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Molecular insights into insulin action and secretion

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Abstract Tightly co-ordinated control of both insulin action and secretion is required in order to maintain glucose homeostasis. Gene knockout experiments have helped to define key signalling molecules that affect insulin action, including insulin and insulin-like growth factor-1 (IGF-1) receptors, insulin receptor substrate (IRS) proteins and various downstream effector proteins. β-cell function is also a tightly regulated process, with numerous factors (including certain signalling molecules) having an impact on insulin production, insulin secretion and β-cell mass. While signalling molecules play important roles in insulin action and secretion under normal circumstances, abnormal insulin signalling in muscle, adipose tissue, liver and pancreas leads to insulin resistance and β-cell dysfunction. In particular, the signalling protein IRS-2 may have a central role in linking these abnormalities, although other factors are likely to be involved.

Keywords diabetes mellitus, noninsulin-dependent; glucose/metabolism; insulin resistance; insulin/secretion; signal transduction
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Introduction

Insulin affects a wide range of physiological processes, although it is best known for its important regulatory role in glucose homeostasis. In response to elevations in plasma glucose, insulin secretion is increased and it stimulates glucose uptake and glycogen synthesis and inhibits glycogenolysis and gluconeogenesis, thus maintaining normoglycaemia. In addition to these well-established short-term actions, insulin exerts a number of other important metabolic effects, many of which are mediated via changes in the expression of more than 100 genes [1]. For example, insulin regulates the expression of genes involved in amino acid uptake, lipid metabolism in muscle and adipose tissue [2] and in cell growth, development and survival [3–7].

Maintenance of normal glucose metabolism requires tightly co-ordinated control of insulin action and secretion. In type 2 diabetes, loss of glycaemic control generally involves impairments in both insulin action (i.e. peripheral insulin resistance) and insulin secretion (i.e. β-cell dysfunction) [8,9]. However, a common underlying molecular mechanism has not yet been identified for the majority of cases of type 2 diabetes.

Our aim in this paper is to review recent studies of insulin signalling mechanisms and, in particular, to discuss how alterations in the functioning of components of the signalling pathway contribute to the development of insulin resistance. Evidence is reviewed that certain key signalling molecules have a common role in both insulin action and production and that one or more of these is disrupted in type 2 diabetes. The identification of these molecular mechanisms and further understanding of their involvement in the pathogenesis of type 2 diabetes might drive the development of rational treatment strategies that effectively address the underlying defects of insulin resistance and β-cell dysfunction.

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The insulin signalling pathway

The diverse effects of insulin are mediated through a multicomponent signalling complex that is strongly conserved across a wide range of species [10]. Binding of insulin to its receptor triggers a cascade of signalling events that ultimately leads to modifications in a number of biological processes (Fig. 1). While more detailed reviews of the insulin signalling cascade are provided elsewhere [11,12], we present a brief overview below, describing the key steps in insulin action. Our understanding of insulin action and its relation to mammalian physiology is clarified greatly by the use of targeted gene disruption experiments (known as gene knockouts) in mice. This technique gives rise to animals lacking specific genes and is used to help elucidate the biological roles of particular proteins.

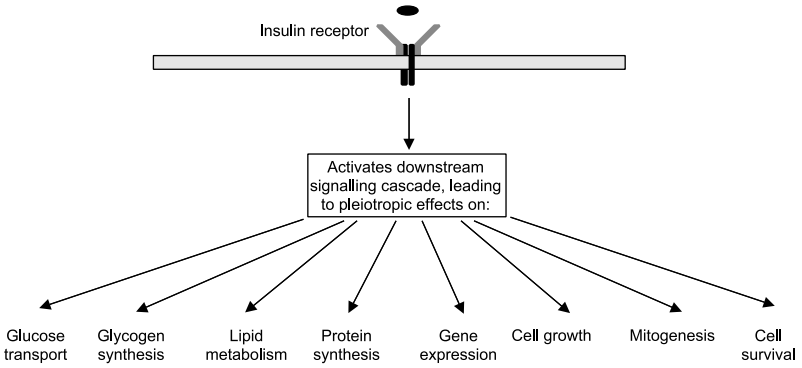

Figure 1 Diverse biological effects of insulin. When insulin binds to its receptor, resultant activation of the insulin signalling cascade leads to multiple effects on several biological processes, including glucose and lipid uptake/metabolism, gene expression/protein synthesis and cell growth, division and survival.

Receptors

Insulin receptors are ubiquitous in vertebrate cells although expression varies significantly between cell types, from as few as 40 receptors per cell on erythrocytes to more than 200 000 on adipocytes and hepatocytes [11]. The receptor consists of two extracellular α-subunits containing the insulin-binding sites and two membrane-spanning β-subunits with intrinsic tyrosine protein kinase activity. The insulin-like growth factor-1 (IGF-1) receptor is structurally related to the insulin receptor, with more than 80% amino acid sequence homology in the kinase domains [11]. As such, insulin and IGF-1 share common signal transduction mechanisms [13]. In contrast, there is little homology between the extracellular domains of the insulin and IGF-1 receptors, consistent with the differing ligand preferences of these two receptors. However, some cross-talk occurs under certain conditions, for example, during fetal development [11].

It is well known that the intrinsic tyrosine kinase activity of the insulin receptor is essential for insulin action. Point mutations in the ATP-binding site that abolish kinase activity also eradicate insulin signalling in cultured cells [14,15], while mutations in humans causing partial kinase inhibition are associated with severe insulin resistance [16,17]. Without insulin receptors, mice die shortly after birth, while people survive for a short time with severe growth retardation and diabetes [7,18–21].

Tissue-specific knockout models

Further insight into the multiple roles of insulin is provided by tissue-specific receptor knockouts (summarized in Table 1). Mice with a muscle-specific insulin receptor knockout (MIRKO) have impaired insulin action in skeletal muscle and abnormalities in lipid metabolism that are reminiscent of the Metabolic Syndrome [22]. Since many believe that muscle represents the primary site of insulin resistance in diabetes, it is surprising that glucose tolerance and plasma insulin levels remain normal in these animals. Clearly other tissues besides muscle are important in insulin-stimulated glucose homeostasis. Liver insulin receptor knockout (LIRKO) mice, on the other hand, are severely insulin resistant and glucose intolerant, confirming that hepatic insulin signalling has a significant role in the regulation of glucose homeostasis and whole body insulin sensitivity, at least in mice [23]. However, these mice do not develop diabetes in the long term owing to compensatory hyperinsulinaemia, indicating once more that defects in the β cell are required for the onset of diabetes.

Type 2 diabetes might be the consequence of insulin resistance in multiple target tissues, including the β cell itself. The β-cell-specific insulin receptor knockout (βIRKO) mouse exhibits a progressive loss of first-phase insulin secretion in response to glucose that causes glucose intolerance [24]. However, it is unclear whether the glucose-intolerant phenotype of the βIRKO mouse is entirely due to eliminating an insulin feedback effect on the β cell itself. Indeed, the notion of an insulin feedback action on the β cell remains controversial [25,26]. The βIRKO mice also develop insulin resistance with obesity and hyperinsulinaemia [27]. Some of the metabolic changes that occur in the βIRKO mice might be a consequence of unintended disruption of the insulin gene in neuronal tissues. The truncated rat insulin promoter used to drive Cre-recombinase expression (an enzyme used to generate tissue-specific gene knockout mice) in pancreatic β cells in order to generate the βIRKO mice [24] also gives marked developmental expression of Cre in certain regions of the brain [28]. Consequently, βIRKO mice might also lack the insulin receptor in parts of the brain that control body weight and energy homeostasis [29]. Intriguingly, a transgenic mouse brain-specific knockout of the insulin receptor (NIRKO) also develops obesity, insulin resistance and glucose intolerance [30]. Thus, some of the βIRKO phenotype might arise from an unintended NIRKO phenotype. Nevertheless, these tissue-specific insulin receptor knockout studies highlight dramatically the contribution of multiple tissue defects to abnormal carbohydrate metabolism and the pathogenesis of type 2 diabetes.

