MECHANISMS OF DISEASE

Molecular and metabolic mechanisms of insulin resistance and β-cell failure in type 2 diabetes

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Abstract | Nearly unlimited supplies of energy-dense foods and technologies that encourage sedentary behaviour have introduced a new threat to the survival of our species: obesity and its co-morbidities. Foremost among the co-morbidities is type 2 diabetes, which is projected to afflict 300 million people worldwide by 2020. Compliance with lifestyle modifications such as reduced caloric intake and increased physical activity has proved to be difficult for the general population, meaning that pharmacological intervention may be the only recourse for some. This epidemiological reality heightens the urgency for gaining a deeper understanding of the processes that cause metabolic failure of key tissues and organ systems in type 2 diabetes, as reviewed here.

Hyperglycaemia
An abnormal elevation in blood glucose levels. The American Diabetes Association currently considers a fasting blood glucose of > 126 mg ml^{-1} as the cut-off for diabetes.

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The purpose of this article is to review the current understanding of molecular, genetic and biochemical factors that cause the loss of metabolic fuel homeostasis in type 2 diabetes. This disease is often associated with obesity and develops when chronic overnutrition conspires with genetic susceptibility to cause impaired insulin signalling, also known as insulin resistance, as well as a relative insulin deficiency of non-autoimmune aetiology. This contrasts with type 1 diabetes, which is caused by the complete absence of insulin secondary to autoimmune destruction of the pancreatic islet β-cells. Insulin normally controls fuel homeostasis through the stimulation of glucose uptake into peripheral tissues and by suppressing the release of stored lipids from adipose tissue. Defective insulin secretion and action therefore leads to multiple metabolic abnormalities in type 2 diabetes, including hyperglycaemia due to impaired insulin-stimulated glucose uptake and uncontrolled hepatic glucose production, and dyslipidaemia, which includes perturbed homeostasis of fatty acids, triglycerides and lipoproteins. These chronic increases in circulating glucose and lipid levels can further impair insulin secretion and action and cause other forms of tissue damage by mechanisms that are discussed in more detail later in the article. As molecular components of the normal insulin signalling pathway have emerged in the past decade (see BOX 1 and recent reviews[1,2]), opportunities have grown for understanding how the environment and genetic susceptibility combine to cause defects in this fundamental pathway that lead to insulin resistance and then type 2 diabetes.

Here, we discuss recent work showing that insulin resistance develops as a consequence of the effects of inflammatory and hormonal factors, endoplasmic reticulum (ER) stress, and accumulation of by-products of nutritional 'overload' in insulin-sensing tissues. Although several of the damaging mechanisms are common across organs and tissues, others may be more specific, which highlights the significant challenges in designing pharmacological interventions for this condition. Meanwhile, in both animals and humans, the triggering factor for the transition from an obese, insulin-resistant state to full-blown type 2 diabetes is β-cell failure, which involves both a partial loss of β-cell mass and a deterioration of β-cell function. Some of the mechanisms that are involved in β-cell failure are similar to the mechanisms of insulin resistance. However, it should be noted that obese and insulin-resistant humans can remain in a state of β-cell compensation that protects them from diabetes for long periods of time before a subset of such individuals ultimately succumb to β-cell failure. Why the β-cell fails suddenly after chronic exposure to the effects of overnutrition is an important question for which answers are beginning to emerge.

Mechanisms of insulin resistance

A widely held notion is that insulin resistance is a direct consequence of obesity-associated exposure of tissues to elevated dietary nutrients, resulting in the accumulation of toxic metabolic by-products. However, recent work has indicated that other factors may also be important,

Box 1 | **An overview of insulin signalling pathways**

Binding of insulin to the insulin receptor (IR) elicits autophosphorylation of the IR, leading to the binding of various scaffold proteins, including the insulin receptor substrate (IRS) proteins, but also Src-homology-2-containing protein (SHC), and the c-Cbl (Cbl) proto-oncogene, among others[1,2]. Phosphorylation of these scaffold proteins by the IR, in turn, engages various signalling pathways. IRS family members seem to have particularly important roles in the control of metabolic fuel homeostasis. Thus, IRS1 is the key mediator of insulin-stimulated glucose uptake and activation of anabolic pathways in muscle and adipose tissue, whereas the anabolic effects of insulin in the liver are mainly directed by IRS2. Phosphorylation of IRS1 and IRS2 leads to their association with the p85 regulatory subunit of phosphatidylinositol 3-kinase (PI3K). This interaction recruits the p110 catalytic subunit of PI3K to the plasma membrane, resulting in conversion of phosphatidylinositol-4,5-bisphosphate to phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P_3). PtdIns(3,4,5)P_3 facilitates additional signalling events by binding to phosphoinositide-dependent protein kinase-1 (PDK1), PDK2 and AKT (also known as protein kinase B (PKB)). Colocalization of PDKs and AKT facilitates activation of AKT by phosphorylation at Thr308 (PDK1) and Ser473 (PDK2), leading to phosphorylation of downstream targets such as glycogen synthase kinase-3 (GSK3) and the AS160 Rab GTPase-activating protein, which in turn interacts with the small GTPase RAB10 to facilitate translocation of glucose transporter-4 (GLUT4)-containing vesicles to the cell surface[113]. These actions of insulin promote glucose uptake and storage under anabolic conditions.

This insulin-triggered signalling cascade can be attenuated or 'tuned down' by various regulators. Thus, the initiating event in the cascade, autophosphorylation of IR, is reversed by the protein Tyr phosphatase-1B (PTB1B, also known as PTPN1), and Tyr kinase activity of the IR is inhibited by suppressor of cytokine signalling-1 (SOCS1) and SOCS2. Tyr phosphorylation and activation of IRS proteins is opposed by Ser phosphorylation of these proteins in response to overnutrition and activation of stress pathways. Accumulation of PtdIns(3,4,5)P_3 and signalling through PDK1–PDK2–AKT can also be downregulated by the activity of the lipid phosphatase PTEN (phosphatase and tensin homologue on chromosome-10), which converts PtdIns(3,4,5)P_3 to PtdIns(4,5)P_2 (REF. 114). The growing understanding of the core elements of the insulin signalling pathway and its modulators provides opportunities for therapy. For example, knockout of *Ptpn1* in mice reduces insulin resistance and improves glucose homeostasis[115,116], and pharmaceutical companies are currently searching for selective inhibitors of this enzyme.

Adipokine
A peptide hormone or cytokine that is produced and secreted by adipocytes, which regulate fuel use and storage in other peripheral tissues.

Hyperphagia
Abnormal or continuous food ingestion.

Hyperlipidaemia
An abnormal elevation of circulating lipids, including triglycerides, free fatty acids and low-density lipoproteins, often accompanied by a decrease in high-density lipoprotein.

Hypoglycaemia
An abnormally low blood glucose level. Humans with blood glucose levels below 50 mg ml^{-1} are considered to be hypoglycaemic and manifest symptoms that are related to an inadequate delivery rate of glucose to the brain.

including inter-organ communication networks that are mediated by peptide hormones and inflammatory molecules (cytokines), and activation of intracellular stress response pathways (discussed below).

Adipokines and insulin resistance. Adipocytes have a regulatory role in the development of insulin resistance because they can produce adipokines (a group of hormones and cytokines) and because their capacity to store excess lipids can become saturated in obesity, resulting in abnormal redistribution of lipids to other organs and tissues. A new appreciation of endocrine functions of adipose tissue began with the discovery that the mutated gene in the *ob*/*ob* mouse, which exhibits hyperphagia, hyperlipidaemia and insulin resistance, is the cytokine-related molecule leptin[3,4]. The ensuing decade of research has revealed that adipose cells also produce other peptide hormones, including adiponectin (ACRP30), retinol-binding protein-4 (RBP4) and resistin, and proinflammatory cytokines such as interleukin (IL)-6 and tumour necrosis factor-α (TNFα)[5,6]. Leptin and adiponectin have been categorized as 'anti-diabetogenic' based on their common capacity to decrease triglyceride (TG) synthesis, stimulate β-oxidation and enhance insulin action in both skeletal muscle and liver. These effects can be explained in part by their common ability to activate 5′-AMP-activated protein kinase (AMPK)[6], an enzyme that responds to a fall in ATP and a rise in AMP levels by activating both glucose and fatty acid oxidation. Interestingly, leptin levels are increased and adiponectin levels are decreased in insulin-resistant obese humans and animals, which suggests that obesity leads to a state of leptin resistance and adiponectin deficiency.

