Coming full circle in diabetes mellitus: from complications to initiation

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Abstract | Glycaemic control, reduction of blood pressure using agents that block the renin–angiotensin system and control of dyslipidaemia are the major strategies used in the clinical management of patients with diabetes mellitus. Each of these approaches interrupts a number of pathological pathways, which directly contributes to the vascular complications of diabetes mellitus, including renal disease, blindness, neuropathy and cardiovascular disease. However, research published over the past few years has indicated that many of the pathological pathways important in the development of the vascular complications of diabetes mellitus are equally relevant to the initiation of diabetes mellitus itself. These pathways include insulin signalling, generation of cellular energy, post-translational modifications and redox imbalances. This Review will examine how the development of diabetes mellitus has come full circle from initiation to complications and suggests that the development of diabetes mellitus and the progression to chronic complications both require the same mechanistic triggers.

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Introduction

Diabetes mellitus refers to a group of heterogeneous disorders that are characterized by impaired glucose metabolism that results in persistent increases in blood levels of glucose (hyperglycaemia) in the context of insulin insufficiency relative to the high levels of glucose in circulation. The two most common forms of diabetes mellitus are type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM). T1DM commonly manifests in children and adolescents as the result of the autoimmune-mediated destruction of pancreatic β cells that leads to absolute insulin dependence and hyperglycaemia.[1] The destruction of β cells is postulated to occur via a combination of environmental factors,[2] such as in children positive for islet cell antibodies,[3] and genetic susceptibility.[4–6] Studies published in 2012 have also highlighted the importance of environmental factors in the development of diabetic nephropathy.[7,8] T1DM accounts for ~10% of cases of diabetes mellitus worldwide, although it is predominantly seen in westernised nations.[9,10]

T2DM accounts for ~85% of diabetes mellitus cases worldwide.[11–13] An increase in the number of diagnoses of T2DM has occurred at the same time as the increase in obesity in Western societies; an estimated 7% of the world's population is affected by T2DM, with young people being increasingly affected.[11,13,14] Insulin resistance in peripheral tissues, such as the skeletal muscle and liver, in combination with abnormal insulin secretion as a result of pancreatic β-cell insufficiency are required to progress to overt T2DM.[15] Therefore, despite the presence of insulin in most patients with T2DM, glucose is unable to be adequately transported and stored intracellularly, which leads to hyperglycaemia. Excessive hepatic output of glucose is also present in patients with T2DM, which exacerbates hyperglycaemia. Although dietary modification and oral hypoglycaemic agents are the first-line strategies to reduce blood concentrations of glucose, patients with T2DM are increasingly treated with exogenous insulin, which is required in up to 40% of patients in some countries.[16,17]

A large proportion of patients with T1DM or T2DM will develop vascular complications, which can be broadly divided into microvascular and macrovascular complications. Microvascular complications affect the body's most intricately vascularized organs, in particular the nervous system, the retina and the kidneys.[18,19] Macrovascular complications encompass disorders of large blood vessels that ultimately lead to early myocardial infarctions, ischaemic events, stroke and premature death.[20]

Excessive exposure to fluctuating concentrations of glucose and insulin is increasingly being recognised as an important pathological factor that contributes to the reduction in the number of β cells and impairments in insulin sensitivity. Changes in the concentrations of glucose and insulin are also postulated to be critical for the development of diabetic vascular complications;[21,22] therefore, these sites of microvascular complications might share common mechanisms of end-organ damage, including changes in the uptake of glucose and fatty acids that affect cellular energy production. Furthermore, activation of several downstream mediators, such as inflammation, excessive generation of reactive oxygen species (ROS) and changes in protein structure and function, which are traditionally identified following

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Key points

- The development of diabetes mellitus and its associated vascular complications share common pathogenic pathways
- Vascular damage is often the result of imbalances in glucose handling at many sites within the cardiovascular system
- Dyslipidaemia and abnormalities in cellular energetics are frequently seen in both the development of diabetes mellitus and its associated vascular complications
- Research programs should now investigate patterns of damage across the body in patients with diabetes mellitus
- Grouping and characterization of patterns in the initiation of diabetes mellitus to enable comparison with patterns in vascular complications of diabetes mellitus might result in superior therapies

