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Hind limb ischemia–reperfusion injury in diet-induced obese mice

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ABSTRACT

Background: Obesity is a major risk factor for the development of diabetes. Limb ischemia–reperfusion injury (IR) is a common clinical problem in diabetics who have compromised lower extremity perfusion. This study compared the histologic, metabolic, and functional outcomes after hind limb IR in diet-induced obese (DIO) and non-diabetic (ND) mice during the acute and the regenerative phases of IR.

Methods: DIO and ND mice were subjected to 1.5 h unilateral hind limb ischemia followed by 1- or 28-d IR. Muscle morphology, metabolic, and genomic stress were evaluated at days 1 and 28 IR; Acute inflammation and thrombosis were only measured at day-1 IR. At day 28, IR, skeletal muscle contractility, and maturation were also assessed.

Results: At day-1 IR, similar levels of acute muscle fiber necrosis were seen in both groups. DIO mice demonstrated substantially greater inflammatory, prothrombotic, and genomic stress responses, which were also associated with a greater reduction in energy substrates and Akt phosphorylation. At 28d, there was no difference in the peak forces generated in the hind limbs for the two groups. DIO mice had reduced fatigue resistance compared with ND and larger areas of fat accumulation although there was no significant difference in muscle fiber maturation.

Conclusions: DIO mice had an exacerbated acute response to IR with enhanced metabolic deficit, fat accumulation, and defective functional recovery during the regenerative phase of IR. These changes in fatigue resistance reflect compromised functional recovery after IR

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Phosphoinositide-3-kinase–protein kinase B/Akt pathway

injury and have relevance for the functional recovery of patients with metabolic syndrome and insulin resistance.

1. Introduction

Obesity and insulin resistance continue to increase worldwide and are considered to be the leading cause for developing type II diabetes, which is a risk factor for the development of peripheral arterial disease [1]. Acute skeletal muscle ischemia–reperfusion injury (IR) frequently occurs in many clinical scenarios including lower extremity arterial disease, surgical interventions, circulatory shock, and trauma. Advances in medical management through thrombolytic therapy or direct operative interventions improved limb salvage and functional recovery rates in most patients except for diabetics [1–4]. Successful muscle regeneration after IR comprised a degeneration phase that includes necrosis and inflammatory response aimed at removing damaged myofibers followed by a regenerative phase manifested by mobilization of the normally quiescent satellite cells. The satellite cells proliferate and migrate to the site of fiber injury, then fuse and differentiate to form new myofibers [5]. However, compromised muscle regeneration in the context of diseased condition, such as aging or diabetes, may lead to impaired healing, permanent loss of muscle mass, and functional deficiency. Pathologically, normal muscle regeneration can be hampered by persistent and robust inflammation followed by fibrosis and significant lipid accumulation [6–8]. Obesity or metabolic syndrome plays a significant role in the development of type II diabetes and cardiovascular disease and the associated comorbidity [9–13].

Recently, experimental model of diet-induced obesity (DIO) and insulin resistance has been developed to study type II diabetes as an alternative to the genetically-manipulated animal models. This is accomplished by feeding rodents diet containing 40%–60% fat for at least 8 wk. However, this model presents insulin resistance without pancreatic beta cell failure, which can be compensated by a marked beta cell proliferation [14,15]. This study was designed to evaluate the effect of the metabolic syndrome and insulin resistance on skeletal muscle acute and regenerative response after IR in DIO mice induced by prolonged high fat diet compared with ND mice.

2. Materials and methods

2.1. Animal protocol

Animal care and experimental procedures were in compliance with the "Principal of Laboratory Animal Care" (Guide for the Care and Use of Laboratory Animals, National Institutes of Health publication 86-23, 1985) and approved by the Institutional Review Committee. Age matched C57BL6 male mice acquired from Jackson Laboratory (Bar Harbor, ME) after being fed either 10% kcal fat diet (ND, $n = 13$) or 60% kcal high-fat diet (DIO, $n = 12$) for 26 wk. The DIO mice were characterized to be obese and have glucose intolerance and moderate hyperinsulinemia, hyperglycemia, and hyperlipidemia. A hind limb murine IR was created as previously described [16]. Briefly, after anesthesia, a calibrated rubber band was applied to the hind limb to induce 1.5 h of ischemia and then removed to initiate reperfusion. At the end of 1- or 28-d reperfusion, hind limbs perfusion was documented with laser Doppler imaging, and the ratio of the injured-to-noninjured contralateral hind limb perfusion was calculated. DIO ($n = 6$) and ND ($n = 6$) Sham mice from each group subjected to anesthesia alone were used as baseline controls. Mice were sacrificed, and hind limb tissues were collected and were fixed with paraformaldehyde and embedded in acrylic or paraffin for histologic analysis or immediately frozen in liquid nitrogen and stored at −80°C until analyzed. A separate group of DIO ($n = 12$) and ND ($n = 12$) mice was subjected to 1.5-h ischemia and 28-d reperfusion or Sham conditions followed by direct muscle electrical stimulation to study hind limb function under Sham condition or after 28-d reperfusion as described in the following.

2.2. Histologic and morphometric analysis

To determine changes in muscle fiber morphology, 2-μm thick acrylic embedded cross sections taken at 1 day after IR from the middle portion of the tibialis anterior (TA) muscle. The muscle sections were stained with Mason's trichrome. Randomized ×200-magnification digital images were recorded and examined by blinded observer as previously described [17]. In the acute IR groups, the injured and noninjured muscle fibers were sorted and expressed as a percentage of the total number of fibers counted per field as previously described [18]. In the 28-d groups, evidence of skeletal muscle fiber maturation in the TA muscle was evaluated by measuring the averages of muscle fiber cross-sectional area (CSA) and adipocytes area in addition to count the number of fibers presenting central nucleus as an indication of muscle fibers regeneration as has been described before [19]. H&E or Oil Red O staining was performed on 2-μm acrylic embedded cross section or 8-μm frozen section from each TA muscle. A minimum of 10 randomized high power fields from each muscle cross section were evaluated by a blinded reviewer.

2.3. Local markers of inflammation

Selected markers of inflammation were measured in solubilized proteins obtained from hind limb muscle after 1-d IR using quantitative sandwich enzyme immunoassays for the CXC chemokine, keratinocyte chemoattractant protein (KC; R&D Systems, Minneapolis, MN), and myeloperoxidase (mouse MPO; Cell Sciences, Canton, MA).

2.4. Assessment of neutrophil granulocytes infiltration

A total of 7-μm cross sections taken from the mid part of paraffin-embedded TA muscles were deparaffinized and rehydrated according to standard protocols. Sections were subjected to immunohistochemistry using monoclonal rat