Perspectives in Diabetes

Insulin Signaling in the Central Nervous System

A Critical Role in Metabolic Homeostasis and Disease From *C. elegans* to Humans

Daniel Porte, Jr.,[1,2,3] Denis G. Baskin,[3] and Michael W. Schwartz[3,4,5]

Insulin and its signaling systems are implicated in both central and peripheral mechanisms governing the ingestion, distribution, metabolism, and storage of nutrients in organisms ranging from worms to humans. Input from the environment regarding the availability and type of nutrients is sensed and integrated with humoral information (provided in part by insulin) regarding the sufficiency of body fat stores. In response to these afferent inputs, neuronal pathways are activated that influence energy flux and nutrient metabolism in the body and ensure reproductive competency. Growing evidence supports the hypothesis that reduced central nervous system insulin signaling from either defective secretion or action contributes to the pathogenesis of common metabolic disorders, including diabetes and obesity, and may therefore help to explain the close association between these two disorders. These considerations implicate insulin action in the brain, an organ previously considered to be insulin independent, as a key determinant of both glucose and energy homeostasis. *Diabetes* 54:1264–1276, 2005

An important function of the central nervous system (CNS) is to ensure a steady supply of energy substrate to maintain the body economy and prepare for reproduction. To accomplish this task, widely divergent afferent signals must be integrated and transduced into homeostatic adjustments of food intake, energy expenditure, and nutrient metabolism. This perspectives article focuses on recent progress in our understanding of neuronal insulin action and the critical role it plays in energy homeostasis. Based on this information, we hypothesize that the close association between diabetes and obesity in humans arises, at least in part, as a consequence of deficient insulin signaling in both the CNS and peripheral tissues.

Studies conducted >50 years ago first implicated the ventral hypothalamus as a key brain area in energy homeostasis, and an explosion of new information has both confirmed this early impression and illuminated many of the critical mechanisms involved. Among the key findings is evidence that insulin functions not only as a peripheral regulator of nutrient storage and release of circulating substrates but as a key afferent signal to the CNS for the control of energy balance. Indeed, neuronal insulin signaling is known to be important for the control of fat storage in animals as primitive as C. *elegans* and *Drosophilia melanogaster*, and the cellular signaling systems mediating these effects bear remarkable homology to those described in mammals. New data have also shown that neuronal insulin signaling is a determinant of lifespan and reproductive function in these organisms, further broadening the importance of this system to diverse areas of physiology and biomedicine.

While we note growing evidence implicating insulin action in the control of cognition and neuronal function in cortical and hippocampal areas important to memory formation and information processing, this is not the focus of this article. However, we point out that considerable work has raised the possibility that abnormal insulin action/function in these brain areas contributes to the pathogenesis of Altzheimer's disease, and the interested reader is referred elsewhere for a discussion of this topic (1,2).

ADIPOSITY SIGNALS TO THE BRAIN

The various afferent inputs that the brain uses to adjust food intake and energy metabolism can be broadly subdivided into two groups: those that communicate information pertaining to body energy stores and those that are generated acutely in response to nutrient ingestion. Among afferent signals indicating the size of body adipose mass, insulin and leptin are the best studied and understood, and both appear to be required by the CNS for the

From the [1]Division of Metabolism, Diabetes, and Endocrinology, University of California San Diego, San Diego, California; the [2]Veterans Affairs San Diego Health Care System, San Diego, California; the [3]Division of Metabolism, Endocrinology, and Nutrition, University of Washington, Seattle, Washington; the [4]Veterans Affairs Puget Sound Health Care System, Seattle, Washington; and the [5]Harborview Medical Center, Seattle, Washington.

Address correspondence and reprint requests to Daniel Porte, Jr, MD, VA San Diego Health Care System (111G), 3350 La Jolla Village Dr., San Diego, CA 92161. E-mail: dporte@ucsd.edu.

Received for publication September 2004 and accepted in revised form 2 January 2005.

AGE, advanced glycation end product; AgRP, Agouti-related peptide; BAT, brown adipose tissue; CART, cocaine and amphetamine-regulated transcript; CNS, central nervous system; ICV, intracerebroventricular; IRS, insulin receptor substrate; JAK-STAT, Janus kinase–signal transducers and activators of transcription; K_{ATP} channel, ATP-sensitive K^+ channel; NPY, neuropeptide Y; PI3K, phosphatidylinositol 3-OH kinase; POMC, precursor proopiomelanocortin; PPY_{3-36}, peptide YY_{3-36}; SNS, sympathetic nervous system; SOCS3, suppressor of cytokine signaling 3.

control of food intake, body weight, and metabolic homeostasis (3). Although leptin is secreted primarily from adipocytes while insulin is released from the endocrine pancreas, both circulate at levels proportionate to body fat mass and exert relatively long-lived inhibitory effects on food intake via actions on a common set of hypothalamic neurons (4).

By comparison, signals responding to recently ingested nutrients are more varied and function primarily on a meal-to-meal basis to control gastric emptying and the timing of meal initiation and termination (5). These short-term signals include afferents transmitted via the vagus nerve from sensory systems intrinsic to the gastrointestinal tract, amines released from gastrointestinal neurons that modulate vagal function, and circulating or locally acting peptides, such as cholecystokinin, that are rapidly secreted from enteroendocrine cells upon nutrient ingestion. Whereas forebrain structures such as the hypothalamic arcuate nucleus are critical sites for sensing adiposity-related input such as leptin and insulin, information provided by these meal-related signals is conveyed primarily to hindbrain areas such as the nucleus of the solitary tract (6), with afferent vagal nerve fibers playing a central role in this process. Unlike adiposity signals, these meal-related signals collectively govern the amount of energy consumed during individual meals but are not generated in proportion to changes in body energy stores.

HYPOTHALAMIC TARGETS OF INSULIN SIGNALING

The arcuate nucleus, situated adjacent to the floor of the third ventricle in the mediobasal hypothalamus, contains neurons that respond to hormonal and nutrient-related afferent signals that are influenced by the size and state of adipose tissue stores and by recent food ingestion. Among them are neurons that coexpress neuropeptide Y (NPY) and Agouti-related peptide (AgRP), two peptides that potently stimulate food intake, reduce energy expenditure, and thus promote weight gain. The orexigenic actions of NPY are mediated via activation of Y1 and/or Y5 receptors (7), while those of AgRP arise from antagonism of neuronal melanocortin receptors (MC3r and MC4r) (8). Because melanocortin signaling reduces food intake and increases energy expenditure, blockade of melanocortin receptors by AgRP increases food intake and decreases energy expenditure, effects that are analogous to those of NPY. The anabolic actions of NPY and AgRP are mediated by "downstream" sites innervated by these "first order" insulin- and leptin-sensitive neurons. The hypothalamic paraventricular nucleus and the lateral hypothalamic area contain neurons that express receptors for both NPY and AgRP and are implicated as critical sites for relaying AgRP- and NPY-mediated signaling to downstream circuits that regulate energy homeostasis (9).

The arcuate nucleus also contains neurons that synthesize α-melanocyte stimulating hormone, a peptide that has powerful anorexic effects in the CNS. As with other melanocortin peptides, α-melanocyte stimulating hormone is derived from the precursor proopiomelanocortin (POMC) by posttranslational processing. Many POMC neurons in the arcuate nucleus also coexpress the cocaine and amphetamine-regulated transcript (CART) peptide. Like α-melanocyte stimulating hormone, CART reduces food intake, although its essential role in the anorexic actions of insulin and leptin is less well defined. These POMC/CART neurons project widely and densely innervate the same hypothalamic areas (e.g., paraventricular nucleus and lateral hypothalamic area) that are supplied by fibers from NPY/AgRP neurons (10). "Second order" neurons located in these downstream areas process signals from the arcuate nucleus and are hypothesized to transduce this afferent input into altered feeding behavior and energy metabolism. As discussed below, one mechanism whereby hypothalamic responses to input from adiposity-related hormones exert their behavioral and metabolic effects is via descending projections to hindbrain areas, such as the nucleus of the solitary tract (11,12), where they modulate the response to input from meal-related satiety signals such as cholecystokinin (13).

Receptors for insulin and leptin are concentrated in the arcuate nucleus, and available evidence suggests that while both hormones inhibit NPY/AgRP neurons, they have the opposite effects on POMC neurons (3). It is via this reciprocal regulation of anabolic and catabolic neuronal circuits that insulin and leptin are hypothesized to mediate their effects on energy balance (4). Similarly, adaptive responses that promote the recovery of lost weight are thought to arise, at least in part, from the activation of NPY/AgRP and inhibition of POMC neurons induced by reduced plasma levels of insulin and leptin that accompany depletion of body fat mass.

GHRELIN AND PEPTIDE $YY_{3–36}$

Two hormones secreted from the gastrointestinal tract exert opposing effects on energy balance via actions in the arcuate nucleus. Ghrelin, an acylated peptide secreted by cells in the gastric mucosa, stimulates food intake and is implicated in meal initiation (14). Peptide $YY_{3–36}$ ($PYY_{3–36}$), a close relative of NPY and member of the pancreatic polypeptide family, is secreted primarily from distal small intestine and colon and appears to paradoxically inhibit feeding (15). Plasma levels of ghrelin, the appetite-stimulating hormone, rise perceptibly in the blood before meals and fall after food is consumed, while the reverse is the case for $PYY_{3–36}$. Interestingly, it has been reported that both ghrelin and $PYY_{3–36}$ exert their feeding effects via a mechanism that involves reciprocal regulation of NPY/AgRP neurons in the ARC. Thus, $PYY_{3–36}$ was found to activate autoinhibitory Y2 receptors on NPY/AgRP cells and thereby reduce the release of anabolic peptides and increase the release of catabolic signals by decreasing inhibitory γ-aminobutyric acid input from NPY to POMC neurons (15). A note of caution is indicated because this weight-reducing effect is reported when $PYY_{3–36}$ is given into the circulation, despite its clear ability to increase food intake when infused directly into the CNS (via stimulation of Y1 and Y5 receptors) (7). By comparison, ghrelin exerts orexigenic effects by binding to its receptor (also known as the growth hormone secretagogue receptor) on NPY/AgRP neurons, thereby activating these cells (16).