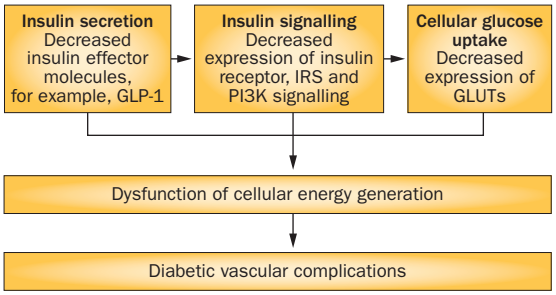

Figure 1 | Pathway leading to the dysfunction of cellular energy generation that results in diabetic complications. Changes in cellular energy generation might also be relevant to the development of diabetes mellitus, creating a feedback loop that could also influence insulin secretion, signalling and glucose uptake. Abbreviations: GLUTs, glucose transporters; GLP-1, glucagon-like peptide 1; IRS, insulin receptor substrate; PI3K, phosphatidylinositol 3-kinase.

long-term exposure to high concentrations of glucose, are also involved in the initiation of diabetes mellitus. Completing the circle, however, are the pathways generally considered as those leading to the onset of diabetes mellitus, which include an altered gut microbiota composition[23] and impaired insulin receptor signalling.[24] This Review suggests that by following the entire circle of diabetes mellitus from initiation through to complications and around again to initiation, we might be able to improve our understanding of how diabetes mellitus develops and the progression of this disease epidemic. This understanding might enable the identification of new therapies that would apply not only to the treatment of patients with diabetes mellitus, but also protect against the onset of vascular complications in these patients, as it is becoming apparent that control of blood glucose levels alone might not be sufficient to prevent complications.[25]

Lessons for vascular complications

Changes in the production, trafficking and signalling of insulin that lead to altered cellular glucose uptake are postulated to ultimately culminate in overt diabetes mellitus via a cascade of downstream signalling events (Figure 1).[26] These pathways are discussed below and how they are involved in the vascular complications of diabetes mellitus has been highlighted, where relevant.

Indeed, one could suggest that abnormalities in insulin signalling and glucose uptake are the key mediators of the vascular complications of diabetes mellitus, as the organs affected by these complications tend to be those that have high metabolic and energy requirements.[27] However, why some patients with diabetes mellitus (60% in the case of diabetic nephropathy) do not develop vascular complications remains a mystery.[27] Perhaps a clue to solving this paradox could be provided by extensive examination of glucose and insulin-dependent signalling in organs prone to complications from patients with diabetes mellitus but no vascular complications. Indeed, two current progressor versus nonprogressor studies will hopefully shed further light on the questions discussed in this Review; the protective genes in diabetes and longevity (PROLONG) study,[28] currently underway in Sweden, is investigating patients who have had diabetes mellitus for 30 years without complications, and the Joslin Medalist Study[29] is providing ongoing analysis of patients who have had diabetes mellitus for 50 years without serious complications.

Insulin secretion

One of the primary mechanisms that control insulin secretion following the ingestion of food is the gut secretion of incretins, namely, glucagon-like peptide 1 (GLP-1) and glucagon inhibitory peptide.[30] Approximately 50% of the insulin secreted acutely after a meal is absorbed is attributable to these molecules; however, their insulinotrophic effects are progressively decreased during the development of T2DM.[31–33] This decrease is attributable to a synergistic combination of decreased incretin secretion, raised circulating levels of the enzyme responsible for its breakdown, dipeptidyl peptidase 4 (DPP4), and resistance to incretin signalling in pancreatic β cells.[32] Indeed, incretins potentiate glucose-stimulated secretion of insulin via binding to specific G-protein coupled receptors that activate the adenylyl cyclase pathway.[34,35] Both GLP-1 and glucagon inhibitory peptide also probably have a number of additional effects on glucose homeostasis via interaction with receptors in tissues and organs, such as skeletal muscle, adipose tissue and the liver, that have not yet been elucidated.

Of the incretins, GLP-1 has received the most attention as its insulinotrophic effect on receptors is preserved in patients with T2DM, thus identifying a potential target for treatment. The insulinotrophic effect was first identified in mice deficient in the GLP-1 receptor. Despite having glucose intolerance during an oral glucose challenge, these mice maintained normal insulin secretory stimulation upon intraperitoneal glucose challenge and therefore maintained normal feeding behaviour.[36] GLP-1 can also increase the mass of β cells and decrease the incidence of apoptosis.[34] Agonists of the GLP-1 receptor, such as the GLP-1 analogues exenatide,[37] albugon[38] and liraglutide,[39] are potent stimulants of insulin secretion and combat hyperglycaemia in humans. Drugs that target GLP-1 are not advised for patients with impaired insulin secretion and T1DM, in whom β cell reserves are severely diminished or absent.

