DR ROBERT W DAVIES (Orcid ID : 0000-0002-2994-7762)

Article type : Original Article

Title: Acute reduction of lower-body contractile function following a microbiopsy of *m. vastus lateralis*

Running Head: Contractile function following muscle microbiopsy

Authorship: Robert W. Davies[1,2], Brian P. Carson[1,2], Joseph J. Bass[1,2,3], Sorcha Holohan[1], Philip M. Jakeman[1,2]

Affiliations: [1] Department of Physical Education and Sport Sciences, University of Limerick, Ireland; [2]Food Health Ireland, Centre for Interventions in Infection, Inflammation & Immunity (4i), University of Limerick, Ireland; [3]MRC/ARUK Centre for Musculoskeletal Ageing Research and National Institute of Health Research, Biomedical Research Centre, School of Medicine, University of Nottingham, Derby, UK

Robert.davies@ul.ie; Brian.carson@ul.ie; Joseph.bass@ul.ie; Sorcha.Holohan@ul.ie; Phil.jakeman@ul.ie

Corresponding Author: Dr. Robert W. Davies

Address: Department of Physical Education and Sport Sciences, University of Limerick, Limerick, Ireland

Telephone: (+353) 061 374 779

Email: Robert.davies@ul.ie

ABSTRACT

Twenty-three resistance trained men 18 to 35 y (23 (3) y, 1.8 (0.1) m, 81 (10) kg body mass, 2.3 (1.1) y resistance training experience; mean (SD)) performed repeated maximal voluntary isometric squats (ISQ) and countermovement jumps (CMJ) pre- and +30 min post a unilateral microbiopsy of *m. vastus lateralis*. ISQ and CMJ were simultaneously measured by two force plates sampling ipsilateral (biopsied) and contralateral (non-biopsied) limb force. Bilateral limb force (ipsilateral + contralateral) and imbalance (ipsilateral · bilateral^{-1}) data are reported as % change from pre-biopsy (mean [95 % CI]). A post-biopsy reduction in bilateral ISQ peak force (-17 [-23, -11] %; $P < 0.001$), ISQ rate of force development (RFD) (-28 [-41, -15] %, $P = 0.002$) and CMJ peak take-off force (-7 [-13,-1] %, $P = 0.019$) occurred. Imbalance was observed for ISQ peak force (3.2 [2.1, 4.3] %, $P < 0.001$), RFD (2.8 [1.6, 4.0] %, $P < 0.001$) and CMJ landing (3.3 [1.0, 5.6] %, $P = 0.009$), resultant of a force transfer from the ipsilateral (biopsied) to the contralateral (non-biopsied) limb. These data suggest that in young, resistance trained men a modulatory influence on maximal voluntary static and dynamic lower-body contractile function is evoked acutely (+30 min) following a microbiopsy of *m. vastus lateralis.*

Keywords: Biopsy, Human, Muscle Contraction, Muscle Strength, Quadriceps Muscle

1. INTRODUCTION

This paper reports an observation from a recent study investigating the effect of protein feeding on the rate of muscle protein synthesis in resistance trained men aged 18 to 35 y (NCT03297151 registered at https://www.clinicaltrials.gov). The protocol for this study required subjects complete measures of lower-body contractile function pre- and +30 min post microbiopsy of *m. vastus lateralis*.

Open [1] and semi-open [2,3] biopsy procedures have been shown previously to reduce contractile function, affecting unilateral isokinetic strength [4] and running mechanics.[5] More recently minimally invasive microbiopsy techniques [6] have also been employed in sport and exercise science research.[7,8] To the authors' knowledge the effect of the microbiopsy procedure on contractile function is not known. Here, we report the change in lower-body static and dynamic maximal voluntary contractile function pre- and +30 min post- a unilateral microbiopsy of *m. vastus lateralis* in resistance trained men 18 to 35 y. Independent measurement of contractile function of the biopsied (ipsilateral) and non-biopsied (contralateral) limb determined potential force imbalance between limbs as a result of the biopsy. These findings may have important implications for sport and exercise science research, particularly when biopsies and measures of contractile function are performed in temporal proximity.