The consequences of the absence of fat (lipodystrophy) further underscore the importance of adipose tissue in the normal regulation of insulin action. Animals lacking white adipose tissue have severe hepatic and muscle insulin resistance, and exhibit large increases in TG stores in both tissues[7]. Transplantation of normal fat tissue into such mice restores insulin sensitivity[8]. Leptin appears to have a crucial role in the 'rescue' of insulin action in these models because leptin infusion ameliorates insulin resistance in lipoatrophic mice[9,10], whereas transplantation of fat from leptin-deficient mice into such animals fails to improve insulin sensitivity[11]. Furthermore, leptin administration to humans with severe lipodystrophy partially reverses their insulin resistance and hyperlipidaemia[12].

The ability of adipose tissue to produce inter-organ regulatory factors is dependent on the metabolic state. Mice with an adipose-specific knockout of the GLUT4 glucose transporter have impaired insulin sensitivity in muscle and liver[13]. The impairment in insulin action is only apparent in tissues *in situ* and not in excised tissue samples, which implies the participation of a blood-borne hormone or metabolite. Furthermore, circulating RBP4 levels are increased in adipose-specific *Glut4*-knockout mice, and infusion or transgenic expression of RBP4 in normal mice causes insulin resistance[14]. Interestingly, food deprivation (fasting) also causes a form of insulin resistance and is associated with a decrease in adipose GLUT4 expression[15]. This raises the possibility that the original purpose of adipocyte-derived insulin-desensitizing molecules, such as RBP4, TNFα and resistin, may have been to prevent hypoglycaemia in the fasted state, which has been subverted to pathophysiology with the advent of overnutrition and sedentary behaviours in modern life[16].

Role of inflammatory mediators. Inter-organ communication leading to insulin resistance may also involve an inflammatory component[17]. Indeed, it has been recognized for the past century that high doses of salicylates (aspirin) reverse insulin resistance and diabetes[18,19], and salicylates also appear to preserve β-cell function by inhibiting prostaglandin formation[20]. High-fat diets or obesity result in activation of the transcription factor NF-κB and its targets in the liver, and salicylates suppress NF-κB activity. Overexpression of a constitutively active version of the NF-κB-activating kinase, IκB kinase catalytic subunit-β (IKKβ), in the liver of normal rodents results in liver and muscle insulin resistance and diabetes. In addition, both high-fat feeding and IKKβ overexpression increase hepatic production of IL-6, IL-1β and TNFα, whereas antibody-mediated

neutralization of IL-6 in animals fed on a high-fat diet partially restores insulin sensitivity[21]. Interestingly, deletion of IKKβ in the liver of mice is protective against diet-induced hepatic insulin resistance, although muscle and adipose insulin resistance still develop. However, mice in which IKKβ is selectively knocked out in myeloid cells remain globally insulin sensitive[22]. In rodents, macrophage infiltration of adipose tissue is stimulated within 1 week of high-fat feeding and appears to involve increased adipocyte expression of monocyte chemotactic protein-1 (MCP1), which recruits monocytes to sites of injury and infection[23,24]. This may be a mechanism by which inflammatory signalling is enhanced during the development of diabetes. Overall, evidence is accumulating that insulin resistance is at least partly caused by changes in hormone and cytokine production by the liver, adipose tissue and infiltrating immune cells in response to chronic exposure to lipids and other metabolic fuels.

Alterations in metabolic function. What are the fundamental changes in metabolism that occur in response to overnutrition that, in turn, cause insulin resistance and type 2 diabetes? Lipid-derived metabolites begin to accumulate outside of the adipose depots (including skeletal muscle, heart and liver) in response to high-fat diets and the onset of obesity. Focusing on insulin resistance, several important questions arise from these observations: are there specific lipid-derived species that make a direct contribution to the development of insulin resistance? If so, are these mechanisms operative in all tissues in which insulin resistance develops? What are the signalling and metabolic mechanisms that link the accumulation of specific lipid metabolites to the loss of insulin sensitivity?

Metabolic overload in the liver. A current popular theory of lipid-induced hepatic insulin resistance is that lipid species accumulate as a result of the impairment of fatty acid oxidation, resulting in the redirection of long-chain acyl CoAs (LC-CoAs) into ER-localized and cytosolic lipid species, such as diacylglycerols (DAGs), ceramides and TGs. This is thought to be regulated mostly by glucose-induced increases in the levels of malonyl CoA, which serves both as the immediate precursor of *de novo* lipogenesis and as an important allosteric inhibitor of carnitine palmitoyltransferase-1 (CPT1), the rate-limiting enzyme for import of LC-CoAs into the mitochondria for β-oxidation[25]. In addition, insulin inhibits the hepatic expression of β-oxidative enzymes by antagonizing the effects of PGC1α (peroxisome proliferator-activated receptor-γ (PPARγ) co-activator-1α)[26]. This role of insulin is maintained even as insulin resistance develops, whereas its role to suppress gluconeogenesis wanes. Together, this sets the stage for development of hepatic steatosis during sustained periods of overfeeding, leading to glucose intolerance. Indeed, infusion of lipids or ingestion of high-fat diets in rodents leads to the accumulation of TGs, LC-CoAs, DAGs and ceramides[27–29]. Suppression of mitochondrial glycerol-3-phosphate acyltransferase-1 (GPAT1, the first enzyme in TG synthesis) or acetyl CoA carboxylase-2 (ACC2) activity results in increased fatty acid oxidation, lowered DAG levels and reversal of hepatic insulin resistance[30–32]. Pharmacological inhibition of Ser palmitoyltransferase-1 (SPT1) or genetic knockout of dihydroceramide desaturase-1 (*Degs1*) — both of which are involved in the synthesis of ceramides from the saturated precursor palmitoyl CoA — prevented hepatic insulin resistance induced by glucocorticoid administration or infusion of saturated (but not unsaturated) fats in rodents[33]. Finally, when obese patients with type 2 diabetes were fed on a hypocaloric low-fat diet, a modest weight loss (8 kg on average) was observed, but a reversal of hepatic insulin resistance was also seen in concert with a large decrease in intrahepatic, but not intramuscular, lipid deposits[34].

Metabolic overload in muscle. As in liver, intramuscular levels of lipid signalling molecules, such as LC-CoAs, DAG and ceramides, positively correlate with TG content and negatively correlate with insulin sensitivity[33,35,36]. However, although a role for these cytosolic lipids in the development of hepatic insulin resistance is well supported, the link with skeletal muscle is still mainly based on circumstantial evidence. The strongest experimental data come from two recent reports in which tissue levels of ceramide and DAG were altered. Thus, pharmacological or genetic inhibition of ceramide biosynthesis in rodents attenuated muscle insulin resistance caused by infusion of high concentrations of palmitate[33]. Inhibition of ceramide synthesis did not prevent insulin resistance in response to linoleic acid, suggesting discrete mechanisms for different fatty acids. It is noteworthy that this report highlighted the role of ceramide in liver and suggested that sphingolipid synthesis in other tissues, including muscle, might not be a factor in the early stages of obesity-induced glucose intolerance. Another study showed that transgenic overexpression of diacylglycerol acyltransferase-1 (DGAT1) in skeletal muscle increased TG content and prevented diet-induced insulin resistance, in association with 20–30% reductions in muscle DAG and ceramide levels[37]. However, muscle β-oxidation was not evaluated, and could have been reduced as a consequence of repartitioning lipids into esterification pathways (see below).