Interestingly, GLP-1 receptors are known to be present at sites of complications of diabetes mellitus.[38,40] Studies published in the past 5 years have identified that the protective effects of GLP-1 receptor agonists are seen in the kidneys,[41,42] atherosclerotic lesions[43–45] and ischaemic heart disease[46,47] in patients with diabetes mellitus and are mostly independent of changes in glucose homeostasis. GLP-1 receptor agonists also affect sodium handling by the kidney,[48,49] which is another important contributor to development and progression of complications of diabetes mellitus by the modulation of haemodynamic pathways. Indeed, the most effective treatments for complications of diabetes mellitus in humans are those targeting haemodynamic pathways via blockade of the renin–angiotensin system.[50–53]

Another way to increase GLP-1 concentration within the circulation is to target its metabolising enzyme, DPP4. DPP4 inhibitors can also regulate glucose homeostasis in humans.[54] Studies have emphasized that this method of targeting the GLP-1 axis also has potential beneficial effects in patients with diabetes mellitus that extend beyond glucose control, such as effects on the kidney,[55] atherosclerosis[56,57] and myocardial infarction.[58] Further data on the efficacy of targeting the GLP-1 axis are expected, as large clinical trials are currently being undertaken, including TECOS,[59] SAVOR[60] and CAROLINA.[61] However, care must be taken with the new line of therapeutics that target GLP-1, as reports are emerging that long-term use might induce pancreatitis, pancreatic cancer and thyroid cancer.[62]

Although hyperinsulinaemia is an important contributor to the development of insulin resistance and T2DM,[63] the specific contribution of hyperinsulinaemia to diabetic complications remains undefined. Little doubt remains that hypoglycaemia, which could occur as a result of hyperinsulinaemia, is a risk factor for cardiovascular disease in patients with diabetes mellitus[64] and is a clear problem in the management of patients with diabetes mellitus who are given antiglycaemic agents. In addition, hyperinsulinaemia is an independent predictor of cardiovascular disease in the absence of overt diabetes mellitus;[65] however, the specific pathogenic pathways responsible for this effect remain to be determined. High levels of circulating insulin might mediate cellular mitogenesis through phosphorylation and other post-translational protein modifications, such as farnesylation. This mediation would affect proteins such as those of the Ras family of GTPases, which have roles in trafficking proteins across cell membranes and in signal transduction of membrane-bound receptors.[66] Insulin also has vasodilatory actions *in vivo* that are dependent on the production of nitric oxide derived from the endothelium.[67] During hyperglycaemia, insulin-dependent production of nitric oxide in skeletal muscle and renal tissues contributes to the progression of vascular complications via the mediation of vascular tone, blood flow and hypertension.[68]

Whether the emerging line of therapeutics that target insulin secretion will prove effective remains unclear. Combination therapies that reduce circulating concentrations of glucose, such as combining an inhibitor of the angiotensin-converting enzyme and one of the emerging DPP4 inhibitors, might instigate further complications by inducing hyperinsulinaemia, which is an independent risk factor for vascular complications.

Insulin signalling

Pertinent to insulin signalling and action is the insulin receptor, which is present in organs that are responsive to insulin, for example pancreatic β cells, liver, skeletal muscle and adipose tissue, and also at sites of diabetes mellitus complications. Mice with a global deletion of the insulin receptor seem to be normal at birth, but develop early postnatal diabetes mellitus.[69] In mice, tissue-specific deletion of the insulin receptor in pancreatic β cells results in defects in insulin secretion similar to those seen in T2DM,[70] whereas tissue-specific deletion of the insulin receptor of skeletal muscle resulted in mice that did not develop hyperglycaemia and never developed diabetes mellitus.[71] This finding is perhaps attributable to the normal function of the insulin receptor in other insulin-responsive tissues, and the glucose uptake in peripheral organs that is mediated by the glucose transporter.[72] In humans, mutations in the gene that encodes the insulin receptor facilitate development of T2DM and associated vascular complications.[24] In support of this finding, an elegant study published in 2010 has demonstrated that a deficiency in insulin receptor signalling in podocytes of the kidney can induce a disease state similar to diabetic nephropathy.[73]

Abnormalities in insulin receptor substrate (IRS) proteins or in second messengers, such as phosphatidylinositol 3-kinase (PI3K), interrupt insulin signalling by changing the docking and downstream signalling of insulin. PI3K is an important kinase that interacts with the IRS proteins to regulate cellular glucose uptake through a series of phosphorylation events.[74] Dysregulation of these kinases is known to be important in the development of progressive defects in insulin secretion, insulin resistance and overt T2DM.[75] Changes in PI3K activity are also responsible for the rapid response to insulin in renal glomerular epithelial and endothelial cells when engaged by IRS.[73,76] However, a gene association study investigated the relationship between T2DM and cardiovascular disease with reference to *IRS1*, which encodes IRS protein 1, and found no association between polymorphisms and pathogenesis.[66,73,76]

The activation of the insulin receptor and IRS proteins is also responsible for a downstream signalling cascade that involves AMP-activated protein kinase (AMPK). AMPK is thought to be a master regulator of cellular energy homeostasis and is activated in response to stresses that deplete cellular ATP supplies, such as low cellular glucose availability, hypoxia and ischaemia.[77] AMPK is believed to be a key therapeutic target for treating obesity and T2DM as a result of its role as a central regulator of both lipid and glucose metabolism. Some evidence suggests that defects in AMPK-mediated phosphorylation might contribute to kidney dysfunction,[78,79] atherosclerosis,[80] retinal damage[81,82] and cardiovascular

disease;[83] however, evidence of a specific causative role in diabetic complications remains to be shown.

