy expenditure, insulin sensitivity, immune responses, inflammation, bone turnover, and glucose homeostasis, through the secretion of hormonal substances acting on distant sites within the organism.

Leptin is a 16-kDa circulating hormone produced and released primarily by adipocytes. Consequently, levels of circulating leptin are directly proportional to total fat mass (3) but also percent body fat and BMI. Leptin exerts regulatory control on food intake and energy expenditure acting on brain centers within the hypothalamus that control satiety and body weight (4). Thus, leptin transfers information to the brain about adipocyte metabolism. This endocrine function couples feeding behavior, metabolism, and endocrine physiology to the nutritional state of the organism. Leptin action is mediated by specific receptors that were initially cloned from complementary DNA derived from the choroid plexus of mice (5). The leptin receptor gene (*db*) was consequently identified by positional cloning. Five alternatively spliced mRNAs are translated into five different protein isoforms of the leptin receptor (ObR) (6). ObRb, the so-called long form of ObR, is essential for the weight-reducing effects of leptin. Distribution of leptin receptors throughout peripheral organs has meanwhile become evident, suggesting multiple peripheral actions of leptin independently of the functions in the central nervous system (7,8). Consequently, leptin has been shown to be involved in diverse physiological functions, such as reproduction (9), angiogenesis (10), hematopoiesis (11), and regulation of bone mass (12).

This report focuses on the specific effects of leptin on insulin-producing pancreatic β -cells and the impact on the pathogenesis of type 2 diabetes in overweight patients suffering from features of the metabolic syndrome.

EXPRESSION OF LEPTIN RECEPTORS ON PANCREATIC β -CELLS

The idea that leptin may directly act on pancreatic β -cells to control insulin secretion was derived from observations in genetically leptin-deficient *ob/ob* mice and leptin receptor-defective *db/db* mice. First, it was noted that defective leptin signaling in these mice strains leads to initial hyperinsulinemia in young animals, even before the development of the obese and diabetic phenotype (13–15). Second, this hyperinsulinemia was ameliorated by treatment of the *ob/ob* mice with recombinant leptin (2), suggesting a direct inhibitory action of leptin on insulin secretion from pancreatic β -cells.

Already at this stage, the idea was postulated that leptin

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GLP, glucagon-like peptide; JAK, janus kinase; K_{ATP} channel, ATP-dependent K^+ channel; ObR, leptin receptor; ObRa, short isoform of ObR; ObRb, long isoform of ObR; PI, phosphatidylinositol; PP-1, protein phosphatase 1; SOCS, suppressor of cytokine signaling; STAT, signal transducer and activator of transcription.

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may represent one part of a bidirectional feedback loop between adipose tissue and the pancreatic β -cell, with the other part consisting of insulin secreted from the pancreas and stimulating in turn leptin biosynthesis and secretion from adipose tissue. The adipogenic actions of insulin are well known, and stimulatory effects of insulin on leptin production in adipocytes were convincingly demonstrated (16,17).

Although the short isoform of the receptor (ObRa) is capable of signaling, the long isoform (ObRb) is currently believed to convey most of the physiological actions of leptin. As the first step to support the hypothesis of direct actions of leptin on pancreatic β -cells, the presence of ObRb on insulin-producing cells was shown. Messenger RNA for ObRb was found in high abundance in murine pancreatic islets but was not detected in the exocrine pancreas (18). Leptin receptor mRNA was detected in rat islets, even in greater abundance than that found in total brain, and was also detected in the pancreatic β -cell line β TC3 by RT-PCR. The presence of functional leptin receptors including ObRb on insulin-producing cells has since been confirmed in other studies and could also be demonstrated in β -cells derived from human pancreatic islets (19).

EFFECTS OF LEPTIN ON PANCREATIC β -CELL FUNCTION

The main function of the pancreatic β -cell is the biosynthesis and adequate secretion of insulin to control blood glucose levels. This function is tightly regulated both by nutrients and hormonal modulators, such as enteroendocrine hormones (glucose-dependent insulinotropic polypeptide and glucagon-like peptide [GLP]-1).

The effects of leptin on insulin secretion have initially yielded conflicting results. In contrast to the pancreatic islets of leptin-deficient *ob/ob* mice, which exhibit a robust reduction of insulin secretion upon leptin stimulation (probably because of higher leptin sensitivity), chronically leptin-exposed pancreatic β -cells display variable results in different experimental conditions. In pancreatic β -cells, leptin exerts a biphasic dose response with respect to insulin secretion. In rat islets, 2 nmol/l leptin significantly and maximally suppressed insulin release, whereas high concentrations were almost ineffective (20,21). Incubation of pancreatic islets isolated from *ob/ob* mice with 6.25–100 nmol/l leptin for 1–2 h robustly inhibits insulin secretion, which was also demonstrated for the perfused pancreas of *ob/ob* mice (18,22). This effect of leptin on insulin release was smaller at higher glucose concentrations and could not be demonstrated when the enteroendocrine hormone GLP-1 was present (22,23), indicating that the more tonic inhibitory leptin effects on insulin secretion could be overcome by acute nutrient- and incretin-stimulated insulin release. Leptin also inhibits insulin release from *ob/ob* islets stimulated by activators of protein kinase C, such as acetylcholine or phorbol ester phorbol-12-myristate 13-acetate (23). In contrast to β -cells of leptin-deficient *ob/ob* mice, initial studies examining the effect of leptin on insulin secretion in normal rodents or cell lines yielded varying results. However, currently it is accepted that under physiological conditions leptin (1–20 nmol/l) significantly reduces insulin release from pancreatic β -cells.

