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Effect of heart failure on skeletal muscle myofibrillar protein content, isoform expression and calcium sensitivity

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Abstract

Background: Alterations in skeletal muscle with heart failure contribute to exercise intolerance and physical disability. The majority of studies to date have examined abnormalities in skeletal muscle oxidative capacity and mitochondrial function. In contrast, less information is available regarding the effect of heart failure on myofibrillar protein metabolism and function. To address this issue, we examined the effect of heart failure on skeletal muscle myofibrillar protein content, isoform distribution and Ca^{2+} sensitivity.

Methods: We measured skeletal muscle myosin heavy chain (MHC) and actin protein content and MHC isoform distribution in soleus (SOL), extensor digitorum longus (EDL), plantaris (PL) and diaphragm (DIA) muscles and myofibrillar Ca^{2+} sensitivity in EDL muscles from Dahl salt-sensitive rats with (high-salt fed: HS; n=10) or without heart failure (low-salt fed: LS; n=8) and assessed the relationship of these variables to markers of disease severity.

Results: No differences in muscle mass were found. Similarly, no differences in MHC (mean±SE; SOL: 1353±29 vs. 1247±52; EDL: 1471±31 vs. 1441±31; PL: 1207±66 vs. 1286±36; DIA: 1166±42 vs. 1239±26 AU/μg protein) or actin (EDL: 348±13 vs. 358±19; PL: 245±20 vs. 242±9; DIA: 383±9 vs. 376±17 AU/μg protein) protein content or the actin-to-MHC ratio were observed, with the exception of lower (P<0.01) actin content in the soleus of LS rats (352±7 vs. 310±8 AU/μg protein). MHC isoform expression (I, IIa, IIx, IIb) did not differ between groups in SOL (I: 89±1% vs. 85±2%; IIa: 11±1% vs. 15±2%), EDL (IIx: 43±10% vs. 38±10%; IIb: 57±10% vs. 62±10%), PL (I: 6±4% vs. 3±3%; IIa: 1±1% vs. 1±1%; IIx: 31±3% vs. 26±4%; IIb: 62±5% vs. 71±6%) or DIA (I: 43±6% vs. 36±6 %; IIa: 9±1% vs. 7±1%; IIx: 47±6% vs. 56±7%; IIb: 2±1% vs. 1±0.5%) muscles. Moreover, heart failure did not affect the Ca^{2+} sensitivity (i.e., pCa_{50}) of extensor digitorum longus myofilaments (5.68±0.11 vs. 5.65±0.09). Finally, MHC and actin content, MHC isoform distribution and myofibrillar Ca^{2+} sensitivity were not related to markers of disease severity.

Conclusions: Our results show that this animal model of heart failure is not characterized by alterations in the quantity or isoform distribution of key skeletal muscle myofibrillar proteins or the Ca^{2+} sensitivity of isometric force production. These findings suggest that alterations in skeletal muscle myofibrillar protein metabolism do not develop in parallel with myocardial failure in the Dahl salt-sensitive rat.

Keywords: Myosin heavy chain; Actin; Isoform; Exercise intolerance

1. Introduction

Skeletal muscle adaptations play an important role in the symptomology of chronic heart failure [1]. The majority of studies that have examined alterations in skeletal muscle have focused on changes in oxidative capacity and mitochondrial function [2–4]. In contrast, relatively less is known about the impact of heart failure on myofibrillar protein metabolism.

Several rodent models have been used to characterize the effect of heart failure on myofibrillar proteins. Most of these investigations have focused on changes in fiber type or myosin heavy chain (MHC) isoform expression.

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Results have been equivocal. Although many studies have observed alterations in fiber type or isoform distribution with heart failure [5–9], no consistent pattern has emerged. In fact, in some muscles, divergent results have been noted between studies [5–7,9]. Findings from Simonini et al. [7] have raised the intriguing possibility that heart failure might also affect the quantity of MHC in skeletal muscle, an alteration that would have clear functional significance. No subsequent studies, however, have measured skeletal muscle MHC protein content. Because of these inconsistencies and the paucity of information on certain aspects of myofibrillar protein, a primary goal of our study was to examine the effect of heart failure on skeletal muscle myofibrillar protein, with a specific emphasis on skeletal muscle MHC protein content and isoform distribution.

