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Review

Mitochondrial dysfunction and complications associated with diabetes ☆

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ABSTRACT

Background: Diabetes is a metabolic syndrome that results in chronically increased blood glucose (hyperglycaemia) due to defects either in insulin secretion consequent to the loss of beta cells in the pancreas (type 1) or to loss of insulin sensitivity in target organs in the presence of normal insulin secretion (type 2). Long term hyperglycaemia can lead to a number of serious health-threatening pathologies, or complications, especially in the kidney, heart, retina and peripheral nervous system.
Scope of review: Here we summarise the current literature on the role of the mitochondria in complications associated with diabetes, and the limitations and potential of rodent models to explore new modalities to limit complication severity.
Major conclusions: Prolonged hyperglycaemia results in perturbation of catabolic pathways and in an over-production of ROS by the mitochondria, which in turn may play a role in the development of diabetic complications. Furthermore, current models don't offer a comprehensive recapitulation of these complications.
General significance: The onset of complications associated with type 1 diabetes can be varied, even with tightly controlled blood glucose levels. The potential role of inherited, mild mitochondrial dysfunction in accelerating diabetic complications, both in type 1 and 2 diabetes, remains unexplored. This article is part of a Special Issue entitled Frontiers of Mitochondrial Research.

1. Introduction

1.1. Hyperglycaemia and diabetes

Diabetes is one of the most common diseases in our society, affecting 285 million people worldwide. This number is expected to increase to 439 million adults by 2030 [1]. Type 1 diabetes results from insulin deficiency and type 2 diabetes is caused by resistance to insulin, with both resulting in a disruption to the equilibrium of energy metabolism. These changes lead to a number of secondary diseases, such as cardiovascular disease, diabetic retinopathy, nephropathy and neuropathy. Here, we first discuss normal blood glucose control and its perturbations consequent to diabetes. We then review the role that the mitochondria play in the pathogenesis of diabetes and associated complications.

1.2. Blood glucose control

Blood glucose levels are a constantly regulated balance between glucose absorption from the gastrointestinal tract and/or glucose production in the liver, and utilisation in peripheral tissues. As glucose is a polar molecule, its transport into the cell must be facilitated and is tightly regulated by a number of hormones, particularly insulin. When the pancreatic beta-cells detect an increase in blood glucose levels, insulin is secreted. Insulin then facilitates the uptake and utilisation of glucose into insulin-sensitive tissues, decreases glucose synthesis from pyruvate (gluconeogenesis) in the liver, increases glucose storage as glycogen (glycogenesis) and increases fatty acid synthesis in the liver. Conversely, pancreatic alpha cells detect lowered glucose levels and respond by secreting glucagon, which increases glycogen breakdown to glucose (glycogenolysis) and gluconeogenesis in the liver and decreases glucose uptake in other insulin-sensitive tissues, such as the adipose tissue and cardiac and skeletal muscles (see Fig. 1) [2].

The mechanism of glucose uptake is also tissue specific and can be separated into insulin-sensitive and insulin-insensitive tissue types.

1.2.1. Insulin-mediated glucose uptake

Insulin-mediated glucose uptake allows the rapid regulation of substrates into the cell for ATP generation. Tissues such as fat, heart and skeletal muscle require controlled access to insulin for glucose influx, glycogen synthesis, glycolysis and fatty acid synthesis. Metabolic switching between glycolysis and other sources of energy is particularly

Abbreviations: AGE, advanced glycation end-products; FADH2, flavin adenine dinucleotide; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; mtDNA, mitochondrial DNA; NADH, nicotinamide adenine dinucleotide; OXPHOS, oxidative phosphorylation; PARP-1, poly(ADP-ribose) polymerase 1; PKC, protein kinase C; ROS, reactive oxygen species; VEGF, vascular endothelial growth factor

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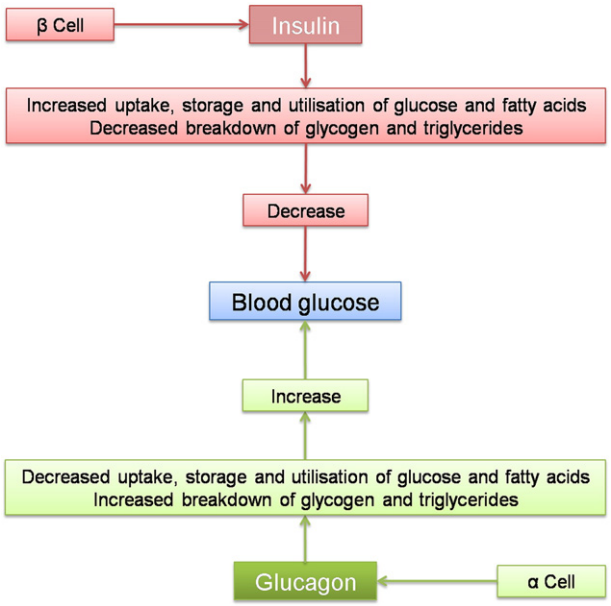

Fig. 1. Control of blood glucose levels. In response to increased hyperglycaemia, insulin is released from pancreatic β cells and mediates the increased utilisation and storage of glucose to decrease blood glucose levels. Glucagon is released from pancreatic α cells and counteracts insulin to increase blood glucose levels.