A number of the effects of AMPK within cells are thought to occur via its interactions with the mammalian target of rapamycin (mTOR). Little doubt exists that interruption of mTOR signalling contributes to the development of insulin resistance and to defects in insulin secretion and damage to β cells.[84] The mTOR protein is a serine/threonine protein kinase that regulates cell growth and survival, in addition to having effects on protein synthesis and transcription.[85] Defects in the phosphorylation of various targets of mTOR, including mTORC1 and mTORC2, contribute directly to the development of diabetic nephropathy.[86,87]

Therapeutics that increase the efficiency of insulin signalling are ideal candidates for stemming both the initiation of diabetes mellitus and the progression of complications. Exercise has been demonstrated to increase the activity of the insulin receptor and IRS proteins in muscle, thus increasing cellular uptake of glucose.[88] As a result 'exercise' has been tested as an effective strategy to prevent the development of diabetes mellitus,[89] as well as lowering HbA_{1c} levels, with obvious beneficial effects.[90] Similarly, metformin works to treat diabetes mellitus by increasing the action of the insulin receptor in areas of fairly low concentrations of insulin,[91] which is an event stimulated by AMPK.[92,93]

Cellular glucose uptake

The cellular uptake and metabolism of glucose is pertinent to the production of cellular ATP and therefore energy homeostasis. AMPK can be a master metabolic switch that regulates cellular uptake of glucose, β-oxidation of fatty acids and expression of glucose transporters.[94] Cellular expression of the glucose transporters, including those of the glucose transporter (GLUT) and sodium–glucose linked transporter (SGLT) families, is specific to particular cells and organs because of the different glucose handling roles of the various tissues and organs.

Although other biological triggers are likely to be involved, high circulating concentrations of insulin facilitate increased uptake of glucose into organs that are sensitive to insulin.[27] As mentioned above, over time these high levels of insulin suppress insulin signalling and result in a decline in insulin-mediated glucose transport.[95] GLUTs actively facilitate the transport of glucose into the cell and have different kinetic properties and tissue-expression profiles. Over 13 different glucose facilitative transporters have been described;[96] however, in organs prone to vascular complications of diabetes mellitus, current research areas focus on GLUT1, GLUT2 and GLUT4. Changes in GLUT expression occur in patients with vascular complications of diabetes mellitus and might exacerbate pathology by further decreasing the energy supply to cells.

The specific decline in the expression of GLUT4 in tissues that are sensitive to insulin has been postulated to be the direct result of impaired insulin receptor signalling.[97] GLUT4 is an insulin-responsive glucose transporter that has an important role in postprandial glucose disposal.[98–100] GLUT4 also translocates to skeletal muscle during exercise.[101,102] Studies in which GLUT4 has been ablated or 'knocked out' provide evidence that GLUT4 is a primary effector molecule that can regulate glucose transport to skeletal muscle and adipose tissue, which maintains glucose homeostasis.[103] Indeed, mice with a homozygous deletion of the gene that encodes GLUT4 have a marked reduction in fat mass and cardiomegaly and a statistically significant decrease in growth and lifespan,[104] whereas mice with a heterozygous deletion of the gene that encodes GLUT4 develop insulin resistance and T2DM.[103] In patients with T2DM, reduced glucose transport to skeletal muscle is also thought to contribute to hyperglycaemia. Skeletal muscle biopsy samples from these patients demonstrate impaired translocation of GLUT4 to cell membranes.[105] Some evidence also exists that diabetic cardiomyopathy can be improved by targeting GLUT4.[106]