This has now been demonstrated by the majority of studies using perfused rat pancreas and isolated rat or mouse islets (19–41). Moreover, these observations derived from rodent β -cell lines or pancreatic islets are now confirmed in isolated human pancreatic islets (19,31,42,43), and there is recent evidence that a physiological increase in serum leptin levels significantly reduces insulin secretion in rats in vivo (44). The fact that leptin is able to inhibit insulin secretion from human islets at concentrations as low as 0.01 nmol/l implies that this regulatory loop between adipose tissue and the endocrine pancreas is also physiologically effective in humans.

EFFECTS OF LEPTIN ON PANCREATIC β -CELL GENE EXPRESSION

The first step of insulin biosynthesis is proinsulin gene expression. It has been shown that the rate-limiting step determining steady-state proinsulin mRNA levels is via transcriptional regulation of the proinsulin gene promoter (45).

The majority of studies report on suppression of preproinsulin mRNA in pancreatic β -cells by leptin. Thereby, leptin has been shown to suppress preproinsulin mRNA expression in mouse β TC6 cells (31) in the rat pancreatic β -cell line INS-1 (38), in isolated primary rat islets (20,31), in *ob/ob* mouse islets, and in human islets (19). Intraperitoneal injection of *ob/ob* mice with 1 μ g/g body wt murine leptin reduced steady-state levels of preproinsulin mRNA in isolated islets by 40% after 24 h (38). In studies of human islets, human leptin (6.25 nmol/l) evokes a time-dependent decrease in preproinsulin mRNA levels in the presence of 11.1 mmol/l glucose but not 5.6 mmol/l glucose (19). When the incretin hormone GLP-1, a known stimulator of the proinsulin gene promoter was used, leptin inhibited GLP-1-stimulated expression of preproinsulin mRNA in human islets at glucose concentrations of 5.6 and 11.1 mmol/l (38). In contrast, in INS-1 β -cells, leptin significantly reduced only preproinsulin mRNA expression stimulated by 25 mmol/l glucose, but not by lower glucose concentrations. These observations imply that the ability of leptin to reduce preproinsulin mRNA expression in pancreatic β -cells may depend on prior stimulation by incretin hormones or stimulatory ambient glucose concentrations. Further, the effects of leptin on steady-state preproinsulin mRNA levels in pancreatic β -cells were only observed after 16 h of incubation and were not seen at shorter incubation periods (38). This observation suggests that the effect of leptin on insulin biosynthesis may represent a more long-term character, and time kinetics imply that gene transcription may be necessary for this effect.

The effect of leptin on the transcriptional activity of the insulin gene promoter has been examined in a consecutive step. A dose of 6.25 nmol/l leptin inhibited a reporter vector expressing the luciferase gene under the control of 410 bp of the rat insulin I gene promoter in INS-1 cells at stimulatory glucose concentrations of 25 mmol/l but not at 5.6 mmol/l glucose (38). In contrast, the induction of transcriptional activity of the insulin promoter by additional stimulatory concentrations of 10 nmol/l GLP-1 at 11.1 mmol/l glucose was also inhibited by leptin. These findings suggest that stimulated insulin promoter activity by either GLP-1 or high glucose (25 mmol/l) represents a

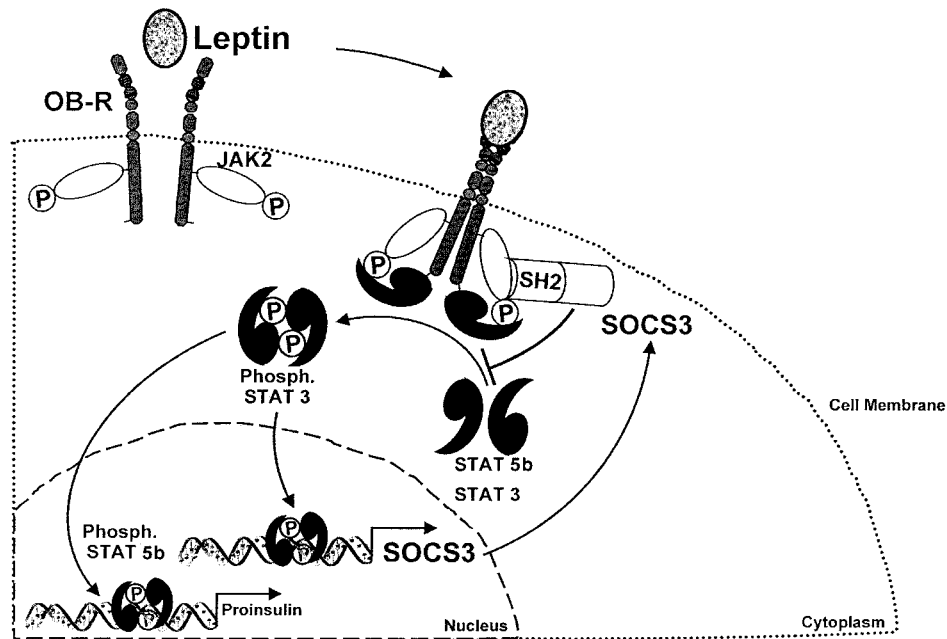


FIG. 1. Leptin signaling and gene regulation in pancreatic β -cells. Upon leptin stimulation, the ObR-associated JAK2 tyrosine kinase becomes activated via transphosphorylation and phosphorylates tyrosine residues of the leptin receptor. STAT3 and STAT5b are now recruited to the leptin receptor and are consecutively tyrosine-phosphorylated via JAK2. Phosphorylated STATs dimerize and translocate to the nucleus to regulate gene transcription. STAT5b transactivates the proinsulin gene promoter, whereas STAT3 may differentially activate the SOCS3 promoter. SOCS3 in turn inhibits the JAK-STAT signaling pathway by binding to the tyrosine-phosphorylated leptin receptor, thereby preventing recruitment of STATs to the leptin receptor. SOCS3 thereby mediates repression of leptin receptor signaling through a negative feedback loop.

prerequisite for the inhibitory actions of leptin on insulin promoter activity (38).

