Assessing Multiple Features of Mitochondrial Function

Esther Phielix[1] and Michael Roden[1,2]

Alterations of energy metabolism are key features of insulin resistance, diabetes, and diabetes-related complications. However, the role of mitochondria—the cell's power plants—in abnormal metabolism is less certain. Mitochondria are responsible for oxidative phosphorylation, which couples substrate oxidation to synthesize ATP. Essentially, the reduction of dinucleotides enables electrons to transfer via the electron transport system across the inner mitochondrial membrane. Energy released from electron transfer is used for proton transport into the matrix, thereby establishing an electrochemical proton gradient ($\Delta\Psi$) (1). Mitochondrial function involves various features, each of which can be examined by different methods. The use of different approaches to assess mitochondrial function, coupled with imprecise terminology regarding "mitochondrial dysfunction" can lead to confusing data interpretation.

Several studies have reported lower mitochondrial function expressed per muscle mass in type 2 diabetes as assessed by enzyme activities (2,3) or by high-resolution respirometry (4–7). Although reduced mitochondrial content could serve as explanation for this observation (3,5,8), some studies have also noted lower intrinsic function (4,6,7,9)—that is, reduced respiratory capacity per mitochondrion. Mitochondrial production of reactive oxygen species (ROS) also leads to the formation of lipid peroxides. In turn, these can induce oxidative stress thereby causing cellular and mitochondrial damage (10,11). In this context, it is of interest to simultaneously monitor $\Delta\Psi$, ATP, and ROS production in a single tissue sample.

In this issue of *Diabetes*, Yu et al. (12) describe a method for evaluating the physiology of isolated mitochondria. They used 2-deoxyglucose (2DOG) clamps, which have been used before to assess mitochondrial-bound hexokinase activity (13). The 2DOG clamp is based on the presence of an excess of hexokinase and 2DOG to clamp $\Delta\Psi$ at defined levels and to quantify ATP production from the conversion of 2DOG to 2DOG-phosphate (2DOGP). Yu et al. report that in vitro ^{1}H- and ^{1}H/^{13}C-magnetic resonance spectroscopy (MRS) provides 30–40-fold greater sensitivity to detect ATP production than classical in vitro ^{31}P-MRS. The method's strength resides in its ability to measure both ATP and ROS production at fixed $\Delta\Psi$. Moreover, the conversion of 2DOG to 2DOGP is an irreversible process occurring in the presence of exogenously added ADP, which effectively controls $\Delta\Psi$. The addition of ADP increases mitochondrial energy demand, not supply, a consideration that is important for detailed evaluation of mitochondrial oxidative capacity. Indeed, this method measures oxidative capacity (not resting mitochondrial function [14]) as ATP production transits from state 4 (i.e., no ATP demand) to state 3 respiration (i.e., high ATP demand by stimulating oxidative phosphorylation coupling to energy production). Although the use of mitochondria deprived from their natural environment is a clear limitation, it also circumvents the influence of substrate supply due to changes in local perfusion and substrate uptake. Studies in humans have tried to quantify intact mitochondrial function in vivo by ^{31}P-MRS, by either applying the magnetization transfer experiment to assess ATP synthase flux with or without insulin stimulation (15,16) or using the postexercise creatine-phosphate (PCr) recovery rate to assess maximal oxidative capacity (4,17). Obviously, these methods cannot evaluate intrinsic mitochondrial features (14).

Using this novel approach, Yu et al. (12) measured $\Delta\Psi$, ATP, and ROS production in muscle of streptozotocin-diabetic rats without (STZ-DM) and with insulin treatment (STZ-INS). The STZ-DM had decreased ATP production and greater ROS production per generated ATP molecule at a lower $\Delta\Psi$ threshold. Whether these findings hold true to for other diabetes models or human type 1 diabetes, needs to be tested. Of note, the STZ-DM rat exhibits β-cell failure without immunological pathogenesis. We recently reported that muscle mitochondrial function also decreases during development of autoimmune diabetes in nonobese diabetic mice (18). Rising blood glucose and lipolysis indicate that glucolipotoxity could possibly underlie abnormal mitochondrial function and insulin resistance also in type 1 diabetes (Fig. 1). Interestingly, Yu et al. showed that the abnormal mitochondrial function improved with prolonged insulin treatment in STZ-INS (12). In near-normoglycemic patients with type 1 diabetes, muscle ATP synthesis is normal during fasting but impaired during insulin stimulation (15) or deprivation (19). One might speculate that this is due to their long-standing disease with previous extended periods of hyperglycemia and lipolysis. As shown in Fig. 1, elevated lipolysis could lead to oxidative and endoplasmic reticulum stress and accumulation of lipotoxic intermediates, such as diacylglycerol, ceramides, or acylcarnitines, thereby contributing to insulin resistance in type 1 diabetes.

