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REVIEW

Pancreatic regulation of glucose homeostasis

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In order to ensure normal body function, the human body is dependent on a tight control of its blood glucose levels. This is accomplished by a highly sophisticated network of various hormones and neuropeptides released mainly from the brain, pancreas, liver, intestine as well as adipose and muscle tissue. Within this network, the pancreas represents a key player by secreting the blood sugar-lowering hormone insulin and its opponent glucagon. However, disturbances in the interplay of the hormones and peptides involved may lead to metabolic disorders such as type 2 diabetes mellitus (T2DM) whose prevalence, comorbidities and medical costs take on a dramatic scale. Therefore, it is of utmost importance to uncover and understand the mechanisms underlying the various interactions to improve existing anti-diabetic therapies and drugs on the one hand and to develop new therapeutic approaches on the other. This review summarizes the interplay of the pancreas with various other organs and tissues that maintain glucose homeostasis. Furthermore, anti-diabetic drugs and their impact on signaling pathways underlying the network will be discussed.
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THE PANCREAS IS AN EXOCRINE AND ENDOCRINE ORGAN

The pancreas has key roles in the regulation of macronutrient digestion and hence metabolism/energy homeostasis by releasing various digestive enzymes and pancreatic hormones. It is located behind the stomach within the left upper abdominal cavity and is partitioned into head, body and tail. The majority of this secretory organ consists of acinar—or exocrine—cells that secrete the pancreatic juice containing digestive enzymes, such as amylase, pancreatic lipase and trypsinogen, into the ducts, that is, the main pancreatic and the accessory pancreatic duct. In contrast, pancreatic hormones are released in an endocrine manner, that is, direct secretion into the blood stream. The endocrine cells are clustered together, thereby forming the so-called islets of Langerhans, which are small, island-like structures within the exocrine pancreatic tissue that account for only 1–2% of the entire organ (Figure 1).[1] There are five different cell types releasing various hormones from the endocrine system: glucagon-producing α-cells,[2] which represent 15–20% of the total islet cells; amylin-, C-peptide- and insulin-producing β-cells,[2] which account for 65–80% of the total cells; pancreatic polypeptide (PP)-producing γ-cells,[3] which comprise 3–5% of the total islet cells; somatostatin-producing δ-cells,[2] which constitute 3–10% of the total cells; and ghrelin-producing ε-cells,[4] which comprise $<1\%$ of the total islet cells. Each of the hormones has distinct functions. Glucagon increases blood glucose levels, whereas insulin decreases them.[5] Somatostatin inhibits both, glucagon and insulin release,[6] whereas PP regulates the exocrine and endocrine secretion activity of the pancreas.[3,7] Altogether, these hormones regulate glucose homeostasis in vertebrates, as described in more detail below. Although the islets have a similar cellular composition among different species, that is, human, rat and mouse, their cytoarchitecture differs greatly. Although islets in rodents are primarily composed of β-cells located in the center with other cell types in the periphery, human islets exhibit interconnected α- and β-cells.[2,8]

Through its various hormones, particularly glucagon and insulin, the pancreas maintains blood glucose levels within a very narrow range of 4–6 mM. This preservation is accomplished by the opposing and balanced actions of glucagon and insulin, referred to as glucose homeostasis. During sleep or in between meals, when blood glucose levels are low, glucagon is released from α-cells to promote hepatic glycogenolysis. In addition, glucagon drives hepatic and renal gluconeogenesis to increase endogenous blood glucose levels[9] during prolonged fasting. In contrast, insulin secretion from β-cells is stimulated by elevated exogenous glucose levels, such as those occurring after a meal.[10] After docking to its receptor on muscle and adipose tissue, insulin enables the insulin-dependent uptake of

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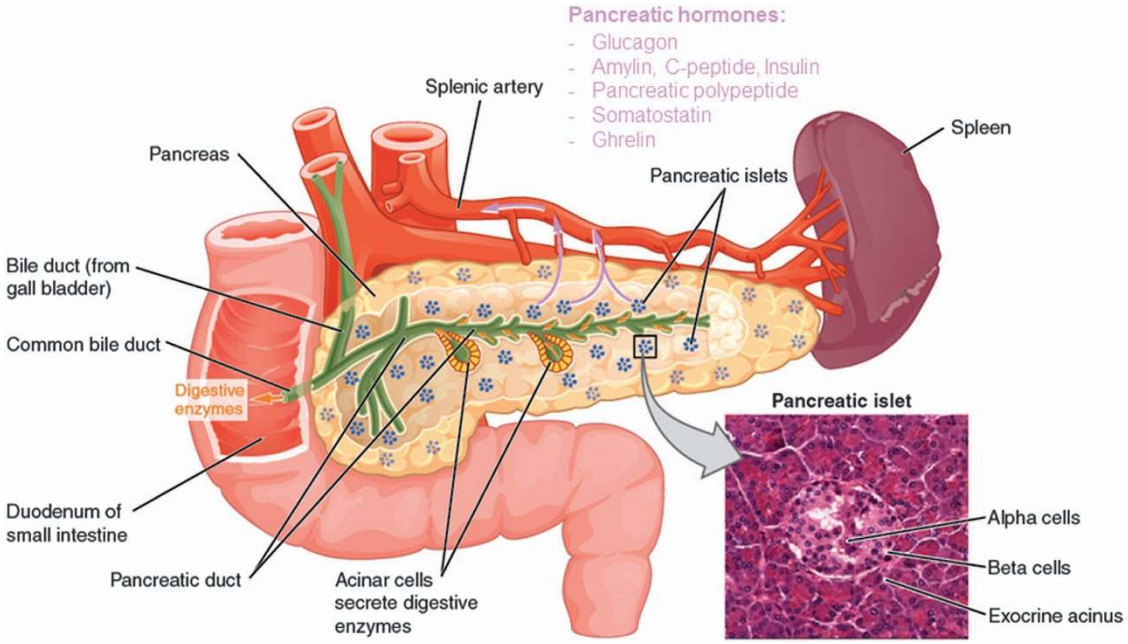

Figure 1 Anatomical organization of the pancreas. The exocrine function of the pancreas is mediated by acinar cells that secrete digestive enzymes into the upper small intestine via the pancreatic duct. Its endocrine function involves the secretion of various hormones from different cell types within the pancreatic islets of Langerhans. The micrograph shows the pancreatic islets. LM × 760 (Micrograph provided by the Regents of University of Michigan Medical School © 2012). Adapted from Human Anatomy and Physiology, an OpenStax College resource.[404]

glucose into these tissues and hence lowers blood glucose levels by removing the exogenous glucose from the blood stream (Figure 2).[11–13] Furthermore, insulin promotes glycogenesis,[14–26] lipogenesis[27,28] and the incorporation of amino acids into proteins;[29] thus, it is an anabolic hormone, in contrast to the catabolic activity of glucagon.

THE INSULIN SECRETION SIGNALING PATHWAY

Endocrine cells secrete their respective hormones in response to external signals, such as nutrient intake or stress, via humoral, neural or hormonal signaling pathways. The underlying molecular process that translates the stimulus into the actual hormone release is called stimulus-secretion coupling which is known as the stimulus-dependent exocytosis of a particular substance, such as glucose-stimulated β-cell insulin release.[30]

In β-cells, the main stimulus for insulin release are elevated blood glucose levels following a meal.[10] The circulating blood glucose is taken up by the facilitative glucose transporter GLUT2 (SLC2A2), which is located on the surface of the β-cells. Once inside the cell, glucose undergoes glycolysis, thereby generating adenosine triphosphate (ATP), resulting in an increased ATP/ADP ratio. This altered ratio then leads to the closure of ATP-sensitive K^+-channels (K_{ATP}-channels). Under non-stimulated conditions, these channels are open to ensure the maintenance of the resting potential by transporting positively charged K^+-ions down their concentration gradient out of the cell. Upon closure, the subsequent decrease in the magnitude of the outwardly directed K^+-current elicits the depolarization of the membrane, followed by the opening of voltage-dependent Ca^+-channels (VDCCs). The increase in intracellular calcium concentrations eventually triggers the fusion of insulin-containing granules with the membrane and the subsequent release of their content.[31] The whole secretory process is biphasic with the first phase peaking around 5 minutes after the glucose stimulus with the majority of insulin being released during this first phase. In the second, somewhat slower, phase, the remaining insulin is secreted.[32–34] Insulin is stored in large dense-core vesicles that are recruited to the proximity of the plasma membrane following stimulation such that insulin is readily available.[35,36] The key molecules that mediate the fusion of the insulin-containing large dense-core vesicles are the synaptosomal-associated protein of 25 kDa (SNAP-25), syntaxin-1 and synaptobrevin 2 (or vesicle-associated membrane protein VAMP2), all of which belong to the superfamily of the soluble *N*-ethylmaleimide-sensitive factor attachment protein (SNAP) receptor proteins (SNAREs). Together with the Sec1/Munc18-like (SM) proteins they form the so-called SNARE complex.[37] To initiate fusion, synaptobrevin 2, a *vesicle (v-)*SNARE that is integrated into the vesicle's membrane, fuses with the *target (t-)*SNAREs syntaxin-1 and SNAP-25, which are located in the target cell membrane,[38,39] with mammalian uncoordinated (munc)-18 playing a key regulatory role (Figure 3).[40,41]

To date, numerous SNARE isoforms, including syntaxin-1, -3 and -4, SNAP-25 and -23, as well as syntaptobrevins 2 and 3 (VAMP2 and 3), have been shown to be involved in glucose-stimulated insulin secretion,[42–46] whereas VAMP8, a non-essential SNARE protein for glucose-stimulated insulin secretion, has a role in the regulation of the glucagon-like

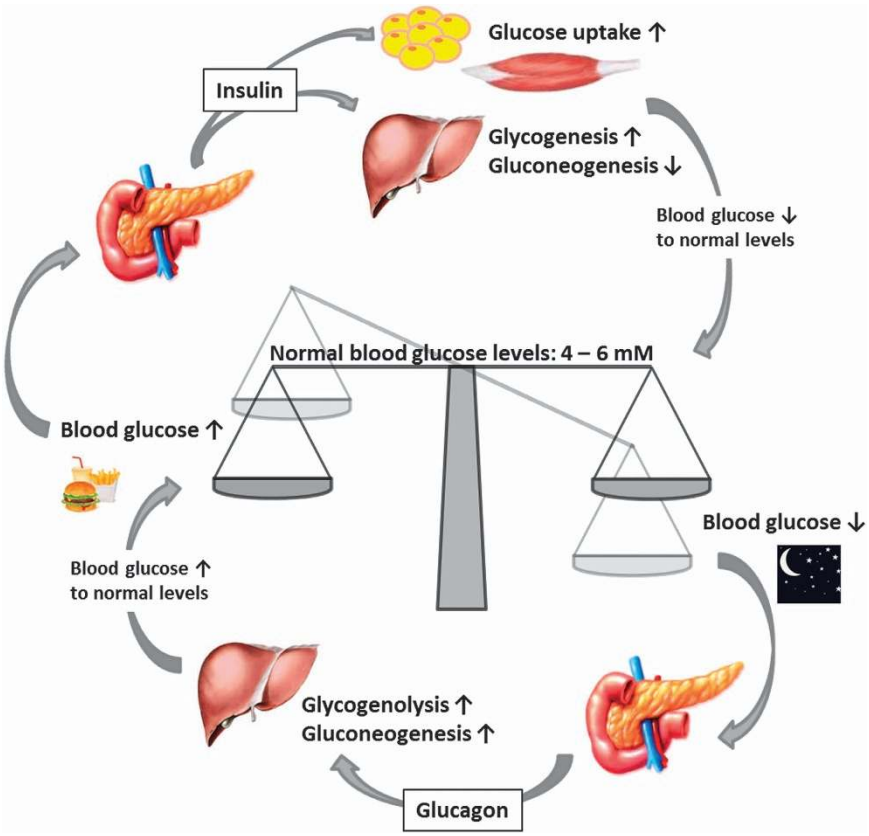

Figure 2 Maintenance of blood glucose levels by glucagon and insulin. When blood glucose levels are low, the pancreas secretes glucagon, which increases endogenous blood glucose levels through glycogenolysis. After a meal, when exogenous blood glucose levels are high, insulin is released to trigger glucose uptake into insulin-dependent muscle and adipose tissues as well as to promote glycogenesis.

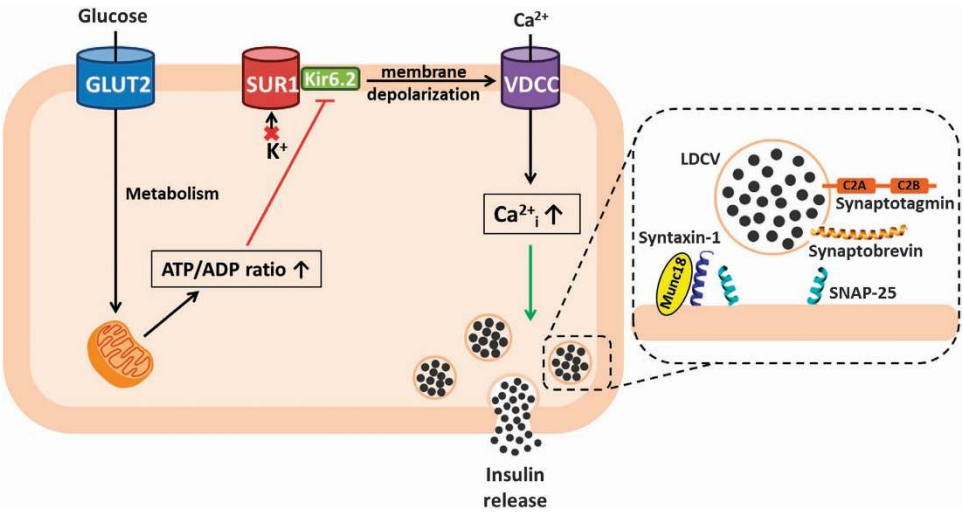

Figure 3 Glucose-stimulated insulin release from a pancreatic β-cell. Exogenous glucose is taken up by GLUT2 and undergoes glycolysis inside the cell. Elevated adenosine triphosphate (ATP) levels alter the ATP/ADP ratio, which in turn leads to the closure of ATP-sensitive K^+-channels. The subsequent membrane depolarization opens voltage-dependent Ca^{2+}-channels in response to increasing intracellular calcium levels, which eventually trigger insulin secretion following vesicle fusion with the membrane.

peptide-1-potentiated insulin secretion.[47] In addition to SNARE and SM proteins, a calcium sensor is required for the initiation of membrane fusion. Synaptotagmins, which are highly expressed in neurons and endocrine cells, were shown to participate in Ca^{2+}-dependent exocytosis processes. To date, 17 synaptotagmins (Syts 1–17) have been identified and only eight of them, namely Syt-1, -2, -3, -5, -6, -7, -9 and -10, are able to bind calcium.[48] Following Ca^{2+}-binding,

synaptotagmins form a complex with the SNAREs to facilitate and trigger the vesicle-membrane fusion process. Among the synaptotagmin family, Syt-3, -5, -7, -8 and -9 are implicated in insulin exocytosis.[49–52]

EXTERNAL FACTORS AFFECTING PANCREATIC HORMONE SECRETION

Metabolism–cAMP coupling

The glucose-triggered stimulus-secretion coupling is an established paradigm of insulin secretion from β-cells and includes a great variety of modulators that trigger, potentiate or inhibit glucose-stimulated insulin secretion, primarily through G-protein-coupled receptors (GPCRs). The most traditional external factor that initiates insulin secretion is glucose. In addition to its trigger function, glucose also induces pathways that amplify insulin secretion through metabolism-cAMP (cyclic adenosine monophosphate) coupling or the incretin hormones glucagon-like peptide (GLP)-1 and glucose-dependent insulinotropic peptide (GIP).[31] Metabolism–cAMP coupling refers to the signaling cascade that occurs after the conversion of ATP, which is generated during intracellular glucose metabolism, into cAMP by adenylate cyclase (AC),[53] which in turn activates protein kinase A (PKA)[54] and cAMP-regulated guanine nucleotide exchange factors, also referred to as exchange protein directly activated by cAMP (Epac) 2.[55,56] Although Epac2 activation amplifies insulin secretion by mobilizing calcium from internal stores to increase Ca^{2+} levels[57,58] and by controlling the granule density in proximity to the plasma membrane,[59] activated PKA exerts its effects by modulating K_{ATP}-channel[60,61] and calcium channel[62,63] activity through phosphorylation, thereby enhancing the number of highly Ca^{2+}-sensitive insulin-containing granules[64] and the probability of releasing secretory vesicles from the readily releasable pool,[65] respectively.