REGULATION OF ENERGY EXPENDITURE

Evidence suggests that many of these same hormones and hypothalamic systems also regulate energy expenditure. This association was first recognized many years ago dur-

ing the study of diet-induced thermogenesis. Diet-induced thermogenesis is a robust phenomenon that arises from activation of the sympathetic nervous system (SNS) with subsequent triglyceride hydrolysis in brown adipose tissue (BAT) (17,18). This in turn stimulates heat production through mitochondrial oxidation of fatty acids that is augmented by uncoupling protein 1, a protein found uniquely in BAT mitochondria that establishes a proton leak favoring energy dissipation as heat. Insulin's importance to diet-induced thermogenesis was originally inferred from a study in rats showing that treatment with diazoxide, a potent inhibitor of insulin secretion, strongly attenuates the thermogenic response to a carbohydrate meal (19). Subsequent pharmacological studies demonstrated increased body temperature and energy expenditure, as well as reduced food intake, when insulin was injected into the hypothalamic ventromedial and paraventricular nuclei (20,21). These pharmacological studies suggested that insulin action in the hypothalamus coordinately reduces food intake while increasing SNS outflow to BAT to produce heat from fatty acid oxidation as a mechanism to increase energy expenditure.

Stemming from these observations is the notion that hyperinsulinemia might mediate the well-documented effect of obesity to increase SNS activity and hence contribute to obesity-associated hypertension (22). This possibility was further supported by evidence that systemic insulin administration increases plasma catecholamine levels (23). Indeed, both insulin and leptin were later shown to increase SNS activity (as determined from recordings of SNS nerve fibers), whether given peripherally or centrally (24,25). Furthermore, genetic mutations that reduced leptin or impaired its receptor signaling decrease SNS outflow, BAT thermogenesis, and resting energy expenditure (26).

A potentially confounding observation for the interpretation of this work is the vasodilatory effect of peripheral insulin administration to activate the SNS. However, the attendant increase of SNS activity appears to be independent of its effects on blood flow (23). An interesting extension of this work is the hypothesis that the elevated levels of free fatty acids common to obesity might arise from insulin-induced activation of the SNS (22). Such a mechanism could contribute to the common association of obesity, hypertension, elevated free fatty acids, and insulin resistance in individuals with type 2 diabetes, a hypothesis that has yet to be critically tested.

CNS INSULIN REGULATION OF HEPATIC GLUCOSE PRODUCTION

Interest in the role of CNS insulin signaling in plasma glucose regulation has increased with the recent demonstration that hepatic glucose production declines sharply during infusion of either insulin or a small-molecule insulin mimetic into the third cerebral ventricle and that this effect occurs independently of any change in circulating levels of insulin or other glucoregulatory hormones (27). This effect appears to depend upon neuronal signal transduction via the insulin receptor substrate (IRS)-phosphatidylinositol 3-OH kinase (PI3K) pathway, as intracerebroventricular (ICV) infusion of a PI3K inhibitor exerts the opposite effect. Adding considerable weight to these findings is evidence that hypothalamic insulin signaling is required for the normal control of glucose production by the liver, based on infusion into the third ventricle of insulin-specific antibodies or antisense oligonucleotides directed against the insulin receptor (28). Both interventions reduced hepatic sensitivity to circulating insulin and thereby increased hepatic glucose production, suggesting that insulin action in the brain is a physiological determinant of liver glucose metabolism. The efferent mechanism coupling insulin action in the brain to hepatic glucose metabolism is unknown but is presumed to involve autonomic innervation of the liver or other tissues. Of fundamental interest is the as yet unanswered question of whether defective hypothalamic insulin signaling contributes to the peripheral tissue insulin resistance and glucose intolerance characteristic of obesity and diabetes.

INSULIN SIGNAL TRANSDUCTION IN THE CNS

Insulin receptor activation in peripheral tissues is coupled to signal transduction pathways via the IRS family of adaptor molecules. IRS-1 was the first of this family to be identified, and while this protein appears to play an essential role in insulin signal transduction in some peripheral tissues, its role in CNS insulin action is uncertain. Despite being expressed diffusely throughout the CNS (29), IRS-1 is not concentrated in ventral hypothalamic nuclei where insulin receptors are found, and mice lacking IRS-1 do not have an abnormal energy homeostasis phenotype (30,31). An unexpected and fortuitous finding from IRS-1 knockout mice was the discovery of IRS-2. This protein is relatively abundant in the arcuate nucleus (32), as are insulin receptors. The hypothesis that IRS-2 is involved in hypothalamic insulin action on energy homeostasis is supported by evidence that insulin administration rapidly induces tyrosine phosphorylation of this protein in association with the production of phosphotidylinositol 3,4,5-trisphosphate, a signaling molecule generated by PI3K that couples IRS proteins to downstream effector molecules (33). Moreover, mice lacking IRS-2, particularly in the hypothalamus, exhibit increased food intake and body fat deposition and a major impairment of reproduction (34–36).

A key function of IRS proteins in peripheral tissues is to couple insulin receptor activation to signaling via the PI3K signal transduction pathway, and in neurons, PI3K is implicated as a key mediator of insulin action as well. In response to insulin administration, hypothalamic PI3K activation was detected within 15 min and shown to occur preferentially within cells of the mediobasal hypothalamus that also contain IRS-2 and phosphotidylinositol 3,4,5-trisphosphate (37). Also notable in response to ICV insulin infusion was the serine phosphorylation of hypothalamic Akt (also known as protein kinase B), a major downstream mediator of PI3K signaling. Insulin's ability to suppress food intake was blocked by administration of either of two PI3K inhibitors—wortmannin and LY294002—into the third cerebral ventricle, supporting a PI3K-dependent mechanism (37). As noted earlier, ICV infusion of LY294002 also causes hepatic insulin resistance, an effect similar to that induced by local blockade of insulin receptor signaling, whereas infusion of an antagonist of the mitogen-activated protein kinase pathway (which is also activated by the insulin receptor, at least in peripheral tissues) was without

effect (27). As in peripheral tissues, therefore, PI3K signaling appears to be critical for hypothalamic insulin action.

Support for the physiologic activation of this pathway has been provided by a study of hyperphagia induced by exposure to cold (in an effort to meet increased energy requirements), which was suggested to arise, at least in part, from diminished insulin signaling in the hypothalamus (32). Cold exposure was found to suppress the inhibitory effect of ICV insulin infusion on food intake compared with control animals maintained at room temperature. In the same study, cold exposure was associated with reduced phosphorylation of the insulin receptor and Akt (protein kinase B) in hypothalamic extracts, although the basal levels of both Akt phosphorylation and food intake were increased. That increased basal signaling via PI3K in the hypothalamus was ineffective in suppressing food intake suggests that cold exposure blocks signal transduction downstream of Akt as well.

CROSS TALK BETWEEN LEPTIN AND INSULIN

As noted earlier, NPY/AgRP neurons in the arcuate nucleus are suppressed by both insulin and leptin, while POMC/CART neurons are activated by these hormones. Therefore, insulin and leptin share in common the ability to suppress anabolic, while activating catabolic, regulatory neurocircuitry (3). Specific examples of this regulation stem from studies in which ICV insulin infusion was shown to block the effects of both fasting and streptozotocin-induced diabetes to increase expression of NPY mRNA in the arcuate nucleus (38,39). Conversely, central insulin administration increases hypothalamic POMC mRNA content, while SHU-9119, a melanocortin receptor antagonist, blocks the ability of ICV insulin to suppress food intake (40). As described for leptin, insulin-induced inhibition of food intake can therefore be explained, at least in part, by the combination of reduced NPY/AgRP signaling and increased melanocortin signaling.

When subthreshold doses of insulin and leptin are administered in combination, they have subadditive effects on short-term food intake, suggesting activation of redundant signaling mechanisms such as the activation of PI3K and of POMC neurons (41). Over time, however, the effects appear to be additive, suggesting activation of independent systems. This assertion appears to be at least partly true, since leptin action on energy homeostasis clearly requires signaling via the Janus kinase–signal transducers and activators of transcription (JAK-STAT) system (particularly JAK-2 and STAT-3) (42). In an effort to isolate the contribution of STAT-3 signaling to leptin action, Bates et al. (43) generated mice in which the native leptin receptor allele was replaced by a mutant that does not activate STAT-3. Mice homozygous for this mutant allele manifest marked hyperphagia and obesity but do not display the pronounced diabetes or infertility seen in *db/db* mice that lack competent leptin signaling capability. Signaling via STAT-3 therefore appears to be crucial for some (e.g., control of food intake and body weight), but not all (e.g., glucose metabolism and reproduction), actions of leptin in the brain. By comparison, JAK-STAT signaling has not been shown to be a critical mediator of neuronal actions of insulin.

While it is widely accepted that divergent cellular mechanisms mediate neuronal responses to insulin and leptin, substantial evidence also suggests that cross talk occurs between cell signaling pathways used by these two hormones. In addition to evidence that both leptin and insulin can increase hypothalamic signaling via PI3K, interaction between leptin and insulin at the level of JAK-STAT signaling has been documented in nonneuronal cells. In rat liver, insulin was shown to activate JAK-2 and to augment leptin-induced activation of STAT-3 signaling when the two hormones were given together (44). Cross talk may also exist in molecular mechanisms terminating signal transduction by insulin and leptin. For example, signaling via both leptin receptors and insulin receptors is terminated by the phosphatase protein tyrosine phosphatase-1B (45), and deficiency of this enzyme results in mice with increased sensitivity to both insulin and leptin (46). Perhaps more importantly, these mice are both lean and resistant to diet-induced obesity. These observations raise the possibility that high-fat feeding causes weight gain, at least in part, via attenuated signaling via adiposity signals, since the inability to attenuate this signaling (via the absence of protein tyrosine phosphatase-1B) confers protection against obesity in this model.

