TITLE: Alterations in whole-body insulin sensitivity resulting from repeated eccentric exercise of a single muscle group: a pilot investigation

SHORT RUNNING TITLE: Muscle damage and insulin resistance

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Abstract

Unaccustomed eccentric exercise using large muscle groups elicits soreness, decrements in physical function and impairs markers of whole-body insulin sensitivity; although these effects are attenuated with a repeated exposure. Eccentric exercise of a small muscle group (elbow flexors) displays similar soreness and damage profiles in response to repeated exposure. However, it is unknown whether damage to small muscle groups impacts upon whole-body insulin sensitivity. This pilot investigation aimed to characterize whole-body insulin sensitivity in response to repeated bouts of eccentric exercise of the elbow flexors. Nine healthy males completed two bouts of eccentric exercise separated by 2 weeks. Insulin resistance (updated homeostasis model of insulin resistance, HOMA2-IR) and muscle damage profiles (soreness and physical function) were assessed before, and 48 h after exercise. Matsuda insulin sensitivity indices ($ISI_{Matsuda}$) were also determined in 6 participants at the same time points as HOMA2-IR. Soreness was elevated, and physical function impaired, by both bouts of exercise (both $P < 0.05$) but to a lesser extent following bout 2 (time x bout interaction, $P < 0.05$). Eccentric exercise decreased $ISI_{Matsuda}$ after the first but not the second bout of eccentric exercise (time x bout interaction $P < 0.05$). Eccentric exercise performed with an isolated upper limb impairs whole-body insulin sensitivity after the first, but not the second, bout.

Keywords: glucose; glycemia; insulin resistance; metabolic control; muscle damage repeated bout.

Abbreviations

GLUT-4: glucose transporter isoform 4

HOMA2-IR: updated homeostasis model of insulin resistance

50 iAUC: incremental area under the curve

51 $ISI_{Matsuda}$: Matsuda insulin sensitivity index

52 MVC: maximal voluntary contraction

53 OGTT: oral glucose tolerance test

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Introduction

Insulin sensitivity indices predict the risk of developing metabolism-related diseases i.e. type 2 diabetes and cardiovascular disease (The DECODE Study Group & The European Diabetes Epidemiology Group, 1999; Zavaroni et al., 1989), even when only the "healthy" range of indices are considered (Ning et al., 2012). Accordingly, understanding how insulin sensitivity responds to stimuli can give insight into metabolic disease risk in currently healthy populations. Whilst regular exercise alongside lifestyle interventions can prevent metabolic disease (Knowler et al., 2002), the acute effects of exercise on whole-body glucose metabolism are equivocal. Following a single bout of exercise, glucose tolerance has been shown to improve (Bonen, Ball-Burnett, & Russel, 1998), deteriorate, or remain stable (Gonzalez, Veasey, Rumbold, & Stevenson, 2013), relative to rest. Numerous factors are postulated to explain these discrepancies (including metabolic and nutritional status' of the population, modality, volume and intensity of exercise), one of which is muscle damage induced by exercise with an eccentric component, and associated impairment of insulin sensitivity (Gonzalez, 2014).

Typically, the exercise paradigms employed to study muscle damage involve large muscle groups or whole-body exercise, i.e., downhill running (Cook, Myers, Kelly, & Willems, 2014; Green et al., 2010), or eccentric exercise of knee flexors (Paschalis et al., 2011). These models produce acute metabolic alterations indicative of reduced insulin sensitivity when measured at 48 h (Green et al., 2010; Paschalis et al., 2011) post-exercise. This effect is only present when exercise is unaccustomed, and is abolished or reversed with multiple bouts (Green et al., 2010; Paschalis et al., 2011). For damaging exercise of a small muscle group, similar profiles of damage, recovery and protection on repeated-bouts have been observed (Howatson, van Someren, & Hortobagyi, 2007), but the effect of damaging exercise of a small muscle group on whole-body insulin sensitivity is unknown. If whole-

body insulin sensitivity can be modified by acute exercise of small muscle groups, such as the elbow flexors of a single limb (constituting <6% of total lean mass (Araujo et al., 2010)), this could reveal an avenue to explore potentially beneficial adaptations with multiple bouts, which may have implications during forced inactivity or immobilization.