important in cardiac tissue as it prevents vascular damage since switching to glucose is a cardiac response to stress [3]. In insulin-sensitive tissues, glucose uptake is achieved by mobilising the glucose transporter receptor GLUT-4 to the cell membrane, allowing the internalisation of the glucose molecules into the cell. Upon insulin binding extracellularly, the intracellular face of the insulin receptor undergoes a conformational change, allowing the autophosphorylation of tyrosine residues and tyrosine kinase activity. This activates the insulin receptor substrate (IRS) proteins and a subsequent phosphorylation cascade which activates PI3 kinases, PDK, PKC, and Akt [4–6]. Simultaneously, the insulin-mediated activation of the insulin receptor leads to autophosphorylation of the Cbl protein, subsequent formation of the CAP–Cbl complex, relocation to lipid rafts and activation of TC-10, CIP4/2 and N-WASP. The convergence of these two pathways leads to the translocation of the GLUT4 transporter to the plasma membrane and successive transport of glucose across the cell membrane [5].

1.2.2. Non-insulin-mediated glucose uptake

Non-insulin-mediated glucose uptake is less tightly regulated than insulin dependent glucose transport. This is important in tissues that depend on a constant supply of glucose. A non-insulin dependent glucose transporter, GLUT-1 is expressed on the surface of cells in almost every tissue and is responsible for low levels of glucose uptake required. It has high affinity for glucose and a very fast reaction rate meaning it functions at near maximum rate [7]. Therefore, glucose uptake through GLUT-1 is regulated by level of surface expression, rather than increased or decreased activity. In neuronal tissue GLUT-1 is particularly important in the blood brain barrier and the blood retinal barrier. GLUT-1 is thought to work in conjunction with GLUT-3, which is the main glucose transporter protein in neurons [7].

In the renal and gut epithelial tissues glucose can be taken up into the cell, independent of insulin, by sodium-dependant transport via the sodium–glucose transporters [8]. This method of glucose uptake is highly sensitive to blood glucose concentration and is highly regulated in the kidney, particularly the proximal tubules where glucose is reabsorbed after being filtered through the glomerulus.

GLUT-2 is a transmembrane carrier protein that enables passive glucose movement across cell membranes. Unlike GLUT4, it does not rely on insulin for facilitated diffusion [9]. In addition to being the transporter of choice to import glucose into beta cells for blood glucose level regulation, GLUT-2 is also present on the basolateral membrane of renal tubular and gut epithelial cells. It facilitates the resorption of glucose (previously taken up through sodium-dependant diffusion) across the basolateral membrane [7]. It is also the primary transporter for transfer of glucose between the liver and blood [9].

2. Complications associated with type 1 diabetes

Poorly managed, long term hyperglycaemia increases the risk of developing complications associated with type 1 diabetes, such as cardiovascular disease, diabetic nephropathy and diabetic retinopathy. Over a six and a half year period, the Diabetes Control and Complications Trial (DCCT) [10] found that intensive blood glucose control reduced the risk of retinal disease by 76%, renal by 50% and neuronal by 60%. The intensive treatment used during the trial period involved multiple blood glucose tests daily, tightly monitoring fuel intake and expenditure and adjusting insulin doses accordingly.

Over a longer time period, it has been shown that tightly controlled blood glucose levels have a positive effect on disease complications even if the intensive control regime is not continued. The DCCT followup study, the Epidemiology of Diabetes Interventions and Complications [11] showed continual decreased risk of a number of complications associated with type 1 diabetes, 11 years after the initial intensive treatment was completed. This phenomenon known as "metabolic memory" has also been described for type 2 diabetes (see [12] for review), where it is hypothesised that the benefits of tightly controlled blood glucose relate to the physiopathology of vascular complications of diabetes. Protection from retinal, renal, cardiovascular and neuronal diseases associated with hyperglycaemia was demonstrated, along with decreased lipid levels and blood pressure.

Despite this, the onset of complications associated with type 1 diabetes can be varied, even with tightly controlled blood glucose levels. The role of genetics in susceptibility to these secondary diseases remains unknown. A study of patients who have had type 1 diabetes for more than 50 years found that glycaemic control was unrelated to complications such as retinopathy, nephropathy or neuropathy [13].

2.1. Microvascular disease

Microvascular disease is very closely associated with complications arising from diabetes. Abnormalities in small arteries manifest themselves as retinopathy, nephropathy and neuropathy. Neovascularisation is triggered by a number of pro- and anti-angiogenic factors, such as VEGF, cytokines and nitric oxide. These factors merge in a complex pathway, resulting in increased vascular permeability, chronic inflammation and vasodilation [14].

2.1.1. Diabetic nephropathy

Diabetic nephropathy is the leading cause of renal failure [10,11]. The clinical manifestations are strongly related to the structural changes observed, which include increased glomerular basement membrane thickness and mesangial expansion associated with nodule formation which compresses local capillaries [15]. These are the result of increased extracellular matrix accumulation, triggered by the activation of profibrotic genes such as TGF-β and collagen IV [16–19] possibly through increased activation of proteins under hyperglycaemic conditions [20]. Vasodilatory proteins increase the dilation of blood vessels supplying the glomerulus, increasing pressure and leading to glomerular hyperfiltration [21].

