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Skeletal muscle mitochondrial dysfunction & diabetes

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Skeletal muscle insulin resistance is a key contributor to the pathophysiology of type 2 diabetes. Recent studies have shown that insulin resistance in a variety of conditions including type 2 diabetes, ageing and in offspring of type 2 diabetes is associated with muscle mitochondrial dysfunction. The important question is whether insulin resistance results from muscle mitochondrial dysfunction or vise versa. Gene array studies from muscle biopsy samples showed that transcript levels of several genes, especially *OXPHOS* genes are altered in type 2 diabetic patients during poor glycaemic control but many of these alterations are normalized by insulin treatment suggesting that reduced insulin action is a factor involved in muscle mitochondrial dysfunction. Moreover, insulin infusion while maintaining glucose and amino acid levels results in increase in muscle mitochondrial gene transcript levels and ATP production indicating that insulin is a key regulator of muscle mitochondrial biogenesis. At a similar post-absorptive insulin levels both type 2 diabetic patients and non diabetic controls have similar muscle mitochondrial ATP production but increasing insulin from low to high levels stimulate ATP production only in non diabetic people but not in the diabetic people. The lack of muscle mitochondrial response to insulin in type 2 diabetic patients is likely to be related to insulin resistance and reduced substrate utilization.

Key words Diabetes - lipid peroxides - mitochondrial dysfunction - skeletal muscle

Type 2 diabetes mellitus is becoming a worldwide problem of epidemic proportions[1-4]. Diabetes and its chronic complications, especially premature cardiovascular diseases, are emerging as a major threat to the welfare of humanity[5]. The problem is almost of pandemic proportion in societies such as in India where there are rapid changes in lifestyle[6] resulting from socio-economic advances. Changes in socio-economic status alone are unlikely to explain the increased prevalence of diabetes among Indians because in places like Singapore where Chinese and Malay populations with similar socio-economic status as Indians have lower prevalence of diabetes than Indian ethnic population[7]. Based on available data, lifestyle changes coupled with genetic predisposition have been proposed as reasons for the high incidence of diabetes among Asian Indians[8]. Diabetes, with its attendant acute and long term complications, and the myriad of disorders associated with it, is a major public health hazard with its major impact on rapidly expanding

urban population. Recently studies reported from south India[9-11] and nationwide studies in India[12] confirmed the high prevalence of diabetes among native Indians. The prevalence rate of diabetes among Indians ranges from 3 per cent in rural areas[9,13] to over 16 per cent in urbanized cities with the highest life expectancy such as Trivandrum (now Thiruvananthapuram), Kerala State in India[14]. Studies have shown that compared with diabetic patients of Caucasian origin, the typical diabetic Indian patient presents earlier in life and demonstrates insulin resistance[15] and develop diabetes with lower body mass index. However, in general the incidence of type 2 diabetes increases with age. Insulin resistance also is known to increase with age[16]. The important question remains to be answered is what is the underlying cause of type 2 diabetes. There is compelling evidence to demonstrate mitochondrial dysfunction with age[17]. Since diabetes is a metabolic problem we have focused our recent research on mitochondria which is the location of most of fuel metabolism in the body. Though skeletal muscle is the predominant site of disposal of glucose and fatty acids following a meal and skeletal muscle insulin resistance has been well established as the beginning event of type 2 diabetes, the underlying mechanism remains to be determined[18,19]. In this article we provide an integrative view on the interrelation between muscle mitochondrial changes resulting in reduced oxidative phosphorylation and insulin resistance and other changes in physical performance in type 2 diabetes.