GLUT2 is a bidirectional concentration-dependent glucose transporter that is expressed in hepatocytes, insulin-secreting pancreatic β cells and gut epithelium, and therefore has a major role in postprandial glucose handling.[107,108] In the gut, GLUT2 is rapidly translocated to the apical brush border surface of cells in response to a meal containing glucose, fructose or glucosamine. In pancreatic β cells, GLUT2 is a critical mediator of insulin secretion stimulated by glucose;[109] loss of GLUT2 expression in these cells is thought to result in hyperglycaemia.[110,111] Not surprisingly, GLUT2-deficient mice develop diabetes mellitus and have impaired secretion of GLP-1, resulting in weakened insulin tolerance and poor insulin secretion stimulated by glucose.[112] Furthermore, invalidating the pathway downstream of GLUT2 using loop technology disrupts glucose homeostasis via adaptations in both pancreatic β cells and in proximal tubular cells of the kidney.[113] Polymorphisms in the gene that encodes GLUT2 (*SLC2A2*) have also been associated with progression from glucose intolerance to overt T2DM.[114] Evidence also indicates that changes in GLUT2 occur within kidney tissues in rat models of diabetes mellitus[115] and in human kidney cells *in vitro*.[116]

Cellular glucose uptake at sites of diabetic complications is thought to become uncontrolled after the onset of overt diabetes mellitus.[117] The expression of cellular glucose transporters at these sites is diverse, with insulin-responsive, insulin-unresponsive and sodium-dependent transporters each represented. GLUT1 is also responsible for the transport of glucose across the blood–brain barrier and into the retina.[118] With hyperglycaemia, the expression of GLUT1 decreases in tissues prone to complications, such as the retina, which is thought to be a protective mechanism.[119] Deficiency in GLUT1 results in severe seizures in infants that impair both behavioural and motor skill development.[120]

From complications to initiation

The overarching pathological mediator that initiates vascular complications in diabetes mellitus is thought to be a high level of glucose. However, the validity of this position has been questioned following studies in

Table 1 | Diabetes-mellitus-related processes that can be targeted by therapeutics

Target	Initiation of diabetes mellitus	Progression of complications
AGEs	Reduction of dietary AGEs has decreased progression to diabetes mellitus	Therapeutic reduction of AGEs in experimental models of diabetes mellitus has reduced vascular complications
ROS	Results are mixed and benefits of antioxidants are minimal Therapeutics that are targeted to mitochondrial proteins might prove beneficial	Minimal and mixed effects of ROS reducing agents on the progression of complications in both T1DM and T2DM have been reported
Dyslipidaemia	Various types of lipids, including phospholipids, ceramide and sphingolipids have been linked to the destruction of β cells	Use of statins and PPARα agonists, which are commonly prescribed therapies, reduced development and progression of complications
Inflammation	Low grade inflammation has a role in the development of diabetes mellitus Warrants further investigation	Therapeutics have had mixed effects, but a clear role for inflammation in the development of complications exists

These processes can lead to both the initiation of diabetes mellitus and the progression of diabetic complications. Each of these pathways is considered as a major pathological mediator. The important question to address is whether we should be considering them as separate events in the time-course of disease initiation and progression in individuals who develop diabetes mellitus and chronic vascular complications. Abbreviations: AGEs, advanced glycation end products; PPARα, peroxisome proliferator-activated receptor α; ROS, reactive oxygen species; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus.

patients with diabetes mellitus who have had the disease for ≥50 years, but have not developed vascular complications.[121,122] These individuals often have residual insulin production[121] and sevenfold lower concentrations of advanced glycation end products (AGEs),[122] but no evidence has been found of long-term improvements in glycaemic control using traditional markers such as HbA_{1c} in these individuals compared with patients who have developed complications. This finding suggests that levels of AGEs could be good predictors of glycaemic control, such as is associated with residual insulin production, in these patients or that factors such as AGEs can be pathological mediators of diabetes mellitus complications irrespective of glucose concentrations.[122] Furthermore, evidence from large-scale clinical trials (such as the ACCORD[123] and ADVANCE[25] studies) suggests that strict glycaemic control alone is not sufficient to abate the risk of cardiovascular disease in patients with diabetes mellitus. Indeed, factors thought to be downstream of glucose, such as AGEs, expression of SGLT2 glucose transporters and ROS, in addition to haemodynamic changes, post-translational changes to proteins and dyslipidaemia, seem to have major roles in the development of diabetes mellitus complications (Table 1). However, it is becoming increasingly apparent that these pathways are also relevant to the development of diabetes mellitus.

