Nil Whey Protein Effect on Glycaemic Control after Intense Mixed-Mode Training in T2D

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Abstract

While intense endurance and resistance exercise training and whey protein supplementation have both been shown to independently improve glycaemic control, no known studies have examined the effect of high-intensity mixed-mode interval training (MMIT) and whey supplementation in adults with Type-2 diabetes (T2D).

Purpose: To determine if peri-training whey protein supplementation combined with MMIT can improve glycaemic control.

Methods: In a double-blind randomised controlled trial, 24 men (55.7±5.6 y) with T2D performed MMIT with whey (20 grams) or placebo control for 10 weeks. Glycaemic control was assessed via glucose disposal rate (GDR) during a euglycaemic insulin clamp, fasting blood glucose concentration (FBG), and HOMA-IR. Changes in peak oxygen consumption (VO_{2peak}), 1-repetition maximum strength (1RM), Vastus lateralis (VL) muscle and subcutaneous adipose thicknesses (SAT), and waist circumference (WC) were also assessed.

Results: 10-weeks of MMIT substantially improved GDR by 27.5% (90%CI 1.2%, 60.7%) and 24.8% (-5.4%, 64.8%) in the whey and control groups, respectively. There were likely and possible reductions in FBG by -17.4% (-30.6%, -1.6) and HOMA-IR by -14.1% (-25.3%, 1.08%) in the whey group, however, whey effects were not clearly beneficial to glycaemic outcomes, relative to control. MMIT also clearly substantially improved 1RM by 20.6% (16.3%, 24.9%) and 22.7% (18.4%, 27.2%), VO_{2peak} by 22.6% (12.0%, 26.2%) and 18.5% (10.5%, 27.4%), VL muscle thickness by 18.9% (12.0%, 26.2%) and 18.6% (10.5%, 27.4%) and possibly reduced WC by -2.1% (-3.1%, -1.0%) and -1.9% (-3.7%, -0.1%) in the control and whey groups respectively, but the whey-control outcome was trivial or unclear.

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Conclusion: A clinically-meaningful enhancement in glycaemic control following 10-weeks of MMIT was not clearly advanced with peri-training whey protein supplementation in middle-aged men with Type-2 diabetes.

Key Words: Milk-protein, exercise, diabetes, interval training, high-intensity, glucose disposal.

Introduction

A central pathology of type-2 diabetes (T2D) is impaired glycaemic control, a condition characterised by a diminished capacity to restore postprandial blood glucose concentrations to homeostatic levels. Skeletal muscle is the major tissue of postprandial glucose disposal (1), and a well-established site of dysfunction in T2D (2). It is well-documented that T2D skeletal muscle displays low expression of proteins contributing to glucose uptake and metabolism, including: contractile, glucose transporter, and mitochondrial proteins (3-5). Exercise has been shown to upregulate the expression of these proteins (6, 7), and it is well-established that the improvements in aerobic capacity, lean mass and strength that follow progressive aerobic or circuit resistance training are also associated with better glycaemic control (8, 9). High-intensity interval training has emerged as an effective low-volume and time-efficient exercise mode for rapidly improving glycaemic control. In middle-aged men with T2D, 2 weeks of high-intensity cycle interval training was shown to significantly increase the expression of glucose transporter 4 (GLUT4) and mitochondrial proteins in the *vastus lateralis* muscle and lower 24-hour blood glucose concentrations (6).