Association between muscle mitochondrial dysfunction and diabetes

There is increasing evidence that muscle mitochondrial dysfunction occurs in many insulin resistant states such as in type 2 diabetes[20-23], offspring of people with type 2 diabetes[24-26] and in obesity[27]. Although a clear association between insulin resistance, type 2 diabetes and muscle mitochondrial dysfunction has been demonstrated, no causal relationship has been established. However, mitochondrial dysfunction may be central to the pathogenesis and the pathophysiology of type 2 diabetes, as it may contribute to insulin resistance[25,28] as well as to impaired insulin secretion, and also to diabetic complications[29]. It has been proposed that reduced muscle mitochondrial activity results in accumulation of intracellular triglyceride accumulation that causes insulin resistance[28]. An alternative hypothesis[23,30] that we pursue is that insulin resistance causes muscle mitochondrial dysfunction. The basis of the above hypothesis is that insulin enhances muscle mitochondrial biogenesis[23]. It has been observed that the activity of the mitochondrial electron transport chain is reduced in the muscles of patients with type 2 diabetes, and that muscle mitochondria are smaller in these patients[20,23].

Mitochondrial oxidative phosphorylation by the electron transport chain provides energy for adenosine triphosphate (ATP) production. This, however, also generates reactive oxygen species (ROS). ROS causes damage to DNA, proteins, and membrane structures. An imbalance between increased ROS and decreased endogenous antioxidants within the mitochondria will enhance the damaging effects of ROS. Further, ROS may lead to increased mutations in mitochondrial DNA (mtDNA), which has a limited repair capacity[29]. Accumulation of point mutations in mtDNA has been reported in ageing humans[31]. The production of ROS also increases with ageing, while defense mechanisms against ROS decrease[29]. We have recently shown that mitochondrial DNA oxidative damage is higher with age and mitochondrial DNA copy numbers decline with age[26]. Moreover, this reduction in mitochondrial DNA copy numbers[31,32] may be the underlying mechanism of reduced mitochondrial mRNA abundance[16], reduced muscle mitochondrial protein synthesis[33], and reduced muscle mitochondrial protein content and ATP production with age[26]. It has been suggested that diabetes, through glucolipotoxicity, causes mitochondrial dysfunction and excess ROS production in a similar way to accelerated tissue aging[29]. During electron transport chain (ETC) reaction oxygen species (ROS) are formed (Fig.). Elevated fatty acids may also result in ROS formation and act in concert with the pathogenic effect of high glucose[34]. Hyperglycaemia-induced overproduction of superoxide by the mitochondrial electron transport

chain appears to play a major role in the pathways leading to diabetic complications[24]. Normalizing levels of mitochondrial ROS with various agents has been shown to prevent glucose-induced activation of the diverse pathways implicated in diabetic complications[35]. However, the impact of these agents in human diabetes or pre-diabetes state has not been established.

Evidence of mitochondrial defects in diabetes

Mitochondrial genome and its potential role in diabetes: Based on recent evidences it has been proposed that a decreased oxidative capacity and mitochondrial aberrations act as a potential contributor to the development of insulin resistance and type 2 diabetes[36]. Mitochondria provide cells with most of the energy in the form of ATP. Mitochondria are complex organelles encoded both by nuclear and mitochondrial DNA. Only about 15 per cent mitochondrial components are encoded by mtDNA, most of the mt-proteins are nuclear DNA encoded. However, all components of protein complexes are critical for mitochondrial functions. Majority of the known mutations leading to a mitochondrial disease have been identified in mtDNA rather than in nuclear DNA. Many of these mutations cause metabolic defects. Recent studies have shown that mitochondrial DNA mutations accelerate aging in mice[37]. One important question is whether oxidative damage to mtDNA is higher or not in diabetes because of the increased ROS formation in diabetic patients. The mitochondrial matrix, which contains DNA, RNA, and numerous enzymes necessary for substrate oxidation, is sensitive to peroxide-induced oxidative damage and needs to be protected against the formation and accumulation of lipids and lipid peroxides. Recent evidence reports that mitochondrial uncoupling is involved in the protection of the mitochondrial matrix against lipid-induced mitochondrial damage. Disturbances in this protection mechanism can contribute to the development of type 2 diabetes[38].