2.1.2. Diabetic retinopathy

One of the leading causes of blindness in the population is diabetic retinopathy. The initial stages of retinopathy, or background retinopathy is characterised by smaller haemorrhages and lipid deposition

[22,23]. Early retinal changes such as pericyte death and decreased growth factors (insulin-like growth factor and VEGF) lead to microvascluar abnormalities, haemorrhage and fluid leakage [24]. Breakdown of the blood–retinal barrier leads to microvascular leakage and can cause oedema. As it progresses, it develops into proliferative retinopathy, where new blood vessels form in the retina, leading to vitreous haemorrhage [25]. Under hyperglycaemia, a number of molecular pathways are affected and can lead to microvascular complications observed in diabetic retinopathy [20].

2.2. Macrovascular disease

Macrovascular disease affects medium to large vessels and therefore has a large role in the pathogenesis of atherosclerosis, where artery walls thicken due to the accumulation of fatty materials such as cholesterol and triglyceride and a chronic inflammatory response in the walls of arteries [14,26].

2.2.1. Cardiovascular disease

Vascular disease primarily manifests as cardiovascular disease in diabetes and is the main cause of death and disability. Studies have shown that type 1 diabetic patients have a higher rate of death due to ischaemic heart disease compared to the non-diabetic population across all ages [10,11].

The pathological mechanism behind this is the development of atherosclerosis resulting from chronic inflammation and abnormalities or injury to endothelial and vascular smooth muscle cells. This encourages the accumulation of lipid particles on the endothelial wall of arteries (atherosclerosis), blocking the flow of blood to the heart, brain and other organs [25,27]. Hyperglycaemia-linked oxidative stress has been shown to contribute to chronic inflammation and endothelial cell abnormalities that lead to atherosclerosis and heart disease [25].

3. Pathophysiology of complications associated with diabetes

Diabetic complications are thought to be mediated by four metabolic pathways that are upregulated or activated by sustained hyperglycaemia. Brownlee [20] proposed a unifying theory where increased substrate availability results in increased oxidative phosphorylation (OXPHOS)-linked reactive oxygen species (ROS) production which causes increased flux through these previously unrelated pathways.

3.1. ROS production in the mitochondria

Small amounts of superoxide, a form of ROS, are routinely released by OXPHOS [28], particularly from complex I and III. Increasing evidence suggests that superoxide normally functions as a signalling molecule [29]. It is thought that some superoxide generation occurs at the site of NADH oxidation of complex I as electrons are transferred through the peripheral matrix-facing arm of the enzyme. However, most superoxide is released during reverse electron flow of complex I which occurs when electrons are transferred back from ubiquinol through the ubiquinone binding site [30]. Complex III also generates superoxide when O_2 reacts with ubiquinone bound at one of the ubiquinone binding sites. Compared to complex I reverse electron flow, complex III superoxide production is much lower under normal physiological conditions. However when the mitochondria are actively generating ATP, superoxide production by complex I is reduced and complex III superoxide production becomes significant [30].

In different physiological states and various tissues, the mitochondria are exposed to variations in oxygen concentration. In addition OXPHOS reactions are tightly regulated, therefore any imbalance or dysfunction can have a detrimental effect on cellular function by decreasing ATP and increasing ROS. In diabetes increased OXPHOS-linked ROS production is believed to be a key mediator of hyperglycaemic tissue injury [17].

3.2. The unique genetics of OXPHOS and association with diabetic complications

OXPHOS is unique in animals in having 13 of approximately 92 structural protein subunits encoded in the mitochondrial DNA (mtDNA) [29]. This adds complexity to genetic association studies where mtDNA is often overlooked. A number of studies have nevertheless reported mtDNA associations with diabetic phenotypes. A heteroplasmic mutation in the mitochondrial DNA (mtDNA) gene for cytochrome b (15059G>A) was shown to associate with increased incidence of hypertension in type 2 diabetes [31]. In addition, some specific mtDNA haplotypes have been suggested to increase risk of developing complications associated with diabetes. In European populations, epidemiological studies have shown increased association between a number of different mtDNA haplotypes and coronary artery disease, diabetic retinopathy, nephropathy and renal failure [32]. In Jewish populations, especially the Ashkenazi Jewish community, other haplotypes are very strongly associated with nephrology, retinopathy and coronary artery disease [33].

Mitochondrial DNA variation may contribute to the severity of complications associated with diabetes. This may go some way to explaining the disparity we see between patients suffering from varying degrees of complications associated with type 1 diabetes [34].

Mismatched nuclear and mitochondrial DNA have been shown to result in mitochondrial dysfunction [35–37]. Nuclear or mtDNA-encoded OXPHOS polymorphisms have the potential to impact on the severity of complication development by contributing to mitochondrial dysfunction and ROS production. Further discussion of diabetes associated with mtDNA mutations is provided below in Section 4.

3.3. The role of increased ROS in type 1 diabetes

Brownlee proposes that under high levels of intracellular glucose, substrate availability is increased, driving the citric acid cycle and pyruvate oxidation [20]. This would lead to an increase in electron-transport intermediates such as NADH and $FADH_2$. It is hypothesised that increased flux through OXPHOS increases the mitochondrial membrane potential beyond a certain threshold, blocking electron transfer in complex III [38]. These electrons then escape the electron transport chain to reduce molecular oxygen, forming superoxide [39] (see Fig. 2).