Accordingly, this pilot investigation aimed to assess whole-body insulin sensitivity during an oral glucose tolerance test (OGTT), in response to two bouts of eccentric exercise of the elbow flexors, separated by 14 days. We hypothesized that damaging exercise of a single muscle group would impair whole-body insulin sensitivity after the first, but not the second bout.

Materials and methods

Participants

Six male participants completed the full protocol, whilst a further three males provided fasting samples only. Thus, postprandial OGTT data are $n = 6$ whilst all other data are $n = 9$ (participant characteristics are presented in Table 1). All participants were naïve to regular resistance exercise.

Study design

Participants visited the laboratory on 6 occasions; twice to complete the eccentric exercise protocol (separated by 2 weeks), and 4 times for blood sampling in line with assessment of physical function and soreness (muscle damage markers). Blood sampling (including OGTT) and damage marker assessments were performed prior to, and 48 h following damaging exercise. The eccentric exercise protocol was performed on an isokinetic dynamometer (System 4 Pro, Biodex Medical Systems Inc. NY, USA) and comprised 8 × 5 maximal eccentric contractions of the left elbow flexors at $30°d.s^{-1}$; each set separated by 90 s rest.

Subjective soreness and physical function

Subjective soreness was determined using 200 mm visual analogue scales during full range of movement of the elbow flexors. Physical function was taken as the peak value attained during 3 isometric maximal voluntary contractions (MVC) of the elbow flexors, each performed with 90° flexion of the elbow, separated by 120 s rest and following a standardized warm-up.

OGTT and blood sampling

Participants were asked to maintain a similar carbohydrate intake throughout to minimize effects of diet on insulin sensitivity. Blood sampling was always performed after a 12-h fast. Participants were instructed to eat their evening meal prior to trials at a standardized time, to eat the same meal before all trials, and to refrain from exercise for 24 h prior to blood sampling in accordance with standardization for postprandial glycemia testing guidelines (Brouns et al., 2005). For those who undertook the OGTT, 75 g of glucose (82 g dextrose monohydrate, corrected for moisture; Myprotein, Cheshire, UK) was dissolved in 300 ml of water and ingested within 5 min. Finger-prick blood samples were taken before (0 min), and 15, 30, 45, 60, 90 and 120 min following ingestion, and analyzed immediately for blood glucose concentration (Biosen C_line, EKF Diagnostics, Magdeberg, Germany), whilst a 250 µL EDTA-microvette was filled with whole blood, before centrifugation (10 min at 3000 rpm). By revisiting glucose data obtained in duplicate from one of our previous studies (Gonzalez & Stevenson, 2012), we are able to report reliability statistics, which include the combined variability of sample collection and analysis. Across 196 pairs of samples (range 3.60-8.81 mmol/L), the standardized typical error was 0.12 mmol/L (95%CI: 0.11, 0.13 mmol/L) and the coefficient of variation was 1.7%. Plasma was stored at −80°C for

subsequent determination of insulin concentrations by commercially available ELISA (IBL International GmbH, Hamburg, Germany; intra-assay coefficient of variation: 6%).

Calculations and statistics

Insulin sensitivity was estimated in the fasted state, using the updated homeostasis model of insulin resistance (HOMA2-IR; reciprocal of insulin sensitivity (Levy, Matthews, & Hermans, 1998)) and in the postprandial state (OGTT), using the Matsuda insulin sensitivity index ($ISI_{Matsuda}$ (Matsuda & DeFronzo, 1999)). Postprandial glucose and insulin concentrations were converted into time-averaged incremental areas under the curve (iAUC) as has been previously used (Gill et al., 2004). All analyses were performed using Prism v6 (Graphpad Software, San Diego, CA). Data were checked for normal distribution (D'Agostino & Pearson omnibus normality test) and log transformed if appropriate, prior to analysis. The difference in work done between bout 1 and bout 2 was assessed by a paired samples t-test. Two-way [time (pre vs. post) x bout (bout 1 vs. bout 2)] repeated measures ANOVA were used to examine differences in fasting blood variables, OGTT data, MVC and soreness ratings. Data are presented as means ± SEM unless stated otherwise, and statistical significance was set at $P < 0.05$.

