Oxidative Stress in Type 2 Diabetes and The Impact of Exercise: From Mitochondria to Glucose Management in Skeletal Muscle

Julia Matzembacher dos Santos¹, Thiago Gomes Heck²

ABSTRACT
Oxidative stress is the result of reactive oxygen species (ROS) overproduction and/or a decline in antioxidant defense mechanisms. Oxidative stress can be marked by deleterious effects to DNA, proteins, and lipids structure, changing cell homeostasis, and contributing to the development of metabolic diseases as type two diabetes (T2D), characterized mainly by insulin resistance in several tissues, as skeletal muscle. The T2D development and its complications are related to mitochondrial dysfunction and oxidative stress, as well as pro-inflammatory state and metabolic unbalance. Acute exercise represents a necessary type of challenge to whole-body homeostasis. Therefore, regular exercise (sum of acute exercise challenges) promotes antioxidant, anti-inflammatory, and metabolic adaptations induced by each stress induced by the exercise session, evoking a hormesis effect (from mitochondria to many tissues) that is beneficial for T2D prevention and treatment. Despite of a considerable research information in the field, the characterization of the sources and pathways of ROS generation in T2D and during exercise still a matter for investigation. Therefore, the multifaceted effects of oxidative stress in T2D and the link of exercise in T2D are discussed in this narrative review.

Keywords: Exercise; Diabetes; Oxidative Stress; Mitochondria; Insulin Resistance.

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ESTRESSE OXIDATIVO NO DIABETES TIPO 2 E O IMPACTO DO EXERCÍCIO FÍSICO: DA MITOCÔNDRIA AO MANEJO DA GLICOSE NO MÚSCULO ESQUELÉTICO

RESUMO
O estresse oxidativo é o resultado da superprodução de espécies reativas de oxigênio (ROS) e/ou um declínio nos mecanismos de defesa antioxidantes. O estresse oxidativo pode levar a efeitos prejudiciais à estrutura do DNA, proteínas e lipídios, alterando a homeostase celular e contribuindo para o desenvolvimento de doenças metabólicas como o diabetes mellitus tipo 2 (DM2), caracterizado principalmente pela resistência à insulina em diversos tecidos, como o músculo esquelético. O desenvolvimento do DM2 e suas complicações estão relacionados à estresse e disfunção mitocondrial, assim como por um estado pró-inflamatório e desequilíbrio metabólico. Em contrapartida, o exercício agudo representa um tipo necessário de desafio para homeostase de todo o corpo. Neste sentido, o exercício regular (soma dos desafios agudos do exercício) promove ação antioxidante, anti-inflamatória, e adaptações metabólicas induzidas por cada sessão de exercício, evocando um efeito hormes (da mitocôndria para muitos tecidos) que é benéfico para a prevenção e tratamento do DM2. Apesar de um considerável nível informação disponível na área, a caracterização das fontes e vias de geração de ROS em DM2 e durante o exercício ainda é motivo de investigação. Portanto, nesta revisão narrativa, discutimos os efeitos multifacetados do estresse oxidativo no DM2 e o impacto do exercício físico neste contexto.

Palavras-chave: Exercício; Diabetes; Estresse Oxidativo; Mitocôndria; Resistência à Insulina

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INTRODUCTION

According to the World Health Organization (WHO), one in eleven people worldwide has diabetes\(^1,2\). Type 2 diabetes (T2D), a slow-developing metabolic disease, accounts for more than 95% of diabetes cases. In T2D, environmental and genetic factors seem involved in insulin resistance development, which increases the secretion of insulin by β-cell of the pancreas\(^3\). The chronic hyperinsulinemia state, if not treated, result in dysfunction of β-cell and, finally, hyperglycemia, a common characteristic of different types of diabetes. Chronic hyperglycemia impairs micro- and macro-vasculature affecting functions of organs and systems, inducing several complications that could result in morbidity and death\(^4\).