Leptin signaling through ObR is intracellularly coupled with the janus kinase (JAK)–signal transducer and activator of transcription (STAT) pathway. Binding of leptin to the receptor (ObR) activates the receptor-associated kinase JAK2 via transphosphorylation and phosphorylates tyrosine residues on ObR. Consecutively, transcription factors of the STAT family are recruited to the receptor and also phosphorylated. Phosphorylated STATs dimerize and translocate to the nucleus to regulate gene transcription. Also in pancreatic β -cells, we have found that repression of insulin promoter activity by leptin was associated with altered binding of the isoform STAT5b to specific DNA sequences within the promoter (38). STATs have mostly been shown to transcriptionally enhance gene expression. In contrast, we have demonstrated that leptin signaling, which activates several STATs in other tissues such as the hypothalamus (46,47), inhibits insulin biosynthesis via transcriptional repression of the proinsulin gene promoter (19,38). In a follow-up study, we sought to characterize this apparent contradiction at the molecular level.

Recently, a new family of molecules, called suppressor of cytokine signaling (SOCS), has been identified. This family of molecules is able to inhibit JAK-STAT signal transduction. These molecules contain a central src homology region 2 domain and a conserved COOH-terminal SOCS box. By their central SH2 domain, they bind directly to tyrosine-phosphorylated residues on the cytokine receptor-associated kinase JAK2. Expression of the SOCS proteins is induced by various cytokines, including interleukin-6, leukemia inhibitory factor, erythropoietin, and growth hormone in a tissue-specific manner, and once expressed, they in turn inhibit cytokine signaling via the JAK-STAT pathway in an intracellular negative feedback loop. Thus, SOCS molecules may function as cytokine inducible negative regulators of cytokine signaling. In the hypothalamus, it has been demonstrated that leptin in-

duces expression of SOCS3 mRNA in areas where ObRb is expressed. Thus, the SOCS molecules may play an important role in the development of leptin resistance that is seen in obesity both in the central nervous system and the endocrine pancreatic β -cell. We examined leptin-mediated signal transduction and gene regulation in insulin-producing pancreatic β -cells more in detail (K. Laubner, T. Kieffer, S. Royer, J. Roller, F. Jacob, J.S., unpublished data).

In brief, by coimmunoprecipitation and Western blot analysis, we found that leptin stimulation (6.25 nmol/l) leads to recruitment and phosphorylation of STAT3 and STAT5b to the receptor-associated JAK2 in INS-1 β -cells. Further, we demonstrated time-dependent nuclear translocation of STAT3 and STAT5b in INS-1 pancreatic β -cells after leptin stimulation by fluorescence immunocytochemistry and transient transfection of expression vectors encoding the STAT5b green-fluorescent protein fusion protein. Moreover, leptin signaling in INS-1 cells and isolated pancreatic islets of *ob/ob* mice induces expression of SOCS3 at the transcriptional level in vitro and in vivo, as revealed by luciferase reporter gene assay and RT-PCR. In electrophoretic mobility shift assays using potential STAT-binding sites within the rat SOCS3 promoter, we found leptin-induced STAT3 DNA binding in INS-1 nuclear extracts. Whereas STAT5b alone transactivates the rat insulin 1 promoter in luciferase reporter gene assays in INS-1 cells, leptin-induced SOCS3 expression inhibits STAT5b-dependent transactivation of the rat insulin 1 promoter. In conclusion, we have acquired evidence that, in pancreatic β -cells, the gene regulatory effects of leptin not only involve transcriptional inhibition of the insulin gene promoter but also transcriptional activation of the promoter of the JAK-STAT inhibitory molecule SOCS3 by transcription factors of the STAT family, thereby constituting a regulatory feedback loop, which in turn inhibits leptin-dependent JAK-STAT activation. Leptin-induced SOCS3 expression may thus mediate the long-term inhibitory effect of leptin action in pancreatic β -cells that is seen in the context of endocrine signaling between adipose tissue

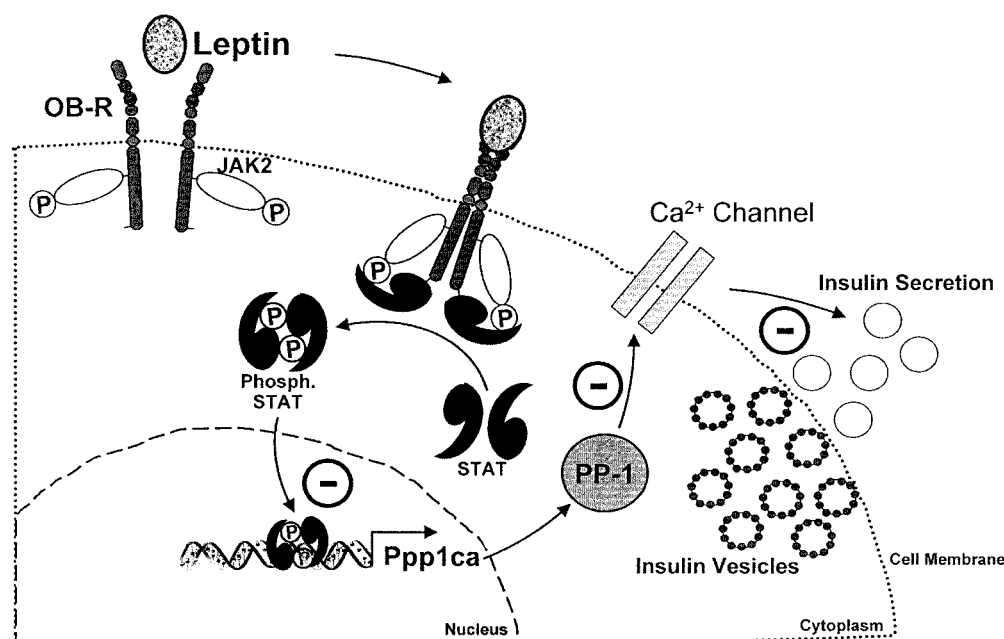


FIG. 2. Leptin inhibition of PP-1 expression and activity in pancreatic β -cells. Upon leptin stimulation, the ObR-associated JAK2 tyrosine kinase becomes activated via transphosphorylation and phosphorylates tyrosine residues of ObR. Phosphorylated STATs dimerize and translocate to the nucleus to regulate gene transcription. The expression of the catalytic subunit Ppp1ca of PP-1 is transcriptionally inhibited by leptin signaling. This leads to reduced PP-1 enzyme activity and consecutive reduction of intracellular calcium concentrations. Consequently, insulin secretion is inhibited by reduced intracellular calcium in pancreatic β -cells.