Results

Total work done during eccentric exercise was similar between bout 1 (2501 ± 205 kJ) and bout 2 (2527 ± 215 kJ; $P = 0.738$). Eccentric exercise elicited increases in soreness on both bouts ($P = 0.003$). Soreness was lower on the second bout vs. the first ($P = 0.001$) and significantly attenuated (time × bout interaction $P = 0.001$; Figure 1A). MVC decreased after both bouts (main effect of time, $P < 0.001$). No significant main effect of bout was detected

155 (P = 0.218), but the reduction in MVC post-damaging exercise was attenuated on repeated
156 bouts (time × bout interaction, P = 0.019; Figure 1B).

157 Fasting indices of insulin sensitivity (glucose and insulin concentrations, and
158 HOMA2-IR) were unaffected by the intervention and neither was the glucose nor insulin
159 iAUC (Table 2 and Figure 2). $ISI_{Matsuda}$ did not display significant main effects for time or
160 bout (both $P > 0.05$) but the reduction in $ISI_{Matsuda}$ observed after bout 1 was abolished after
161 bout 2 (time x bout interaction, P = 0.030, Figure 1C) indicating preserved insulin sensitivity
162 after the second bout.

163

164 **Discussion**

165 These data indicate that: 1) unaccustomed eccentric exercise of a single upper-body
166 limb reduces insulin sensitivity at the whole-body level, detectable in the postprandial state;
167 2) the impairment in insulin sensitivity is abolished following a second bout of damaging
168 eccentric exercise.

169 Previous work has demonstrated acute reductions in insulin sensitivity following
170 downhill running are absent following a second bout (Green et al., 2010), and others have
171 shown that after 8 bouts, eccentric exercise of the knee flexors *increases* fasting insulin
172 sensitivity indices (Paschalis et al., 2011). Here we demonstrate that a single exposure to
173 eccentric exercise of a single, small muscle group (left elbow flexors) induces an adaptive
174 response, whereby full protection from acute impairment of insulin sensitivity is observed.
175 Whether eccentric exercise of an upper limb has the capacity to positively influence insulin
176 sensitivity over a longer time-course however, warrants further investigation. If this is the
177 case, then one can envisage potential application during imposed inactivity or immobilization
178 of lower limbs.