Oxidative stress is the result of reactive oxygen species (ROS) overproduction and/or a decline in antioxidant defense mechanisms. ROS production can be beneficial in some cases, as used by the immune system to activate antioxidant defense and cell communications. However, excessive generation of ROS can result in deleterious effects causing damage to DNA, proteins, and lipids structure, ultimately leading to change in cell homeostasis and cell death. Therefore, the current understanding of the complex role of ROS is required for developing strategies to manage or inhibit T2D and diabetic complications.

For several decades, researchers around the world almost unanimous demonstrated that muscle contraction increases glucose uptake in skeletal muscle in the absence of insulin-mediated signaling, a single bout of exercise decrease blood glucose, and that regular exercise can inhibit or manage T2D\(^5\)-\(^8\). Due to the significant number of data describing the beneficial effects of exercise in diabetess, world-renowned organizations such as WHO, American Diabetic Association (ADA), and American College of Sports Medicine (ACSM) included regular exercise in their guidelines for preventing T2D or controlling hyperglycemia\(^1,9\). Interestingly, the aerobic is, at the same time, an efficient type of exercise for blood glucose management and one type of effort that can acutely promotes a great increase in ROS production. However, chronically, via ROS, exercise can modify cell phenotype, promoting cellular protection against pathophysiological concentrations of ROS and hyperglycemia\(^10\)-\(^13\). Despite cutting-edge research in the field, the characterization of the sources and pathways of ROS generation in T2D and during exercise still a matter for investigation. Therefore, the multifaceted effects of oxidative stress in T2D and the link of exercise in T2D are discussed in this review.
as atypical protein kinase C (aPKC), and protein kinase B (AKT). The substrate of 160 kD of AKT (AS160) associates GLUT4 vesicle into the cytosol, and when phosphorylated, AS160 releases vesicles to translocate towards the cell membrane. Simultaneously, aPKC activates motor proteins that translocate GLUT4 vesicles via microtubules to the cell membrane.

Disruption of the complex pathway by which insulin induces GLUT4 translocation (insulin resistance) causes glucose intolerance. Several factors might underlie insulin resistance, such as dyslipidemia associated with obesity, air pollution exposure, the consumption of industrialized food, and genetic and epigenetic factors. It seems like the accumulation of more than one of these factors is involved in insulin resistance and T2D development.

On a molecular perspective, studies have suggested that initially, insulin resistance is linked with several defects within the insulin cascade, namely reduced IRS and PI3K, whereas on the late state of the T2D decrease in GLUT4 expression could add to this condition. Various pathways have been associated with the impairment of insulin-induced glucose uptake, and oxidative stress is an important piece on a puzzle to understand this process that leads to insulin resistance and T2D.

In T2D, ROS can be generated by several sources and can mediate pathways that can either promote or attenuate insulin resistance in the skeletal muscles and adipocytes. A considerable number of different mechanisms have been described to induce ROS in diabetes, including autooxidation of glucose, increased superoxide production in the cytosol and mitochondria, and decreased scavenging defense.

Nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase or NOX) is a membrane-bound enzyme complex that faces the extracellular space. NOX translocates to the membrane upon activation, forming a complex along with gp91phox and p22phox in the membranous core and p47phox, p40phox, p67phox, and ras-related C3 botulinum toxin substrate 1 (RAC1) as the cytosolic core. Hyperglycemia was shown in-vitro and in animal studies to activate NOX in endothelial cells and further induce cytosolic production of ROS. Further studies have suggested that increased Nox-derived ROS implicates insulin pathway inducing insulin resistance. The exact mechanism by which NOX induce insulin resistance is under investigation; however, Nox-derived ROS appears to affect mitochondria homeostasis as a result of hyperglycemia.