The incretins GLP-1 and GIP

The gut-derived hormones GLP-1 and GIP, which are secreted from enteroendocrine L-cells[66] and K-cells,[67] respectively, upon glucose,[66,68] fructose,[69] amino acid[70] and free fatty acid (FFA)[71,72] ingestion, also potentiate insulin release through the so-called incretin effect. This effect describes the observation that orally, but not intravenously, administered glucose enhances insulin secretion by triggering GLP-1 and GIP secretion;[73–75] the resulting potentiation of insulin secretion may account for up to 50% of the total release. The underlying mechanism includes GLP-1 and GIP binding to their GPCRs (GLP-1R and GIPR), both of which are expressed in pancreatic β-cells.[76] The binding induces a conformational change in the receptors' structure, followed by the exchange of guanosine diphosphate for guanosine triphosphate and the subsequent dissociation of the $G_s\alpha$-subunit from the receptors. This subunit, in turn, activates adenylate cyclase to convert ATP into cAMP, thereby stimulating the cAMP signaling pathway described above.[77–82] Furthermore, GLP-1 increases intracellular calcium concentrations by mobilizing Ca^{2+} from ryanodine-sensitive stores[83,84] or, similar to GIP, by acting on voltage-dependent Ca^{2+}-channels,[85] thereby potentiating insulin release.[85–87] Recent studies have also shown that GLP-1R agonists, such as exendin-4[88], induce the PKA-mediated phosphorylation of Snapin or Synaptotagmin-7, which in turn enhances GSIS by Snapin interacting with SNAP-25[89] or by directly enhancing glucose- and Ca^{2+}-triggered insulin release.[90]

Free Fatty Acids

FFAs not only stimulate incretin secretion but are also known to modulate insulin release through fatty acid metabolism. Although long-chain FFAs augment insulin secretion, short-chain FFAs inhibit it. The binding and subsequent interaction of long-chain FFAs with the G-protein-coupled free fatty acid receptor (FFAR) 1 in the pancreatic β-cells leads to the activation of phospholipase C. PLC then hydrolyzes phosphatidylinositol-4,5-bisphosphate (PIP_2) to diacylglycerol and inositol-1,4,5-triphosphate (IP_3), with the latter docking on a calcium channel in the endoplasmic reticulum. The subsequent release of Ca^{2+} into the cytosol increases the intracellular Ca^{2+} concentration, which eventually triggers insulin secretion.[91–94] In contrast, short-chain FFAs inhibit glucose-stimulated insulin secretion due to decreased glucose oxidation and the subsequently decreased ATP/ADP ratio.[95] Another inhibitor of insulin release is stress, specifically norepinephrine (noradrenaline) produced in response to stress.[96] Norepinephrine binds to its α_2-adrenergic receptors, which are linked to GPCRs, resulting in the inhibition of AC as well as in hyperpolarization. This prevents an increase in the cytosolic Ca^{2+} concentration and, subsequently, insulin secretion.[97,98]

INTERPLAY BETWEEN THE PANCREATIC ISLETS AND OTHER ORGANS

The brain–islet axis

Just as insulin exerts its effects on other organs and tissues, other organs interact with the pancreas to modulate insulin secretion (Figure 4). One of these interacting organs is the brain, which comprises the mutual brain–islet axis that interacts with the pancreas and vice versa. The pancreas is highly innervated with both, parasympathetic[99,100] and sympathetic[100,101] nerve fibers from the autonomic nervous system. At the same time, insulin receptors are widely distributed within the brain, including the hypothalamus, cerebral cortex, cerebellum[102] and hippocampal formation[103] in humans, as well as the olfactory and limbic areas,[104,105] hypothalamus[106]—particularly the periventricular nucleus[107] and the arcuate nucleus[108,109]—hippocampus and the choroid plexus[105] in rat brains. Lesions in various brain regions were shown to affect pancreatic hormone secretion. The destruction of the ventromedial hypothalamus results not only in insulin hypersecretion[110–112] due to loss of the ventromedial hypothalamus-mediated inhibitory impact on pancreatic β-cells[113] but also in higher glucagon levels.[111,112] Glucagon secretion may also be modulated by the hypothalamic brain-derived neurotrophic factor[114] via efferent nerves,[115] whereas the melanocortin system directly reduces basal insulin levels by

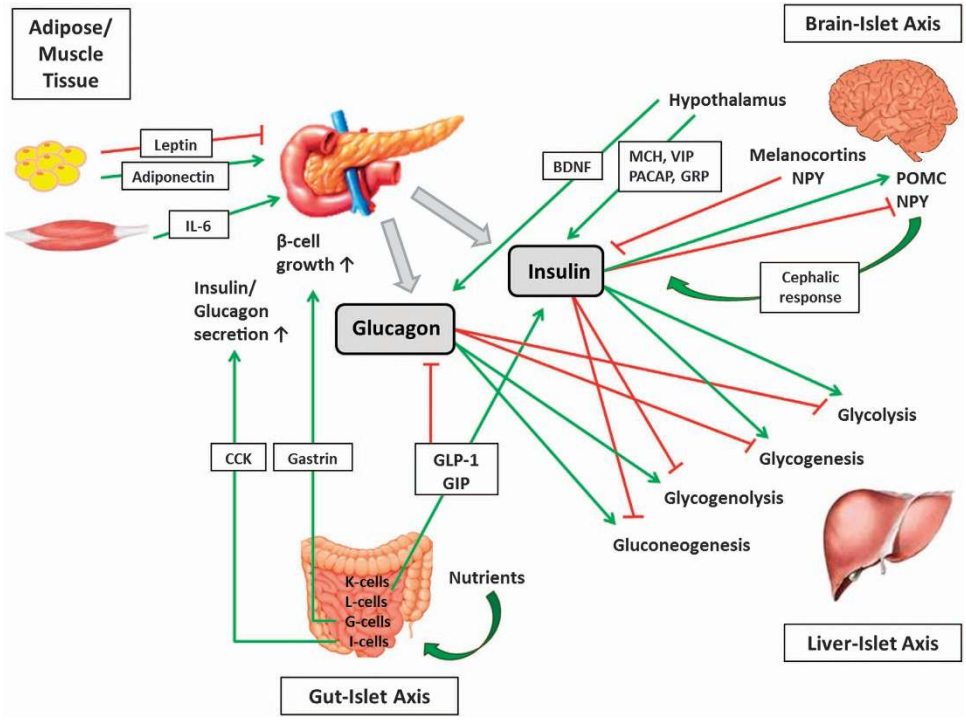

Figure 4 The interplay of the pancreas with the brain, liver, gut as well as adipose and muscle tissue. The pancreas interacts with the brain, liver, gut and adipose and muscle tissue in a highly sophisticated network via various hormones, neurotransmitters and cytokines. BNDF, brain-derived neurotrophic factor; CCK, cholecystokinin; GIP, glucose-dependent insulinotropic peptide; GLP-1, glucagon-like peptide 1; GRP, gastrin-releasing peptide; IL-6, Interleukin 6; MCH, melanin concentrating hormone; NPY, neuropeptide Y; PACAP, pituitary adenylate cyclase-activating polypeptide; POMC, pro-opiomelanocortin; VIP, vasoactive intestinal peptide.

stimulating sympathetic nerve fibers via α-adrenoceptors.[116] Acting via α-adrenoceptors,[117] norepinephrine also inhibits insulin secretion,[96] which is an important aspect of the fight-or-flight response. The neurotransmitter Neuropeptide Y (NPY), which is mainly expressed in the sympathetic nerve fibers of the autonomic nervous system, also blunts insulin release,[118,119] and the loss of NPY's inhibitory action results in elevated basal and glucose-stimulated insulin secretion as well as in increased islet mass.[120] NPY binding to its GPCR Y_1 causes the activated $G_i\alpha$-subunit to block adenylate cyclase activation, which in turn inhibits the cAMP pathway.[121] Furthermore, the NPY-mediated inhibition was shown to be $G_{\beta\gamma}$- and Ca^{2+}-independent.[122] In addition to the well-known insulin stimulator acetylcholine, which exerts its effects via M_3 muscarinic receptors,[123] melanin concentrating hormone, vasoactive intestinal peptide (VIP), its close relative pituitary adenylate cyclase-activating polypeptide (PACAP) and gastrin-releasing peptide also promote insulin and, in the case of VIP[124] and PACAP,[125] glucagon release. The various neuropeptides exert their effects through various pathways, including the extracellular signal-regulated kinase (ERK)/Akt pathway, and modulation of Ca^{2+}-influx (melanin concentrating hormone),[126] cAMP and, to a lesser extent, PI3K signaling (VIP and PACAP),[127,128] muscarinic/β-adrenoceptors signaling, PI3K/PKC signaling and Ca^{2+}-mobilization from intracellular stores (gastrin-releasing peptide).[129,130]

Likewise, insulin release is stimulated by the so-called cephalic phase, which represents the conditioned reflex of increased hormone secretion, referred to as cephalic phase insulin response,[131] even in the absence of nutrients/glucose as a trigger,[132–134] such as when anticipating a meal, to prepare the organism to adequately respond to incoming nutrients.[135] Moreover, cephalic phase insulin response is pivotal for ensuring normal postprandial glucose management.[136] The neural mechanism underlying cephalic phase insulin response was found to include cholinergic and non-cholinergic processes[136] as well as the dorsal vagal complex located in the medulla oblongata.[137] Conversely, insulin released in response to a meal enters the brain via the blood–brain–barrier[138] to decrease food intake[139,140] by stimulating hypothalamic pro-opiomelanocortin neurons[141] and initiating the PI3K signaling pathway[142] in these pro-opiomelanocortin neurons.[143] In contrast to its pro-opiomelanocortin-stimulating action, insulin inhibits NPY expression[144] in Agouti-related peptide (AgRP/NPY) neurons, which are known to secrete the orexigenic neuropeptides NPY[145–147] and AgRP.[148,149] Both, peripheral and central insulin signaling are impaired in obese or diabetic states.[150–154]

The liver–islet axis

The second group represents the liver–islet axis. The liver has a key role in glucose homeostasis by storing (glycogenesis) or releasing (glycogenolysis/gluconeogenesis) glucose upon

interaction with insulin and glucagon, respectively. The binding of glucagon to its hepatic GPCR evokes the signaling cascade described under 'External factors affecting pancreatic hormone secretion', eventually resulting in the activation of PKA, which in turn stimulates two processes; one promotes glycogenolysis/gluconeogenesis and the other inhibits glycolysis/glycogenesis.[155,156] Glycogenolysis is a multistep process that includes the PKA-mediated phosphorylation of phosphorylase kinase,[157] cleavage of glucose-1-phosphate (G-1-P) from glycogen by activated glycogen phosphorylase a[158] and the conversion of G-1-P into G-6-P,[159] eventually resulting in phosphate and free glucose. Hepatic gluconeogenesis is promoted by the PKA-mediated phosphorylation of the cAMP response element-binding protein, which in turn upregulates peroxisome proliferator-activated receptor-γ coactivator (PGC)-1.[160] Together with the hepatocyte nuclear factor (HNF)-4, PGC-1 induces the transcription of phosphoenolpyruvate carboxykinase,[161] which catalyzes the conversion of oxaloacetate into phosphoenolpyruvate, a rate-limiting step in gluconeogenesis. This is followed by reversed glycolysis, during which stimulation of the bifunctional PFK-2/FBPase-2 leads to both, enhanced gluconeogenesis through the abrogation of disabled fructose-1,6-bisphosphatase (FBPase)-1, which facilitates the successive conversion of substrates into G-6-P, and to suppressed glycolysis.[162,163] Glycolysis is further inhibited by the PKA-mediated inactivation of pyruvate kinase,[164–166] resulting in the production of glucose instead of pyruvate. In addition, glucagon was found to suppress pyruvate kinase gene expression as well as to enhance pyruvate kinase mRNA degradation.[167,168] Finally, the PKA-induced inactivation of hepatic glycogen synthase[169–171] decreases glycogen synthesis and concomitantly increases the hepatic glucose pool.

As glucagon's opponent, insulin stimulates glycolysis via enhanced expression of the hepatic glucokinase gene,[14,15] a key enzyme that converts glucose into G-6-P. This increase is mediated by the sterol regulatory element binding protein-1c[15] and requires the absence of cAMP.[14] Furthermore, insulin inactivates glycogen phosphorylase and glycogen synthase kinase (GSK)-3[172] through the PI3K pathway, which in turn activates glycogen synthase.[18–20] The second liver-specific effect of insulin is to repress the expression of the phosphoenolpyruvate carboxykinase and G-6-Pase genes; the first by disrupting the association of cAMP response element-binding protein and RNA polymerase II with the phosphoenolpyruvate carboxykinase gene promoter,[23] whereas G-6-P suppression requires PKBα/Akt and forkhead transcription factor (FOXO1),[24,25] whose expression was shown to be diminished by the inhibition of GSK-3.[26]

It is not only insulin and glucagon acting on the liver; hepatocyte-derived factors conversely influence the pancreas and/or insulin secretion. Although HNF3β was proposed to be pivotal for the transcription of the pancreatic and duodenal homeobox 1 (pdx1 or insulin promotor factor 1 (IPF-1)) gene, a transcription factor regulating pancreatic development[173,174175], it is the loss of HNF1α resulting in an almost abolished insulin secretion, likely due to a decreased response to intracellular calcium. These findings support the importance of HNF1α in maintaining β-cell function[176] and its involvement in maturity-onset diabetes of the young (MODY3).[177]

The hepatokine betatrophin, also known as TD26, re-feeding induced fat and liver (RIFL), lipasin or angiopoietin-like (ANGPTL) 8, was first identified as a factor that drives β-cell proliferation and thus increases β-cell mass in a murine model of insulin resistance.[178] Subsequent studies, however, did not reveal impairments in glucose homeostasis[179] or β-cell expansion in *Angptl8* knockout mice.[180] Moreover, betatrophin does not have an effect on human β-cell replication, challenging its usefulness in diabetes therapy.[181] This is substantiated by the fact that betatrophin levels are higher in T2DM patients,[182–184] although they were lower in one study.[185] However, this is likely to be due to technical issues.[186]

The gut–islet axis

Another important axis is the gut–islet axis. The gut releases various hormones upon nutrient ingestion, including GLP-1 and GIP, that bind to their respective receptors on pancreatic β-cells to potentiate insulin secretion, as described under 'External factors affecting pancreatic hormone secretion'. Furthermore, both hormones exert pancreatic effects, such as GLP-1-stimulated insulin gene expression,[77,187] incretin-induced β-cell neogenesis, proliferation[188–191] and survival,[192] the prevention of β-cell apoptosis in general[193,194] and in response to glucolipotoxicity.[195] The extrapancreatic actions of GLP-1 include suppression of endogenous glucose production[196]/glycogenolysis,[197] glucagon secretion,[197,198] appetite,[199,200] a delay in gastric emptying[198,199] and improved β-cell insulin sensitivity[199,201,202] and glucose disposal,[203,204] whereas GIP positively affects lipid[205–207] and bone metabolism.[208–211] Thus, GLP-1 and GIP mediate insulin secretion and concomitantly, insulin modulates GIP[212] and GLP-1 release; the latter ocurring through the PI3K/Akt- and mitogen-activated protein kinase kinase (MAPKK or MEK)/ERK1/2 pathway.[213] The importance of this interplay is also demonstrated by defective insulin responses and consequent glucose intolerance in *GLP-1R*$^{-/-}$ and *GIPR*$^{-/-}$ mice[214–218] as well as in the pathogenesis of T2DM.[219–223]

In addition to incretins, there are the so-called decretins, namely limostatin and Neuromedin U (NmU), which are secreted during fasting to suppress insulin release. NmU, a (neuro)peptide that mediates the contraction of smooth muscles in the uterus (hence the 'U') among others, was first isolated from the pig spinal cord.[224] Further mRNA expression studies, however, revealed NmU to be highly expressed in the gastrointestinal (GI) tract with the highest levels found in the upper GI, that is, duodenum and jejunum.[225,226] Within the GI structure, NmU is mainly located in submucosal and myenteric cells,[227,228] indicating its possible involvement in the neuronal control of GI function.[229] In addition to this, NmU is likely to regulate insulin secretion; the G-protein-coupled NmU receptor 1 (NmUR1) is expressed in pancreatic islets and its simulation dose dependently decreased insulin release.[230,231]

The underlying mechanism involves the simultaneous release of somatostatin—a known modulator of insulin secretion[6]—upon NmUR1 activation.[232] A very recent study showed[231] that the peptide hormone limostatin, which is expressed in Drosophila melanogaster, also reduces insulin secretion and its absence caused hyperinsulinemia, hypoglycemia and obesity. Moreover, knockdown of the fly NmUR orthologue not only reproduced the consequences of limostatin deficiency but also diminished its insulin-suppressing ability. Limostatin release is initiated by food depletion and hence may represent a novel mechanism for modulating insulin secretion during fasting.

Other gastrointestinal hormones that interact with the pancreas are gastrin and cholecystokinin (CCK). Gastrin, which is secreted from G-cells in the stomach and duodenum, acts as an islet growth factor, together with transforming growth factor-α, by promoting differentiation of ductular precursor cells[233] and β-cell neogenesis as well as by enhancing the islet mass from transdifferentiated exocrine pancreatic tissue.[234] Furthermore, it induces the expression of glucagon genes in α-cells.[235] Along the same lines, CCK, which is synthesized and released from duodenal I-cells, potentiates basal, glucose-[236,237] and amino acid-induced insulin secretion,[238] and augments glucagon secretion.[237,239] The pivotal role of CCK in modulating glucose homeostasis is reflected in postprandial hyperglycemia, which is due to reduced CCK plasma levels in noninsulin-dependent diabetes mellitus.[240]

Another important factor that is related to metabolic disorders such as obesity, T2DM and type 1 DM (T1DM) is the gut microbiota. Obesity, T2DM and T1DM patients display alterations in the composition of their microbiota that may initiate and/or promote the respective disorder. Recent findings linked an aberrant microbiome, which is generally represented by diminished diversity, including fewer butyrate-producing (butyrate was shown to trigger mucin production and hence gut integrity) and mucin-degrading bacteria,[241] to the development of autoimmunity in T1DM.[242] An altered microbiota composition may also contribute to obesity[243,244] as well as to T2DM[245–247] and 'correction' by antibiotics,[248] probiotics[249] or prebiotics, the last of which causing a short-chain FFA-stimulated increase in GLP-1,[250] may improve the disease condition.[251]

The adipocytes/myocytes–islet axis

On one hand, insulin's interplay with adipose and muscle tissues is broadly based on facilitating insulin-dependent glucose uptake through the glucose transporter 4 (GLUT4).[11–13] On the other hand, adipokines and myokines secreted from the adipose and muscle tissue, respectively, modulate insulin release. As part of the so-called adipoinsular axis,[252] leptin, the most famous adipokine, mainly acts on its receptors in the hypothalamic arcuate nucleus to inhibit food intake and control whole body homeostasis.[253] However, leptin receptor (Ob-R) mRNA expression was also observed in pancreatic islets[254] and its stimulation caused a reduction in insulin secretion[255–257] due to the activation of K_{ATP}-channels, which in turn prevented Ca^{2+}-influx[258] and the subsequent signaling pathway. Furthermore, leptin was shown to suppress insulin gene expression,[259,260] representing a negative feedback loop. Conversely, insulin enhances *ob* gene expression and leptin secretion.[261–264] Likewise, insulin modulates the expression of adiponectin, another well-known adipokine, the abundance of its receptor in adipose and muscle tissue[265,266] as well as its secretion.[267,268] Adiponectin is not only involved in glucose and fatty acid metabolism[269] but it also forestalls β-cell apoptosis and induces insulin gene expression and release;[270] the latter was mediated by the ERK/Akt pathway in one study[270] and by the AMPK pathway in another study.[271] Other adipokines, such as apelin,[272,273] chemerin,[274–276] omentin,[277,278] resistin[279] and visfatin,[280,281] were also shown to directly interact with insulin, whereas retinol-binding protein 4, tumor necrosis factor-α and vaspin are related to insulin in an indirect manner.[282] In addition to adipokine secretion by adipocytes, myocytes release cytokines, which are referred to as myokines. Fibroblast growth factor-21 is a widely expressed protein with a broad mode of action, including the regulation of carbohydrate and fatty acid metabolism[283] and may be considered as a myokine due to its secretion from muscle cells.[284] Fibroblast growth factor-21 is regulated by insulin[285] through the PI3K/Akt1 signaling pathway.[286] Interleukin
(IL)-6, which is both an adipokine and myokine,[287] was shown to influence the pancreas by controlling the expression of pro-glucagon mRNA as well as glucagon secretion. It also increases α-cell proliferation and islet mass while protecting the pancreas from metabolic stress-induced apoptosis.[288] Furthermore, IL-6 increased GLP-1 production from proglucagon in pancreatic α-cells and its secretion from α-cells and intestinal L-cells, eventually resulting in a GLP-1-mediated increase in insulin secretion.[289]

MODULATING INSULIN SECRETION AS A MEANS OF DIABETES THERAPY

Due to the worldwide, still spreading epidemic of T2DM, there is an urgent need for (new) anti-diabetic drugs and therapies that are more effective and have fewer side effects. Currently, the most commonly used drugs can be classified into agents that enhance insulin secretion (secretagogues such as sulfonylureas (SUs) and incretin mimetics), sensitize the target organs of insulin (for example, metformin from the class of biguanides or thiazolidinediones), or reduce glucose absorption from the gastrointestinal tract (inhibitors of gastrointestinal α-glucosidase). Different therapies address different problems and stages of T2DM and may be prescribed in combination to exert synergistic effects.