179 It has been suggested that due to relatively low insulin concentrations used to
180 calculate HOMA2-IR (fasting vs. a clamp procedure or postprandial), this measure represents
181 a different balance of sensitivity (hepatic vs. peripheral) than the $ISI_{Matsuda}$ (Matsuda &
182 DeFronzo, 1999; Radziuk, 2014). Accordingly, the reduction in $ISI_{Matsuda}$ seen in the present
183 study, when viewed in light of the lack of change in HOMA2-IR, suggests that eccentric
184 exercise reduced peripheral (but not hepatic) insulin sensitivity.
185 Numerous mechanisms have been proposed to underlie muscle damage-induced
186 reductions in insulin sensitivity. These include, a decrease in glucose transporter isoform 4
187 (GLUT-4) at the plasma membrane due to reduced GLUT-4 transcription and thus GLUT-4
188 protein content (Kristiansen, Jones, Handberg, Dohm, & Richter, 1997), associated with
189 reduced muscle glucose transport manifest under hyperinsulinaemia but, intriguingly,
190 elevated glucose transport when not exposed to insulin (Asp & Richter, 1996). This provides
191 another potential explanation for the detectable reductions in $ISI_{Matsuda}$ but not in HOMA2-IR.
192 Secondly, systemic factors released by damaged muscle including cytokines such as tumor
193 necrosis factor-α may also be implicated an impaired ability of insulin to stimulate insulin
194 receptor substrate-1, phosphatidylinositol 3-kinase and Akt (protein kinase B) (Asp,
195 Daugaard, Kristiansen, Kiens, & Richter, 1996; Del Aguila et al., 2000; Krogh-Madsen,
196 Plomgaard, Moller, Mittendorfer, & Pedersen, 2006; Liao, Zhou, Ji, & Zhang, 2010). Whilst
197 our data are unable to give insight into which of these mechanisms is responsible, given the
198 relatively small muscle group used (<6% of total lean mass (Araujo et al., 2010)), the impact
199 at the systemic level is noteworthy. This indicates that, either a very small decrease in total
200 GLUT-4 content has implications for insulin sensitivity at the whole body level, and/or
201 damage to small muscle groups produces adequate release of systemic factors (*ie.* cytokines)
202 to impair the action of a sufficient mass of insulin sensitive tissue to influence whole-body
203 metabolism.

In conclusion, these data indicate that eccentric exercise of a single upper limb, inhibits whole-body insulin sensitivity 48 h after the first bout, and such a reduction is not apparent after a second bout.

Novelty statement: Eccentric exercise of large muscle groups (leg flexors of both legs, or whole-body exercise) is known to impair whole-body insulin sensitivity after an initial exposure, with protection from this effect demonstrated with subsequent bouts. This is the first study to demonstrate that eccentric exercise with a single small muscle group (elbow flexors of a single arm) impairs insulin sensitivity following the first bout, but not following a second bout.

Practical application statement: In developing strategies to modulate insulin sensitivity, activating large muscle groups may not necessarily be required to elicit a response at the whole-body level. Eccentric exercise using upper limbs is likely sufficient to influence whole-body insulin sensitivity and this pilot work highlights a new strategy to potentially influence metabolism.

Author contributions

All authors contributed to study design, data collection and analysis, drafting, editing and approved the final article.

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Conflict of interest

The authors declare no conflict of interest.

References

Araujo, A. B., Chiu, G. R., Kupelian, V., Hall, S. A., Williams, R. E., Clark, R. V., & McKinlay, J. B. (2010). Lean mass, muscle strength, and physical function in a diverse population of men: a population-based cross-sectional study. *BMC Public Health, 10*, 508. doi: 10.1186/1471-2458-10-508

Asp, S., Daugaard, J. R., Kristiansen, S., Kiens, B., & Richter, E. A. (1996). Eccentric exercise decreases maximal insulin action in humans: muscle and systemic effects. *The Journal of Physiology, 494 (Pt 3)*, 891-898.

Asp, S., & Richter, E. A. (1996). Decreased insulin action on muscle glucose transport after eccentric contractions in rats. *J Appl Physiol (1985), 81*(5), 1924-1928.

Bonen, A., Ball-Burnett, M., & Russel, C. (1998). Glucose tolerance is improved after low- and high-intensity exercise in middle-age men and women. *Canadian Journal of Applied Physiology, 23*(6), 583-593.

Brouns, F., Bjorck, I., Frayn, K. N., Gibbs, A. L., Lang, V., Slama, G., & Wolever, T. M. (2005). Glycaemic index methodology. *Nutr Res Rev, 18*(1), 145-171. doi: 10.1079/NRR2005100

Cook, M. D., Myers, S. D., Kelly, J. S., & Willems, M. E. (2014). Acute Post-Exercise Effects of Concentric and Eccentric Exercise on Glucose Tolerance. *Int J Sport Nutr Exerc Metab*. doi: 10.1123/ijsnem.2013-0261

Del Aguila, L. F., Krishnan, R. K., Ulbrecht, J. S., Farrell, P. A., Correll, P. H., Lang, C. H., . . . Kirwan, J. P. (2000). Muscle damage impairs insulin stimulation of IRS-1, PI 3-kinase, and Akt-kinase in human skeletal muscle. *American Journal of Physiology - Endocrinology and Metabolism, 279*(1), E206-212.