A second molecule implicated in termination of leptin signal transduction is suppressor of cytokine signaling 3 (SOCS3). Induced in the arcuate nucleus and other tissues following the binding of leptin to its receptor (via activation of the JAK-STAT pathway), SOCS3 binds to and inactivates both the leptin receptor and JAK-2, thus blocking further activation of STAT-3 (47). However, recent data implicate this mechanism in the termination of signaling by insulin as well. Specifically, SOCS3 can induce cellular insulin resistance by modification of the insulin receptor and IRS proteins, thereby impairing insulin signaling via PI3K (48). In addition, a recent selective brain knockout of SOCS3 demonstrated a loss in body weight associated with an enhanced leptin-induced STAT-3 phosphorylation and POMC induction. The mice were resistant to high-fat diet–induced weight gain and had increased sensitivity to insulin. While weight loss may have contributed, the simultaneous increase in leptin and insulin sensitivity is consistent with a combined direct CNS effect (49). A supportive study for a direct effect for SOCS3 to regulate insulin signaling was subsequently shown in adipose tissue in vitro (50). These considerations illustrate the potential of leptin-induced SOCS3 expression to downregulate hypothalamic signaling via both STAT-3 and IRS-PI3K. Moreover, insulin was shown to activate SOCS3 expression in a cell-based system via activation of Janus kinase and subsequent activation of STAT-5B (51), although whether insulin induces SOCS3 in vivo is unknown. Induction of SOCS3 is therefore an attractive potential mediator of hypothalamic resistance to the actions of both insulin and leptin and may contribute to the pathogenesis of common forms of obesity.

INSULIN, LEPTIN AND THE CONTROL OF NEURONAL ION CHANNELS

Work from Spanswick et al. (52) suggests that insulin action in at least a subset of hypothalamic neurons involves regulation of ATP-sensitive K^+ channels (K_{ATP} channels). As in pancreatic β-cells, these channels are inactivated (i.e., closed) by increased intracellular ATP

levels in response to oxidation of glucose or other substrates. K_{ATP} channel closure in turn raises intracellular concentrations of K^+, leading to membrane depolarization and increased firing rate. Thus, glucose-excited neurons are those that are activated (i.e., depolarized) by increased local concentrations of glucose, and these cells are relatively abundant in mediobasal hypothalamus. In response to insulin, K_{ATP} channels are activated (e.g., opened) within a few minutes, resulting in reduced neuronal firing owing to membrane hyperpolarization and increased K^+ conductance. This insulin effect is blocked by either of two inhibitors of PI3K, LY249002 and wortmannin, but not by several other enzyme inhibitors. However, a recent study by Wang et al. (53) indicates that these effects are not seen at a lower, more physiological glucose level and are completely opposite at hypoglycemic levels. They conclude that these neurons are downstream of NPY and POMC neurons and potentially play an integrating role for peripheral and central energy homeostasis.

Compelling evidence of cross talk between insulin and leptin was provided with the demonstration that leptin, like insulin, activates K_{ATP} channels in glucose-responsive hypothalamic neurons and that this activity is also blocked by PI3K inhibitors (54,55). Moreover, glucose-responsive neurons from Zucker fatty (*fa/fa*) rats that develop obesity as a result of a leptin receptor mutation are insensitive to both insulin and leptin. Thus, congenital leptin insensitivity is associated with hypothalamic resistance to insulin as well as leptin, a possibility originally suggested by the observation that ICV insulin inhibits neither food intake (56) nor NPY gene expression in these *fa/fa* rats (57).

That insulin and leptin act on the same pool of neurons is further demonstrated by studies showing that both hormones inhibit large conductance calcium-activated potassium channels in a hippocampal slice preparation (58,59). However, leptin's effect on these channels is largely related to activation of PI3K, whereas the insulin effect appeared to be more dependent on mitogen-activated protein kinase activation. Nevertheless, some of insulin's effects on these neurons were mimicked by the K_{ATP} channel opener diazoxide and were partially blocked by inhibitors of PI3K.

GLUCOSE UTILIZATION IN CNS INSULIN ACTION

While overall brain glucose utilization appears to be independent of insulin action and is unaffected by its administration in both animals and humans, the insulin-sensitive glucose transporter GLUT-4 is reported to be expressed in several brain areas. The highest levels are found in the cerebellum, olfactory bulb, and hippocampus, while lower amounts are present in the lateral hypothalamic area, the arcuate nucleus, and globus pallidus (60). Although the functional significance of this protein in the hypothalamus has been questioned, GLUT-4 mRNA has been identified in glucose-excited, glucose-inhibited, and nonglucosensing ventromedial nucleus neurons along with mRNA for glucokinase and the insulin receptor (61). However, studies have yet to demonstrate insulin-stimulated glucose transport in hypothalamic neurons, so the question of whether GLUT-4 participates in neuronal insulin signaling or in glucose-sensing mechanisms underlying food intake and body weight regulation remains uncertain.

A new transporter, GLUT-8, has also identified in the brain and localized specifically to the hippocampus, the cerebral cortex, and the hypothalamus (62). In hippocampus, this molecule is synthesized exclusively in neurons and has a novel intracellular distribution such that under basal conditions it is associated with intracellular organelles, but upon peripheral glucose administration it is redistributed to the plasma membrane (63). Based on these studies and on the absence of GLUT-8 trafficking under hyperglycemic-insulinopenic conditions in animals with streptozotocin-induced diabetes, insulin (but not glucose) is proposed to serve as the stimulus for GLUT-8 translocation in the rat hippocampus. The postulated role for this transporter revolves around the idea that glucose is liberated from oligosaccharides during protein glycosylation, events that occur in the rough endoplasmic reticulum, and that GLUT-8 transports glucose from this organelle into the cytoplasm.

Glucokinase, the glucose sensor of the β-cell, is also present in the CNS. In the arcuate nucleus, >75% of NPY-positive neurons express glucokinase (64), and intracarotid glucose infusions increased hypothalamic C-*fos* gene expression, which paralleled glucokinase expression (65). Glucokinase may therefore be a mediator of glucose-sensing in both glucose-responsive (also referred to as glucose-excited) and glucose-sensitive (also referred to as glucose-inhibited) neurons. Many glucokinase-expressing neurons coexpress K_{ATP} channels, and coexpression of GLUT-4 with insulin receptor mRNA is also reported in glucose-responsive neurons (61). Interactions among glucose-sensing, ion channel function, neuropeptide gene expression, and neuropeptide release are therefore likely but are complex and poorly understood. Future research is required to dissect the mechanisms underlying these processes and their contribution to the control of food intake and energy homeostasis.

BRAIN INSULIN IN WORMS AND FLIES: BIOLOGY EXPANDED

By 1997, molecules and signaling pathways homologous to the CNS insulin signaling system in mammals had been discovered in two very simple organisms: the nematode *Caenorhabitis elegans* and the fruit fly *Drosophila melanogaster* (66,67). In these invertebrates, insulin-related signaling is implicated not only in the regulation of fat storage and reproduction but surprisingly as a determinant of lifespan as well. The initial discovery in *C. elegans* was the cloning of *DAF-2*, the gene that encodes a homologue of the mammalian insulin receptor, containing both ligand binding and tyrosine kinase domains (68).

The relevance of DAF-2 to *C. elegans* physiology was initially based on its association with a stage of diapause arrest called "dauer." Entry into this developmental stage, characterized by inhibition of reproduction and reduced metabolism and growth that resembles suspended animation or hibernation, is normally triggered by periods of reduced food availability. Mutations of DAF-2 were shown to produce the dauer state and also revealed DAF-2 as the first step in a signal transduction cascade homologous to the insulin pathway described in mammals. DAF-2–mediated signaling is present in neurons, gastrointestinal tract cells, and muscles of this organism, as are other proteins

in this signal transduction pathway. One of these is a protein called advanced glycation end product (AGE)-1, a homologue of mammalian PI3K whose knockout induces the same dauer stage phenotype with increased longevity seen with mutation of DAF-2 (68). Another key protein is DAF-16, a member of the forkhead transcription factor family related to mammalian HNF-3 and FOXO1. The finding that DAF-16 mutation completely reversed the phenotype arising from DAF-2 or AGE-1 knockout (69) (a phenomenon referred to as genetic complementation) suggests that this forkhead protein functions downstream of the more proximal DAF-2 and AGE-1 proteins, that its activity is normally inhibited by activation of the upstream DAF-2/AGE-1 cascade, and that this inhibition is a dominant component of signaling in this cascade.

In their original report in 1997, Kimura et al. (68) suggested that following binding of an insulin-like ligand to DAF-2, AGE-1 is activated, and signaling downstream of this PI3K-like molecule (e.g., inhibition of DAF-16) is required to prevent dauer formation and to thereby limit lifespan. They further suggested that increased longevity associated with the DAF-2 knockout is analogous to the effect of caloric restriction to increase mammalian longevity, since calorically restricted animals experience decreases of both circulating insulin (and hence reduced insulin receptor signal transduction) and fertility. Three years later, this group restored DAF-2 signaling specifically to neurons, muscle, or intestine of animals that otherwise lack this protein, using promoters that selectively express DAF-2 cDNAs in each of these cell types (70).

Interestingly, only restoration of DAF-2 in neurons was sufficient to restore lifespan and reproduction of DAF-2 knockouts to wild-type values, and neuron-specific restoration of AGE-1 in animals that otherwise lack this protein produced the same effect. These neuron-specific "genetic rescue" experiments also reversed the pattern of excessive intestinal triglyceride deposition and associated metabolic defects. By comparison, restoration of DAF-2 or AGE-1 in muscle regulated metabolism but did not reverse the dauer stage or lifespan, and restoration in the intestine had relatively minor effects. Thus, neuronal insulin-like signaling appears to be a key regulator of various essential functions in *C. elegans*, and the metabolic and reproductive defects induced by whole-body deletion of DAF-2 appear to be attributable in large part to abnormalities specific to the absence of insulin-like signaling in the nervous system.