The increased superoxide partially inhibits the glycolytic enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH) [39] by triggering its increased ribosylation [40]. Ribosylation is performed by poly(ADP-ribose) polymerase-1 (PARP-1), an enzyme that produces ADP-ribose by splitting NAD^+ into nicotinic acid and ADP-ribose, resulting in ADP-ribose polymers that accumulate on proteins such as GAPDH. It is thought that increased superoxide induces DNA strand breaks, activating PARP-1 [3,41]. Experimentally, increased hyperglycaemia-linked superoxide production results in increased poly(ADP-ribose) polymerase-1 (PARP-1) activity, increased GAPDH ribosylation and decreased GAPDH activity in aortic endothelial cells [40]. The effect was ameliorated by increased expression of uncoupling protein (UCP-1), superoxide dismutase 2 (MnSOD) or the PARP inhibitor PJ34, demonstrating that mitochondrial superoxide production contributes to PARP activation and GAPDH inhibition [40].

The inhibition of GAPDH results in the accumulation of metabolites that feed into glycolytic pathways. These metabolites are shunted into alternative pathways such as the polyol, hexosamine, PKC activation and AGE product pathways, as shown in Fig. 3.

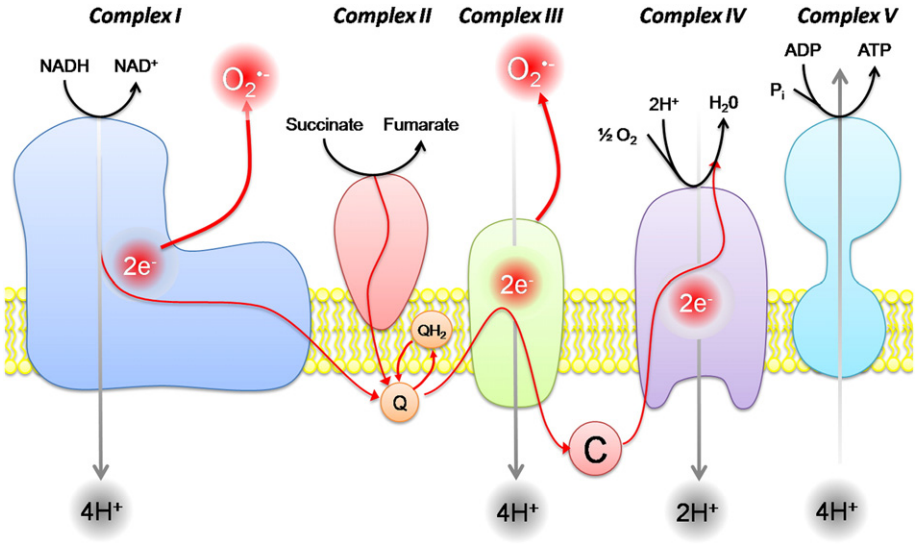

Fig. 2. The production of mitochondrial superoxide. Superoxide is routinely produced by oxidative phosphorylation when O_2 binds electrons leaked from complex I (NADH:ubiquinone oxidoreductase) or complex III (ubiquinol:cytochrome c oxidoreductase; cytochrome bc (1) complex). In hyperglycaemia, it is believed that increased flux through OXPHOS results in increased production of superoxide which exacerbates complications associated with diabetes.

3.4. Polyol pathway

An alternative in the first stages of glucose metabolism is the polyol pathway in which aldose reductase catalyses the reduction of glucose to sorbitol in a NADPH-dependant reaction. The resultant increase in $NADP^+$ drives oxidation of glutathione (GSH) to glutathione disulphide (GSSG). The increased flux through the polyol pathway is thought to drive damaging oxidative stress because the decrease in GSH results in decreased superoxide scavenging [20,42]. This has greater impact in non-insulin dependent cells, such as nerve, retinal and vascular cells, than insulin dependent cells such as endothelial cells [43].

3.5. Hexosamine pathway

Another alternate glucose metabolism pathway under hyperglycaemia is the hexosamine pathway where the increased conversion of excess glucose to glucosamine occurs through glutamine:fructose-6-phosphate amidotransferase (GFAT) [20]. The increased hexosamine variant UDP-