Alternatively, recent work has suggested that mitochondrially derived by-products of lipid oxidation (rather than diversion of lipid metabolites into biosynthetic pathways) may have a key role in the development of insulin resistance in skeletal muscle[38]. Chronic exposure of muscle to elevated lipids induces an increase rather than a decrease in expression of genes of the fatty acid β-oxidative pathway[38,39]. In addition, lipid-induced upregulation of the enzymatic machinery for β-oxidation in muscle is not coordinated with upregulation of downstream metabolic pathways such as the tricarboxylic acid (TCA) cycle and electron transport chain. This could lead to incomplete metabolism of fatty acids in the β-oxidation pathway and accumulation of lipid-derived metabolites in the mitochondria[38,39]. Higher rates of incomplete fat oxidation were observed in isolated mitochondria from insulin-resistant rat skeletal muscles compared with insulin-sensitive muscles,

Hepatic steatosis
The accumulation of stored lipids, most notably triglycerides, to abnormally high levels in the liver.

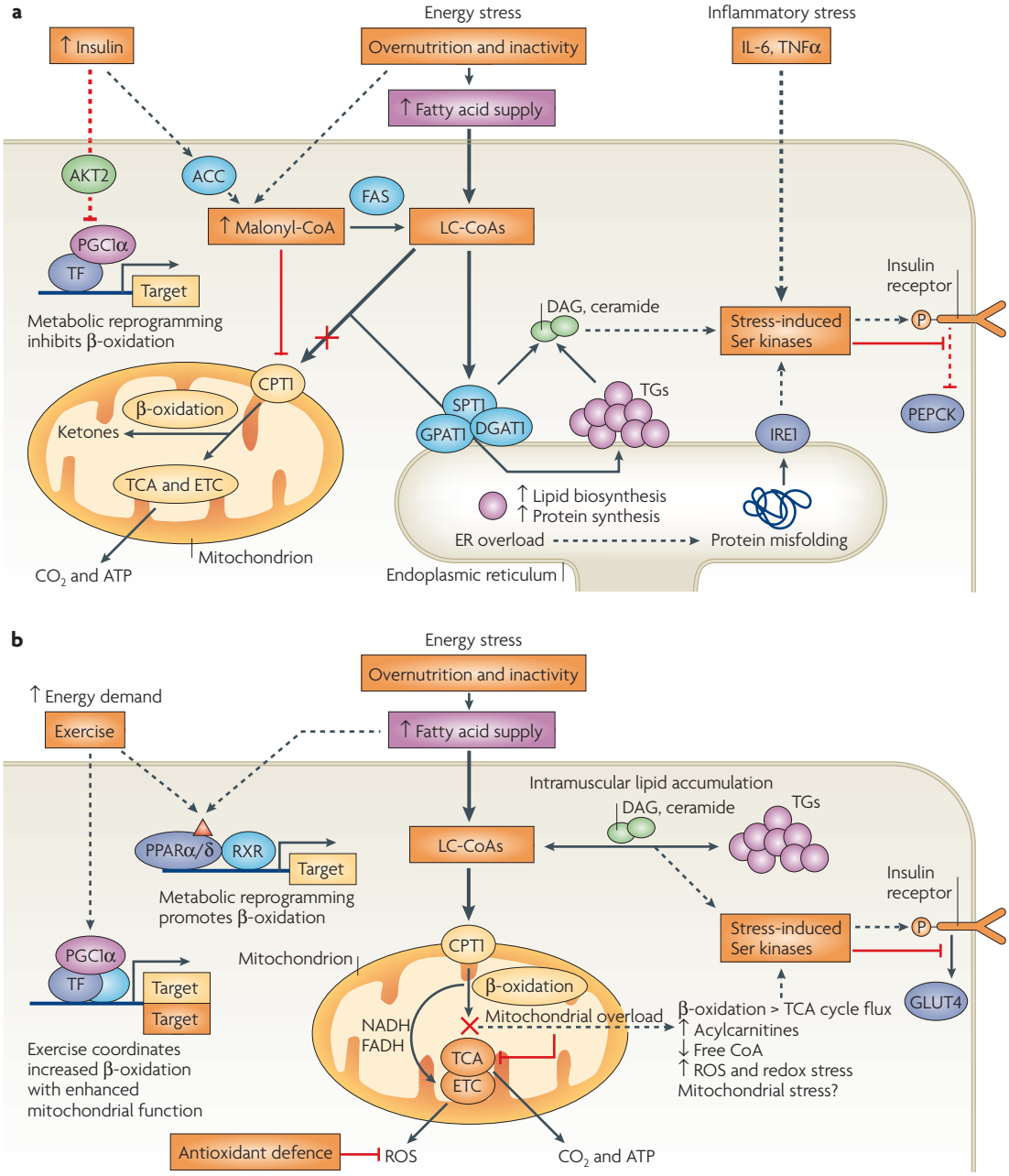

Figure 1 | **Metabolic overload in the liver and skeletal muscle. a** | In the liver, chronic overfeeding increases malonyl CoA levels (see main text), which promotes *de novo* fatty acid synthesis and inhibits CPT1 activity. As a result, LC-CoAs are diverted away from mitochondrial oxidation (the TCA cycle and the ETC) towards biosynthetic enzymes (for example, GPAT1, DGAT1 and SPT1) that produce TGs and signalling intermediates, such as DAG and ceramide. Overnutrition also imposes a heavy anabolic burden on the ER, causing protein misfolding and activation of IRE1. Collectively, these adverse events converge on a family of stress-induced Ser kinases that impede insulin-mediated suppression of gluconeogenesis, while allowing lipid synthesis and restricting β-oxidation. **b** | Metabolic overload in skeletal muscle. During conditions of overnutrition, fatty acid influx and PPARα/δ-mediated activation of target genes (yellow) promote β-oxidation without a coordinated increase in TCA cycle flux. As a result, metabolic by-products of incomplete fat oxidation (acylcarnitines, ROS) accumulate in the mitochondria. These stresses might activate Ser kinases that impede insulin signalling and GLUT4 translocation (blue). Exercise combats lipid stress by increasing TCA cycle flux and by coupling ligand-induced PPARα/δ activity with PGC1α-mediated remodelling of downstream metabolic pathways (orange). Enhanced mitochondrial performance then restores insulin sensitivity. ACC, acetyl CoA carboxylase; AKT2, Ser/Thr protein kinase; CPT1, carnitine palmitoyltransferase-1; DAG, diacylglycerol; DGAT1, diacylglycerol acyltransferase-1; ER, endoplasmic reticulum; ETC, electron transport chain; FAS, fatty acid synthase; GLUT4, glucose transporter-4; GPAT1, glycerol-3-phosphate acyltransferase-1; IL-6, interleukin-6; IRE1, inositol requiring kinase-1; LC-CoAs, long-chain acyl CoAs; PEPCK, phosphoenolpyruvate carboxykinase; PGC1α, PPARγ co-activator-1α; PPARγ, peroxisome proliferator-activated receptor-γ; ROS, reactive oxygen species; RXR, retinoid X receptor; SPT1, serine palmitoyltransferase-1; TCA, tricarboxylic acid cycle; TF, transcription factor; TGs, triglycerides; TNFα, tumour necrosis factor-α.

Box 2 | **Special role of the liver in whole-body insulin sensitivity**

Why do drugs like metformin, which activate 5′-AMP-activated protein kinase (AMPK) and stimulate fatty acid oxidation, contribute to improved insulin action? Importantly, the improvement in insulin sensitivity caused by metformin was completely abolished upon liver-specific deletion of LKB1, the upstream activator of AMPK[117]. Likewise, acetyl CoA carboxylase-2 (*Acaca*)-null mice, which resist diet-induced glucose intolerance, exhibit only minor adjustments in muscle lipid metabolism but show profound increases in hepatic fat oxidation and ketogenesis[31]. In these and other studies[40,54], the liver appears to serve as a catabolic sink that defends against systemic hyperlipidaemia.