The effect of insulin on mitochondria and ROS is also of interest for obesity and type 2 diabetes because oxidative stress can activate c-Jun N-terminal kinase (JNK) via nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) and protein kinase C (PKC). Both of these have been linked to insulin resistance (20,21) (Fig. 1). Indeed, insulin can stimulate muscle mitochondrial function in lean healthy subjects but not in type 2 diabetic patients (16,22). However, ROS production was not measured in these studies. Reduction of H_2O_2 production by lipid lowering

From the [1]Institute for Clinical Diabetology, German Diabetes Center, Düsseldorf, Germany; and the [2]Department of Endocrinology and Diabetology, University Clinics Düsseldorf, Heinrich-Heine University, Düsseldorf, Germany.
Corresponding author: Michael Roden, michael.roden@ddz.uni-duesseldorf.de.
DOI: 10.2337/db13-0303

See accompanying original article, p. 1833.

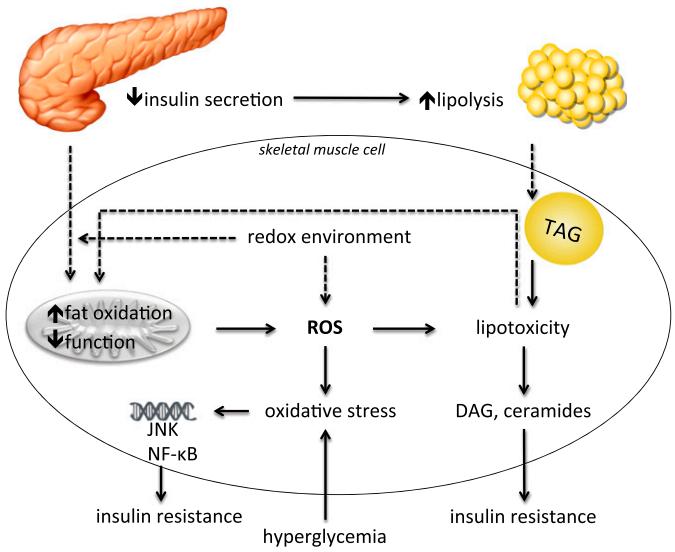

FIG. 1. Hypothesis on the alterations of muscle energy metabolism in type 1 diabetes. Insulin deficiency leads to hyperglycemia and elevated lipolysis with increased free fatty acids, which are oxidized and stored as TAG or converted to lipotoxic DAG or ceramides, which can induce insulin resistance. Together with augmented ROS production, possibly due to leakage from the mitochondria and altered redox status, this might result in the formation of lipid peroxides. Oxidative stress can further activate JNK and NF-κB signaling and thereby contribute to insulin resistance. Insulin resistance would impair glucose uptake and so reduce mitochondrial function. The cellular redox environment may stimulate ROS production and alter insulin action. Dashed lines indicate hypothetical pathways to be investigated. DAG, diacylglycerol; TAG, triacylglycerol.

likely improves insulin sensitivity by affecting mitochondrial oxidative capacity in type 2 diabetes (23), which renders the relationship between oxidative stress and insulin action uncertain.

Finally, Yu et al. (12) describe three processes for the generation and use of $\Delta\Psi$: *1*) generation via proton pumping via the electron transport system complexes, *2*) consumption through proton leaks, and *3*) ATP synthesis. However, the $\Delta\Psi$ driving force also significantly depends on the cellular redox environment (24). Moreover, the ratio of reduced glutathione to the oxidized disulfide form of glutathione has recently been suggested to link mitochondrial hydrogen peroxide emission to the control of redox-sensitive phosphatases that may target insulin signaling (24,25) (Fig. 1). Mitochondria are plastic organelles, rapidly adapting to environmental changes (14,26) with altered cellular redox states. Whether this affects insulin signaling or ROS production in the STZ-INS requires further investigation.

Taken together, the new methodology for measuring mitochondrial ATP production from titration of ADP levels at fixed $\Delta\Psi$ values is a promising tool to explore the interaction between oxidative pathways and insulin action in the fields of insulin resistance, type 1, and type 2 diabetes.

ACKNOWLEDGMENTS

The research of M.R. is funded in part by the German Ministry of Health and North-Rhine Westfalia Ministry for Innovation, Science, Research and Technology to the Leibniz Center for Diabetes Research and by a grant from the German Federal Ministry of Education and Research to the German Diabetes Center for Diabetes Research (DZD e. V.). The work of E.P. is currently supported by a VENI grant for innovative research from the Netherlands Organization for Scientific Research and a grant from the European Foundation for the Study of Diabetes.

No potential conflicts of interest relevant to this article were reported.