and the endocrine pancreas within the adipoinular axis (Fig. 1).

As described above, leptin exerts bifunctional gene regulatory effects on the proinsulin and SOCS3 genes in pancreatic β -cells. Thus, we hypothesized that additional genes may be regulated by leptin in insulin-producing cells. Using a subtractive PCR approach with cDNA pools derived from leptin and vehicle-treated INS-1 β -cells, we identified the catalytic subunit of protein phosphatase 1 (PP-1) as a leptin-regulated gene in pancreatic β -cells (P. Kühn, K. Laubner, J. Roller, F. Jakob, J.S., unpublished data). In brief, PP-1 has recently been characterized genetically as a candidate gene for type 2 diabetes (48,49). Further, serine/threonine phosphatase PP-1 is a key enzyme in the insulin-signaling cascade in muscle and liver. Through RT-PCR and fluorescence immunocytochemistry, we found coexpression of PP-1 and insulin in the pancreatic β -cell line INS-1 and in primary β -cells from human pancreatic islets. Moreover, RT-PCR and Northern and Western blot analysis demonstrated time-dependent inhibition of PP-1 mRNA and protein expression by leptin (100 nmol/l) within 48 h in pancreatic β -cells. Using a PP-1-specific assay, leptin reduced functional PP-1 enzyme activity by 64% in pancreatic β -cells, an effect that could be mimicked by the PP-1-specific inhibitors calyculin A and okadaic acid. Finally, leptin-, calyculin A-, or okadaic acid-mediated reduction of PP-1 activity leads to profound inhibition of both glucose- and glucagon-induced insulin secretion in pancreatic β -cells (Fig. 2).

Taken together, leptin exerts gene regulatory effects on multiple genes within insulin-producing pancreatic β -cells. Current knowledge, however, suggests that all gene regulatory effects concomitantly culminate to physiologically reduce insulin secretion and biosynthesis.

MULTIPLE MOLECULAR EFFECTS OF LEPTIN IN PANCREATIC β -CELLS

Insulin secretion from pancreatic β -cells relies in part on the activity of ATP-dependent K^+ (K_{ATP}) channels. Closure

(inactivation) of K_{ATP} channels in response to glucose or other insulin secretagogues depolarizes β -cells, resulting in the activation of voltage-dependent Ca^{2+} channels, a rise in cytosolic calcium concentration, and a rise in insulin secretion (50). Glucose-induced insulin secretion is further potentiated by hormone-mediated elevation of the intracellular second messengers cAMP/protein kinase A and phospholipase C/protein kinase C. Electrophysiological characterization of the effects of leptin on pancreatic β -cells revealed that leptin hyperpolarized the cell membrane, which led to inhibition of insulin secretion (22,27). This hyperpolarization has been demonstrated to be due to an increase in membrane conductance caused by the opening (activation) of K_{ATP} channels. Thus, the K_{ATP} channel is a molecular target of leptin in pancreatic β -cells for inhibition of insulin secretion. Consequently, activation of K_{ATP} channels in pancreatic β -cells by leptin reduces cytosolic calcium concentration, and this fall can be overcome by coincubation with 20 mmol/l glucose and GLP-1 (10 nmol/l). Although the molecular mechanism by which leptin activates K_{ATP} channels is not fully understood, studies indicate that phosphorylation or dephosphorylation of proteins may be involved (51), and treatment of insulinoma HIT-T15 cells with murine leptin results in a threefold activation of phosphatidylinositol (PI) 3-kinase (21). Phosphodiesterase 3B, which reduces the cellular content of cAMP, is also activated by leptin, and leptin (1–5 nmol/l) suppressed the elevation of cAMP induced by GLP-1 in HIT-T15 cells (5 nmol/l) (21). These findings suggest that the inhibitory actions of leptin on insulin secretion are primarily mediated through the PI 3-kinase-dependent activation of phosphodiesterase 3B and a subsequent reduction of intracellular cAMP.

The inhibitory effects of leptin on preproinsulin gene expression, however, appear to be independent of the activation of K_{ATP} channels. The K_{ATP} channel opener diazoxide did not affect both leptin suppression of preproinsulin mRNA levels and inhibition of insulin promoter activity in INS-1 cells (38), indicating that gene regulatory

TABLE 1
Leptin effects on pancreatic β -cells

	Leptin effect	Reference
Insulin secretion	Inhibition (human, rat/mouse pancreatic islets, pancreatic β -cell lines, perfused pancreas in vivo)	8, 18–28, 30, 31, 33–40, 42–44, 51–58
	No effect	59, 60
	Stimulation	61
Gene expression	Reduction of insulin mRNA	19, 20, 31, 38
	Inhibition of insulin promoter	38
	Uncoupling protein 2 expression	62, 63
	Reduction of PP-1 expression	Unpublished data*
	Transcriptional induction of SOCS3 expression	Unpublished data†
Signal transduction	JAK-STAT signaling	38
	Activation of K_{ATP} channel	22, 27, 51, 54
	Interference with cAMP pathway	21, 64
	Activation of PI 3-kinase	21
	Inhibition of PP-1 activity	Unpublished data*

Authors of unpublished data: *P. Kühnen, K. Laubner, J. Roller, F. Jakob, J.S.; †K. Laubner, T. Kieffer, S. Royer, J. Roller, F. Jakob, J.S.

effects of leptin use signal transduction pathways different from those that mediate the effect on insulin secretion.