2. METHODS

2.1 Ethical Approval

The University of Limerick Research Ethics Committee (2016_12_09 EHS) approved the study design and procedures, which conformed to the standards set by the Declaration of Helsinki. Subjects were informed fully of the risks and benefits associated with participation before providing written informed consent. The study was registered at https://www.clinicaltrials.gov (NCT03297151).

2.2 Subjects and Experimental Protocol

Eligibility criteria was: (i) men (ii) 18 to 35 y; (iii) resistance trained (*i.e.* reporting > 0.5 y continuous resistance training > 3 h per wk, prior to the study), (iv) no current injury, illness or disease, (v) not currently taking any medication. Twenty-three men (age 23 (3) y**,** 1.8 (0.1) m, 81 (10) kg body mass, 2.3 (1.1) y resistance training experience; mean (SD)) were recruited to the study. Subjects reported to the laboratory for three consecutive days, the same time each day (between 06.00 and 09.00), postabsorptive (~ 10 h), and having performed no other formal exercise for 3 d prior to testing. On day 1 subjects were familiarised with the isometric squat (ISQ) and countermovement jump (CMJ) tests. On day 2, 24 h prior to the biopsy, ISQ and CMJ measurement were repeated providing pre-biopsy test data. On day 3 ISQ and CMJ was measured +30 min following a unilateral microbiopsy of *m. vastus lateralis.* Contractile function was not measured immediately prior to the biopsy as it may interfere with primary aim of the study.

2.3 Measures of Lower Body Contractile Function

The measurement of maximal voluntary ISQ force was undertaken by a custom made squat rack with a fixed barbell, adjustable in height, positioned above two force plates (AMTI, Watertown MA) and bolted to the floor. Squat posture was fixed (110° knee angle) and left and right foot positioning on the plates was marked, recorded and repeated for each subsequent measurement. Once positioned in contact with the barbell, force was tared and on an audible cue, subjects were given standard verbal encouragement to push into the barbell as fast and forcefully as possible for at least 3 s. This was repeated three times with 3 min rest between attempts. Ground reaction force data was sampled at 1 kHz and excluded if any countermovement was evident. Peak isometric force (PIF) was defined as the highest 1 s average ground reaction force attained during the three attempts (Figure 1). The rate of force development (RFD) was derived from the slope of the force-time trace

(Δforce·Δtime^{-1} 0 to 150ms from the onset of the contraction; Figure 1).[9] Root mean square error (RMSE) and coefficient of variation (CV) calculated from the repeat attempts on day 2 was: 29 N (4.4 %) for PIF and 435 N·s^{-1} (16.2 %) for RFD.

A countermovement jump (CMJ) was used to measure lower-body dynamic force. To prevent non-vertical movement between take-off and landing, subjects were instructed to place hands on hips and keep their body straight throughout, landing in the same upright toe-first position as for take-off. Subjects repeated tests 3 times with 3 min rest between attempts. Peak jump height (*i.e.* vertical displacement calculated from flight time [10]), peak and average force during the upward phase of the jump (pre-take off), and ground reaction forces (GRF) on landing were used as criterion measures (Figure 2). RMSE (CV) was: 1.4 cm (3.3 %) for vertical displacement, 71N (5.8 %) for peak force, 56 N (9.1 %) for average force and 431 N (14.7 %) for GRF.

Adopting two force plates (one under each foot) with resting mass equally distributed, enabled simultaneous measurement of ipsilateral and contralateral limb force. Bilateral force was defined as the sum of forces generated by each limb, *i.e.* ipsilateral force + contralateral force. Force-balance, or imbalance, was calculated as the difference between the pre- to post-biopsy ipsilateral and contralateral limb forces expressed as a % distribution of the total bilateral force, *i.e.* (ipsilateral force·100) · (bilateral force)$^{-1}$. Measurement order was the same for all subjects each day (*i.e.* ISQ test - 3 min rest – CMJ test).

2.4 Measurement of Muscle Pain

Immediately following ISQ subjects were asked to quantify general subjective feelings of muscle pain during the ISQ test, using a 10 cm visual analog scale (VAS). The VAS descriptors were 'no pain' (0 cm), to 'worst possible pain' (10 cm).