Recently, knockout of *FOXO1* in the mouse was reported to produce effects resembling those of DAF-16 deletion in *C. elegans* (71,72), indicating conservation of the function of these homologues over the course of evolution. While activation of DAF-2 can potentially occur via binding by any of several insulin-like ligands present in *C. elegans*, studies using DNA microarrays describe INS-7 as an insulin-like molecule that stimulates the DAF-2 pathway and in turn inhibits DAF-16 (73). This leads to suppression of a variety of antioxidant enzymes and heat shock proteins related to stress, while at the same time producing more egg yolk protein for reproduction and, surprisingly, stimulation of INS-7, which is released and hypothesized to stimulate and coordinate the adjacent cells of the animal (74). Thus, a feed-forward signaling mechanism may exist to coordinate the cells of *C. elegans* whereby INS-7 is released from neuronal cells to act upon adjacent cells and amplify the INS-7 signal. (Fig. 1*A*).

INSULIN-LIKE SIGNALING IN *DROSOPHILA*

In 2001, an analogous set of studies investigated the insulin receptor–like signaling system in the fruit fly, *Drosophila*. While the insulin-like receptor was first reported in this species in 1996, knockouts were found to be lethal; hence, insulin receptor–like activity is absolutely essential for life during development. However, mutations of either an IRS homologue termed CHICO, or complex heterozygotes of the insulin-like receptor, were shown to extend lifespan and reduce reproduction in a manner similar to that induced by DAF-2 mutants in *C. elegans* (75). As in *C. elegans*, lifespan extension was associated with a general growth deficiency and a decrease in cell number and size, and insulin-like signaling was shown to depend on a PI3K homologue. Additionally, the CHICO mutation increased lifespan and triglyceride storage and decreased body size (76). A downstream FOXO-like molecule (d FOXO) is described as well and, analogous to DAF-16 in *C. elegans*, it exerts a negative effect on feeding, growth, and organism size (77) (Fig. 1*B*).

While the role of insulin-like signaling in the regulation of carbohydrate storage and mobilization has not been studied in *Drosophilia*, surgical removal of the medial neurosecretory glands in the blowfly brain, which contain an insulin-like peptide, leads to hyperglycemia (78). Since these cells are also present in *Drosophila* brain, insulin-like molecules appear to be synthesized and secreted from neurosecretory cells and, via effects in brain, regulate lifespan and reproduction in flies, as in *C. elegans*. The findings in *C. elegans*, which possess neither adipose cells nor leptin-like molecules, are compatible with the hypothesis that the insulin signaling system for regulating energy homeostasis is evolutionarily older than leptin. These considerations also suggest that an early evolutionary role for insulin may have been to regulate metabolism through neuronal control of nutrient storage, a process tightly coupled with control of reproduction and lifespan, since both energy storage and reproduction depend upon nutrient availability. According to this hypothesis, the emergence of insulin as a key regulator of carbohydrate metabolism in vertebrates was a more recent evolutionary development.

INSULIN AND THE CONTROL OF REPRODUCTION AND LIFESPAN IN MAMMALS

Insulin signaling in the CNS of mammals, including humans, seems to have many biochemical, molecular, and physiological parallels with its role in invertebrates. For example, caloric restriction in mammals is associated with reduced secretion of insulin and reproductive hormones (e.g., follicle-stimulating hormone and leutinizing hormone) and, if prolonged, with extension of lifespan (79). Energy-restricted states are also associated with decreased adipose tissue mass and, consequently, of plasma leptin and insulin concentrations as well. Since insulin is a major regulator of leptin biosynthesis and release from adipocytes, low plasma concentrations of both hormones are usually found together and are associated with similar

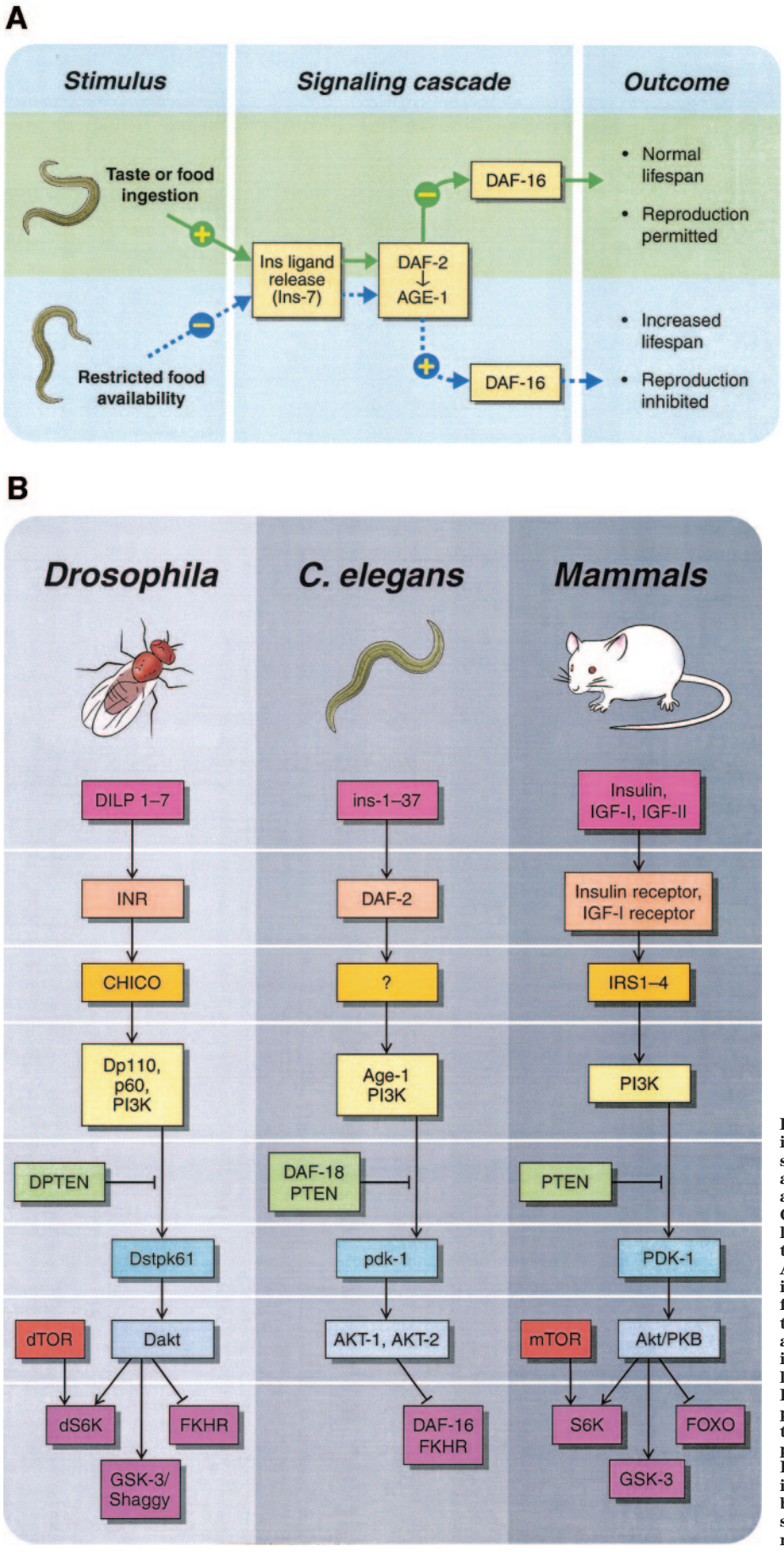

FIG. 1. ***A*****: Proposed role of insulin-like signaling in the control of lifespan, reproduction, and fat storage in** ***C. elegans*****. In response to the taste and/or ingestion of food, one or more INS ligands are released from neurosecretory cells in the CNS. Activation of the insulin receptor homologue, DAF-2, in CNS and elsewhere triggers intracellular signaling via the PI3K homologue AGE-1. The ultimate consequence of these events is the inactivation (by phosphorylation) of the forkhead transcription factor, DAF-16, an effect that permits normal growth, aging, reproduction, and fat storage. This cascade of events can be interrupted by inadequate nutrient availability, leading to reduced insulin signaling, activation of DAF-16, and subsequent entry into the dauer phase of development. Such animals are characterized by reduced growth and reproductive capacity and increased longevity and fat mass. Inactivating mutations of DAF-2 or AGE-1 also induce the dauer condition, and these effects are blocked by inactivation of DAF-16.** ***B*****: Insulin-like signaling in** ***C. elegans*****,** ***D. melanogaster*****, and mammals (adapted from 61).**

outcomes. However, in the fat cell–specific insulin receptor knockout mouse, lifespan extension occurs in an animal with a reduced fat mass, an increase in food intake, and an increase in plasma leptin but not in insulin compared with control animals. Therefore, low levels of insulin may be the critical factor in prolonging life in mammals as well as invertebrates (80,81).

These observations provide a compelling framework within which to consider the well-documented effect of caloric restriction to delay the onset of puberty. Women participating in vigorous exercise, with its attendant reduction in body fat stores, experience reductions of both circulating leptin and insulin and of pituitary reproductive hormones and consequently can have delayed puberty menstrual disturbances and reduced reproductive capacity (82,83). Recent studies (84) suggest that physiological leptin replacement during a short-term fast in men is sufficient to restore testosterone completely with improved pulsatile gonadotrophin release, suggesting that leptin deficiency plays an important role in the reproductive consequences of a deficient fat mass in humans. While the extent to which reduced insulin signaling might contribute to these reproductive consequences of deficient energy stores awaits further study, an effect seems likely given the redundancy in hypothalamic signaling mechanisms. Direct evidence in support of this assertion is provided by mice with selective knockout of the brain insulin receptor. These mice exhibit increased body fat and, interestingly, impaired reproductive capacity due to low pituitary leutinizing hormone and follicle-stimulating hormone secretion (85). Excessive weight gain due to reduced brain insulin signaling was also observed following selective deletion of the insulin receptor in rat hypothalamus (28).