Advanced glycation

AGEs are modifications formed on the amino groups of proteins that alter the structure and function of the proteins. AGEs result from nonenzymatic biochemical reactions that involve reactive carbonyls and sugars and are thought to be a physiological labelling system for senescent proteins that are processed and then excreted by the kidney.[124] As a result, these modifications were traditionally associated with the complications of diabetes mellitus, as high levels of sugars and metabolic intermediates of sugar metabolism are present in this condition.[125–127] However, a number of studies published in the past decade have shown that AGEs might also be relevant contributors to the development of diabetes mellitus in the absence of hyperglycaemia.[128–130] Furthermore, the symptoms of obesity-related disorders have been shown to improve following dietary restriction of AGEs.[130] The ligation of AGEs and other ligands to the receptor for AGEs (RAGE) is also thought to be required for the development and progression of vascular complications of diabetes mellitus. Interestingly, changes to RAGE have been associated with increased susceptibility to T1DM[127,131] and to increased insulin receptor signalling in skeletal muscle cells.[132]

Sodium glucose transporters

SGLT proteins are expressed in the proximal tubules of kidneys and in the small intestine. These proteins have a central role in the absorption of glucose and galactose from food and the reabsorption of glucose from the urinary filtrate. In proximal tubular cells, Na^+/K^+ ATPase pumps that are located on the basolateral membrane actively transport Na^+ into the peritubular capillary, which creates a concentration gradient across the cell membrane. The energy created by this gradient is used to transport glucose across the apical membrane of the proximal tubular cell from an area of low glucose concentration into an area of high glucose concentration.[133] Changes in the expression of SGLT2 have been shown in the kidneys of patients with diabetes mellitus in a number of studies.[116,134,135] In the past 2 years, the advent of therapies that inhibit the SGLT2 transporters has facilitated their clinical translation from targets for complications of diabetes mellitus to new approaches to control blood levels of glucose via glycosuria.[136,137]

Reactive oxygen species

ROS are highly reactive radicals containing oxygen that are thought to be important signalling molecules. ROS are produced within cells as a by-product of normal metabolism, for example during oxidative phosphorylation. Enzyme complexes (such as NADPH) can produce vast quantities of ROS to facilitate processes such as the killing of cells mediated by the immune system.[138] A number of evolutionary processes exist to combat excess generation of ROS, including dismutation of superoxide (O_2^-)

via its conversion to H_2O_2 and, in a family of superoxide dismutases, conversion to water by enzymes such as catalase, thioredoxin reductase and glutathione peroxidase. Although H_2O_2 is necessary for cell function up to a threshold, it can become detrimental at high concentrations.[139] Essential intracellular H_2O_2 is a product of NADPH oxidase.[140] At sites of diabetes mellitus complications, however, the overproduction of H_2O_2 can result from dismutation of superoxide NADPH oxidase.[141,142] Controversy remains as to how excess generation of ROS contributes to the onset of diabetes mellitus, as data show that ROS signalling is required for insulin secretion;[143] however, this area warrants further investigation.[144]

Dyslipidaemia

Dyslipidaemia is one of the major risk factors for cardiovascular disease as a result of diabetes mellitus. The characteristic features of dyslipidaemia in patients with diabetes mellitus are high plasma concentrations of triglycerides in the context of low circulating levels of HDL cholesterol. Unlike in T2DM, this feature is not commonly seen in patients with T1DM until renal disease manifests.[145] These changes in the lipid profile, which are associated with diabetes mellitus, are generally attributed to increased free fatty acid flux that is secondary to insulin resistance.[146] Interestingly, this association suggests that this type of dyslipidaemia might not be as relevant for the development of T1DM as it is for T2DM. However, some evidence indicates that changes in the concentrations of other types of lipids, such as phospholipids,[147] might be relevant to the development of T1DM. Furthermore, ceramide and sphingolipids are involved in the destruction of pancreatic β cells.[148]

Two major classes of pharmacological agents are used to treat dyslipidaemia in patients with diabetes mellitus; agents that are inhibitors of 3-hydroxy-3-methyl-glutaryl-CoA reductase (that is, statins[149]) and those that target peroxisome proliferator-activated receptor α, such as the agonist fenofibrate.[150] Each of these approaches have shown marked benefits for delaying the development and progression of diabetic complications, in particular atherosclerosis and myocardial infarction.[151]

Toxicity as a result of excess lipid accumulation and storage is also thought to be a potent contributor to the progression of insulin resistance to overt T2DM, possibly via effects on insulin secretion and further impairment of peripheral insulin sensitivity.[152] In simple terms, lipotoxicity is the result of over-accumulation of fatty acids within cells, which can then enter deleterious pathways, such as production of ceramide and generation of excess ROS. These pathways then activate signalling molecules such as protein kinase C, the ultimate result of which is cell death. Whether excessive consumption of calories from sources such as saturated fats or problems in the handling of fatty acids that are independent of obesity are the penultimate facilitators of dyslipidaemia and T2DM remains controversial. To further complicate this area of active research, a study published in 2012 has shown that long-term use of statins in postmenopausal women actually increases their risk of developing diabetes mellitus, in particular for those women with a BMI >25 kg/m^2.[153] Similar results have also been reported in large meta-analyses of incident diabetes mellitus, which demonstrated that statins increased plasma concentrations of glucose and increased the risk of developing diabetes mellitus.[154,155] Given that these agents are widely used in ageing populations it might be important to define if these findings are independent of the effects of statins on lowering levels of lipids, which was not discerned in the above meta-analyses. Clearly, this area is of great importance and warrants further investigation.