Mitochondria is the power source of the cells by using the products of glucose, lipids and protein reaction, together with oxygen to produce ATP. Dysfunction of mitochondria can induce impairment on these organic component’s metabolism as well as overproduction of superoxide, a primary ROS. In mitochondria, superoxide is produced in the last phase of ATP generation, the electron transport chain (ETC), more precisely on complex I and III. Overproduction of superoxide damage mitochondrial DNA (mtDNA) impairs mtDNA transcription affecting translational proteins of ETC, producing ROS in a vicious cycle. The impairment in lipid metabolism induced by mitochondrial dysfunction could induce accumulation of lipid intermediates such as diacylglycerol and ceramide that directly affect the activation of PI3K via IRS, leading to insulin resistance.
For the management of ROS, cells are equipped with a powerful antioxidant defense system to neutralize free radicals. This antioxidant defense system is comprised of several cytosolic and mitochondrial enzymes, including superoxide scavenging enzymes (SOD), glutathione peroxidase and reductase (GPX) and catalase. Mitochondrial ROS do not readily cross membranes, but manganese superoxide dismutase (MnSOD), a superoxide scavenging enzyme, converts in-tramitochondrial superoxide to hydrogen peroxide, which can diffuse out of mitochondria and converted to H₂O and O₂. Also, intracellular antioxidants, such as glutathione, vitamin E and ascorbic help to neutralize the detrimental effect of free radicals. Data from a recent study performed by Mohsen and colleagues demonstrated that Mn-SOD and CAT gene expression was significantly lower in the obese children (prone to develop insulin resistance) when compared to non-obese children. Moreover, it was identified in T2D patients polymorphic variations in MnSOD, GPX1, CAT, which together with other results suggest that not only increased ROS underlies insulin resistance and T2D by also decreased antioxidant capacity.

EXERCISE, T2D AND OXIDATIVE STRESS

The research field “exercise and oxidative stress” emerged from human and animal studies from the end of the 70’s years. It was discovered that exercise activity (endurance exercise in humans at 50% of VO₂max) increased ROS production and may produce an increase in the lipid oxidative damage markers (in rats) stared a worldwide investigative process about the relationship of exercise type, duration, intensity, and oxidative stress. Therefore, subsequent findings showed that muscle and other organs (e.g., Liver) increased ROS production during exercise but also increased antioxidant enzyme activities. Consequently, it was proposed that regular exercise training (also named as the chronic effect of exercise) induces antioxidant adaptations, which nowadays are related to the health benefits of exercise in terms of prevention and treatment of several diseases, including T2D. In this way, exercise benefits may be described by the “hormesis theory”, which is an adaptive response of cells to stressors (in this case, exercise per se induces a ROS production) that results in a biphasic dose-response relationship such that low dose stimulation (adequate exercise training for each subject) results in a beneficial adaptation (antioxidant adaptations). In contrast, a high dose results in a toxic effect (e.g., overtraining and oxidative stress). Therefore, the redox signaling induced by ROS is closely related to exercise-induced hormesis. These concepts were closely related to the well-known general adaptation syndrome proposed in 1936 by Hans Selye and the “J” curve profile of immune response to exercise proposed in 1994 by David Nieman. Although we shall never truly understand completely the relationship between exercise, ROS and health, there are many degrees of information, from basic sciences to clinical application results, that allow us to propose exercise as an antioxidant strategy against T2D.

Experimental research performed with different approaches together with epidemiological and retrospective studies have well dementated that regular exercise may be preventive and inhibits the development of T2D by increa-
sing glucose uptake in skeletal muscle\textsuperscript{5,7,8}. This process is critical on T2D because skeletal muscle is the leading site for glucose transport under insulin stimulation\textsuperscript{4}. A bout of physical exercise significantly benefits insulin sensitivity in T2D adults, and these benefits may persist beyond 72 hours after an exercise session\textsuperscript{8}. Besides, studies have shown that even when insulin secretion is deficient, in type 1 diabetic (T1D) subjects, aerobic exercise can decrease blood glucose \textsuperscript{5,6}. These results go along with in-vitro and ex-vivo studies demonstrating that muscle contraction by itself can induce GLUT4 translocation to the cell membrane and increasing glucose uptake in skeletal muscle.