Insulin receptor substrate proteins

As described above, binding of insulin to its receptor activates tyrosine kinase, resulting in autophosphorylation of tyrosine residues on the receptor β-subunit. This in turn leads to phosphorylation of several protein substrates,

Table 1 Summary of key knockout mouse models

Mutant	Phenotype	Reference
Insulin receptor knockouts		
Complete	Normal intrauterine growth and development	[7]
	Severe hyperglycaemia and hyperketonaemia develops shortly after birth, leading to death after 48–72 h	
Muscle	Elevated fat mass, serum triglycerides and free fatty acids (FFA)	[22]
(MIRKO)	Normal blood glucose, serum insulin and glucose tolerance	
Liver (LIRKO)	Insulin resistance, severe glucose intolerance, insulin fails to suppress hepatic glucose output	[23]
	Marked hyperinsulinaemia caused by increased insulin secretion/decreased insulin clearance	
	Metabolic phenotype improves with ageing	
β cell (βIRKO)	Reduced insulin secretion in response to glucose	[24]
	Progressive impairment of glucose tolerance and mild obesity	[27]
Brain (NIRKO)	Develop diet-sensitive obesity and insulin resistance	[30]
	Hyperinsulinaemia and hypertriglyceridaemia	
	Impaired spermatogenesis and ovarian follicle maturation	
IGF-1 receptor knockout		
Complete	Lethal at birth owing to respiratory failure	[110]
	Severe growth deficiency and widespread developmental defects	
IRS protein knockouts		
IRS-1	Significant growth inhibition	[36]
	Mild insulin resistance and glucose intolerance but diabetes does not develop owing to compensatory hyperinsulinaemia	[35]
IRS-2	Insulin resistance in muscle and liver coupled with abnormal β-cell function lead to diabetes	[40]
	Males develop dehydration and hyperosmolar coma leading to death	
IRS-3	Body weight and plasma glucose/insulin levels comparable to wild type	[44]
	Insulin-stimulated glucose uptake in adipocytes from IRS-3 knockout mice similar to wild type	
IRS-4	Mild defects in growth in male mice	[47]
	Mild defects in reproduction and slight impairments in glucose homeostasis	
Insulin/IGF-1 signalling protein knockout		
Knockout of PI 3-kinase	Increased insulin sensitivity, hypoglycaemia and increased glucose transport caused by switch to alternative pathway (p50)	
p85 regulatory subunit	Demonstrates role for PI 3-kinase in glucose homeostasis	[111]
Akt/PKB-2	Insulin resistance in muscle and liver coupled with increased pancreatic islet mass	[52]
(Complete)	Glucose intolerant and hyperinsulinaemic	
$p70^{s6K}$-1	No insulin resistance	[88]
(Complete)	Reduced β-cell size coupled to decreased β-cell mass, insulin content and secretion	
Glucose transporter knockouts		
GLUT4	Insulin resistant with mild impairment of glucose tolerance, growth retardation and decreased fat tissue deposition	[56]
(Complete)	Hyperinsulinaemia, cardiac hypertrophy, decreased levels of lactate and FFA	
GLUT4	Insulin resistant, fasting hyperglycaemia, glucose intolerance – effects more severe than in muscle-specific insulin receptor knockout	[59]
(Muscle)		
GLUT4	Markedly impaired insulin-stimulated glucose uptake in adipocytes	[60]
(Adipose)	Insulin resistance in muscle and liver leading to glucose intolerance and hyperinsulinaemia	

primarily the insulin receptor substrate (IRS) proteins (Fig. 2). These proteins have an important regulatory role, providing an interface between insulin receptors and downstream effector molecules. To date, four mammalian IRS proteins have been identified [31–34]. Based on work with transgenic mice, IRS-1 is primarily involved in somatic cell growth and insulin action in muscle and adipose tissue [35–38], whereas IRS-2 plays important roles in β-cell survival/growth, insulin action in the liver, brain growth, reproduction and food intake [39–43]. IRS-3 and IRS-4 are predominantly expressed in adipose and neuroendocrine tissue, respectively [44,45], although their precise roles are still under investigation.

Gene knockout experiments demonstrate the critical roles that IRS-1 and IRS-2 play in activating multiple signalling pathways. Although mice deficient in IRS-1 are viable, they exhibit marked defects in both embryonic and postnatal growth [35,36]. These mice are also insulin resistant, with impaired glucose tolerance coupled with other features of the Metabolic Syndrome, such as hypertriglyceridaemia and hypertension [35,36,46]. However, despite insulin resistance, diabetes never develops in

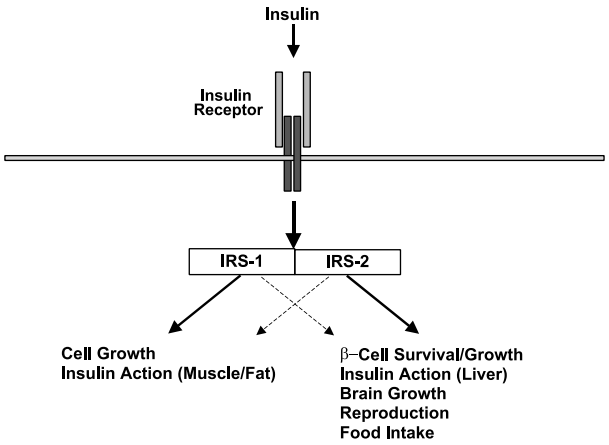

Figure 2 Interaction between insulin and the insulin receptor substrate (IRS) proteins. Insulin activates the insulin signalling cascade largely through its interaction with the IRS proteins. Phosphorylation of IRS proteins results in a number of downstream effects. The best characterized IRS proteins, IRS-1 and IRS-2, have different but overlapping functions. While IRS-1 has a predominant role in cell growth and insulin action in muscle and adipose tissue, the effects of IRS-2 are better defined in the β-cell and liver, in addition to its roles in brain growth, reproduction and food intake.

IRS-1-deficient mice because of compensatory hypersecretion of insulin [35]. Disruption of IRS-2, conversely, causes diabetes in mice and is ultimately fatal in young male mice and middle-aged female mice [40]. In contrast, mice deficient in IRS-3 or IRS-4 do not have a marked phenotype [44,47], although mice lacking IRS-4 exhibit small changes in growth, reproduction and glucose homeostasis compared with wild-type animals [47].

Euglycaemic–hyperinsulinaemic clamp studies in IRS-1- and IRS-2-deficient mice indicate a broader role for IRS-2 in liver, adipose tissue and muscle, whereas IRS-1 plays a more limited role in metabolic regulation that is focused mainly in skeletal muscle [42]. Further studies indicate a central role for IRS-1 in the regulation of protein synthesis in muscle and of glucose transport in both muscle and adipose tissue [37,48]. IRS-2, on the other hand, appears to have numerous functions in peripheral insulin-sensitive tissues plus in β-cell survival/expansion and neuroendocrine regulation of reproduction and energy homeostasis [39–43]. Overall, however, studies have shown that there is not a simple separation of function between IRS-1 and IRS-2 in tissues but that it is the balance between these proteins that is important and the ability to compensate for IRS-1 deficiency in these mouse models depends ultimately on the level of IRS-2 [37,38].