As discussed, apart from producing ATP, mitochondria are also a major source of ROS[39]. These ROS products have a very short half-life and react rapidly with DNA, protein, and lipids, thereby leading to oxidative damage which leads to oxidative stress. Oxidative stress occurs when the balance between the

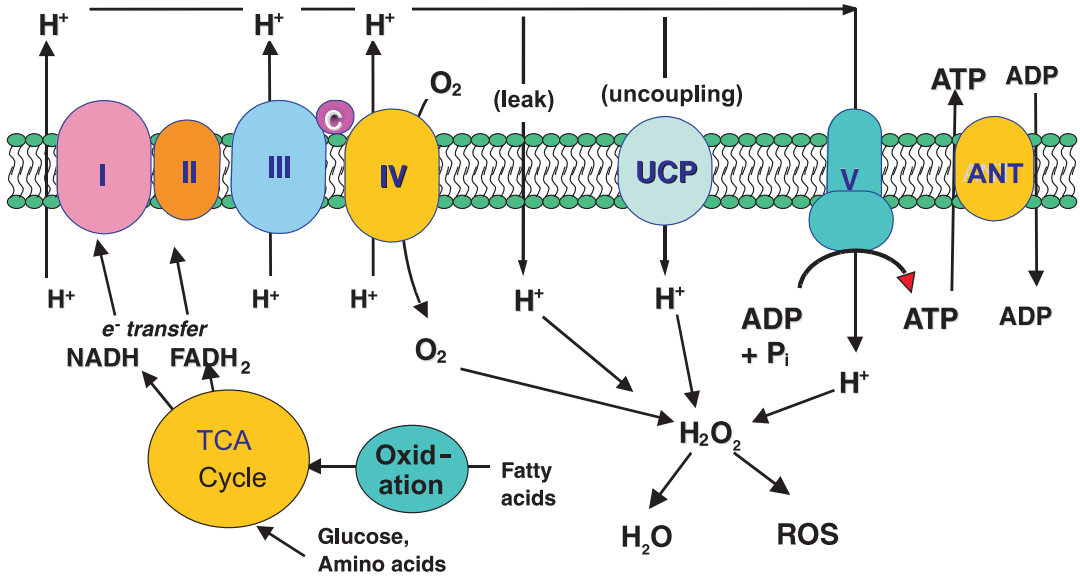

Fig. Oxidative phosphorylation pathways in mitochondria. Reactive oxygen species (ROS) is the toxic byproduct of ATP production and is involved in developing oxidative stress in diabetes. TCA, Tricarboxytic acid; ANT, Adenine Nucleotide Translocase.

production of oxidation products and the ability of antioxidant mechanisms to neutralize these products is tilted in the favour of the former. The production of reactive oxygen species increases in patients with diabetes[40]. The possible sources for the overproduction of reactive oxygen species are widespread and include enzymatic pathways, autoxidation of glucose and the mitochondria. In diabetes, the overproduction of O^{2-} has been attributed to increase in the activity of several enzymes including nitric oxide synthase and NADH/ NAD(P)H oxidase[40]. In conditions related to diabetes, high glucose and free fatty acid levels have been shown to stimulate ROS production in cultured vascular cells through a protein kinase C (PKC)-dependent activation of NAD(P)H oxidase[41]. Activation of NAD(P)H oxidase has also been linked to the increased production of advanced glycation end-products(AGEs)[42]. Exposing cultured human endothelial cells to increased concentrations of AGEs caused an increase in intracellular formation of H_2O_2 and expression of vascular cell adhesion molecule-1, which was suppressed by diphenyliodonium[42]. In aortas from diabetic rats Hink *et al*[43] found an activation of NAD(P)H oxidase and a 7-fold increase in gp91phox mRNA levels, a subunit of the NAD(P)H complex. Angiotensin II, which is increased in diabetes, through activation of angiotensin-1 receptors has also been demonstrated to upregulate several subunits of NAD(P)H oxidase and increase intracellular levels of O^{2-} [44].