Milk protein supplementation has shown promise as a complementary therapeutic agent to exercise for improving glycaemic control. Milk proteins are rich in amino acids that stimulate protein synthesis in skeletal muscle (10), which may, like exercise training, lead to

69 better glycaemic control. Independently, whey supplementation was shown to improve
70 glucose tolerance and FBG after 8 weeks in insulin resistant rats (11, 12) and HOMA-IR after
71 12 weeks in overweight and obese adults (13). As an adjunct therapy to exercise and
72 compared to carbohydrate consumption alone, milk-protein supplementation for 6 weeks was
73 reported to improve VO_{2max} in treadmill trained sedentary men (14) and lean mass and 1RM
74 bench press strength after 8 weeks in mixed-mode trained female college basketball players
75 (15). As each of those outcomes has been previously associated with improved glycaemic
76 control (9, 16, 17) combined treatments may also provide better therapeutic outcomes than
77 exercise alone in populations with T2D.
78 The aim of this study was to determine whether whey supplementation for 10 weeks
79 would improve glycaemic control in a population with T2D performing high-intensity mixed-
80 mode interval training. We hypothesised that whey supplementation would enhance
81 glycaemic control to a greater extent than exercise alone. If effective, this may provide a
82 practical adjunct therapy to exercise for improving T2D rehabilitation outcomes.
83
84 **Methods**
85 *Participants*
86 Men with T2D (*n*=24) were recruited from local medical centres in Wellington, NZ.
87 Inclusion characteristics were age 40-65 y, BMI<40, not requiring insulin therapy, and not
88 meeting the ACSM guidelines for exercise for T2D (18). Ethics was approved by the
89 Northern B Health and Disability Ethics Committee, Ministry of Health, Wellington NZ
90 (13/NTB/69). Participants provided written informed consent.
91
92 *Experimental Design*

93 The design was a double blind, randomized (Research Randomizer, Version 4.0,
94 http://www.randomizer.org), placebo controlled trial (http://www.anzctr.org.au/, Registration
95 number ACTRN12613000340730). At early stages of data collection, the original intended
96 third group: whey without MMIT, was removed from the study design because recruited
97 eligible participants declined to participate if not randomised to an exercise group creating
98 sampling bias. In the two-group design, participants consumed a whey-protein beverage or
99 carbohydrate placebo before and after 45 early-morning MMIT sessions over 10 weeks.
100 Participants were encouraged to maintain dietary and medication habits throughout the
101 experimental period and not to participate in strenuous activity within 2 days of testing
102 sessions. Participants were familiarised with all testing procedures except the euglycaemic
103 insulin clamp prior to baseline testing. Cardiac screening via ECG was performed at
104 familiarisation during a VO_{2peak} cycling test. Baseline testing occurred 5-10 days prior to
105 commencement of the intervention with post-testing 2 days after 45 exercise sessions. The
106 post glucose clamp was performed 48 hours after maximal cycling and strength tests to
107 provide a washout period that would allow for the bulk of the acute effects of intense exercise
108 on glycaemia to return to pre-exercise levels without inducing a period of deconditioning (19,
109 20).

110

111 *Exercise Protocol*

112 Participants completed 27 cycling and 18 resistance training sessions (4-5 sessions each
113 week). Sessions included a 5-minute warm-up at low intensity on a cycle ergometer or
114 rowing machine followed by 20 minutes of 1-minute interval style cycling or resistance
115 exercise. Pre-programmed cycling sessions (VeloTron Racer Mate, Seattle, WA) included 10

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intervals at 70%-90% (increased 5% every 2 weeks) of the participants' peak oxygen consumption volume (VO_{2peak}) obtained from baseline and fortnightly cycle testing (SensorMedics Vmax, YorbaLinda, CA), with 1-minute active recovery intervals at 40% of peak workload. Resistance training included 5 sets of 30 repetitions of each exercise (Day 1: bench press and seated rows), and (Day 2: lateral pulldowns and barbell upright rows) with 1 minute of crunches on a fitball (Hart Sport, Auckland, NZ) as active recovery. Intensity was set at 20% of 1-repetition maximum (1RM) during weeks 1-2 and increased to 25% of 1RM to elicit a high-intensity workload, for the remainder of the intervention based upon baseline and fortnightly testing. If participants were unable to maintain a set cycling or strength workload for a full minute the subsequent interval was reduced by 10%. All exercise and testing sessions were supervised by the researchers.

