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Original article

High-fat diet induces skeletal muscle oxidative stress in a fiber type-dependent manner in rats

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ABSTRACT

This study investigated the effects of high-fat (HF) diet on parameters of oxidative stress among muscles with distinct fiber type composition and oxidative capacities. To accomplish that, male Wistar rats were fed either a low-fat standard chow (SC) or a HF diet for 8 weeks. Soleus, extensor digitorum longus (EDL), and epitrochlearis muscles were collected and mitochondrial H_2O_2 (mtH_2O_2) emission, palmitate oxidation, and gene expression and antioxidant system were measured. Chronic HF feeding enhanced fat oxidation in oxidative and glycolytic muscles. It also caused a significant reduction in mtH_2O_2 emission in the EDL muscle, although a tendency towards a reduction was also found in the soleus and epitrochlearis muscles. In the epitrochlearis, HF diet increased mRNA expression of the NADPH oxidase complex; however, this muscle also showed an increase in the expression of antioxidant proteins, suggesting a higher capacity to generate and buffer ROS. The soleus muscle, despite being highly oxidative, elicited H_2O_2 emission rates equivalent to only 20% and 35% of the values obtained for EDL and epitrochlearis muscles, respectively. Furthermore, the Epi muscle with the lowest oxidative capacity was the second highest in H_2O_2 emission. In conclusion, it appears that intrinsic differences related to the distribution of type I and type II fibers, rather than oxidative capacity, drove the activity of the anti- and pro-oxidant systems and determine ROS production in different skeletal muscles. This also suggests that the impact of potentially deleterious effects of ROS production on skeletal muscle metabolism/function under lipotoxic conditions is fiber type-specific.

1. Introduction

Under obesogenic conditions (e.g. high fat diet), the excessive abundance of fat surpasses the ability of all tissues to oxidize this substrate. This leads to intramyocellular (IMCL) accumulation and disruption of normal muscle function, a condition that develops as a consequence of the toxic effects of intracellular overload of lipid intermediates (lipotoxicity) [1]. These deleterious effects of IMCL accumulation have been partially attributed to increased ROS production that can compromise the antioxidant defense system of muscle cells [2]. Reactive oxygen species (ROS) play an important role in the maintenance of normal cellular function, but this seems to be dependent on the amount and site of production. For instance, low levels of ROS regulate the activity of important signaling pathways that modulate the antioxidant system and mitochondrial function [3]. Conversely, high levels of ROS accompanied by a decline in the antioxidant defense system (a process known as oxidative stress) can damage organelles, proteins, lipids, and DNA [4,5]. In addition, ROS overproduction and/or impaired antioxidant capacity alters the cellular redox balance and induces systemic oxidative stress, which can aggravate certain pathological conditions [4].

Increased ROS production in obesity has also been associated with the increased activation of immune cells that increase the local presence of pro-inflammatory mediators [6]. The chronic, low-grade inflammatory response triggered by obesity includes systemic increases in circulating cytokines and acute phase proteins, recruitment and activation of leukocytes, and generation of reparative tissue responses [7]. Under such conditions, a variety of lipid products are generated that are able to activate the NADPH oxidase system. The latter is a multi-subunit enzymatic complex present in membranes of cells and organelles that

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plays a key role in maintaining normal cellular functions [8]. Following stimuli and cell activation, translocation of the regulatory subunits $p47^{phox}$ and $p67^{phox}$ occurs and superoxide production proceeds by one-electron reduction of oxygen via the $gp91^{phox}$ subunit using reduced NADPH as the electron donor [8]. If NADPH becomes dysregulated, the system can produce excessive amounts of ROS, leading to potential damage of the tissue.

Myocytes have the capacity to respond to the obesity-induced inflammatory stimulus and ROS production via pattern recognition receptors such as Toll-like receptor-4 (TLR4) [9] and/or through an efficient antioxidant system that includes several antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) [10], causing direct metabolic effects. Importantly, skeletal muscles are composed of heterogeneous populations of fiber types that display adaptive responses to different physiological stimuli that are fiber-type specific. Mitochondria-rich slow twitch type I fibers exhibit oxidative metabolism, are fatigue resistant and are classically identified by their expression of type I myosin heavy chain. Conversely, fast twitch type IIa, IIx/d, and IIb myofibers exert quick contractions, tend to fatigue rapidly, contain fewer mitochondria, are glycolytic, and are characterized by their expression of fast myosin heavy chain isoforms (Myh2, Myh1, and Myh4, respectively) [11–13]. Besides being crucial for ATP production, mitochondria are also major sites for ROS production [14]. Therefore, it is possible that the pattern of fiber type distribution in skeletal muscles could exert an important role in determining tissue susceptibility to ROS-induced tissue damage. This could be, at least in part, because mitochondrial content, ROS-generating ability, and antioxidant capacity varies significantly between oxidative and glycolytic skeletal muscles [15]. In fact, previous studies have reported that muscles with high content of type IIb fibers posses unique properties that seem to potentiate mitochondrial ROS production/emission [15]. Furthermore, high fat (HF) diet has been shown to increase mitochondrial biogenesis and enhance oxidative capacity in skeletal muscles [16]; such adaptive responses to lipid overload could significantly affect the ability of the muscle tissue to produce and deal with ROS. In fact, either 3 days or 3 weeks of HF diet have been reported to increase the H_2O_2-emitting potential of mitochondria, shift the cellular redox environment to a more oxidized state, and decrease the redox-buffering capacity of skeletal muscles [2]. However, limited information is available regarding whether or not increased ROS production in skeletal muscles is sustained after longer periods of lipid overload. It could be that skeletal muscles adjust their redox-buffering capacity under chronic HF feeding to cope with the overabundance of lipids. Additionally, it still remains to be determined if the oxidative stress response differs between skeletal muscles with different fiber type composition under conditions of prolonged lipid overload such as diet-induced obesity. Therefore, the aim of this study was to compare the effects of HF diet on parameters of oxidative stress in skeletal muscles with distinct patterns of fiber type composition. To accomplish that, we fed rats a HF diet for 8 weeks and performed a detailed analysis of the redox response of skeletal muscles that are either predominately oxidative or glycolytic. Here, we provide evidence that 8 weeks of HF feeding increased the oxidative capacity of all muscles, but reduced H_2O_2 emission and affected the antioxidant system in a fiber type dependent manner. These findings indicate that fiber type distribution plays a major role in determining the redox state of skeletal muscles and likely dictates the propensity of different muscles to be affected by ROS under conditions of diet-induced obesity.

