EFFECT OF WHEY PROTEIN QUALITY ON PHYSIOLOGICAL RESPONSE TO CHRONIC RESISTANCE EXERCISE IN TRAINED MEN: A DOUBLE-BLIND, PLACEBO-CONTROLLED, RANDOMIZED TRIAL

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By

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EFFECT OF WHEY PROTEIN QUALITY ON PHYSIOLOGICAL RESPONSE TO CHRONIC RESISTANCE EXERCISE IN TRAINED MEN: A DOUBLE-BLIND, PLACEBO-CONTROLLED, RANDOMIZED TRIAL

A DISSERTATION APPROVED FOR THE
DEPARTMENT OF HEALTH AND EXERCISE SCIENCE

BY

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DEDICATION

To my wife, Beth, whom I committed on our wedding day that I would always strive to make proud. You have been patient, supportive and loving throughout all that has been sacrificed of our time together to accomplish this goal. May our lives begin anew as a family, a couple, as parents, as best of friends, and as God's promise of loving companions with which to best serve His purpose on this earth....until death do us part.

To Avery, whom I would sacrifice everything of myself to provide a life filled with God's promise of joy and abundance of opportunity to His children. May you NEVER doubt in yourself, God's love for and forgiveness of you, the truth that you are not defined by the events of your life or others but rather how you respond to those events and serve others....and may you never doubt in the unconditional love I will always have for, and bestow upon you.

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ABSTRACT

Acute studies have revealed that insulin and possibly incretin hormone [e.g., glucagon-like peptide-1 (GLP-1)] response in humans is significantly affected by whey protein (WP) form [e.g., whey protein isolate (WPI) versus hydrolysate (WPH)], whereas extensive hydrolysates of casein protein, versus native casein, were recently shown to promote a potentially greater (p=0.10) acute muscle protein synthesis response. Similarly, fractions and specific peptides from WP have been identified that may potentiate exercise recovery and/or the muscle protein synthesis response from heavy resistance training. However, to date, no study has compared the chronic effects WP form or molecular distribution may have when consumed in combination with heavy resistance training. **PURPOSE**: Therefore, the primary purpose of this investigation was to compare the effects of three different variations of a WP on the physiological response to weight training in previously resistance trained, healthy males. **METHODS**: Fifty-six resistance trained men (21.40±0.36 yrs; 79.46±1.04 kg; 178.59±0.66 cm; 1.24±0.03 1RM bench press-to-body mass ratio) were randomly assigned to receive one of four double-blinded treatments: 30 g/serving carbohydrate (PLA) or 30 g/serving protein from either a) 80% whey protein concentrate (WPC80), b) high lactoferrin containing 80% WPC (WPC80+), or c) extensively hydrolyzed WPC80 (WPH). All subjects participated in eight weeks of a split-body, linear periodized resistance training program, and consumed two servings of treatment per day (one immediately pre- and post-exercise on training days; twice between meals on non-training days). Body composition, upper- and lower-body strength [1RM Bench Press (1RM BP) and 1RM Hack Squat (1RM HS),

respectively] and anaerobic endurance [80% of 1RM for maximal repetitions (80RM BP and 80RM HS, respectively)], and fasted blood measures were assessed before (PRE) and after (POST) the 8-week intervention. Twenty-four hour muscle damage (CK) and immune (WBC) response to lower-body resistance training was assessed during Week 1 and 8. Also, total repetitions to failure, CK and WBC were assessed during POST, prior to and in response to repeated daily (x3) bouts of 80RM HS. Two-way repeated measures ANCOVAs were used for statistical analyses. Significance was set at $\alpha = 0.05$. **RESULTS**: No significant differences ($p>0.05$) were observed between groups for total training volume (kg/min), or relative energy (kcals/kg/d), protein (g/kg/d), carbohydrate (g/kg/d), or lipid (g/kg/d) during the 8-week intervention. All groups increased ($p<0.0125$) 1RM BP (kg), 1RM HS (kg), 80RM BP (reps) and 80RM HS (reps) from PRE to POST, however, no significant ($p>0.05$) between-group effects were observed. For repeated 80RM HS tests, only WPC80+ realized a significant difference for total repetitions completed between any of the three days of testing (+15.56% more repetitions for $80RM_{24}$ versus $80RM_{POST}$; $p<0.0125$). No significant between- or within-group ($p>0.05$ and $p>0.0125$, respectively) changes were observed for 12-hour fasted blood lipids, glucose, WBC or CK from PRE to POST; however, all groups reduced ($p<0.0125$) creatinine, and WPH creatinine at POST was shown to be significantly different from WPC80+ (-14.218%Δ; $p<0.05$). Urea nitrogen (BUN) was also shown to decrease significantly ($p<0.0125$) from PRE to POST for WPH (-18.064%), which differed significantly ($p<0.05$) from WPC80 (+16.908%; $p<0.0125$). CK response to Week 1 versus Week 8 lower-body exercise, however, decreased significantly in all groups except WPH (-60.327%; $p=0.073$), and no significant differences occurred between- or

within-groups for WBC. Likewise, repeated 80RM HS resulted in no significant between group differences (p>0.05) for either CK or WBC. Lean body mass and total body muscle mass increased (p<0.0125) in all groups, as did body mass in all groups except WPH (+0.641 kg; p=0.114). However, WPH realized a significant PRE to POST reduction in fat mass (-5.942%) and percent body fat (-1.601%), which was significantly different (p<0.05) from PLA (+9.100% and +0.640%, respectively). **CONCLUSION**: In previously trained, college-aged men, 60 g/d of WPC80, WPC80+, WPH or PLA provide similar responses to an 8-week heavy resistance training program on measures of total body muscle mass, strength, anaerobic endurance and blood lipids. However, WPH appears to significantly augment lipolysis and may increase nitrogen retention.

INTRODUCTION

Resistance exercise, both in the fasted and postprandial state, and protein (PRO) ingestion independent of exercise have each been shown to stimulate muscle protein synthesis (MPS).(1-3) However, combined strenuous resistance training or recovery from exercise in the presence of increased essential amino acid (EAA) availability has been shown to significantly increase exercise-induced MPS and more dramatically affect measures of anabolic response and adaptation to training than exercise alone or exercise plus carbohydrate. Carbohydrate (CHO) intake, however, does not appear to directly influence MPS, but rather, via insulin, has been shown to act as an antagonist to muscle protein breakdown (MPB) but cannot ultimately raise exercise-induced MPS in the absence of increased EAA availability.(1-4) Optimally, when MPS > MPB then net protein balance is positive; when repeated chronically over time, the cumulative effect can lead to increases in muscle fiber hypertrophy.

Whether PRO should be consumed prior to, following, or both pre- and post-exercise to most effectively stimulate chronic changes in muscle hypertrophy and strength is less clear.(5) Acute data indicates that MPS will be significantly elevated so long as resistance exercise is performed in the presence of increased EAA availability or provision of EAA are made available within 1-2 hours, and not beyond 4-5 hours post-exercise.(6-8) Furthermore, Bohe et al. (9) concluded that it is the extracellular concentration of EAA rather than intracellular availability that significantly affects MPS. Assuming peak EAA concentrations occur between 20-90 minutes post-ingestion of fast-

absorbing proteins (e.g., whey) (10-13), and exercise duration lasts 45-90 minutes, consuming PRO both pre- and post-exercise, as opposed to any one feeding time alone, may elicit the most significant effects on resistance training adaptations.

Specifically, the magnitude of myofibrillar protein synthesis arising from acute resistance exercise performed under fasted conditions indicates that the response is intensity dependent at low training intensities (relative to one-rep maximum, or 1RM), but plateaus when training involves 60-90% 1RM loading. Additionally, myofibrillar protein synthesis, regardless of age, appears to return to near baseline levels by 2-4 hours post-exercise under fasted conditions and thus establishes some basis for the need to increase nutrient availability within this optimal post-workout "window."(6) For example, when whey protein isolate (WPI) was provided immediate post-exercise, myofibrillar protein synthesis has been reported to remain significantly elevated (+229%) by up to five hours post-exercise.(7) An intriguing finding of the aforementioned Moore et al. (7) study was that sarcoplasmic protein synthesis was significantly elevated at three hours post-WPI ingestion alone, and was not further increased in response to combined resistance training and PRO ingestion. In myofibrillar protein, a significantly additive response was observed beginning at one hour and continuing through five hours post-exercise WPI ingestion. Such a finding would seem to support the hypothesis that chronic PRO ingestion at intervals of approximately every 2-3 hours may optimally maintain a positive anabolic state.

Tipton et al. (14) concluded that 20 g of whey PRO (WP), consumed either immediately

before or one hour following ~25 minutes of heavy, intermittent knee extension training at 80% 1RM, in previously inactive, but healthy subjects, resulted in equivocal net muscle protein balance; each trial raising arterial AA concentrations by ~50% above baseline. These results may, however, be specific to the population (previously untrained), protein source (WP) and/or duration of the fasting period prior to subjects receiving the pre-exercise PRO bolus.(15) Specifically, with regards to population training status, acute exercise bouts have been shown to result in a more rapid, but short-lived rise in MPS in response to training; whereas peak MPS and its decline occurs more gradually in previously untrained muscle. For example, Tang et al. (8) reported that, under fasted conditions, a 162% increase in MPS occurred in response to training by four hours post-exercise, which was significantly greater ($p<0.01$) than the 108% rise in MPS at the same timepoint in previously untrained. Furthermore, MPS appeared to peak at 16 hours post-exercise in previously untrained muscle, whereby MPS under trained conditions was not significantly different from baseline at 16 hours post. By 28 hours post-exercise, MPS was still significantly elevated in the untrained group (+70%; $p<0.01$).

In light of the aforementioned data, it is of little surprise that no significant differences were observed for pre- versus post-exercise PRO ingestion in the Tipton et al. study (14): subjects had not engaged in regular resistance training for at least five years, and PRO balance was assessed for only four hours post-exercise. Thus, the anabolic response to combined resistance training and timed PRO ingestion may be more predicative in previously trained populations. For example, Hulmi et al. (16) reported that 15 g of WP,

consumed immediately prior to and following twice weekly resistance training in previously untrained males, significantly increased acute (1- and 48-hour post exercise) and chronic (after 21 weeks) mammalian target of rapamycin (mTOR) signaling response versus nonenergetic placebo. Despite significant increases in mTOR signaling, which can stimulate MPS and hypertrophy, type I (slow-twitch) and type II (fast twitch) muscle fiber cross-sectional area of the vastus lateralis, as assessed by immunohistochemical staining, was no different between WP and placebo. Body mass and muscle thickness, as assessed by ultrasonography, was however significantly greater in the WP group by 10.5 weeks and again after 21 weeks. Non-energetic placebo plus exercise resulted in only a statistically significant within-group change in muscle thickness which was not significantly different from non-exercise control. In response to 10 weeks combined heavy resistance training (4 d/wk x 6-8 reps/set x 3 sets @ 85-90% 1RM) and 20g PRO or CHO supplementation consumed one hour prior to and following exercise, in previously untrained men, Willoughby et al. (17) did, however, report greater gains in markers of MPS, anabolism and performance from PRO. In resistance-trained subjects, Kerksick et al. (18) reported that 48 g/d of a PRO blend consisting of WP (40 g/d) and casein (CP; 8 g/d), versus a blend of WP (40 g/d), branched chain amino acids (BCAA; 3 g/d) and glutamine (5 g/d), or 48 g/d CHO, resulted in the most significant improvements on body composition following a similar 10-wk training program as was used in the aforementioned Willoughby et al. (17) study. Changes in maximal upper- and lower-body strength and anaerobic capacity were, however, equal amongst groups in the Kerksick et al. trial. It appears then, from the above studies, that, 1) combined strenuous resistance training and WP supplementation differentially affects the physiological

benefits observed in trained versus untrained males (i.e., metabolic adaptations may be more affected in trained subjects, whereas the response in untrained may be more global), and 2) PRO source appears to influence physiological response (e.g., higher total dose of native PRO was more effective than partial substitution with free amino acids).

To further elaborate on the potential contribution of PRO source on physiological adaptations to resistance exercise, Cribb and colleagues (19) provided evidence that 1.5 g/kg b.w./d x 10 wks of whey protein hydrolysate (WPH; as derived from WPI) increased fat-free mass (FFM), reduced fat mass (FM), and resulted in significantly greater (p<0.05) gains in absolute and relative strength in recreational bodybuilders, when compared to consuming an hydrolysate of CP (CPH). Similarly, Tang et al. (20) recently reported that, in healthy but untrained males, an acute dose of 10 g of EAA from WPH increased MPS significantly more than an equivalent dose of EAA from soy protein isolate (SPI) or CP at rest (+18% and +93%, respectively) and in response to resistance exercise (+31% and +122%, respectively). Blood EAA, total BCAA and leucine concentrations were also significantly higher in response to WPH. One limitation to the study, however, was that WP quality [using WPH as opposed to a native whey protein concentrate (WPC) or WPI] may have been a significant contributing factor in eliciting the improved effects when compared to native CP or SPI. For example, Tipton et al. (21) found no significant differences in net phenylalanine balance for up to five hrs post-exercise in response to consuming 20 g of either CP or WPI one hour after exercise, in healthy but untrained men and women. The WPI did, however, result in significantly higher peak and total area under the curve for insulin, intracellular leucine concentrations,

and net leucine balance, compared to CP and placebo. Eighteen grams of milk protein isolate (MPI; a mixture of 20% WP and 80% CP) did, however, significantly increase MPS and total area under the curve for net protein balance when consumed immediately post-exercise and compared to the effects of 18 g SPI in healthy, young male subjects.(22) Anabolic superiority of dairy protein over soy was not observed, however, when compared on a macro physiological level in response to six weeks of combined resistance training and PRO supplementation.(23) Candow et al. (23) concluded that both WP and SP provided comparable improvements above exercise alone, and that these results were independent of PRO source. Two methodological concerns arise in interpreting the findings of this study, though: 1) 18 female and 9 male subjects were divided across three groups which may confound sex specific responses and statistical power, and 2) study duration may have been too abbreviated to observe mean differences between groups of such a small sample size. It has also been reported that SP preferentially increases splanchic protein synthesis as opposed to peripheral tissue protein synthesis (e.g., MPS), and that conversion to urea is greater for soy than from dairy.(24, 25) Exceptions notwithstanding, it appears then that dairy protein, and possibly WPH in particular may elicit the most profound effect on MPS and EAA availability; most probably during acute feeding or in response to heavy resistance training. If true, the combination of effects may improve chronic adaptations to exercise.