Supplement

Participants appeared each morning to the exercise laboratory in a fasted state. A chocolate flavoured whey protein isolate (WPI-895, Fonterra, Auckland, New Zealand) beverage (20 grams protein/10 grams carbohydrate/3 grams milk-fat) or an identically-flavoured but non-protein formulated isocaloric beverage (30 grams carbohydrate/3 grams milk-fat) was consumed immediately before and after each exercise session. Each drink contained 175 calories (731 kilojoules). To reduce hunger and provide opportunity for a clear peri-training whey compared to carbohydrate consumption effect to be observed, each participant consumed a low-protein snack bar (Nature Valley, General Mills, Auckland, NZ) 1 hour after exercise and resumed normal eating habits after 2 hours.

Glycaemic Measures

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Glucose disposal rate (GDR) for each individual was determined via a modified euglycaemic insulin clamp as described previously (21). Briefly, participants appeared for testing between 7 and 9 am after an overnight fast and at least 48 hours after the last exercise testing session. A catheter was placed at the antecubital vein for insulin and glucose infusion, and dorsally at the hand for blood draws. Arterialised blood was obtained by placing the hand in a heater box at 50 °C. Participants received priming insulin doses of 160 $mU \cdot m^2 \cdot min^{-1}$ for 4 minutes and 80 $mU \cdot m^2 \cdot min^{-1}$ for 3 minutes, after which the dosage was reduced to 40 $mU \cdot m^2 \cdot min^{-1}$ for the remainder of the clamp. A 25% glucose infusion was initiated at 15 minutes or sooner if fasting blood glucose levels were below 6.5 $mmol \cdot L^{-1}$and adjusted after 5-minute blood glucose readings until stabilised at 5 $mmol \cdot L^{-1}$. As this method elevated blood insulin concentrations within a physiological rather than a supraphysiological range, the time to stabilisation was variable between participants. GDR was calculated from the average rate of glucose infused ($mg \cdot kg^{-1} \cdot min^{-1}$) during a 60 minute stabilisation phase. Fasting blood samples were obtained to determine FBG and HOMA-IR.

Physical Exercise Capacity

Participants completed a continuous ramp protocol to volitional exhaustion on a cycle ergometer commencing at 40 Watts for 3 minutes and increasing 1 Watt every 4 seconds. Participants were encouraged to maintain a cadence of 70 rpm during the ramp. Peak oxygen consumption ($mL \cdot kg^{-1} \cdot min^{-1}$) was measured as the average of the highest 30-second consumption rate during the test. Acceptance of a maximal effort was dependent upon the participant achieving a maximal Borg Scale (1-20) rating and/or an RER>1.15. Estimated 1 repetition-maximum (1RM) tests were completed at baseline and every 2 weeks for smith

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machine bench press, lateral pulldown, seated row and barbell upright row during a maximal-effort of 3-6 repetitions and predicted via the Brzycki Formula (22).

Body Composition

Body composition measures were taken in a fasted state prior to exercise testing. VL thickness and subcutaneous adipose tissue (SAT) were measured after lying supine for 15 minutes via B-mode ultrasound (Terason T32000, Teratech Corp., Burlington, MA) using previously validated protocols (23, 24) modified to include measurement of SAT at the biceps and cross-section diameter at the VL muscle. Measurements were taken in a supine position after participants had been lying relaxed for 15 minutes and then analysed using ImageJ software (National Institute of Health, Bethesda, Maryland). SAT was determined from the sum of adipose thickness at 4 standard calliper sites: thigh, calf, biceps, and triceps and VL thickness from the maximal cross-sectional diameter measured at 1/3 the distance from the centre of the patella to the tubercle of the anterior superior iliac spine.

Statistical Methods

Sample size estimation was based upon the primary outcome GDR using the test-retest values reported by Defronzo et al (25) in a healthy adult population and upon sample size estimations for magnitude based clinical inference (26, 27). The typical error of measurement was doubled to allow for uncertainty in variability in a T2D population and *n* was increased by 10% to allow for potential dropouts, which brought the required sample to 24. The threshold for smallest worthwhile clinical change in GDR was 5.4% based upon the effect of 3 months of hypoglycaemic therapy (Metformin) on naïve Type-2 diabetics (28).