Direct evidence that increased NAD(P)H oxidase activity impairs vascular function comes from studies by Hamilton *et al*[45]. They demonstrated that inhibition of NAD(P)H activity with apocynin decreased O^{2-} production by human mammary arteries and saphenous veins, increased NO production and induced vasodilation[45]. Increased formation of O^{2-} in diabetes has also been linked to increased activity of xanthine oxidase[46]. The activity of xanthine oxidase is increased in liver and plasma of diabetic animals, and in diabetic rabbits, increased O^{2-} formation has been demonstrated to be blocked by allopurinol, an inhibitor of xanthine oxidase[46]. Another enzyme system that produces ROS in the vascular wall is nitric oxide synthase (NOS). In diabetes the generation and/or bioactivity of NO by endothelial NOS (eNOS) is reduced[47]. However, a decrease in substrate and co-factor availability, arginine and tetrahydrobiopterin (TH_4), respectively, may also contribute to this deficit. The administration of arginine has been demonstrated to improve vascular function in diabetic patients and animal models[47]. Likewise, the administration of tetrahydrobiopterin derivative has been shown to improve diabetes-impaired vascular function[48]. In diabetes a suboptimal concentration of tetrahydrobiopterin reduces the formation of NO and favours "uncoupling" of NOS leading to NOS-mediated reduction of oxygen and formation of O^{2-} and H_2O_2[49]. Excessive amounts of ROS may also arise from dysregulation of the mitochondrial electron transport chain. Four main molecular mechanisms *viz*., increased flux of glucose through the polyol pathway, increased formation of AGEs, increased activity of PKC, and increased flux through the hexosamine pathway have been linked to hyperglycaemia-induced vascular dysfunction[50]. These mechanisms have in common one feature which is the overproduction of O^{2-} by the mitochondrial electron-transport chain[50].

Several other studies support the paradigm that mitochondria are a major source for O^{2-} production in the vasculature of diabetic rats[51]. Using cultured bovine aortic endothelial cells, Brownlee *et al*[50] demonstrated that hyperglycaemia increased O^{2-} production. Hyperglycaemia, due to an overproduction of electron donors derived from glycolysis and the TCA cycle, has been demonstrated to increase the proton gradient across the mitochondrial inner membrane above a threshold level causing a prolonged period of O^{2-} generation[52,53]. It has been shown that overexpression of Mn-SOD abolishes the signal generated by ROS, and overexpression of uncoupling protein-1 collapses the proton electrochemical gradient, thereby preventing the overproduction of ROS by endothelial cells[35]. Overall, these studies demonstrate that multiple sources exist for overproduction of ROS in diabetes and illustrate the challenges facing investigators in designing strategies to prevent the development of oxidative stress.

Mitochondrial damage: a role for lipid peroxides and hyperglycaemia: Fatty acids are particularly prone to oxidative damage, resulting in the formation of lipid peroxides. These peroxide products are cytotoxic and highly reactive, leading to free-radical damage to proteins and DNA. Therefore, accumulation of fatty acids in the vicinity of the mitochondrial matrix, where oxidative processes take place, makes them prone to lipid peroxidation, which eventually may result in damaged mitochondrial proteins and reduced oxidative capacity as a consequence. Such a situation might be the case in type 2 diabetes. Patients with type 2 diabetes are characterized by high plasma FFA levels[54] and a reduced fat oxidative capacity[55]. Under such conditions, fatty acids that cannot be oxidized will accumulate in the muscle cell[38,56], and the increased load of fatty acids on the mitochondrial membrane will lead to the entrance of neutral fatty acids into the mitochondrial matrix[57], where they are prone to peroxidation. When mitochondrial ROS production becomes excessive, and lipid peroxides are formed, mitochondrial uncoupling would reduce ROS production, thereby reducing the formation of lipid peroxides. In skeletal muscle, 4-hydroxy-2-nonenal-induced uncoupling was shown to be accomplished by the mitochondrial uncoupling protein (UCP)-3[58], which is in accordance with the earlier notion that UCP3 plays a role in the prevention of excessive ROS[59]. This negative feedback loop appears to be interrupted in the absence of UCP3, as UCP3-ablated mice have been shown to have increased skeletal muscle ROS production[59], increased lipid peroxidation, and damage to mitochondrial proteins[60]. Consistent with this idea is the recent finding that skeletal muscle of obese insulin-resistant subjects contained a higher amount of intramyocellular lipids and, more importantly, these lipids showed a higher degree of lipid peroxidation[61]. Also, in the elderly it was shown that muscular lipid accumulation is related to mitochondrial dysfunction and insulin resistance[25], and aging is associated with accumulation of ROS-induced mutations in control sites of mtDNA replication[31]. Together, these results suggest that lipid accumulation in muscle cells, as observed in type 2 diabetes[38,56], could impair mitochondrial oxidative capacity due to lipid peroxidation-induced damage to mitochondria. In turn, the reduced mitochondrial oxidative capacity would further exacerbate the storage of lipids inside the muscle cell. Together, with a suggested reduced peroxisome proliferator-activated receptor (PPAR)-γ co-activator (PGC1) activity, which would limit mitochondrial biogenesis[62], a positive feedback loop would exist in which mitochondrial functioning would rapidly deteriorate.