The role of inflammation in hyperglycaemia

The co-ordinated sequence of events that leads to the activation of the immune system is tightly regulated. Activation of the immune system results in the temporal recruitment of immune cells to sites of pathogenic invasion or damage and culminates in the resolution of the inflammatory infiltrate via processes such as apoptosis. Chronic low-grade activation of the immune system might contribute to the development and progression of diabetes mellitus and to its vascular complications. Interestingly, an emerging body of evidence has shown that insulin is a potent suppressor of inflammation.[156,157] Indeed, in both major forms of diabetes mellitus, insulin insufficiency is present, which might partly explain why chronic inflammation is seen during the development of diabetes mellitus[156] and in patients with diabetes mellitus who have poor glycaemic control.[157] In addition, patients with diabetes mellitus who do not develop complications seem to have residual insulin-producing β cells,[122] despite little evidence for improved glycaemic control measured by levels of HbA_{1c}, which also suggests that insulin might be an important factor in the prevention of vascular complications.

Inflammatory cytokines are commonly secreted from adipose tissue and other sites, including the endothelium and skeletal muscle, in response to stimuli such as excess free fatty acids. Tumour necrosis factor (TNF), IL-6, retinol binding protein 4 (RBP4) and resistin are adipokines secreted from adipose tissue deposits, although whether the specific cellular source of these adipokines is the adipocytes themselves or infiltrating macrophages is not fully understood. The receptor signalling cascades activated by these cytokines include IκB kinase and nuclear factor κB (NFκB), c-Jun N terminal kinase 1 (JNK1) and production of ROS.

However, it is difficult to determine whether obesity, and hence the release of adipokines, is a critical process required for inflammation and/or whether inflammation is perpetuated by changes within the gut microbiota.[158] If this inflammation is truly pathological, particularly for the development of vascular complications in patients with diabetes mellitus, in whom anti-inflammatory drugs have had mixed success, remains to be determined.[159,160] These anti-inflammatory agents include salicylic acid, cyclooxygenase 2 inhibitors[161,162] and fish oil.[163] Uncertainty over the use of this class of agents is also compounded by the finding that most patients with diabetes mellitus have impaired immune-cell recruitment

and wound healing; therefore, assessing the effect of chronic use of anti-inflammatory agents in this group of patients is difficult.

Lessons from other body systems

Adiposity

Adipose tissue is a major controller of glucose homeostasis. The best evidence for this contribution comes from lipodystrophic animals and humans, who develop severe insulin resistance and diabetes mellitus despite having very little adiposity.[164,165] These findings suggest that secretions from adipose tissue exist that assist in maintaining glucose homeostasis in an endocrine-dependent manner. Control of caloric intake or weight loss as a result of increases in physical activity are widely implemented clinical strategies to reduce the incidence of diabetes mellitus.[166] Interestingly, some rodent studies have shown that restriction of calories[167] and physical activity[168,169] can improve diabetic kidney disease.

Two of the most widely studied adipose-derived hormones in insulin resistance are leptin and adiponectin.[170,171] Leptin controls satiety by interaction with receptors within the hypothalamus,[172] whereas adiponectin modulates a number of metabolic processes, mostly processes associated with glucose homeostasis and catabolism of fatty acids.[173] Mice with mutations in the leptin receptor (db/db) or with leptin abnormalities (ob/ob) are widely studied as models of the contributors to the development of diabetes mellitus. Db/db mice on the KsJ background (BKS.Cg-*Dock7m* +/+ *Leprdb*/J) are gaining popularity for examining diabetic kidney and cardiovascular disease, as they develop many of the comorbidities seen in humans, including dyslipidaemia, obesity and raised systolic blood pressure.[174] However, many paradoxes are seen with leptin with respect to complications of diabetes mellitus. In the context of the development of diabetes mellitus, one could suggest that returning leptin concentrations to usual levels or activating the leptin receptor seems to improve obesity,[175] and deletion of the leptin receptor worsens the symptoms of autonomic neuropathy in db/db mice.[176] However, increasing the concentration of leptin at sites of diabetic complications has been shown to increase retinal neovascularization[177] and worsen renal function.[178]