Downstream effector molecules

While the IRS proteins are early components of the insulin signalling pathway, it is the subsequent specific recruitment of multiple downstream signalling proteins that ultimately generates the unique insulin responses in various cells and tissues. During insulin stimulation, tyrosine phosphorylation sites in the IRS proteins bind specifically to the Src-homology-2 (SH2) domains in various downstream signalling molecules, including phosphatidylinositol 3-kinase (PI 3-kinase), growth factor receptor binding protein 2 (Grb-2) and SH2-containing protein-tyrosine phosphatase-2 (SHP-2; Fig. 3). The outcome of insulin action in any cell depends on which of these effector molecules are expressed and recruited into the signalling complex and the pathways that are activated as a result [10]. In skeletal muscle and adipose tissue, insulin stimulation of the PI 3-kinase pathway enhances glucose utilization by regulating the expression or subcellular localization of glucose transporters, GLUT4 and GLUT1, and stimulates the storage of glucose as glycogen or fat [49–51]. In pancreatic β cells, the PI 3-kinase cascade probably promotes survival of β cells [41,52,53]. Moreover, insulin stimulation of PI 3-kinase

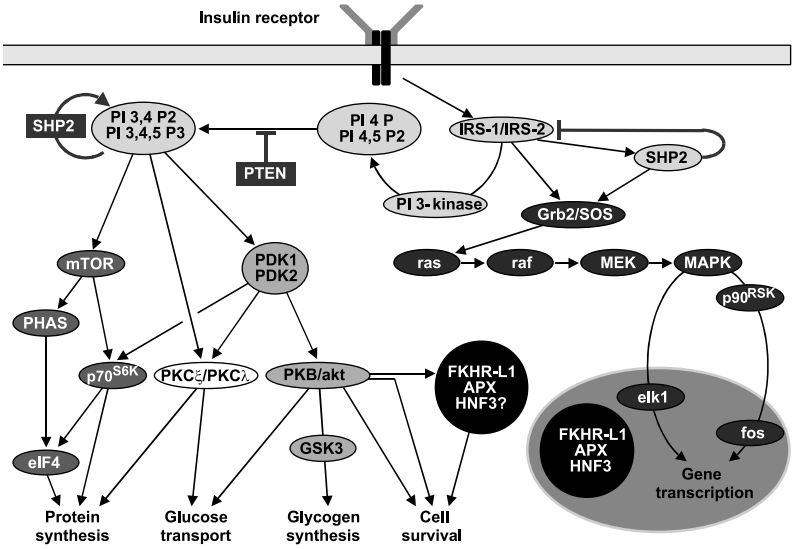

Figure 3 The insulin signalling cascade. When insulin binds to its receptor and activates the IRS proteins, this in turn triggers multiple downstream events that ultimately cause a unique insulin response, depending on the cells or tissues involved. Key molecules involved in this process are shown below, indicating some of the biological processes that are affected by changes in the insulin signalling cascade.

strongly activates total protein synthesis in most cell types that are regulated by the mammalian target of rapamycin (mTOR) pathway. Alternatively, activation of the adapter protein Grb-2 stimulates gene transcription through the mitogen-activated protein kinase (MAPK) cascade [54].

Glucose transporter knockout mice provide further information concerning the insulin signalling pathway downstream of the IRS proteins. The main insulin-responsive glucose transporter, GLUT4, is located primarily in muscle cells and adipocytes [55]. Perhaps surprisingly, complete disruption of GLUT4 in mice produces only mild glucose intolerance [56], although subsequent studies indicate severe insulin resistance and frank diabetes in some of the male animals [57,58]. Selective inactivation of GLUT4 in muscle or adipose tissue, however, has significant effects on glucose tolerance, leading to substantial reductions in insulin-stimulated glucose uptake in these tissues [59,60].

Regulation of β-cell function

β-cell function is regulated primarily by plasma glucose concentrations although numerous other signals are involved, including other sugars, amino acids, free fatty acids (FFA), hormones, growth factors and certain pharmacological agents (Fig. 4). Presented below is a brief overview of the many processes that together regulate β-cell function.

Regulation of insulin secretion

Glucose is a key regulator of insulin secretion. Regulation occurs via the process of stimulus–secretion coupling (Fig. 5), outlined below and reviewed in more detail elsewhere [61–63]. In brief, exposure to glucose increases the ATP : ADP ratio and triggers closure of ATP-sensitive K^+

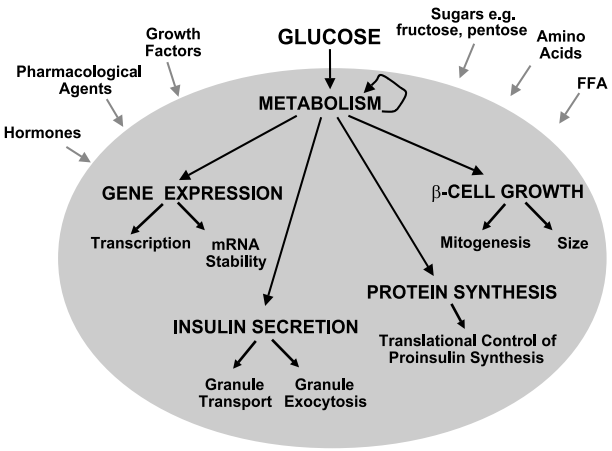

Figure 4 Regulators of β-cell function. Although β-cell function is primarily regulated by changes in plasma glucose concentrations, other factors may also have an effect. For example, other sugars, amino acids, free fatty acids, growth factors, hormones and pharmacological agents all influence β-cell function.

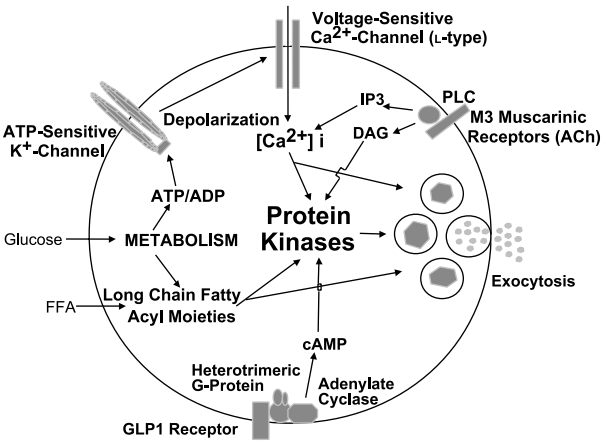

Figure 5 Stimulus–secretion coupling in the β cell. During stimulus-secretion coupling, rising plasma glucose concentrations result in an increased ratio of ATP to ADP and lead to closure of ATP-sensitive K^+-channels, which in turn triggers membrane depolarization and opening of voltage-sensitive Ca^{2+}-channels. The influx of Ca^{2+} that follows causes protein kinase activation, resulting in exocytosis of insulin secretory particles. Insulin secretion is also triggered via other pathways and several other agents besides glucose influence stimulus–secretion coupling, for example, free fatty acids, glucagon-like polypeptide-1 (GLP-1) and cholinergic agents.

channels. This in turn causes membrane depolarization and stimulates the opening of voltage-dependent Ca^{2+} channels. The resultant Ca^{2+} influx leads to increased cytosolic Ca^{2+} concentrations and promotes exocytosis – an effect mediated through protein kinase C or through direct stimulation of secretory granules.

Although glucose provides the primary stimulus, several other molecules such as FFA, amino acids and keto acids also influence stimulus–secretion coupling [64,65]. In addition, a number of hormones and neuromodulators stimulate insulin secretion, including glucagon-like polypeptide-1 (GLP-1) that increases cAMP levels and activates protein kinase C through specific G-protein-coupled receptors [66]. Another pathway operates via binding of cholinergic agents to muscarinic receptors, which stimulates the production of inositol triphosphate (IP_3) and diacylglycerol (DAG) and thus increases intracellular calcium concentrations and promotes protein kinase C activity [67–69]. The exact mechanism by which Ca^{2+} and its associated protein kinases induce transport of secretory granules to the plasma membrane and subsequently stimulate granule exocytosis is currently unclear.