No data, however, is available to directly assess the chronic impact of combined resistance training and WP form or molecular composition. Instead, hypothetical extrapolation from sparse acute observations involving WP and other PRO sources is

currently necessary. From work involving acute bouts of high-intensity resistance exercise in trained, healthy men, and employing a 4-hour primed constant infusion (of radiolabeled l-leucine), Moore et al. (26) revealed, for example, a curvilinear rise in MPS from immediate post-exercise ingestion of 0, 5, 10, 20 and 40 g of egg albumin PRO. MPS increased under fasting conditions (0 g PRO) and was approximately 37%, 56% and 93% greater than the fasted response for 5, 10 and 20 g PRO trials, respectively. The rise in MPS from 20 to 40 g was not significantly different and there was no significant effect, at any PRO dose, on downstream signaling proteins of mTOR, insulin or glucose concentrations over time. Therefore, collectively supporting the widely held conclusion that stimulation of MPS is most affected by EAA availability, independent of insulin or glucose. Cuthbertson et al. (27) came to a similar conclusion when comparing the effects of consuming a 0, 2.5, 5, 10, or 20 g EAA solution in healthy young versus elderly men after a 12-hour fast. Here, insulin and growth hormone were both clamped to maintain baseline plasma insulin concentrations (~10 mIU/L) throughout the 4-hour duration and across all EAA doses. Again, a dose-dependent rise as well as a ceiling effect was observed in the ability to stimulate MPS. Specifically, a dose-dependent increase in myofibrillar protein synthesis was observed up to 10 g, and was not significantly different for 10 versus 20 g ingested EAA in the younger males. Sarcoplasmic protein synthesis followed the same pattern, but to a lesser extent. The patterns observed in the elderly subjects yielded similar curvilinear slopes, albeit shifted down and to the right; indicative of decreased MPS stimulatory sensitivity and responsiveness with aging. The lack of an insulinotropic response to PRO ingestion as well as the lower relative changes in MPS observed by Moore et al. (26) may likely be the result of PRO source and/or quality. For

example, Koopman et al. (13) showed that CPH was significantly more effective than intact CP at increasing insulin and MPS response, as well as increasing plasma AA concentrations. Deglaire et al. (28), however, recently showed otherwise. CPH indeed resulted in a faster rate of absorption, eliciting both an earlier and stronger rise in plasma insulin and AA concentrations, but there was no significant difference between CP and CPH for whole-body nitrogen retention. Power et al. (11), however, presented evidence that ingestion of 45 g WPH provides a significantly higher and total (as measured by area under the curve) insulin response when compared to consuming an equal dose of WPI. In fact, Claessens et al. (29) showed that both plasma insulin and glucagon rises, and glucose falls in a dose-dependent (0.3 to 0.6 g/kg b.w.) fashion for WPI, SPI and their extensively hydrolyzed proteins (WPH and SPH, respectively) in healthy, fasted males. However, no such dose-dependent effect on glucose response was observed for SPI and dose-dependent flux across all proteins and doses tested was most profound for glucagon. Interestingly, SPI resulted in higher total area under the curve responses for both insulin and glucagon compared to SPH, whereas no significant differences were noted between WPI and WPH. The latter may be due to the high concentration of insulinotropic BCAAs present in WPI, though. *In vitro* and *in situ* data, for example, revealed that of the seven identified BCAA-containing dipeptides isolated (by extensive hydrolysis) from WP, all of the identified dipeptides caused significant uptake of glucose into myotubes. The principle BCAA dipeptide present in WPH, Isoleucine-Leucine, also was shown to stimulate glucose uptake into, and increase glycogen content in isolated skeletal muscle.(30) Similarly, when a 9.3 g PRO/L solution containing either 80% whey protein concentrate (WPC80) or CP, or their respective hydrolysates (WPH and CPH), was

consumed in random cross-over fashion by healthy male volunteers after an overnight fast, the hydrolysates resulted in 50% greater gastric secretions than their native PRO counterparts. Directly in support of the insulinotropic response of hydrolysates versus intact PRO, hydrolysates significantly increased glucose-dependent insulinotropic polipeptide (GIP) secretion within the first 20 minutes of gastric emptying. GIP, as its name suggests, stimulates insulin release. Despite no significant differences for rate of gastric emptying, there was an observed faster rate of appearance and peak concentration for BCAA, EAA and total amino acids from hydrolysates. Specifically, concentrations peaked at about 20 minutes post-prandial, but only the concentrations for CPH versus CP reached significance.(10)

Of note, Claessens et al. (12) reported that hydrolyzed protein from pea, rice, soy, gluten, whey or egg all provide significant increases, but not significantly different effects (with only one exception) on plasma concentrations of insulin and glucagon response in overnight fasted, healthy but sedentary adult males. Of specific attention in the methods, however, is that the degree of hydrolysis (DH) across all PRO sources reportedly varied by 9 to 27%, which has been shown to have a significant effect on absorption kinetics due to the molecular weight distribution of the resulting PRO fractions.(31, 32) Controlling for total PRO concentration, but unfortunately not DH, Foltz et al. (33) reported greater stimulation of receptors involved in controlling satiety *in vitro*: hydrolysates of soy>potato>casein, whereas WPH and pea protein hydrolysates were ineffective. In young men provided 50 g of WPI, SPI or egg albumen one hour prior to a meal, researchers found that only WPI and SPI significantly reduced total energy intake

in the subsequent meal. This effect wasn't maintained, however, when the treatment occurred late in the morning (~1100h) as opposed to early (~900h) – when the treatment was conducted in the late morning hours, both SPI and egg increased subsequent meal energy intake. Replacing 25 g of SPI with 25 g of a low or high glycemic CHO (amylose or glucose, respectively) also ameliorated the suppression of subsequent meal energy intake from SPI preloading. And lastly, when 50 g of WPI was compared to 50 g of its hydrolysate (WPH), duration of satiety was extended by 50%.(34) These results support findings observed in rodents (35) fed an *ad libitum* diet consisting of 55% of total energy from WPI, WPC or milk protein concentrate (MPC) for 25 days. It was determined that WPI>WPC>MPC decreased energy intake and bodyweight gain, with WPI providing the most significant energy efficiency (weight gain / energy intake). Fasting blood lipids, insulin and fat mass were also significantly lower for WPI than for the other groups (WPI>WPC>MPC). The importance of these seemingly tangential observations is that, again, the speed at which AA enter the small intestine and can increase extracellular AA concentrations appears to have widespread effects on a number of physiological outcomes with specific application to changes in body composition and, potentially, exercise recovery.

Amino acids and PRO are in fact amongst the most potent secretagogues within the gastrointestinal tract. The faster their (AA) rate of appearance into the small intestine and at the brush-border membrane, the more profound the response that could be expected from an increase in nitrogen or peptide availability. Extensively hydrolyzed proteins – characterized as the majority (>80%) of PRO fractions as ≤1 kD molecular weight, or

typically equal to or less than ~8 AA in length – would theoretically provide the greatest benefit toward improved rate of availability and increased nitrogen retention. However, very little evidence has directly assessed this research question in humans. Calbet and Holst (10) indeed reported significantly faster rises in plasma AA concentrations from CPH as opposed to intact CP, yet no significant effect was observed from WPH versus WPI though the slope of their respective absorption curves tend to support the faster absorption of AA from hydrolysates. The average peptide length reported for the hydrolysates used in the Calbet and Holst trial was extremely well controlled across both hydrolysates and reported to be an average of 3.8 AA in length. Using direct gastric infusion into the small intestine of male volunteers, Grimble et al. (36) reported that low molecular weight versus high (M.W.) significantly increased absorption and retention. Nitrogen retention in starved rats re-fed WP or CP versus their respective hydrolysates have also shown significant benefit from hydrolysates.(37) However, in the aforementioned study, PRO fraction average molecular weight varied greatly between hydrolysates. PRO hydrolysates with the greatest concentration of PRO fractions occurring between 0.2-5 kDa have also been reported to increase plasma concentrations of AA, nitrogen retention and PRO efficiency, when compared to providing free AA to starved rats.(38) However, AA profile of the two diets was not controlled, but rather only total energy from PRO. *In vitro* evidence to determine the most hypoallergenic PRO formulas for use in non-breast fed infants or in persons with dairy allergies has similarly concluded that providing peptides of <1.4 kD results in minimal antigen binding; below 0.97 kD resulted in no antigen binding.(39) Such findings highlight the importance of the H+-oligopeptide cotransporter, Pept-1, specific to the absorption of di- and tri-peptides

(note: Pept-2 transporters are also present within the body, and both Pept-1 and Pept-2 are active beyond intestinal absorption of AA; however, such a discussion is beyond the scope of this paper). These transporters, located predominantly on the brush-border membrane of the intestinal mucosa and basolateral membrane have been reported to transport some 400 known dipeptides and 8,000 tripeptides (40), and have become major areas of focus in drug discovery and delivery because of the Pept-1 transporter's high capacity and low substrate specificity.(40-42) This contrasts greatly to the low capacity, high substrate specificity for uptake of free amino acids. In fact, bioactive peptides derived from hydrolyzed dairy PRO may hold great promise for a number of metabolic conditions, such as Type 2 diabetes or insulin-resistance, as well as metabolic wasting diseases and aging. For example, the *in vitro* and *in situ* data presented previously (30) and involving BCAA-containing dipeptides derived from WPH, may have application in diseases affecting glucose metabolism. Similarly, in human patients with advanced HIV-infection, 45 g/d WPH significantly increased glutathione levels by two weeks and remained significantly elevated after six months of chronic ingestion. Also of note, body weight, T-cell counts and other clinical measures did not deteriorate or change over the six-month intervention.(43) In another group of HIV-positive subjects (44), 40 g WPI x 2/d x 12 weeks resulted in no change in body mass and significantly increased immune response (as characterized by a increase in CD4 lymphocytes), and reduced fasting triglycerides. An isocalorically matched CHO solution, on the other hand, significantly reduced CD4 lymphocytes and increased cardiovascular risk factors by raising triglycerides. Whey PRO and WPH, as well as specific bioactive peptides from WP and WPH have also been reported to increase free radical scavenging and antioxidant

capacity (45-48), function as angiotensin I-converting enzyme (ACE) inhibitors and antihypertensives (49), aid in the treatment of diarrhea, thrombosis, mineral malabsorption, immunodeficiency, and function as antimicrobials (50) as well as having been shown to increase glutathione response in sedentary and trained male subjects.(51) For example, Mulder and colleagues (48) reported that 200 mg/d x 7d, but not 100 mg/d of lactoferrin (isolated from whey) significantly increased total T-cell activation and antioxidant capacity in 30-55 year old, healthy male subjects. Therefore, increased provision of extensively hydrolyzed WP (WPH) or a high lactoferrin containing WPC, in combination with strenuous resistance training, may significantly improve workout recovery and facilitate more dramatic physiological adaptations in response to exercise.

To date, however, no studies have compared the chronic effects of different forms of WPC (e.g., extensively hydrolyzed WPC versus its native WPC source, or comparison of two forms of WPC) for maximizing physiological adaptation to heavy resistance exercise in previously trained subjects. Therefore, the primary purpose of this investigation was to compare the effects of heavy resistance exercise plus an extensively hydrolyzed 80% WPC (WPH) versus its native WPC80, on body composition, muscle mass, upper- and lower-body strength and anaerobic endurance, and clinical measures of exercise recovery and adaptation in previously trained, healthy males. A second purpose of this investigation was to compare the effects of WP of different macro-fractional concentrations [WPC80 versus WPH versus a high-lactoferrin containing WPC80 produced by a different WP supplier (WPC80+)], on the physiological response to heavy resistance training.

PURPOSE

The primary purpose of this investigation was to evaluate the physiological effects of eight weeks of linear, periodized resistance training in combination with 60 g/d (30 g x 2/d) of one of three forms of whey protein – 80% whey protein concentrate (WPC80), an extensively hydrolyzed form of the WPC80 (WPH), and a high-lactoferrin containing 80% whey protein concentrate from another raw material supplier (WPC80+) – to assess the effects of WP quality (as defined by average molecular weight distribution) and macro-fraction concentration on body composition, human performance and health in previously trained males. The dependant variables under investigation included body composition (BC), muscle mass (MM), upper- and lower-body strength (1RM), upper- and lower-body anaerobic endurance (80RM to failure), repeated lower-body 80RM bouts, and clinical response and physiological adaptations as determined by blood and plasma analyses.