The different functional, molecular, and gene regulatory effects of leptin involving insulin secretion (8,18–28, 30,31,33–40,42–44,51–61), pancreatic β -cell gene expression (19,20,31,38,62,63), and signal transduction (21,22,27, 38,51,54,64) are summarized in Table 1.

CONCLUSIONS

A growing body of evidence has been accumulated showing that the adipose tissue–derived hormone leptin directly acts on pancreatic β -cells in addition to its effects in the hypothalamus to reduce food intake and increase energy expenditure. At the cellular level, inhibitory effects of leptin on both insulin secretion and insulin biosynthesis, mainly represented by the inhibition of preproinsulin gene expression, have been demonstrated. In this context, leptin affects different signal transduction pathways and molecular targets.

Taken together, all effects of leptin in pancreatic β -cells that are known to date act concomitantly to exert a

physiological long-term control of insulin secretion from the pancreatic β -cell, which adapts the amount of insulin secretion to the amount of body fat stores. It is important to note that this tonic restriction of insulin secretion by leptin generally does not seem to interfere with the short-term stimulatory actions of nutrients and hormones, such as glucose- and incretin-dependent insulin secretion.

Leptin seems to represent a signaling molecule from the adipose tissue to the endocrine pancreas to restrict insulin secretion according to the needs that are determined by body fat stores. As such, this effect establishes an “adipo-insular” transmission of signals from the adipocyte to the pancreatic β -cell. Vice versa, the lipogenic action of insulin has long been well known, and, consequently, it has been demonstrated that insulin stimulates both leptin biosynthesis and secretion from white adipose tissue. This establishes a classic endocrine adipo-insular feedback loop—the so-called “adipo-insular axis” (Fig. 3A). To date, most evidence to support this concept has come from experimental animal models and in vitro studies. A growing body of arguments, however, indicates that the adipo-insular

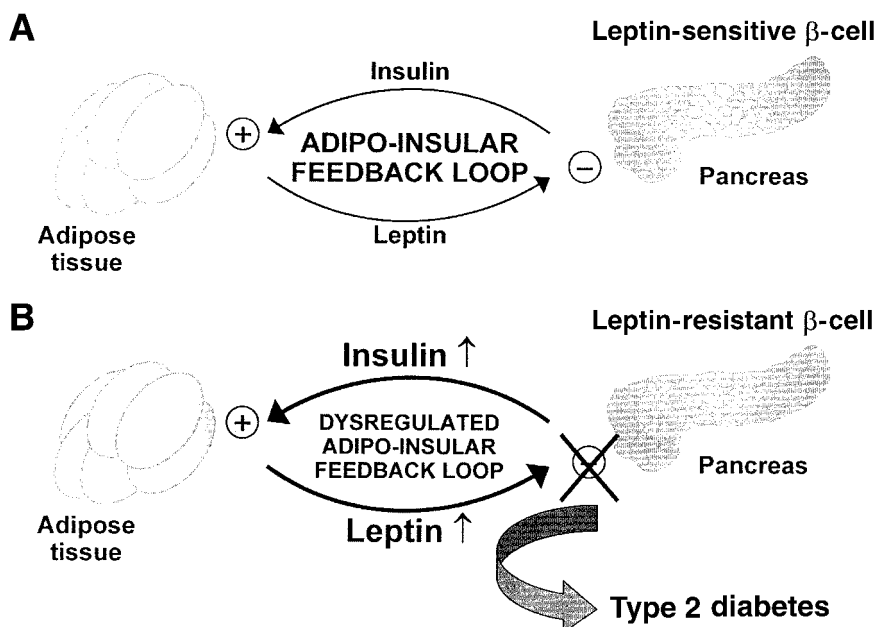


FIG. 3. Dysregulation of the adipo-insular axis and pathogenesis of type 2 diabetes. **A**: In leptin-sensitive individuals, leptin secretion from the adipose tissue restricts insulin secretion from pancreatic β -cells to adapt glucose homeostasis to the body fat stores. **B**: In leptin-resistant overweight individuals, diminished leptin signaling in pancreatic β -cells leads to chronic hypersecretion of insulin (hyperinsulinemia). Elevated insulin levels promote both insulin resistance and increased leptin biosynthesis and secretion from adipose tissue, which may further desensitize leptin signaling in the endocrine pancreas and increase leptin resistance. Chronic hypersecretion of insulin by the pancreatic β -cell because of a lack of tonic inhibition by leptin may contribute to ultimate pancreatic β -cell failure and eventual manifestation of type 2 diabetes in overweight patients.

axis also physiologically regulates insulin secretion and leptin production in humans (65). Leptin effects detected so far on pancreatic β -cells are generally delayed and involve more long-term cellular events. In contrast, acute stimulation of insulin secretion by nutrients and postprandially secreted hormones overcame most of the leptin effects on insulin secretion, but not on gene regulation, in the majority of studies. Thus, it is tempting to speculate that the inhibitory effect of leptin on insulin secretion within the adipo-insular axis is designed to adapt the set point of pancreatic insulin release to the body fat stores during the fasting state.

The adipo-insular axis may, however, play an important role during the development of type 2 diabetes in obese patients. During the development of this disease, initial hyperinsulinemia is believed to represent a simple compensatory response of the pancreatic β -cell to insulin resistance (66,67), and hyperglycemia is the consequence of pancreatic β -cell failure. However, in recent studies, it became obvious that hyperinsulinemia frequently precedes the development of insulin resistance, arguing at least in part for the existence of an early functional defect in insulin secretion and against obesity-induced insulin resistance fully explaining the development of type 2 diabetes.