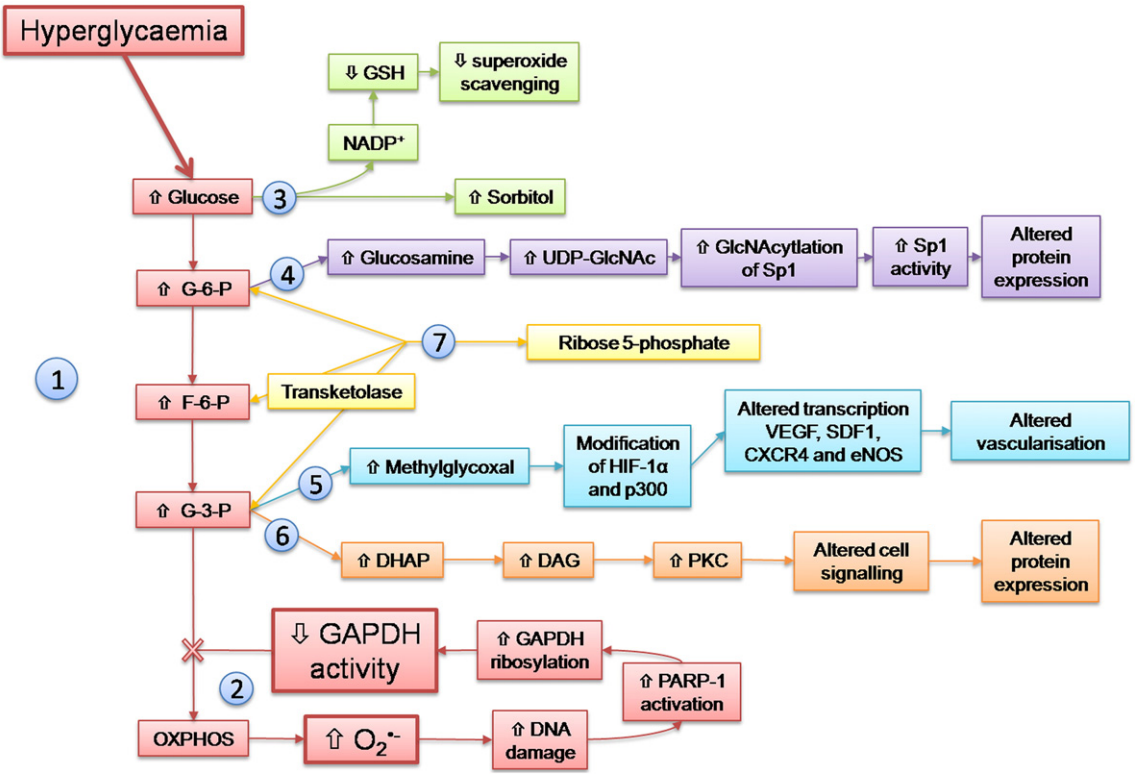

Fig. 3. The role of mitochondrially-driven ROS in complications associated with diabetes. Hyperglycaemia drives increased flux through glycolysis (1) and OXPHOS, resulting in increased superoxide production which leads to decreased GAPDH activity (2). Glycolytic metabolites are shunted into alternate pathways; the polyol pathway (3), the hexosamine pathway (4), increased AGE production (5) and increased PKC activation (6). The pentose phosphate pathway (7) may be protective by diverting metabolites away from these pathways.

N-acetylglucosamine is thought to lead to the O-GlcNAcytlation of transcription factors such as Sp1 making them more transcriptionally active, thereby resulting in hyperglycaemia-mediated systemic increase in protein expression [20,42].

3.6. Advanced glycation end-products pathway

Increased glucose over euglycaemic levels can result in the conversion of glucose to glyoxal, methylglyoxal and 3-deoxyglucose via other metabolic intermediates. These precursors react with amine groups on proteins to form advanced glycation end products (AGE) (for review see [44]). AGE accumulation results in oxidative damage [45] and contributes to the increased expression of cytokines, growth factors, pro-inflammatory factors and pro-coagulatory factors. AGE also modify collagen-type proteins to decrease vessel elasticity, cell adhesion and matrix–matrix interaction [20,44].

3.7. Protein kinase C activation pathway

Another cellular mechanism contributing to diabetic-related macrovascular pathology is the activation of protein kinase C (PKC) through increased de novo synthesis of diacylglycerol (DAG) via the glycolytic intermediate dihydroxyacetone phosphate (DHAP) in the PKC pathway. Increased DAG can activate a large number of pathways leading to detrimental changes in blood flow and vessel permeability and increased fibrosis [20,42].

Increased AGE formation can also result in activation of the PKC and MAPK pathways, leading to the dephosphorylation of PDGF and signalling pericyte apoptosis [46]. Pericyte loss is one of the earliest signs of diabetic retinopathy [21].

3.8. Pentose phosphate pathway

The pentose phosphate pathway (PPP) is an anabolic pathway, responsible for generating pentoses and reducing equivalents in the form of NADPH. In a pathway that runs parallel to glycolysis, glucose-6-phosphate is oxidised to ribose 5-phosphate and back via transketolase and transaldolase [47]. The direction of the reaction is thought to be determined by substrate availability [48]. Fructose-6-phosphate and glyceraldehyde-3-phosphate are also generated in this pathway [47]. Under hyperglycaemia, it is thought that the PPP could be protective by diverting these excess glycolytic metabolites away from the hexosamine, AGE and PKC pathways and towards the production of less damaging endpoints [48,49].

Increased flux through the PPP could result in an increased supply of NADPH to the GSH redox cycle, thereby increasing the anti-oxidant capacity of the cell. However, in high glucose conditions, glucose-6-phosphate dehydrogenase (G6PD) has been shown to be inhibited [50]. G6PD is the major component of the PPP responsible for NADPH production and its inhibition would decrease supply to glutathione reductase to reduce GSSG, a major ROS scavenger and antioxidant.

4. Mitochondria and the pathophysiology of diabetes

4.1. Mitochondria and type 2 diabetes

Type 2 diabetes results from a combination of reduced tissue sensitivity to insulin and inadequate insulin secretion. It usually develops in adults and is thought to result from a complex interaction between obesity, physical inactivity, diet and genes [51]. Increased fat mass leads to several factors that inhibit insulin action including decreased GLUT4, increased free fatty acids and other circulating molecules [52,53]. Due to decreased insulin response in sensitive tissues, excess glucose accumulates leading to chronic hyperglycaemia [51].