A secondary purpose of this investigation was to quantitatively assess the validity of hypotheses that have been proposed in response to acute muscle protein synthesis (MPS) and WP data. That is, do the statistically significant differences that have been observed under acute conditions summate into statistically significant effects over time? Lastly, on a consumer level, WPC is the most widely used WP in sports nutrition, largely due to its affordable cost. However, WP substantiation derives predominantly from studies involving WPI or WPH. Thus, a third purpose of this study was to quantify the target consumer benefit of consuming WPC versus WPH, versus a carbohydrate placebo (PLA).

To simulate real world application, this study involved a minimal nutrition intervention, *ad libitum* diet and was conducted on healthy, college-aged males with a minimum of three months uninterrupted bodybuilding and/or strength training experience.

Research Questions

- Does supplementation with WP of predominantly low molecular weight peptides (WPH) improve BC, MM, 1RM and 80RM more than native WPC80 or WPC80+, when consumed in combination with eight weeks of heavy resistance training?
- Does supplementation with WP of predominantly low molecular weight peptides (WPH) improve clinical response to training, as measured by blood and plasma analytes, more than WPC80 or WPC80+?
- Does WP macro-fraction concentration (WPC80 versus WPC80+ versus WPH) differentially affect the physiological response to eight weeks of heavy resistance training?
- Does WP supplementation, in combination with eight weeks of heavy resistance training, improve BC, MM, 1RM and 80RM more than PLA?
- Does WP supplementation improve clinical response to training, as measured by blood and plasma analytes, more than PLA?

Hypotheses Tested

- H_0: WP supplementation provides no additional benefit to a linear, periodized resistance training program in previously trained men (WPH = WPC80 =

WPC80+ = PLA).

- H_{A1}: WP supplementation enhances the effects of resistance exercise by reducing FM and %FAT more than PLA, and further increases TBMM, as well as improves 1RM, 80RM, repeated 80RM, and clinical adaptations to training (WPH = WPC80 = WPC80+ > PLA).
- H_{A2}: WPH and WPC80+ supplementation enhances the effects of resistance exercise by reducing FM and %FAT more than WPC80 or PLA, and further increases TBMM, 1RM, 80RM, repeated 80RM, and clinical adaptations to training (WPH = WPC80+ > WPC80 > PLA).
- H_{A3}: WPH augments the physiological response to heavy resistance exercise observed by native WP (WPC80 and WPC80+) versus PLA (WPH > WPC80+ = WPC80 > PLA).

DELIMITATIONS

- 68 healthy, college-aged (18-35) males were accepted into the study.
- Qualified participants possessed a minimum of three months uninterrupted, chronic (≥3 d/wk) bodybuilding and/or strength training experience.
- The study duration lasted approximately 10 weeks, consisting of two weeks baseline and post-testing (PRE and POST, respectively) and eight weeks of heavy resistance training.
- Subjects participated in 4 d/wk, supervised heavy resistance training using an individualized, split-body, linear, periodized program previously shown to elicit improvements in body composition and strength in previously trained men.(18)

- Participants were recruited from the general public using fliers and direct recruitment (e.g., announcements in class and to university fraternities).
- Participants abstained from consuming ergogenic aids within two weeks preceding baseline testing.
- All participants were required to complete a health history questionnaire and sign an informed consent prior to testing.
- Testing took place within the Human Performance Laboratory, in the Department of Health and Exercise Science at the University of Oklahoma (Norman, OK), and all blood and serum analyses were conducted by Diagnostic Labs of Oklahoma (Oklahoma City, OK).
- All training sessions were directly supervised, total training volume (load x reps x sets) and duration were recorded by subjects, and subjects' weekly training loads were prescribed by the study coordinator based upon subject progress.
- Supplement and placebo servings were double-blinded for packaging, taste, texture, solubility in water, and visual characteristics, and individually packaged in single serving foil packets. Each subject was randomly assigned an individual case (124 servings) of their randomly assigned group supplement, from which all of the subject's servings were pulled.
- Supplement and placebo ingestion was distributed and supervised by investigators on training days (4 x/wk). Between training sessions, subjects were required to consume two packets per day and were only provided enough individually marked packets to last until the next scheduled training session.
- All participants were required to maintain pre-testing dietary habits, and were

additionally required to provide three-day nutrition diaries prior to (PRE) and during weeks 1, 4-5, and 8 of the study.

- Dependant Variables measured included:
  - Body Mass (BM) was estimated by electronic clinical scale, and fat mass (FM), percent body fat (%FAT) and lean body mass (LBM) were estimated by dual-energy X-ray absorptiometry (DXA).
  - Total body muscle mass (TBMM) was estimated using the validated DXA-derived prediction equation by Kim et al.(52)
  - Upper- and lower-body strength were determined from one-repetition maximum (1RM) testing on the barbell flat bench press (BP) and plate-loaded incline hack squat machine (HS), respectively.
  - Upper- and lower-body anaerobic endurance were determined from 80% 1RM repetitions to volitional failure (80RM) on the BP and HS, respectively.
  - Twenty-four- and 48-hour repeated lower-body anaerobic endurance (80RM, $80RM_{24}$ and $80RM_{48}$, respectively) was measured on the HS.
  - Clinical adaptations and response to supplementation and heavy resistance training were determined from blood and serum assays.
  - Nutritional analysis were determined from three-day (x4) nutrition diaries.

LIMITATIONS

- Study lacked a genuine random sample [i.e., predominance of undergraduate, Caucasian, fraternity members and University of Oklahoma (Norman) students].

- Limited control over subject compliance to all requirements (e.g., prior resistance training experience, health status, use of antibiotics, ergogenic aids, anabolic steroids, supplemental exercise between training sessions, maintenance of pre-test dietary habits, etc.).
- Dependence upon subject accuracy for providing reliable dietary recall diaries.
- Subject withdrawal or removal rate was 16.2%, with 72.7% of all subject drop-outs (or removals) occurring by the end of the first week of the intervention.
- Dependence upon DXA as the sole measure to assess changes in body composition.(53)
- Mechanical malfunctioning, during the course of the eight-week intervention, of the HS machines used to train and assess lower-body 1RM, 80RM and repeated 80RM.
- Did not control for post-exercise nutrition intake beyond delivery of the post-workout supplement.

ASSUMPTIONS

Theoretical:

- Participants answered all recruiting information and health history questionnaire questions honestly.
- Participants observed a 12-hour fast prior to PRE and POST body composition testing and blood draws.
- Participants observed a 4-hour fast prior to week 1 and 8 blood draws.
- Participants consumed a nearly identical meal, 90-120 minutes prior to both PRE

and POST strength and anaerobic endurance tests, as well as prior to 24- and 48-hr repeated anaerobic endurance tests.

- ❖ Participants did not consume ergogenic aids prior to testing or at any time during the eight-week intervention; nor did subjects engage in any supplemental exercise outside of regularly scheduled training sessions.
- ❖ Participants did not deviate significantly from pre-study dietary habits, nor did subjects use the twice daily shakes to replace regularly scheduled meals.
- ❖ Participants reported any and all adverse events to the investigators.
- ❖ Subjects provided maximal effort on all PRE, POST and repeated exercise tests.
- ❖ Subjects provided maximal effort on all exercise sets during the eight-week resistance training intervention.
- ❖ Participants were compliant with the supplementation intervention on non-training days.
- ❖ The four supplement interventions remained blinded to both the subjects and investigators until the study was complete and the statistical analyses had been conducted.
- ❖ Equal verbal encouragement and motivation were provided to all participants during testing and throughout the eight-week resistance training intervention.

Statistical:

- ❖ The population from which the subjects were drawn was normally distributed, and the data parametric (interval or ratio).
- ❖ The assignment to treatment group was random and the observations obtained are independent.

- ❖ The variability of means between groups were equal or nearly so (homogeneity of variance).
- ❖ The correlations between trials were equal or nearly so (homogeneity of covariance, or sphericity).
- ❖ Selected covariates used for ANCOVA analysis share a linear relationship with the dependent variables (linearity).

STUDY IMPLICATIONS

Whey protein concentrates (WPC; 34-80% protein per total weight) are the most readily used form of WP in sports nutrition products, largely because of their inexpensive cost compared to whey protein isolates (WPI; ≥90% protein per total weight) and hydrolysates (WPH). Generally speaking, the lower the percent PRO per total weight, the lower the price of the PRO and the higher the CHO and fat content. Similarly, the lower the percent PRO per total weight, the sweeter and more flavorful the taste of the WP. Therefore, it should be of little surprise that to attract the greatest number of customers, and to do so at the lowest possible cost to remain competitive, has created a commercial environment in which the WP market is one dominated by WPC; many times, existing as the first PRO ingredient within a host of other proteins and amino acids as part of a company's "proprietary protein blend." Paradoxically, structure/function claims used to market the majority of WP products available to consumers utilize data derived almost exclusively from studies that have involved WPI or WPH within the methods. Thus, one implication of this study is that the differences between chronic supplementation of a predominantly low versus high molecular weight WP (WPH vs. WPC, respectively) has

been quantified within the target consumer demographic – college-aged males involved in bodybuilding and/or strength training exercise. Secondly, results from this chronic study help elucidate hypotheses that have arisen from acute studies reporting significant transient differences in anabolic response to hydrolysates versus ingestion of the native PRO, in combination with resistance training. Specifically, data from acute studies would indicate that chronic ingestion of WPH may result in more significant increases in protein accretion and muscle cross-sectional area over time. Additionally, this study addressed the question of whether different effects are elicited by supplementing with different brands of WP of similar PRO concentration (i.e., WPC80 versus WPC80+). Lastly, this study adds to the body of literature specific to the efficacy and safety of WP supplementation when combined with exercise.

REVIEW OF LITERATURE

It has generally been reported that acute protein (PRO) ingestion significantly increases muscle protein synthesis (MPS) and provides an augmented anabolic response when consumed in combination with strenuous resistance exercise.(1-3) For example, Dreyer et al. (54) reported that acute heavy resistance exercise alone increased MPS by 41% above baseline levels, whereas a 145% increase in MPS was observed when a leucine-rich essential amino acid (EAA) solution was consumed immediately post-exercise. It has not been concluded, however, as to whether consuming PRO prior to, following, or when consumed both pre- and post-exercise is most effective at stimulating chronic changes in muscle hypertrophy and strength.(5) Hypotheses generated from acute data indicate that MPS will be significantly elevated so long as resistance exercise is performed in the presence of increased EAA availability or provision of EAA are made available within 1-2 hours, but not greater than 4-5 hours post-exercise.(6-8) Assuming peak AA concentrations occur between 20-90 minutes post-ingestion of fast-absorbing PRO (e.g., whey) (10-13), and exercise duration lasts 45-90 minutes, consuming PRO both pre- and post-exercise, as opposed to any one feeding time alone, may elicit more significant effects on resistance training adaptations.

Results such as those reported by Dreyer et al. (54) are of little surprise then, considering that in the post-exercise state insulin sensitivity is increased and there is an increased concentration of the major glucose transporter, glucose transporter type-4 (GLUT4), present in skeletal muscle membranes. Therefore, simultaneously eliciting a rise in

insulin and increasing AA availability decreases proteolysis and further increases MPS as gluconeogenic pathways are inhibited by increased glucose (exogenous or hepatic) uptake into muscle. Whey PRO (WP) and hydrolysates have each been shown to stimulate insulin release in the absence of glucose, and MPS may also be upregulated via direct anabolic signaling by the essential and branched-chain amino acid (BCAA), leucine.(10, 55, 56) Whether or not leucine, which is present in high concentrations in WP, functions in such a capacity is yet to be determined. Collectively, the data involving MPS concludes that it is EAA availability, not glucose, that potentiates the anabolic response to exercise.(1, 57) Furthermore, Bohe et al. (9) concluded that it is the extracellular concentration of EAA rather than intracellular EAA availability that significantly affects MPS. Additionally, a curvilinear relationship between PRO ingestion and MPS response appears to exist, such that a threshold dose of PRO has been postulated to occur at the equivalent of between 8.5-20 g of EAA.(26, 27)

Hypothetically then, combining heavy resistance exercise and its recovery period with ingestion of a fast absorbing, insulinotropic PRO source, such as an extensively hydrolyzed whey protein (WPH), may augment the exercise-induced MPS response more than either carbohydrate (CHO) alone or slower absorbing PRO sources. As evidence, Tang et al. (20) found that when the equivalent of 10 g EAA from either WPH (dairy-fast), soy protein isolate (SPI; vegetable-fast) or micellar casein (CAS; dairy-slow) were consumed immediately post-resistance exercise, that WPH>SPI>CAS stimulated MPS to in both trained (post-exercise) and untrained (rest) legs. From this and similar such acute studies, dairy proteins, and possibly WP in particular has largely been accepted as

providing the most anabolic response in combination with heavy resistance exercise.(3, 4, 58, 59)

Comparative studies between the various forms of WP (concentrates, isolates, and hydrolysates), however, leaves many research questions unanswered. Power et al. (11), for example, showed that under fasting conditions consumption of ~45 g of WPH peaked insulin concentrations higher than an equal dose of whey protein isolate (WPI) in healthy male subjects. A 28% and 43% greater peak and area under the curve for insulin response from WPH, respectively, coincided with a 15% lower area under the curve for plasma BCAAs which may be indicative of more rapid uptake of BCAAs into myocytes. However, no significant differences existed between WPH and WPI for rates of appearance for any of the plasma AA investigated. Casein hydrolysates (CPH), on the other hand, have more consistently been shown to increase the rate of AA absorption and appearance when compared to intact CAS.(10, 13) For example, Koopman et al.(13) recently reported that, in elderly men, 35 g of CPH significantly increased PRO digestion, absorption, insulin response and plasma AA availability, and resulted in a trend (p=0.10) toward increased MPS, versus an equal dose of CAS when measured over a 6-hour postprandial period. A plausible explanation for the significant differences observed between native CAS and its hydrolysates, versus WP and its hydrolysates, may simply have to do with native WP already possessing a rapid rate of absorption.