2.5 Microbiopsy

Unilateral microbiopsy samples were taken mid-belly of *m. vastus lateralis* following a standard procedure.[6] Briefly, anaesthetic (5ml 1 % lidocaine (Braun, Dublin)) was injected superficial to the facia and the skin was then punctured with a 14-gauge insertion cannula perpendicular to the limb and advanced through the facia into the muscle. Muscle tissue was then sampled using a 14-gauge spring-loaded microbiopsy needle. Samples were then: washed in ice-cold saline, frozen in liquid N_2 and weighed. Samples were 51 (SD 12) mg, ranging from 29 to 78 mg.

2.6 Statistics

Data are expressed as mean (SD) in standard units and mean % change [low, high] 95 % confidence interval (CI) pre- to post-biopsy. Ipsilateral and contralateral limb imbalance is expressed as % force distribution of the ipsilateral (biopsied) limb to the total bilateral force (*i.e.* 50 % indicates equal force distribution between limbs). There was no control group in this study. Therefore, to assess the effect of the microbiopsy, direct comparison was made pre- to post-biopsy (*i.e.* day 2 *vs.* day 3) via student's t-tests ($\alpha = 0.05$), effect size (*d*) [11] and 95 % CIs (RStudio 1.1.383). Normality and homogeneity of variance was confirmed prior to statistical analysis.

3. RESULTS

A reduction in bilateral static (ISQ PIF and RFD) and dynamic (CMJ) lower-body contractile function was observed post-biopsy ($P < 0.05$) (Table 1, Figure 1, Figure 2). Imbalance between the ipsilateral (biopsied) and contralateral (non-biopsied) limb was noted for CMJ GRF, ISQ PIF and ISQ RFD, with force transfer from the ipsilateral (biopsied) to the contralateral (non-biopsied) limb (Table 2). Although bilateral dynamic contractile function decreased pre- to post-biopsy, no discernible imbalance was noted prior to take-off during the CMJ (Table 2).

An increase in pain was reported post-biopsy (22 [15, 29] %, $d = 1.4$, $P < 0.001$).

4. DISCUSSION

In sport and exercise science research muscle biopsies are often performed in temporal proximity to tasks requiring contractile function,[3,12] which has enabled a greater understanding between the phenotype,[7,8,13,14] metabolism, [3,15] energy status [16] and the mechanical properties of the muscle. In the present study we report that in resistance trained men aged 18 to 35 y, +30 min following a microbiopsy of *m. vastus lateralis* an acute inhibition of static and dynamic lower-body contractile function, with force-transfer shifting away from the biopsied limb during measurement. It has been shown previously that biopsy of *m.vastus lateralis* reduces running performance [5] and isokinetic knee extensor strength [4] causing muscle pain,[6] muscle damage [19] and inflammation.[20] Based on the findings from the present study we assert that biopsy of *m.vastus lateralis* also reduces measurement accuracy and performance for tasks requiring maximal voluntary (static and dynamic) contractile function. Thus, we urge caution when planning and designing experiments using these measures and highlight the importance of an appropriate control during acute biopsy studies (*e.g.* independently measured contralateral limb or an independent subject group), as potentially pre-biopsy assessment of contractile function may prompt physiological change for some more sensitive measures (*e.g.* mRNA, protein phosphorylation, enzyme activity, substrate availability and metabolite accrual).

From these data we cannot discern the cause of the force deficit following the biopsy, however, studies investigating the effect of nociceptive stimulation have reported perceptual increases in pain disrupting force transmission during sustained voluntary contraction.[17,18] Indeed, in the present study all subjects reported a consistent increase in muscle pain following the procedure, with the force deficit and limb imbalance most evident during the sustained static contraction (*i.e.* ISQ *vs.* dynamic CMJ). Acute inhibition of force has been reported previously following more invasive biopsy procedures, [4,5] which are known to cause pain and discomfort. Given the absence of any other likely

cause arising as a result of the procedure, we posit that in the present study, the biopsy-induced pain is still most plausible explanation for the acute loss of force.

Although here we are only able to report an acute inhibition of contractile function +30 min following the procedure, other acute biochemical [20] and morphological [19] changes have been reported following muscle biopsy. In order to ascertain when accurate measures can be made, it may be pertinent for future research to outline the full temporal recovery profile of the muscle post-biopsy.