Since the phenotype of leptin-deficient *ob/ob* and leptin-resistant *db/db* mice is also characterized by hypothalamic hypogonadism, deficiency of either leptin or insulin action in the CNS is sufficient to impair reproduction. These observations suggest that both the ability to store fat and to regulate the reproductive axis depend on signals informing the CNS that fat has been stored. This concept is further supported by studies of leptin-deficient children entering puberty. Available data suggest that hypogonadotrophic hypogonadism is a characteristic of such individuals and that this phenotype is ameliorated by leptin replacement (86). As a whole, this body of work suggests that genetic dissection of the insulin signaling system in *Drosophila* and *C. elegans* may lead to the identification of new metabolic end points and potential targets for future therapeutic intervention in diabetes, obesity, reproductive disorders, and/or lifespan extension.

Because insulin resistance is commonplace in peripheral tissues, it seems reasonable to hypothesize that a similar resistance phenomenon might occur in the brain. Were this to occur, the hypothalamus and pituitary might then perform their evolutionarily conserved roles and adjust peripheral physiology to the perceived deficit in fat stores, increasing energy storage while inhibiting reproduction. While this response might serve the organism well in situations of low nutrient availability, reduced CNS insulin action due to impaired signal transduction could potentially contribute to weight gain and reproductive complications in insulin resistance syndromes, such as polycystic ovarian disease (87). Clearly, the impact of such resistance would be magnified if the same biochemical defect were to affect leptin signal transduction as well, and the previous discussion highlighting cross talk between leptin and insulin signaling pathways emphasizes the feasibility of this possibility.

Especially intriguing in this regard is evidence that the downstream pathways controlling longevity and reproduction may be independent of one another (88). This conclusion is based on the suppression of DAF-2 activity in adult *C. elegans* (after the development of reproductive capacity) via application of RNA*i*. In these animals, lifespan is extended despite suppression of insulin-like signaling that was intact until a late stage of development. Furthermore, reproductive timing was specified independently of the dauer decision, suggesting that the DAF-2 pathway can function late in development to affect the timing of reproduction (88). Thus, while extension of longevity appeared at first to be invariably associated with impaired growth or reproduction, selective manipulation of this pathway may permit youthfulness and lifespan extension without affecting these other processes.

The report (89) and evaluation (90,91) of the Snell dwarf mouse suggest a similar extension of lifespan in this mammalian model, and its association with reduced insulin/IGF-1 signaling additionally supports the lifespan mechanisms uncovered in *C. elegans* and *Drosophila*. Recent work suggests that this phenomenon can be induced without the need for reduced food intake, as mice with selective knockout of the insulin receptor in adipose tissue have normal food intake, a 25% reduction in adipose mass, elevated leptin (relative to fat mass), relatively low plasma insulin, and normal fertility with an extended lifespan (81).

IMPLICATIONS FOR THE ASSOCIATION OF TYPE 2 DIABETES WITH OBESITY

A major mechanism linking obesity and type 2 diabetes is the effect of excessive body fat deposition to induce peripheral tissue insulin resistance, thereby increasing the demand on the β-cell, and if the increased secretory demand cannot be met, hyperglycemia ensues. However, this mechanism in and of itself may not fully explain the close association between obesity and diabetes, and viable alternatives warrant careful consideration. It is possible, for example, that a shared defect contributes to the pathogenesis of both disorders. According to this hypothesis, an underlying genetic defect or set of defects that are sensitive to environmental factors predispose first to positive energy balance and weight gain and, as the disorder progresses, to impaired glucose homeostasis and diabetes (Fig. 2). Since glucose intolerance is clearly exacerbated by obesity-induced peripheral insulin resistance, this model proposes that a feed-forward process is set in motion, whereby the more weight is gained the greater the deterioration of glucose homeostasis. Here, we suggest that although several factors can set this pathophysiological sequence in motion, impaired insulin signal transduction in tissues throughout the body, including the CNS, plays a fundamental role.

As should now be evident, both body weight regulation and glucose homeostasis rely in part on a common set of

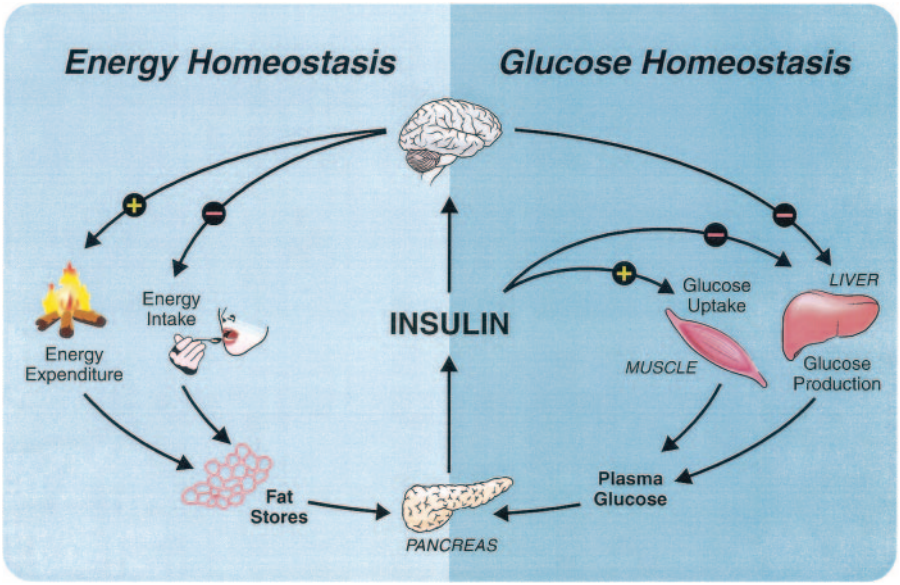

FIG. 2. Model for the link between diabetes and obesity related to insulin. Obesity (increased adipose mass) and diabetes (increased plasma glucose) are linked by a common dependence on insulin action and secretion in peripheral tissues and brain.

intracellular signaling mechanisms. It therefore follows that both processes would be impaired if reduced insulin signaling were to occur in tissues throughout the body. Due to defects in either insulin secretion or cellular insulin sensitivity (or both), insulin action is by definition reduced in tissues of individuals with type 2 diabetes. It therefore follows that unless tissue-specific defects are present in type 2 diabetes that spare the brain, CNS insulin action is likely to be reduced in affected individuals as well. If this were to occur early in the natural history of this disorder, excessive weight gain would be expected and could therefore serve as an initiating event (92).

Any of several mechanisms—defective insulin secretion, reduced blood-brain barrier insulin transport, or reduced neuronal responsiveness to insulin—can be invoked to explain how insulin action in the brain of affected individuals might be attenuated. Perhaps because of their overlapping roles in reproduction and nutrition, the same neuronal targets and signal transduction mechanisms used by insulin are also used by leptin. Hence, disruption of this signaling system is predicted to compromise negative feedback from all known adiposity signals, and increased caloric intake and storage is the predicted outcome.

Based on these considerations, impaired insulin secretion can also be viewed as a primary event, at least in some cases, since it is expected to favor weight gain, with other factors remaining unchanged. Because deficient insulin signaling in peripheral tissues increases hepatic glucose production and decreases glucose utilization, such weight gain should be coupled to a predisposition to hyperglycemia, depending on the magnitude of the secretory defect and the ability of islet β-cells to compensate.

Examples from the clinical literature are instructive for the evaluation of this hypothesis. Among them are patients with maturity-onset diabetes of the young type 2, characterized by a mild isolated defect of insulin secretion due to a coding region mutation of the glucokinase gene. Because this defect is readily and almost completely compensated for by hyperglycemia, at least initially, the underlying defect is difficult to detect (93). In such individuals, the contribution of an isolated mild impairment of insulin secretion to the development of obesity is inherently limited, since insulin levels remain near normal as glucose levels increase promptly. However, if the demand placed on the islet increases due to environmental factors (e.g., consumption of a high-fat diet) or when present in combination with a genetic propensity toward obesity, then the need to increase insulin secretion rises in a curvilinear fashion to compensate for the associated insulin resistance (94). Under these conditions, the insulin secretory defect may become more overt, and food intake and body weight will increase as a result. As weight increases, so does insulin resistance, and while this response may initially help to normalize plasma insulin levels and limit further weight gain, hyperglycemia will result if this β-cell compensation is incomplete. These considerations highlight the interwoven nature of consequences arising from reduced insulin signaling in both CNS and periphery that favor the association of diabetes with obesity.

The association between these two disorders may be further strengthened by peripheral metabolic consequences of reduced neuronal insulin signaling. For example, recent studies (27,28) demonstrate that reduced hypothalamic neuronal insulin signaling causes hepatic insulin resistance. If β-cell function is intact, increased insulin output can compensate completely or almost completely for insulin resistance, and both hyperglycemia and excessive storage of body fat will be minimized. However, any limitation to β-cell compensation will permit hyperglycemia to develop once insulin secretion falls below that needed to suppress hepatic glucose output and maintain normal glucose levels, and the propensity for obesity will again be increased. This vicious cycle can theoretically progress until insulin deficiency becomes severe, at which point glycosuria and unrestrained lipolysis become prominent and prevent further weight gain. Thus, whether the initial defect lies in the capacity to secrete insulin or to activate insulin signal transduction (in brain or periphery), food intake and weight will rise and the associated insulin resistance will lead to the development of hyperglycemia if β-cell compensation is impaired.