In obese animal models and also in most obese patients, it has been shown that despite high levels of circulating leptin according to the increased fat mass, leptin seems to fail exerting its effect on the hypothalamus (i.e., reducing food intake and increasing energy expenditure). This observation has been termed "leptin resistance" and was attributed to several molecular alterations in postreceptor leptin signal transduction in the hypothalamus (68). Given the almost identical cellular targets and signal transduction pathways that are used by leptin in both the hypothalamus and the pancreatic β -cell, one may postulate the existence of leptin resistance also at the level of the pancreatic islet (8,69). Reduced leptin sensitivity of the pancreatic β -cell, however, leads to dysregulation of the adipo-insular axis, resulting in increased insulin release, which is then no longer under controlled repression by leptin. Thereby, leptin resistance at the level of the pancreatic β -cell may promote hyperinsulinemia in obese patients prone to developing type 2 diabetes (Fig. 3B). Elevated insulin concentrations promote both insulin resistance and further stimulation of leptin production and secretion from the adipose tissue, which may in turn enhance leptin resistance of the endocrine pancreas by further desensitizing leptin signal transduction pathways and constituting a vicious circle that promotes manifestation of type 2 diabetes in obese people (Fig. 3B).

Thus, identification of the molecular determinants of the adipo-insular axis and especially leptin resistance in pancreatic β -cells may provide novel targets for the development of therapeutic strategies. This process may prevent the early steps in the pathogenesis of type 2 diabetes and ultimately the manifestation of the disease in susceptible overweight patients.

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REFERENCES

- Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM: Positional cloning of the mouse obese gene and its human homologue. *Nature* 372:425–432, 1994
- Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, Collins F: Effects of the obese gene product on body weight regulation in ob/ob mice. *Science* 269:540–543, 1995
- Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL: Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med* 334:292–295, 1996
- Flier JS: Leptin expression and action: new experimental paradigms. *Proc Natl Acad Sci U S A* 94:4242–4245, 1997
- Tartaglia LA, Dembski M, Weng X, Deng N, Culpepper J, Devos R, Richards GJ, Campfield LA, Clark FT, Deeds J: Identification and expression cloning of a leptin receptor, OB-R. *Cell* 83:1263–1271, 1995
- Lee GH, Proenca R, Montez JM, Carroll KM, Darvishzadeh JG, Lee JJ, Friedman JM: Abnormal splicing of the leptin receptor in diabetic mice. *Nature* 379:632–635, 1996
- Fei H, Okano HJ, Li C, Lee GH, Zhao C, Darnell R, Friedman JM: Anatomic localization of alternatively spliced leptin receptors (Ob-R) in mouse brain and other tissues. *Proc Natl Acad Sci U S A* 94:7001–7005, 1997
- Kieffer TJ, Habener JF: The adipoinsular axis: effects of leptin on pancreatic beta-cells. *Am J Physiol Endocrinol Metab* 278:E1–E14, 2000
- Chehab FF, Mounzih K, Lu R, Lim ME: Early onset of reproductive function in normal female mice treated with leptin. *Science* 275:88–90, 1997
- Sierra-Honigmann MR, Nath AK, Murakami C, Garcia-Cardena G, Papatropoulos A, Sessa WC, Madge LA, Schechner JS, Schwabb MB, Polverini PJ, Flores-Riveros JR: Biological action of leptin as an angiogenic factor. *Science* 281:1683–1686, 1998
- Cioffi JA, Shafer AW, Zupancic TJ, Smith-Gbur J, Mikhail A, Platika D, Snodgrass HR: Novel B219/OB receptor isoforms: possible role of leptin in hematopoiesis and reproduction. *Nat Med* 2:585–589, 1996
- Takeda S, Eleftheriou F, Levasseur R, Liu X, Zhao L, Parker KL, Armstrong D, Ducy P, Karsenty G: Leptin regulates bone formation via the sympathetic nervous system. *Cell* 111:305–317, 2002
- Coleman DL: Diabetes-obesity syndromes in mice. *Diabetes* 31:1–6, 1982
- Coleman DL: Obese and diabetes: two mutant genes causing diabetes-obesity syndromes in mice. *Diabetologia* 14:141–148, 1978
- Chen NG, Romsos DR: Enhanced sensitivity of pancreatic islets from preobese 2-week-old ob/ob mice to neurohormonal stimulation of insulin secretion. *Endocrinology* 136:505–511, 1995
- Laferrere B, Caixas A, Fried SK, Bashore C, Kim J, Pi-Sunyer FX: A pulse of insulin and dexamethasone stimulates serum leptin in fasting human subjects. *Eur J Endocrinol* 146:839–845, 2002
- Moreno-Aliaga MJ, Stanhope KL, Havel PJ: Transcriptional regulation of the leptin promoter by insulin-stimulated glucose metabolism in 3t3-L1 adipocytes. *Biochem Biophys Res Commun* 283:544–548, 2001
- Emilsson V, Liu YL, Cawthorne MA, Morton NM, Davenport M: Expression of the functional leptin receptor mRNA in pancreatic islets and direct inhibitory action of leptin on insulin secretion. *Diabetes* 46:313–316, 1997
- Seufert J, Kieffer TJ, Leech CA, Holz GG, Moritz W, Ricordi C, Habener JF: Leptin suppression of insulin secretion and gene expression in human pancreatic islets: implications for the development of adipogenic diabetes mellitus. *J Clin Endocrinol Metab* 84:670–676, 1999
- Pallett AL, Morton NM, Cawthorne MA, Emilsson V: Leptin inhibits insulin secretion and reduces insulin mRNA levels in rat isolated pancreatic islets. *Biochem Biophys Res Commun* 238:267–270, 1997
- Zhao AZ, Bornfeldt KE, Beavo JA: Leptin inhibits insulin secretion by activation of phosphodiesterase 3B. *J Clin Invest* 102:869–873, 1998
- Kieffer TJ, Heller RS, Leech CA, Holz GG, Habener JF: Leptin suppression of insulin secretion by the activation of ATP-sensitive K⁺ channels in pancreatic beta-cells. *Diabetes* 46:1087–1093, 1997
- Chen NG, Swick AG, Romsos DR: Leptin constrains acetylcholine-induced