Extensively hydrolyzed proteins – characterized as the majority (>80%) of PRO fractions as ≤1 kD molecular weight, or typically less than about eight AA in length – may

theoretically provide improved rates of EAA availability to support increased nitrogen retention, compared to native PRO. However, very little evidence has directly assessed this research question in humans. Calbet and Holst (10) indeed reported significantly faster rises in plasma AA concentrations from CPH as opposed to intact CP, yet no significant effect was observed for WPH versus WPI. However, the slopes of the WP absorption curves tend to support the faster absorption of AA from hydrolysates. Using direct gastric infusion into the small intestine of male volunteers, Grimble et al. (36) reported that low molecular weight PRO fractions (55% of fractions as <4 AA in length) significantly increased AA and nitrogen absorption, compared to larger fractions (98% of fractions as >4 AA in length), and nitrogen retention in previously starved rats that were re-fed WP or CP or their respective hydrolysates has also shown significant benefit from hydrolysates.(37) Such findings appear to highlight the importance of the H+-oligopeptide cotransporter, Pept-1, specific to the absorption of di- and tri-peptides. These transporters, located predominantly on the brush-border membrane of the intestinal mucosa and basolateral membrane have been reported to transport some 400 known dipeptides and 8,000 tripeptides (40), and have become a major area of focus in drug discovery and delivery because of the Pept-1 transporter's high capacity and low substrate specificity.(40-42) This contrasts greatly to the low capacity, high substrate specificity for uptake of free amino acids. In fact, bioactive peptides derived from hydrolyzed dairy PRO appear to hold great promise for a number of metabolic conditions, such as Type 2 diabetes, insulin-resistance, or metabolic wasting diseases and aging. For example, WP and WPH, as well as specific bioactive peptides and PRO fractions from WP and WPH have been reported to increase free radical scavenging and antioxidant capacity (45-47),

function as angiotensin I-converting enzyme (ACE) inhibitors and antihypertensives (49), aid in the treatment of diarrhea, thrombosis, mineral malabsorption, immunodeficiency, and function as antimicrobials and antibacterials (50, 60) as well as having been shown to increase glutathione response in sedentary and trained male subjects.(51) Therefore, increased provision of extensively hydrolyzed WP (WPH), in combination with strenuous resistance training, may significantly improve workout recovery and facilitate more dramatic physiological adaptations in response to exercise. However, large PRO fractions, though apparently not absorbed as readily as low molecular weight fractions, may instead serve other supporting roles to speed tissue repair and promote improved physiological adaptations to exercise. For example, *in vitro* and *in vivo* evidence in osteoblast cell cultures and in mice, respectively, have shown the iron-chelating WP macro-fraction, lactoferrin, to promote significant osteogenic responses, which may have specific application for use in the elderly and female athletes, as well as reducing wound-healing time.(61)

Therefore, the primary purpose of the current investigation was to determine if chronic ingestion of either a WPC or its extensive hydrolysate (WPH), in combination with strenuous resistance exercise, resulted in any significant differences on measures of body composition, human performance and health in previously trained, healthy males. A secondary purpose of the current investigation was to assess if different physiological adaptations to exercise occurred in response to whey proteins of varying PRO fraction concentration.

The following selected studies are specific to those variables that have direct implication(s) on either the proposed methods or hypothetical outcome(s) of the current investigation.

EFFECT OF PROTEIN AND PROTEIN SOURCE ON PHYSIOLOGICAL RESPONSE AT REST AND IN RESPONSE TO EXERCISE

Biolo G, Tipton KD, Klein S, Wolfe RR. An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein. *Am J Physiol* 1997 Jul;273(1 Pt 1):E122-9.(62) Six previously untrained males received intravenous infusion of 0.15 g/kg/h x 3h of AA at rest and immediately after exercise. AA uptake and effect on muscle was determined by isotopically labeled AA presence in arteriovenous blood samples and muscle biopsies. Leg blood flow, in response to exercise and increased AA availability, increased by 64% above resting conditions, and amino acid transport increased by 30-100% above resting conditions for the four labeled AA under investigation. MPS, but not MPB was significantly affected by combined exercise and AA availability; MPS increased by 291% in response to exercise plus AA, versus a 141% increase in MPS in response to AA alone under resting conditions. *IMPLICATION*: This study was among the first to suggest that AA availability, immediately subsequent exercise, provides an increased anabolic response to exercise.

Hayes A, Cribb PJ. Effect of whey protein isolate on strength, body composition and muscle hypertrophy during resistance training. *Curr Opin Clin Nutr Metab Care* 2008 Jan;11(1):40-4.(58) *IMPLICATION*: This review paper suggests PRO supplementation

supports at least three roles in augmenting the muscle hypertrophy response to heavy resistance exercise: 1) PRO ingestion close to training increases the anabolic response to exercise, 2) frequent PRO feeding appears to stimulate repeated increases in MPS and thus can promote higher net gains in muscle PRO accretion, and 3) PRO high in EAAs, and particularly leucine (ie., whey), may maintain or restore the acute anabolic response to PRO feeding that typically declines with age.

Moore DR, Tang JE, Burd NA, Rerecich T, Tarnopolsky MA, Phillips SM. Differential stimulation of myofibrillar and sarcoplasmic protein synthesis with protein ingestion at rest and after resistance exercise. *J Physiol* 2009 Feb 15;587(Pt 4):897-904.(7) Healthy but untrained male volunteers were studied, using muscle biopsies and primed constant infusion, to assess plasma AA concentrations, and myofibrillar and sarcoplasmic protein synthesis under fasting conditions and for five hours subsequent heavy unilateral resistance training; the non-exercised leg served as the rested state control. Immediately post-exercise, subjects ingested 25 g WPI. Both myofibrillar and sarcoplasmic protein synthesis were significantly (p<0.01) elevated in response to WPI feeding at "rest" and immediate post-exercise. Myofibrillar protein synthesis increased in the trained leg compared to the untrained control at 1 (~100%), 3 (~216%) and peaked at 5 (~229%) hours post-exercise WPI ingestion. Compared to a 103% rise in myofibrillar protein synthesis at five hours post-WPI alone (i.e., in the rested control leg), WPI+exercise raised protein synthesis 204% (p<0.01) by the same time point. At three hours post-exercise, it was observed that WPI alone or in combination with exercise significantly increased both myofibrillar and sarcoplasmic protein synthesis. Exercise, however, was

shown to have no significant effect on sarcoplasmic rates of protein synthesis at any time point. *IMPLICATION*: These findings suggest that, 1) exercise only affects myofibrillar protein synthesis and that AA availability is required to stimulate sarcoplasmic protein synthesis, 2) that AA availability significantly increases the exercise-induced affect on myofibrillar protein synthesis, 3) PRO alone significantly triggers myofibrillar protein synthesis by three hours postprandial, and 4) combined exercise and PRO significantly increases protein synthesis for up to five hours post-exercise in previously untrained males. Whether these effects are unique for WPI and WP, possibly because of their high concentration of BCAA and leucine, is yet to be determined. However, these findings do support the hypotheses of regular protein feeding at 2-3-hour intervals to continually support MPS, and does suggest that WP may elicit a more anabolic response than other protein sources when combined with exercise.

Hulmi JJ, Tannerstedt J, Selanne H, Kainulainen H, Kovanen V, Mero AA. Resistance exercise with whey protein ingestion affects mTOR signaling pathway and myostatin in men. *J Appl Physiol* 2009 May;106(5):1720-9.(16) Previously untrained, college-aged male subjects were baseline tested for body composition by five-site skinfold and vastus lateralis muscle thickness, as assessed by ultrasound, prior to an acute bout of lower body resistance training and following 21 weeks of twice weekly exercise plus non-energetic placebo or 30 g/d WPI (15 g immediately pre- and post-exercise during both acute and chronic interventions). Muscle biopsies were collected prior to, as well as one and 48 hours post-exercise (acute intervention only), and 4-5 days subsequent the 21-day training intervention. WPI resulted in significantly ($p<0.05$) elevated mTOR signaling at

1 and 48 hours post exercise, and sustained the mTOR response to exercise as observed after 48 hours acute and 21 weeks of repeated training. Muscle thickness was also significantly (p=0.003) increased in the WPI group at both 10.5 and after 21 weeks of training. Fiber size, as determined by immunohistochemical staining, revealed that both WPI and placebo significantly increased muscle cross-sectional area in both Type I and Type II fibers in response to the 21 weeks of twice weekly training, but no significant (p>0.05) differences were observed between groups (WPI = +41.55% and +51.34%; placebo = +40.78% and +50.55%). No significant (p>0.05) within- or between-group changes were observed for %FAT, either. *IMPLICATION*: Total daily energy intake, including PRO intake, as averaged across the entire 21-week intervention, was shown to not be significantly different between the WPI and placebo groups (WPI = 1.5±0.3 g/kg b.w./d; placebo = 1.4±0.4 g/kg b.w./d; p=0.57). Therefore, 1) it may not be surprising that little difference occurred between groups shown to be consuming statistically similar diets and engaged in the same exercise routine, 2) the significant increase, both at baseline and after 21 weeks, of acute mTOR response from 15 g WPI consumed pre- and post-exercise, but no long-term differences between groups in muscle mass cross-sectional area may be suggestive of a need for significantly higher chronic and/or daily doses of PRO, and 3) the non-significant changes in muscle cross-sectional area between the placebo and non-exercise control (sedentary, no WPI) group supports both the large variability likely to result from the use of previously untrained subjects or potentially the need for higher training volume and/or frequency.

Hoffman JR, Ratamess NA, Tranchina CP, Rashti SL, Kang J, Faigenbaum AD. Effect of

protein-supplement timing on strength, power, and body-composition changes in resistance-trained men. *Int J Sport Nutr Exerc Metab* 2009 Apr;19(2):172-85.(63) Resistance trained men (intercollegiate athletes and competitive powerlifters) participated in a 4 d/wk x 10 wks, periodized resistance program, either with no supplemental intervention (control) or while consuming 84 g/d PRO (blend of hydrolyzed collagen protein isolate, WPI, CPI + 250 mg BCAA) in two divided doses: either immediately pre- and post-exercise (Pre/Post), or in the morning and evening regardless of training time (AM/PM). Both PRO groups consumed two servings of PRO on non-training days. All groups significantly ($p<0.05$) increased 1RM squat and both PRO groups significantly ($p<0.05$) increased 1RM bench press, however none of the changes were significantly ($p>0.05$) different between groups. There were also no significant ($p>0.05$) differences within or between groups for BM, FM, %FAT or FFM as determined by DXA. A significant ($p<0.05$) increase in daily protein intake was only observed in the AM/PM group, whereas relative PRO intake (g/kg b.w./d) increased, but non-significantly ($p>0.05$) in Pre/Post. Relative intake of PRO as a percent of total macronutrient intake did, however, increase significantly in both AM/PM and Pre/Post. No significant within- or between-group changes were observed for total energy ($p=0.70$), CHO ($p=0.73$) or FAT ($p=0.73$) intake during the investigation. *IMPLICATION*: 1) Four days per week split-body periodized training was shown to significantly increase human performance measures in a highly trained population, and 2) no significant increase in daily PRO intake in Pre/Post coincided with an observed, albeit not significant reduction in total daily energy intake which may explain the significant increase observed in percent of total energy from PRO within this group (Pre/Post). The latter observation, in light of

such high daily supplemental doses being added to the athletes' diets (84 g/d) may indicate prolonged satiety arising secondary to an augmented MPS response from combined resistance training and AA availability.

Hoffman JR, Ratamess NA, Tranchina CP, Rashti SL, Kang J, Faigenbaum AD. Effect of a proprietary protein supplement on recovery indices following resistance exercise in strength/power athletes. *Amino Acids* 2009 Apr 4.(64) Subjects were of the same population as the aforementioned Hoffman et al. study. (63) Subjects consumed either 42 g PRO (a PRO blend, as previously described) or an equal amount of maltodextrin (placebo) 10 minutes prior to and 15 minutes following an acute heavy resistance training bout. The training on day one consisted of four sets of the back squat, deadlift and barbell lunge, utilizing an 80% 1RM load and allowing for 90 seconds of rest between sets. Subjects returned 24 and 48 hours later to repeat pre- and post-exercise supplementation while performing only four sets of the back squat for maximal repetitions and following the same loading and rest interval lengths as on the day prior. No between-group differences ($p>0.05$) were observed for total training volume or hormonal response (e.g., testosterone, cortisol, creatine kinase, testosterone:cortisol) to the acute lower-body exercise session (Day 0). A significant difference ($p<0.05$) was, however, observed between groups for total training volume at 24- and 48-hr repeated tests, with the PRO group performing more total reps (+30.4% and +40.9% more than placebo, respectively). Additionally, whereas resting creatine kinase was significantly ($p<0.05$) elevated in both groups prior to the 24-hour back squat test, creatine kinase was not increased further in the PRO group prior to 48-hour testing but had increased

significantly (p<0.05) for the placebo group. *IMPLICATION*: These results support an improved recovery response from pre- and post-exercise PRO, as opposed to CHO ingestion in strength/power athletes.