It should also be noted that the storage of lipids in liver can trigger inter-organ communication systems that affect insulin sensitivity in muscle. For example, hepatic expression of malonyl CoA decarboxylase (MCD) partially resolved hepatic steatosis and caused near-complete reversal of whole animal, hepatic and muscle insulin resistance in rats that were fed a high-fat diet[40]. Conversely, adenovirus-mediated overexpression of glycerol-3-phosphate acyltransferase-1 (GPAT1) in the liver of normal rats induced hepatic steatosis and both hepatic and muscle insulin resistance[54]. Furthermore, overexpression of peroxisome proliferator-activated receptor-γ2 (PPARγ2) in mice resulted in marked hepatic steatosis that was associated with an increase in energy expenditure, reduced adiposity and improved systemic insulin sensitivity[118]. This effect of PPARγ2 was abrogated by vagotomy or selective afferent blockage of the hepatic vagus nerve. The results of the PPARγ2 studies may, at first, seem at odds with those reported in the GPAT1 and MCD studies. We propose that these effects are mediated by distinct mechanisms, with the effect of PPARγ2 occurring as a result of a more severe hepatic steatosis that triggers activation of signalling in the central nervous system (CNS). However, the effects of GPAT1 and MCD, studied in the context of less severe steatosis, may be secondary to increased and decreased rates of lipid delivery to muscle, respectively, which in turn modulate β-oxidative flux and affect the extent of incomplete fatty acid oxidation and mitochondrial overload. Both of these signalling mechanisms may be complemented by direct sensing of changes in glucose and fatty acid levels within the CNS, leading to alterations in the control of hepatic glucose and lipid metabolism[119,120]. Further testing of these ideas is clearly required.

and several long- and medium-chain acylcarnitines accumulated in the muscle of obese rats compared with lean rats[39,40]. These abnormalities were reversed by exercise intervention in mice that were fed on a high-fat diet, in association with increased TCA cycle activity and restoration of insulin sensitivity and glucose tolerance[39].

Other work has shown that obesity results in impaired switching from fatty acid to carbohydrate substrates during the fasting-to-fed transition and in a coincident reduction in levels of several TCA cycle intermediates[41]. This phenomenon, known as metabolic inflexibility[42], was apparent at both the whole-body level and in isolated muscle mitochondria. Moreover, knockout mice lacking malonyl CoA decarboxylase (MCD), which promotes CPT1 activity and β-oxidation, had markedly lower acylcarnitine levels in muscle and were protected against diet-induced insulin resistance, despite high levels of LC-CoA[41]. Conversely, transgenic mice with muscle-specific overexpression of PPARα, a nuclear receptor that activates β-oxidative genes, developed both local and systemic glucose intolerance[43].

A unifying hypothesis of metabolic overload. These recent studies led us to propose a tissue-dependent model for the metabolic mechanisms that underlie insulin resistance (FIG. 1). A large body of evidence supports the idea that the impairment of mitochondrial fatty acid oxidation and diversion of lipid species into cytosolic by-products has a major role in the development of hepatic insulin resistance (see above and REF. 44) (FIG. 1a). In contrast, oversupplying lipids to muscle can result both in increased diversion of LC-CoA species into cytosolic products such as TG, DAG and ceramide, and in enhanced incomplete fatty acid oxidation owing to transcriptional regulation and increased substrate supply (FIG. 1b). In the absence of exercise, this increase in fatty acid oxidation is not matched by an increase in TCA cycle activity. As a result, lipid-derived intermediates accumulate in mitochondria, possibly contributing to mitochondrial stress, and ultimately to insulin resistance.

Muscle insulin resistance has been linked to a form of mitochondrial dysfunction that is characterized by morphological and metabolic abnormalities, diminished oxidative phosphorylation capacity, reduced activity of the electron transport chain and low expression levels of PGC1α, a master transcriptional regulator of mitochondrial biogenesis[39,45–48]. These traits have been observed in association with ageing, inactivity, obesity and type 2 diabetes, and are also evident in young insulin-resistant offspring of parents with diabetes (reviewed in REFS 38, 49). Thus, mitochondrial insufficiencies of various origins appear to occur in close association with impaired insulin action. An important remaining question is whether impairment of mitochondrial function is a necessary prerequisite for the development of muscle insulin resistance. Conversely, overload of muscle mitochondria with metabolic fuels could be sufficient to launch a distress signal that eventually halts glucose uptake in individuals with robust mitochondrial function. Clearly, these models are not mutually exclusive, and it would not be surprising if the highest susceptibility exists in cases of low intrinsic mitochondrial function and poor dietary practices.

Assuming that excessive incomplete fat oxidation contributes to both mitochondrial malfunction and insulin resistance in skeletal muscle, why then do some pharmacological agents that promote β-oxidation also improve glucose tolerance? We propose that such drugs act primarily in the liver. Evidence in support of this idea, and the role of inter-organ communication networks in mediating whole-body insulin sensitivity, are discussed in BOX 2.

Vagotomy
Division of fibres of the vagus nerve by surgery, a technique that is used to diminish acid secretion of the stomach.

Acylcarnitine
One of a family of carnitine esters that are derived from acetyl CoA and acyl CoA intermediates of fatty acid and amino acid catabolism.

Relating metabolic overload to insulin signalling. Regardless of the lipid species involved, the next logical question is what are the connection points between the accumulation of lipid-derived metabolites and the core insulin signalling pathway? Members of the protein kinase C (PKC) family are regulated by lipid-derived by-products such as DAG and are therefore implicated. These kinases have been shown to phosphorylate Ser on the insulin receptor and/or its immediate targets, insulin receptor substrate-1 (IRS1) and -2 (IRS2), thereby impairing insulin-receptor-mediated Tyr phosphorylation of IRS1 and, as a consequence, interfering with insulin signalling[1,2,50–53]. Moreover, hepatic steatosis induced by overexpression of GPAT1 in rat liver is associated with increased hepatic DAG levels and activation of PKCε[54], whereas *Gpat1*-knockout mice exhibit decreased PKCε activity in concert with enhanced hepatic insulin sensitivity[55]. Furthermore, silencing of hepatic PKCε in liver is sufficient to prevent hepatic insulin resistance caused by short-term high-fat feeding[56].

In skeletal muscle, PKCθ has emerged as a candidate mediator of fatty-acid-induced insulin resistance, based on findings that this enzyme is activated by lipid infusion in both humans and rodents, and that *Pkcθ*-knockout mice are protected from insulin resistance that is caused by acute lipid infusion[27,57]. However, other reports suggest that PKCθ is required for the maintenance of normal skeletal muscle insulin sensitivity[58,59]. Thus, there is more to learn about signalling pathways in lipid-induced muscle insulin resistance, including whether increased fat oxidation in muscle mitochondria leads to oxidative stress and activation of kinases other than PKCs to interfere with insulin signalling.

Lipids are not the only mediators of the effects of overnutrition on insulin action. The concentrations of several amino acids are elevated in obese patients with type 2 diabetes[60], and infusion of amino acids in rodent models or humans impairs skeletal muscle glucose uptake, increases hepatic gluconeogenesis and impairs insulin action[61–64]. Recent work suggests that the interfering effects of amino acids on insulin signalling involve a particular activity of branched-chain amino acids that activates mTOR (mammalian target of rapamycin) and ribosomal protein S6 kinase-1 (S6K1) through the phosphatidylinositol 3-kinase (PI3K) hVps34, leading to phosphorylation of IRS1 (REF. 64). Consistent with this idea, *S6k1*-knockout mice have increased insulin sensitivity and are protected against diet-induced insulin resistance. Because modern overnutrition includes increased ingestion of protein as well as fat, amino-acid-induced pathways might synergize with lipid-induced mechanisms to bring about the full syndrome of obesity-associated impairment of insulin action.

Finally, an important and recently emergent idea is that excess lipids and other metabolic changes that are associated with overnutrition may trigger stress responses in the ER[65]. In genetic or diet-induced forms of obesity, markers of ER stress are elevated in the liver and adipose tissue (FIG. 1a). These changes are linked to activation of c-jun N-terminal kinases (JNKs), which phosphorylate IRS1, thereby interfering with insulin action. In fibroblasts from mice that lack inositol-requiring kinase-1 (IRE1), a proximal ER stress sensor, chemical activators of ER stress fail to activate JNK. Overexpression of *Xbp1* (a downstream transcription factor that modulates the unfolded protein response (UPR)) prevents JNK activation in liver cells that are treated with agents that cause ER stress, and heterozygous deletion of XBP1 increases the susceptibility of mice to diet-induced insulin resistance and diabetes[65]. Moreover, treatment of obese and diabetic mice with 4-phenyl butyric acid and taurine-conjugated ursodeoxycholic acid (which are both small molecule chemical chaperones, or ER-stress-relieving agents) resolves hepatic steatosis and restores peripheral insulin sensitivity[66]. Thus, diet-induced insulin resistance appears to be controlled in part by the induction of ER and inflammatory stress responses that connect to the core insulin signalling pathways. Interestingly, several of the enzymes that are involved in lipid esterification pathways are localized to the ER, providing a possible link between hepatic lipid overstorage and the development of ER stress.