Sulfonylureas

A-glucosidase inhibitors and sensitizers do not target the pancreas or insulin secretion itself but instead target the upstream (slowed intestinal glucose absorption) or downstream (improved insulin sensitivity) processes. In contrast, insulin secretagogues directly modulate insulin release. The SUs are the

first broadly applied oral anti-hyperglycemic drugs. To date, there are two generations of agents: acetohexamide, chlorpropamide, tolazamide and tolbutamide, which constitute the first generation and glibenclamide/glyburide, gliclazide, glimepiride, glipizide and gliquidone, which comprise the second generation. First-generation SUs are rarely used these days since tolbutamide intake was associated with an increase in lethal cardiac events.[290,291] More importantly, the second-generation SUs are more potent due to modifications in their side chains' structure, resulting in improved SUR-affinity, accompanied by lower effective plasma levels, which in turn may reduce undesirable drug-protein interactions.

All SUs share a central SU backbone but differ in their side chains. Despite having different pharmacokinetics, they work in the same way, namely by triggering endogenous insulin release by blocking K_{ATP}-channels and hence activating the insulin signaling pathway. More precisely, SUs bind to the sulfonylurea receptor (SUR) subunit of the K_{ATP}-channel with high affinity.[292,293] SUR, together with the pore-forming subunit Kir6.x, forms a hetero–octameric complex consisting of four inner Kir6.x subunits surrounded by four SUR subunits (4:4 stoichiometry).[294,295] Moreover, different isoforms of the two subunits are expressed, depending on the tissue-specific expression of the K_{ATP}-channels: SUR1 and Kir6.2 are expressed in the pancreas and brain,[296] Kir6.2 and SUR2A are expressed in the heart and skeletal muscle,[297] while SUR2B is expressed in the brain and smooth muscle,[298] and Kir6.1 and SUR2B are expressed in vascular smooth muscle.[299] Although SUs bind to both, SURs and Kir6.2, the interactions with the latter are of low affinity[300,301] and hence only SUR-interacting agents are used for diabetes treatment. In addition to their mode of action as inhibitors of K_{ATP}-channels, SUs were shown to improve glucose uptake into insulin-dependent tissues and glucose disposal as well as to reduce hepatic glycogenolysis/gluconeogenesis.[302–304]

In contrast to SUs inactivating the K_{ATP}-channels by binding to the SUR1 subunit, ATP closes them by interacting with Kir6.2.[305] Moreover, while the binding of only one ATP molecule is sufficient to completely close the channel,[306] inhibition by SUs is incomplete as the channel might still open even when SUs are bound to SUR1.[299] Nonetheless, second-generation SUs reduce the glycated hemoglobin or HbA_{1c}, which represent the average plasma glucose concentrations over time and thus serve as a diagnostic measure for diabetes mellitus, by 1.0–2.0%. In addition to the weight gain attributed to the anabolic effects of increased insulin secretion, the main side effect of SUs is hypoglycemia[307,308] due to excess circulating insulin levels and due to the fact that SUs evoke insulin secretion in a glucose-independent manner.[309]

Although they are not SUs *per se*, meglitinides, that is, repaglinide and nateglinide, share their mode of action of inhibiting K_{ATP}-channels.[310] However, meglitinides and some of the second-generation SUs, for example, glibenclamide, interact with both, the SUR1 and the SUR2A or B isoforms.[311] Despite the possible disadvantage of this generalized binding that may cause undesirable effects on other K_{ATP}-channel types, for example, those in the heart,[312] meglitinides, namely nateglinide, have an earlier onset of action and a faster dissociation rate from the sulfonylurea receptor,[313–315] resulting in a diminished risk of hypoglycemia.[316] Like SUs, meglitinides also cause weight gain.[317,318]

Incretin mimetics

Another group of insulin secretagogues is comprised of the incretins GLP-1 and GIP. As both incretins are rapidly inactivated by the enzyme dipeptidyl peptidase IV (DPP-IV),[319] their application in T2DM treatment focuses on modified analogues[320–325] or receptor agonists, including the well-known, short-acting exenatide.[326–328] The long-lasting agonists exenatide LAR,[329,330] liraglutide[331,332] and lixisenatide[333–335] are currently under investigation. However, based on the lipogenetic properties[205–207] of GIP, insufficient insulin-potentiating effects in T2DM patients[220,336] and a possible worsening effect by GIP,[337,338] the focus is on GLP-1 analogues/receptor agonists for T2DM treatment. By acting on its receptor, GLP-1 induces the signaling cascade described under 'External factors affecting pancreatic hormone secretion', resulting in its main effect: potentiating insulin secretion. In addition to reducing the HbA_{1C} levels, GLP-1 analogues/receptor agonists promote weight loss and, more importantly, do not evoke hypoglycemia, as do SUs,[326–334] due to the glucose-dependent mode of action and the self-regulating mechanism of GLP-1.[68,336,339] When blood glucose levels are lowered to physiological levels, GLP-1 is incapable of enhancing insulin secretion, thereby preventing hypoglycemia.[79,340] In addition, GLP-1 (analogues/receptor agonists) exerts further pancreatic and extrapancreatic actions, as mentioned under 'Interplay between the pancreatic islets and other organs'. Although GLP-1 (analogues/receptor agonists) exhibits some minor side effects, including nausea, vomiting or gastrointestinal impairments,[326–335] the beneficial properties outweigh the negative effects, and thus, GLP-1 is a promising anti-diabetic agent.

Insulin sensitizers

Metformin, which is generally the most widely used first-line anti-diabetic medication,[341] is a so-called (insulin) sensitizer. It not only diminishes hepatic glucose output due to glycogenolysis/gluconeogenesis[342] but it also enhances glucose uptake into peripheral tissues, such as skeletal muscle, by activating 5′-adenosine monophosphate-activated protein kinase (AMPK-α2).[343] Furthermore, it supports weight loss[344] by reducing food consumption.[345] With respect to its effects on β-cell function, metformin was shown to increase insulin gene expression,[346] possibly by nuclear accumulation of pdx1 and its subsequently improved DNA-binding activity.[347] Interestingly, metformin exerts opposing effects on β-cell proliferation and/or apoptosis; on the one hand, it suppresses β-cell proliferation and enhances apoptosis through an AMPK-dependent and autophagy-mediated mechanism[348] following the metformin-induced activation of c-Jun-N-terminal kinase and caspase-3.[349] On the other hand, metformin reduces

caspase-3- and -8-mediated apoptosis in isolated islets from T2DM patients[350] and protects against lipotoxicity-induced β-cell defects.[348,351]

The other members of the sensitizer group include the thiazolidinediones (or glitazones). Currently, only pioglitazone is available; troglitazone was withdrawn from the market in 2000 and rosiglitazone was withdrawn in 2010 due to liver toxicity, drug-induced hepatitis[352–354] and the increased risk of cardiovascular events, respectively.[355] Their mode of action involves activation of the peroxisome proliferator-activated receptor (PPARγ), a nuclear transcription factor that is highly expressed in adipose tissue, and the subsequent regulation of genes that are involved in glucose and fat metabolism.[356–358] By promoting lipogenesis, FFAs are removed from the blood stream, whereupon cells become dependent on glucose as an energy substrate. However, enhanced lipogenesis also leads to the weight gain observed in thiazolidinedione-treated T2DM patients.[359] In contrast to metformin, pioglitazone prevents (oxidative stress-induced) apoptosis[360,361] by decreasing the expression of apoptosis-promoting genes, while increasing anti-apoptotic and anti-oxidative gene expression. However, this may depend on the disease state.[362,363] Furthermore, pioglitazone increases β-cell mass by upregulating cell differentiation/proliferation genes.[364] Although they have partially different modes of action, both groups of sensitizers cause a reduction in the HbA_{1c} level by 1.5–2.0%.

A-glucosidase inhibitors

A-glucosidase inhibitors, such as acarbose, miglitol and voglibose, not only decelerate the breakdown of starch into glucose in the small intestine but also decrease its bioavailability, resulting in reduced levels of glucose entering the blood stream and hence attenuated postprandial glucose excursions.[365–370] In addition, they support weight loss[371,372] and ameliorate blood pressure,[373] insulin sensitivity[367,368] and triglyceride levels.[369,370] Similar to pioglitazone, α-glucosidase inhibitors attenuate reductions in β-cell mass, which may delay the onset of diabetes.[374–376] As α-glucosidase inhibitors only mildly reduce HbA_{1c} levels (0.5–1.0%), they are usually only used in the early stage of T2DM, that is, impaired glucose tolerance or in combination with other drugs.[377]

CONCLUSIONS AND OUTLOOK

The pancreas has key roles in maintaining normal blood glucose levels by producing and releasing insulin and glucagon. These opponents interact not only with each other through the intra-islet insulin axis[378–381] but also with other organs/tissues, that is, the brain, liver, gut as well as insulin-dependent adipose and muscle tissues. Altogether, the islet–organ/tissues axes described here form a highly sophisticated network that includes, but is not limited to, various signaling molecules, that is, neuropeptides (brain-derived neurotrophic factor, NPY, melanin concentrating hormone, gastrin-releasing peptide, VIP and PACAP), hepatokines (betatrophin and HNFs), enteroendocrine hormones (the incretins GLP-1 and GIP, the decretins NmU and limostatin, gastrin and CCK) as well as adipokines (leptin and adiponectin) and myokines (fibroblast growth factor-21 and IL-6) that mainly interact through GPCR signaling pathways, such as the cAMP cascade. In good health, the well-functioning interactions between all of the organs and tissues involved ensure glucose homeostasis. However, impairments in the secretion of and/or sensitivity to insulin may result in metabolic diseases, such as T2DM. Referring to the American Diabetes Association, T2DM and noninsulin-dependent diabetes mellitus are characterized by insulin resistance, hyperglycemia and a relative insulin deficiency. Furthermore, T2DM is associated with low-grade inflammation,[382,383] cardiovascular disease,[384,385] nephropathy[386,387] and alterations in the secretion of various hormones, including IL-6, IL-18, tumor necrosis factor-α,[388] adiponectin and leptin,[389] neuropeptides,[390] ghrelin[391,392] and the incretins GLP-1 and GIP.[219–223] Although lifestyle interventions[393] and weight loss[394] reverse T2DM in early stages, when insulin is still secreted, T2DM patients may become dependent on anti-diabetic drugs in later stages. Currently, there are three classes of agents: insulin secretatogues, insulin sensitizers and α-glucosidase inhibitors, all of which have different modes of action and hence target different stages and symptoms of T2DM. Treatments that modulate insulin release—on condition of an appropriate insulin sensitivity of the target organs—appear to be promising approaches. Current research is unveiling new molecules, enzymes and interactions that are involved in the signaling pathways underlying insulin secretion, among others, and is likely to introduce new therapeutic approaches. Strategies that target these mediating molecules may include, but are not limited to, the calcium sensor Syt-7,[90,395] the SNARE-associated protein Snapin,[89] the *t*-SNARE SNAP-25,[396] cyclin-dependent kinase (Cdk) 5,[397] ryanodine receptor (RyR) 2,[398] the nucleotide exchange factor and intracellular cAMP sensor Epac2,[57–59,399] mammalian uncoordinated proteins (munc)13[400,401] and munc18[40,41] as well as the Ras-related proteins (Rab) 3A[402] and 27A.[403]

CONFLICT OF INTEREST

The authors declare no conflict of interest.

1 Chandra R, Liddle RA. Neural and hormonal regulation of pancreatic secretion. *Curr Opin Gastroenterol* 2009; **25**: 441–446.
2 Brissova M, Fowler MJ, Nicholson WE, Chu A, Hirshberg B, Harlan DM *et al.* Assessment of human pancreatic islet architecture and composition by laser scanning confocal microscopy. *J Histochem Cytochem* 2005; **53**: 1087–1097.
3 Katsuura G, Asakawa A, Inui A. Roles of pancreatic polypeptide in regulation of food intake. *Peptides* 2002; **23**: 323–329.
4 Wierup N, Svensson H, Mulder H, Sundler F. The ghrelin cell: a novel developmentally regulated islet cell in the human pancreas. *Regul Pept* 2002; **107**: 63–69.
5 Goke B. Islet cell function: alpha and beta cells–partners towards normoglycaemia. *Int J Clin Pract Suppl* 2008; **159**: 2–7.
6 Hauge-Evans AC, King AJ, Carmignac D, Richardson CC, Robinson IC, Low MJ *et al.* Somatostatin secreted by islet delta-cells fulfills multiple roles as a paracrine regulator of islet function. *Diabetes* 2009; **58**: 403–411.
7 Batterham RL, Le Roux CW, Cohen MA, Park AJ, Ellis SM, Patterson M *et al.* Pancreatic polypeptide reduces appetite and food intake in humans. *J Clin Endocrinol Metab* 2003; **88**: 3989–3992.

8 Cabrera O, Berman DM, Kenyon NS, Ricordi C, Berggren PO, Caicedo A. The unique cytoarchitecture of human pancreatic islets has implications for islet cell function. *Proc Natl Acad Sci USA* 2006; **103**: 2334–2339.
9 Freychet L, Rizkalla SW, Desplanque N, Basdevant A, Zirinis P, Tchobroutsky G *et al.* Effect of intranasal glucagon on blood glucose levels in healthy subjects and hypoglycaemic patients with insulin-dependent diabetes. *Lancet* 1988; **1**: 1364–1366.
10 Komatsu M, Takei M, Ishii H, Sato Y. Glucose-stimulated insulin secretion: a newer perspective. *J Diabetes Investig* 2013; **4**: 511–516.
11 Khan AH, Pessin JE. Insulin regulation of glucose uptake: a complex interplay of intracellular signalling pathways. *Diabetologia* 2002; **45**: 1475–1483.
12 Kohn AD, Summers SA, Birnbaum MJ, Roth RA. Expression of a constitutively active Akt Ser/Thr kinase in 3T3-L1 adipocytes stimulates glucose uptake and glucose transporter 4 translocation. *J Biol Chem* 1996; **271**: 31372–31378.
13 Zisman A, Peroni OD, Abel ED, Michael MD, Mauvais-Jarvis F, Lowell BB *et al.* Targeted disruption of the glucose transporter 4 selectively in muscle causes insulin resistance and glucose intolerance. *Nat Med* 2000; **6**: 924–928.
14 Sibrowski W, Seitz HJ. Rapid action of insulin and cyclic AMP in the regulation of functional messenger RNA coding for glucokinase in rat liver. *J Biol Chem* 1984; **259**: 343–346.
15 Kim SY, Kim HI, Kim TH, Im SS, Park SK, Lee IK *et al.* SREBP-1c mediates the insulin-dependent hepatic glucokinase expression. *J Biol Chem* 2004; **279**: 30823–30829.
16 Aiston S, Hampson LJ, Arden C, Iynedjian PB, Agius L. The role of protein kinase B/Akt in insulin-induced inactivation of phosphorylase in rat hepatocytes. *Diabetologia* 2006; **49**: 174–182.
17 Syed NA, Khandelwal RL. Reciprocal regulation of glycogen phosphorylase and glycogen synthase by insulin involving phosphatidylinositol-3 kinase and protein phosphatase-1 in HepG2 cells. *Mol Cell Biochem* 2000; **211**: 123–136.
18 Miller TB Jr, Larner J. Mechanism of control of hepatic glycogenesis by insulin. *J Biol Chem* 1973; **248**: 3483–3488.
19 Akpan JO, Gardner R, Wagle SR. Studies on the effects of insulin and acetylcholine on activation of glycogen synthase and on glycogenesis in hepatocytes. *Biochem Biophys Res Commun* 1974; **61**: 222–229.
20 Stalmans W, De Wulf H, Hue L, Hers HG. The sequential inactivation of glycogen phosphorylase and activation of glycogen synthetase in liver after the administration of glucose to mice and rats. The mechanism of the hepatic threshold to glucose. *Eur J Biochem* 1974; **41**: 127–134.
21 O'Brien RM, Lucas PC, Forest CD, Magnuson MA, Granner DK. Identification of a sequence in the PEPCK gene that mediates a negative effect of insulin on transcription. *Science* 1990; **249**: 533–537.
22 Streeper RS, Svitek CA, Chapman S, Greenbaum LE, Taub R, O'Brien RM. A multicomponent insulin response sequence mediates a strong repression of mouse glucose-6-phosphatase gene transcription by insulin. *J Biol Chem* 1997; **272**: 11698–11701.
23 Duong DT, Waltner-Law ME, Sears R, Sealy L, Granner DK. Insulin inhibits hepatocellular glucose production by utilizing liver-enriched transcriptional inhibitory protein to disrupt the association of CREB-binding protein and RNA polymerase II with the phosphoenolpyruvate carboxykinase gene promoter. *J Biol Chem* 2002; **277**: 32234–32242.
24 Nakae J, Kitamura T, Silver DL, Accili D. The forkhead transcription factor Foxo1 (Fkhr) confers insulin sensitivity onto glucose-6-phosphatase expression. *J Clin Invest* 2001; **108**: 1359–1367.
25 Schmoll D, Walker KS, Alessi DR, Grempler R, Burchell A, Guo S *et al.* Regulation of glucose-6-phosphatase gene expression by protein kinase Balpha and the forkhead transcription factor FKHR. Evidence for insulin response unit-dependent and -independent effects of insulin on promoter activity. *J Biol Chem* 2000; **275**: 36324–36333.
26 Lochhead PA, Coghlan M, Rice SQ, Sutherland C. Inhibition of GSK-3 selectively reduces glucose-6-phosphatase and phosphatase and phosphoenolypyruvate carboxykinase gene expression. *Diabetes* 2001; **50**: 937–946.
27 Walton PE, Etherton TD. Stimulation of lipogenesis by insulin in swine adipose tissue: antagonism by porcine growth hormone. *J Anim Sci* 1986; **62**: 1584–1595.
28 McTernan PG, Harte AL, Anderson LA, Green A, Smith SA, Holder JC *et al.* Insulin and rosiglitazone regulation of lipolysis and lipogenesis in human adipose tissue *in vitro*. *Diabetes* 2002; **51**: 1493–1498.
29 Biolo G, Declan Fleming RY, Wolfe RR. Physiologic hyperinsulinemia stimulates protein synthesis and enhances transport of selected amino acids in human skeletal muscle. *J Clin Invest* 1995; **95**: 811–819.
30 Ashcroft FM, Proks P, Smith PA, Ammala C, Bokvist K, Rorsman P. Stimulus-secretion coupling in pancreatic beta cells. *J Cell Biochem* 1994; **55 Suppl**: 54–65.
31 Henquin JC. Triggering and amplifying pathways of regulation of insulin secretion by glucose. *Diabetes* 2000; **49**: 1751–1760.
32 Cerasi E, Luft R. The plasma insulin response to glucose infusion in healthy subjects and in diabetes mellitus. *Acta Endocrinol (Copenh)* 1967; **55**: 278–304.
33 Porte D Jr, Pupo AA. Insulin responses to glucose: evidence for a two pool system in man. *J Clin Invest* 1969; **48**: 2309–2319.
34 Curry DL, Bennett LL, Grodsky GM. Dynamics of insulin secretion by the perfused rat pancreas. *Endocrinology* 1968; **83**: 572–584.
35 Hao M, Li X, Rizzo MA, Rocheleau JV, Dawant BM, Piston DW. Regulation of two insulin granule populations within the reserve pool by distinct calcium sources. *J Cell Sci* 2005; **118**(Pt 24): 5873–5884.
36 Olofsson CS, Gopel SO, Barg S, Galvanovskis J, Ma X, Salehi A *et al.* Fast insulin secretion reflects exocytosis of docked granules in mouse pancreatic B-cells. *Pflugers Arch* 2002; **444**: 43–51.
37 Thurmond DC. *Regulation of Insulin Action and Insulin Secretion by SNARE-Mediated Vesicle Exocytosis*. Landes Bioscience: Austin, TX, USA, 2000.
38 Chapman ER, An S, Barton N, Jahn R. SNAP-25, a t-SNARE which binds to both syntaxin and synaptobrevin via domains that may form coiled coils. *J Biol Chem* 1994; **269**: 27427–27432.
39 Fasshauer D, Otto H, Eliason WK, Jahn R, Brunger AT. Structural changes are associated with soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptor complex formation. *J Biol Chem* 1997; **272**: 28036–28041.
40 Voets T, Toonen RF, Brian EC, de Wit H, Moser T, Rettig J *et al.* Munc18-1 promotes large dense-core vesicle docking. *Neuron* 2001; **31**: 581–591.
41 Lam PP, Ohno M, Dolai S, He Y, Qin T, Liang T *et al.* Munc18b is a major mediator of insulin exocytosis in rat pancreatic beta-cells. *Diabetes* 2013; **62**: 2416–2428.
42 Foster LJ, Yeung B, Mohtashami M, Ross K, Trimble WS, Klip A. Binary interactions of the SNARE proteins syntaxin-4, SNAP23, and VAMP-2 and their regulation by phosphorylation. *Biochemistry* 1998; **37**: 11089–11096.
43 Ravichandran V, Chawla A, Roche PA. Identification of a novel syntaxin- and synaptobrevin/VAMP-binding protein, SNAP-23, expressed in non-neuronal tissues. *J Biol Chem* 1996; **271**: 13300–13303.
44 Leung YM, Kwan EP, Ng B, Kang Y, Gaisano HY. SNAREing voltage-gated K+ and ATP-sensitive K+ channels: tuning beta-cell excitability with syntaxin-1A and other exocytotic proteins. *Endocr Rev* 2007; **28**: 653–663.
45 Regazzi R, Wollheim CB, Lang J, Theler JM, Rossetto O, Montecucco C *et al.* VAMP-2 and cellubrevin are expressed in pancreatic beta-cells and are essential for Ca(2+)-but not for GTP gamma S-induced insulin secretion. *EMBO J* 1995; **14**: 2723–2730.
46 Zhu D, Koo E, Kwan E, Kang Y, Park S, Xie H *et al.* Syntaxin-3 regulates newcomer insulin granule exocytosis and compound fusion in pancreatic beta cells. *Diabetologia* 2013; **56**: 359–369.
47 Zhu D, Zhang Y, Lam PP, Dolai S, Liu Y, Cai EP *et al.* Dual role of VAMP8 in regulating insulin exocytosis and islet beta cell growth. *Cell Metab* 2012; **16**: 238–249.
48 Gustavsson N, Han W. Calcium-sensing beyond neurotransmitters: functions of synaptotagmins in neuroendocrine and endocrine secretion. *Biosci Rep* 2009; **29**: 245–259.
49 Gut A, Kiraly CE, Fukuda M, Mikoshiba K, Wollheim CB, Lang J. Expression and localisation of synaptotagmin isoforms in endocrine beta-cells: their function in insulin exocytosis. *J Cell Sci* 2001; **114**(Pt 9): 1709–1716.
50 Gustavsson N, Wei SH, Hoang DN, Lao Y, Zhang Q, Radda GK *et al.* Synaptotagmin-7 is a principal Ca^{2+} sensor for Ca^{2+} -induced glucagon exocytosis in pancreas. *J Physiol* 2009; **587**(Pt 6): 1169–1178.
51 Mizuta M, Kurose T, Miki T, Shoji-Kasai Y, Takahashi M, Seino S *et al.* Localization and functional role of synaptotagmin III in insulin secretory vesicles in pancreatic beta-cells. *Diabetes* 1997; **46**: 2002–2006.
52 Iezzi M, Kouri G, Fukuda M, Wollheim CB. Synaptotagmin V and IX isoforms control Ca^{2+} -dependent insulin exocytosis. *J Cell Sci* 2004; **117** (Pt 15): 3119–3127.