Cribb PJ, Williams AD, Carey MF, Hayes A. The effect of whey isolate and resistance training on strength, body composition, and plasma glutamine. *Int J Sport Nutr Exerc Metab* 2006 Oct;16(5):494-509.(19) Previously trained male subjects consumed, in double-blind manner, equal loads of PRO as either hydrolyzed whey protein isolate (WPH) or casein (CPH) in combination with 10 weeks supervised resistance training. WPH was determined to be significantly more effective than CPH at increasing 1RM strength (p<0.05) for the barbell bench press, parallel squat and lat pull-down, and at increasing FFM (+5.0±0.3 kg versus +0.8±0.4 kg; p<0.01) and reducing FM (-1.5±0.5 kg versus +0.2±0.3 kg; p<0.05) as assessed by DXA. When strength was compared relative to BM, WPH was still significantly (p<0.05) more effective than CPH. *IMPLICATION*: This study supports the hypothesis that WPH appears to improve physiological response to chronic resistance exercise in previously trained male subjects.

Tang JE, Moore DR, Kujbida GW, Tarnopolsky MA, Phillips SM. Ingestion of whey hydrolysate, casein, or soy protein isolate: effects on mixed muscle protein synthesis at rest and following resistance exercise in young men. *J Appl Physiol* 2009,107(3):987-92.(20) The effects of consuming the equivalent of 10 g EAA as either WPH (21.4 g), micellar CP (21.9 g) or SPI (22.2 g) were assessed during rest and immediately following strenuous unilateral lower body resistance exercise in previously trained young (22.8±3.9

yrs) men. Plasma insulin and blood amino acid concentrations, and mixed muscle fractional synthetic rate (FSR), a measure of muscle protein synthesis (MPS) were determined via blood draws and primed constant infusion, respectively. Subjects' non-exercise leg served as the control for assessing resting conditions. Plasma insulin increased significantly (p<0.05) and comparably at 60 minutes post-supplementation for WPH and SPI, but not CP (p=0.43). EAA and leucine concentrations increased (p<0.05) across all treatments by 30 minutes post-supplementation with WPH≈SPI>CP and WPH>SPI>CP, respectively. Aminoacidemia at 30 minutes post was also significantly (p<0.05) greater for WPH than both SPI and CP (WPH>SPI>CP). Similarly, at 60 minutes post, EAA and leucine concentrations were significantly greater for WPH than SPI or CP (WPH>SPI>CP; p<0.05). Leucine concentration AUC for the complete 180 minutes post-supplementation period was 73% and 200% greater (p<0.05) than SPI and CAS, respectively. At 180 minutes post-supplementation, MPS was significantly higher for WPH than CAS (93%; p<0.01) and tended to be higher than SPI (18%; p=0.067) under resting conditions. In response to exercise, all groups significantly (p<0.05) increased MPS, but WPH resulted in significantly greater MPS than both SPI and CAS [31% (p<0.05) and 122% (p<0.01) greater, respectively]. Under both conditions, SPI resulted in significantly higher MPS than CAS [rest = 64% (p<0.01); exercise = 69% (p<0.01)]. *IMPLICATION*: 1) WPH>SPI>CP increases the absorption rate and availability of AA critical to support anabolism, 2) WPH>SPI>CP increases total leucine availability during the 3-hour period following combined heavy resistance exercise and PRO intake, 3) WPH>CP, and possibly greater than SPI, increases MPS in the absence of exercise, and 4) WPH>SPI>CP increases MPS response to exercise as assessed over the

3-hour period post-exercise. Collectively, these findings support the hypothesis that fast absorbing PRO increases anabolism more than "slow" proteins both at rest and in response to exercise, and that PRO from WP supports a more anabolic environment than PRO from either native SPI or CP. Also, significant MPS occurring three hours subsequent ingestion of WPH, under resting conditions, confirms similar findings presented earlier involving WP (7) and supports the dietary implementation of WP feedings occurring approximately every 2-3 hours to support a chronic anabolic environment.

EFFECT OF PROTEIN QUALITY ON PHYSIOLOGICAL RESPONSE AT REST AND IN RESPONSE TO EXERCISE

Tello PG, Camacho F, Jurado E, Paez MP, Guadix EM. Enzymatic hydrolysis of whey proteins. II. Molecular-weight range. *Biotechnol Bioeng* 1994 Aug 5;44(4):529-32.(65) This paper describes techniques used to hydrolyze PRO to achieve peptide hydrolysates of varying molecular weights. A degree of hydrolysis (DH) of ≥20% was found to be required to achieve 65-95% of the hydrolysates as peptides of <1 kD in molecular weight. Similarly, this paper served as a review of the data on peptide chain length and absorption kinetics to date. Specifically, that 1) free AA absorption appears to occur at a slower rate than for low molecular weight peptides because free AA share selective transporters, 2) di- and tri-peptides can be absorbed intact and hydrolyzed within the cell, and 3) to minimize potential allergenicity of ingested PRO and potentially increase AA kinetics, a PRO should contain a high biological value (e.g., WP) and be hydrolyzed such that the average molecular weight of its peptides is ~0.5 kD, but no more than 1 kD.

Calbet JA, Holst JJ. Gastric emptying, gastric secretion and enterogastrone response after administration of milk proteins or their peptide hydrolysates in humans. *Eur J Nutr* 2004 Jun;43(3):127-39.(10) In a cross-over design, six healthy adult males were randomly assigned to receive, via nasogastric tube, one of four double-blind PRO (9.3 g/L) solutions, equally matched for volume, nitrogen content, energy density, osmolality, pH and temperature: 80% WPC, hydrolysate of WPC80 (WPH), CP and CPH. Before subject testing was conducted, it was determined that 94% of the peptides within WPH were an average of 3.7 residues in length, with only 6% of total nitrogen as free AA. CPH contained 93% of its peptides as chain lengths averaging 3.8 residues, with only 7% of its total nitrogen as free AA. Gastric emptying and secretions were determined using a tritiated water technique that was reported to have good agreement with accepted methods (r=0.93 and r=0.86, respectively). Rate of gastric emptying was shown to be similar for all PRO solutions. Gastric secretions were elevated by approximately 50% in the first hour for WPH and CPH, compared to WPC and CP ($p<0.05$). Hydrolysates maintained a significantly ($p<0.05$) higher gastric pH for up to 60 minutes post-feeding, despite all solutions being of equal pH when delivered nasogastrically. Hydrolysis significantly affected the rate of appearance and magnitude of BCAA, EAA and total AA plasma concentrations for CP (CPH>CP; $p<0.05$) but not WP. Peak AA concentrations occurred between 20-25 minutes for hydrolysates and 30-90 minutes for native PRO. Plasma glucose-dependent insulinotropic polipeptide (GIP) was significantly ($p<0.05$) and positively affected by hydrolysates. *IMPLICATION*: Despite the requisite mode of delivery to assess gastric measures, it would appear that extensively hydrolyzed dairy

protein significantly increases absorption kinetics when compared to their native PRO source. Also, hydrolysates may significantly affect insulin response more than their native PRO.

Koopman R, Crombach N, Gijsen AP, Walrand S, Fauquant J, Kies AK, Lemosquet S, Saris WHM, Boirie Y, van Loon LJC. Ingestion of a protein hydrolysate is accompanied by an accelerated in vivo digestion and absorption rate when compared with its intact protein. *Am J Clin Nutr* 2009;90:106-15.(13) Reported significantly (p<0.001) increased AA digestion and absorption, and a trend toward higher MPS (33±16%; p=0.10) and total net protein balance (+18.4%; p=0.08) when elderly male subjects consumed 35 g PRO as intrinsically L-[1-^{13}C]phenylalanine labeled enzymatically hydrolyzed casein (CPH) versus intact casein (CP), under overnight fasted and rested conditions. Subjects were chronically sedentary and this trial was of a double-blind, repeated measures, crossover design. Total exogenous phenylalanine AUC, over the 6-hour period, was an average of 27±6% (p<0.001) higher for CPH than CP, and total net protein balance over the 6-hour period tended (p=0.08) to be higher for CPH than CP. Average total leucine flux (rate of appearance and rate of disappearance) was also significantly (p<0.05) higher for CPH than CP over the 6-hour period (7±1% and 8±2% higher, respectively). Additionally, both peak and AUC plasma insulin concentrations were significantly greater for CPH than CP [~92% (p<0.01) and ~5x (p<0.05) higher, respectively], and peak AA concentrations for phenylalanine, tyrosine, leucine, valine and isoleucine were all significantly (p<0.05) greater for CPH than CP by ~25-50% each. A nadir for each plasma AA was reached between the 4th and 6th hour of testing, such that each AA

became transiently, albeit significantly (p<0.05) lower for CPH than CAS. *IMPLICATION*: This study confirms the insulinotropic response, increased absorption rate and magnitude of plasma AA concentrations arising from hydrolysates versus the native PRO source. The AA concentration nadir observed for hydrolysates, between four and six hours post ingestion would also seem to support previous non-exercise data that indicates a potential need for frequent PRO feeding intervals. Lastly, hydrolysates of a PRO appear more effective at increasing muscle hypertrophy; however, work needs to be done with exercise and using trained subjects to validate.

Power O, Hallihan A, Jakeman P. Human insulinotropic response to oral ingestion of native and hydrolysed whey protein. *Amino Acids* 2008 Aug 5.(11) Sixteen healthy young men participated in a double-blind, repeated measures, cross-over trial to assess the effects of an acute dose of 45 g WP as either WPI or an extensively hydrolyzed 80% WPC (WPH). Testing was conducted after an overnight fast and under resting conditions. WPH was reported as a 30% DH and contained 93% of its peptides as ≤1 kD in molecular weight; by contrast, 83% of the peptides in the WPI were >5 kD. Plasma insulin increased under both conditions but peaked between 30-50 minutes for WPI, whereas insulin continued to rise with WPH (+28% above WPI; p=0.018) and peaked between 50-90 minutes post-PRO load (p=0.20). Area under the curve for plasma insulin concentration, over the 3-hour postprandial period, was also higher for WPH (+43%; p=0.21) but did not reach significance. Likewise, there was no difference between groups for changes in plasma glucose. A non-significant (p=0.15) trend toward faster gastric emptying was observed for WPH, however, both WPH and WPI had completely

emptied from the stomach by 120 minutes postprandial, which led the researchers to conclude WP yields an average gastric emptying rate of 1.5286 kcal/min. BCAA rate of appearance increased steeply but there was no difference for BCAA rate of appearance between groups for up to 40 minutes postprandial. Peak BCAA and total area under the curve was, however, 8% (p=0.176) and 15% (p=0.07) higher for WPI than WPH. Phenylalanine response revealed a 10% (p=0.003) higher peak concentration and 22% (p=0.23) greater area under the curve for WPH versus WPI. Regression analysis revealed no correlation between BCAA or phenylalanine concentrations and insulin response. *IMPLICATION*: Physiological response to, and amino acid kinetics arising from WPH and WPI differ in healthy males under fasted, rested conditions. Also, a significantly higher, and possibly total insulin response can likely be expected from WPH versus WPI, and complete clearance of 45 g "fast" WP occurs by approximately three hours postprandial (under prior fasting and rested conditions).

Buckley JD, Thomson RL, Coates AM, Howe PR, Denichilo MO, Rowney MK. Supplementation with a whey protein hydrolysate enhances recovery of muscle force-generating capacity following eccentric exercise. *J Sci Med Sport* 2008 Sep 1.(66) Knee extensor peak isometric torque, perceived muscle soreness, and muscle damage (serum creatine kinase activity) and inflammation (plasma TNFα concentrations) were assessed after an overnight fast (baseline) and repeated 1, 2, 6 and 24 hours after performing 100 maximal eccentric contractions on an isokinetic dynamometer set at an angular velocity of 40°/s and performed through an 80° range of motion. Immediately after baseline testing, and again after the 6th and prior to the 24th hour follow-up tests, subjects –

healthy, sedentary college-age males – consumed one of three double-blind drinks: 25 g PRO from WPI or hydrolyzed WPI (WPH), or flavored water (placebo). Thus, a total of 75 g PRO was supplemented to the WPI and WPH subjects' 24-hour diets. Peak isometric torque decreased across all groups immediately post-exercise (-23%) but returned to baseline levels by hour-6 in response to WPH. However, peak isometric torque remained suppressed over the 24-hour period for both WPI and placebo (ANOVA treatment x time interaction; p=0.006). No changes within or between groups occurred for muscle soreness, damage or inflammation. *IMPLICATION*: WPH consumed post-exercise overload may have significantly augmented the anabolic response of combined exercise and increased AA availability via increased maximal and total insulin response. These findings may indicate faster recovery from high-intensity, high-volume training, and therefore greater time under tension of muscle to stimulate increases in muscle hypertrophy.