Regulation of insulin production

Production of proinsulin, the precursor molecule of insulin, is regulated by glucose at both the transcriptional and post-transcriptional levels [70]. Such multifaceted gene regulation allows rapid replenishment of intracellular hormone stores during periods of high insulin secretion [71–73]. The stimulatory effects of glucose are selective for insulin, as

proinsulin biosynthesis can be stimulated up to 30-fold at the translational level, while general protein synthesis increases only twofold [72,74–76].

Numerous reports demonstrate the importance of untranslated regions of certain mRNAs in the specific regulation of that protein's synthesis. Recently, it has been indicated that a secondary 'stem loop' structure in the 5′-untranslated region and a short primary sequence in the 3′-untranslated region of preproinsulin mRNA confers a specific regulation of preproinsulin translation in response to glucose [76]. A glucose-induced rise in preproinsulin mRNA translation is paralleled by specific increases in the biosynthesis of proinsulin-converting enzymes PC2 and PC3 [77,78]. Increased synthesis of these proteins has also been attributed to changes at the level of mRNA translation [72,73,77–79], although whether this occurs through the same mechanism as for proinsulin gene expression remains to be confirmed [76]. Nonetheless, increased biosynthesis of PC2 and PC3 in parallel to their proinsulin substrate provides a means whereby proinsulin to insulin conversion in the β-cell adapts to changes in glucose homeostasis.

Under normal circumstances, glucose stimulates both proinsulin biosynthesis and insulin secretion in a co-ordinated manner. Other nutrients may also impact on these processes, for example, elevated concentrations of FFA promote increased basal insulin secretion in rats [80]. However, since elevated FFA do not stimulate proinsulin synthesis to a corresponding extent in these animals, the net result is a decrease in β-cell insulin content [80]. These data are supported by findings in isolated β cells [81]. This suggests a mechanism by which chronically elevated FFA may contribute to β-cell dysfunction in the pathogenesis of type 2 diabetes, i.e. by increasing basal insulin secretion without inducing a compensatory increase in proinsulin biosynthesis [80]. Further insight comes from a glucose-infusion rat model of type 2 diabetes [82]. In hyperglycaemic animals, chronically elevated glucose levels induce a substantial increase in proinsulin biosynthesis leading to hyperproinsulinaemia [83]. This appears to be due to premature secretion of proinsulin rather than to inefficient processing of proinsulin to insulin as a result of an acquired deficiency in PC2 and PC3 enzyme activities [83]. For a further discussion of the characteristic elevation in proinsulin levels in individuals with type 2 diabetes, see accompanying article in this supplement by Bergman *et al.* [84].

Regulation of β-cell mass

The net rate of β-cell turnover is an important factor in regulating glucose homeostasis. Since the β-cell mass exists in a dynamic state, compensatory changes, for example to correct for changes in insulin sensitivity, ensure maintenance of euglycaemia in healthy individuals. A number of factors affect β-cell mass, for example, changes in the rate of replication, neogenesis and cell death (both necrosis and apoptosis), as well as β-cell volume [85]. Although adult β cells are generally well-differentiated, with only around 0·5% of cells undergoing mitosis at any one time, β-cell proliferation

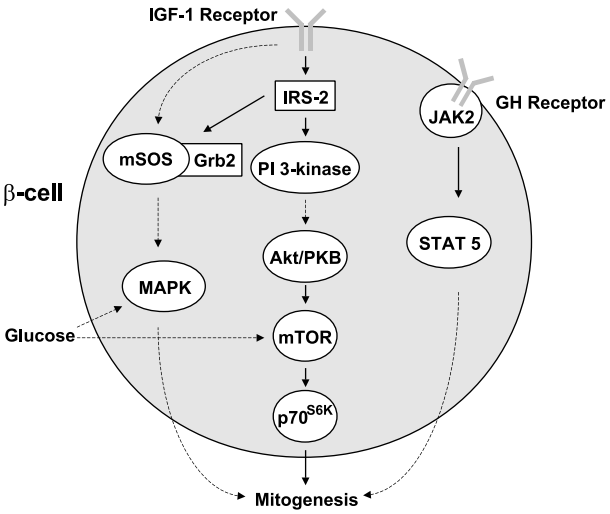

Figure 6 IGF-1 and GH signal transduction pathways in the β cell. An overview of key events occurring in the IGF-1 and GH signalling pathways that lead to stimulation of β-cell mitogenesis. Note that increased glucose metabolism can lead to activation of elements in the signal transduction pathway downstream of IRS-2. A solid arrow indicates a single step while a broken arrow is indicative of several steps. For a more detailed review, see ref. [93].

can be increased by nutrients such as glucose and amino acids [86,87]. Normal cell growth is influenced by glucose on a number of levels, including mitogenesis, DNA synthesis and cell proliferation, while the regulation of β-cell size is also considered glucose-dependent. Recently, Akt/PKB and $p70^{s6K}$ transgenic mouse models have indicated that β-cell size can be controlled via Akt/PKB, mTOR and $p70^{s6K}$ signal transduction pathways, downstream of IRS-2/PI 3-kinase activation [52,53,88].

The role of insulin signalling molecules such as the IRS proteins in the regulation of β-cell mitogenesis is of particular interest (Fig. 6). For example, IGF-1 stimulates β-cell proliferation, giving up to a 50-fold stimulation of mitogenesis in pancreatic β-cell line models [89]. The increased β-cell proliferation is glucose-dependent, but also requires the recruitment of PI 3-kinase and Grb2 to IRS-2, resulting in the activation of MAP kinase and $p70^{s6K}$, and, ultimately, increased β-cell proliferation [89]. These findings support the important part that IRS-2 plays in regulating β-cell mass as found in studies of IRS-2 knockout mice [40,41]. In addition to the effects of IGF-1, growth hormone (GH) is also important in potentiating glucose-dependent β-cell mitogenesis, although this occurs via a JAK2/STAT5 mitogenic signalling pathway independent of IRS [90].

Recent experiments have helped to elucidate the nature of the glucose-dependency of IGF-1-induced β-cell proliferation. Glucose can independently cause MAPK activation in a Ca^{2+}-dependent manner that requires glucose metabolism [89,91]. Although glucose can modestly increase PI 3-kinase activity [92,93] this surprisingly does not result in a glucose-induced activation of Akt/PKB in β cells [94]. In addition, Akt/PKB can be activated by IGF-1 independently of glucose in the β cell. However, glucose does act

downstream of Akt/PKB by independently activating the mTOR/$p70^{s6K}$ pathway [94]. There is a fine balance between the MAPK and PI 3-kinase signalling pathways in the β cell that highlights the complex nutrient and growth factor regulation of mitogenesis (discussed in detail in Dickson *et al.* [94]). By gaining further understanding of the mechanism by which β-cell proliferation and survival is stimulated, we may get one step closer to learning how we can preserve, or even enhance, β-cell mass in subjects with type 2 diabetes as a means to compensate for insulin resistance.

Abnormal insulin signalling: insulin resistance and β-cell dysfunction

We have outlined above the critical parts played by signalling molecules in regulating insulin action and secretion under normal circumstances. What happens when signalling pathways are disrupted, for example, in type 2 diabetes? In this case, abnormal insulin signalling in muscle, adipose tissue, liver and pancreas leads to insulin resistance and β-cell dysfunction.