The rapid increase in obesity prevalence over the past 10–15 years in our society is, not unexpectedly, paralleled by an alarming increase in the prevalence of type 2

diabetes. Among many factors implicated in this trend is the ready availability of high-density, highly palatable foods of relatively low cost (95,96) and a lifestyle that demands little in the way of physical activity. The opportunity to study a population of Japanese Americans in Seattle, who have maintained their genetic identity by intermarriage into the second and third generation, allowed us to evaluate this progression prospectively to identify risk factors for the development of type 2 diabetes and the associated risk of cardiovascular disease (97). Among the potential risk factors in this population was a polymorphism in the β-cell glucokinase promoter that was both common (frequency 25%) and associated with a 30% reduction in the early insulin response to glucose and a significantly increased risk of impaired glucose tolerance (98,99). The functional significance of this −30 G/A polymorphism in Caucasians was recently confirmed and extended by Weedon et al. (100).

In studies by Fujimoto and colleagues (97,101), Japanese Americans progressing to type 2 diabetes were compared with those who did not at baseline and after 2.5 and 5 years of observation. While both progressor groups had impaired early insulin secretion at baseline compared with nonprogressors, increased abdominal obesity was present at baseline only among those progressing to diabetes within 2.5 years, while those who developed diabetes at 5 years did not develop excess intra-abdominal fat until that assessment. Thus, this population is characterized by a genetic risk factor for impaired insulin secretion whose progression from normal glucose tolerance to diabetes was associated with impaired insulin secretion 5 years before the development of intra-abdominal fat, the best obesity-related correlate of insulin resistance identified so far (102).

Based on the high prevalence of the glucokinase promoter polymorphism in this population, a high proportion of the population is hypothesized to have inherited a mild defect of insulin secretion that was not expressed clinically as long as body weight was not excessive. As the population aged and consumed an increasingly high-fat, highly palatable western diet, the frequency of obesity and impaired glucose tolerance increased. While a high-fat diet may induce a reduction of central insulin action to increase food intake and lead to obesity (103), we hypothesize that increased calorie ingestion in this population was also facilitated by relatively impaired insulin secretion, which in turn favored increased deposition of intra-abdominal fat and subsequent insulin resistance. Increased body fat in this situation can therefore be interpreted as compensation, in part, for a genetic defect in insulin secretion leading to increased insulin levels until a new steady state of obesity and hyperglycemia is reached. Final progression from impaired glucose tolerance or early type 2 diabetes to overt clinical hyperglycemia is associated with a progressive deterioration of β-cell function. This delayed deterioration is well described and may relate to toxic factors secondary to lipid (104) or glucose excess (105) and/or to amyloid deposition in islet β-cells (106, 107), leading to β-cell death and further impairment of insulin secretion despite treatment (108).

These observations support a model in which diabetes and obesity can be functionally linked to one another by β-cell abnormalities that predispose to both weight gain and hyperglycemia. This progression is exacerbated by environmental factors and common gene variants that favor weight gain and further increase the demand on the β-cell. Genetic defects that can contribute to this pathophysiological sequence are likely to be common, varied in nature, and modest with respect to their functional consequences. Inheritance of one or more of these gene variants, in combination with environmental factors, is presumably required to produce the obesity and insulin resistance syndromes. In addition to primary lesions affecting insulin secretion, candidate genes could also include those predisposing to insulin resistance. In isolation, such defects may have a limited impact, but in the presence of environmental factors that predispose to β-cell stress and/or damage (such as high fat feeding [104], mild chronic hyperglycemia [105], or peripheral insulin resistance) could suffice to set this pathophysiological sequence in motion. Regardless of whether underlying gene defects affect insulin secretion or action, those individuals with impaired β-cell responses would tend to be the most obese and therefore have the greatest risk for the development of the obesity-diabetes syndrome.

From this perspective, preventive treatments aimed at either insulin resistance or impaired insulin secretion should slow the progression of hyperglycemia. While supporting this hypothesis, recent experience with the thiazolidinedione class of insulin-sensitizing drugs, which reduce hyperglycemia and hyperinsulinemia, demonstrates a key problem: a high prevalence of undesirable weight gain (109). This weight gain may be related to the propensity of these agents to differentiate preadipocytes into mature cells and to favor fat deposition. However, the insulin- and leptin-lowering effects of these drugs might aggravate obesity by reducing adiposity-related signaling in the hypothalamus.

ACKNOWLEDGMENTS

This work was supported by the Department of Veterans Affairs Career Development Program, Merit Review Research Program, Research Enhancement Award Program, Research Career Scientist Award, and National Institutes of Health Grants DK17046, DK12829, DK52989, NS32273, and DK035816.

The authors thank Mark Baskin for preparation of the figures.

REFERENCES

1. Hoyer S: Glucose metabolism and insulin receptor signal transduction in Alzheimer disease. *Eur J Pharmacol* 490:115–125, 2004
2. Craft S, Watson GS: Insulin and neurodegenerative disease: shared and specific mechanisms. *Lancet Neurol* 3:169–178, 2004
3. Schwartz MW: Central nervous system control of food intake. *Nature* 404:661–671, 2000
4. Porte D Jr, Baskin DG, Schwartz MW: Leptin and insulin action in the central nervous system. *Nutr Rev* 60:S20–S29, 2002 [discussion S68–S84, S85–S87]
5. Woods SC: Gastrointestinal satiety signals I. An overview of gastrointestinal signals that influence food intake. *Am J Physiol Gastrointest Liver Physiol* 286:G7–G13, 2004
6. Grill HJ, Kaplan JM: The neuroanatomical axis for control of energy balance. *Front Neuroendocrinol* 23:2–40, 2002
7. Raposinho PD, Pedrazzini T, White RB, Palmiter RD, Aubert ML: Chronic neuropeptide Y infusion into the lateral ventricle induces sustained