Heart failure is characterized by skeletal muscle contractile dysfunction [1]. Several studies have identified defects in excitation–contraction coupling in skeletal muscle from rats with heart failure [10–12] which might contribute to contractile dysfunction. These alterations are similar to those observed in cardiac muscle [13,14], leading some investigators to hypothesize that heart failure is characterized by a generalized myopathy of striated muscle [10,11]. In addition to defects in excitation–contraction coupling, heart failure is characterized by changes in cardiac myofibrillar protein function; most notably, an increase in Ca^{2+} sensitivity [15,16]. Thus, if a generalized myopathy of striated muscle exists, skeletal muscle myofibrillar Ca^{2+} sensitivity should be increased similarly. Whether skeletal muscle myofibrillar Ca^{2+} sensitivity is affected by heart failure, however, is not clear. Thus, a secondary goal of our study was to examine the effect of heart failure on skeletal muscle myofibrillar Ca^{2+} sensitivity.

The overall goal of our study was to evaluate the effect of heart failure on skeletal muscle myofibrillar protein metabolism and calcium sensitivity. To accomplish our objectives, we measured MHC and actin protein content, MHC isoform distribution and myofibrillar Ca^{2+} sensitivity in the Dahl salt-sensitive rat model of failure [17,18]. Myofibrillar protein content and isoform distribution measurements were performed on a variety of muscles (soleus, extensor digitorum longus, plantaris and diaphragm) that differ in both functional demands and isoform distribution. Myofibrillar Ca^{2+} sensitivity measurements were performed on the extensor digitorum longus muscle. This muscle was chosen because previous studies have shown that it exhibits altered excitation–contraction coupling similar to cardiac muscle [10,11]; whereas, other muscles, such as soleus, flexor digitorum brevis and gastrocnemius, do not [19,20]. An ancillary goal of our study was to examine whether adaptations in skeletal muscle myofibrillar protein metabolism with heart failure are related to markers of disease severity.

2. Materials and methods

2.1. Animals

Dahl salt-sensitive rats were obtained (Taconic Inc.; Germantown, NY) at 6 weeks of age. All rats were in plastic-bottomed cages, two or three to a cage and were maintained on a 12-h:12-h light/dark cycle in a temperature-controlled room. Tap water and a low-salt (0.6%) chow were available ad libitum prior to and during the study. At 7 weeks of age, rats were divided into populations that received either high-salt (HS, 8% NaCl; n=10; 9 male, 1 female) or low-salt (LS, 0.6% NaCl; n=8; 4 male, 4 female) food pellets of similar macronutrient content. Six weeks following the initiation of diet, echocardiography was performed once per week in HS rats to assess left ventricular (LV) function. Myocardial failure was considered to be present if LV fractional shortening was $\leq$30% [16,18]. Skeletal muscle tissue was removed within 1 week of detection of heart failure. Echocardiography was performed on LS rats prior to collection of muscle tissue. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Vermont and studies were conducted in accordance with the Guide for the Care and Use of Laboratory Animals. Data from a sub-set of these animals (n=4 HS rats) have been published previously [21].

2.2. Muscle tissue

Rats were anesthetized using sodium pentobarbital (90 mg/kg, IP), a tracheotomy was performed and ventilation initiated with a respirator. Muscles were removed approximately 25 min following initiation of anesthesia. Soleus, extensor digitorum longus and plantaris muscles for myofibrillar protein content and MHC isoform distribution measurements were obtained from the left hindlimb, weighed and frozen in liquid N_2. A sample of diaphragm muscle was removed and frozen immediately in liquid N_2 for myofibrillar protein content and isoform distribution measurements. All four muscles were available from 8 HS and 7 LS rats for myofibrillar protein content and MHC isoform determinations. The extensor digitorum longus muscle from the right hindlimb was removed for functional measurements, as described below (HS: n=6; LS: n=7). Diaphragm muscle was carefully excised from a separate group of 15 rats (HS: n=7; LS: n=8) for muscle weight determinations.

2.3. Echocardiography

Rats were anaesthetized with isoflurane (1%), placed in the supine position on top of a warming pad and their precordium shaved. Echocardiography was performed using a Sequoia system with a 15L8 linear array transducer (15.0-MHz; Acuson Corp., Mountain View, CA) [16].