The mechanism by which contraction-induced glucose uptake is partially identified. During muscle contraction, ATP is hydrolyzed to ADP + Pi, and levels of Ca\textsuperscript{2+} raise to facilitate the cross-bridge between actin and myosin. Activation of 5\textsuperscript{′} AMP-activated protein kinase (AMPK), induced by decreases in ATP, and calcium-mediated second messengers Ca\textsuperscript{2+}/calmodulin-dependent protein kinase (CaMK), appear to be involved in the mechanism by which contraction induces glucose uptake. In fact, AMPK is also the target on the first line of antidiabetic medication, metformin\textsuperscript{9}. It has been shown that both CaMK and AMPK might be involved in the activation of glucokinase and AS160, increasing GLUT4 translocation and glucose uptake \textsuperscript{7}. Therefore, it was suggested by Santos et al.\textsuperscript{4} that AMPK and AS160 might represent the common link between the pathway by which insulin and contraction induce glucose uptake.

Regular exercise can induce changes in skeletal muscle phenotype that increases its capacity to uptake glucose. Studies have demonstrated that chronic aerobic exercise increases skeletal muscle GLUT4 expression in laboratory animal and T2D patients\textsuperscript{26}. Indeed, muscle atrophy induced by immobilization protocols, which mimic physical inactivity in experimental settings, leads to a significant reduction in skeletal muscle GLUT4 expression\textsuperscript{27}. Moreover, Horii and colleagues (2020) demonstrated that resistance exercise was effective in activating the AKT/AS160/GLUT4 pathways and increase insulin sensitivity\textsuperscript{28}. Epigenetic modification on proteins involved in insulin pathways, as well as GLUT4, might be involved in this process \textsuperscript{3}. As a result, regular exercise, either aerobic or resistance, can improve GLUT4 content in skeletal muscle, which increases the capacity of skeletal muscle to uptake glucose in a rest state and under insulin stimulation.

Also, exercise induces adaptations in the antioxidant systems (enzymatic and non-enzymatic), activation of uncoupling proteins, and promotes anti-inflammatory effects. These effects may be attributed to ROS signaling induced by exercise\textsuperscript{25}, which at a molecular level, activates redox circuits related to stress adaptation involving AMPK, Mitogen Activated Protein kinases (MAPK) and NF-\textsuperscript{κ}B pathways\textsuperscript{29}. In this way, dependent on exercise duration and intensity, exercise is able to increase the activity antioxidant enzyme activities (SOD, catalase and GPX) in different organs by activation of NF-\textsuperscript{κ}B. An acute bout of exercise activates NF\textsuperscript{κ}B binding in rat skeletal muscle (and other tissues and cells), and both IkB and IxB kinase (IKK) are phosphorylated after exercise, whereas P50/P65 is transported into the nucleus\textsuperscript{25,30}. Besides the antioxidant enzymes, proteins and enzymes that require consensus binding of p65 in their promoters, or AMPK and MAP kinases activation, are iNOS and IL-6 and heat shock proteins (HSPs), which
are involved in a wide variety of biological functions such as antioxidant defense, inflammation, immunity and proteolysis\textsuperscript{25}. Also, the benefits of exercise mediated by muscle-derived secretory proteins as myokines (e.g., IL-6 and Irisin)\textsuperscript{31} or “chaperokines” (e.g., extracellular 70kDa HSPs, a.k.a. eHSP70)\textsuperscript{30,32}, are evoked by transient elevations in oxidative stress induced by exercise, and thus elicit auto/paracrine but also endocrine effects on organs such as liver, adipose tissue, pancreas and immune cells. However, activation of NF-κB should be carefully considered as a therapeutic approach in T2D, since several laboratories suggest that constant activation of NF-κB induce insulin resistance and T2D development.

