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Differential acute and chronic effects of burn trauma on murine skeletal muscle bioenergetics

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ABSTRACT

Altered skeletal muscle mitochondrial function contributes to the pathophysiological stress response to burns. However, the acute and chronic impact of burn trauma on skeletal muscle bioenergetics remains poorly understood. Here, we determined the temporal relationship between burn trauma and mitochondrial function in murine skeletal muscle local to and distal from burn wounds.

Male BALB/c mice (8–10 weeks old) were burned by submersion of the dorsum in water (~95 °C) to create a full thickness burn on ~30% of the body. Skeletal muscle was harvested spinotrapezius underneath burn wounds (local) and the quadriceps (distal) of sham and burn treated mice at 3 h, 24 h, 4 d and 10 d post-injury. Mitochondrial respiration was determined in permeabilized myofiber bundles by high-resolution respirometry. Caspase 9 and caspase 3 protein concentration were determined by western blot.

In muscle local to burn wounds, respiration coupled to ATP production was significantly diminished at 3 h and 24 h post-injury ($P < 0.001$), as was mitochondrial coupling control ($P < 0.001$). There was a 5- ($P < 0.05$) and 8-fold ($P < 0.001$) increase in respiration in response to cytochrome at 3 h and 24 h post burn, respectively, indicating damage to the outer mitochondrial membranes. Moreover, we also observed greater active caspase 9 and caspase 3 in muscle local to burn wounds, indicating the induction of apoptosis. Distal muscle mitochondrial function was unaltered by burn trauma until 10 d post burn, where both respiratory capacity ($P < 0.05$) and coupling control ($P < 0.05$) were significantly lower than sham.

These data highlight a differential response in muscle mitochondrial function to burn trauma, where the timing, degree and mode of dysfunction are dependent on whether the muscle is local or distal to the burn wound.

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1. Introduction

Altered mitochondrial function is a hallmark of the pathophysiological stress response to burns [1–11]. We recently demonstrated that skeletal muscle mitochondrial respiratory capacity and function were diminished at 2 and 3 weeks post-injury in humans with burns covering approximately 70% of the total body surface area (TBSA) [2]. In these patients, greater skeletal muscle mitochondrial thermogenesis was associated with profound hypermetabolism (increased metabolic rate) [2]. Further, we have recently reported similar findings in severely burned children, where altered mitochondrial function in skeletal muscle persists for up to 2 years post-injury [11]. Collectively, these data support a putative role for skeletal muscle mitochondrial thermogenesis in the hypermetabolic stress response to burns. In agreement with our findings in humans, Righi and colleagues reported greater TCA cycle flux but lower ATP production rates in murine skeletal muscle directly under burn wounds, suggesting a reduction in the coupling of TCA cycle flux to ATP production [12], indicative of mitochondrial uncoupling and thus thermogenesis.

An important difference in our human study [2] and the rodent study of Righi and colleagues [12] is the timing and site of muscle sampling. We biopsied tissue under the skin and from area which was not directly under full-thickness burn wounds from humans approximately 2 and 3 weeks post-injury [2]. In contrast, Righi et al. [12] studied muscle directly under a comparatively small burn (5% TBSA) a matter of hours post injury. While we concluded that our data reflected a chronic adaptation where muscle mitochondria become uncoupled post burn in order to support thermoregulation [2], the data of Righi and co-workers [12] may reflect a more acute response to burn trauma, where reduced ATP production observed by these researchers may reflect apoptosis and/or mitophagy, at least in part. In support of this, Yasuhara and colleagues demonstrated altered mitochondrial membrane potential and release of mitochondrial cytochrome C into the cytosol as early as 15 min post burn in murine skeletal muscle harvested from under burn wounds [13]. Cytochrome C activates cytosolic caspase enzymes responsible for apoptosis [14]. Indeed, from 6 h to 7 d post injury, Yasuhara et at. [13] showed that caspase 3 activity was augmented in skeletal muscle directly beneath burn wounds, suggesting acute alterations in skeletal muscle mitochondrial function following direct burn trauma reflect an apoptotic response.

It appears that there may be differential acute and chronic effects of burn on skeletal muscle bioenergetics, which may be broadly characterized as an acute response to the blunt trauma of full-thickness burns, followed by a more chronic adaption in skeletal muscle mitochondria to the systemic milieu accompanying a large burn. Here, we set out to delineate the acute and chronic impact of severe burn trauma on skeletal muscle mitochondrial function local to and distal from full-thickness burn wounds. Our working hypothesis is that the timing and nature of altered bioenergetics of muscle underlying burn wounds will be distinct from that of skeletal muscle distal to burn wounds.

2. Materials and methods

2.1. Animal procedures

We used a murine model of full-thickness burn trauma in the current study [15–18]. Like human burn victims, hypermetabolism has been documented in similar murine models of burn trauma [19]. All animal experiments were performed in line with the National Institutes of Health guidelines on the use of animals for research. This study was approved by the Institutional Animal Care and Use Committee at the University of Texas Medical Brach.

Briefly, male BALB/c mice (8–10 weeks old) were housed on a 12:12 light:dark cycle at 24–26 °C throughout the study period. Following the administration of buprenorphine (0.1 mg/kg i.p.), mice were anesthetized by inhalation of a 3–5% isoflurane mixture and remained fully anesthetized throughout the surgical procedure. Approximately 40% of the dorsum was shaved with electrical clippers before ~1 cc of lactated ringers (LR) was injected s.c. in both burn and sham treated animals to protect the spinal column. Thereafter, the dorsum of burn treated animals was exposed to ~95 °C water for 10 s in a custom made cast to produce a full-thickness scald wound covering approximately 25–30% of the TBSA. Both burn and sham treated mice were then resuscitated with and i.p. injection of 1–2 cc of LR. All mice were housed individually following burn or sham treatment. Cohorts of mice were sacrificed at 3 h, 24 h, 4 d and 10 d post injury. The spinotrapezius muscle directly under burn wounds and the quadriceps muscle group distal to burn wounds were excised from burn and sham treated animals for biochemical analysis.

2.2. Tissue analysis

Skeletal muscle samples were split into three portions following collection from animals. One portion of tissue from both the spinotrapezius and the quadriceps was immediately placed in an ice-cold mitochondrial preservation buffer containing 10 mM CaK_2-EGTA; 7.23 mM K_2-EGTA; 20 mM imidazole; 20 mM taurine 50 mM K-MES; 0.5 mM dithiothreitol; 6.56 mM $MgCl_2$; 5.77 mM ATP and 15 mM creatine phosphate (pH 7.1). This tissue was transferred to the laboratory for mitochondrial respiration measurements. The remaining muscle tissue was frozen in liquid nitrogen and stored at −80 °C for future western blot analysis or fixed in 10% formalin for histochemical analysis.

2.3. Muscle fiber preparation

Approximately ten mgs of skeletal muscle was permeabilized by agitating myofiber bundles in 2 ml of mitochondrial preservation buffer containing 20 μM saponin at 4 °C for approximately 30 min. Thereafter, myofiber bundles were washed in 2 ml of mitochondrial respiration buffer containing 0.5 mM EGTA; 3 mM $MgCl_2$; 60 mM K-lactobionate; 20 mM taurine; 10 mM KH_2PO_4; 20 mM HEPES; 110 mM sucrose; 1 mg/ml essential fatty acid free bovine serum albumin (pH 7.1) at 4 °C for approximately 15 min. Then, approximately 2–4 mg of myofiber bundles were blotted on filter paper and weighed on