53 Sadana R, Dessauer CW. Physiological roles for G protein-regulated adenylyl cyclase isoforms: insights from knockout and overexpression studies. *Neurosignals* 2009; **17**: 5–22.
54 Das R, Esposito V, Abu-Abed M, Anand GS, Taylor SS, Melacini G. cAMP activation of PKA defines an ancient signaling mechanism. *Proc Natl Acad Sci USA* 2007; **104**: 93–98.
55 Li S, Tsalkova T, White MA, Mei FC, Liu T, Wang D *et al*. Mechanism of intracellular cAMP sensor Epac2 activation: cAMP-induced conformational changes identified by amide hydrogen/deuterium exchange mass spectrometry (DXMS). *J Biol Chem* 2011; **286**: 17889–17897.
56 Leech CA, Chepurny OG, Holz GG. Epac2-dependent rap1 activation and the control of islet insulin secretion by glucagon-like peptide-1. *Vitam Horm* 2010; **84**: 279–302.
57 Kang G, Joseph JW, Chepurny OG, Monaco M, Wheeler MB, Bos JL *et al*. Epac-selective cAMP analog 8-pCPT-2'-O-Me-cAMP as a stimulus for Ca^{2+}-induced Ca^{2+} release and exocytosis in pancreatic beta-cells. *J Biol Chem* 2003; **278**: 8279–8285.
58 Dzhura I, Chepurny OG, Kelley GG, Leech CA, Roe MW, Dzhura E *et al*. Epac2-dependent mobilization of intracellular Ca(2)+ by glucagon-like peptide-1 receptor agonist exendin-4 is disrupted in beta-cells of phospholipase C-epsilon knockout mice. *J Physiol* 2010; **588**(Pt 24): 4871–4889.
59 Shibasaki T, Takahashi H, Miki T, Sunaga Y, Matsumura K, Yamanaka M *et al*. Essential role of Epac2/Rap1 signaling in regulation of insulin granule dynamics by cAMP. *Proc Natl Acad Sci USA* 2007; **104**: 19333–19338.
60 Beguin P, Nagashima K, Nishimura M, Gonoi T, Seino S. PKA-mediated phosphorylation of the human K(ATP) channel: separate roles of Kir6.2 and SUR1 subunit phosphorylation. *EMBO J* 1999; **18**: 4722–4732.
61 Ribalet B, Ciani S, Eddlestone GT. ATP mediates both activation and inhibition of K(ATP) channel activity via cAMP-dependent protein kinase in insulin-secreting cell lines. *J Gen Physiol* 1989; **94**: 693–717.
62 Kanno T, Suga S, Wu J, Kimura M, Wakui M. Intracellular cAMP potentiates voltage-dependent activation of L-type Ca^{2+} channels in rat islet beta-cells. *Pflugers Arch* 1998; **435**: 578–580.
63 Safayhi H, Haase H, Kramer U, Bihlmayer A, Roenfeldt M, Ammon HP *et al*. L-type calcium channels in insulin-secreting cells: biochemical characterization and phosphorylation in RINm5F cells. *Mol Endocrinol* 1997; **11**: 619–629.
64 Wan QF, Dong Y, Yang H, Lou X, Ding J, Xu T. Protein kinase activation increases insulin secretion by sensitizing the secretory machinery to Ca^{2+}. *J Gen Physiol* 2004; **124**: 653–662.
65 Renstrom E, Eliasson L, Rorsman P. Protein kinase A-dependent and -independent stimulation of exocytosis by cAMP in mouse pancreatic B-cells. *J Physiol* 1997; **502**(Pt 1): 105–118.
66 Reimann F, Habib AM, Tolhurst G, Parker HE, Rogers GJ, Gribble FM. Glucose sensing in L cells: a primary cell study. *Cell Metab* 2008; **8**: 532–539.
67 Parker HE, Habib AM, Rogers GJ, Gribble FM, Reimann F. Nutrient-dependent secretion of glucose-dependent insulinotropic polypeptide from primary murine K cells. *Diabetologia* 2009; **52**: 289–298.
68 Elliott RM, Morgan LM, Tredger JA, Deacon S, Wright J, Marks V. Glucagon-like peptide-1 (7-36)amide and glucose-dependent insulinotropic polypeptide secretion in response to nutrient ingestion in man: acute post-prandial and 24-h secretion patterns. *J Endocrinol* 1993; **138**: 159–166.
69 Kuhre RE, Gribble FM, Hartmann B, Reimann F, Windelov JA, Rehfeld JF *et al*. Fructose stimulates GLP-1 but not GIP secretion in mice, rats, and humans. *Am J Physiol Gastrointest Liver Physiol* 2014; **306**: G622–G630.
70 Reimann F, Williams L, da Silva Xavier G, Rutter GA, Gribble FM. Glutamine potently stimulates glucagon-like peptide-1 secretion from GLUTag cells. *Diabetologia* 2004; **47**: 1592–1601.
71 Tolhurst G, Heffron H, Lam YS, Parker HE, Habib AM, Diakogiannaki E *et al*. Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein-coupled receptor FFAR2. *Diabetes* 2012; **61**: 364–371.
72 Hirasawa A, Tsumaya K, Awaji T, Katsuma S, Adachi T, Yamada M *et al*. Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through GPR120. *Nat Med* 2005; **11**: 90–94.
73 Schauder P, Brown JC, Frerichs H, Creutzfeldt W. Gastric inhibitory polypeptide: effect on glucose-induced insulin release from isolated rat pancreatic islets *in vitro*. *Diabetologia* 1975; **11**: 483–484.
74 Dupre J, Ross SA, Watson D, Brown JC. Stimulation of insulin secretion by gastric inhibitory polypeptide in man. *J Clin Endocrinol Metab* 1973; **37**: 826–828.
75 Kreymann B, Williams G, Ghatei MA, Bloom SR. Glucagon-like peptide-1 7-36: a physiological incretin in man. *Lancet* 1987; **2**: 1300–1304.
76 Moens K, Heimberg H, Flamez D, Huypens P, Quartier E, Ling Z *et al*. Expression and functional activity of glucagon, glucagon-like peptide I, and glucose-dependent insulinotropic peptide receptors in rat pancreatic islet cells. *Diabetes* 1996; **45**: 257–261.
77 Drucker DJ, Philippe J, Mojsov S, Chick WL, Habener JF. Glucagon-like peptide I stimulates insulin gene expression and increases cyclic AMP levels in a rat islet cell line. *Proc Natl Acad Sci USA* 1987; **84**: 3434–3438.
78 Ramos LS, Zippin JH, Kamenetsky M, Buck J, Levin LR. Glucose and GLP-1 stimulate cAMP production via distinct adenylyl cyclases in INS-1E insulinoma cells. *J Gen Physiol* 2008; **132**: 329–338.
79 Holz GGt 4th, Leech CA, Habener JF. Activation of a cAMP-regulated Ca(2+)-signaling pathway in pancreatic beta-cells by the insulinotropic hormone glucagon-like peptide-1. *J Biol Chem* 1995; **270**: 17749–17757.
80 Wheeler MB, Gelling RW, McIntosh CH, Georgiou J, Brown JC, Pederson RA. Functional expression of the rat pancreatic islet glucose-dependent insulinotropic polypeptide receptor: ligand binding and intracellular signaling properties. *Endocrinology* 1995; **136**: 4629–4639.
81 Wheeler MB, Lu M, Dillon JS, Leng XH, Chen C, Boyd AE 3rd. Functional expression of the rat glucagon-like peptide-I receptor, evidence for coupling to both adenylyl cyclase and phospholipase-C. *Endocrinology* 1993; **133**: 57–62.
82 Volz A, Goke R, Lankat-Buttgereit B, Fehmann HC, Bode HP, Goke B. Molecular cloning, functional expression, and signal transduction of the GIP-receptor cloned from a human insulinoma. *FEBS Lett* 1995; **373**: 23–29.
83 Holz GG, Leech CA, Heller RS, Castonguay M, Habener JF. cAMP-dependent mobilization of intracellular Ca^{2+} stores by activation of ryanodine receptors in pancreatic beta-cells. A Ca^{2+} signaling system stimulated by the insulinotropic hormone glucagon-like peptide-1-(7-37). *J Biol Chem* 1999; **274**: 14147–14156.
84 Gromada J, Dissing S, Bokvist K, Renstrom E, Frokjaer-Jensen J, Wulff BS *et al*. Glucagon-like peptide I increases cytoplasmic calcium in insulin-secreting beta TC3-cells by enhancement of intracellular calcium mobilization. *Diabetes* 1995; **44**: 767–774.
85 Lu M, Wheeler MB, Leng XH, Boyd AE 3rd. The role of the free cytosolic calcium level in beta-cell signal transduction by gastric inhibitory polypeptide and glucagon-like peptide I(7-37). *Endocrinology* 1993; **132**: 94–100.
86 Fridolf T, Ahren B. Effects of glucagon like peptide-1(7-36) amide on the cytoplasmic Ca(2+)-concentration in rat islet cells. *Mol Cell Endocrinol* 1993; **96**: 85–90.
87 Cullinan CA, Brady EJ, Saperstein R, Leibowitz MD. Glucose-dependent alterations of intracellular free calcium by glucagon-like peptide-1 (7-36amide) in individual ob/ob mouse beta-cells. *Cell Calcium* 1994; **15**: 391–400.
88 Thorens B, Porret A, Buhler L, Deng SP, Morel P, Widmann C. Cloning and functional expression of the human islet GLP-1 receptor. Demonstration that exendin-4 is an agonist and exendin-(9-39) an antagonist of the receptor. *Diabetes* 1993; **42**: 1678–1682.
89 Song WJ, Seshadri M, Ashraf U, Mdluli T, Mondal P, Keil M *et al*. Snapin mediates incretin action and augments glucose-dependent insulin secretion. *Cell Metab* 2011; **13**: 308–319.
90 Wu B, Wei S, Petersen N, Ali Y, Wang X, Bacaj T *et al*. Synaptotagmin-7 phosphorylation mediates GLP-1-dependent potentiation of insulin secretion from beta-cells. *Proc Natl Acad Sci USA* 2015; **112**: 9996–10001.
91 Shapiro H, Shachar S, Sekler I, Hershfinkel M, Walker MD. Role of GPR40 in fatty acid action on the beta cell line INS-1E. *Biochem Biophys Res Commun* 2005; **335**: 97–104.
92 Fujiwara K, Maekawa F, Yada T. Oleic acid interacts with GPR40 to induce Ca^{2+} signaling in rat islet beta-cells: mediation by PLC and L-type Ca^{2+} channel and link to insulin release. *Am J Physiol Endocrinol Metab* 2005; **289**: E670–E677.
93 Salehi A, Flodgren E, Nilsson NE, Jimenez-Feltstrom J, Miyazaki J, Owman C *et al*. Free fatty acid receptor 1 (FFA(1)R/GPR40) and its involvement in fatty-acid-stimulated insulin secretion. *Cell Tissue Res* 2005; **322**: 207–215.