Unfolded protein response
A transcriptional program that functions to slow protein synthesis and promote protein degradation in response to the accumulation of unfolded or misfolded proteins in the endoplasmic reticulum.

β-cell failure in type 2 diabetes

Although obesity often leads to insulin resistance, only a subset of obese, insulin-resistant individuals progress to type 2 diabetes. In both animal models and humans, the triggering factor is β-cell failure, which involves a decrease in β-cell mass and deterioration of key β-cell functions such as glucose-stimulated insulin secretion (GSIS). Obesity-related β-cell failure has both similar and distinct mechanistic elements compared with the development of insulin resistance in liver and muscle, as summarized below.

Regulation of insulin secretion in normal islets. In all mammals, including humans, postprandial insulin secretion is regulated in a biphasic manner by nutritional and hormonal signals, but the primary regulator is glucose. Other secretagogues such as free fatty acids, amino acids or the incretin regulator glucagon-like peptide-1 (GLP1) serve as potentiators, requiring a threshold stimulatory level of glucose in the bloodstream before their effects are engaged (BOX 3).

Insulin secretion from pancreatic islet β-cells is stimulated by glucose metabolism, which leads to a rise in the ATP:ADP ratio, resulting in closure of ATP-sensitive K^+ (K_{ATP}) channels, plasma membrane depolarization, activation of voltage-gated Ca^{2+} channels, and Ca^{2+}-mediated stimulation of granule exocytosis[67,68]. The K_{ATP} channel-dependent mechanism, also known as the triggering signal, appears to be particularly important for the first, acute phase of insulin release that occurs in the first 10 minutes following glucose stimulation[69]. By contrast, in the second and sustained phase of insulin secretion, ATP and Ca^{2+} may have more limited or permissive roles, allowing other glucose-derived second messengers (otherwise known as amplifying signals) to come to the forefront[69,70].

The mitochondrial metabolism of glucose generates signals other than changes in the ATP:ADP ratio that are important for the control of insulin release.

Box 3 | **Potentiators of glucose-stimulated insulin secretion**

The regulation of insulin secretion by glucose is complemented by various physiological potentiators. For example, as food is digested and absorbed, enteroendocrine cells of the small and large intestine (L cells) are stimulated to release the incretin hormones glucagon-like peptide-1 (GLP1) and gastrointestinal inhibitory polypeptide (GIP), both of which potentiate insulin secretion[121]. These hormones activate adenylate cyclase and increase levels of cyclic AMP in β-cells. GLP1 modulates the activities of three ion channels in the β-cell: the K_{ATP} channels, the voltage-gated Ca^{2+} channels, and the voltage-dependent K^+ channels (Kv channels). Kv channels are thought to repolarize glucose-stimulated action potentials and inhibit Ca^{2+} entry through voltage-gated Ca^{2+} channels; therefore, Kv channels serve as negative regulators of insulin secretion, and Kv channel antagonists such as GLP1 are insulinotropic in a glucose-dependent manner[122]. In addition, GLP1 may mediate its actions through the activation of cAMP-dependent protein kinase (PKA), through subsequent phosphorylation and activation of the Rab3A-interacting molecule-1 (RIM1), and by a PKA-independent mechanism that involves cAMP guanine nucleotide exchange factor-II (cAMP-GEFII, also known as EPAC2)[123,124].

Glucose-stimulated insulin secretion (GSIS) is also potentiated by other metabolic fuels, most notably fatty acids and amino acids[67,68]. Recently, several groups showed that the orphan G-protein-coupled receptor GPR40 (also known as free fatty acid receptor-1; FFAR1), is a fatty acid receptor that is preferentially expressed in islet β-cells within the pancreas, and stimulation of FFAR1 with medium- or long-chain fatty acids results in increased intracellular Ca^{2+} and potentiation of GSIS[125,126]. A role for these receptors has also been suggested in lipid-mediated β-cell dysfunction. In one study involving *Ffar1*-knockout mice, *Ffar1*$^{-/-}$ islets secreted less insulin in response to stimulatory glucose plus fatty acids, whereas exposure of *Ffar1*$^{-/-}$ islets to elevated levels of palmitate for 48 h caused a smaller decrease in GSIS than was observed in wild-type islets[127]. In contrast, in an independent strain of *Ffar1*-knockout mice, a role for FFAR1 in mediating lipid-induced β-cell dysfunction was not observed, although its role in the acute potentiation of GSIS by fatty acids was confirmed[128]. Arginine is a potent insulin secretagogue that probably directly affects membrane polarization, analogous to the effects of high concentrations of K^+. Leucine is transaminated to form α-ketoisocaproate, a secretagogue in its own right, and is then further catabolized to acetyl CoA, which can enter the tricarboxylic acid cycle and contribute to ATP production. Leucine also serves as an allosteric activator of glutamate dehydrogenase, thereby potentiating the insulinotropic effect of glutamine.

Understanding the nature of all of the glucose-derived signals for insulin secretion (both triggering and amplifying) is required for gaining insight into the functional failure of the β-cell in diabetes and the development of new drugs for correcting this problem. Recent studies in this area have focused on understanding the metabolic fates of pyruvate in the mitochondria. Islet β-cells express both pyruvate carboxylase (PC) and pyruvate dehydrogenase (PDH) in abundance, such that in the fed state, pyruvate flows into mitochondrial metabolic pathways in roughly equal proportions through the anaplerotic (PC) and oxidative (PDH) entry points[71–74]. An important clue to the role of anaplerotic metabolism of pyruvate in β-cells came with the discovery that these cells express enzymes that allow cycling of pyruvate through its PC-catalysed conversion to oxaloacetate (OXA), metabolism of OXA to malate, citrate or isocitrate in the TCA cycle, and subsequent reconversion of these metabolites to pyruvate through several possible combinations of cytosolic and mitochondrial pathways[75] (FIG. 2). It is now clear that these pathways are important in GSIS regulation[74,76–78]. Initial insights came from studies revealing that GSIS was correlated with PC-catalysed pyruvate cycling, but not with PDH-catalysed glucose oxidation in variously glucose-responsive INS1-derived insulinoma cell lines[74].

More recent studies have focused on the identification of specific pyruvate cycling pathways that may be involved in signal generation for insulin secretion (FIG. 2). One important pathway appears to involve export of citrate and/or isocitrate from the mitochondria through the citrate–isocitrate carrier (CIC), and subsequent conversion of isocitrate to α-ketoglutarate (α-KG) by the cytosolic NADP-dependent isoform of isocitrate dehydrogenase (ICDc)[79,80]. Small interfering RNA (siRNA)-mediated suppression of CIC or ICDc causes substantial impairment of GSIS in both insulinoma cell lines and primary rat islets. By contrast, siRNA-mediated suppression of ATP-citrate lyase in cell lines or rat islets has no effect on GSIS[81]. Citrate lyase catalyses an alternative metabolic fate of citrate in which it is cleaved to acetyl CoA and OXA, which can be used for lipogenesis or recycled to pyruvate, respectively. Consistent with the idea that these pathways are not crucial for GSIS, suppression of fatty acid synthase also has no effect on GSIS[81]. These studies suggest that a metabolic by-product of pyruvate–isocitrate cycling may be important amplifying signals for the control of GSIS (FIG. 2). Possible mediators that are under investigation include NADPH, α-KG or its metabolites, or GTP generated by the succinyl CoA dehydrogenase reaction, all of which are direct or downstream products of the ICDc reaction[79,80,82–84].