Insulin resistance and type 2 diabetes have been linked to alterations in mitochondrial metabolism. Patients with insulin resistance or type 2 diabetes have shown decreased mitochondrial density and ATP production [54,55] and reduced mitochondrial mRNA levels [56–58]. It has also been shown that ROS levels have a significant role in the pathogenesis of insulin resistance [59]. In patients with a family history of type 2 diabetes, a short-term high calorie diet resulted in increased markers for oxidative stress and a transient increase in OXPHOS enzyme protein expression. This led the authors to speculate that, with prolonged overfeeding, the sustained increases in ROS may lead to the impairment of mitochondrial proteins resulting in mitochondrial dysfunction and the lipid accumulation that typifies type 2 diabetes [60].

Mitochondrial DNA is particularly susceptible to oxidative damage due to its close proximity to the production of OXPHOS-linked ROS [61]. Type 2 diabetes is commonly seen in mtDNA disease patients. Maternally-inherited, insulin-independent diabetes in conjunction with deafness (MIDD) was first associated with a 10.4 kb deletion of mitochondrial DNA and significantly decreased OXPHOS activity by Ballinger and colleagues in 1992 [62]. The proband was heteroplasmic for the deletion (69% in the muscle, 53% in the lymphoblasts and 17% in the myoblasts) and also showed decreased mitochondrial protein. Other pedigree members also showed similar heteroplasmy (between 38 and 44%) with maternal inheritance [62]. A single point mtDNA mutation in the gene for $tRNA^{Leu}$ (A3543G) was also shown to cosegregate with MIDD in a large pedigree. Again, the proband was heteroplasmic for the mutation (38% in the muscle, 41% in the fibroblasts and 4% in the blood) and had decreased OXPHOS activity. Interestingly, the same mutation, at higher heteroplasmic levels, causes mitochondrial encephalomyopathy with lactic-acidosis and stroke-like episodes (MELAS) suggesting it is a more severe phenotype of the same disease [63].

A model for mitochondrial DNA variation using a non-obese rat model for type 2 diabetes with mitochondrial DNA from FHH rats and nuclear DNA from Wistar rats ($T2DN^{mtFHH}$) has been developed. Sequencing data showed that the major variants in the mitochondrial DNA were located in genes for subunits for complex I. The $T2DN^{mtFHH}$ rats showed cardiac remodelling due to increased myocyte size in 12 month old rats. They also showed decreased complex I activity in a complex I + III linked assay and complex I-linked respiration in the cardiac tissue, even though OXPHOS protein levels were unchanged [64].

4.2. Mitochondria and type 1 diabetes

There are numerous factors that are associated with the development of type 1 diabetes. Antibody mediated autoimmunity is hypothesised to alter immune function and is potentially triggered by viruses such as rubella or coxackie. Environmental toxins such as nitrates or nitrites and early exposure to other antigens present in food such as cow's milk or wheat have also been linked to the autoimmune dysfunction that leads to type 1 diabetes. Mutations of the MHC Class II histocompatibility complex HLA-DR/DQ region and IDDM (insulin-dependent diabetes mellitus) genes also confer an increased susceptibility to the disease. The combination of genetic susceptibilities and environmental factors somehow contributes to the pathological mechanism of beta cell loss underlying the disease.

Beta cells are responsible for releasing insulin from the pancreas into the body. The pancreas is both an exocrine (excretes via a duct) and endocrine (excretes via diffusion) gland. The endocrine function is performed by the islets of Langerhans, which are comprised of several cell populations; alpha cells which release glucagon and proglucagon, gamma cells secreting somatostain-14, epsilon cells secreting ghrelin, PP cells secreting pancreatic polypeptide and, most crucial to the pathology of diabetes, beta cells. Beta cells comprise approximately 55% of the pancreas and produce several hormones, including insulin. They are particularly sensitive to ROS damage because they have deceased antioxidant capacity [65].

Irrespective of the primary insult, the pathogenesis of type 1 diabetes is characterised by progressive loss of beta cells in the pancreas,

thought to involve T-cell mediated autoimmunity. In the pancreatic lymph node, T-cells are presented with antigens specific to the islets of Langerhans. The T-cells infiltrate the islets, leading to the targeted destruction of beta cells [66] through mitochondrial-driven apoptosis [67].

While there are no mitochondrial polymorphisms specifically associated with type 1 diabetes, Uchigata and colleagues [68] showed that the frequency of Mt5178C polymorphism was more common among patients with type 1 diabetes and was significantly higher than that among healthy control subjects. This suggests that Mt5178C is associated with genetic susceptibility to type 1 diabetes [68]. Natural sequence variation has also been associated with resistance against type 1 diabetes. A SNP in the gene for complex I (*mt-Nd2*^{a}) has shown to be associated with resistance to beta cell damage by cytotoxic T cell attack and TNFα injury [69], leading the authors to hypothesise that *mt-Nd2*^{a} mutants may generate less ROS, thereby inhibiting beta-cell apoptosis.

5. Mitochondrial dysfunction in type 1 diabetic models

Mitochondrial function in insulin-dependent diabetes has been investigated extensively, however these studies are contradictory. It seems that the mitochondria from diabetic models are functionally impaired and biogenesis is increased, but this is heavily dependent on the model and particularly the tissue or cell type being assessed.