- insulin secretion from pancreatic islets of ob/ob mice. *J Clin Invest* 100:1174–1179, 1997
24. Ahren B, Havel PJ: Leptin inhibits insulin secretion induced by cellular cAMP in a pancreatic B cell line (INS-1 cells). *Am J Physiol* 277:R959–R966, 1999
 25. Ceddia RB, William WN Jr, Carpinelli AR, Curi R: Modulation of insulin secretion by leptin. *Gen Pharmacol* 32:233–237, 1999
 26. Fehmann HC, Peiser C, Bode HP, Stamm M, Staats P, Hedetoft C, Lang RE, Goke B: Leptin: a potent inhibitor of insulin secretion. *Peptides* 18:1267–1273, 1997
 27. Harvey J, McKenna F, Herson PS, Spanswick D, Ashford ML: Leptin activates ATP-sensitive potassium channels in the rat insulin-secreting cell line, CRI-G1. *J Physiol (Lond)* 504:527–535, 1997
 28. Ishida K, Murakami T, Mizuno A, Iida M, Kuwajima M, Shima K: Leptin suppresses basal insulin secretion from rat pancreatic islets. *Regul Pept* 70:179–182, 1997
 29. Kawai T, Hirose H, Seto Y, Fujita H, Saruta T: Chronic effects of different fatty acids and leptin in INS-1 cells. *Diabetes Res Clin Pract* 51:1–8, 2001
 30. Koyama K, Chen G, Wang MY, Lee Y, Shimabukuro M, Newgard CB, Unger RH: Beta-cell function in normal rats made chronically hyperleptinemic by adenovirus-leptin gene therapy. *Diabetes* 46:1276–1280, 1997
 31. Kulkarni RN, Wang ZL, Wang RM, Hurley JD, Smith DM, Gbatei MA, Withers DJ, Gardiner JV, Bailey CJ, Bloom SR: Leptin rapidly suppresses insulin release from insulinoma cells, rat and human islets and, in vivo, in mice. *J Clin Invest* 100:2729–2736, 1997
 32. Lee JW, Romsos DR: Leptin administration normalizes insulin secretion from islets of Lep(ob)/Lep(ob) mice by food intake-dependent and -independent mechanisms. *Exp Biol Med (Maywood)* 228:183–187, 2003
 33. Lee JW, Swick AG, Romsos DR: Leptin constrains phospholipase C-protein kinase C-induced insulin secretion via a phosphatidylinositol 3-kinase-dependent pathway. *Exp Biol Med (Maywood)* 228:175–182, 2003
 34. Mackowiak P, Nogowski L, Fabis M, Nowak KW: Leptin perfusion affects insulin secretion but not insulin receptors in rats. *Arch Physiol Biochem* 109:63–68, 2001
 35. Ookuma M, Ookuma K, York DA: Effects of leptin on insulin secretion from isolated rat pancreatic islets. *Diabetes* 47:219–223, 1998
 36. Poutout V, Rouault C, Guerre-Millo M, Briaud I, Reach G: Inhibition of insulin secretion by leptin in normal rodent islets of Langerhans. *Endocrinology* 139:822–826, 1998
 37. Roduit R, Thorens B: Inhibition of glucose-induced insulin secretion by long-term preexposure of pancreatic islets to leptin. *FEBS Lett* 415:179–182, 1997
 38. Seufert J, Kieffer TJ, Habener JF: Leptin inhibits insulin gene transcription and reverses hyperinsulinemia in leptin-deficient ob/ob mice. *Proc Natl Acad Sci U S A* 96:674–679, 1999
 39. Silvestre RA, Rodriguez-Gallardo J, Egado EM, Marco J: Effect of leptin on insulin, glucagon and somatostatin secretion in the perfused rat pancreas. *Horm Metab Res* 33:207–212, 2001
 40. Widdup G, Bryson JM, Pawlak D, Phuyal JL, Denyer GS, Caterson ID: In vivo and in vitro suppression by leptin of glucose-stimulated insulin hypersecretion in high glucose-fed rats. *Eur J Endocrinol* 143:431–437, 2000
 41. Zhou YT, Shimabukuro M, Lee Y, Koyama K, Trieu F, Unger RH: Leptin normalizes the impaired response of proinsulin mRNA to long chain fatty acids in heterozygous Zucker diabetic fatty rats. *J Biol Chem* 272:25648–25651, 1997
 42. Fehmann HC, Berghofer P, Brandhorst D, Brandhorst H, Hering B, Bretzel RG, Goke B: Leptin inhibition of insulin secretion from isolated human islets. *Acta Diabetol* 34:249–252, 1997
 43. Lupi R, Marchetti P, Maffei M, Del Guerra S, Benzi L, Marselli L, Bertacca A, Navalesi R: Effects of acute or prolonged exposure to human leptin on isolated human islet function. *Biochem Biophys Res Commun* 256:637–641, 1999
 44. Cases JA, Gabrieli I, Ma XH, Yang XM, Michaeli T, Fleischer N, Rossetti L, Barzilai N: Physiological increase in plasma leptin markedly inhibits insulin secretion in vivo. *Diabetes* 50:348–352, 2001
 45. Docherty K, Clark AR, Scott V, Knight SW: Metabolic control of insulin gene expression and biosynthesis. *Proc Nutr Soc* 50:553–558, 1991
 46. Vaisse C, Halaas JL, Horvath CM, Darnell JEJ, Stoffel M, Friedman JM: Leptin activation of Stat3 in the hypothalamus of wild-type and ob/ob mice but not db/db mice. *Nat Genet* 14:95–97, 1996