Genetic susceptibility to β-cell failure. In Zucker diabetic fatty (ZDF) rats, β-cell mass shows a dramatic compensatory increase at the onset of obesity, but as body weight continues to increase and insulin resistance worsens, β-cell mass and function ultimately decline[85]. The decrease in β-cell mass in these animals has been ascribed to increased apoptosis[86], whereas multiple theories have been proposed for the underlying cause of their loss of function (GSIS). Similar mechanisms of β-cell failure appear to occur in human diabetes. β-cell mass is increased in obese non-diabetic humans compared with lean controls, and is decreased in obese patients with impaired fasting glucose or type 2 diabetes[87]. Similarly, β-cell apoptosis is increased in obese humans with glucose intolerance or diabetes.

Genetic background has an important role in determining the susceptibility of β-cells to decompensation and progression to type 2 diabetes. This is demonstrated using rodent models. For example, BTBR/leptin^{ob} mice exhibit defective islet proliferation in response to obesity, leading to severe diabetes, whereas C57BL6/leptin^{ob} mice are able to expand β-cell mass and are protected against hyperglycaemia[88]. In humans, several forms of maturity-onset diabetes of the young (MODY), which are generally considered to be a subclass of type 2 diabetes, are monogenic diseases that involve mutations in important β-cell transcription factors or metabolic regulatory proteins, including hepatocyte nuclear factor-4α (HNF4α; resulting in MODY1), glucokinase (resulting in MODY2), and pancreatic and duodenal homeobox-1 (PDX1; resulting in MODY4)[89]. These diseases are generally characterized by impaired GSIS and the onset of diabetes at an early age, but represent only 1–2% of the total population of type 2 diabetes cases worldwide. The remaining population with typical or obesity-related type 2 diabetes is thought

Anaplerotic
Repletion of the tricarboxylic acid cycle with intermediates that can condense with acetyl CoA to form citrate.

Pyruvate cycling
The exchange of pyruvate with intermediates from the tricarboxylic acid cycle.

Insulinoma cell line
A cell line that is derived from rodent pancreatic islet β-cells that are transformed by oncogene expression or another means of transformation.

to comprise a cluster of genetic variations that confer enhanced susceptibility to environmental factors such as overnutrition, obesity and stress. Current evidence suggests that β-cell failure occurs as a combined consequence of metabolic overload, oxidative stress, increased rates of apoptosis, and loss of expression of fundamental components of the insulin granule secretory machinery, but specific genetic mutations that predispose to these events in patients with non-MODY type 2 diabetes remain to be identified.

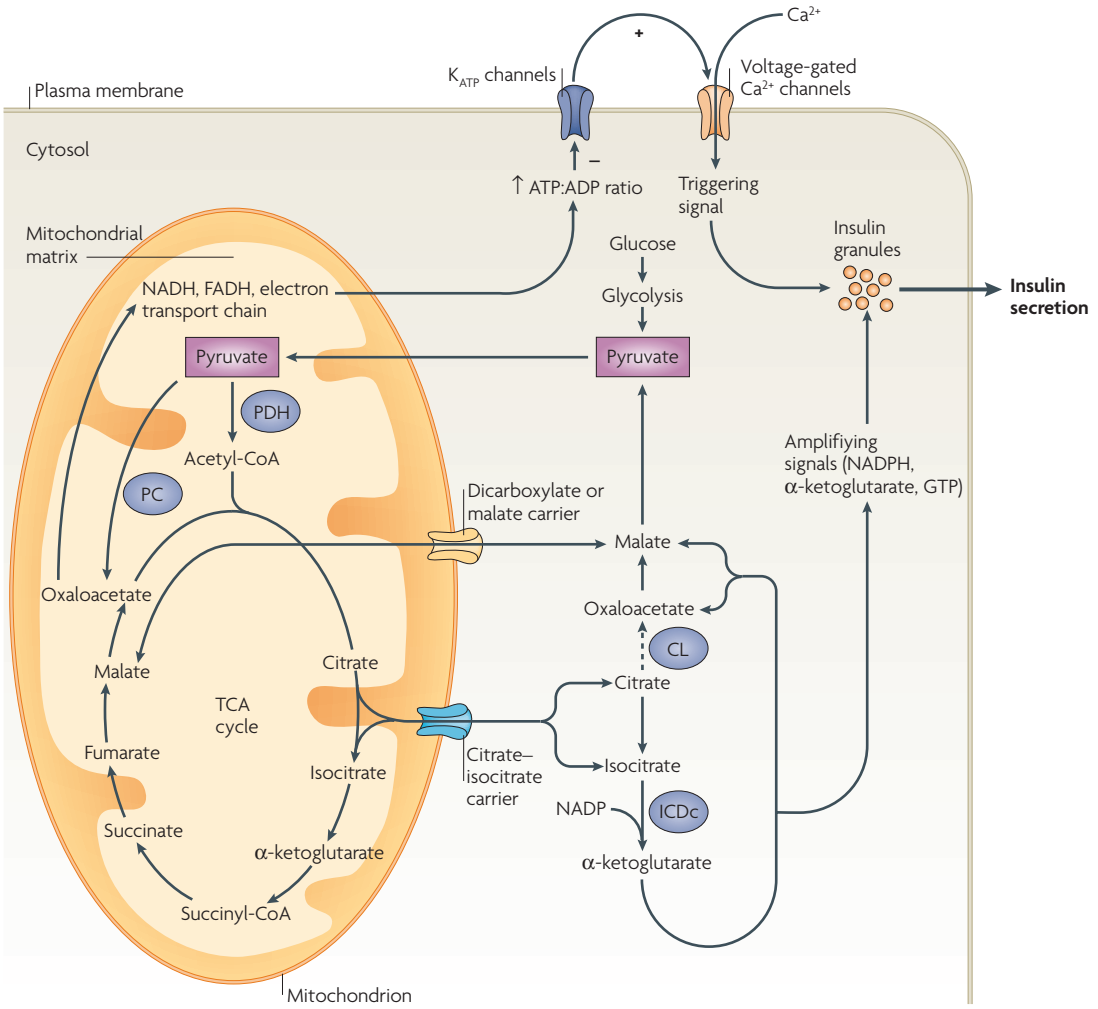

Figure 2 | **Biochemical mechanisms of glucose-stimulated insulin secretion, including roles of the pyruvate cycling pathways of the β-cell.** Glucose metabolism generates ATP from glycolysis, reducing equivalent shuttles (for example, the glycerol phosphate shuttle shown here) and mitochondrial oxidation. The rise in the ATP:ADP ratio inhibits ATP-sensitive K^+ channels (K_{ATP} channels), resulting in membrane depolarization, activation of voltage-gated Ca^{2+} channels, influx of Ca^{2+} and stimulation of insulin granule exocytosis (referred to as the triggering signal[69]). Although ATP:ADP ratio changes are an important initiating event, growing evidence indicates that mitochondrially derived signals other than changes in this ratio also have a crucial role in glucose-stimulated insulin secretion (GSIS). Pyruvate that is derived from glucose through glycolysis can enter mitochondrial metabolism through the oxidative entry point (pyruvate dehydrogenase (PDH)) or the anaplerotic enzyme pyruvate carboxylase (PC). Islet β-cells also exhibit active 'pyruvate cycling', involving the anaplerotic entry of pyruvate or other substrates into the tricarboxylic acid (TCA) cycle to generate excess intermediates that then exit the mitochondria to engage in various cytosolic pathways that lead back to pyruvate. Recent evidence suggests that a pyruvate–isocitrate cycling pathway has particular relevance for the generation of amplifying signals that enhance the Ca^{2+}-mediated triggering signal for exocytosis. In this pathway, pyruvate enters mitochondrial metabolism through PDH and PC, and oxaloacetate and acetyl CoA condense to form citrate and isocitrate. Citrate or isocitrate can exit the mitochondria through the citrate–isocitrate carrier (CIC). Citrate is converted to isocitrate in the cytosol, and isocitrate is converted to α-ketoglutarate by the cytosolic, NADP-dependent isocitrate dehydrogenase (ICDc). α-ketoglutarate can then recycle to pyruvate through a series of mitochondrial and/or cytosolic reactions, including the generation of malate, which can traverse the mitochondrial membrane through the dicarboxylate or malate carrier. NADPH, GTP and α-ketoglutarate are possible signalling factors that are generated from this cycle and serve as amplifying signals for GSIS. In support of an important role of this pathway in GSIS, small interfering RNA-mediated suppression of CIC or ICDc causes strong impairment of GSIS[79,80], whereas suppression of citrate lyase (CL) has no effect[81].