In addition, adipose tissue is a site of lipid storage and breakdown (lipolysis), which is mediated primarily by circulating concentrations of insulin. When free fatty acids are released by adipose tissue, hepatic gluconeogenesis occurs and glucose is released into the circulation. Adiponectin might be able to inhibit hepatic gluconeogenesis.[179] Mice with deficiencies in adiponectin develop clinically relevant kidney injury and the authors postulated a direct role for adiponectin in mediating the behaviour of kidney cells.[180]

Cytokines are secreted from many sites, including adipose tissue, in response to a range of stimuli such as dyslipidaemia[181] and hyperinsulinaemia.[182] TNF, IL-6, RBP4, and resistin are adipokines secreted from adipocytes or infiltrating macrophages.[183] These cytokines are thought to initiate a signalling cascade through mediators such as NF-κB and JNK1. Some clinical trials in humans have examined the utility of blocking these signalling cascades using salicylates[184,185] and other agents, which have in general yielded lukewarm results. JNK inhibitors[186] and inhibitors of NF-κB have also been tested in experimental mouse models of diabetes mellitus, with mostly disappointing results.[187]

Treatment of obesity with weight loss surgery (for example, bariatric surgery) has proven effective in stopping the progression to T2DM,[188] and can also result in remission of T2DM.[189] Further improvements are seen in renal function[190] and in cardiovascular function following bariatric surgery.[191]

The digestive system and gut microbiota

The digestive system extracts nutrients from our food with the help of a complex milieu of micro-organisms, extreme acid/base conditions and enzymes. Indeed, the gut microbiota seems to have a critical role in energy homeostasis and fat storage and in concerted regulation of metabolic pathways, in addition to presentation of antigens to the immune system at sites such as Payer's patches. Nutrient 'overload' is thought to have a number of detrimental consequences for metabolic pathways. These pathways include release, *de novo* genesis and impaired oxidation of free fatty acids, liberation of endotoxin from bacteria and impairment of incretin secretion leading to insulin secretory abnormalities. Ultimately, patients with diabetes mellitus also have slowed gastric emptying and more profound changes in the function of the gut barrier than patients without diabetes mellitus.

Mounting evidence indicates that changes in the composition of the gut microbiota contribute to both susceptibility and progression to T1DM.[23] Germ-free mice have a notably increased incidence of T1DM, which is thought to be the result of a naive immune system.[192] By contrast, germ-free mice fed Western-style diets containing high quantities of fats and carbohydrates gain considerably less weight[193,194] than conventionally colonised mice.[182,183] However, marked benefits were also seen in these mice and in humans given prebiotics and probiotics, which improve insulin sensitivity and decrease inflammation.[195]

In T1DM, the activity of serum lipopolysaccharide is associated with the progression of kidney disease.[196] Furthermore, a study in rats has indicated that changing the composition of the gut microbiota might influence kidney fibrosis in diabetes mellitus.[197] Dietary metabolism of phosphatidyl choline by the intestinal microbiota can induce atherosclerosis,[198] which would also be an important consequence of consumption of high-fat diets. In any case, the composition of our intestinal microbiota and the influence that our westernised diet has on it is becoming an important question in investigating the pathogenesis of both diabetes mellitus and its complications.

Conclusions

Little doubt exists that changes in the way we live have had a dramatic influence on the development of diabetes mellitus and the development of the vascular complications of this condition. Evolutionary processes within

our bodies are not keeping up with our changing lifestyles and we therefore need to seriously consider how to combat this major disease epidemic. The question remains as to whether we can design improved targets to stop or prevent the development of diabetes mellitus and its devastating complications or whether we should be focusing efforts on facilitating changes in our lifestyle through public policy. Perhaps we have been focusing too closely on the effects of pathways within specific organs rather than assessing the body as a whole. Furthermore, studying diabetes mellitus or its vascular complications in isolation might not provide the answers we seek, given that many pathways exist that mechanistically contribute to the development of diabetes mellitus and then to complications. In particular, the capacity to protect insulin signalling pathways not just at traditional sites seems to be important, as evidence is emerging that good glycaemic control might not be sufficient to prevent cardiovascular disease in patients with diabetes mellitus.[25,123]

Review criteria

A search for original articles listed in PubMed and focusing on the development of diabetes mellitus and complications of diabetes mellitus was performed. The search terms used were "diabetes", "initiation", "complications", "hyperglycaemia", "clean hypothesis", "insulin resistance", "insulin sensitivity" and "inflammation". All articles identified were English-language, full-text, peer-reviewed articles published between 1980 and 2012. In addition, the professional opinion and understanding of all authors was considered. All cited material was considered and reviewed in the preparation of the manuscript.

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Author contributions

B. E. Harcourt and J. M. Forbes contributed to researching data for the article, discussion of content, writing the article and reviewing/editing the article before submission. S. A. Penfold contributed to researching data for the article and writing the article.