Data from knockout mouse models indicate that deficiencies in a number of key signalling proteins, such as IRS-1 and IRS-2, lead to insulin resistance in peripheral tissues [35,36,40]. This is reinforced by additional evidence implicating decreased insulin signalling in the development of peripheral insulin resistance and supporting a role for elevated FFA concentrations and/or accumulation of intracellular lipid in reducing insulin sensitivity, particularly in skeletal muscle [95]. More specifically, increased circulating FFA levels have been linked with multiple abnormalities in the insulin-signalling pathway, including decreased IRS-1 tyrosine phosphorylation, reduced PI 3-kinase activity and increased activation of PKC [96,97]. The ultimate consequence is a decrease in GLUT4 translocation, leading to significant reductions in muscle glucose transport [96]. Preliminary data indicate that thiazolidinediones may, at least in part, exert their insulin sensitizing effects by reducing elevated plasma FFA and intracellular lipid concentrations, thus restoring insulin signalling in skeletal muscle [98,99]. For example, there is evidence that rosiglitazone modifies muscle PKCθ and PKCε activity in high-fat-fed rats [100], which may provide a potential mechanism to explain these effects. More details on the role of elevated FFA in the development of insulin resistance are given in another article in this supplement by Boden and Shulman [101].

Although the concept of 'insulin resistance' in the β cell is relatively new, evidence from IRS-2, Akt/PKB and $p70^{s6K}$ knockout mice for example suggest that defective IGF-1 signalling in the endocrine pancreas contributes to β-cell dysfunction in type 2 diabetes [24,40,41,52,53,88]. While there is a mounting body of evidence indicating that elevated fat significantly inhibits insulin signalling in skeletal muscle, it is not yet known if accumulation of fat in β cells reduces insulin signalling in the pancreas. However, elevated plasma FFA concentrations, as commonly seen in individuals with type 2 diabetes, appear to have a lipotoxic effect on the pancreas and there is evidence from rodent models that increased FFA contribute to β-cell dysfunction by increasing β-cell apoptosis [102,103]. Furthermore, *in vitro* data indicate an inhibitory effect of long-chain FFA on glucose- and IGF-1-induced DNA synthesis, leading to alterations in the activity of several protein kinases (especially inhibition of Akt/PKB activation) involved in the insulin/IGF-1 signalling pathway [91]. A hypothesis has recently been put forward suggesting that intracellular fat accumulation in β cells during obesity may lead to inhibition of β-cell mass expansion and thus failure to compensate for peripheral insulin resistance, which in turn leads to type 2 diabetes [93]. In addition to the effects of fat on β-cell insulin/IGF-1 signalling, there is evidence that high glucose concentrations are also toxic to β cells (glucotoxicity) and induce apoptosis [104]. At elevated glucose concentrations, glucose-dependent IGF-1-induced β-cell proliferative pathways are also reduced, again indicating the adverse effects of hyperglycaemia on β-cell function [89,90]. Preliminary data from Zucker Diabetic Fatty (ZDF) rats treated with rosiglitazone indicate that the thiazolidinediones, which have been shown to reduce plasma FFA and glucose levels, may also prevent the characteristic decline in β-cell mass seen in untreated animals [105,106].

While defective insulin signalling appears to be important in peripheral insulin resistance, it is becoming clearer that defective IGF-1 signalling also contributes to β-cell dysfunction. This raises the possibility of a common signalling molecule linking insulin/IGF-1 action and insulin secretory deficiencies that is disrupted in type 2 diabetes. Although IRS-1 has been put forward as providing a novel functional link between the insulin signalling and insulin secretion pathways [107], there is more evidence supporting IRS-2 as the key molecule since mice deficient in this protein develop both insulin resistance and β-cell dysfunction [39–42]. Unlike IRS-1 knockout mice, which are hyperinsulinaemic owing to higher-than-normal β-cell mass that allows them to compensate for insulin resistance, IRS-2 knockouts have a characteristic >50% reduction in β-cell mass [108]. Further clarification of the specific role of the IGF-1 → IRS-2 signalling pathway will help to elucidate whether it plays a central part in regulating both insulin action and secretion and, in particular, the development of a β-cell-specific IRS-2 knockout may provide further insight. Research is also underway to investigate whether the thiazolidinediones, which have been shown to improve β-cell function as well as insulin sensitivity, have an impact on insulin signalling in β cells as well as peripheral tissues [109].

Conclusion

Since the IRS-2 branch of the insulin/IGF-1 signalling pathway is such a fundamental process in both insulin action and β-cell function, abnormalities might contribute to both the insulin resistance and β-cell dysfunction seen in type 2 diabetes.

If this is the case, what is the likelihood that a single molecular defect can give rise to both insulin resistance and β-cell dysfunction? Although we have discussed evidence supporting the central role of IRS-2 in linking these two abnormalities, it is likely that other factors, especially those that counter-regulate the IGF-1 → IRS-2 pathway, might be involved. While this remains a subject for debate, it is clear that any molecules linking peripheral insulin action and β-cell secretory function are of interest as potential targets for therapeutic intervention because such an intervention might delay disease progression.