Metabolic overload in β-cells. Chronic exposure of pancreatic islets to elevated levels of nutrients induces β-cell dysfunction and ultimately triggers β-cell death. Exposure of isolated rodent islets to hyperglycaemia for several days raises basal insulin secretion but abrogates insulin secretion in response to stimulatory glucose[90,91]. Similarly, exposure of islets to elevated levels of fatty acids does not impair GSIS unless the islets are cultured at or above a threshold concentration of glucose (usually ~8 mM)[92,93]. These and other findings have led to the concept of β-cell functional impairment as a consequence of 'glucolipotoxicity' rather than as a consequence of exposure to either nutrient alone[92,93]. In this model, glucose increases malonyl CoA levels, thereby leading to inhibition of CPT1 and fatty acid oxidation, and diversion of lipid metabolites into cytosolic products such as ceramides or esterified lipids, similar to what was described earlier as the commonly held metabolic mechanism of insulin resistance. However, once again, recent studies suggest that the deleterious effects of fatty acids on β-cell function may actually occur as a consequence of increased rather than decreased fatty acid oxidation.

Chronic exposure of rodent islets to elevated fatty acids has been reported to decrease PDH activity and glucose oxidation, and it was suggested that this inhibition could prevent the normal glucose-induced increase in ATP:ADP ratio, thereby impairing GSIS[94,95]. However, several recent studies have arrived at different conclusions. Long-term treatment of INS1 cells with oleate caused a modest decrease in glucose oxidation, but glycolytic flux, citrate levels and PDH activity were unchanged[96]. Furthermore, in the INS1-derived 832/13 cell line and in rat islets, chronic fatty acid treatment did not alter glucose oxidation[76,97], but instead induced β-oxidative enzymes and stimulated fatty acid oxidation[76,98].

PC enzymatic activity has been reported to increase in islets from insulin-resistant, pre-diabetic ZDF rats, leading to the suggestion that this would result in increased pyruvate cycling and increased insulin secretion in these animals[99]. Because the static measurement of PC activity does not provide information about metabolic flux, ^{13}C NMR was used to measure flux through the relevant metabolic pathways in β-cells that were exposed to elevated fatty acids[76]. Chronic exposure of 832/13 cells to fatty acids caused an increase in pyruvate cycling activity at basal glucose levels that paralleled increased insulin release. Moreover, the increase in basal cycling activity eliminated the normal glucose-induced increment in pyruvate cycling flux, which could explain the impairment in insulin secretion that is observed at stimulatory glucose concentrations in lipid-cultured cells. Thus, rather than suppression of PDH, the emergent mechanism for lipid-induced impairment of GSIS involves activation of PC by its allosteric activator acetyl CoA, which rises as a consequence of upregulated fatty acid oxidation in lipid cultured cells[76] (FIG. 3). Further evidence for the importance of dysregulated pyruvate cycling in mediating lipid-induced β-cell dysfunction comes from studies using a membrane-permeant ester of malate, dimethylmalate (DMM). Addition of DMM to β-cell lines and rat islets potentiates GSIS and increases pyruvate cycling activity[76]. Inclusion of DMM during insulin secretion assays that were performed on glucose insensitive islets from ZDF rats or lipid-impaired 832/13 cells caused a remarkable improvement in GSIS in both cases.

Changes in mitochondrial metabolism may synchronize with other effects of chronic lipid exposure in β-cells. Exposure of islets or insulinoma cell lines to elevated fatty acid levels increases uncoupling protein-2 (UCP2) expression[100,101], whereas lipid-induced impairment of β-cell function is prevented in islets from *Ucp2*$^{-/-}$ mice[101]. Furthermore, UCP2 expression is increased in islets from *ob/ob* mice, and breeding of *Ucp2*$^{-/-}$ mice with *ob/ob* mice results in the restoration of first-phase insulin secretion and normalization of blood glucose levels[102]. However, the mechanism by which UCP2 might impact β-cell function is not fully resolved. One idea is that UCP2 overexpression causes mitochondrial proton leakage, resulting in impaired ATP production during glucose stimulation[103,104]. It has also been reported that palmitate increases the production of reactive oxygen species (ROS) in normal islets, but not in *Ucp2*$^{-/-}$ islets[104]. However, another recent study showed no increase in ROS (peroxide) or nitric oxide species (NOS) in rat islets that were chronically exposed to elevated glucose and fatty acid[105]. Moreover, the addition of antioxidants such as *N*-acetyl-cysteine (NAC) or pyridoxamine failed to correct lipid-induced impairment of GSIS in these studies, although NAC blocks the toxic effects of chronic exposure of islets to severe hyperglycaemia[106]. Finally, if UCP2 expression is specifically increased by fatty acids, why do islets in normal rodents exhibit sustained compensation for insulin resistance induced by high-fat feeding, even in the face of hyperlipidaemia? These questions remain to be answered before a clear role can be assigned for UCP2 and ROS in β-cell failure of type 2 diabetes.

The role of ER stress pathways in β-cell failure. Mechanisms that underlie the increased rate of β-cell apoptosis and the decline in β-cell mass in type 2 diabetes are incompletely understood. However, several important clues have been revealed from recent work, and the outlines of a possible pathway are emerging (FIG. 3). Interestingly, ER stress could have a role. The protein kinase RNA (PKR)-like ER-associated kinase (PERK) is an important regulator of protein translation in mammalian cells because it phosphorylates and inhibits eukaryotic translation initiation factor-2a (eIF2a). Regulation of PERK–eIF2a is important for linking ER stress to the control of protein translation. Humans and mice that lack PERK have profound β-cell dysfunction and are severely diabetic[107], whereas mice with a mutation in the PERK phosphorylation site in eIF2a have fewer β-cells and are diabetic as a result of insulin deficiency[108]. Moreover, feeding of heterozygous *Eif2a*-mutant mice on a high-fat diet causes distension of the ER lumen in β-cells, a reduction in islet insulin content and diabetes[109].

Although mutations in PERK–eIF2a or other components of the ER stress pathway have not been described in human diabetes, the studies mentioned above suggest a pathway by which chronic exposure of