The effect of treatment and time on all dependent variables was estimated from mixed models (Proc Mixed, SAS Version 9.1; SAS Institute, Cary, NC). Data were log transformed prior to analysis. Total 1RM strength was expressed as the back log-transformed average of 4 log-transformed lift scores. Uncertainty was presented as 90% confidence limits or *P* value. Magnitude-based inference was employed to infer clinical and mechanistic outcome effects (27, 29). The probability that a contrast was at least greater than the clinical threshold or smallest Cohen's *d* standardized difference (0.2 × baseline SD) was: 25-75% possible, 75-95% likely, 95-99.5% very likely, >99.5% almost certain (27). In the case where the majority (>50%) of the CI lay between the thresholds for positive and negative substantiveness, the effect was qualified trivial (negligible) with the respective probabilities as above (30). The terms *benefit*, *trivial (negligible)*, and *harm* refer to the most likely directional outcome, relative to the smallest effect threshold. The terms *unclear, inconclusive* refers to outcomes where the likelihood of both benefit and harm exceeded 5%. The likelihood of a clinical benefit of intervention was expressed as the benefit:harm odds ratio, with 66:1 the smallest adoption threshold (27). Pre- and post-intervention scores are presented in figures as raw means and standard deviations.

Results

Twenty-four men with T2D were recruited to the study (Figure 1). There were no clear differences between group characteristics at baseline (Table 1). All participants completed the 45 exercise sessions within the 10-week period. The glycaemic control outcomes and statistical summary for all parameter measures are in Figure 2 and Table 2, respectively. Ten weeks of MMIT produced a clinically meaningful enhancement in GDR in

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the whey and control groups, respectively, relative to the smallest threshold change (5.4%); however, the whey-control difference was unclear. The secondary outcome measures of glycaemic control (FBG and HOMA-IR) showed a likely and possible benefit of whey supplementation on FBG, and possible and unclear benefits on HOMA-IR in the whey and control groups respectively, reaching the adoption threshold (OR>66:1) only in the Whey group; however, there was also no clear difference in the Whey-Control contrast. Very likely and almost certain improvements in VO_{2peak}, 1RM strength and VL muscle thickness in response to 10-weeks of MMIT in both the Whey and Control groups were observed (Figure 3), but the whey-control differences were also negligible and unclear. There was a possible decrease in WC in both groups and a possible decrease in SAT in the whey group only (Table 2).

Discussion

The current study showed that consumption of 20 grams of whey protein before and after MMIT for 10 weeks did not enhance glycaemic control in a T2D population assessed via measures of glucose disposal rate, fasting blood glucose, and HOMA-IR. Similarly, whey supplementation did not enhance any of the exercise performance adaptations accruing in response to MMIT, including VO_{2peak}, 1RM strength, and muscle thickness. While previous evidence indicates that whey supplementation and high-intensity interval training independently improve glycaemic control (6, 13), no clear benefit of combined therapies was observed.

Previously, consumption of 10 grams of whey protein hydrolysate before and after resistance training for 10 weeks was shown to significantly increase quadriceps cross-

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sectional area in healthy trained men (31). In addition, consumption of a single-dose of a mixed milk-protein (20 grams) carbohydrate beverage after treadmill training for 6 weeks significantly increased VO_{2max} in sedentary middle-aged men compared to an isocaloric carbohydrate control (14). As both the increase in mid-thigh muscle cross-sectional area and VO_{2peak} following exercise intervention have been previously associated with improved HbA1c in populations with Type-2 diabetics (9, 17), we predicted that peri-training whey supplementation for 10 weeks would lead to better glycaemic control than the MMIT alone. Our observation that whey supplementation did not clearly increase muscle thickness at the VL or VO_{2peak} suggests that adaptive responses previously seen in exercising healthy populations may be lost with the development of T2D and may explain why we saw no effect of whey protein on GDR, FBG or HOMA-IR.