Tissues highly dependent on oxygen such as the cardiac, skeletal and smooth muscles, central and peripheral nervous systems, kidney and insulin-producing pancreatic β-cell are particularly susceptible to impaired OXPHOS [70]. As discussed previously, glucose uptake varies between tissue types, therefore in hyperglycaemic conditions, OXPHOS substrate availability varies between different tissues and so does the significance of mitochondrial dysfunction.

Under prolonged hyperglycaemia, kidney mitochondria from streptozotocin-treated rats showed increased superoxide production in conjunction with decreased complex III activity and increased AGE on complex III proteins [71]. Mitochondria isolated from the kidney of streptozotocin-treated rats had increased complex I and complex IV activities and decreased complex II and III activities [72]. This was accompanied by increased reactive oxygen and nitrogen species and carbonylated proteins. However, there were no morphological differences or changes in ATP synthesis and respiration in the kidney mitochondria of Akita mice [73], despite increased expression of fatty acid oxidation and TCA enzymes.

Replication of this study in the liver mitochondria revealed similar findings [72,73]. Using streptozotocin-treated mice Liu et al. [74] also found increased mitochondrial gene transcripts and evidence for increased mitochondrial biogenesis.

In the heart of the OVE26 mouse model for diabetes, mitochondrial abundance increased in conjunction with increased mitochondrial DNA copy number and mitochondrial proteins [75]. Using primary cardiac endothelial cell cultures from streptozotocin treated mice, increased expression of the mitochondrial fusion protein OPA1 and decreased fission protein DRP1 were demonstrated [76]. This finding was confirmed in human cardiac endothelial cells [76]. This study also showed decreased respiratory control rate in conjunction with increased mitochondrial and cellular superoxide. Decreased state 3 respiration and ATP synthesis have been demonstrated in the heart of Akita mice [73] and streptozotocin-treated rats, along with TCA cycle activity and heart function [77]. Therefore, the increase in mitochondrial density does not seem to translate into increased functionality or efficiency.

In the brain of streptozotocin-treated rats with short term hyperglycaemia, Moreira and colleagues found no changes in any of the measured parameters [78]. Decreased OXPHOS enzyme activity and protein levels were described in the dorsal root ganglia of streptozotocin-treated rats, however this tissue had increased respiration [79]. Mitochondrial dysfunction in these neurons has been linked to adenosine monophosphate-activated protein kinase (AMPK) signalling and was corrected by resveratrol treatment [80].

In streptozotocin-treated mice, the retina had increased expression of OXPHOS proteins, where all the mitochondrially-encoded OXPHOS protein subunits and some nuclear-encoded subunits were increased [81]. Mitochondria have been shown to be enlarged in rats with poor glycaemic control and further analysis has established alterations in proteins involved in mitochondrial biogenesis [82]. Interestingly, streptozotocin-treated mice were protected from mitochondrial DNA damage in the retina by overexpression of a superoxide scavenger [83].

Mitochondria are likely to play a key role in the pathology of diabetes. A general tissue response may be to increase mitochondrial content in diabetic tissues, but mitochondrial function appears not as efficient, suggesting that increased number of the mitochondria is a compensation for poor performance. However the evidence is conflicting. There is currently a poor understanding of pathology of secondary diseases associated with diabetes and a lack of good representation of these diseases in current rodent models. Given the central role played by mitochondrial dysfunction, understanding the pathological mechanisms may reveal disease pathways and therapeutic targets.

5.1. The effect of therapeutics on the mitochondria

Medications used in to treat type 1 and 2 diabetes focus on reducing blood glucose levels. In type 1 diabetes this is primarily achieved through the replacement of insulin. Oral anti-hyperglycaemic medication is used in type 2 diabetes to increase organ sensitivity, increase insulin secretion, or decrease glucose absorption in digestion. Insulin and metformin are the first line of treatment in type 1 and type 2 diabetes respectively. Their effects on mitochondrial function may contribute to the mechanism underlying dysfunction in diabetes.

5.1.1. Insulin

Mitochondria are, in part, regulated by insulin. Insulin stimulates mitochondrial transcription, protein synthesis and function [84]. Although a direct mechanism between insulin stimulation and mitochondrial function has not been firmly established, numerous studies have shown elevated mitochondrial protein synthesis under high levels of insulin [84] and decreased synthesis when deprived of insulin [85].

Stump and colleagues have shown that insulin infusion in the muscle increases mitochondrial enzyme activity (citrate synthase and complex IV) and ATP production in the skeletal muscle of non-diabetic patients [84]. mRNA transcripts of nuclear and mitochondrial genes for OXPHOS and mitochondrial protein synthesis were also elevated, but this was not observed in type 2 diabetic patients in the same study. However in another study by the same group, nuclear-encoded electron transport chain mRNA transcripts were increased, even though overall gene transcripts for mitochondrial proteins were decreased and mitochondrial copy numbers remained unaltered in the muscle of type 2 diabetic patients undergoing low-dose insulin infusion [86].

Other studies have shown altered mitochondrial levels and function. In miniature swine with high doses of insulin, mitochondrial and nuclear protein synthesis rates were increased in the skeletal muscle but remained unchanged in the liver and heart tissues [87]. Whereas in the brain of streptozotocin-treated mice with high doses of insulin, decreased mitochondrial membrane potential, ATP levels and respiration were observed [88]. In vitro studies showed that mouse primary hepatocytes exposed directly to insulin had decreased mitochondrial mass and mitochondrial mRNA particularly genes associated with mitochondrial ATP production and O_2 consumption [74].