PERSPECTIVES

In resistance trained men 18 to 35 y, capable of producing forceful/powerful muscular contractions, a minimally invasive unilateral microbiopsy of *m. vastus lateralis* causes an acute inhibition of lower-body bilateral contractile function and an imbalance between limbs. In sport and exercise science research biopsies are frequently performed alongside measures of contractile function. Based on these observations, authors recommend caution when planning and designing experiments using both of these measures in temporal proximity.

FUNDING

Funding for this study was provided by Food for Health Ireland (FHI) (Grant Number [TC20130001]). Authors disclose no conflict of interest.

COMPETING INTERESTS

Authors declare no competing interests.

AUTHOR CONTRIBUTIONS

R.W.D, B.P.C, J.J.B, and P.M.J conceived the study design and hypothesis. R.W.D and S.H completed the data acquisition and analysis. R.W.D, B.P.C, S.H, and P.M.J interpreted the data. R.W.D and S.H drafted the manuscript. R.W.D, B.P.C, J.J.B, S.H, and P.M.J revised the manuscript critically for its intellectual content. All authors approved the final version of the manuscript agreeing to be accountable for all aspects of the work.

ACKNOWLEDGEMENTS

We wish to acknowledge researchers Arthur Lynch, Timothy Locke, Dr. Emmet Kerin, Roisin McGinley and Dr. Jason McMahon for their technical expertise.

REFERENCES

1. Bergström J. Percutaneous needle biopsy of skeletal muscle in physiological and clinical research. Scand J Lab Invest 1975;34:609-616.
2. Henriksson KG. "Semi-open" muscle biopsy technique. Acta Neurol Scand 1979;59:317-323.
3. Shanely RA, Zwetsloot KA, Triplett NT, Meaney MP, Farris GE, Nieman DC. Human skeletal muscle biopsy procedures using the modified Bergström technique. J Vis Exp 2014;91.
4. Laskin J, Bell G, Burnham R. The effect of a needle biopsy of the vastus lateralis on isokinetic muscle performance. Clin J Sport Med 1998;8;221-223.
5. Morin JB, Samozino P, Féasson L, Geyssant A, Millet G. Effects of muscular biopsy on the mechanics of running. Eur J Appl Physiol 2009;105:185-190.
6. Hayot M, Michaud A, Koechlin C, Caron MA, LeBlanc P, Prefaut C, Maltais F. Skeletal muscle microbiopsy: a validation study of a minimally invasive technique. Eur Respir J 2005;25:431-440.
7. Townsend JR, Hoffman JR, Fragala MS, Oliveira LP, Jajtner AR, Fukuda DH, Stout JR. A microbiopsy method for immunohistological and morphological analysis: a pilot study. Med Sci Sports Exerc 2016;48:331-335.
8. Seitz LB, Trajano GS, Haff GG, Dumke CC, Tufano JJ, Blazevich AJ. Relationships between maximal strength, muscle size, and myosin heavy chain isoform composition and postactivation potentiation. Appl Physiol Nutr Metab 2016;41:491-497.
9. Aagaard P, Simonsen EB, Andersen JL, Magnusson P, Dyhre-Poulsen P. Increased rate of force development and neural drive of human skeletal muscle following resistance training. J Appl Physiol 2002;93:1318-1326.
10. Komi PV, Bosco C. Utilization of stored elastic energy in leg extensor muscle by men and women. Med Sci Sports 1978;10:261-265.
11. Cohen J. A power primer. Psychol Bull 1992;112;155.
12. Gaitanos GC, Williams C, Boobis LH, Brooks S. Human muscle metabolism during intermittent maximal exercise. J Appl Physiol 1993;75:712-719.