Both type 1[63-65] and type 2[66,67] diabetes is characterized by hypermetabolic states. While glucose and fatty acid fluxes and oxidation are increased in type 2 diabetes, amino acid oxidation is increased in type 1 diabetes[68,69]. However, the hypermetabolic state in type 2 diabetes is reversible by treatment. In remains to be determined whether untreated diabetes and hypermetabolic state contribute to mitochondrial damage and dysfunction and treatment prevents the mitochondrial damage. Oxidation of glucose, fatty acids, and amino acids ultimately produces acetyl CoA that enters Kreb's cycle and produce electron carriers-NADH and FADH that enter into mitochondrial respiratory chain. High flux of substrate oxidation results in high superoxide formation that may damage mitochondrial DNA or even the integrity of mitochondrial inner membrane. The impact of these are not only in diabetic complications but also on further deterioration of mitochondrial function.

Mitochondrial DNA mutations and depletion in type 2 diabetes: Research in this field is largely focused on the analysis of insulin receptors and pancreatic beta-cell dysfunction, it has become apparent that mutations in mtDNA can also lead to type 2 diabetes. A total of about 40 mutations have been catalogued that result in diabetes mellitus[70]. Many of these mutations are associated with type 1 diabetes, yet a lesser number of mutations leads to type 2 diabetes. Maternally inherited type 2 diabetes was one of the first mitochondrially inherited diabetic conditions discovered by Ballinger *et al*[71]. It is the most common form of mitochondrial type 2 diabetes accounting for 1-2 per cent of diabetes cases and is caused by an adenine to guanine mutation in the $tRNA_{LEU}$[72]. It is reported that a strong association

exists between the level of mutational heteroplasmy and type 2 diabetes diagnosis[73]. Several other mutations on mtDNA found to correspond to the type 2 diabetes are spread throughout the mitochondrial genome[74].

Mitochondrial dysfunction as seen in the depletion of mtDNA may play an important role in pathogenesis of type 2 diabetes. It is shown in rat model of type 2 diabetes with impaired insulin secretion that the mitochondria of beta cells were decreased in volume along with a decrease in mtDNA copies[75]. This decrease in mtDNA was not associated with any major mutations or deletions and was observed only in adults, not in foetal tissue. These results suggest a connection between glucose stimulated insulin secretion and mtDNA depletion. Another example of exogenously induced oxidative damage to mtDNA is illustrated with streptozotocin to create diabetes in animals[76].

mRNA abundance of mitochondrial/nuclear proteins in type 2 diabetes: Increasing evidences support that mtDNA damage accumulates with ageing, particularly in skeletal muscle where insulin resistance occurs in type 2 diabetes. However, the question that remains is whether these changes affect mitochondrial function in type 2 diabetes. Mitochondrial DNA damage could limit mitochondrial gene expression at the level of transcription and eventually at translation level. We have shown that in skeletal muscle transcript levels of about 85 genes, including several mitochondrial genes, were altered in type 2 diabetes and 10 days of intensive insulin treatment improved the transcripts level in all but 11 genes[21]. The 11 genes that remained unaltered by insulin treatment include some of the genes involved in muscle mitochondrial function. However, insulin treatment altered the transcription of 29 additional genes involved in different metabolic functions including energy metabolism. This study and several other follow up studies[22,77] identified some of the candidate genes for muscle insulin resistance, complications associated with poor glycaemic control in people with type 2 diabetes. These follow up studies used larger number of subjects and more sophisticated bioinformatics system, but did not consider the potential interactions of glucose and insulin levels on muscle gene transcript levels. Since insulin has been shown to affect muscle gene transcript levels, especially of those mitochondrial genes[21,23], it is difficult to interpret the muscle gene transcript expression data of type 2 diabetic patients in comparison with non diabetic people unless insulin and glucose levels are controlled to match each group.