It is possible that adults with T2D require a larger dose of milk-protein to induce clinically meaningful outcomes. 20 grams of protein has been reported to be the optimal dosage for improving protein synthetic responses in the skeletal muscle of healthy young men (32). While we also provided a total of 40 g of protein as 20 g before and 20 g after MITT, in another study in healthy elderly individuals (71±4 y), 40 grams of whey protein increased muscle protein synthesis after resistance training compared to a 20 gram dose (33). The cohort in the current study was middle-aged (55.6±5.7 y), however, T2D skeletal muscle has been shown to display characteristics of aged tissue, including: accelerated muscle wasting (34); lower contractile strength to muscle volume (35), and decreased mitochondrial density (36). Future investigations should test dosage effects on muscle protein synthetic responses in a population with T2D.

While we saw no clear benefits of whey supplementation on glycaemic control in this study, there was some evidence that the protein exposure produced a more pronounced effect

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on each of the glycaemic measures compared to exercise alone, as suggested through the observation of a substantially larger clinically-beneficial odds ratios for GDR, FBG, and HOMA-IR in the whey compared to the control group. We also observed that the adoption threshold (odds ratio >66) was reached for FBG, HOMA-IR and SAT only in the whey group, suggesting that the magnitude of the improvements in those secondary outcomes was sufficient to justify treatment use only when therapies were combined. It is important to acknowledge, however, that the full placebo-control adjusted outcome (whey-control), which takes into account the on-study effect, left a statistically unclear whey-protein effect. We suggest that a longer intervention or a larger cohort (to increase study power) may have clarified whether whey supplementation was enhancing the pattern for improvement in clinical outcomes.

An inherent potential confounder of investigations with control of energy intake was that the control group was consuming substantially more carbohydrate each training morning than the whey group (60 compared to 20 grams). We reasoned that while there was potential for the control group to be consuming more carbohydrate than their normal dietary intake, which could be deleterious to glycaemic control, we expected that the metabolic demands of 20 minutes of MMIT would obviate any effect on post-exercise blood glucose concentration in a previously sedentary population. In addition, 6 x 20 minute sessions of high-intensity interval cycling was previously shown to significantly improve postprandial and 24-hour blood glucose regulation in middle-aged adults with T2D (6). Our findings confirm that chronic intense interval training is effective for improving glycaemic control in populations with T2D. We also found that the 5-days per week, mixed-mode training regime was well-adhered to by a previously sedentary, middle-aged T2D population, improved glucose disposal rates by a 4-5-fold greater magnitude than an equivalent duration of

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pharmacotherapy (Metformin) alone (28), and negated the potentially deleterious impact of consuming 2 × 30 grams of a carbohydrate beverage each morning. Therefore, the MMIT mode of exercise training may prove to be highly effective for improving T2D health outcomes in long-term rehabilitation programs where high intensity exercise is appropriate.

In conclusion, consumption of 20 grams of whey protein before and after high-intensity mixed-mode interval training for 10 weeks, compared to isocaloric non-protein control, did not clearly enhance glycaemic control, VO_{2peak}, 1RM strength, or VL muscle cross-section diameter in middle-aged men with T2D. These findings suggest that over short-term interventions, populations with T2D may be resistant to nutritional stimulation of this nature. However, recent dose response data, and patterns for greater gains in some clinical parameters in the whey group support further investigation of the nutritional intervention, possibly increasing the supplement dose or the intervention period.