13. Gollnick PD, Matoba H. The muscle fiber composition of skeletal muscle as a predictor of athletic success. An overview. Am J Sports Med 1984;12:212-217.
14. Harridge SD, Bottinelli R, Canepari M, Pellegrino MA, Reggiani C, Esbjörnsson M, Saltin B. Whole-muscle and single-fibre contractile properties and myosin heavy chain isoforms in humans. Pflügers Archiv 1996;432:913-20.
15. Zierath JR, Hawley JA. Skeletal muscle fibre type: influence of contractile and metabolic properties. PLoS Biol 2004;2:e348.
16. Gollnick PD, Piehl K, Saltin B. Selective glycogen depletion pattern in human muscle fibres after exercise of varying intensity and at varying pedalling rates. J Physiol 1974;241:45-57.
17. Kennedy DS, McNeil CJ, Gandevia SC, Taylor JL. Fatigue-related firing of distal muscle nociceptors reduces voluntary activation of proximal muscles of the same limb. J Appl Physiol 2014;116:385-394.
18. Martin PG, Weerakkody N, Gandevia SC, Taylor JL. Group III and IV muscle afferents differentially affect the motor cortex and motoneurons in humans. J Physiol 2008;586:1277-1289.
19. Staron RS, Hikida RS, Murray TF, Nelson MM, Johnson P, Hagerman F. Assessment of skeletal muscle damage in successive biopsies from strength-trained and untrained men and women. Eur J Appl Physiol Occup Physiol 1992;65:258-264.
20. Malm C, Nyberg P, Engström M, Sjödin B, Lenkei R, Ekblom B, Lundberg I. Immunological changes in human skeletal muscle and blood after eccentric exercise and multiple biopsies. J Physiol 2000;529;243-262.

TABLES

Table 1: Contractile Function pre- and post-biopsy.

	Pre-biopsy Mean (SD)	Post-biopsy Mean (SD)	% Mean Difference [CI]	*d*	*P*
Isometric Squat					
PIF (N)	701 (177)	627 (168)	-10 [-16, -4]	0.7	0.002
RFD ($N \cdot s^{-1}$)	2472 (1325)	2078 (1276)	-28 [-44, -12]	0.8	0.002
Countermovement Jump					
Peak Force (N)	1068 (480)	997 (485)	-7 [-14, 0]	0.5	0.019
Average Force (N)	596 (175)	547 (143)	-11 [-23, 1]	0.5	0.044
Displacement (cm)	35.2 (5.6)	33.8 (5.9)	-6 [-10, -2]	0.7	0.004
GRF (N)	2823 (619)	2512 (589)	-8 [-19, 3]	0.4	0.069

Change pre- to post-biopsy. Peak Isometric Force (PIF); Rate of Force Development (RFD); Ground Reaction Force (GRF). % mean difference is percent change pre- to post-biopsy and corresponding 95 % confidence interval (CI); Cohen's d (*d)*; p-value (*P)*.

Table 2: Force-imbalance pre- and post-biopsy

	Pre-biopsy Mean (SD)	Post-biopsy	Mean Balance Change [CI]	*d*	*P*
Isometric Squat					
PIF Balance (%)	52 (3)	48 (3)	+3.2 [1.9,, 4.5]	0.8	< 0.001
RFD Balance (%)	52 (2)	48 (3)	+2.8 [1.4, 4.2]	1.4	< 0.001
Countermovement Jump					
Peak Force Balance (%)	48 (3)	48 (1)	+1.0 [-3.0, 2.7]	< 0.1	0.578
Average Force Balance (%)	50 (5)	48 (2)	+3.0 [-4.4, 10.4]	0.1	0.727
GRF Balance (%)	49 (3)	44 (3)	+3.3 [0.5, 6.1]	0.6	0.009

Data are biopsied limb % force distribution where 50 % indicates equal balance between limbs. Peak Isometric Force (PIF); Rate of Force Development (RFD); Countermovement Jump (CMJ); Ground Reaction Force (GRF). For mean balance change (pre- to post-biopsy (%)) and 95 % confidence interval (CI); Cohen's d (*d)*; p-value (*P)*; +ve indicates force transfer away from the biopsied limb, -ve indicates force transfer toward the biopsied limb.

Figure 1. Isometric Squat force-time trace. Pre-biopsy (grey line) and post-biopsy (black line) isometric squat (ISQ) bilateral force-time trace from a typical subject. Corresponding dashed lines signify the change (Δ) pre- (grey) to post-biopsy (black) for peak isometric force (PIF) and the rate of force development (0 to 150 ms) (RFD).

Figure 2. Countermovement Jump force-time trace. Pre-biopsy (grey line) and post-biopsy (black line) countermovement jump (CMJ) bilateral force-time trace from a typical subject. Corresponding dashed lines signify the change pre- (grey) to post-biopsy (black) for CMJ variables (ground reaction force (A), peak force (B), average force (C), flight time (D)).