EFFECT OF PROTEIN DOSE ON PHYSIOLOGICAL RESPONSE AT REST AND IN RESPONSE TO EXERCISE

Moore DR, Robinson MJ, Fry JL, Tang JE, Glover EI, Wilkinson SB, Prior T, Tarnopolsky MA, Phillips SM. Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men. *Am J Clin Nutr* 2009 Jan;89(1):161-8.(26) Young adult males with a minimum of four months prior weight training experience performed an acute bout of heavy leg training under overnight fasted conditions on five occasions, separated by 1-week wash-out intervals. Immediately following each exercise bout, subjects were randomly assigned to consume varying doses

of egg albumin PRO – 0, 5, 10, 20 or 40 g. Primed constant infusion of [1-13C]leucine and muscle biopsies were used to assess whole-body leucine oxidation, MPS, albumin protein synthesis (APS) and blood amino acid concentrations over a 4-hour postprandial period. MPS has been shown to increase in response to PRO plus exercise, whereas APS does not respond to exercise but is a hepatic-derived plasma protein that is stimulated by increased AA availability and may function to store excess AAs. MPS and APS increased in a dose-dependent manner up to the 20 g dose, with a higher absolute but not statistically significant mean response for the 40 g dose. Specifically, MPS increased by approximately 37 and 56% for the 5 and 10 g doses, respectively, and approximately 93% for both the 20 and 40 g doses. APS followed the same curvilinear pattern: 0 g<5 g<10 g<20 g≈40 g. Only the 40 g dose significantly (p<0.01) increased EAA, BCAA and leucine concentrations at all time points, and these values were significantly (p<0.01) different from all other PRO doses. Insulin concentration was not significantly different for time x treatment, however, total insulin area under the curve was significantly (p<0.01) greater for the 40 g than for the 5 and 10 g doses, and tended (p=0.09) to be higher than what was observed in response to the 20 g dose. *IMPLICATION*: The researchers concluded that 20 g PRO is the uppermost, optimal dose for maximizing protein synthesis, and that above this amount AA will be lost to irreversible oxidation. Similarly, the researchers suggest that more than 5-6 servings of 20 g PRO, per serving, would result in oxidative loss and could lead to downregulation of the protein synthetic response. However, leucine oxidation under both the 20 and 40 g dosing was significantly higher than for 0, 5 and 10 g, but there was no significant difference between the oxidation rates observed between 20 and 40 g. Similarly, a 50% increase in

PRO exists between doses of 20 and 40 g, and thus it is possible that the optimal dose per serving may lie between these two values. Also, as has been shown rather convincingly out of the same lab, PRO source has a significant effect on MPS, both at rest and in response to exercise.(20) Thus, it remains to be seen if the same absolute dose responses occurs following the ingestion of other PRO sources and/or different training protocols.

Claessens M, Saris WH, van Baak MA. Glucagon and insulin responses after ingestion of different amounts of intact and hydrolysed proteins. *Br J Nutr* 2008 Jul;100(1):61-9.(29) In sedentary, but non-obese adult males, plasma insulin and glucagon response to 0.3, 0.4 and 0.6 g/kg b.w PRO was evaluated. The effects of PRO from WPI, SPI, and their respective hydrolysates (WPH and SPH, respectively) was assessed under resting conditions and after a 10-hour fast by single-blind, crossover, repeated measures design. Blood was sampled at baseline and 15, 30, 60, 90 and 120 minutes postprandial. WPH contained 82% of its fractions as low molecular weight peptides (≤1 kD) and SPH was reported to contain 77% of its fractions as ≤1 kD. Increasing SP dose, whether SPI or SPH, increased insulin total area under the curve response in a dose-dependent manner (p=0.001). Significant within-group differences for insulin response also existed for both SPI and SPH: 0.6>0.4≈0.3 g/kg b.w. dose. Total area under the curve for both insulin and glucagon were significantly greater for SPI than SPH (p=0.018 and p=0.001, respectively), and in both cases SPI resulted in a faster increase in both hormones. Significant differences (p<0.05) were also present for glucagon response for both SPI and SPH, between 0.3 and 0.4 g/kg b.w. doses, however, only SPH realized a significant difference (p<0.05) between 0.4 and 0.6 g/kg b.w. doses. WPI and WPH also resulted in

significant dose-dependent increases in insulin response area under the curve ($p<0.001$); however, within-group differences were not observed for WPH but were significantly different for WPI (0.6≈0.4>0.3 g/kg b.w.; $p\leq0.002$). A significant ($p=0.002$) interaction effect was observed for glucagon (WPH>WPI; $p\leq0.004$), such that plasma glucagon rose and decreased faster with increasing doses of WPH. Additionally, SPI and SPH provided similar and non-significant effects ($p>0.05$) on plasma glucose, whereas total area under the curve for glucose decreased significantly ($p=0.001$) with increasing WP loads. *IMPLICATION*: The primary finding of this study is that, 1) WPH and WPI appear to differentially affect plasma insulin and glucagon response, and 2) the effects are generally dose-dependent up to 0.6 g/kg b.w. PRO dose.

EFFECT OF AGE AND TRAINING EXPERIENCE ON PHYSIOLOGICAL RESPONSE TO COMBINED PROTEIN INTAKE AND EXERCISE

Kumar V, Selby A, Rankin D, Patel R, Atherton P, Hildebrandt W, Williams J, Smith K, Seynnes O, Hiscock N, Rennie MJ. Age-related differences in the dose-response relationship of muscle protein synthesis to resistance exercise in young and old men. *J Physiol* 2009 Jan 15;587(Pt 1):211-7.(6) Twenty-five young (24±6 yrs) and older (70±5 yrs) males were studied to assess MPS and anabolic signaling in response to varying resistance exercise intensities (20-90% 1RM). Total training volume was maintained across all exercise intensities. A significant ($p<0.05$) difference was observed for MPS between young and older men, both in response to each exercise intensity and at 1-2 hours post-exercise; most notably in response to training intensities between 60-90% 1RM [MPS was 30±6% higher ($p<0.04$) in younger males]. *IMPLICATION*: It was

concluded that MPS increases in a dose-dependent manner with exercise intensity up to 60% 1RM, and that older men appear to have a blunted anabolic response to resistance training across all intensities when compared to younger males.

Drummond MJ, Dreyer HC, Pennings B, Fry CS, Dhanani S, Dillon EL, Sheffield-Moore M, Volpi E, Rasmussen BB. Skeletal muscle protein anabolic response to resistance exercise and essential amino acids is delayed with aging. *J Appl Physiol* 2008 May;104(5):1452-61.(67) Young and older (29.7±1.7 and 70.0±2.1 yrs, respectively), healthy males were studied to assess MPS, anabolic signaling, and plasma AA, hormone and glucose:lactate concentrations in response to 8 sets x 10 reps/set of 70% 1RM knee extension training and immediate post-exercise ingestion of 20 g EAA. MPS was significantly elevated in the younger males by 1-3 hours post, and this MPS response was significantly greater than that of the older males at the same time point ($p<0.05$). A significant within-group increase in MPS for the older males was only observed between 3-6 hours post, however there was no significant difference between younger and older males during that same period. Significant within- and between-group differences were also observed for AMP-activated protein kinase-alpha (AMPKα) phosphorylation, a negative regular of protein synthesis. Specifically, AMPKα was significantly elevated in older males at 1 and 3 hours post ($p<0.05$), which was significantly higher than AMPKα phosphorylation observed in the younger males ($p<0.05$). *IMPLICATION*: Older subjects appear to have a delayed MPS response to combined resistance training and AA availability, and therefore subject recruitment for the purposes of the current investigation was drawn from a younger population.

Micke P, Beeh KM, Buhl R. Effects of long-term supplementation with whey proteins on plasma glutathione levels of HIV-infected patients. *Eur J Nutr* 2002 Feb;41(1):12-8.(43) Thirty HIV-infected patients consumed 45 g WP as either double-blinded WPC or WPH, twice per day x 2 weeks. Plasma total glutathione increased by 44% (p=0.004) and 24.5% (p=0.43) for WPH and WPC, respectively. All patients were switched to WPH and continued consuming 90 g supplemental PRO, per day, for an additional six months. Plasma glutathione remained significantly elevated (+26.7%; p=0.033), while there was no significant change in BM, T-cell counts or other clinical measures of interest. IMPLICATION: Clinical application notwithstanding, WPH appears to be more effective than WPC at increasing circulating concentrations of the body's primary antioxidant, glutathione. This may have an effect on training recovery in healthy populations engaged in strenuous weight training.

Sattler FR, Rajicic N, Mulligan K, Yarasheski KE, Koletar SL, Zolopa A, Alston Smith B, Zackin R, Bistrian B. Evaluation of high-protein supplementation in weight-stable HIV-positive subjects with a history of weight loss: a randomized, double-blind, multicenter trial. *Am J Clin Nutr* 2008 Nov;88(5):1313-21.(44) Fifty-nine weight stable HIV-infected patients with prior weight loss of greater than 3% were randomly assigned to receive one of two double-blinded 280 kcal/serv supplements, twice per day x 12 weeks – 40 g WPI or isocalorically matched CHO placebo. No changes in BM, FFM or self-selected food intake occurred, however fasting triglycerides decreased in response to

WPI (-16 mg/dL; p=0.03) and increased with CHO (+39 mg/dL; p=0.025). CD4 lymphocytes, which decline as a result of HIV and are indicative of immune function, increased in response to WPI (+31 cells/mm^3; p=0.03) and decreased with CHO (-5 cells/mm^3; p=0.03). *IMPLICATION*: Aside from CHO ingestion increasing cardiovascular risk factors in HIV-positive patients, this study indicates that WP may improve exercise recovery and aid in preventing over-reaching and over-training due to an increase in training volume combined with high-intensity exercise.

Marshall K. Therapeutic applications of whey protein. *Altern Med Rev* 2004 Jun;9(2):136-56.(68) This review article describes the whey protein fractions and biologically active peptides that had, at the time, been found to yield immune-enhancing effects. Brief study summaries provide evidence that bioactive peptides, or other fractional elements deriving from WP may increase antioxidant and ACE-inhibiting effects, and possess antihypertensive, hypolipidemic, antiviral, antibacterial and chelating actions. Clinical trials involving the successful use of WP in the treatment of osteoporosis, HIV, hepatitis B, cardiovascular disease and cancer are also discussed. *IMPLICATION*: Many of the immune-enhancing properties present in dairy, derive from WP. WPC maintains the native structure of the peptides and thus may provide an improved effect on immune response and recovery, independent of an increased insulin or MPS response that may occur in response to WPH. Similarly, WPC of higher lactoferrin concentration – a widely recognized immune-enhancing WP fraction – may augment exercise recovery.

Adibi SA. Regulation of expression of the intestinal oligopeptide transporter (Pept-1) in health and disease. Am J Physiol Gastrointest Liver Physiol2003 Nov;285(5):G779-88.(40) This paper provides a thorough review of the independent variables that affect Pept-1 transporter expression and action in the context of human health and disease. Of the independent variables that affect Pept-1 transporter expression: high PRO intake has been shown to increase Pept-1 gene expression 1.5-2x compared to low PRO, in as little as three days and for up to two weeks; insulin rapidly stimulates Pept-1; triiodothyronine (T3) hormone may directly or indirectly downregulate peptide uptake by Pept-1; diurnal rhythm; developmental age; fasting – both acute or prolonged – increases Pept-1 expression; diabetes increases Pept-1 expression, independent of insulin; short-bowel syndrome, Crohn's disease and ulcerative colitis have been shown to increase Pept-1 expression within the colon (Pept-1 is predominant in the small intestine); antibiotics downregulate Pept-1 expression; and α_2-adrenergic agonists may upregulate Pept-1. It has also been identified that l-valine ester containing di- and tri-peptides possess very high bioavailability and are currently being exploited for use in drug development and transport. *IMPLICATION*: Inclusion/exclusion criteria included disease states shown to affect Pept-1 transporters; known α_2-adrenergic agonists, such as yohimbine, were not allowed for use by participants within this study; and subjects were excused from the study in the event they required the use of antibiotics.

Morifuji M, Koga J, Kawanaka K, Higuchi M. Branched-chain amino acid-containing dipeptides, identified from whey protein hydrolysates, stimulate glucose uptake rate in L6 myotubes and isolated skeletal muscles. *J Nutr Sci Vitaminol (Tokyo)* 2009 Feb;55(1):81-

6.(30) This study presented *in vitro* and *in situ* analyses of the effects of BCAA-containing dipeptides, derived from enzymatically hydrolyzed WP (WPH), on rat skeletal muscle glucose uptake and storage. Seven BCAA dipeptides were detected, with Isoleucine-Leucine and Valine-Leucine being the most prevalent (3.69 and 3.62 mg/g, respectively); almost all of the dipeptides deriving from β-lactoglobulin. All dipeptides resulted in significant ($p<0.05$) uptake of glucose into skeletal muscle cells by a minimum of 33% (Isoleucine-Leucine dipeptide) and up to 55% (Isoleucine-Isoleucine). By comparison, insulin acted as a positive control and was found to yield 75% greater uptake of glucose in myotubes. Only Isoleucine-Leucine was further assessed for its affects on glycogen concentration and was found to significantly ($p<0.05$) increase glycogen concentration by 18%. *IMPLICATION*: It is possible that the increased insulin response observed from the consumption of fast-absorbing WP (BCAA concentration for WPI>WPC) is being affected by a high concentration of these insulinotropic BCAA-containing dipeptides. Theoretically, enzymatic hydrolysis of a high-β-lactoglobulin containing WP could be engineered to deliver an abundance of these dipeptides in high concentration and thereby promote a more dramatic insulin response.