Gill, J. M., Al-Mamari, A., Ferrell, W. R., Cleland, S. J., Packard, C. J., Sattar, N., . . . Caslake, M. J. (2004). Effects of prior moderate exercise on postprandial metabolism and vascular function in lean and centrally obese men. *J Am Coll Cardiol, 44*(12), 2375-2382. doi: 10.1016/j.jacc.2004.09.035

Gonzalez, J. T. (2014). Paradoxical second-meal phenomenon in the acute post-exercise period. *Nutrition, 30*(9), 961-967. doi: 10.1016/j.nut.2013.12.001

Gonzalez, J. T., & Stevenson, E. J. (2012). Postprandial glycemia and appetite sensations in response to porridge made with rolled and pinhead oats. *J Am Coll Nutr, 31*(2), 111-116.

Gonzalez, J. T., Veasey, R. C., Rumbold, P. L., & Stevenson, E. J. (2013). Breakfast and exercise contingently affect postprandial metabolism and energy balance in physically active males. *British Journal of Nutrition, 110*(4), 721-732. doi: 10.1017/S0007114512005582

Green, M. S., Doyle, J. A., Ingalls, C. P., Benardot, D., Rupp, J. C., & Corona, B. T. (2010). Adaptation of insulin-resistance indicators to a repeated bout of eccentric exercise in human skeletal muscle. *Int J Sport Nutr Exerc Metab, 20*(3), 181-190.

The DECODE Study Group & The European Diabetes Epidemiology Group. (1999). Glucose tolerance and mortality: comparison of WHO and American Diabetes Association diagnostic criteria. Diabetes Epidemiology: Collaborative analysis Of Diagnostic criteria in Europe. *Lancet, 354*(9179), 617-621.

Howatson, G., van Someren, K., & Hortobagyi, T. (2007). Repeated bout effect after maximal eccentric exercise. *Int J Sports Med, 28*(7), 557-563. doi: 10.1055/s-2007-964866

Knowler, W. C., Barrett-Connor, E., Fowler, S. E., Hamman, R. F., Lachin, J. M., Walker, E. A., . . . Diabetes Prevention Program Research, G. (2002). Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med, 346*(6), 393-403. doi: 10.1056/NEJMoa012512

Kristiansen, S., Jones, J., Handberg, A., Dohm, G. L., & Richter, E. A. (1997). Eccentric contractions decrease glucose transporter transcription rate, mRNA, and protein in skeletal muscle. *American Journal of Physiology, 272*(5 Pt 1), C1734-1738.

Krogh-Madsen, R., Plomgaard, P., Moller, K., Mittendorfer, B., & Pedersen, B. K. (2006). Influence of TNF-alpha and IL-6 infusions on insulin sensitivity and expression of IL-18 in humans. *American Journal of Physiology - Endocrinology and Metabolism, 291*(1), E108-114. doi: 10.1152/ajpendo.00471.2005

Levy, J. C., Matthews, D. R., & Hermans, M. P. (1998). Correct homeostasis model assessment (HOMA) evaluation uses the computer program. *Diabetes Care, 21*(12), 2191-2192.

Liao, P., Zhou, J., Ji, L. L., & Zhang, Y. (2010). Eccentric contraction induces inflammatory responses in rat skeletal muscle: role of tumor necrosis factor-alpha. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology, 298*(3), R599-607. doi: 10.1152/ajpregu.00480.2009

Matsuda, M., & DeFronzo, R. A. (1999). Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care, 22*(9), 1462-1470.