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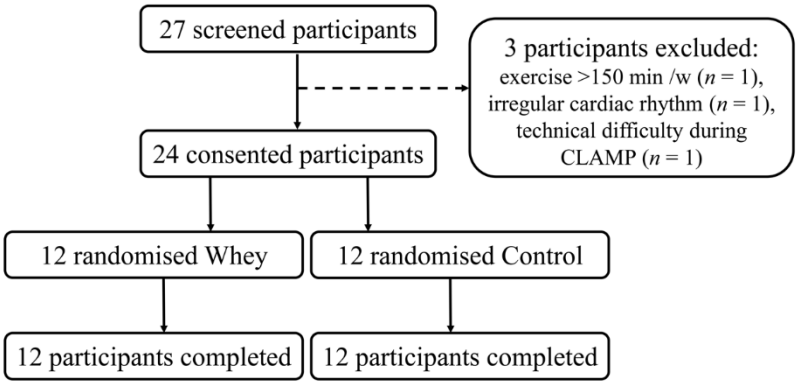

Figure 1. Recruitment flowchart.

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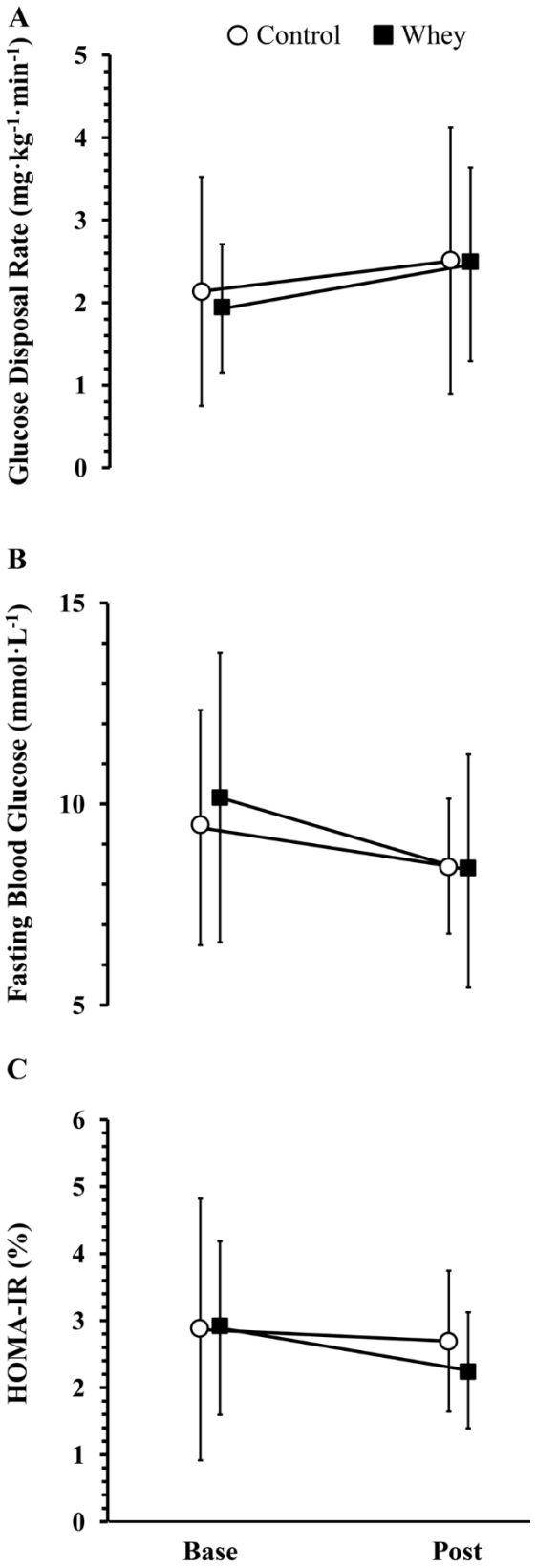