94 Itoh Y, Kawamata Y, Harada M, Kobayashi M, Fujii R, Fukusumi S *et al.* Free fatty acids regulate insulin secretion from pancreatic beta cells through GPR40. *Nature* 2003; **422**: 173–176.
95 Ximenes HM, Hirata AE, Rocha MS, Curi R, Carpinelli AR. Propionate inhibits glucose-induced insulin secretion in isolated rat pancreatic islets. *Cell Biochem Funct* 2007; **25**: 173–178.
96 Porte D Jr, Williams RH. Inhibition of insulin release by norepinephrine in man. *Science* 1966; **152**: 1248–1250.
97 Peterhoff M, Sieg A, Brede M, Chao CM, Hein L, Ullrich S. Inhibition of insulin secretion via distinct signaling pathways in alpha2-adrenoceptor knockout mice. *Eur J Endocrinol* 2003; **149**: 343–350.
98 Porte D Jr. A receptor mechanism for the inhibition of insulin release by epinephrine in man. *J Clin Invest* 1967; **46**: 86–94.
99 Rossi J, Santamaki P, Airaksinen MS, Herzig KH. Parasympathetic innervation and function of endocrine pancreas requires the glial cell line-derived factor family receptor alpha2 (GFRalpha2). *Diabetes* 2005; **54**: 1324–1330.
100 Yoshimatsu H, Niijima A, Oomura Y, Yamabe K, Katafuchi T. Effects of hypothalamic lesion on pancreatic autonomic nerve activity in the rat. *Brain Res* 1984; **303**: 147–152.
101 Borden P, Houtz J, Leach SD, Kuruvilla R. Sympathetic innervation during development is necessary for pancreatic islet architecture and functional maturation. *Cell Rep* 2013; **4**: 287–301.
102 Hopkins DF, Williams G. Insulin receptors are widely distributed in human brain and bind human and porcine insulin with equal affinity. *Diabet Med* 1997; **14**: 1044–1050.
103 Ho L, Yemul S, Knable L, Katsel P, Zhao R, Haroutunian V *et al.* Insulin receptor expression and activity in the brains of nondiabetic sporadic Alzheimer's disease cases. *Int J Alzheimers Dis* 2012; **2012**: 321280.
104 Hill JM, Lesniak MA, Pert CB, Roth J. Autoradiographic localization of insulin receptors in rat brain: prominence in olfactory and limbic areas. *Neuroscience* 1986; **17**: 1127–1138.
105 Marks JL, Porte D Jr, Stahl WL, Baskin DG. Localization of insulin receptor mRNA in rat brain by in situ hybridization. *Endocrinology* 1990; **127**: 3234–3236.
106 Obici S, Feng Z, Karkanias G, Baskin DG, Rossetti L. Decreasing hypothalamic insulin receptors causes hyperphagia and insulin resistance in rats. *Nat Neurosci* 2002; **5**: 566–572.
107 Young WS 3rd. Periventricular hypothalamic cells in the rat brain contain insulin mRNA. *Neuropeptides* 1986; **8**: 93–97.
108 Mirshamsi S, Laidlaw HA, Ning K, Anderson E, Burgess LA, Gray A *et al.* Leptin and insulin stimulation of signalling pathways in arcuate nucleus neurones: PI3K dependent actin reorganization and KATP channel activation. *BMC Neurosci* 2004; **5**: 54.
109 Wang R, Liu X, Hentges ST, Dunn-Meynell AA, Levin BE, Wang W *et al.* The regulation of glucose-excited neurons in the hypothalamic arcuate nucleus by glucose and feeding-relevant peptides. *Diabetes* 2004; **53**: 1959–1965.
110 Berthoud HR, Jeanrenaud B. Acute hyperinsulinemia and its reversal by vagotomy after lesions of the ventromedial hypothalamus in anesthetized rats. *Endocrinology* 1979; **105**: 146–151.
111 Rohner-Jeanrenaud F, Jeanrenaud B. Consequences of ventromedial hypothalamic lesions upon insulin and glucagon secretion by subsequently isolated perfused pancreases in the rat. *J Clin Invest* 1980; **65**: 902–910.
112 Goto Y, Carpenter RG, Berelowitz M, Frohman LA. Effect of ventromedial hypothalamic lesions on the secretion of somatostatin, insulin, and glucagon by the perfused rat pancreas. *Metabolism* 1980; **29**: 986–990.
113 Rohner F, Dufour AC, Karakash C, Le Marchand Y, Ruf KB, Jeanrenaud B. Immediate effect of lesion of the ventromedial hypothalamic area upon glucose-induced insulin secretion in anaesthetized rats. *Diabetologia* 1977; **13**: 239–242.
114 Hanyu O, Yamatani K, Ikarashi T, Soda S, Maruyama S, Kamimura T *et al.* Brain-derived neurotrophic factor modulates glucagon secretion from pancreatic alpha cells: its contribution to glucose metabolism. *Diabetes Obes Metab* 2003; **5**: 27–37.
115 Gotoh K, Masaki T, Chiba S, Ando H, Fujiwara K, Shimasaki T *et al.* Hypothalamic brain-derived neurotrophic factor regulates glucagon secretion mediated by pancreatic efferent nerves. *J Neuroendocrinol* 2013; **25**: 302–311.
116 Fan W, Dinulescu DM, Butler AA, Zhou J, Marks DL, Cone RD. The central melanocortin system can directly regulate serum insulin levels. *Endocrinology* 2000; **141**: 3072–3079.
117 Malaisse W, Malaisse-Lagae F, Wright PH, Ashmore J. Effects of adrenergic and cholinergic agents upon insulin secretion *in vitro*. *Endocrinology* 1967; **80**: 975–978.
118 Vettor R, Pagano C, Granzotto M, Englaro P, Angeli P, Blum WF *et al.* Effects of intravenous neuropeptide Y on insulin secretion and insulin sensitivity in skeletal muscle in normal rats. *Diabetologia* 1998; **41**: 1361–1367.
119 Moltz JH, McDonald JK. Neuropeptide Y: direct and indirect action on insulin secretion in the rat. *Peptides* 1985; **6**: 1155–1159.
120 Imai Y, Patel HR, Hawkins EJ, Doliba NM, Matschinsky FM, Ahima RS. Insulin secretion is increased in pancreatic islets of neuropeptide Y-deficient mice. *Endocrinology* 2007; **148**: 5716–5723.
121 Morgan DG, Kulkarni RN, Hurley JD, Wang ZL, Wang RM, Ghatei MA *et al.* Inhibition of glucose stimulated insulin secretion by neuropeptide Y is mediated via the Y1 receptor and inhibition of adenylyl cyclase in RIN 5AH rat insulinoma cells. *Diabetologia* 1998; **41**: 1482–1491.
122 Schwetz TA, Ustione A, Piston DW. Neuropeptide Y and somatostatin inhibit insulin secretion through different mechanisms. *Am J Physiol Endocrinol Metab* 2013; **304**: E211–E221.
123 Gautam D, Han SJ, Hamdan FF, Jeon J, Li B, Li JH *et al.* A critical role for beta cell M3 muscarinic acetylcholine receptors in regulating insulin release and blood glucose homeostasis *in vivo*. *Cell Metab* 2006; **3**: 449–461.
124 Schebalin M, Said SI, Makhlouf GM. Stimulation of insulin and glucagon secretion by vasoactive intestinal peptide. *Am J Physiol* 1977; **232**: E197–E200.
125 Filipsson K, Tornoe K, Holst J, Ahren B. Pituitary adenylate cyclase-activating polypeptide stimulates insulin and glucagon secretion in humans. *J Clin Endocrinol Metab* 1997; **82**: 3093–3098.
126 Pissios P, Ozcan U, Kokkotou E, Okada T, Liew CW, Liu S *et al.* Melanin concentrating hormone is a novel regulator of islet function and growth. *Diabetes* 2007; **56**: 311–319.
127 Borboni P, Porzio O, Pierucci D, Cicconi S, Magnaterra R, Federici M *et al.* Molecular and functional characterization of pituitary adenylate cyclase-activating polypeptide (PACAP-38)/vasoactive intestinal polypeptide receptors in pancreatic beta-cells and effects of PACAP-38 on components of the insulin secretory system. *Endocrinology* 1999; **140**: 5530–5537.
128 Straub SG, Sharp GW. A wortmannin-sensitive signal transduction pathway is involved in the stimulation of insulin release by vasoactive intestinal polypeptide and pituitary adenylate cyclase-activating polypeptide. *J Biol Chem* 1996; **271**: 1660–1668.
129 Gregersen S, Ahren B. Studies on the mechanisms by which gastrin releasing peptide potentiates glucose-induced insulin secretion from mouse islets. *Pancreas* 1996; **12**: 48–57.
130 Pettersson M, Ahren B. Gastrin releasing peptide (GRP): effects on basal and stimulated insulin and glucagon secretion in the mouse. *Peptides* 1987; **8**: 55–60.
131 Bellisle F, Louis-Sylvestre J, Demozay F, Blazy D, Le Magnen J. Cephalic phase of insulin secretion and food stimulation in humans: a new perspective. *Am J Physiol* 1985; **249**(6 Pt 1): E639–E645.
132 Woods SC, Alexander KR, Porte D Jr. Conditioned insulin secretion and hypoglycemia following repeated injections of tolbutamide in rats. *Endocrinology* 1972; **90**: 227–231.
133 Stockhorst U, Steingruber HJ, Scherbaum WA. Classically conditioned responses following repeated insulin and glucose administration in humans. *Behav Brain Res* 2000; **110**: 143–159.
134 Berthoud HR, Jeanrenaud B. Sham feeding-induced cephalic phase insulin release in the rat. *Am J Physiol* 1982; **242**: E280–E285.
135 Power ML, Schulkin J. Anticipatory physiological regulation in feeding biology: cephalic phase responses. *Appetite* 2008; **50**: 194–206.
136 Ahren B, Holst JJ. The cephalic insulin response to meal ingestion in humans is dependent on both cholinergic and noncholinergic mechanisms and is important for postprandial glycemia. *Diabetes* 2001; **50**: 1030–1038.
137 Powley TL. Vagal circuitry mediating cephalic-phase responses to food. *Appetite* 2000; **34**: 184–188.
138 Banks WA, Jaspan JB, Kastin AJ. Selective, physiological transport of insulin across the blood-brain barrier: novel demonstration by species-specific radioimmunoassays. *Peptides* 1997; **18**: 1257–1262.
139 Woods SC, Lotter EC, McKay LD, Porte D Jr. Chronic intracerebroventricular infusion of insulin reduces food intake and body weight of baboons. *Nature* 1979; **282**: 503–505.

140 Woods SC, Stein LJ, McKay LD, Porte D Jr. Suppression of food intake by intravenous nutrients and insulin in the baboon. *Am J Physiol* 1984; **247** (2 Pt 2): R393–R401.
141 Benoit SC, Air EL, Coolen LM, Strauss R, Jackman A, Clegg DJ *et al.* The catabolic action of insulin in the brain is mediated by melanocortins. *J Neurosci* 2002; **22**: 9048–9052.
142 Niswender KD, Morrison CD, Clegg DJ, Olson R, Baskin DG, Myers MG Jr *et al.* Insulin activation of phosphatidylinositol 3-kinase in the hypothalamic arcuate nucleus: a key mediator of insulin-induced anorexia. *Diabetes* 2003; **52**: 227–231.
143 Xu AW, Kaelin CB, Takeda K, Akira S, Schwartz MW, Barsh GS. PI3K integrates the action of insulin and leptin on hypothalamic neurons. *J Clin Invest* 2005; **115**: 951–958.
144 Schwartz MW, Sipols AJ, Marks JL, Sanacora G, White JD, Scheurink A *et al.* Inhibition of hypothalamic neuropeptide Y gene expression by insulin. *Endocrinology* 1992; **130**: 3608–3616.
145 Flood JF, Morley JE. Increased food intake by neuropeptide Y is due to an increased motivation to eat. *Peptides* 1991; **12**: 1329–1332.
146 Morley JE, Hernandez EN, Flood JF. Neuropeptide Y increases food intake in mice. *Am J Physiol* 1987; **253**(3 Pt 2): R516–R522.
147 Dube MG, Xu B, Crowley WR, Kalra PS, Kalra SP. Evidence that neuropeptide Y is a physiological signal for normal food intake. *Brain Res* 1994; **646**: 341–344.
148 Tang-Christensen M, Vrang N, Ortmann S, Bidlingmaier M, Horvath TL, Tschop M. Central administration of ghrelin and agouti-related protein (83-132) increases food intake and decreases spontaneous locomotor activity in rats. *Endocrinology* 2004; **145**: 4645–4652.
149 Rossi M, Kim MS, Morgan DG, Small CJ, Edwards CM, Sunter D *et al.* A C-terminal fragment of Agouti-related protein increases feeding and antagonizes the effect of alpha-melanocyte stimulating hormone *in vivo*. *Endocrinology* 1998; **139**: 4428–4431.
150 Schwartz MW, Marks JL, Sipols AJ, Baskin DG, Woods SC, Kahn SE *et al.* 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* 1991; **128**: 2645–2647.
151 Carvalheira JB, Ribeiro EB, Araujo EP, Guimaraes RB, Telles MM, Torsoni M *et al.* Selective impairment of insulin signalling in the hypothalamus of obese Zucker rats. *Diabetologia* 2003; **46**: 1629–1640.
152 Ikeda H, West DB, Pustek JJ, Figlewicz DP, Greenwood MR, Porte D Jr *et al.* Intraventricular insulin reduces food intake and body weight of lean but not obese Zucker rats. *Appetite* 1986; **7**: 381–386.
153 Obici S, Zhang BB, Karkanias G, Rossetti L. Hypothalamic insulin signaling is required for inhibition of glucose production. *Nat Med* 2002; **8**: 1376–1382.
154 Gelling RW, Morton GJ, Morrison CD, Niswender KD, Myers MG Jr, Rhodes CJ *et al.* Insulin action in the brain contributes to glucose lowering during insulin treatment of diabetes. *Cell Metab* 2006; **3**: 67–73.
155 Jiang G, Zhang BB. Glucagon and regulation of glucose metabolism. *Am J Physiol Endocrinol Metab* 2003; **284**: E671–E678.
156 Hers HG. Mechanisms of blood glucose homeostasis. *J Inherit Metab Dis* 1990; **13**: 395–410.
157 Ramachandran C, Goris J, Waelkens E, Merlevede W, Walsh DA. The interrelationship between cAMP-dependent alpha and beta subunit phosphorylation in the regulation of phosphorylase kinase activity. Studies using subunit specific phosphatases. *J Biol Chem* 1987; **262**: 3210–3218.
158 Rath VL, Ammirati M, LeMotte PK, Fennell KF, Mansour MN, Danley DE *et al.* Activation of human liver glycogen phosphorylase by alteration of the secondary structure and packing of the catalytic core. *Mol Cell* 2000; **6**: 139–148.
159 Gao H, Leary JA. Kinetic measurements of phosphoglucomutase by direct analysis of glucose-1-phosphate and glucose-6-phosphate using ion/molecule reactions and Fourier transform ion cyclotron resonance mass spectrometry. *Anal Biochem* 2004; **329**: 269–275.
160 Herzig S, Long F, Jhala US, Hedrick S, Quinn R, Bauer A *et al.* CREB regulates hepatic gluconeogenesis through the coactivator PGC-1. *Nature* 2001; **413**: 179–183.
161 Yoon JC, Puigserver P, Chen G, Donovan J, Wu Z, Rhee J *et al.* Control of hepatic gluconeogenesis through the transcriptional coactivator PGC-1. *Nature* 2001; **413**: 131–138.
162 Pilkis SJ, El-Maghrabi MR, McGrane M, Pilkis J, Claus TH. Regulation by glucagon of hepatic pyruvate kinase, 6-phosphofructo 1-kinase, and fructose-1,6-bisphosphatase. *Fed Proc* 1982; **41**: 2623–2628.
163 Rosa JL, Ventura F, Tauler A, Bartrons R. Regulation of hepatic 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase gene expression by glucagon. *J Biol Chem* 1993; **268**: 22540–22545.
164 Blair JB, Cimbala MA, Foster JL, Morgan RA. Hepatic pyruvate kinase. Regulation by glucagon, cyclic adenosine 3'-5'-monophosphate, and insulin in the perfused rat liver. *J Biol Chem* 1976; **251**: 3756–3762.
165 Feliu JE, Hue L, Hers HG. Hormonal control of pyruvate kinase activity and of gluconeogenesis in isolated hepatocytes. *Proc Natl Acad Sci USA* 1976; **73**: 2762–2766.
166 Riou JP, Claus TH, Pilkis SJ. Control of pyruvate kinase activity by glucagon in isolated hepatocytes. *Biochem Biophys Res Commun* 1976; **73**: 591–599.
167 Decaux JF, Antoine B, Kahn A. Regulation of the expression of the L-type pyruvate kinase gene in adult rat hepatocytes in primary culture. *J Biol Chem* 1989; **264**: 11584–11590.
168 Vaulont S, Munnich A, Decaux JF, Kahn A. Transcriptional and post-transcriptional regulation of L-type pyruvate kinase gene expression in rat liver. *J Biol Chem* 1986; **261**: 7621–7625.
169 de Wulf H, Hers HG. The role of glucose, glucagon and glucocorticoids in the regulation of liver glycogen synthesis. *Eur J Biochem* 1968; **6**: 558–564.
170 Hutson NJ, Brumley FT, Assimacopoulos FD, Harper SC, Exton JH. Studies on the alpha-adrenergic activation of hepatic glucose output. I. Studies on the alpha-adrenergic activation of phosphorylase and gluconeogenesis and inactivation of glycogen synthase in isolated rat liver parenchymal cells. *J Biol Chem* 1976; **251**: 5200–5208.
171 Ciudad C, Camici M, Ahmad Z, Wang Y, DePaoli-Roach AA, Roach PJ. Control of glycogen synthase phosphorylation in isolated rat hepatocytes by epinephrine, vasopressin and glucagon. *Eur J Biochem* 1984; **142**: 511–520.
172 Aiston S, Coghlan MP, Agius L. Inactivation of phosphorylase is a major component of the mechanism by which insulin stimulates hepatic glycogen synthesis. *Eur J Biochem* 2003; **270**: 2773–2781.
173 Hale MA, Kagami H, Shi L, Holland AM, Elsasser HP, Hammer RE *et al.* The homeodomain protein PDX1 is required at mid-pancreatic development for the formation of the exocrine pancreas. *Dev Biol* 2005; **286**: 225–237.
174 Ahlgren U, Jonsson J, Jonsson L, Simu K, Edlund H. beta-cell-specific inactivation of the mouse Ipf1/Pdx1 gene results in loss of the beta-cell phenotype and maturity onset diabetes. *Genes Dev* 1998; **12**: 1763–1768.
175 Wu KL, Gannon M, Peshavaria M, Offield MF, Henderson E, Ray M *et al.* Hepatocyte nuclear factor 3beta is involved in pancreatic beta-cell-specific transcription of the pdx-1 gene. *Mol Cell Biol* 1997; **17**: 6002–6013.
176 Pontoglio M, Sreenan S, Roe M, Pugh W, Ostrega D, Doyen A *et al.* Defective insulin secretion in hepatocyte nuclear factor 1alpha-deficient mice. *J Clin Invest* 1998; **101**: 2215–2222.
177 Vaxillaire M, Rouard M, Yamagata K, Oda N, Kaisaki PJ, Boriraj VV *et al.* Identification of nine novel mutations in the hepatocyte nuclear factor 1 alpha gene associated with maturity-onset diabetes of the young (MODY3). *Hum Mol Genet* 1997; **6**: 583–586.
178 Yi P, Park JS, Melton DA. Betatrophin: a hormone that controls pancreatic beta cell proliferation. *Cell* 2013; **153**: 747–758.
179 Wang Y, Quagliarini F, Gusarova V, Gromada J, Valenzuela DM, Cohen JC *et al.* Mice lacking ANGPTL8 (Betatrophin) manifest disrupted triglyceride metabolism without impaired glucose homeostasis. *Proc Natl Acad Sci USA* 2013; **110**: 16109–16114.
180 Gusarova V, Alexa CA, Na E, Stevis PE, Xin Y, Bonner-Weir S *et al.* ANGPTL8/betatrophin does not control pancreatic beta cell expansion. *Cell* 2014; **159**: 691–696.
181 Jiao Y, Le Lay J, Yu M, Naji A, Kaestner KH. Elevated mouse hepatic betatrophin expression does not increase human beta-cell replication in the transplant setting. *Diabetes* 2014; **63**: 1283–1288.
182 Hu H, Sun W, Yu S, Hong X, Qian W, Tang B *et al.* Increased circulating levels of betatrophin in newly diagnosed type 2 diabetic patients. *Diabetes Care* 2014; **37**: 2718–2722.
183 Chen X, Lu P, He W, Zhang J, Liu L, Yang Y *et al.* Circulating betatrophin levels are increased in patients with type 2 diabetes and associated with insulin resistance. *J Clin Endocrinol Metab* 2015; **100**: E96–E100.
184 Fu Z, Berhane F, Fite A, Seyoum B, Abou-Samra AB, Zhang R. Elevated circulating lipasin/betatrophin in human type 2 diabetes and obesity. *Sci Rep* 2014; **4**: 5013.