2. Material and methods

2.1. Animals and diets

Male rats (Wistar strain) weighing ~ 250 g were maintained on a 12/12 h light/dark cycle at 22 °C and fed ad libitum either a low-fat standard chow diet (SC; 27.0%, 13.0%, and 60.0% of calories provided by protein, fat, and carbohydrates, respectively) with energy density of 3.43 kcal/g, or HF diet (20.0%, 60.0%. and 20.0% of calories provided by protein [casein], fat [lard/soybean oil], and carbohydrates [maltodextrin/sucrose], respectively) with energy density of 5.16 kcal/g. SC diet was purchased from Lab Diet (catalogue #5012, St. Louis, MO, USA) and HF diet was purchased from Research Diets (catalogue # D12492, New Brunswick, NJ, USA). Following 8 weeks of diet intervention, rats were anesthetized with ketamine/xylazine (150 mg/kg:10 mg/kg) prior to removal of the soleus (Sol), extensor digitorum longus (EDL), and epitrochlearis (Epi) muscles. These muscles were chosen because of their wide range of reported fiber-type distributions with distinct mitochondrial contents and oxidative capacities. The percentages of type I, type IIa, and type IIb in Sol, EDL, and Epi muscles are 84/16/0, 3/57/40 [17], and 15/20/65 [18], respectively. All experimental procedures were approved by the York University Animal Care Ethics Committee (York University Animal Care Committee (YUACC) permit #2012-03) and performed strictly in accordance with YUACC guidelines.

2.2. Measurement of energy intake (EI), body weight (BW), respiratory exchange ratio (RER), and fat mass

The animals were housed in individual cages in order to manually measure EI and BW daily during the study. Food intake was also monitored by using the comprehensive laboratory animal monitoring system (CLAMS) from Columbus Instruments (Columbus, OH) and the values were identical to those obtained with manual determinations. RER was measured in the CLAMS at the end of week 8 of the diet intervention. The animals were placed in the CLAMS and monitoring of in vivo parameters started at 11:00 a.m. The 1st h of data collected in the CLAMS was discarded, because it is the time required for the rats to acclimatize to the cage environment [19]. The rats were monitored for a 24-h period encompassing the light (07:00–19:00 h) and dark (19:00–07:00 h) cycles. Fat mass was measured at the end of the study with the rats anesthetized (ketamine/xylazine 150 mg/kg:10 mg/kg). The retroperitoneal (Retro), epididymal (Epid), and subcutaneous (SC) inguinal (Ing) fat depots were thoroughly removed and weighed as previously described [20].

2.3. Preparation of permeabilized muscle fiber bundles (PMFB)

This technique is partially adapted from previous methods [21,22] and has been described in detail previously [23]. Briefly, small portions (~ 25 mg) of muscle were dissected and placed in ice-cold BIOPS [24]. The muscles were trimmed of connective tissue, blood vessels, and fat and divided into several small muscle bundles (~ 2.7 mm, 1.0–2.5 mg wet weight). Each bundle was gently separated along its longitudinal axis with a pair of anti-magnetic needle-tipped forceps under magnification (using a Zeiss Stemi microscope). Bundles were then treated with 40 μg/ml of saponin [25] in BIOPS and incubated in a rotor for 30 min at 4 °C. Following permeabilization, PMFB were washed at 4 °C (< 30 min) in Buffer Z until experiments were initiated.

2.4. Mitochondrial H_2O_2 (mtH_2O_2) emission potential

Muscle mtH_2O_2 emission potential was measured in PMFB bundles prepared as described previously [15]. mtH_2O_2 emission potential was measured at 37 °C in response to 10 mM succinate by continuously monitoring oxidation of 10 μM Amplex UltraRed (Life Technologies) in Buffer Z [2] supplemented with 0.5 mg/ml BSA. The reaction was catalyzed by 1 U/ml horseradish peroxidase in a quartz cuvette using continuous stirring with a PTI Quantamaster 40 spectrofluorometer. Total mitochondrial superoxide production was converted to H_2O_2 emission through endogenous matrix superoxide dismutase and exogenously added 25 U/ml Cu/Zn superoxide dismutase [26]. The rate of mtH_2O_2 emission potential was expressed as pmol/min/mg dry muscle