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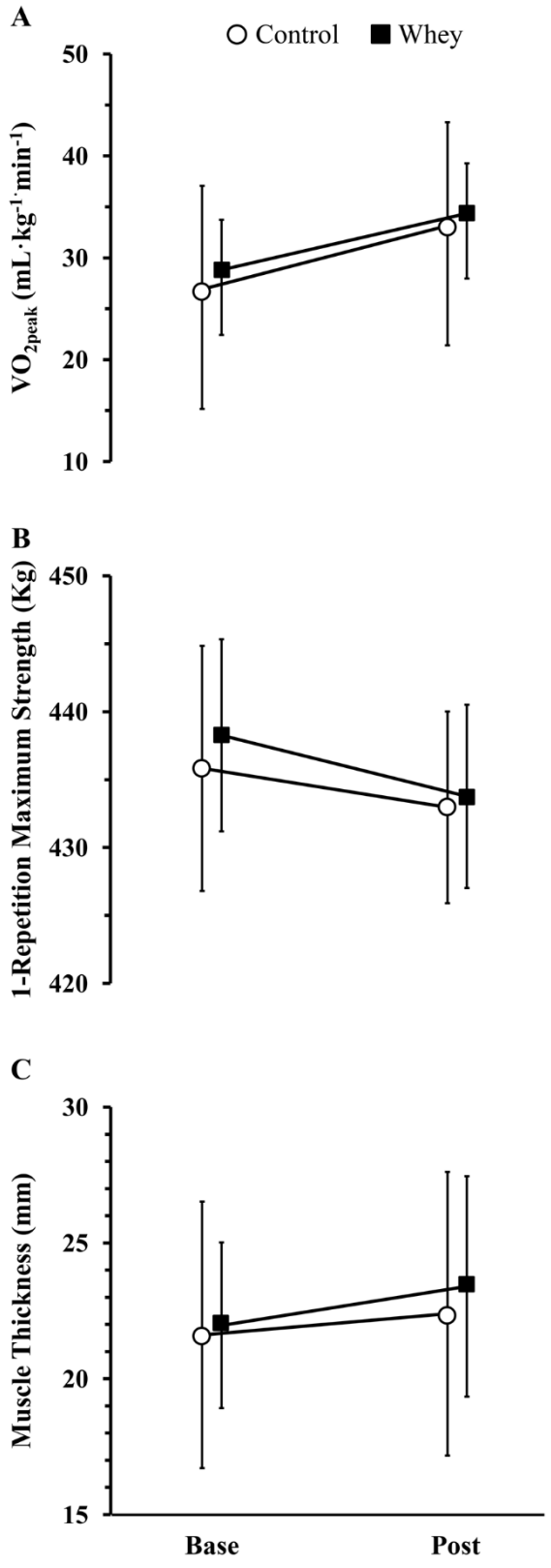
409 **Figure 2.** Effect of 10 weeks of peri-training whey supplementation on: A) glucose disposal
410 rate; B) fasting blood glucose concentration; and, C) HOMA-IR. Data are raw means and SD
411 for the Pre (baseline) and Post testing time points.



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413 **Figure 3.** Effect of 10 weeks of peri-training whey supplementation on: A) VO_{2peak}; B) 1RM
414 strength (the back log-transformed average of 4 log-transformed lift scores); and, C) *vastus*
415 *lateralis* muscle thickness. Data are raw means and SD for the Pre (baseline) and Post testing
416 time points.

417

Table 1. Baseline characteristics of the Control and Whey groups.

	Control *n*=12	**Whey** *n*=12
Parameter	Mean SD	Mean SD
Age (y)	57.8 ± 5.2	53.5 ± 5.6
Height (cm)	174.6 ± 7.1	177.1 ± 8.7
Weight (kg)	91.9 ± 15.5	92.8 ± 11.0
BMI ($kg \cdot m^{2}$)	30.1 ± 4.9	29.6 ± 2.7
VO_{2peak} ($mL \cdot kg^{-1} \cdot min^{-1}$)	26.9 ± 10.2	28.7 ± 4.9
FBG ($mmol \cdot L^{-1}$)	9.4 ± 2.9	10.2 ± 3.6
GDR ($mg \cdot kg^{-1} \cdot L^{-1}$)	2.11 ± 1.4	1.93 ± 0.8
Time to euglycaemia (min)	106.3 ± 67.2	106.7 ± 53.9

Data are presented as means and standard deviations.