Ning, F., Zhang, L., Dekker, J. M., Onat, A., Stehouwer, C. D., Yudkin, J. S., . . . Swedish Study, I. (2012). Development of coronary heart disease and ischemic stroke in relation to fasting and 2-hour plasma glucose levels in the normal range. *Cardiovascular Diabetology, 11*, 76. doi: 10.1186/1475-2840-11-76

Paschalis, V., Nikolaidis, M. G., Theodorou, A. A., Panayiotou, G., Fatouros, I. G., Koutedakis, Y., & Jamurtas, A. Z. (2011). A weekly bout of eccentric exercise is sufficient to induce health-promoting effects. *Medicine & Science in Sports & Exercise, 43*(1), 64-73. doi: 10.1249/MSS.0b013e3181e91d90

Radziuk, J. (2014). Homeostastic model assessment and insulin sensitivity/resistance. *Diabetes, 63*(6), 1850-1854. doi: 10.2337/db14-0116

Zavaroni, I., Bonora, E., Pagliara, M., Dall'Aglio, E., Luchetti, L., Buonanno, G., . . . et al. (1989). Risk factors for coronary artery disease in healthy persons with hyperinsulinemia and normal glucose tolerance. *N Engl J Med, 320*(11), 702-706. doi: 10.1056/NEJM198903163201105

316 **Figure legends**

317 **Figure 1.** Subjective soreness (A), maximal voluntary force production (B) and insulin
318 sensitivity indices (C) before and 48 h after 2 bouts of eccentric exercise using the elbow
319 flexors of an upper limb in males. MVC, maximum voluntary contraction force; $ISI_{Matsuda}$,
320 Matsuda insulin sensitivity index (Matsuda & DeFronzo, 1999). Data expressed as means ±
321 SEM. *, significant main effect of time; #, significant main effect of bout; ^, significant time
322 x bout interaction effect ($P < 0.05$).

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324

325 **Figure 2.** Blood glucose (A, B) and plasma insulin (C, D) concentrations during an OGTT
326 prior to and 48 h following, an initial (A, C) and second (B, D) bout of eccentric exercise
327 using the elbow flexors of an upper limb in males. Data expressed as means ± SEM.

List of tables

Table 1. Participant characteristics.

Variable	Fasting Data[1]		OGTT Data[2]		Independent t-test (*P*)
	Mean ± SEM	Range	Mean ± SEM	Range	
Age (y)	21 ± 1	19 – 26	21 ± 1	19 – 26	0.749
Stature (cm)	180 ± 2	173 – 188	181 ± 2	173 – 186	0.845
Body mass (kg)	76.9 ± 2.8	65 – 89.2	77.0 ± 3.1	68.7 – 86.2	0.997
Body mass index (kg/m^2)	23.6 ± 0.6	19.9 – 26.0	23.5 ± 0.8	19.9 – 26.0	0.923

[1], $n = 9$; [2], $n = 6$.

Table 2. Indices of insulin sensitivity in response to acute and repeated exposure to eccentric exercise.

Variable	Bout 1		Bout 2		ANOVA
	Pre	48 h post	Pre	48 h post	time x bout interaction (*P*)
Fasting glucose[1] (mmol/L)	4.45 ± 0.13	4.46 ± 0.20	4.46 ± 0.14	4.57 ± 0.18	0.756
Fasting insulin[1] (pmol/L)	128 ± 36	149 ± 54	147 ± 40	160 ± 46	0.910
HOMA2-IR[1]	2.25 ± 0.62	2.60 ± 0.92	2.54 ± 0.65	2.75 ± 0.73	0.874
Glucose iAUC[2] (mmol/L)	1.47 ± 0.23	1.29 ± 0.19	1.91 ± 0.22	1.27 ± 0.33	0.285
Insulin iAUC[2] (pmol/L)	139 ± 36	153 ± 26	158 ± 17	144 ± 38	0.160

Data expressed as means ± SEM. [1], $n = 9$; [2], $n = 6$; HOMA2-IR, updated homeostasis model of insulin resistance (Levy et al., 1998); iAUC, incremental time-averaged area under the curve.