References

1 O'Brien RM, Granner DK. Regulation of gene expression by insulin. *Physiol Rev* 1996;**76**:1109–61.
2 Yenush L, White MF. The IRS-signalling system during insulin and cytokine action. *Bioessays* 1997;**19**:491–500.
3 Kimball SR, Vary TC, Jefferson LS. Regulation of protein synthesis by insulin. *Annu Rev Physiol* 1994;**56**:321–48.
4 Randazzo PA, Morey VA, Polishook AK, Jarett L. Characterization of the growth of murine fibroblasts that express human insulin receptors. I. The effect of insulin in the absence of other growth factors. *Exp Cell Res* 1990;**190**:25–30.
5 Taub R, Roy A, Dieter R, Koontz J. Insulin as a growth factor in rat hepatoma cells. Stimulation of proto-oncogene expression. *J Biol Chem* 1987;**262**:10893–7.
6 Sell SM, Reese D, Ossowski VM. Insulin-inducible changes in insulin receptor mRNA splice variants. *J Biol Chem* 1994;**269**:30769–72.
7 Accili D, Drago J, Lee EJ, Johnson MD, Cool MH, Salvatore P *et al.* Early neonatal death in mice homozygous for a null allele of the insulin receptor gene. *Nat Genet* 1996;**12**:106–9.
8 Osei K, Gaillard T, Schuster DP. Pathogenetic mechanisms of impaired glucose tolerance and type II diabetes in African-Americans. The significance of insulin secretion, insulin sensitivity, and glucose effectiveness. *Diabetes Care* 1997;**20**:396–404.
9 Tripathy D, Carlsson M, Almgren P, Isomaa B, Taskinen MR, Tuomi T *et al.* Insulin secretion and insulin sensitivity in relation to glucose tolerance: lessons from the Botnia Study. *Diabetes* 2000;**49**:975–80.
10 Withers DJ, White M. Perspective. The insulin signaling system – a common link in the pathogenesis of type 2 diabetes. *Endocrinology* 2000;**141**:1917–21.
11 White MF. The insulin signalling system and the IRS proteins. *Diabetologia* 1997;**40** (Suppl. 2):S2–17.
12 Avruch J. Insulin signal transduction through protein kinase cascades. *Mol Cell Biochem* 1998;**182**:31–48.
13 Benito M, Valverde AM, Lorenzo M. IGF-I: a mitogen also involved in differentiation processes in mammalian cells. *Int J Biochem Cell Biol* 1996;**28**:499–510.
14 Chou CK, Dull TJ, Russell DS, Gherzi R, Lebwohl D, Ullrich A *et al.* Human insulin receptors mutated at the ATP-binding site lack protein tyrosine kinase activity and fail to mediate postreceptor effects of insulin. *J Biol Chem* 1987;**262**:1842–7.
15 McClain DA, Maegawa H, Lee J, Dull TJ, Ulrich A, Olefsky JM. A mutant insulin receptor with defective tyrosine kinase displays no biologic activity and does not undergo endocytosis. *J Biol Chem* 1987;**262**:14663–71.
16 Odawara M, Kadowaki T, Yamamoto R, Shibasaki Y, Tobe K, Accili D *et al.* Human diabetes associated with a mutation in the tyrosine kinase domain of the insulin receptor. *Science* 1989;**245**:66–8.
17 Moller DE, Yokota A, White MF, Pazianos AG, Flier JS. A naturally occurring mutation of insulin receptor alanine 1134 impairs tyrosine kinase function and is associated with dominantly inherited insulin resistance. *J Biol Chem* 1990;**265**:14979–85.
18 Wertheimer E, Lu SP, Backeljauw PF, Davenport ML, Taylor SI. Homozygous deletion of the human insulin receptor gene results in leprechaunism. *Nat Genet* 1993;**5**:71–3.
19 Krook A, Brueton L, O'Rahilly S. Homozygous nonsense mutation in the insulin receptor gene in infant with leprechaunism. *Lancet* 1993;**342**:277–8.
20 Hone J, Accili D, Psiachou H, Alghband-Zadeh J, Mitton S, Wertheimer E *et al.* Homozygosity for a null allele of the insulin receptor gene in a patient with leprechaunism. *Hum Mutat* 1995;**6**:17–22.
21 Jospe N, Kaplowitz PB, Furlanetto RW. Homozygous nonsense mutation in the insulin receptor gene of a patient with severe congenital insulin resistance: leprechaunism and the role of the insulin-like growth factor receptor. *Clin Endocrinol (Oxf)* 1996;**45**:229–35.
22 Bruning JC, Michael MD, Winnay JN, Hayashi T, Horsch D, Accili D *et al.* A muscle-specific insulin receptor knockout exhibits features of the metabolic syndrome of NIDDM without altering glucose tolerance. *Mol Cell* 1998;**2**:559–69.
23 Michael MD, Kulkarni RN, Postic C, Previs SF, Shulman GI, Magnuson MA *et al.* Loss of insulin signaling in hepatocytes leads to severe insulin resistance and progressive hepatic dysfunction. *Mol Cell* 2000;**6**:87–97.
24 Kulkarni RN, Bruning JC, Winnay JN, Postic C, Magnuson MA, Kahn CR. Tissue-specific knockout of the insulin receptor in pancreatic beta cells creates an insulin secretory defect similar to that in type 2 diabetes. *Cell* 1999;**96**:329–39.
25 Aspinwall CA, Lakey JR, Kennedy RT. Insulin-stimulated insulin secretion in single pancreatic beta cells. *J Biol Chem* 1999;**274**:6360–5.
26 Elahi D, Nagulesparan M, Hershcopf RJ, Muller DC, Tobin JD, Blix PM *et al.* Feedback inhibition of insulin secretion by insulin: relation to the hyperinsulinemia of obesity. *N Engl J Med* 1982;**306**:1196–202.
27 Mauvais-Jarvis F, Virkamaki A, Michael MD, Winnay JN, Zisman A, Kulkarni RN *et al.* A model to explore the interaction between muscle insulin resistance and beta-cell dysfunction in the development of type 2 diabetes. *Diabetes* 2000;**49**:2126–34.
28 Gannon M, Shiota C, Postic C, Wright CV, Magnuson M. Analysis of the Cre-mediated recombination driven by rat insulin promoter in embryonic and adult mouse pancreas. *Genesis* 2000;**26**:139–42.
29 Schwartz MW, Woods SC, Porte DJ, Seeley RJ, Baskin DG. Central nervous system control of food intake. *Nature* 2000;**404**:661–71.
30 Bruning JC, Gautam D, Burks DJ, Gillette J, Schubert M, Orban PC *et al.* Role of brain insulin receptor in control of body weight and reproduction. *Science* 2000;**289**:2122–5.
31 Sun XJ, Rothenberg P, Kahn CR, Backer JM, Araki E, Wilden PA *et al.* Structure of the insulin receptor substrate IRS-1 defines a unique signal transduction protein. *Nature* 1991;**352**:73–7.
32 Sun XJ, Wang LM, Zhang Y, Yenush L, Myers MGJ, Glasheen E *et al.* Role of IRS-2 in insulin and cytokine signalling. *Nature* 1995;**377**:173–7.

33 Lavan BE, Lane WS, Lienhard GE. The 60-kDa phosphotyrosine protein in insulin-treated adipocytes is a new member of the insulin receptor substrate family. *J Biol Chem* 1997;**272**:11439–43.

34 Lavan BE, Fantin VR, Chang ET, Lane WS, Keller SR, Lienhard GE. A novel 160-kDa phosphotyrosine protein in insulin-treated embryonic kidney cells is a new member of the insulin receptor substrate family. *J Biol Chem* 1997;**272**:21403–7.

35 Araki E, Lipes MA, Patti ME, Bruning JC, Haag B, Johnson RS *et al.* Alternative pathway of insulin signalling in mice with targeted disruption of the IRS-1 gene. *Nature* 1994;**372**:186–90.

36 Tamemoto H, Kadowaki T, Tobe K, Yagi T, Sakura H, Hayakawa T *et al.* Insulin resistance and growth retardation in mice lacking insulin receptor substrate-1. *Nature* 1994;**372**:182–6.

37 Yamauchi T, Tobe K, Tamemoto H, Ueki K, Kaburagi Y, Yamamoto-Honda R *et al.* Insulin signalling and insulin actions in the muscles and livers of insulin-resistant, insulin receptor substrate 1-deficient mice. *Mol Cell Biol* 1996;**16**:3074–84.

38 Kido Y, Burks DJ, Withers D, Bruning JC, Kahn CR, White MF *et al.* Tissue-specific insulin resistance in mice with mutations in the insulin receptor, IRS-1, and IRS-2. *J Clin Invest* 2000;**105**:199–205.

39 Rother KI, Imai Y, Caruso M, Beguinot F, Formisano P, Accili D. Evidence that IRS-2 phosphorylation is required for insulin action in hepatocytes. *J Biol Chem* 1998;**273**:17491–7.

40 Withers DJ, Gutierrez JS, Towery H, Burks DJ, Ren JM, Previs S *et al.* Disruption of IRS-2 causes type 2 diabetes in mice. *Nature* 1998;**391**:900–4.

41 Withers DJ, Burks DJ, Towery HH, Altamuro SL, Flint CL, White MF. IRS-2 coordinates IGF-1 receptor-mediated beta-cell development and peripheral insulin signalling. *Nat General* 1999;**23**:32–40.

42 Previs SF, Withers DJ, Ren JM, White MF, Shulman GI. Contrasting effects of IRS-1 *versus* IRS-2 gene disruption on carbohydrate and lipid metabolism *in vivo*. *J Biol Chem* 2000;**275**:38990–4.

43 Burks DJ, de Mora JF, Schubert M, Withers DJ, Myers MG, Towery HH *et al.* IRS-2 pathways integrate female reproduction and energy homeostasis. *Nature* 2000;**407**:377–82.

44 Liu SC, Wang Q, Lienhard GE, Keller SR. Insulin receptor substrate 3 is not essential for growth or glucose homeostasis. *J Biol Chem* 1999;**274**:18093–9.

45 Numan S, Russell DS. Discrete expression of insulin receptor substrate-4 mRNA in adult rat brain. *Brain Res Mol Brain Res* 1999;**72**:97–102.

46 Abe H, Yamada N, Kamata K, Kuwaki T, Shimada M, Osuga J *et al.* Hypertension, hypertriglyceridemia, and impaired endothelium-dependent vascular relaxation in mice lacking insulin receptor substrate-1. *J Clin Invest* 1998;**101**:1784–8.