418

Table 2. The effect of 10-weeks peri-training whey-protein supplementation on established clinical measures of glycaemic control, exercise performance, and body composition.

Contrast[a]	**% Change**	**Upper CI**	**Lower CI**	**Likelihood (%) benefit/trivial/harm**[b]	**Qualitative**[b]	**Benefit odds**[b]
			Glucose Disposal Rate			
Control	24.8	64.8	-5.4	90.1/7.1/2.8	Benefit likely	318
Whey	27.5	60.7	1.2	95.6/3.5/0.9	Benefit very likely	2424
Whey-Control	2.2	44.8	-28.0	42.6/24.4/33.0	Unclear	2
			Fasting Blood Glucose			
Control	-8.1	10.7	-23.7	50.4/45.8/3.8	Benefit possible	26
Whey	-17.4	-1.6	-30.6	88.8/11.0/0.2	Benefit likely	3291
Whey-Control	-10	15.3	-29.8	57.3/35.9/6.8	Unclear	19
			HOMA-IR			
Control	-5.3	28.3	-30.1	23.7/68.8/7.6	Unclear	4
Whey	-14.1	1.08	-25.3	42.0/58/0.0	Benefit possible	3331

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Whey-Control	9.2	25.4	-34.2	35.0/59.1/6.0	Unclear	8
				VO_{2peak}		
Control	22.6	26.2	12.0	99.8/0.2/0.0	Benefit almost certain	5.05E+07
Whey	18.5	27.4	10.5	99.1/0.9/0.0	Benefit very likely	2.81E+06
Whey-Control	-3.3	9.07	-8.75	4.4/69.1/26.5	Trivial possible	0
				1-Repetition Maximum Strength[c]		
Control	20.6	24.9	16.3	100/0.0/0.0	Benefit almost certain	3.29E+31
Whey	22.7	27.2	18.4	100/0.0/0.0	Benefit almost certain	7.80E+35
Whey-Control	1.8	7.1	-3.2	0.1/99.8/0.0	Trivial almost certain	11
				Muscle Thickness		
Control	18.9	26.2	12.0	100/0.0/0.0	Benefit almost certain	1.78E+09
Whey	18.6	27.4	10.5	99.89/0.02/0.0	Benefit almost certain	6.62E+07
Whey-Control	-0.2	9.1	-8.8	13.6/70.6/15.9	Unclear	1
				Waist Circumference		
Control	-2.1	-1.0	-3.1	41.0/59.1/0.0	Benefit possible	7.44E+05
Whey	-1.9	-0.1	-3.7	28.6/71.4/0.0	Benefit possible	2888
Whey-Control	0.1	2.1	-1.8	0.3/99.5/0.2	Trivial very likely	2
				Subcutaneous Adipose Tissue[d]		
Control	-1	6.9	-8.3	6.7/90.8/2.5	Trivial likely	3
Whey	-6.9	3.5	-16.2	43.7/55.8/0.5	Benefit possible	151
Whey-Control	-6.0	6.7	-17.1	40.1/57.9/2.0	Benefit possible	32

[a] Data for each contrast are post-pre. [b] The threshold for smallest clinical effect for glucose disposal rate was 5.4% (28); and for all other measures the smallest standardised difference (0.2xSD). The likelihood that a contrast was at least greater than the clinical threshold was: 25-75% possible, 75-95% likely, 95-99.5% very likely, >99.5% almost certain. Unclear refers to outcomes where the likelihood of both benefit and harm exceeded 5%. The clinical adoption threshold was expressed as a benefit: harm odds ratio >66:1. [c] Total 1-Repetition Maximum strength was expressed as the back log-transformed average of 4 log-transformed lift scores. [d] Subcutaneous Adipose Tissue was expressed as the sum of 4 sites.

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