feeding and obesity in mice lacking either Npy1r or Npy5r expression. *Endocrinology* 145:304–310, 2004
8. Ellacott KL, Cone RD: The central melanocortin system and the integration of short- and long-term regulators of energy homeostasis. *Recent Prog Horm Res* 59:395–408, 2004
9. Cowley MA, Pronchuk N, Fan W, Dinulescu DM, Colmers WF, Cone RD: Integration of NPY, AGRp, and melanocortin signals in the hypothalamic paraventricular nucleus: evidence of a cellular basis for the adipostat. *Neuron* 24:155–163, 1999
10. Fekete C, Wittmann G, Liposits Z, Lechan RM: Origin of cocaine- and amphetamine-regulated transcript (CART)-immunoreactive innervation of the hypothalamic paraventricular nucleus. *J Comp Neurol* 469:340–350, 2004
11. Blevins JE, Schwartz MW, Baskin DG: Evidence that paraventricular nucleus oxytocin neurons link hypothalamic leptin action to caudal brain stem nuclei controlling meal size. *Am J Physiol Regul Integr Comp Physiol* 287:R87–R96, 2004
12. Blevins JE, Eakin TJ, Murphy JA, Schwartz MW, Baskin DG: Oxytocin innervation of caudal brainstem nuclei activated by cholecystokinin. *Brain Res* 993:30–41, 2003
13. Schwartz GJ, Moran TH: Leptin and neuropeptide y have opposing modulatory effects on nucleus of the solitary tract neurophysiological responses to gastric loads: implications for the control of food intake. *Endocrinology* 143:3779–3784, 2002
14. Cummings DE, Foster KE: Ghrelin-leptin tango in body-weight regulation. *Gastroenterology* 124:1532–1535, 2003
15. Batterham RL, Cowley MA, Small CJ, Herzog H, Cohen MA, Dakin CL, Wren AM, Brynes AE, Low MJ, Ghatei MA, Cone RD, Bloom SR: Gut hormone PYY(3–36) physiologically inhibits food intake. *Nature* 418:650–654, 2002
16. Willesen MG, Kristensen P, Romer J: Co-localization of growth hormone secretagogue receptor and NPY mRNA in the arcuate nucleus of the rat. *Neuroendocrinology* 70:306–316, 1999
17. Rothwell NJ, Stock MJ: Brown adipose tissue and diet-induced thermogenesis. In *Brown Adipose Tissue*. Trayhurn P, Nicholls DG, Eds. New York, Edward Arnold, 1986, p. 269–298
18. Stock MJ: Gluttony and thermogenesis revisited. *Int J Obes Relat Metab Disord* 23:1105–1117, 1999
19. Rothwell NJ, Saville ME, Stock MJ: Role of insulin in thermogenic responses to refeeding in 3-day-fasted rats. *Am J Physiol* 245:E160–E165, 1983
20. Menendez JA, Atrens DM: Insulin and the paraventricular hypothalamus: modulation of energy balance. *Brain Res* 555:193–201, 1991
21. McGowan MK, Andrews KM, Grossman SP: Chronic intrahyphothalamic infusions of insulin or insulin antibodies alter body weight and food intake in the rat. *Physol Behav* 51:753–766, 1992
22. Landsberg L: Insulin-mediated sympathetic stimulation: role in the pathogenesis of obesity-related hypertension (or, how insulin affects blood pressure, and why). *J Hypertens* 19:523–528, 2001
23. Scherrer U, Sartori C: Insulin as a vascular and sympathoexcitatory hormone: implications for blood pressure regulation, insulin sensitivity, and cardiovascular morbidity. *Circulation* 96:4104–4113, 1997
24. Dunbar JC, Lu H: Leptin-induced increase in sympathetic nervous and cardiovascular tone is mediated by proopiomelanocortin (POMC) products. *Brain Res Bull* 50:215–221, 1999
25. Muntzell MS, Anderson EA, Johnson AK, Mark AL: Mechanisms of insulin action on sympathetic nerve activity. *Clin Exp Hypertens* 17:39–50, 1995
26. Himms-Hagen J: Defective brown adipose tissue thermogenesis in obese mice. *Int J Obes* (Suppl. 2):17–24, 1985
27. Obici S, Zhang BB, Karkanias G, Rossetti L: Hypothalamic insulin signaling is required for inhibition of glucose production. *Nat Med* 8:1376–1382, 2002
28. Obici S, Feng Z, Karkanias G, Baskin DG, Rossetti L: Decreasing hypothalamic insulin receptors causes hyperphagia and insulin resistance in rats. *Nat Neurosci* 5:566–572, 2002
29. Baskin DG, Sipols AJ, Schwartz MW, White MF: Immunocytochemical detection of insulin receptor substrate-1 (IRS-1) in rat brain: colocalization with phosphotyrosine. *Regul Pept* 48:257–266, 1993
30. Araki E, Lipes MA, Patti ME, Bruning JC, Haag B 3rd, Johnson RS, Kahn CR: Alternative pathway of insulin signaling in mice with targeted disruption of the IRS-1 gene. *Nature* 372:186–190, 1994
31. Tamemoto H, Kadowaki T, Tobe K, Yagi T, Sakura H, Hayakawa T, Terauchi Y, Ueki K, Kaburagi Y, Satoh S, et al.: Insulin resistance and growth retardation in mice lacking insulin receptor substrate-1. *Nature* 372:182–186, 1994
32. Torsoni MA, Carvalheira JB, Pereira-Da-Silva M, de Carvalho-Filho MA, Saad MJ, Velloso LA: Molecular and functional resistance to insulin in hypothalamus of rats exposed to cold. *Am J Physiol Endocrinol Metab* 285:E216–E223, 2003
33. Niswender KD, Schwartz MW: Insulin and leptin revisited: adiposity signals with overlapping physiological and intracellular signaling capabilities. *Front Neuroendocrinol* 24:1–10, 2003
34. Kubota N, Terauchi Y, Tobe K, Yano W, Suzuki R, Ueki K, Takamoto I, Satoh H, Maki T, Kubota T, Moroi M, Okada-Iwabu M, Ezaki O, Nagai R, Ueta Y, Kadowaki T, Noda T: Insulin receptor substrate 2 plays a crucial role in beta cells and the hypothalamus. *J Clin Invest* 114:917–927, 2004
35. Lin X, Taguchi A, Park S, Kushner JA, Li F, Li Y, White MF: Dysregulation of insulin receptor substrate 2 in beta cells and brain causes obesity and diabetes. *J Clin Invest* 114:908–916, 2004
36. Burks DJ, de Mora JF, Schubert M, Withers DJ, Myers MG, Towery HH, Altamuro SL, Flint CL, White MF: IRS-2 pathways integrate female reproduction and energy homeostasis. *Nature* 407:377–382, 2000
37. Niswender KD, Morrison CD, Clegg DJ, Olson R, Baskin DG, Myers MG Jr, Seeley RJ, Schwartz MW: Insulin activation of phosphatidylinositol 3-kinase in the hypothalamic arcuate nucleus: a key mediator of insulin-induced anorexia. *Diabetes* 52:227–231, 2003
38. Schwartz MW, Sipols AJ, Marks JL, Sanacora G, White JD, Scheurinck A, Kahn SE, Baskin DG, Woods SC, Figlewicz DP, Porte D Jr: Inhibition of hypothalamic neuropeptide Y gene expression by insulin. *Endocrinology* 130:3608–3616, 1992
39. Sipols AJ, Baskin DG, Schwartz MW: Effect of intracerebroventricular insulin infusion on diabetic hyperphagia and hypothalamic neuropeptide gene expression. *Diabetes* 44:147–151, 1995
40. Benoit SC, Air EL, Coolen LM, Strauss R, Jackman A, Clegg DJ, Seeley RJ, Woods SC: The catabolic action of insulin in the brain is mediated by melanocortins. *J Neurosci* 22:9048–9052, 2002
41. Air EL, Benoit SC, Clegg DJ, Seeley RJ, Woods SC: Insulin and leptin combine additively to reduce food intake and body weight in rats. *Endocrinology* 143:2449–2452, 2002
42. Bates SH, Myers MG: The role of leptin→STAT3 signaling in neuroendocrine function: an integrative perspective. *J Mol Med* 82:12–20, 2004
43. Bates SH, Stearns WH, Dundon TA, Schubert M, Tso AW, Wang Y, Banks AS, Lavery HJ, Haq AK, Maratos-Flier E, Neel BG, Schwartz MW, Myers MG: STAT3 signalling is required for leptin regulation of energy balance but not reproduction. *Nature* 421:856–859, 2003
44. Carvalheira JB, Ribeiro EB, Folli F, Velloso LA, Saad MJ: Interaction between leptin and insulin signaling pathways differentially affects JAK-STAT and PI 3-kinase-mediated signaling in rat liver. *Biol Chem* 384:151–159, 2003
45. Zabolotny JM, Bence-Hanulec KK, Stricker-Krongrad A, Haj F, Wang Y, Minokoshi Y, Kim YB, Elmquist JK, Tartaglia LA, Kahn BB, Neel BG: PTP1B regulates leptin signal transduction in vivo. *Dev Cell* 2:489–495, 2002
46. Elchebly M, Cheng A, Tremblay ML: Modulation of insulin signaling by protein tyrosine phosphatases. *J Mol Med* 78:473–482, 2000
47. Pirola L, Johnston AM, Van Obberghen E: Modulation of insulin action. *Diabetologia* 47:170–184, 2004
48. Ueki K, Kondo T, Kahn CR: Suppressor of cytokine signaling 1 (SOCS-1) and SOCS-3 cause insulin resistance through inhibition of tyrosine phosphorylation of insulin receptor substrate proteins by discrete mechanisms. *Mol Cell Biol* 24:5434–5446, 2004
49. Mori H, Hanada R, Hanada T, Aki D, Mashima R, Nishinakamura H, Torisu T, Chien KR, Yasukawa H, Yoshimura A: Socs3 deficiency in the brain elevates leptin sensitivity and confers resistance to diet-induced obesity. *Nat Med* 10:739–743, 2004
50. Shi H, Tzameli I, Bjorbaek C, Flier JS: Suppressor of cytokine signaling 3 is a physiological regulator of adipocyte insulin signaling. *J Biol Chem* 279:34733–34740, 2004
51. Sadowski CL, Choi TS, Le M, Wheeler TT, Wang LH, Sadowski HB: Insulin induction of SOCS-2 and SOCS-3 mRNA expression in C2C12 skeletal muscle cells is mediated by Stat5*. *J Biol Chem* 276:20703–20710, 2001
52. Spanswick D, Smith MA, Mirshamsi S, Routh VH, Ashford ML: Insulin activates ATP-sensitive K+ channels in hypothalamic neurons of lean, but not obese rats. *Nat Neurosci* 3:757–758, 2000
53. Wang R, Liu X, Hentges ST, Dunn-Meynell AA, Levin BE, Wang W, Routh VH: The regulation of glucose-excited neurons in the hypothalamic arcuate nucleus by glucose and feeding-relevant peptides. *Diabetes* 53:1959–1965, 2004
54. Harvey J, Ashford ML: Leptin in the CNS: much more than a satiety signal. *Neuropharmacology* 44:845–854, 2003
55. Mirshamsi S, Laidlaw HA, Ning K, Anderson E, Burgess LA, Gray A, Sutherland C, Ashford ML: Leptin and insulin stimulation of signalling