REFERENCES

1. Nicholls D. Mitochondrial bioenergetics, aging, and aging-related disease. Sci SAGE KE 2002;2002:pe12
2. Ritov VB, Menshikova EV, He J, Ferrell RE, Goodpaster BH, Kelley DE. Deficiency of subsarcolemmal mitochondria in obesity and type 2 diabetes. Diabetes 2005;54:8–14
3. Kelley DE, He J, Menshikova EV, Ritov VB. Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. Diabetes 2002;51:2944–2950
4. Phielix E, Schrauwen-Hinderling VB, Mensink M, et al. Lower intrinsic ADP-stimulated mitochondrial respiration underlies in vivo mitochondrial dysfunction in muscle of male type 2 diabetic patients. Diabetes 2008;57:2943–2949
5. Boushel R, Gnaiger E, Schjerling P, Skovbro M, Kraunsøe R, Dela F. Patients with type 2 diabetes have normal mitochondrial function in skeletal muscle. Diabetologia 2007;50:790–796
6. Mogensen M, Sahlin K, Fernström M, et al. Mitochondrial respiration is decreased in skeletal muscle of patients with type 2 diabetes. Diabetes 2007;56:1592–1599
7. Phielix E, Meex R, Moonen-Kornips E, Hesselink MK, Schrauwen P. Exercise training increases mitochondrial content and ex vivo mitochondrial function similarly in patients with type 2 diabetes and in control individuals. Diabetologia 2010;53:1714–1721
8. Hey-Mogensen M, Højlund K, Vind BF, et al. Effect of physical training on mitochondrial respiration and reactive oxygen species release in skeletal muscle in patients with obesity and type 2 diabetes. Diabetologia 2010;53:1976–1985
9. Abdul-Ghani MA, Jani R, Chavez A, Molina-Carrion M, Tripathy D, Defronzo RA. Mitochondrial reactive oxygen species generation in obese non-diabetic and type 2 diabetic participants. Diabetologia 2009;52:574–582
10. Lowell BB, Shulman GI. Mitochondrial dysfunction and type 2 diabetes. Science 2005;307:384–387
11. Roden M. Muscle triglycerides and mitochondrial function: possible mechanisms for the development of type 2 diabetes. Int J Obes (Lond) 2005;29(Suppl. 2):S111–S115
12. Yu L, Fink BD, Herlein JA, Sivitz WI. Mitochondrial function in diabetes: novel methodology and new insight. Diabetes 2013;62:1833–1842
13. da-Silva WS, Gómez-Puyou A, de Gómez-Puyou MT, et al. Mitochondrial bound hexokinase activity as a preventive antioxidant defense: steady-state ADP formation as a regulatory mechanism of membrane potential and reactive oxygen species generation in mitochondria. J Biol Chem 2004;279:39846–39855
14. Szendroedi J, Phielix E, Roden M. The role of mitochondria in insulin resistance and type 2 diabetes mellitus. Nat Rev Endocrinol 2012;8:92–103
15. Kacerovsky M, Brehm A, Chmelik M, et al. Impaired insulin stimulation of muscular ATP production in patients with type 1 diabetes. J Intern Med 2011;269:189–199
16. Szendroedi J, Schmid AI, Chmelik M, et al. Muscle mitochondrial ATP synthesis and glucose transport/phosphorylation in type 2 diabetes. PLoS Med 2007;4:e154
17. Meex RC, Schrauwen-Hinderling VB, Moonen-Kornips E, et al. Restoration of muscle mitochondrial function and metabolic flexibility in type 2 diabetes by exercise training is paralleled by increased myocellular fat storage and improved insulin sensitivity. Diabetes 2010;59:572–579
18. Sequaris G, Jelenik T, Phielix E, et al. Effects of hyperglycemia on muscular and hepatic mitochondrial function and insulin sensitivity in mice (Abstract). Diabetes 2012;61(Suppl. 1):A414
19. Karakelides H, Asmann YW, Bigelow ML, et al. Effect of insulin deprivation on muscle mitochondrial ATP production and gene transcript levels in type 1 diabetic subjects. Diabetes 2007;56:2683–2689
20. Bloch-Damti A, Bashan N. Proposed mechanisms for the induction of insulin resistance by oxidative stress. Antioxid Redox Signal 2005;7:1553–1567
21. Chakraborti S, Chakraborti T. Oxidant-mediated activation of mitogen-activated protein kinases and nuclear transcription factors in the cardiovascular system: a brief overview. Cell Signal 1998;10:675–683

22. Stump CS, Short KR, Bigelow ML, Schimke JM, Nair KS. Effect of insulin on human skeletal muscle mitochondrial ATP production, protein synthesis, and mRNA transcripts. Proc Natl Acad Sci USA 2003;100:7996–8001
23. Phielix E, Szendroedi J, Sequaris G, Jelenik T, Zeppetzauer M, Roden M. Insulin-dependent effect on mitochondrial capacity in obese and lean subjects (Abstract). Diabetes 2012;61(Suppl. 1):A62
24. Muoio DM, Neufer PD. Lipid-induced mitochondrial stress and insulin action in muscle. Cell Metab 2012;15:595–605
25. James SJ, Rose S, Melnyk S, et al. Cellular and mitochondrial glutathione redox imbalance in lymphoblastoid cells derived from children with autism. FASEB J 2009;23:2374–2383
26. Jelenik T, Roden M. Mitochondrial plasticity in obesity and diabetes mellitus. Antioxid Redox Signal. 9 October 2012 [Epub ahead of print]