Mulder AM, Connellan PA, Oliver CJ, Morris CA, Stevenson LM. Bovine lactoferrin supplementation supports immune and antioxidant status in healthy human males. Nutr Res 2008;28(9):583-9.(48) Healthy, male adults were administered a placebo, and 100 mg and 200 mg of whey-derived lactoferrin for seven days (each arm), in a repeated measures design. Blood lymphocytes, T-cell activation, natural killer cell cytotoxicity, and serum cytokine levels (e.g., tumor necrosis factor-alpha) and antioxidant status were

measured prior to and after each intervention period. No significant effect ($p>0.05$) was observed in response to either the placebo or 100 mg/d interventions for any of the blood measures analyzed. However, T-cell activation (total, helper and cytotoxic) was found to be significantly elevated ($p<0.001$; compared to baseline and post-placebo intervention) after one and seven days of repeated use of the 200 mg/d lactoferrin dose. Hydrophilic antioxidant capacity also was found to be significantly different from baseline, though not significantly different ($p>0.05$) from post-placebo measures. *IMPLICATION*: The results of this study may support the supplemental use of 200 mg/d lactoferrin, or a whey protein manufactured to contain comparably high concentrations of lactoferrin, to improve immune function and possibly aid exercise recovery (or least decrease the likelihood of over-reaching/over-training onset). However, complicating this conclusion is that the three treatment arms were not provided in random, cross-over design and nor was a washout period included between interventions. Instead, placebo and then 100 mg/d always preceded the 200 mg/d treatment.

METHODS

STUDY DESIGN

This study involved a minimal nutrition (*ad libitum*) intervention, double-blind, placebo-controlled, randomized, repeated measures design. The study design was selected to simulate "real world" application of consuming 30 g of whey protein (WP), 2x/d x 8 wks, in combination with 4 d/wk heavy resistance training in healthy, college-aged, resistance-trained males. Subjects were randomly assigned into 1 of 4 treatment groups: exercise + dextrose placebo control (PLA), exercise + whey protein concentrate 80% (WPC80), exercise + high lactoferrin-containing whey protein concentrate 80% (WPC80+), or exercise + extensively hydrolyzed whey protein concentrate 80% (WPH). All subjects consumed two supplements per day; immediately pre- and post-exercise on training days, and twice daily between meals on non-training days. Body composition testing occurred on day 1 of week 0 (PRE) and week 9 (POST), following a 12-hour fast (water only) and a minimum of 48 hours without participating in strenuous exercise. Nine blood draws occurred: immediately following body composition testing in PRE and POST; 24 hours after the first and final lower body workout in weeks 1 and 8, and following a 4-hour fast; immediately following POST strength (1RM) and anaerobic endurance testing (80RM); and, immediately prior to and following 24- and 48-hour repeated anaerobic endurance testing ($80RM_{24}$ and $80RM_{48}$, respectively) in POST. Upper- and lower-body 1RM and 80RM testing was initiated 48 hours after body composition testing in PRE and POST, with upper- and lower-body 1RM tests preceding upper- and lower-body 80RM testing. Twenty-four and 48-hour repeated 80RM testing only occurred during POST. Three-day

nutritional diaries were recorded before PRE (baseline), and again during weeks 1, 4-5 and 8 of the intervention. Subjects also recorded nutritional intake for the 24 hours preceding strength and anaerobic endurance tests. For testing and training day sequence of events, refer to **Figure 1** in Appendix B.

SUBJECTS

Sixty-eight (N≥68) healthy, resistance-trained (≥ 3 months uninterrupted, ≥ 3 d/wk resistance training) men, 18-35 years of age (21.40±0.36), volunteered to participate in the study. Each participant was assessed for inclusion into, or exclusion from the study via responses provided during verbal interviews as well as written (and signed) health history and related documents. One subject from WPC80+ withdrew because of a shoulder injury (received outside of the study), two subjects (1 WPH and 1 WPC80) were removed for non-compliance and missed workouts, two subjects from WPH were removed because of viral infections requiring the use of antibiotics, four subjects (1 PLA, 2 WPH, and 1 WPC80) withdrew because of the training being of too high an intensity, and two subjects (1 PLA and 1 WPH) withdrew because of headaches brought about during lower-body training. Additionally, data from one subject within WPC80 was removed from final analysis on the basis of being an extreme (>3 SD) outlier for PRE body mass (BM), percent body fat (%FAT), one repetition maximum on the bench press (1RM BP), and height. Therefore, 57 subjects completed the study, and data from 56 subjects were used for analyses [refer to **Table 1** in Appendix A]. This study was approved by the University of Oklahoma Health Sciences Center Institutional Review Board for Human Subjects, and written informed consent was obtained from each

participant prior to testing.

Inclusion and Exclusion Criteria

Inclusion into the current study was in accordance with previous research methods used within the University of Oklahoma, Department of Health and Exercise Science Human Performance Laboratory, and entailed that each subject meet the following criteria:

- College-aged male between the ages of 18-35
- ≥ 3 months of continuous resistance training (≥ 3 d/wk) experience, for the period immediately prior to the start of the investigation
- Apparently healthy and free from disease as determined by a health history questionnaire
- Provided written consent and agreed to all of the conditions of the protocol
- Had not used dietary supplements that may confound the results of the study (e.g., creatine, stimulants, thermogenics, etc.) within 14 days of PRE testing, and agreed to not engage in supplementation of such products or additional protein supplementation during the course of the study
- Agreed to not engage in supplemental resistance or aerobic exercise during the course of the study

Participants were excluded from participation in the study if they reported or exhibited any of the following:

- Participated in another clinical trial or had received an investigational product within 30 days prior to enrollment

- Lost or gained >10 lbs of bodyweight during the previous six months, and had maintained the change in weight
- Did not eat meals at regular intervals
- History of drug or alcohol abuse within two years prior to enrollment
- Regular use of tobacco products (i.e., cigarettes, dip, snuff, chew, cigars, etc.)
- Significant history, or existing presence of a treated or untreated bleeding disorder, diabetes mellitus, high blood pressure (systolic > 140 and/or diastolic > 90 mmHg), thyroid disease, tachyarrhythmia, heart disease, kidney disease or liver disease
- Having had an abnormal electrocardiogram
- Existing sleep disorder and/or being treated for (or a known history of) clinical depression, eating disorder(s) or any other psychiatric condition(s) that may confound the results of the study
- Known allergy or sensitivity to any ingredient contained within either of the three test formulas or placebo (inclusive of persons with phenylketonuresis, lactose intolerance or dairy food allergies)
- Any findings on the health status questionnaire that represented a clinically significant deviation from normal/acceptable
- A medical condition or use of any medication that may place the subject at risk or confound the results of the study
- Use of any androgenic anabolic steroids, “pro-hormones”, or related precursors or salts within one year prior to enrollment
- Missed > 1 training session per week or was not complying with the study

guidelines or controls (e.g., consuming additional supplements, engaging in supplemental exercise, etc.)

- ❖ Identified as a moderate-to-high risk individual as described by the American College of Sports Medicine (69) (i.e., possessing > 1 of the following):
  - ➢ Father or brother, or mother or sister that had a sudden death before 55 or 65 years of age, respectively
  - ➢ Current cigarette smoker or quit smoking < 6 months prior to enrollment
  - ➢ On hypertensive medication or had a confirmed systolic or diastolic blood pressure ≥ 140 or 90 mmHg, respectively
  - ➢ On lipid lowering medication or had a total cholesterol level ≥ 200 mg/dL
  - ➢ A confirmed fasting blood glucose of ≥ 100 mg/dL
  - ➢ Clinically obese (> 32% body fat)
  - ➢ Sedentary

NUTRITIONAL ANALYSIS

All participants were instructed to maintain pre-study, *ad libitum* dietary habits and asked to provide three-day nutrition logs for the week prior to baseline (PRE) testing, as well as for weeks 1, 4-5, and 8 of the intervention, for a total of four weeks of nutrition logs. Each log included two non-consecutive weekdays and one weekend day, and was used to represent subjects' average weekly diets. Logs were analyzed for total energy (CALS; kcals/d and kcals/kg/d), macronutrient [FAT (g/d, g/kg/d, % of kcals/d, and Unsaturated, Saturated and *Trans*-), CHO (g/d, g/kg/d, % of kcals/d, and Sugar and Fiber), and PRO (g/d, g/kg/d, and % kcals/d)], essential vitamins and minerals, caffeine and alcohol intake

per day, using Food Processor Version 8.6.0 (ESHA Research, Salem, Oregon). Results obtained for weeks 1, 4-5, and 8 were combined to provide an average daily value across each nutritional variable for the 8-week intervention. Subjects also recorded nutritional intake for the 24 hours preceding baseline (PRE) 1RM and 80RM testing. For $1RM_{POST}$, $80RM_{POST}$ and repeated 80RM testing, subjects were provided a copy of their baseline 24-hour nutrition log and required to replicate (as closely as possible) the same nutritional intake prior to and during all 1RM, 80RM and repeated 80RM testing days (with the addition of twice daily supplementation occurring during POST testing).

EXERCISE PROTOCOL

The resistance training intervention involved an 8-week, split-body, linear periodized program as used previously by Kerksick et al.(70) Subjects participated in supervised upper- and lower-body heavy resistance training 2x/wk, for a total of four workouts per week x 8 wks. Training and recovery days followed a 2-on/1-off/2-on/2-off schedule (e.g., Monday-UPPER, Tuesday-LOWER, Wednesday-OFF, Thursday-Upper, Friday-Lower, Saturday-OFF, Sunday-OFF, repeat). A 5-minute moderate intensity, continuous motor recruitment warm-up (e.g., stationary cycling or treadmill jogging) preceded each workout session. Resistance exercises targeted all major muscle groups and consisted primarily of multi-joint movements. After the 5-minute warm-up, bench press and hack squat were always performed first on upper- and lower-body training days, respectively. Exercise order for the remaining exercises was not controlled. Subjects completed three sets per exercise, allowing one-minute rest between sets and two-minute rest periods between exercises, using a 10-12RM and 5-8RM load for weeks 1-4 and 5-8,

respectively. Refer to **Figure 2** in Appendix B, for the resistance training program used during the 8-week intervention. Subjects were instructed to complete each set to volitional muscle failure, adjusting the load lifted accordingly to ensure all sets were completed within the requisite repetition range. All subjects were provided a stopwatch to ensure accuracy of rest period duration and to track total workout duration. All subjects recorded loads used and successfully completed repetitions per set, and total training time and sets on individually marked training logs. Upper- and lower-body, as well as total training volume were calculated for each subject, week and total over the entire eight weeks as follows: *Absolute Volume (Kg) = (load x reps) x sets*; *Relative Volume (Kg/min) = Absolute Volume / time*. Subject training logs were assessed weekly, and prescriptive loads provided for the subsequent week's workouts. All participant workouts were supervised by an ACSM or National Strength and Conditioning Association certified strength and conditioning specialist or trainer.

DIETARY SUPPLEMENT INTERVENTION

Beginning 48 hours after completion of $1RM_{PRE}$ and $80RM_{PRE}$ testing, participants began consuming 1 of 4 double-blind treatments – PLA, WPH, WPC80+ or WPC80 – twice daily for 62 consecutive days (i.e. each day of the 8-week training intervention and POST testing period). All treatments were formulated to contain similar amounts of total energy and lipid, and all treatments were double-blinded for appearance, taste, texture and packaging. Supplements were consumed immediately pre- and post-exercise on training days, and twice daily between regularly scheduled meals on non-training days. On training days, subjects were provided their supplements, pulled from individually and

randomly assigned cases of their randomly assigned within-group product allotment. Prior to non-training day periods, subjects were allocated enough product for full compliance until the next scheduled training day. Subjects were instructed to consume the supplement on an empty stomach (i.e., no closers than 90 minutes after a prior meal) and not to consume food or other energy-containing items within 30 minutes after supplement consumption. To mix the supplement, subjects were instructed to fill a shaker cup with 6-8 fl ozs of water, empty the contents of their individually labeled packet into the water, add ~2 fl ozs of water to the empty packet, mix and pour the remaining contents of the packet into the shaker cup, shake contents of the cup vigorously for ~1-2 minutes, let the solution settle for ~2-3 minutes, consume within 5 minutes, add an additional 2-4 fl ozs of water to the cup, shake vigorously, and consume the remaining dilute.

The placebo (PLA) was formulated with 30 g of dextrose anhydrous per serving, as well as minor amounts of reduced-fat dairy creamer and xanthan gum to both equilibrate the lipid content across all treatments and to double-blind the treatments for viscosity and appearance. The WPC80 group consumed 30 g of PRO from an 80% whey protein concentrate (Carbelac®, Carbery, Cork, Ireland), whereas subjects in the WPC80+ group consumed 30 g of PRO from a high-lactoferrin containing 80% whey protein concentrate (Progenex Dairy Bioactives, Inc, Costa Mesa, CA). Subjects in WPH consumed 30 g of PRO from an extensively hydrolyzed (32% degree of hydrolysis) 80% whey protein concentrate (Optipep™, Carbery, Cork, Ireland), designed to provide greater than 80% of its protein fractions as <1 kD in molecular weight (see **Figure 3**, in Appendix B, for the

complete molecular weight distributions/profiles of each of the whey proteins used in this study). All treatments were formulated with sucralose, orange flavoring and citric acid, whereas the WPH treatment additionally required the use of a mint-based masking agent to reduce bitterness (see **Figure 4**, in Appendix B, for complete nutritional profiles across all treatments). All groups consumed two supplements per day, for a total of 60 g/d of active material or placebo. Final formulation, packaging and double-blinding was conducted at and by a cGMP compliant manufacturing facility (CSB Nutrition, Lindon, UT), and un-blinding was provided by the manufacturer's representative agent upon request by the study coordinator after all statistical analysis had been completed.