pathways in arcuate nucleus neurones: PI3K dependent actin reorganization and KATP channel activation. *BMC Neurosci* 5:54, 2004
56. Ikeda H, West DB, Pustek JJ, Figlewicz DP, Greenwood MRC, Porte D Jr, Woods SC: Intraventricular insulin reduces food intake and body weight of lean but not obese Zucker rats. *Appetite* 7:381–386, 1986
57. Schwartz MW, Marks J, Sipols AJ, Baskin DG, Woods SC, Kahn SE, Porte D Jr: Central insulin administration reduces neuropeptide Y mRNA expression in the arcuate nucleus of food-deprived lean (Fa/Fa) but not obese (fa/fa) Zucker rats. *Endocrinology* 128:2645–2647, 1991
58. O'Malley D, Shanley LJ, Harvey J: Insulin inhibits rat hippocampal neurones via activation of ATP-sensitive K+ and large conductance Ca2+-activated K+ channels. *Neuropharmacology* 44:855–863, 2003
59. Shanley LJ, Irving AJ, Rae MG, Ashford ML, Harvey J: Leptin inhibits rat hippocampal neurons via activation of large conductance calcium-activated K+ channels. *Nat Neurosci* 5:299–300, 2002
60. Choeiri C, Staines W, Messier C: Immunohistochemical localization and quantification of glucose transporters in the mouse brain. *Neuroscience* 111:19–34, 2002
61. Kang L, Routh VH, Kuzhikandathil EV, Gaspers LD, Levin BE: Physiological and molecular characteristics of rat hypothalamic ventromedial nucleus glucosensing neurons. *Diabetes* 53:549–559, 2004
62. Ibberson M, Uldry M, Thorens B: GLUTX1: a novel mammalian glucose transporter expressed in the central nervous system and insulin-sensitive tissues. *J Biol Chem* 275:4607–4612, 2000
63. Piroli GG, Grillo CA, Hoskin EK, Znamensky V, Katz EB, Milner TA, McEwen BS, Charron MJ, Reagan LP: Peripheral glucose administration stimulates the translocation of GLUT8 glucose transporter to the endoplasmic reticulum in the rat hippocampus. *J Comp Neurol* 452:103–114, 2002
64. Lynch RM, Tompkins LS, Brooks HL, Dunn-Meynell AA, Levin BE: Localization of glucokinase gene expression in the rat brain. *Diabetes* 49:693–700, 2000
65. Dunn-Meynell AA, Routh VH, Kang L, Gaspers L, Levin BE: Glucokinase is the likely mediator of glucosensing in both glucose-excited and glucose-inhibited central neurons. *Diabetes* 51:2056–2065, 2002
66. Guarente L, Kenyon C: Genetic pathways that regulate ageing in model organisms. *Nature* 408:255–262, 2000
67. Garofalo RS: Genetic analysis of insulin signaling in Drosophila. *Trends Endocrinol Metab* 13:156–162, 2002
68. Kimura KD, Tissenbaum HA, Liu Y, Ruvkun G: daf-2, an insulin receptor-like gene that regulates longevity and diapause in Caenorhabditis elegans. *Science* 277:942–946, 1997
69. Ogg S, Paradis S, Gottlieb S, Patterson GI, Lee L, Tissenbaum HA, Ruvkun G: The fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in *C. elegans*. *Nature* 389:994–999, 1997
70. Wolkow CA, Kimura KD, Lee MS, Ruvkun G: Regulation of *C. elegans* life-span by insulinlike signaling in the nervous system. *Science* 290:147–150, 2000
71. Kitamura T, Nakae J, Kitamura Y, Kido Y, Biggs WH 3rd, Wright CV, White MF, Arden KC, Accili D: The forkhead transcription factor Foxo1 links insulin signaling to Pdx1 regulation of pancreatic beta cell growth. *J Clin Invest* 110:1839–1847, 2002
72. Nakae J, Biggs WH 3rd, Kitamura T, Cavenee WK, Wright CV, Arden KC, Accili D: Regulation of insulin action and pancreatic beta-cell function by mutated alleles of the gene encoding forkhead transcription factor Foxo1. *Nat Genet* 32:245–253, 2002
73. Murphy CT, McCarroll SA, Bargmann CI, Fraser A, Kamath RS, Ahringer J, Li H, Kenyon C: Genes that act downstream of DAF-16 to influence the lifespan of Caenorhabditis elegans. *Nature* 424:277–283, 2003
74. Gems D, McElwee JJ: Ageing: microarraying mortality. *Nature* 424:259–261, 2003
75. Tatar M, Kopelman A, Epstein D, Tu MP, Yin CM, Garofalo RS: A mutant Drosophila insulin receptor homolog that extends life-span and impairs neuroendocrine function. *Science* 292:107–110, 2001
76. Bohni R, Riesgo-Escovar J, Oldham S, Brogiolo W, Stocker H, Andruss BF, Beckingham K, Hafen E: Autonomous control of cell and organ size by CHICO, a Drosophila homolog of vertebrate IRS1-4. *Cell* 97:865–875, 1999
77. Puig O, Marr MT, Ruhf ML, Tjian R: Control of cell number by Drosophila FOXO: downstream and feedback regulation of the insulin receptor pathway. *Genes Dev* 17:2006–2020, 2003
78. Rulifson EJ, Kim SK, Nusse R: Ablation of insulin-producing neurons in flies: growth and diabetic phenotypes. *Science* 296:1118–1120, 2002
79. Weindruch R, Sohal RS: Seminars in medicine of the Beth Israel Deaconess Medical Center: caloric intake and aging. *N Engl J Med* 337:986–994, 1997
80. Bluher M, Michael MD, Peroni OD, Ueki K, Carter N, Kahn BB, Kahn CR: Adipose tissue selective insulin receptor knockout protects against obesity and obesity-related glucose intolerance. *Dev Cell* 3:25–38, 2002
81. Bluher M, Kahn BB, Kahn CR: Extended longevity in mice lacking the insulin receptor in adipose tissue. *Science* 299:572–574, 2003
82. Frisch RE, Gotz-Welbergen AV, McArthur JW, Albright T, Witschi J, Bullen B, Birnholz J, Reed RB, Hermann H: Delayed menarche and amenorrhea of college athletes in relation to age of onset of training. *JAMA* 246:1559–1563, 1981
83. Frisch RE, McArthur JW: Menstrual cycles: fatness as a determinant of minimum weight for height necessary for their maintenance or onset. *Science* 185:949–951, 1974
84. Chan JL, Heist K, DePaoli AM, Veldhuis JD, Mantzoros CS: The role of falling leptin levels in the neuroendocrine and metabolic adaptation to short-term starvation in healthy men. *J Clin Invest* 111:1409–1421, 2003
85. Bruning JC, Gautam D, Burks DJ, Gillette J, Schubert M, Orban PC, Klein R, Krone W, Muller-Wieland D, Kahn CR: Role of brain insulin receptor in control of body weight and reproduction. *Science* 289:2122–2125, 2000
86. Farooqi IS, Matarese G, Lord GM, Keogh JM, Lawrence E, Agwu C, Sanna V, Jebb SA, Perna F, Fontana S, Lechler RI, DePaoli AM, O'Rahilly S: Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. *J Clin Invest* 110:1093–1103, 2002
87. Dunaif A, Thomas A: Current concepts in the polycystic ovary syndrome. *Annu Rev Med* 52:401–419, 2001
88. Dillin A, Crawford DK, Kenyon C: Timing requirements for insulin/IGF-1 signaling in *C. elegans*. *Science* 298:830–834, 2002
89. Flurkey K, Papaconstantinou J, Harrison DE: The Snell dwarf mutation Pit1(dw) can increase life span in mice. *Mech Ageing Dev* 123:121–130, 2002
90. Hsieh CC, DeFord JH, Flurkey K, Harrison DE, Papaconstantinou J: Effects of the Pit1 mutation on the insulin signaling pathway: implications on the longevity of the long-lived Snell dwarf mouse. *Mech Ageing Dev* 123:1245–1255, 2002
91. Hsieh CC, DeFord JH, Flurkey K, Harrison DE, Papaconstantinou J: Implications for the insulin signaling pathway in Snell dwarf mouse longevity: a similarity with the *C. elegans* longevity paradigm. *Mech Ageing Dev* 123:1229–1244, 2002
92. Porte D Jr, Seeley RJ, Woods SC, Baskin DG, Figlewicz DP, Schwartz MW: Obesity, diabetes and the central nervous system. *Diabetologia* 41:863–881, 1998
93. Kahn SE, Porte D Jr: Pathophysiology and genetics of type II diabetes mellitus. In *Diabetes Mellitus*. 6th ed. Porte D Jr, Sherwin RS, Baron A, Eds. New York, McGraw Hill, 2003, p. 331–366
94. Kahn SE, Prigeon RL, McCulloch DK, Boyko EJ, Bergman RN, Schwartz MW, Neifing JL, Ward WK, Beard JC, Palmer JP, Porte D Jr: Quantification of the relationship between insulin sensitivity and B-cell function in human subjects: evidence for a hyperbolic function. *Diabetes* 42:1663–1672, 1993
95. Kawamori R: Diabetes trends in Japan. *Diabetes Metab Res Rev* (Suppl. 3):S9–S13, 2002
96. van Dam RM, Rimm EB, Willett WC, Stampfer MJ, Hu FB: Dietary patterns and risk for type 2 diabetes mellitus in U.S. men. *Ann Intern Med* 136:201–209, 2002
97. Fujimoto WY, Bergstrom RW, Boyko EJ, Chen K, Kahn SE, Leonetti DL, McNeely MJ, Newell LL, Shofer JB, Wahl PW: Type 2 diabetes and the metabolic syndrome in Japanese Americans. *Diabetes Res Clin Pract* (Suppl. 2):S73–S76, 2000
98. Stone LM, Kahn SE, Deeb SS, Fujimoto WY, Porte D Jr: Glucokinase gene variations in Japanese-Americans with a family history of NIDDM. *Diabetes Care* 17:1480–1483, 1994
99. Stone LM, Kahn SE, Deeb SS, Fujimoto WY, Porte D Jr: A variation at position −30 of the B-cell glucokinase gene promoter is associated with reduced B-cell function in middle-aged Japanese-American men. *Diabetes* 45:422–428, 1996
100. Weedon MN, Frayling TM, Shields B, Knight B, Turner T, Metcalf BS, Voss L, Wilkin TJ, McCarthy A, Ben-Shlomo Y, Davey Smith G, Ring S, Jones R, Golding J, Byberg L, Mann V, Axelsson T, Syvanen AC, Leon D, Hattersley AT: Genetic regulation of birth weight and fasting glucose by a common polymorphism in the islet cell promoter of the glucokinase gene. *Diabetes* 54:576–581, 2005
101. Chen K, Boyko EJ, Bergstrom RW, Leonetti DL, Newell-Morris L, Wahl PW, Fujimoto WY: Earlier appearance of impaired insulin secretion than visceral adiposity in the pathogenesis of NIDDM: 5-year follow-up of initially nondiabetic Japanese-American men. *Diabetes Care* 18:747–753, 1995
102. Cnop M, Landchild MJ, Vidal J, Havel PJ, Knowles NG, Carr DR, Wang F,

Hull RL, Boyko EJ, Retzlaff BM, Walden CE, Knopp RH, Kahn SE: The concurrent accumulation of intra-abdominal and subcutaneous fat explains the association between insulin resistance and plasma leptin concentrations: distinct metabolic effects of two fat compartments. *Diabetes* 51:1005–1015, 2002
103. Chavez M, Riedy CA, Van Dijk G, Woods SC: Central insulin and macronutrient intake in the rat. *Am J Physiol* 271:R727–R731, 1996
104. Unger RH: Lipotoxic diseases. *Annu Rev Med* 53:319–336, 2002
105. Poitout V, Robertson RP: Minireview: secondary beta-cell failure in type 2 diabetes: a convergence of glucotoxicity and lipotoxicity. *Endocrinology* 143:339–342, 2002
106. Kahn SE, Andrikopoulos S, Verchere CB: Islet amyloid: a long-recognized but underappreciated pathological feature of type 2 diabetes. *Diabetes* 48:241–253, 1999
107. Porte D Jr, Kahn SE: β-Cell dysfunction and failure in type 2 diabetes: potential mechanisms. *Diabetes* (Suppl. 1):S160–S163, 2001
108. Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC: β-Cell deficit and increased β-cell apoptosis in humans with type 2 diabetes. *Diabetes* 52:102–110, 2003
109. Chilcott J, Tappenden P, Jones ML, Wight JP: A systematic review of the clinical effectiveness of pioglitazone in the treatment of type 2 diabetes mellitus. *Clin Ther* 23:1792–1823, 2001 [discussion 1791]