185 Gomez-Ambrosi J, Pascual E, Catalan V, Rodriguez A, Ramirez B, Silva C *et al.* Circulating betatrophin concentrations are decreased in human obesity and type 2 diabetes. *J Clin Endocrinol Metab* 2014; **99**: E2004–E2009.
186 Fu Z, Abou-Samra AB, Zhang R. An explanation for recent discrepancies in levels of human circulating betatrophin. *Diabetologia* 2014; **57**: 2232–2234.
187 Buteau J, Roduit R, Susini S, Prentki M. Glucagon-like peptide-1 promotes DNA synthesis, activates phosphatidylinositol 3-kinase and increases transcription factor pancreatic and duodenal homeobox gene 1 (PDX-1) DNA binding activity in beta (INS-1)-cells. *Diabetologia* 1999; **42**: 856–864.
188 Buteau J, Foisy S, Joly E, Prentki M. Glucagon-like peptide 1 induces pancreatic beta-cell proliferation via transactivation of the epidermal growth factor receptor. *Diabetes* 2003; **52**: 124–132.
189 Perfetti R, Zhou J, Doyle ME, Egan JM. Glucagon-like peptide-1 induces cell proliferation and pancreatic-duodenum homeobox-1 expression and increases endocrine cell mass in the pancreas of old, glucose-intolerant rats. *Endocrinology* 2000; **141**: 4600–4605.
190 Trumper A, Trumper K, Trusheim H, Arnold R, Goke B, Horsch D. Glucose-dependent insulinotropic polypeptide is a growth factor for beta (INS-1) cells by pleiotropic signaling. *Mol Endocrinol* 2001; **15**: 1559–1570.
191 Ehses JA, Pelech SL, Pederson RA, McIntosh CH. Glucose-dependent insulinotropic polypeptide activates the Raf-Mek1/2-ERK1/2 module via a cyclic AMP/cAMP-dependent protein kinase/Rap1-mediated pathway. *J Biol Chem* 2002; **277**: 37088–37097.
192 Kim SJ, Winter K, Nian C, Tsuneoka M, Koda Y, McIntosh CH. Glucose-dependent insulinotropic polypeptide (GIP) stimulation of pancreatic beta-cell survival is dependent upon phosphatidylinositol 3-kinase (PI3K)/protein kinase B (PKB) signaling, inactivation of the forkhead transcription factor Foxo1, and down-regulation of bax expression. *J Biol Chem* 2005; **280**: 22297–22307.
193 Li Y, Hansotia T, Yusta B, Ris F, Halban PA, Drucker DJ. Glucagon-like peptide-1 receptor signaling modulates beta cell apoptosis. *J Biol Chem* 2003; **278**: 471–478.
194 Trumper A, Trumper K, Horsch D. Mechanisms of mitogenic and anti-apoptotic signaling by glucose-dependent insulinotropic polypeptide in beta(INS-1)-cells. *J Endocrinol* 2002; **174**: 233–246.
195 Buteau J, El-Assaad W, Rhodes CJ, Rosenberg L, Joly E, Prentki M. Glucagon-like peptide-1 prevents beta cell glucolipotoxicity. *Diabetologia* 2004; **47**: 806–815.
196 Prigeon RL, Quddusi S, Paty B, D'Alessio DA. Suppression of glucose production by GLP-1 independent of islet hormones: a novel extrapancreatic effect. *Am J Physiol Endocrinol Metab* 2003; **285**: E701–E707.
197 Degn KB, Juhl CB, Sturis J, Jakobsen G, Brock B, Chandramouli V *et al.* ONE week's treatment with the long-acting glucagon-like peptide 1 derivative liraglutide (NN2211) markedly improves 24-h glycemia and alpha- and beta-cell function and reduces endogenous glucose release in patients with type 2 diabetes. *Diabetes* 2004; **53**: 1187–1194.
198 Willms B, Werner J, Holst JJ, Orskov C, Creutzfeldt W, Nauck MA. Gastric emptying, glucose responses, and insulin secretion after a liquid test meal: effects of exogenous glucagon-like peptide-1 (GLP-1)-(7-36) amide in type 2 (noninsulin-dependent) diabetic patients. *J Clin Endocrinol Metab* 1996; **81**: 327–332.
199 Zander M, Madsbad S, Madsen JL, Holst JJ. Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and beta-cell function in type 2 diabetes: a parallel-group study. *Lancet* 2002; **359**: 824–830.
200 Gutzwiller JP, Drewe J, Goke B, Schmidt H, Rohrer B, Lareida J *et al.* Glucagon-like peptide-1 promotes satiety and reduces food intake in patients with diabetes mellitus type 2. *Am J Physiol* 1999; **276**(5 Pt 2): R1541–R1544.
201 Kjems LL, Holst JJ, Volund A, Madsbad S. The influence of GLP-1 on glucose-stimulated insulin secretion: effects on beta-cell sensitivity in type 2 and nondiabetic subjects. *Diabetes* 2003; **52**: 380–386.
202 Chang AM, Jakobsen G, Sturis J, Smith MJ, Bloem CJ, An B *et al.* The GLP-1 derivative NN2211 restores beta-cell sensitivity to glucose in type 2 diabetic patients after a single dose. *Diabetes* 2003; **52**: 1786–1791.
203 Egan JM, Meneilly GS, Habener JF, Elahi D. Glucagon-like peptide-1 augments insulin-mediated glucose uptake in the obese state. *J Clin Endocrinol Metab* 2002; **87**: 3768–3773.
204 Meneilly GS, McIntosh CH, Pederson RA, Habener JF, Gingerich R, Egan JM *et al.* Effect of glucagon-like peptide 1 on non-insulin-mediated glucose uptake in the elderly patient with diabetes. *Diabetes Care* 2001; **24**: 1951–1956.
205 Eckel RH, Fujimoto WY, Brunzell JD. Gastric inhibitory polypeptide enhanced lipoprotein lipase activity in cultured preadipocytes. *Diabetes* 1979; **28**: 1141–1142.
206 Song DH, Getty-Kaushik L, Tseng E, Simon J, Corkey BE, Wolfe MM. Glucose-dependent insulinotropic polypeptide enhances adipocyte development and glucose uptake in part through Akt activation. *Gastroenterology* 2007; **133**: 1796–1805.
207 Kim SJ, Nian C, McIntosh CH. Activation of lipoprotein lipase by glucose-dependent insulinotropic polypeptide in adipocytes. A role for a protein kinase B, LKB1, and AMP-activated protein kinase cascade. *J Biol Chem* 2007; **282**: 8557–8567.
208 Bollag RJ, Zhong Q, Ding KH, Phillips P, Zhong L, Qin F *et al.* Glucose-dependent insulinotropic peptide is an integrative hormone with osteotropic effects. *Mol Cell Endocrinol* 2001; **177**: 35–41.
209 Xie D, Cheng H, Hamrick M, Zhong Q, Ding KH, Correa D *et al.* Glucose-dependent insulinotropic polypeptide receptor knockout mice have altered bone turnover. *Bone* 2005; **37**: 759–769.
210 Xie D, Zhong Q, Ding KH, Cheng H, Williams S, Correa D *et al.* Glucose-dependent insulinotropic peptide-overexpressing transgenic mice have increased bone mass. *Bone* 2007; **40**: 1352–1360.
211 Zhong Q, Itokawa T, Sridhar S, Ding KH, Xie D, Kang B *et al.* Effects of glucose-dependent insulinotropic peptide on osteoclast function. *Am J Physiol Endocrinol Metab* 2007; **292**: E543–E548.
212 Lynn FC, Thompson SA, Pospisilik JA, Ehses JA, Hinke SA, Pamir N *et al.* A novel pathway for regulation of glucose-dependent insulinotropic polypeptide (GIP) receptor expression in beta cells. *Faseb J* 2003; **17**: 91–93.
213 Lim GE, Huang GJ, Flora N, LeRoith D, Rhodes CJ, Brubaker PL. Insulin regulates glucagon-like peptide-1 secretion from the enteroendocrine L cell. *Endocrinology* 2009; **150**: 580–591.
214 Scrocchi LA, Brown TJ, MaClusky N, Brubaker PL, Auerbach AB, Joyner AL *et al.* Glucose intolerance but normal satiety in mice with a null mutation in the glucagon-like peptide 1 receptor gene. *Nat Med* 1996; **2**: 1254–1258.
215 Hansotia T, Baggio LL, Delmeire D, Hinke SA, Yamada Y, Tsukiyama K *et al.* Double incretin receptor knockout (DIRKO) mice reveal an essential role for the enteroinsular axis in transducing the glucoregulatory actions of DPP-IV inhibitors. *Diabetes* 2004; **53**: 1326–1335.
216 Miyawaki K, Yamada Y, Yano H, Niwa H, Ban N, Ihara Y *et al.* Glucose intolerance caused by a defect in the entero-insular axis: a study in gastric inhibitory polypeptide receptor knockout mice. *Proc Natl Acad Sci USA* 1999; **96**: 14843–14847.
217 Pamir N, Lynn FC, Buchan AM, Ehses J, Hinke SA, Pospisilik JA *et al.* Glucose-dependent insulinotropic polypeptide receptor null mice exhibit compensatory changes in the enteroinsular axis. *Am J Physiol Endocrinol Metab* 2003; **284**: E931–E939.
218 Preitner F, Ibberson M, Franklin I, Binnert C, Pende M, Gjinovci A *et al.* Gluco-incretins control insulin secretion at multiple levels as revealed in mice lacking GLP-1 and GIP receptors. *J Clin Invest* 2004; **113**: 635–645.
219 Nauck M, Stockmann F, Ebert R, Creutzfeldt W. Reduced incretin effect in type 2 (non-insulin-dependent) diabetes. *Diabetologia* 1986; **29**: 46–52.
220 Nauck MA, Heimesaat MM, Orskov C, Holst JJ, Ebert R, Creutzfeldt W. Preserved incretin activity of glucagon-like peptide 1 [7-36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. *J Clin Invest* 1993; **91**: 301–307.
221 Toft-Nielsen MB, Damholt MB, Madsbad S, Hilsted LM, Hughes TE, Michelsen BK *et al.* Determinants of the impaired secretion of glucagon-like peptide-1 in type 2 diabetic patients. *J Clin Endocrinol Metab* 2001; **86**: 3717–3723.
222 Vilsboll T, Krarup T, Deacon CF, Madsbad S, Holst JJ. Reduced postprandial concentrations of intact biologically active glucagon-like peptide 1 in type 2 diabetic patients. *Diabetes* 2001; **50**: 609–613.
223 Vilsboll T, Krarup T, Madsbad S, Holst JJ. Defective amplification of the late phase insulin response to glucose by GIP in obese Type II diabetic patients. *Diabetologia* 2002; **45**: 1111–1119.
224 Minamino N, Kangawa K, Matsuo H. Neuromedin U-8 and U-25: novel uterus stimulating and hypertensive peptides identified in porcine spinal cord. *Biochem Biophys Res Commun* 1985; **130**: 1078–1085.

225 Austin C, Lo G, Nandha KA, Meleagros L, Bloom SR. Cloning and characterization of the cDNA encoding the human neuromedin U (NmU) precursor: NmU expression in the human gastrointestinal tract. *J Mol Endocrinol* 1995; **14**: 157–169.
226 Austin C, Oka M, Nandha KA, Legon S, Khandan-Nia N, Lo G *et al.* Distribution and developmental pattern of neuromedin U expression in the rat gastrointestinal tract. *J Mol Endocrinol* 1994; **12**: 257–263.
227 Augood SJ, Keast JR, Emson PC. Distribution and characterisation of neuromedin U-like immunoreactivity in rat brain and intestine and in guinea pig intestine. *Regul Pept* 1988; **20**: 281–292.
228 Ballesta J, Carlei F, Bishop AE, Steel JH, Gibson SJ, Fahey M *et al.* Occurrence and developmental pattern of neuromedin U-immunoreactive nerves in the gastrointestinal tract and brain of the rat. *Neuroscience* 1988; **25**: 797–816.
229 Honzawa M, Sudoh T, Minamino N, Kangawa K, Matsuo H. Neuromedin U-like immunoreactivity in rat intestine: regional distribution and immunohistochemical study. *Neuropeptides* 1990; **15**: 1–9.
230 Kaczmarek P, Malendowicz LK, Pruszynska-Oszmalek E, Wojciechowicz T, Szczepankiewicz D, Szkudelski T *et al.* Neuromedin U receptor 1 expression in the rat endocrine pancreas and evidence suggesting neuromedin U suppressive effect on insulin secretion from isolated rat pancreatic islets. *Int J Mol Med* 2006; **18**: 951–955.
231 Alfa RW, Park S, Skelly KR, Poffenberger G, Jain N, Gu X *et al.* Suppression of insulin production and secretion by a decretin hormone. *Cell Metab* 2015; **21**: 323–333.
232 Kaczmarek P, Malendowicz LK, Fabis M, Ziolkowska A, Pruszynska-Oszmalek E, Sassek M *et al.* Does somatostatin confer insulinostatic effects of neuromedin u in the rat pancreas? *Pancreas* 2009; **38**: 208–212.
233 Wang TC, Bonner-Weir S, Oates PS, Chulak M, Simon B, Merlino GT *et al.* Pancreatic gastrin stimulates islet differentiation of transforming growth factor alpha-induced ductular precursor cells. *J Clin Invest* 1993; **92**: 1349–1356.
234 Rooman I, Lardon J, Bouwens L. Gastrin stimulates beta-cell neogenesis and increases islet mass from transdifferentiated but not from normal exocrine pancreas tissue. *Diabetes* 2002; **51**: 686–690.
235 Leung-Theung-Long S, Roulet E, Clerc P, Escrieut C, Marchal-Victorion S, Ritz-Laser B *et al.* Essential interaction of Egr-1 at an islet-specific response element for basal and gastrin-dependent glucagon gene transactivation in pancreatic alpha-cells. *J Biol Chem* 2005; **280**: 7976–7984.
236 Ahren B, Pettersson M, Uvnas-Moberg K, Gutniak M, Efendic S. Effects of cholecystokinin (CCK)-8, CCK-33, and gastric inhibitory polypeptide (GIP) on basal and meal-stimulated pancreatic hormone secretion in man. *Diabetes Res Clin Pract* 1991; **13**: 153–161.
237 Karlsson S, Ahren B. Effects of three different cholecystokinin receptor antagonists on basal and stimulated insulin and glucagon secretion in mice. *Acta Physiol Scand* 1989; **135**: 271–278.
238 Rushakoff RJ, Goldfine ID, Carter JD, Liddle RA. Physiological concentrations of cholecystokinin stimulate amino acid-induced insulin release in humans. *J Clin Endocrinol Metab* 1987; **65**: 395–401.
239 Verspohl EJ, Ammon HP. Cholecystokinin (CCK8) regulates glucagon, insulin, and somatostatin secretion from isolated rat pancreatic islets: interaction with glucose. *Pflugers Arch* 1987; **410**: 284–287.
240 Rushakoff RA, Goldfine ID, Beccaria LJ, Mathur A, Brand RJ, Liddle RA. Reduced postprandial cholecystokinin (CCK) secretion in patients with noninsulin-dependent diabetes mellitus: evidence for a role for CCK in regulating postprandial hyperglycemia. *J Clin Endocrinol Metab* 1993; **76**: 489–493.
241 Brown CT, Davis-Richardson AG, Giongo A, Gano KA, Crabb DB, Mukherjee N *et al.* Gut microbiome metagenomics analysis suggests a functional model for the development of autoimmunity for type 1 diabetes. *PLoS ONE* 2011; **6**: e25792.
242 Giongo A, Gano KA, Crabb DB, Mukherjee N, Novelo LL, Casella G *et al.* Toward defining the autoimmune microbiome for type 1 diabetes. *ISME J* 2011; **5**: 82–91.
243 Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. *Nature* 2006; **444**: 1022–1023.
244 Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE *et al.* A core gut microbiome in obese and lean twins. *Nature* 2009; **457**: 480–484.
245 Karlsson FH, Tremaroli V, Nookaew I, Bergstrom G, Behre CJ, Fagerberg B *et al.* Gut metagenome in European women with normal, impaired and diabetic glucose control. *Nature* 2013; **498**: 99–103.
246 Larsen N, Vogensen FK, van den Berg FW, Nielsen DS, Andreasen AS, Pedersen BK *et al.* Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PLoS ONE* 2010; **5**: e9085.
247 Moreno-Indias I, Cardona F, Tinahones FJ, Queipo-Ortuno MI. Impact of the gut microbiota on the development of obesity and type 2 diabetes mellitus. *Front Microbiol* 2014; **5**: 190.
248 Hwang I, Park YJ, Kim YR, Kim YN, Ka S, Lee HY *et al.* Alteration of gut microbiota by vancomycin and bacitracin improves insulin resistance via glucagon-like peptide 1 in diet-induced obesity. *FASEB J* 2015; **29**: 2397–2411.
249 Simon MC, Strassburger K, Nowotny B, Kolb H, Nowotny P, Burkart V *et al.* Intake of Lactobacillus reuteri improves incretin and insulin secretion in glucose-tolerant humans: a proof of concept. *Diabetes Care* 2015; **38**: 1827–1834.
250 Cani PD, Lecourt E, Dewulf EM, Sohet FM, Pachikian BD, Naslain D *et al.* Gut microbiota fermentation of prebiotics increases satietogenic and incretin gut peptide production with consequences for appetite sensation and glucose response after a meal. *Am J Clin Nutr* 2009; **90**: 1236–1243.
251 Membrez M, Blancher F, Jaquet M, Bibiloni R, Cani PD, Burcelin RG *et al.* Gut microbiota modulation with norfloxacin and ampicillin enhances glucose tolerance in mice. *FASEB J* 2008; **22**: 2416–2426.
252 Kieffer TJ, Habener JF. The adipoinsular axis: effects of leptin on pancreatic beta-cells. *Am J Physiol Endocrinol Metab* 2000; **278**: E1–E14.
253 Elmquist JK, Elias CF, Saper CB. From lesions to leptin: hypothalamic control of food intake and body weight. *Neuron* 1999; **22**: 221–232.
254 Kieffer TJ, Heller RS, Habener JF. Leptin receptors expressed on pancreatic beta-cells. *Biochem Biophys Res Commun* 1996; **224**: 522–527.
255 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* 1997; **46**: 313–316.
256 Kulkarni RN, Wang ZL, Wang RM, Hurley JD, Smith DM, Ghatei MA *et al.* Leptin rapidly suppresses insulin release from insulinoma cells, rat and human islets and, *in vivo*, in mice. *J Clin Invest* 1997; **100**: 2729–2736.
257 Cases JA, Gabriely I, Ma XH, Yang XM, Michaeli T, Fleischer N *et al.* Physiological increase in plasma leptin markedly inhibits insulin secretion *in vivo*. *Diabetes* 2001; **50**: 348–352.
258 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* 1997; **46**: 1087–1093.
259 Seufert J, Kieffer TJ, Habener JF. Leptin inhibits insulin gene transcription and reverses hyperinsulinemia in leptin-deficient ob/ob mice. *Proc Natl Acad Sci USA* 1999; **96**: 674–679.
260 Seufert J, Kieffer TJ, Leech CA, Holz GG, Moritz W, Ricordi C *et al.* Leptin suppression of insulin secretion and gene expression in human pancreatic islets: implications for the development of adipogenic diabetes mellitus. *J Clin Endocrinol Metab* 1999; **84**: 670–676.
261 Bradley RL, Cheatham B. Regulation of ob gene expression and leptin secretion by insulin and dexamethasone in rat adipocytes. *Diabetes* 1999; **48**: 272–278.
262 Kolaczynski JW, Nyce MR, Considine RV, Boden G, Nolan JJ, Henry R *et al.* Acute and chronic effects of insulin on leptin production in humans: Studies *in vivo* and *in vitro*. *Diabetes* 1996; **45**: 699–701.
263 Zeigerer A, Rodeheffer MS, McGraw TE, Friedman JM. Insulin regulates leptin secretion from 3T3-L1 adipocytes by a PI 3 kinase independent mechanism. *Exp Cell Res* 2008; **314**: 2249–2256.
264 Barr VA, Malide D, Zarnowski MJ, Taylor SI, Cushman SW. Insulin stimulates both leptin secretion and production by rat white adipose tissue. *Endocrinology* 1997; **138**: 4463–4472.
265 Sattar AA, Sattar R. Insulin-regulated expression of adiponectin receptors in muscle and fat cells. *Cell Biol Int* 2012; **36**: 1293–1297.
266 Tsuchida A, Yamauchi T, Ito Y, Hada Y, Maki T, Takekawa S *et al.* Insulin/Foxo1 pathway regulates expression levels of adiponectin receptors and adiponectin sensitivity. *J Biol Chem* 2004; **279**: 30817–30822.
267 Cong L, Chen K, Li J, Gao P, Li Q, Mi S *et al.* Regulation of adiponectin and leptin secretion and expression by insulin through a PI3K-PDE3B dependent mechanism in rat primary adipocytes. *Biochem J* 2007; **403**: 519–525.
268 Liu BH, Wang YC, Wu SC, Mersmann HJ, Cheng WT, Ding ST. Insulin regulates the expression of adiponectin and adiponectin receptors in porcine adipocytes. *Domest Anim Endocrinol* 2008; **34**: 352–359.