47 Fantin VR, Wang Q, Lienhard GE, Keller SR. Mice lacking insulin receptor substrate 4 exhibit mild defects in growth, reproduction, and glucose homeostasis. *Am J Physiol Endocrinol Metab* 2000;**278**:E127–33.

48 Kaburagi Y, Satoh S, Tamemoto H, Yamamoto-Honda R, Tobe K, Veki K *et al.* Role of insulin receptor substrate-1 and pp60 in the regulation of insulin-induced glucose transport and GLUT4 translocation in primary adipocytes. *J Biol Chem* 1997;**272**:25839–44.

49 Robinson LJ, Razzack ZF, Lawrence JCJ, James DE. Mitogen-activated protein kinase activation is not sufficient for stimulation of glucose transport or glycogen synthase in 3T3-L1 adipocytes. *J Biol Chem* 1993;**268**:26422–7.

50 Clarke JF, Young PW, Yonezawa K, Kasuga M, Holman GD. Inhibition of the translocation of GLUT1 and GLUT4 in 3T3-L1 cells by the phosphatidylinositol 3-kinase inhibitor, wortmannin. *Biochem J* 1994;**300**:631–5.

51 Okada T, Kawano Y, Sakakibara T, Hazeki O, Ui M. Essential role of phosphatidylinositol 3-kinase in insulin-induced glucose transport and antilipolysis in rat adipocytes. Studies with a selective inhibitor wortmannin. *J Biol Chem* 1994;**269**:3568–73.

52 Cho H, Mu J, Kim JK, Thorvaldsen JL, Chu Q, Crenshaw EB *et al.* Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKB beta). *Science* 2001;**292**:1728–31.

53 Tuttle RL, Gill NS, Pugh W, Lee JP, Koeberlein B, Furth EE *et al.* Regulation of pancreatic beta-cell growth and survival by the serine/threonine protein kinase Akt1/PKBalpha. *Nat Med* 2001;**7**:1133–7.

54 Skolnik EY, Lee CH, Batzer A, Vicentini LM, Zhou M, Daly R *et al.* The SH2/SH3 domain-containing protein GRB2 interacts with tyrosine-phosphorylated IRS1 and Shc: implications for insulin control of ras signalling. *EMBO J* 1993;**12**:1929–36.

55 Shepherd PR, Kahn BB. Glucose transporters and insulin action – implications for insulin resistance and diabetes mellitus. *N Engl J Med* 1999;**341**:248–57.

56 Katz EB, Stenbit AE, Hatton K, DePinho R, Charron MJ. Cardiac and adipose tissue abnormalities but not diabetes in mice deficient in GLUT4. *Nature* 1995;**377**:151–5.

57 Rossetti L, Stenbit AE, Chen W, Hu M, Barzilai N, Katz EB *et al.* Peripheral but not hepatic insulin resistance in mice with one disrupted allele of the glucose transporter type 4 (GLUT4) gene. *J Clin Invest* 1997;**100**:1831–9.

58 Stenbit AE, Tsao TS, Li J, Burcelin R, Geenen DL, Factor SM *et al.* GLUT4 heterozygous knockout mice develop muscle insulin resistance and diabetes. *Nat Med* 1997;**3**:1096–101.

59 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**:924–8.

60 Abel ED, Peroni O, Kim JK, Kim Y-B, Boss O, Hadro E *et al.* Adipose-selective targeting of the GLUT4 gene impairs insulin action in muscle and liver. *Nature* 2001;**409**:729–33.

61 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.

62 Sjoholm A. Aspects of novel sites of regulation of the insulin stimulus-secretion coupling in normal and diabetic pancreatic islets. *Endocrine* 1998;**9**:1–13.

63 Satin LS. Localized calcium influx in pancreatic beta-cells. its significance for Ca^{2+}-dependent insulin secretion from the islets of Langerhans. *Endocrine* 2000;**13**:251–62.

64 Prentki M, Tornheim K, Corkey BE. Signal transduction mechanisms in nutrient-induced insulin secretion. *Diabetologia* 1997;**40** (Suppl. 2):S32–41.

65 Deeney JT, Prentki M, Corkey BE. Metabolic control of beta-cell function. *Semin Cell Dev Biol* 2000;**11**:267–75.

66 Thorens B. Glucagon-like peptide-1 and control of insulin secretion. *Diabete Metab* 1995;**21**:311–18.

67 Wollheim CB, Biden TJ. Second messenger function of inositol 1,4,5-trisphosphate. Early changes in inositol phosphates, cytosolic Ca^{2+}, and insulin release in