In general, it appears that multiple gene transcript levels are altered in skeletal muscle of type 2 diabetic patients but many of them, especially of those of mitochondrial genes are normalized by insulin. What remains to be determined is the proportion of genes whose transcripts remain unaltered by insulin. The important question is whether these genes are involved in insulin resistance and muscle mitochondrial dysfunctions. An alternative explanation is that these genes that remain unaltered by insulin treatment, may represent the effect of insulin resistance in type 2 diabetic patients. In addition, a number of other genes are shown to be involved in insulin resistance in skeletal muscle of people with type 2 diabetes which included insulin receptor substrate 1[78], glycogen synthetase[79], uncoupling protein-3 (UCP-3)[80], glucose transporter-4 (GLUT-4)[81], hexokinase II[82], PI3-kinase[83], MAP kinase[84], serine-threonine kinase[85], *rad* genes[84], calpain-10[87] and mitochondrial transcription factor A (mtTFA)[88]. Peroxisome proliferator-activated receptor gamma co-activator 1 alpha is emerging as a pivotal regulator of mitochondrial biogenesis in skeletal muscle[89,90], and its transcript levels are reduced in skeletal muscle from type 2 diabetic and insulin-resistant individuals[22,77], suggesting a potential common signaling pathway linking insulin sensitivity and mitochondrial function.

Mitochondrial protein synthesis and function in skeletal muscle in type 2 diabetes: Impaired insulin action to stimulate tissue substrate utilization with particular regard to glucose is a common metabolic defect and a defining feature of insulin resistance in both type 2 diabetes and ageing[91,92]. Skeletal muscle is a key metabolic organ and the major site of insulin-mediated glucose disposal, and has been shown to play a pivotal

role in the metabolic alterations of type 2 diabetes. Since proteins are the key functional molecules we determined whether synthesis rates of muscle proteins, especially mitochondrial proteins are altered in type 2 diabetes.

In recent years, acute increments in the plasma insulin concentration have been demonstrated to stimulate skeletal muscle mitochondrial gene expression, mitochondrial protein synthesis and function *in vivo* in healthy individuals. In a miniature pig model, acute intravenous insulin infusion was shown to enhance the mitochondrial protein synthesis rate in a tissue-specific fashion[93]. Mitochondrial protein synthesis was increased in skeletal muscle, but not in cardiac muscle and liver. Indeed, liver mitochondrial protein synthesis tended to be lower during hyperinsulinaemia, indicating that the stimulatory effect of insulin is specific to skeletal muscle under these experimental conditions[91]. The insulin effects were similar in the presence of near-basal or moderately increased plasma amino acid concentrations. Importantly, increased mitochondrial protein synthesis was not associated with increments in the synthesis rates of other muscle protein pools, including sarcoplasmic protein and major contractile protein myosin heavy chain[93]. This study indicated a novel effect of insulin in selectively enhancing muscle mitochondrial protein synthesis *in vivo*.

Skeletal muscle has also emerged as a target of acute insulin effects on muscle mitochondria in humans. Hyperinsulinaemia in the high physiological range increased transcript levels of complex I and complex IV subunits of the respiratory chain[94]. Interestingly, increments in mitochondrial transcripts were positively related to those of insulin-mediated glucose disposal[94], supporting the concept that muscle mitochondria are responsive to insulin action to increase fuel utilization. A recent study[23] further characterized the role of insulin in the acute regulation of skeletal muscle mitochondrial function. When insulin was infused into healthy men and women, mitochondrial transcript levels, protein synthesis, respiratory chain enzyme activity and the ATP production rate all significantly increased after 7 h, with a trend towards an increment also at 4 h. This study introduced the theory that insulin is a stimulant of muscle mitochondrial biogenesis and function *in vivo* in humans.