269 Yamauchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S *et al.* Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med* 2002; **8**: 1288–1295.
270 Wijesekara N, Krishnamurthy M, Bhattacharjee A, Suhail A, Sweeney G, Wheeler MB. Adiponectin-induced ERK and Akt phosphorylation protects against pancreatic beta cell apoptosis and increases insulin gene expression and secretion. *J Biol Chem* 2010; **285**: 33623–33631.
271 Gu W, Li X, Liu C, Yang J, Ye L, Tang J *et al.* Globular adiponectin augments insulin secretion from pancreatic islet beta cells at high glucose concentrations. *Endocrine* 2006; **30**: 217–221.
272 Boucher J, Masri B, Daviaud D, Gesta S, Guigne C, Mazzucotelli A *et al.* Apelin, a newly identified adipokine up-regulated by insulin and obesity. *Endocrinology* 2005; **146**: 1764–1771.
273 Wei L, Hou X, Tatemoto K. Regulation of apelin mRNA expression by insulin and glucocorticoids in mouse 3T3-L1 adipocytes. *Regul Pept* 2005; **132**: 27–32.
274 Takahashi M, Okimura Y, Iguchi G, Nishizawa H, Yamamoto M, Suda K *et al.* Chemerin regulates beta-cell function in mice. *Sci Rep* 2011; **1**: 123.
275 Takahashi M, Takahashi Y, Takahashi K, Zolotaryov FN, Hong KS, Kitazawa R *et al.* Chemerin enhances insulin signaling and potentiates insulin-stimulated glucose uptake in 3T3-L1 adipocytes. *FEBS Lett* 2008; **582**: 573–578.
276 Sell H, Laurencikiene J, Taube A, Eckardt K, Cramer A, Horrighs A *et al.* Chemerin is a novel adipocyte-derived factor inducing insulin resistance in primary human skeletal muscle cells. *Diabetes* 2009; **58**: 2731–2740.
277 Tan BK, Adya R, Farhatullah S, Lewandowski KC, O'Hare P, Lehnert H *et al.* Omentin-1, a novel adipokine, is decreased in overweight insulin-resistant women with polycystic ovary syndrome: *ex vivo* and *in vivo* regulation of omentin-1 by insulin and glucose. *Diabetes* 2008; **57**: 801–808.
278 Yang RZ, Lee MJ, Hu H, Pray J, Wu HB, Hansen BC *et al.* Identification of omentin as a novel depot-specific adipokine in human adipose tissue: possible role in modulating insulin action. *Am J Physiol Endocrinol Metab* 2006; **290**: E1253–E1261.
279 McTernan PG, Fisher FM, Valsamakis G, Chetty R, Harte A, McTernan CL *et al.* Resistin and type 2 diabetes: regulation of resistin expression by insulin and rosiglitazone and the effects of recombinant resistin on lipid and glucose metabolism in human differentiated adipocytes. *J Clin Endocrinol Metab* 2003; **88**: 6098–6106.
280 Haider DG, Schaller G, Kapiotis S, Maier C, Luger A, Wolzt M. The release of the adipocytokine visfatin is regulated by glucose and insulin. *Diabetologia* 2006; **49**: 1909–1914.
281 Brown JE, Onyango DJ, Ramanjaneya M, Conner AC, Patel ST, Dunmore SJ *et al.* Visfatin regulates insulin secretion, insulin receptor signalling and mRNA expression of diabetes-related genes in mouse pancreatic beta-cells. *J Mol Endocrinol* 2010; **44**: 171–178.
282 Rabe K, Lehrke M, Parhofer KG, Broedl UC. Adipokines and insulin resistance. *Mol Med* 2008; **14**: 741–751.
283 Potthoff MJ, Inagaki T, Satapati S, Ding X, He T, Goetz R *et al.* FGF21 induces PGC-1alpha and regulates carbohydrate and fatty acid metabolism during the adaptive starvation response. *Proc Natl Acad Sci USA* 2009; **106**: 10853–10858.
284 Ribas F, Villarroya J, Hondares E, Giralt M, Villarroya F. FGF21 expression and release in muscle cells: involvement of MyoD and regulation by mitochondria-driven signalling. *Biochem J* 2014; **463**: 191–199.
285 Hojman P, Pedersen M, Nielsen AR, Krogh-Madsen R, Yfanti C, Akerstrom T *et al.* Fibroblast growth factor-21 is induced in human skeletal muscles by hyperinsulinemia. *Diabetes* 2009; **58**: 2797–2801.
286 Izumiya Y, Bina HA, Ouchi N, Akasaki Y, Kharitonenkov A, Walsh K. FGF21 is an Akt-regulated myokine. *FEBS Lett* 2008; **582**: 3805–3810.
287 Trayhurn P, Drevon CA, Eckel J. Secreted proteins from adipose tissue and skeletal muscle - adipokines, myokines and adipose/muscle cross-talk. *Arch Physiol Biochem* 2011; **117**: 47–56.
288 Ellingsgaard H, Ehses JA, Hammar EB, Van Lommel L, Quintens R, Martens G *et al.* Interleukin-6 regulates pancreatic alpha-cell mass expansion. *Proc Natl Acad Sci USA* 2008; **105**: 13163–13168.
289 Ellingsgaard H, Hauselmann I, Schuler B, Habib AM, Baggio LL, Meier DT *et al.* Interleukin-6 enhances insulin secretion by increasing glucagon-like peptide-1 secretion from L cells and alpha cells. *Nat Med* 2011; **17**: 1481–1489.
290 Meinert CL, Knatterud GL, Prout TE, Klimt CR. A study of the effects of hypoglycemic agents on vascular complications in patients with adult-onset diabetes. II. Mortality results. *Diabetes* 1970; **19** (Suppl) 789–830.
291 Schwartz TB, Meinert CL. The UGDP controversy: thirty-four years of contentious ambiguity laid to rest. *Perspect Biol Med* 2004; **47**: 564–574.
292 Babenko AP, Gonzalez G, Bryan J. The tolbutamide site of SUR1 and a mechanism for its functional coupling to K(ATP) channel closure. *FEBS Lett* 1999; **459**: 367–376.
293 Koster JC, Sha Q, Nichols CG. Sulfonylurea and K(+)-channel opener sensitivity of K(ATP) channels. Functional coupling of Kir6.2 and SUR1 subunits. *J Gen Physiol* 1999; **114**: 203–213.
294 Clement JPt 4th, Kunjilwar K, Gonzalez G, Schwanstecher M, Panten U, Aguilar-Bryan L *et al.* Association and stoichiometry of K(ATP) channel subunits. *Neuron* 1997; **18**: 827–838.
295 Miki T, Nagashima K, Seino S. The structure and function of the ATP-sensitive K+ channel in insulin-secreting pancreatic beta-cells. *J Mol Endocrinol* 1999; **22**: 113–123.
296 Gloyn AL, Weedon MN, Owen KR, Turner MJ, Knight BA, Hitman G *et al.* Large-scale association studies of variants in genes encoding the pancreatic beta-cell KATP channel subunits Kir6.2 (KCNJ11) and SUR1 (ABCC8) confirm that the KCNJ11 E23K variant is associated with type 2 diabetes. *Diabetes* 2003; **52**: 568–572.
297 Inagaki N, Gonoi T, Clement JP, Wang CZ, Aguilar-Bryan L, Bryan J *et al.* A family of sulfonylurea receptors determines the pharmacological properties of ATP-sensitive K+ channels. *Neuron* 1996; **16**: 1011–1017.
298 Isomoto S, Kondo C, Yamada M, Matsumoto S, Higashiguchi O, Horio Y *et al.* A novel sulfonylurea receptor forms with BIR (Kir6.2) a smooth muscle type ATP-sensitive K+ channel. *J Biol Chem* 1996; **271**: 24321–24324.
299 Proks P, Reimann F, Green N, Gribble F, Ashcroft F. Sulfonylurea stimulation of insulin secretion. *Diabetes* 2002; **51** (Suppl 3): S368–S376.
300 Gros L, Virsolvy A, Salazar G, Bataille D, Blache P. Characterization of low-affinity binding sites for glibenclamide on the Kir6.2 subunit of the beta-cell KATP channel. *Biochem Biophys Res Commun* 1999; **257**: 766–770.
301 Gribble FM, Tucker SJ, Ashcroft FM. The interaction of nucleotides with the tolbutamide block of cloned ATP-sensitive K+ channel currents expressed in Xenopus oocytes: a reinterpretation. *J Physiol* 1997; **504** (Pt 1): 35–45.
302 Overkamp D, Volk A, Maerker E, Heide PE, Wahl HG, Rett K *et al.* Acute effect of glimepiride on insulin-stimulated glucose metabolism in glucose-tolerant insulin-resistant offspring of patients with type 2 diabetes. *Diabetes Care* 2002; **25**: 2065–2073.
303 Best JD, Judzewitsch RG, Pfeifer MA, Beard JC, Halter JB, Porte D Jr. The effect of chronic sulfonylurea therapy on hepatic glucose production in non-insulin-dependent diabetes. *Diabetes* 1982; **31**(4 Pt 1): 333–338.
304 Kolterman OG, Gray RS, Shapiro G, Scarlett JA, Griffin J, Olefsky JM. The acute and chronic effects of sulfonylurea therapy in type II diabetic subjects. *Diabetes* 1984; **33**: 346–354.
305 Craig TJ, Ashcroft FM, Proks P. How ATP inhibits the open K(ATP) channel. *J Gen Physiol* 2008; **132**: 131–144.
306 Markworth E, Schwanstecher C, Schwanstecher M. ATP4- mediates closure of pancreatic beta-cell ATP-sensitive potassium channels by interaction with 1 of 4 identical sites. *Diabetes* 2000; **49**: 1413–1418.
307 Gangji AS, Cukierman T, Gerstein HC, Goldsmith CH, Clase CM. A systematic review and meta-analysis of hypoglycemia and cardiovascular events: a comparison of glyburide with other secretagogues and with insulin. *Diabetes Care* 2007; **30**: 389–394.
308 van Staa T, Abenhaim L, Monette J. Rates of hypoglycemia in users of sulfonylureas. *J Clin Epidemiol* 1997; **50**: 735–741.
309 Ahren B. Avoiding hypoglycemia: a key to success for glucose-lowering therapy in type 2 diabetes. *Vasc Health Risk Manag* 2013; **9**: 155–163.
310 Fuhlendorff J, Rorsman P, Kofod H, Brand CL, Rolin B, MacKay P *et al.* Stimulation of insulin release by repaglinide and glibenclamide involves both common and distinct processes. *Diabetes* 1998; **47**: 345–351.
311 Gribble FM, Tucker SJ, Seino S, Ashcroft FM. Tissue specificity of sulfonylureas: studies on cloned cardiac and beta-cell K(ATP) channels. *Diabetes* 1998; **47**: 1412–1418.
312 Hu S, Wang S, Dunning BE. Tissue selectivity of antidiabetic agent nateglinide: study on cardiovascular and beta-cell K(ATP) channels. *J Pharmacol Exp Ther* 1999; **291**: 1372–1379.
313 Hu S, Wang S, Fanelli B, Bell PA, Dunning BE, Geisse S *et al.* Pancreatic beta-cell K(ATP) channel activity and membrane-binding studies with nateglinide: a comparison with sulfonylureas and repaglinide. *J Pharmacol Exp Ther* 2000; **293**: 444–452.

314 Seto Y, Fujita H, Dan K, Fujita T, Kato R. Stimulating activity of A-4166 on insulin release in in situ hamster pancreatic perfusion. *Pharmacology* 1995; **51**: 245–253.
315 Ikenoue T, Akiyoshi M, Fujitani S, Okazaki K, Kondo N, Maki T. Hypoglycaemic and insulinotropic effects of a novel oral anti-diabetic agent, (-)-N-(trans-4-isopropylcyclohexanecarbonyl)-D-phenylalanine (A-4166). *Br J Pharmacol* 1997; **120**: 137–145.
316 Melander A. Kinetics-effect relations of insulin-releasing drugs in patients with type 2 diabetes: brief overview. *Diabetes* 2004; **53** (Suppl 3) S151–S155.
317 Van Gaal LF, Van Acker KL, De Leeuw IH. Repaglinide improves blood glucose control in sulphonylurea-naive type 2 diabetes. *Diabetes Res Clin Pract* 2001; **53**: 141–148.
318 Furlong NJ, Hulme SA, O'Brien SV, Hardy KJ. Repaglinide versus metformin in combination with bedtime NPH insulin in patients with type 2 diabetes established on insulin/metformin combination therapy. *Diabetes Care* 2002; **25**: 1685–1690.
319 Meier JJ, Nauck MA, Kranz D, Holst JJ, Deacon CF, Gaeckler D *et al.* Secretion, degradation, and elimination of glucagon-like peptide 1 and gastric inhibitory polypeptide in patients with chronic renal insufficiency and healthy control subjects. *Diabetes* 2004; **53**: 654–662.
320 Green BD, Gault VA, O'Harte FP, Flatt PR. Structurally modified analogues of glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) as future antidiabetic agents. *Curr Pharm Des* 2004; **10**: 3651–3662.
321 Green BD, Lavery KS, Irwin N, O'Harte FP, Harriott P, Greer B *et al.* Novel glucagon-like peptide-1 (GLP-1) analog (Val8)GLP-1 results in significant improvements of glucose tolerance and pancreatic beta-cell function after 3-week daily administration in obese diabetic (ob/ob) mice. *J Pharmacol Exp Ther* 2006; **318**: 914–921.
322 Green BD, Mooney MH, Gault VA, Irwin N, Bailey CJ, Harriott P *et al.* N-terminal His(7)-modification of glucagon-like peptide-1(7-36) amide generates dipeptidyl peptidase IV-stable analogues with potent antihyperglycaemic activity. *J Endocrinol* 2004; **180**: 379–388.
323 Irwin N, Gault VA, Green BD, Greer B, Harriott P, Bailey CJ *et al.* Antidiabetic potential of two novel fatty acid derivatised, N-terminally modified analogues of glucose-dependent insulinotropic polypeptide (GIP): N-AcGIP(LysPAL16) and N-AcGIP(LysPAL37). *Biol Chem* 2005; **386**: 679–687.
324 Irwin N, Green BD, Mooney MH, Greer B, Harriott P, Bailey CJ *et al.* A novel, long-acting agonist of glucose-dependent insulinotropic polypeptide suitable for once-daily administration in type 2 diabetes. *J Pharmacol Exp Ther* 2005; **314**: 1187–1194.
325 Siegel EG, Gallwitz B, Scharf G, Mentlein R, Morys-Wortmann C, Folsch UR *et al.* Biological activity of GLP-1-analogues with N-terminal modifications. *Regul Pept* 1999; **79**: 93–102.
326 Ratner RE, Maggs D, Nielsen LL, Stonehouse AH, Poon T, Zhang B *et al.* Long-term effects of exenatide therapy over 82 weeks on glycaemic control and weight in over-weight metformin-treated patients with type 2 diabetes mellitus. *Diabetes Obes Metab* 2006; **8**: 419–428.
327 Moretto TJ, Milton DR, Ridge TD, Macconell LA, Okerson T, Wolka AM *et al.* Efficacy and tolerability of exenatide monotherapy over 24 weeks in antidiabetic drug-naive patients with type 2 diabetes: a randomized, double-blind, placebo-controlled, parallel-group study. *Clin Ther* 2008; **30**: 1448–1460.
328 Kendall DM, Riddle MC, Rosenstock J, Zhuang D, Kim DD, Fineman MS *et al.* Effects of exenatide (exendin-4) on glycemic control over 30 weeks in patients with type 2 diabetes treated with metformin and a sulfonylurea. *Diabetes Care* 2005; **28**: 1083–1091.
329 Buse JB, Drucker DJ, Taylor KL, Kim T, Walsh B, Hu H *et al.* DURATION-1: exenatide once weekly produces sustained glycemic control and weight loss over 52 weeks. *Diabetes Care* 2010; **33**: 1255–1261.
330 Kim D, MacConell L, Zhuang D, Kothare PA, Trautmann M, Fineman M *et al.* Effects of once-weekly dosing of a long-acting release formulation of exenatide on glucose control and body weight in subjects with type 2 diabetes. *Diabetes Care* 2007; **30**: 1487–1493.
331 Buse JB, Rosenstock J, Sesti G, Schmidt WE, Montanya E, Brett JH *et al.* Liraglutide once a day versus exenatide twice a day for type 2 diabetes: a 26-week randomised, parallel-group, multinational, open-label trial (LEAD-6). *Lancet* 2009; **374**: 39–47.
332 Zinman B, Gerich J, Buse JB, Lewin A, Schwartz S, Raskin P *et al.* Efficacy and safety of the human glucagon-like peptide-1 analog liraglutide in combination with metformin and thiazolidinedione in patients with type 2 diabetes (LEAD-4 Met+TZD). *Diabetes Care* 2009; **32**: 1224–1230.
333 Fonseca VA, Alvarado-Ruiz R, Raccah D, Boka G, Miossec P, Gerich JE. Efficacy and safety of the once-daily GLP-1 receptor agonist lixisenatide in monotherapy: a randomized, double-blind, placebo-controlled trial in patients with type 2 diabetes (GetGoal-Mono). *Diabetes Care* 2012; **35**: 1225–1231.
334 Ratner RE, Rosenstock J, Boka G. Dose-dependent effects of the once-daily GLP-1 receptor agonist lixisenatide in patients with Type 2 diabetes inadequately controlled with metformin: a randomized, double-blind, placebo-controlled trial. *Diabet Med* 2010; **27**: 1024–1032.
335 Rosenstock J, Hanefeld M, Shamanna P, Min KW, Boka G, Miossec P *et al.* Beneficial effects of once-daily lixisenatide on overall and post-prandial glycemic levels without significant excess of hypoglycemia in type 2 diabetes inadequately controlled on a sulfonylurea with or without metformin (GetGoal-S). *J Diabetes Complications* 2014; **28**: 386–392.
336 Elahi D, McAloon-Dyke M, Fukagawa NK, Meneilly GS, Sclater AL, Minaker KL *et al.* The insulinotropic actions of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (7-37) in normal and diabetic subjects. *Regul Pept* 1994; **51**: 63–74.
337 Chia CW, Carlson OD, Kim W, Shin YK, Charles CP, Kim HS *et al.* Exogenous glucose-dependent insulinotropic polypeptide worsens post prandial hyperglycemia in type 2 diabetes. *Diabetes* 2009; **58**: 1342–1349.
338 Mentis N, Vardarli I, Kothe LD, Holst JJ, Deacon CF, Theodorakis M *et al.* GIP does not potentiate the antidiabetic effects of GLP-1 in hyperglycemic patients with type 2 diabetes. *Diabetes* 2011; **60**: 1270–1276.
339 Herrmann C, Goke R, Richter G, Fehmann HC, Arnold R, Goke B. Glucagon-like peptide-1 and glucose-dependent insulin-releasing polypeptide plasma levels in response to nutrients. *Digestion* 1995; **56**: 117–126.
340 Nathan DM, Schreiber E, Fogel H, Mojsov S, Habener JF. Insulinotropic action of glucagonlike peptide-I-(7-37) in diabetic and nondiabetic subjects. *Diabetes Care* 1992; **15**: 270–276.
341 Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group. *Lancet* 1998; **352**: 854–865.
342 Hundal RS, Krssak M, Dufour S, Laurent D, Lebon V, Chandramouli V *et al.* Mechanism by which metformin reduces glucose production in type 2 diabetes. *Diabetes* 2000; **49**: 2063–2069.
343 Musi N, Hirshman MF, Nygren J, Svanfeldt M, Bavenholm P, Rooyackers O *et al.* Metformin increases AMP-activated protein kinase activity in skeletal muscle of subjects with type 2 diabetes. *Diabetes* 2002; **51**: 2074–2081.
344 Seifarth C, Schehler B, Schneider HJ. Effectiveness of metformin on weight loss in non-diabetic individuals with obesity. *Exp Clin Endocrinol Diabetes* 2013; **121**: 27–31.
345 Lee A, Morley JE. Metformin decreases food consumption and induces weight loss in subjects with obesity with type II non-insulin-dependent diabetes. *Obes Res* 1998; **6**: 47–53.
346 Hashemitabar M, Bahramzadeh S, Saremy S, Nejaddehbashi F. Glucose plus metformin compared with glucose alone on beta-cell function in mouse pancreatic islets. *Biomed Rep* 2015; **3**: 721–725.
347 Richardson H, Campbell SC, Smith SA, Macfarlane WM. Effects of rosiglitazone and metformin on pancreatic beta cell gene expression. *Diabetologia* 2006; **49**: 685–696.
348 Jiang Y, Huang W, Wang J, Xu Z, He J, Lin X *et al.* Metformin plays a dual role in MIN6 pancreatic beta cell function through AMPK-dependent autophagy. *Int J Biol Sci* 2014; **10**: 268–277.
349 Kefas BA, Cai Y, Kerckhofs K, Ling Z, Martens G, Heimberg H *et al.* Metformin-induced stimulation of AMP-activated protein kinase in beta-cells impairs their glucose responsiveness and can lead to apoptosis. *Biochem Pharmacol* 2004; **68**: 409–416.
350 Marchetti P, Del Guerra S, Marselli L, Lupi R, Masini M, Pollera M *et al.* Pancreatic islets from type 2 diabetic patients have functional defects and increased apoptosis that are ameliorated by metformin. *J Clin Endocrinol Metab* 2004; **89**: 5535–5541.
351 Lupi R, Del Guerra S, Fierabracci V, Marselli L, Novelli M, Patane G *et al.* Lipotoxicity in human pancreatic islets and the protective effect of metformin. *Diabetes* 2002; **51**(Suppl 1): S134–S137.
352 Murphy EJ, Davern TJ, Shakil AO, Shick L, Masharani U, Chow H *et al.* Troglitazone-induced fulminant hepatic failure. Acute Liver Failure Study Group. *Dig Dis Sci* 2000; **45**: 549–553.