The effect of regular exercise on ROS induced by NOX complex activity was also investigated. A protocol of resistance or endurance exercise of 8-weeks was effective to affect MnSOD expression but no effect in Nox in skeletal muscle\textsuperscript{33}. On the other hand, La Favor et al.\textsuperscript{34} found that eight weeks of interval exercise was sufficient to decrease protein expression Nox subunits complex in obese subjects. Therefore, the effect of exercise on the Nox complex and its involvement in insulin resistance waits for further clarifications.

Since mitochondria are essential for the generation of energy and tightly connected to oxidative stress, it is possible to believe that the benefits of exercise are also associated with some type of hormesis in this organelle. Thus, according to this scenario, adaptive improvements induced by regular exercise is required a “mitohormesis” action. For example, endurance training leads to an increased mitochondrial density and oxidative capacity, and peroxisome proliferator-activated receptor-γ coactivator (PGC-1α) is a key regulator of skeletal muscle mitochondrial biogenesis: This is activated by several intracellular sensors and signaling pathways of contractile activity, including sensors of energy lack such as AMPK. Consequently, proteins are secreted from skeletal muscle after PGC-1α overexpression, linking the potential positive health effects of exercise to myokines secretion induced by altered redox state in the mitochondria\textsuperscript{35}. Although people with T2D have higher daily variability of both oxidative stress and inflammatory biomarkers, if T2D subjects incorporate an intensive lifestyle intervention, with high volumes of exercise, it is possible to improve beta-cell function, associated with a decrease in low-grade inflammation and/or body weight\textsuperscript{36}. Thus, it is possible to promote the discontinuation of glucose-lowering medication treatment in a dose-dependent manner of exercise\textsuperscript{37}.

PGC-1α activates a pathway mediated by nuclear respiratory factor 1 (NRF1) and mitochondria transcription factor A (TFAM) that controls mtDNA transcription. TFAM is chaperoned from the cytosol to the mitochondria by HSP70 and re-folded by HSP60 in the mitochondria matrix\textsuperscript{13}. TFAM binds to the promoter area of mtDNA, D-loop, controlling either mtDNA transcription and replication\textsuperscript{38}. Cytochrome b (cytb), transcribed in mtDNA under TFAM activation, plays a key role in complex III of ETC, and decreased transcription of cytb accounts for the generation of ROS by complex III\textsuperscript{10}. A group of recent publications suggested that endurance training improves intrinsic mitochondrial respiration and mtDNA transcription on cytb in T2D without alterations in mitochondrial DNA copy number\textsuperscript{39,40}. Together, these data suggest that exercise has a protective
effect of mtDNA, and that attenuates mitochondria dysfunction, mitochondrial ROS, and further insulin resistance.

Although this review showed molecular aspects of T2D development and the impact of exercise in this process, we recommended future studies that aim to discuss and test the effects of exercise considering the a) the wide range of possibilities in terms of exercise type; b) different effects in graduated intensities of exercise; c) the bottom and upper limit of the dose-effect impact of the duration of exercise and d) the impact of regular frequency of exercise on this markers.

CONCLUSION

The T2D development and its complications are related to mitochondrial dysfunction and oxidative stress, as well as pro-inflammatory state and metabolic unbalance. Acute exercise represents a necessary type of challenge to whole-body homeostasis. Therefore, regular exercise (sum of acute exercise challenges) promotes antioxidant, anti-inflammatory, and metabolic adaptations induced by each stress induced by the exercise session, evoking a hormesis effect (from mitochondria to many tissues) that is beneficial for T2D prevention and treatment (Figure 1).

Figure 1 – The Type 2 Diabetes development and its complications are related to mitochondrial dysfunction and the impact of exercise.

Source: Figure created by authors in biorender.com
REFERENCES

13 Santos JM, Kowluru RA. Role of mitochondria biogenesis in the metabolic memory associated with the continued progression of diabetic retinopathy and its regulation by lipoic acid. Investigative Ophthalmology and Visual Science. 2011. doi: 10.1167/ iovs.11-8203
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