In type 1 diabetic patients, insulin deprivation leads to decreased OXPHOS mRNA and muscle ATP production [85]. Physiological measures have shown that insulin deprivation leads to increased energy expenditure and O_2 consumption in type 1 diabetic patients [85,89] and decreased exercise capacity and VO_2 max in type 2 diabetics [90].

By suggesting that increased mitochondrially-driven ROS has an upstream role in the mechanisms behind micro- and macrovascular diseases associated with diabetes, Brownlee has provided a theory that underlies mitochondrial involvement in diabetes. However, the exact mechanism by which the mitochondria produce ROS under hyperglycaemic conditions remains unclear. Mitochondrial function in insulin-dependent diabetes has been investigated extensively, however these studies are contradictory. Results and interpretations depend heavily on tissue type and insulin-dependency.

Overall, insulin may stimulate OXPHOS in the skeletal muscle and possibly other tissues by increasing mitochondrial protein transcription and elevating mitochondrial function. Studies that investigate the direct effect of insulin on mitochondrial ROS production may reveal that the pathological role of the mitochondria in complications associated with diabetes is exacerbated by the action of insulin.

5.1.2. Metformin

Metformin is the most commonly used anti-hyperglycaemic in the treatment of type 2 diabetes. One of its most important functions is to suppress gluconeogenesis in the liver. However its mechanism of action has not been firmly established. It is thought that AMP-activated protein kinase (AMPK) plays a central role in this process [91], but there is increasing evidence that AMPK-independent pathways may contribute significantly to metformin's anti-hyperglycaemic action [92] (reviewed in [93]).

It has been shown that, once transported into the cell, metformin inhibits mitochondrial function. Different studies have shown that rat hepatoma cells exposed to metformin had decreased state 3 complex I-driven respiration, while complex II-driven respiration was normal [94,95]. The same investigations showed that complex I activity was decreased in the mitochondria isolated from the liver of rats that were treated with metformin [94,95]. Further experiments established that metformin exposure led to decreased gluconeogenesis in isolated hepatocytes and patch-clamped livers, in addition to altered metabolites associated with gluconeogenesis [95]. Significantly, this study showed decreased ATP/ADP, which may prove to be a key step in the mechanism of metformin action.

Rather than directly interacting with AMPK, it has been demonstrated that metformin increases the ratio of AMP:ATP and ADP:ATP [96] resulting in reduced gluconeogenesis through a number of pathways [92]. In addition to the increased availability of AMP contributing to the increased activation of AMPK, AMP may also inhibit fructose-6-phosphate (F6P) production in gluconeogenesis by inhibiting fructose-1,6-biphosphatase (FBPase) [95]. Thus, metformin may ameliorate hyperglycaemia by reducing the effect of increased ATP in the cell.

Importantly for long term effects of hyperglycaemia and associated complications, metformin has been shown to decrease levels of mitochondrially produced ROS. ROS production by reverse electron flow into complex I can be ameliorated by metformin [97] and when bovine aortic endothelial cells grown in high glucose conditions were exposed to metformin, they produced less mitochondrial-linked ROS [98]. This indicates a direct link between mitochondrial ROS production and the action of metformin.

6. Conclusion

In conclusion, it is apparent that mitochondrially-produced superoxide plays an important role in diseases that are associated with type 1 diabetes. Increased flux through the polyol and hexosamine pathways and increased PKC activation and AGE formation are ameliorated by uncoupling proteins, superoxide scavenging and PARP-1 inhibitors. These studies link superoxide production and GAPDH inhibition to altered glycolytic pathways in hyperglycaemic conditions and have shown a causal relationship between mitochondrial overproduction of ROS and diabetes, especially in *in vivo* models.

Tissues have different insulin dependent and independent modes of glucose uptake. OXPHOS substrates will vary and this may have an impact in the pathological mechanism of ROS production, and therefore diseases associated with prolonged hyperglycaemia. Insulin also has a role in stimulating mitochondrial protein transcription and function. These factors may contribute to the contradictory findings in models for type 1 diabetes, especially in the presence and absence of insulin treatment.

The exact mechanism by which OXPHOS overproduces ROS in hyperglycaemia and the role that insulin plays is unclear. It is our hypothesis that mitochondrial DNA mutations may play a role in the heterogeneity of the incidence of complications associated with type 1 diabetes in the face of controlled glucose levels. Mouse models for inherited mitochondrial DNA variation are rare and their generation is subject to numerous technical difficulties [99]. Our group has developed a unique homoplasmic xenomitochondrial mouse, with evolutionarily divergent mitochondrial DNA (*Mus terricolor*) and nuclear DNA (C57Bl/6J *Mus domesticus*) [37,100] which allows us to test the hypothetical link of mildly impaired OXPHOS driving increased complications. Preliminary findings using the streptozotocin-induced type 1 diabetes model in the xenomitochondrial mouse suggest that worse cardiac and kidney complications evolve in this mouse [101]. C57Bl/6 mice are relatively resistant to development of diabetic complications [102]; provision of a new model with more fulminant development of the complications seen in human diabetes will be a valuable addition to the field.

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