353 Neuschwander-Tetri BA, Isley WL, Oki JC, Ramrakhiani S, Quiason SG, Phillips NJ *et al.* Troglitazone-induced hepatic failure leading to liver transplantation. A case report. *Ann Intern Med* 1998; **129**: 38–41.
354 Kohlroser J, Mathai J, Reichheld J, Banner BF, Bonkovsky HL. Hepatotoxicity due to troglitazone: report of two cases and review of adverse events reported to the United States Food and Drug Administration. *Am J Gastroenterol* 2000; **95**: 272–276.
355 Nissen SE, Wolski K. Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. *N Engl J Med* 2007; **356**: 2457–2471.
356 Berger J, Bailey P, Biswas C, Cullinan CA, Doebber TW, Hayes NS *et al.* Thiazolidinediones produce a conformational change in peroxisomal proliferator-activated receptor-gamma: binding and activation correlate with antidiabetic actions in db/db mice. *Endocrinology* 1996; **137**: 4189–4195.
357 Schoonjans K, Peinado-Onsurbe J, Lefebvre AM, Heyman RA, Briggs M, Deeb S *et al.* PPARalpha and PPARgamma activators direct a distinct tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene. *EMBO J* 1996; **15**: 5336–5348.
358 Dumasia R, Eagle KA, Kline-Rogers E, May N, Cho L, Mukherjee D. Role of PPAR- gamma agonist thiazolidinediones in treatment of pre-diabetic and diabetic individuals: a cardiovascular perspective. *Curr Drug Targets Cardiovasc Haematol Disord* 2005; **5**: 377–386.
359 Smith SR, De Jonge L, Volaufova J, Li Y, Xie H, Bray GA. Effect of pioglitazone on body composition and energy expenditure: a randomized controlled trial. *Metabolism* 2005; **54**: 24–32.
360 Ishida H, Takizawa M, Ozawa S, Nakamichi Y, Yamaguchi S, Katsuta H *et al.* Pioglitazone improves insulin secretory capacity and prevents the loss of beta-cell mass in obese diabetic db/db mice: Possible protection of beta cells from oxidative stress. *Metabolism* 2004; **53**: 488–494.
361 Diani AR, Sawada G, Wyse B, Murray FT, Khan M. Pioglitazone preserves pancreatic islet structure and insulin secretory function in three murine models of type 2 diabetes. *Am J Physiol Endocrinol Metab* 2004; **286**: E116–E122.
362 Kawasaki F, Matsuda M, Kanda Y, Inoue H, Kaku K. Structural and functional analysis of pancreatic islets preserved by pioglitazone in db/db mice. *Am J Physiol Endocrinol Metab* 2005; **288**: E510–E518.
363 Kimura T, Kaneto H, Shimoda M, Hirukawa H, Okauchi S, Kohara K *et al.* Protective effects of pioglitazone and/or liraglutide on pancreatic beta-cells in db/db mice: comparison of their effects between in an early and advanced stage of diabetes. *Mol Cell Endocrinol* 2015; **400**: 78–89.
364 Kanda Y, Shimoda M, Hamamoto S, Tawaramoto K, Kawasaki F, Hashiramoto M *et al.* Molecular mechanism by which pioglitazone preserves pancreatic beta-cells in obese diabetic mice: evidence for acute and chronic actions as a PPARgamma agonist. *Am J Physiol Endocrinol Metab* 2010; **298**: E278–E286.
365 Wachters-Hagedoorn RE, Priebe MG, Heimweg JA, Heiner AM, Elzinga H, Stellaard F *et al.* Low-dose acarbose does not delay digestion of starch but reduces its bioavailability. *Diabet Med* 2007; **24**: 600–606.
366 Gerard J, Luyckx AS, Lefebvre PJ. Improvement of metabolic control in insulin dependent diabetics treated with the alpha-glucosidase inhibitor acarbose for two months. *Diabetologia* 1981; **21**: 446–451.
367 Josse RG, Chiasson JL, Ryan EA, Lau DC, Ross SA, Yale JF *et al.* Acarbose in the treatment of elderly patients with type 2 diabetes. *Diabetes Res Clin Pract* 2003; **59**: 37–42.
368 Meneilly GS, Ryan EA, Radziuk J, Lau DC, Yale JF, Morais J *et al.* Effect of acarbose on insulin sensitivity in elderly patients with diabetes. *Diabetes Care* 2000; **23**: 1162–1167.
369 Baron AD, Eckel RH, Schmeiser L, Kolterman OG. The effect of short-term alpha-glucosidase inhibition on carbohydrate and lipid metabolism in type II (noninsulin-dependent) diabetics. *Metabolism* 1987; **36**: 409–415.
370 Hillebrand I, Boehme K, Frank G, Fink H, Berchtold P. The effects of the alpha-glucosidase inhibitor BAY g 5421 (Acarbose) on postprandial blood glucose, serum insulin, and triglyceride levels: dose-time-response relationships in man. *Res Exp Med (Berl)* 1979; **175**: 87–94.
371 Wolever TM, Chiasson JL, Josse RG, Hunt JA, Palmason C, Rodger NW *et al.* Small weight loss on long-term acarbose therapy with no change in dietary pattern or nutrient intake of individuals with non-insulin-dependent diabetes. *Int J Obes Relat Metab Disord* 1997; **21**: 756–763.
372 Nakhaee A, Sanjari M. Evaluation of effect of acarbose consumption on weight losing in non-diabetic overweight or obese patients in Kerman. *J Res Med Sci* 2013; **18**: 391–394.
373 Chiasson JL, Josse RG, Gomis R, Hanefeld M, Karasik A, Laakso M. Acarbose treatment and the risk of cardiovascular disease and hypertension in patients with impaired glucose tolerance: the STOP-NIDDM trial. *JAMA* 2003; **290**: 486–494.
374 Goda T, Suruga K, Komori A, Kuranuki S, Mochizuki K, Makita Y *et al.* Effects of miglitol, an alpha-glucosidase inhibitor, on glycaemic status and histopathological changes in islets in non-obese, non-insulin-dependent diabetic Goto-Kakizaki rats. *Br J Nutr* 2007; **98**: 702–710.
375 Koyama M, Wada R, Mizukami H, Sakuraba H, Odaka H, Ikeda H *et al.* Inhibition of progressive reduction of islet beta-cell mass in spontaneously diabetic Goto-Kakizaki rats by alpha-glucosidase inhibitor. *Metabolism* 2000; **49**: 347–352.
376 Fukaya N, Mochizuki K, Tanaka Y, Kumazawa T, Jiuxin Z, Fuchigami M *et al.* The alpha-glucosidase inhibitor miglitol delays the development of diabetes and dysfunctional insulin secretion in pancreatic beta-cells in OLETF rats. *Eur J Pharmacol* 2009; **624**: 51–57.
377 Hanefeld M, Schaper F. Acarbose: oral anti-diabetes drug with additional cardiovascular benefits. *Expert Rev Cardiovasc Ther* 2008; **6**: 153–163.
378 Hussain MA, Daniel PB, Habener JF. Glucagon stimulates expression of the inducible cAMP early repressor and suppresses insulin gene expression in pancreatic beta-cells. *Diabetes* 2000; **49**: 1681–1690.
379 Greenbaum CJ, Havel PJ, Taborsky GJ Jr, Klaff LJ. Intra-islet insulin permits glucose to directly suppress pancreatic A cell function. *J Clin Invest* 1991; **88**: 767–773.
380 Xu E, Kumar M, Zhang Y, Ju W, Obata T, Zhang N *et al.* Intra-islet insulin suppresses glucagon release via GABA-GABAA receptor system. *Cell Metab* 2006; **3**: 47–58.
381 Meier JJ, Kjems LL, Veldhuis JD, Lefebvre P, Butler PC. Postprandial suppression of glucagon secretion depends on intact pulsatile insulin secretion: further evidence for the intraislet insulin hypothesis. *Diabetes* 2006; **55**: 1051–1056.
382 Duncan BB, Schmidt MI, Pankow JS, Ballantyne CM, Couper D, Vigo A *et al.* Low-grade systemic inflammation and the development of type 2 diabetes: the atherosclerosis risk in communities study. *Diabetes* 2003; **52**: 1799–1805.
383 de Jager J, Dekker JM, Kooy A, Kostense PJ, Nijpels G, Heine RJ *et al.* Endothelial dysfunction and low-grade inflammation explain much of the excess cardiovascular mortality in individuals with type 2 diabetes: the Hoorn Study. *Arterioscler Thromb Vasc Biol* 2006; **26**: 1086–1093.
384 Haffner SM, Lehto S, Ronnemaa T, Pyorala K, Laakso M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *N Engl J Med* 1998; **339**: 229–234.
385 Almdal T, Scharling H, Jensen JS, Vestergaard H. The independent effect of type 2 diabetes mellitus on ischemic heart disease, stroke, and death: a population-based study of 13,000 men and women with 20 years of follow-up. *Arch Intern Med* 2004; **164**: 1422–1426.
386 Adler AI, Stevens RJ, Manley SE, Bilous RW, Cull CA, Holman RR. Development and progression of nephropathy in type 2 diabetes: the United Kingdom Prospective Diabetes Study (UKPDS 64). *Kidney Int* 2003; **63**: 225–232.
387 Ravid M, Brosh D, Ravid-Safran D, Levy Z, Rachmani R. Main risk factors for nephropathy in type 2 diabetes mellitus are plasma cholesterol levels, mean blood pressure, and hyperglycemia. *Arch Intern Med* 1998; **158**: 998–1004.
388 Moriwaki Y, Yamamoto T, Shibutani Y, Aoki E, Tsutsumi Z, Takahashi S *et al.* Elevated levels of interleukin-18 and tumor necrosis factor-alpha in serum of patients with type 2 diabetes mellitus: relationship with diabetic nephropathy. *Metabolism* 2003; **52**: 605–608.
389 Wannamethee SG, Lowe GD, Rumley A, Cherry L, Whincup PH, Sattar N. Adipokines and risk of type 2 diabetes in older men. *Diabetes Care* 2007; **30**: 1200–1205.
390 Milewicz A, Mikulski E, Bidzinska B. Plasma insulin, cholecystokinin, galanin, neuropeptide Y and leptin levels in obese women with and without type 2 diabetes mellitus. *Int J Obes Relat Metab Disord* 2000; **24 Suppl 2**: S152–S153.
391 Katsuki A, Urakawa H, Gabazza EC, Murashima S, Nakatani K, Togashi K *et al.* Circulating levels of active ghrelin is associated with abdominal adiposity, hyperinsulinemia and insulin resistance in patients with type 2 diabetes mellitus. *Eur J Endocrinol* 2004; **151**: 573–577.
392 Poykko SM, Kellokoski E, Horkko S, Kauma H, Kesaniemi YA, Ukkola O. Low plasma ghrelin is associated with insulin resistance, hypertension, and the prevalence of type 2 diabetes. *Diabetes* 2003; **52**: 2546–2553.

393 Tuomilehto J, Lindstrom J, Eriksson JG, Valle TT, Hamalainen H, Ilanne-Parikka P *et al.* Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N Engl J Med* 2001; **344**: 1343–1350.
394 Lim EL, Hollingsworth KG, Aribisala BS, Chen MJ, Mathers JC, Taylor R. Reversal of type 2 diabetes: normalisation of beta cell function in association with decreased pancreas and liver triacylglycerol. *Diabetologia* 2011; **54**: 2506–2514.
395 Gustavsson N, Lao Y, Maximov A, Chuang JC, Kostromina E, Repa JJ *et al.* Impaired insulin secretion and glucose intolerance in synaptotagmin-7 null mutant mice. *Proc Natl Acad Sci USA* 2008; **105**: 3992–3997.
396 Shu Y, Liu X, Yang Y, Takahashi M, Gillis KD. Phosphorylation of SNAP-25 at Ser187 mediates enhancement of exocytosis by a phorbol ester in INS-1 cells. *J Neurosci* 2008; **28**: 21–30.
397 Lilja L, Johansson JU, Gromada J, Mandic SA, Fried G, Berggren PO *et al.* Cyclin-dependent kinase 5 associated with p39 promotes Munc18-1 phosphorylation and Ca(2+)-dependent exocytosis. *J Biol Chem* 2004; **279**: 29534–29541.
398 Dixit SS, Wang T, Manzano EJ, Yoo S, Lee J, Chiang DY *et al.* Effects of CaMKII-mediated phosphorylation of ryanodine receptor type 2 on islet calcium handling, insulin secretion, and glucose tolerance. *PLoS ONE* 2013; **8**: e58655.
399 Dzhura I, Chepurny OG, Leech CA, Roe MW, Dzhura E, Xu X *et al.* Phospholipase C-epsilon links Epac2 activation to the potentiation of glucose-stimulated insulin secretion from mouse islets of Langerhans. *Islets* 2011; **3**: 121–128.
400 Kang L, He Z, Xu P, Fan J, Betz A, Brose N *et al.* Munc13-1 is required for the sustained release of insulin from pancreatic beta cells. *Cell Metab* 2006; **3**: 463–468.
401 Kwan EP, Xie L, Sheu L, Nolan CJ, Prentki M, Betz A *et al.* Munc13-1 deficiency reduces insulin secretion and causes abnormal glucose tolerance. *Diabetes* 2006; **55**: 1421–1429.
402 Yaekura K, Julyan R, Wicksteed BL, Hays LB, Alarcon C, Sommers S *et al.* Insulin secretory deficiency and glucose intolerance in Rab3A null mice. *J Biol Chem* 2003; **278**: 9715–9721.
403 Torii S, Takeuchi T, Nagamatsu S, Izumi T. Rab27 effector granuphilin promotes the plasma membrane targeting of insulin granules via interaction with syntaxin 1a. *J Biol Chem* 2004; **279**: 22532–22538.
404 Betts GJ, Desaix P, Johnson E, Korol O, Kruse D, Poe B *et al. Human Anatomy and Physiology*. OpenStax College: Houston, TX, USA, 2013.