 47. Rosenblum CI, Tota M, Cully D, Smith T, Collum R, Qureshi S, Hess JF, Phillips MS, Hey PJ, Vongs A, Fong TM, Xu L, Chen HY, Smith RG, Schindler C, Van der Ploeg LH: Functional STAT 1 and 3 signaling by the leptin receptor (OB-R): reduced expression of the rat fatty leptin receptor in transfected cells. *Endocrinology* 137:5178–5181, 1996
 48. Bjorbak C, Lavery HJ, Bates SH, Olson RK, Davis SM, Flier JS, Myers MG Jr: SOCS3 mediates feedback inhibition of the leptin receptor via Tyr985. *J Biol Chem* 275:40649–40657, 2000
 49. Savage DB, Agostini M, Barroso I, Gurnell M, Luan J, Meirhaeghe A, Harding AH, Ihrke G, Rajanayagam O, Soos MA, George S, Berger D, Thomas EL, Bell JD, Meeran K, Ross RJ, Vidal-Puig A, Wareham NJ, O'Rahilly S, Chatterjee VK, Schafer AJ: Digenic inheritance of severe insulin resistance in a human pedigree. *Nat Genet* 31:379–384, 2002
 50. Ashcroft SJ: The beta-cell K(ATP) channel. *J Membr Biol* 176:187–206, 2000
 51. Harvey J, Ashford ML: Role of tyrosine phosphorylation in leptin activation of ATP-sensitive K⁺ channels in the rat insulinoma cell line CRI-G1. *J Physiol (Lond)* 510:47–61, 1998
 52. Ahren B, Larsson H: Leptin: a regulator of islet function? Its plasma levels correlate with glucagon and insulin secretion in healthy women. *Metabolism* 46:1477–1481, 1997
 53. Emilsson V, O'Dowd J, Nolan AL, Cawthorne MA: Hexosamines and nutrient excess induce leptin production and leptin receptor activation in pancreatic islets and clonal beta-cells. *Endocrinology* 142:4414–4419, 2001
 54. Harvey J, Ashford ML: Insulin occludes leptin activation of ATP-sensitive K⁺ channels in rat CRI-G1 insulin secreting cells. *J Physiol (Lond)* 511:695–706, 1998
 55. Kieffer TJ, Heller RS, Habener JF: Leptin receptors expressed on pancreatic beta-cells. *Biochem Biophys Res Commun* 224:522–527, 1996
 56. Larsson H, Elmstahl S, Ahren B: Plasma leptin levels correlate to islet function independently of body fat in postmenopausal women. *Diabetes* 45:1580–1584, 1996
 57. Morton NM, Emilsson V, de Groot P, Pallett AL, Cawthorne MA: Leptin signalling in pancreatic islets and clonal insulin-secreting cells. *J Mol Endocrinol* 22:173–184, 1999
 58. Vickers MH, Reddy S, Ikenasio BA, Breier BH: Dysregulation of the adipoinular axis: a mechanism for the pathogenesis of hyperleptinemia and adipogenic diabetes induced by fetal programming. *J Endocrinol* 170:323–332, 2001
 59. Karlsson E, Stridsberg M, Sandler S: Leptin regulation of islet amyloid polypeptide secretion from mouse pancreatic islets. *Biochem Pharmacol* 56:1339–1346, 1998
 60. Leclercq-Meyer V, Considine RV, Sener A, Malaisse WJ: Do leptin receptors play a functional role in the endocrine pancreas? *Biochem Biophys Res Commun* 229:794–798, 1996
 61. Tanizawa Y, Okuya S, Ishihara H, Asano T, Yada T, Oka Y: Direct stimulation of basal insulin secretion by physiological concentrations of leptin in pancreatic beta cells. *Endocrinology* 138:4513–4516, 1997
 62. Brown JE, Thomas S, Digby JE, Dunmore SJ: Glucose induces and leptin decreases expression of uncoupling protein-2 mRNA in human islets. *FEBS Lett* 513:189–192, 2002
 63. Zhou YT, Shimabukuro M, Koyama K, Lee Y, Wang MY, Trieu F, Newgard CB, Unger RH: Induction by leptin of uncoupling protein-2 and enzymes of fatty acid oxidation. *Proc Natl Acad Sci U S A* 94:6386–6390, 1997
 64. Fehmann HC, Bode HP, Ebert T, Karl A, Goke B: Interaction of GLP-I and leptin at rat pancreatic B-cells: effects on insulin secretion and signal transduction. *Horm Metab Res* 29:572–576, 1997
 65. Guldstrand M, Ahren B, Adamson U: Improved beta-cell function after standardized weight reduction in severely obese subjects. *Am J Physiol Endocrinol Metab* 284:E557–E565, 2003
 66. Cerasi E: Insulin deficiency and insulin resistance in the pathogenesis of NIDDM: is a divorce possible? *Diabetologia* 38:992–997, 1995
 67. DeFronzo RA: Pathogenesis of type 2 (non-insulin dependent) diabetes mellitus: a balanced overview. *Diabetologia* 35:389–397, 1992
 68. Bjorbaek C, Elmquist JK, Frantz JD, Shoelson SE, Flier JS: Identification of SOCS-3 as a potential mediator of central leptin resistance. *Mol Cell* 1:619–625, 1998
 69. Seufert J: The significance of leptin for the pathogenesis of diabetes mellitus type 2: direct effects on endocrine pancreas (in German). *Internist (Berl)* 40:894–897, 1999