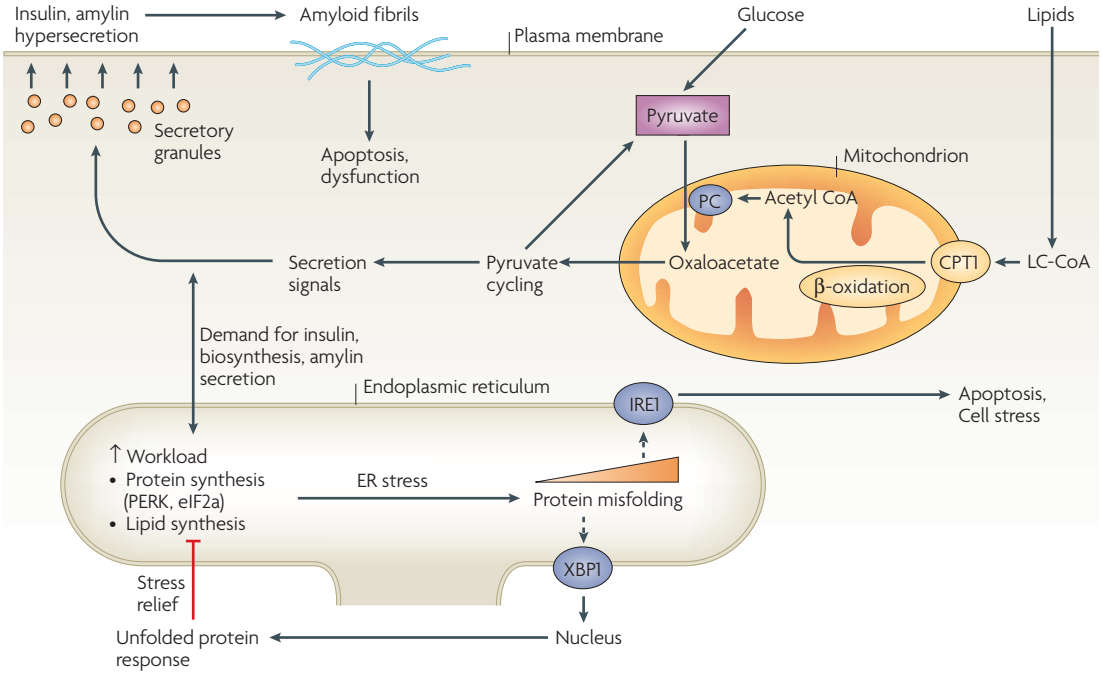

Figure 3| **Mechanisms of β-cell failure in type 2 diabetes.** The model includes metabolic overload (mitochondria), endoplasmic reticulum (ER) stress and deposition of harmful amyloid fibrils. Overnutrition and increased lipid supply induce enzymes of β-oxidation, such as carnitine palmitoyltransferase-1 (CPT1), resulting in increased acetyl CoA levels, allosteric activation of pyruvate carboxylase (PC) and constitutive upregulation of pyruvate cycling. This leads to basal insulin hypersecretion and loss of the glucose-stimulated increment in pyruvate cycling flux, thereby blunting glucose-stimulated insulin secretion. The increased demand for insulin biosynthesis increases demand (workload) in the ER, gradually leading to ER stress and increased protein misfolding. ER stress is initially relieved by the unfolded protein response (UPR), mediated by the transcription factor XBP1, but over time, the UPR becomes less effective and the deleterious effects of ER stress lead to cell death, mediated by IRE1. Finally, insulin hypersecretion is accompanied by amylin secretion, which in humans can form amyloid fibrils that accumulate at the surface of β-cells to induce dysfunction and apoptotic death. eIF2a, eukaryotic translation initiation factor-2a; IRE1, inositol-requiring kinase-1; LC-CoA, long-chain acyl CoA; PERK, protein kinase RNA (PKR)-like ER-associated kinase.

islets to increased nutrient levels could cause the gradual demise of the β-cell. Thus, ingestion of excess calories and increased body weight will require an increase in insulin biosynthesis and secretion in order to maintain fuel homeostasis. As this condition becomes chronic, the biosynthetic demand may eventually overload the protein folding capacity of the ER, leading to activation of the UPR, which in turn leads to activation of PERK and inhibition of protein translation (FIG. 3). Continued exposure to overnutrition could then lead to desensitization of the UPR, ultimately uncoupling translational control from protein folding capacity, exactly as occurs in heterozygous *Eif2a*-mutant mice that are fed on a high-fat diet[109]. The slow, cumulative damage feature of this model is attractive because it could help to explain why humans can remain obese and insulin resistant for long periods of time before β-cell decompensation finally causes the transition to full-blown diabetes.

Role of amyloid fibrils in β-cell failure. Finally, the deposition of toxic amyloid fibrils may be a further mechanism that links overnutrition and hyperstimulation of the islet β-cell to eventual β-cell decompensation and failure. Sections of islets taken from humans with type 2 diabetes contain amyloid fibril deposits, which are now known to be comprised of islet amyloid polypeptide (IAPP), also known as amylin[110,111]. Amylin is synthesized and secreted from islet β- and δ-cells; in humans, non-human primates and cats, it has a propensity to form amyloid fibrils owing, in large part, to the hydrophobicity of amino acids 20–29 in the protein. By contrast, rodents have three Pro substitutions in this region of amylin and, therefore, rodent amylin does not form amyloid fibrils. Thus, early studies with transgenic mice overexpressing rodent amylins were not revealing, but more recent studies in rodent models of human amylin overexpression demonstrate the development of an islet pathology that is similar to that of human diabetes. In one recent example, human amylin overexpression led to increased rates of β-cell apoptosis, diminished first-phase insulin secretion and decreased β-cell mass, ultimately resulting in the onset of glucose intolerance and then diabetes[112]. Again, the gradual accumulation of amyloid deposits would be consistent with the observation of prolonged β-cell compensation in many obese and insulin-resistant individuals before the transition to diabetes as the β-cell fails. We propose that this mechanism could work in concert with metabolically induced impairment of glucose sensing and cumulative ER stress to create a 'perfect storm' that causes β-cell decompensation (FIG. 3).

Box 4 | **Evaluation of current therapies**

A universal antidote does not exist for all of the metabolic abnormalities that are embodied in type 2 diabetes. Several drugs that target the K_{ATP} channel complex to stimulate insulin secretion are available for the treatment of β-cell dysfunction, but these compounds tend to be transiently efficacious and are associated with hypoglycaemia. Therapies that are based on glucagon-like peptide-1 (GLP1) — including more efficacious or long-acting analogues of the native peptide or inhibitors of the GLP1-degrading enzyme DPP-IV — are attractive because of the low risk of hypoglycaemia, given that GLP1 is entirely glucose-dependent as an insulin secretagogue. Recent studies comparing islets from type 2 diabetic individuals and normal controls have revealed drastically decreased expression of multiple proteins of the insulin exocytosis pathway, and strongly impaired glucose-stimulated insulin secretion (GSIS). Remarkably, GLP1 can almost completely restore GSIS in the diabetic islets[129]. GLP1 has also been ascribed an anti-apoptotic and β-cell-regenerative function[121]. The extent to which GLP1 administration to humans engages these regenerative effects remains to be established.

In the realm of insulin resistance, the biguanide metformin and the thiazolidinediones are the most commonly used medications. Metformin activates 5′-AMP-activated protein kinase (AMPK) to stimulate glycolysis and fatty acid oxidation. Thiazolidinediones are PPARγ ligands and, in addition to activating AMPK, stimulate adipogenesis and the redistribution of lipids from liver and muscle into adipose tissue. However, weight gain and fluid retention are common side-effects; hepatotoxicity[130] and an increased risk of heart disease[131] have also been associated with members of this class of compound.

Can a universal drug that combats the diverse metabolic lesions of type 2 diabetes emerge? Because overnutrition is a central driver of the disease, one potential strategy is to reduce food intake. It has been suggested that antagonists of the cannabinoid receptor-1, which regulates central appetite control in response to endogenous ligands, may fulfil this purpose[132]. However, these antagonist drugs have incompletely characterized peripheral effects, and have been associated with increased nausea, dizziness and depressed mood. Nevertheless, strategies that restore energy balance, or that combat fundamental pathogenic mechanisms such as ER stress[66], may ultimately prove to be globally efficacious and deserve further investigation.

Conclusions

The recent studies summarized in this article have greatly advanced our understanding of the molecular and biochemical mechanisms that are involved in the development of type 2 diabetes. Although these advances are both satisfying and thrilling, they also raise new concerns about the complexity of this disease and the challenges of developing new pharmacological therapies (BOX 4). For example, drugs aimed at metabolic targets, such as the enhancement of fatty acid oxidation, may have desirable effects on hepatic insulin action and steatosis, but possible deleterious effects in muscle or islet β-cells. Moreover, drugs that stimulate insulin secretion beyond the already elevated levels of the obese and insulin-resistant state may eventually cause β-cell stress and permanent damage. These complexities may force a focus on therapeutic strategies that combat the root causes of this disease — overnutrition, energy imbalance and cellular stress responses that are induced by metabolic overload. Progress in these areas will require additional insights into these signalling pathways, including a better understanding of the neuronal circuitry that controls feeding behaviours, and the development of methods to intervene in such pathways without causing side-effects such as depression (BOX 4). A better understanding of the mechanisms that link metabolic fuel excess to changes in the activity of key insulin signalling proteins is also required. The need for progress is urgent because there is currently no single drug therapy that can provide long-term, complete normalization of function in patients with type 2 diabetes.

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DATABASES

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OMIM: http://www.ncbi.nlm.nih.gov/sites/entrez?db=OMIM
MODY1 | MODY2 | MODY4
UniProtKB: http://beta.uniprot.org/uniprot
ACC2 | ACRP30 | AMPK | CPT1 | DGAT1 | eIF2a | GLUT4 | GPAT1 | HNF4α | IL-6 | IRS1 | IRS2 | MCD | MCP1 | PDX1 | PERK | PGC1α | PPARα | RBP4 | resistin | SPT1 | TNFα

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