As insulin resistance characterizes type 2 diabetes, it is conceivable that mitochondrial defects occur in this disease, in particular in skeletal muscle. Stump *et al*[23] provided further important evidence in this regard by demonstrating that the acute stimulation of mitochondrial ATP production when insulin infusion was increased from low to a higher level observed in healthy individuals, was not achieved in age-matched individuals with type 2 diabetes. A defective response of skeletal muscle mitochondria to acute insulin-induced stimulation thus occurs in humans with type 2 diabetes. An altered mitochondrial response to insulin may contribute to impaired substrate utilization, as an integral component of insulin resistance in this disease. These results are particularly relevant to the post-prandial period, when plasma insulin concentrations increase in an acute fashion to levels comparable to those achieved in the study. It remains to be determined whether reduced insulin action or glucose disposal caused muscle mitochondrial defect or mitochondrial defect caused impaired glucose disposal.

Reports of mitochondrial oxidative capacity in skeletal muscle from individuals with type 2 diabetes under basal post-absorptive conditions have been somewhat conflicting. Some studies observed reduced post-absorptive mitochondrial citrate synthase activity in obese individuals with type 2 diabetes after treatment withdrawal compared with non diabetic lean[95] or obese non diabetic control individuals[20,96]. Other studies did not confirm these findings, reporting comparable activities of oxidative marker enzymes in human diabetic and non diabetic muscle[97,98]. One of the reports[98] however, demonstrated an increased fatigability of leg muscle, defined as reduced strength after repeated contractions, a potential indicator of reduced muscle mitochondrial ability to generate ATP. A general relationship between muscle oxidative capacities and glucose disposal has been confirmed in several studies[20,95-97]. In the only study measuring muscle mitochondrial protein fractional synthesis rate[98], no

differences were observed under basal conditions between obese (body mass index 30 kg/m^2) individuals with type 2 diabetes studied after treatment withdrawal and either obese or lean non diabetic control groups in the presence of similar mitochondrial oxidative capacities. The same study reported a slight (10-12%) but statistically significant selective increment of muscle citrate synthase, but not of cytochrome c oxidase activity, in individuals with diabetes after 10 days of intensive insulin treatment, in the absence of changes in the mitochondrial protein synthesis rate[98]. The reasons for some discrepancies in basal muscle oxidative capacities reported in different studies are not completely clear, although they could be at least partly accounted for by heterogeneity in type 2 diabetic populations and treatment status. Chronic moderate increments of the post-absorptive plasma insulin concentration can be observed in type 2 diabetes and were reported in some[20,95], although not in all[98] studies of muscle mitochondrial function. Reduced oxidative capacities in the presence of increased insulin levels raise the possibility that resistance to potential insulin effects also occurs in the presence of moderate hormone increments under post-absorptive conditions.

Taken together, available reports support impaired mitochondrial function in skeletal muscle in type 2 diabetes. Acute effects of high physiological hyperinsulinaemia to stimulate muscle protein synthesis and function suggest that either mitochondrial stimulation is involved in insulin-mediated post-prandial substrate disposal or reduced substrate disposal results from mitochondrial dysfunction. Post-absorptive mitochondrial defects have been reported[20,95,98], and could also contribute to altered substrate utilization in individuals with type 2 diabetes.

Conclusion

It is generally agreed that muscle mitochondrial capacity to produce ATP is reduced in type 2 diabetes. It remains to be determined whether this is a functional defect secondary to the diabetic state or insulin resistance *per se* is secondary to muscle mitochondrial dysfunction. Evidences indicate that insulin itself stimulates mitochondrial biogenesis and capacity to produce ATP in skeletal muscle. It is therefore possible that reduced insulin action may contribute to muscle mitochondrial function. It remains to be determined whether poor glycaemic control and hypermetabolic state in diabetes increase mitochondrial damage ultimately contributing to reduced mitochondrial function in people with diabetes. A reduced muscle mitochondrial function could cause reduction in endurance and thus reduce spontaneous physical activity levels further aggrevating insulin resistance.

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