carbamylcholine-stimulated RINm5F cells. *J Biol Chem* 1986;**261**:8314–19.
68 Hughes SJ, Chalk JG, Ashcroft SJ. The role of cytosolic free Ca^{2+} and protein kinase C in acetylcholine-induced insulin release in the clonal beta-cell line, HIT-T15. *Biochem J* 1990;**267**:227–32.
69 Persaud SJ, Jones PM, Sugden D, Howell SL. The role of protein kinase C in cholinergic stimulation of insulin secretion from rat islets of Langerhans. *Biochem J* 1989;**264**:753–8.
70 Vaulont S, Vasseur-Cognet M, Kahn A. Glucose regulation of gene transcription. *J Biol Chem* 2000;**275**:31555–8.
71 Pipeleers DG, Marichal M, Malaisse WJ. The stimulus-secretion coupling of glucose-induced insulin release. XIV. Glucose regulation of insular biosynthetic activity. *Endocrinology* 1973;**93**:1001–11.
72 Alarcón C, Lincoln B, Rhodes CJ. The biosynthesis of the subtilisin-related proprotein convertase PC3, but not that of the PC2 convertase, is regulated by glucose in parallel to proinsulin biosynthesis in rat pancreatic islets. *J Biol Chem* 1993;**268**:4276–80.
73 Itoh N, Okamoto H. Translational control of proinsulin synthesis by glucose. *Nature* 1980;**283**:100–2.
74 Guest PC, Bailyes EM, Rutherford NG, Hutton JC. Insulin secretory granule biogenesis. Co-ordinate regulation of the biosynthesis of the majority of constituent proteins. *Biochem J* 1991;**274** (1):73–8.
75 Grimaldi KA, Siddle K, Hutton JC. Biosynthesis of insulin secretory granule membrane proteins. Control by glucose. *Biochem J* 1987;**245**:567–73.
76 Wicksteed B, Herbert TP, Alarcon C, Lingohr MK, Moss LG, Rhodes CJ. Cooperativity between the preproinsulin mRNA untranslated regions is necessary for glucose-stimulated translation. *J Biol Chem* 2001;**276**:22553–8.
77 Skelly RH, Schuppin GT, Ishihara H, Oka Y, Rhodes CJ. Glucose-regulated translational control of proinsulin biosynthesis with that of the proinsulin endopeptidases PC2 and PC3 in the insulin-producing MIN6 cell line. *Diabetes* 1996;**45**:37–43.
78 Schuppin GT, Rhodes CJ. Specific co-ordinated regulation of PC3 and PC2 gene expression with that of preproinsulin in insulin-producing beta TC3 cells. *Biochem J* 1996;**313** (1):259–68.
79 Welsh M, Scherberg N, Gilmore R, Steiner DF. Translational control of insulin biosynthesis. Evidence for regulation of elongation, initiation and signal-recognition-particle-mediated translational arrest by glucose. *Biochem J* 1986;**235**:459–67.
80 Bollheimer LC, Skelly RH, Chester MW, McGarry JD, Rhodes CJ. Chronic exposure to free fatty acid reduces pancreatic β cell insulin content by increasing basal insulin secretion that is not compensated for by a corresponding increase in proinsulin biosynthesis translation. *J Clin Invest* 1998;**101**:1094–101.
81 Skelly RH, Bollheimer LC, Wicksteed BL, Corkey BE, Rhodes CJ. A distinct difference in the metabolic stimulus-response coupling pathways for regulating proinsulin biosynthesis and insulin secretion that lies at the level of a requirement for fatty acyl moieties. *Biochem J* 1998;**331** (2):553–61.
82 Leahy JL, Weir GC. Evolution of abnormal insulin secretory responses during 48-h in vivo hyperglycemia. *Diabetes* 1988;**37**:217–22.
83 Alarcón C, Leahy JL, Schuppin GT, Rhodes CJ. Increased secretory demand rather than a defect in the proinsulin conversion mechanism causes hyperproinsulinemia in a glucose-infusion rat model of non-insulin-dependent diabetes mellitus. *J Clin Invest* 1995;**95**:1032–9.
84 Bergman RN, Finegood DT, Kahn SE. The evolution of β-cell dysfunction and insulin resistance in type 2 diabetes. *Eur J Clin Invest* 2002;**32** (Suppl. 3):3–13.
85 Bonner-Weir S. Beta-cell turnover. its assessment and implications. *Diabetes* 2001;**50** (Suppl. 1):S20–4.
86 Brockenbrough JS, Weir GC, Bonner-Weir S. Discordance of exocrine and endocrine growth after 90% pancreatectomy in rats. *Diabetes* 1988;**37**:232–6.
87 Swenne I. Pancreatic beta-cell growth and diabetes mellitus. *Diabetologia* 1992;**35**:193–201.
88 Pende M, Kozma SC, Jaquet M, Oorschot V, Burcelin R, Le Marchand-Brustel Y *et al.* Hypoinsulinaemia, glucose intolerance and diminished beta-cell size in S6K1-deficient mice. *Nature* 2000;**408**:994–7.
89 Hugl SR, White MF, Rhodes CJ. Insulin-like growth factor I (IGF-I) -stimulated pancreatic beta-cell growth is glucose-dependent. Synergistic activation of insulin receptor substrate-mediated signal transduction pathways by glucose and IGF-I in INS-1 cells. *J Biol Chem* 1998;**273**:17771–9.
90 Cousin SP, Hugl SR, Myers MGJ, White MF, Reifel-Miller A, Rhodes CJ. Stimulation of pancreatic beta-cell proliferation by growth hormone is glucose-dependent. signal transduction via janus kinase 2 (JAK2) /signal transducer and activator of transcription 5 (STAT5) with no crosstalk to insulin receptor substrate-mediated mitogenic signalling. *Biochem J* 1999;**344** (3):649–58.
91 Khoo S, Cobb MH. Activation of mitogen-activating protein kinase by glucose is not required for insulin secretion. *Proc Natl Acad Sci USA* 1997;**94**:5599–604.
92 Cousin SP, Hugl SR, Wrede CE, Kajio H, Myers MGJ, Rhodes CJ. Free fatty acid-induced inhibition of glucose and insulin-like growth factor I-induced deoxyribonucleic acid synthesis in the pancreatic beta-cell line INS-1. *Endocrinology* 2001;**142**:229–40.
93 Rhodes CJ. IGF-I and GH post-receptor signaling mechanisms for pancreatic beta-cell replication. *J Mol Endocrinol* 2000;**24**:303–11.
94 Dickson LM, Lingohr MK, McCuaig J, Hugl SR, Snow L, Kahn BB *et al.* Differential activation of protein kinase B and $p70^{S6K}$ by glucose and insulin-like growth factor 1 in pancreatic beta-cells (INS-1). *J Biol Chem* 2001;**276**:21110–20.
95 Shulman GI. Cellular mechanisms of insulin resistance. *J Clin Invest* 2000;**106**:171–6.
96 Dresner A, Laurent D, Marcucci M, Griffin ME, Dufour S, Cline GW *et al.* Effects of free fatty acids on glucose transport and IRS-1-associated phosphatidylinositol 3-kinase activity. *J Clin Invest* 1999;**103**:253–9.
97 Griffin ME, Marcucci MJ, Cline GW, Bell K, Barucci N, Lee D *et al.* Free fatty acid-induced insulin resistance is associated with activation of protein kinase C theta and alterations in the insulin signaling cascade. *Diabetes* 1999;**48**:1270–4.
98 Oakes ND, Kennedy CJ, Jenkins AB, Laybutt DR, Chisholm DJ, Kraegen EW. A new antidiabetic agent, BRL 49653, reduces lipid availability and improves insulin action and glucoregulation in the rat. *Diabetes* 1994;**43**:1203–10.
99 Oakes ND, Camilleri S, Furler SM, Chisholm DJ, Kraegen EW. The insulin sensitizer, BRL 49653, reduces systemic fatty acid supply and utilization and tissue lipid availability in the rat. *Metabolism* 1997;**46**:935–42.
100 Schmitz-Peiffer C, Oakes ND, Browne CL, Kraegen EW, Biden TJ. Reversal of chronic alterations of skeletal muscle

protein kinase C from fat-fed rats by BRL-49653. *Am J Physiol* 1997;**273**:E915–21.
101 Boden G, Shulman GI. Free fatty acids in obesity and type 2 diabetes: defining their role in the development of insulin resistance and β-cell dysfunction. *Eur J Clin Invest* 2002;**32** (Suppl. 3):14–23.
102 Unger RH. Lipotoxicity in the pathogenesis of obesity-dependent NIDDM. Genetic and clinical implications. *Diabetes* 1995;**44**:863–70.
103 Unger RH, Zhou YT. Lipotoxicity of beta-cells in obesity and in other causes of fatty acid spillover. *Diabetes* 2001;**50** (Suppl. 1):S118–21.
104 Efanova IB, Zaitsev SV, Zhivotovsky B, Kohler M, Efendic S, Orrenius S *et al.* Glucose and tolbutamide induce apoptosis in pancreatic beta-cells. A process dependent on intracellular Ca^{2+} concentration. *J Biol Chem* 1998;**273**:33501–7.
105 Finegood DT, Topp B. β-cell deterioration – prospects for reversal or prevention. *Diabetes Obes Metab* 2001;**3** (Suppl. 1):S20–7.
106 Finegood DT, McArthur MD, Kojwang D, Thomas D, Topp B, Leonard T *et al.* Beta-cell mass dynamics in zucker diabetic fatty rats. Rosiglitazone prevents the rise in net cell death. *Diabetes* 2001;**50**:1021–9.
107 Kulkarni RN, Winnay JN, Daniels M, Brüning JC, Flier SN, Hanahan D *et al.* Altered function of insulin receptor substrate-1-deficient mouse islets and cultured β-cell lines. *J Clin Invest* 1999;**104**:R69–75.
108 Burks DJ, White MF. IRS proteins and β-cell function. *Diabetes* 2001;**50** (Suppl. 1):S140–5.
109 Burks DJ, Withers DJ, Towery HH, Flint CL, Altamuro SL, White MF. Rosiglitazone modulates glucose metabolism in IRS-2 knockout mice. *Diabetes* 2000;**49** (Suppl. 1):A279.
110 Liu JP, Baker J, Perkins AS, Robertson EJ, Efstratiadis A. Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf-1) and type 1 IGF receptor (Igf1r). *Cell* 1993;**75**:59–72.
111 Terauchi Y, Tsuji Y, Satoh S, Minoura H, Murakami K, Okuno A *et al.* Increased insulin sensitivity and hypoglycaemia in mice lacking the p85 alpha subunit of phosphoinositide 3-kinase. *Nat Genet* 1999;**21**:230–5.