Upper- and Lower-Body Strength

One-repetition maximum (1RM) upper- and lower-body strength was determined using the barbell flat bench press (1RM BP) and incline plate-loaded hack squat machine (1RM HS), respectively. Subjects performed two warm-up sets prior to 1RM attempts. The first warm-up allowed subjects to perform 10 repetitions of an estimated 50% 1RM, whereas the second warm-up set utilized an estimated 80% 1RM load for 2-3 repetitions. Warm-up sets and all subsequent 1RM attempts were separated by 3-minute recovery periods, (71) and no more than five 1RM attempts were allowed for either the BP or HS. Subjects performed 1RM BP attempts prior to 1RM HS testing, and only correctly performed repetitions were accepted for data collection purposes. During all BP and HS attempts, a spotter (or spotters) assisted in un-racking the weight and to ensure subject safety.

Upper- and Lower-Body Anaerobic Endurance

Upper- and lower-body anaerobic endurance (80RM) was determined using the barbell flat bench press (80RM BP) and incline plate-loaded hack squat machine (80RM HS), respectively. Five to seven minutes recovery was provided between 1RM testing and initiation of 80RM tests.(72) Subjects performed one maximal effort set to volitional exhaustion (for total number of repetitions completed), of both the BP and HS, using a 80% 1RM load of the respective exercise. Subjects performed 80RM BP testing prior to 80RM HS. Three to five minutes recovery was allowed between 80RM BP and 80RM HS attempts. Repetitions that were not performed using proper form/acceptable range of motion were deducted from the total repetitions completed, and subjects were not allowed to pause for greater than two seconds after the completion of each repetition while performing 80RM attempts. During POST testing, subjects consumed a supplement immediately pre- and post-testing, and used the same 80RM load as determined during PRE testing.

Repeated Lower-Body Anaerobic Endurance

Twenty-four and 48 hours after 80RM HS testing, in week 9 (POST), subjects returned to the laboratory to repeat 80RM HS attempts. Upon arrival, blood was collected to assess pre-exercise (or, 24-hr recovery) creatine kinase and white blood cells. Following blood collection, subjects consumed one serving of their assigned supplement and then performed two progressive warm-up sets on the HS. The first warm-up set was performed for 8-10 repetitions at 50% of PRE-testing 1RM HS load, whereas the second warm-up set was performed for 2-3 repetitions at 70% of PRE-testing 1RM HS load.

Warm-up sets were separated by one-minute rest periods; a 3-minute rest period was provided between the second warm-up set and the full effort 80RM HS attempt. Blood was again collected immediately post-exercise, which was then followed by consumption of a post-workout supplement.

BODY COMPOSITION

All body composition assessments were performed on the same day, following a 12-hour fast (water intake was allowed up to one hour prior to testing). No exercise or diuretic-enhancing products (e.g., caffeine) were allowed 48 hours prior to testing, and subjects were instructed to remain well hydrated prior to testing. Hydration status was determined immediately prior to body composition testing, using specific gravity via handheld refractometry (Model CLX-1, precision = 0.001±0.001, VEE GEE Scientific, Inc., Kirkland, WA).(73) Subjects with urine specific concentrations $\geq$ 1.029 ppm were asked to consume 8 fl ozs of drinking water, every 15 minutes, and were retested every 30 minutes until an acceptable hydration status was achieved. Subjects with urine specific concentrations $\leq$ 1.005 ppm were asked to pedal slowly on an upright cycle ergometer for 15 mins and were retested every 30 mins until an acceptable hydration status was achieved.

Body mass (BM) was measured using a calibrated clinical scale to the nearest 0.001 kg, with subjects wearing only tight-fitting compression shorts; height (HT) was measured to the nearest 0.5 cm using a calibrated stadiometer. Fat mass (FM) and lean body mass (LBM) were estimated using dual-energy X-ray absorptiometry (DXA; enCORE™ 2006,

software version 10.50.086, Lunar Prodigy Advance, Madison, WI). Percent body fat (%FAT) was calculated as: *%FAT = (FM/BM) x 100*. The sum of lean soft tissue for both arms and legs (ALST), as measured by DXA, was used to estimate total body skeletal muscle mass (TBMM) from the validated equation of Kim et al.(52): *TBMM = (1.13 x ALST) – (0.02 x age) + 0.97*.

Each day, prior to testing, a quality assurance phantom was performed to ensure calibration of the DXA machine. Subjects were positioned supine on the DXA table, subjects' arms extend at their sides and hands pronated and flat on the table. Subjects' HT, BM, sex, date of birth, and race were entered into the software program, total body mode was selected for each scan, and scanning thickness was determined by the DXA software. All DXA assessments were conducted, and all DXA machine-provided regions of interest (ROIs) were manually checked and adjusted (if necessary) by the same researcher.(74) Test-retest measurements of 11 men and women, measured 24-48 hours apart, for %FAT and TBMM resulted in intraclass correlation coefficients (ICC) greater than 0.99, and standard error of measurements (SEM) of 0.75% and 0.04 kg, respectively.(75)

Clinical Adaptations

Blood was collected a total of nine times (T1-T9) over the course of the 8-week intervention and two weeks of testing. Specifically, T1 and T4 blood samples were collected following a 12-hour fast (water only) and minimum 48-hour abstinence from strenuous exercise, immediately following body composition testing during PRE and

POST testing to assess blood lipid, hepatic, immune and renal response to the intervention. T2 and T3 blood samples were collected following a 4-hour fast (water only), 24 hours after the first and final lower-body exercise session in weeks 1 and 8, respectively, to assess creatine kinase and white blood cells. T6-T9 were collected immediately pre- and post-24- and 48-hour repeated 80RM HS testing during POST. T4 served as baseline pre-exercise (day 1) of the repeated 80RM HS testing, whereas T5 served as baseline post-exercise (day 1) and was withdrawn immediately after completion of all $1RM_{POST}$ and $80RM_{POST}$ testing. Samples were individually labeled by subject code, separated by centrifugation, stored in refrigeration, and collected daily by Diagnostic Labs of Oklahoma (Oklahoma City, OK) for analysis.

STATISTICS

Separate 2x4 or 6x4 two-way repeated measures ANCOVAs [time (PRE vs. POST, or WEEK 1 vs. WEEK 8, or $POST_{PRE\ \&\ POST}$ vs. $24HR_{PRE\ \&\ POST}$ vs. $48HR_{PRE\ \&\ POST}$, respectively) x group (PLA vs. WPH vs. WPC80+ vs. WPC80)] were used to identify main effects for time and time*group interactions. Subject's baseline upper-body strength ranking (i.e., fitness level), as assessed by the ACSM's adapted percentile rankings for 1RM BP-to-BM ratio (69), total 8-week relative training volume (kg/min), and average 8-week relative protein intake (g/kg/d) were selected as covariates. For statistical analyses of clinical measures, blood collection time also served as a covariate. In the event of sphericity violations, Greenhouse-Geisser *F*-tests were used to analyze main effects. If a significant interaction was observed, the statistical model was decomposed by examining simple main effects with one-way repeated measures

ANCOVAs across groups and one-way factorial ANCOVAs across time. In the event of a simple main effect, Tukey *post-hoc* comparisons were performed among groups; all pair-wise comparison dependent samples t-tests with Bonferroni corrections were performed across time ($p \leq 0.0125$). If there were no interaction, main effects were analyzed by collapsing across the non-interacting variable as described above for simple main effects. In the event of significant baseline differences of a dependent variable, as determined by multiple one-way ANOVAs, homogeneity-of-slopes tests were used to determine the interaction between the covariate and factor, and to assess the appropriateness of including the variable as a covariate within subsequent ANCOVA analyses. No other variables (other than those pre-selected) were necessary to serve as covariates. Additionally, no significant ($p > 0.05$) violations of linearity were observed. Using an *a priori* level of significance of $p \leq 0.05$ and medium effect size (ES) of 0.25 for within-between interactions for repeated measures ANOVA analysis across four groups and with statistical power (1-β) of 0.80, total sample size (N) was determined to be 42. *Post-hoc* ANCOVA analysis for fixed effects, main effects and interactions *F*-tests, using $p \leq 0.05$ level of significance, N=56, observed partial correlation coefficient of 0.180 (mean value for FM and TBMM between-groups effects), and three covariates, yielded an ES=0.47, critical *F* statistic of 4.03, and observed 1-β=0.93. All *a priori* and *post-hoc* sample size and power analyses were performed using G*Power Version 3.1.1 (Franz Faul, Universitat Kiel, Germany), and all statistical analyses were performed using SPSS 17.0 (SPSS Inc. Chicago, IL).

RESULTS

DESCRIPTIVE ANALYSES

Data from 56 of the 57 subjects that completed the study were used for analysis (PLA, n=15; WPH, n=13; WPC80+, n=15; and WPC80, n=13). Baseline (PRE) measures for age, height, percent body fat (%FAT), upper- and lower-body maximal strength (1RM BP and 1RM HS, respectively), training status ratio (1RM BP/BM), and relative daily energy (CALS), protein (PRO), carbohydrate (CHO) and lipid (FAT) intake did not differ (p>0.05) between groups (refer to **Table 1**, in Appendix A). A trend toward significant differences (p=0.079) at PRE was, however, observed for body mass (BM). Post hoc analyses revealed the effect arose from a significant difference (p=0.050) between WPC80 and PLA. Homogeneity of slopes tests did not, however, conclude BM_{PRE} to be a significant (p>0.05) covariate for further analysis. Additionally, there were no significant differences (p>0.05) between groups for any dietary measures assessed for the 24 hours prior to $1RM_{PRE}$ and $80RM_{PRE}$ testing.

NUTRITIONAL ANALYSES

No significant (p>0.05) interactions or main effects for time (PRE versus the average of weeks 1-8) were observed for adjusted average means for relative CALS (kcals/kg/d), CHO (g/kg/d or % of kcals/d), or FAT (g/kg/d or % of kcals/d), however, significant within-group differences across time (p<0.0125) were observed for CALS (WPC80+ = +14.79%; WPC80 = +23.33%), CHO [% of kcals/d (WPC80+ = -13.08%; WPC80 = -14.45%)] and FAT [g/kg/d (WPC80 = +20.59%); % of kcals/d (WPH = -16.48%); see

Table 2, in Appendix A]. Interestingly, no significant (p>0.05) interaction or main effect for time was observed for relative PRO (g/kg/d), though within-group differences across time were significant (p<0.0125) for each of the three WP groups. When PRO was expressed as a % of kcals/d, however, significant (p<0.05) interaction and main effects for time were present, and *post hoc* comparisons revealed significant differences (p<0.05) between PLA and each of the three WP groups.

Training Volume

Adjusted average means for total relative training volume (kg/min) revealed PLA>WPC80>WPH>WPC80+, however no differences were observed between groups (p>0.05). Specifically, total relative training volume for PLA (553,956.26±23,003.15 kg/min) was 1.06% greater than WPC80 (548,128.12±20,143.12 kg/min), 5.43% greater than WPH (525,421.60±21,021.41 kg/min), and 8.31% greater than WPC80+ (511,454.05±19,511.84 kg/min). Repeated measures [4x8 (Group x Time)] analysis also resulted in no significant (p>0.05) interaction or main effects for time for relative total training volume (time: p=0.194, ES=0.030, 1-β=0.465; time*group: p=0.594, ES=0.049, 1-β=0.485), nor were any significant differences (p>0.05) observed between groups when total relative training volume was decomposed by one-way factorial ANCOVAs across time (see **Figure 5**, in Appendix B).

Strength and Anaerobic Endurance

Results from 1RM, 80RM and repeated 80RM testing are presented in **Table 3**, in Appendix A, and **Figures 6** and **7** in Appendix B. Briefly, there were no significant

(p>0.05) interaction effects or observable trends (refer to **Figure 7**, "Individual responses for strength and anaerobic endurance changes from PRE to POST and for repeated 80RM", in Appendix B) for any of the upper- or lower-body 1RM, 80RM, or repeated 80RM dependent variables. Main effects for time were, however, significant (p<0.05) for both upper- and lower-body 1RM and 80RM. Collapsing across groups revealed that all groups increased upper- and lower-body 1RM and 80RM significantly (p<0.0125) from PRE to POST. No significant (p>0.05) main effect for time was observed for repeated lower-body 80RM, though. Collapsing across groups did, however, reveal a significant (p<0.0125) within-group effect for WPC80+, between repeated 80RM reps completed during POST and 24 hours post-testing (24HR; +15.56%).

Body Composition

Body composition results and individual response graphs are presented in **Table 4**, in Appendix A, and **Figures 8** and **9** in Appendix B. Notably, *post hoc* analyses revealed no significant differences (p>0.05) between changes observed in PLA versus any of the three WP groups (or, between WP groups), except for changes in FM and %FAT between PLA and WPH [+0.861 kg (p=0.057) and +0.640%, versus -1.126 kg and -1.601%, respectively]. Similarly, though no significant (p>0.05) interaction effects were observed for changes in LBM or TBMM, and all groups significantly (p<0.0125) increased LBM and TBMM from PRE to POST, individual response plots would seem to indicate that WPH provided the most consistent positive response across these two variables (92.31% and 100.00% positive responders for changes in LBM and TBMM, respectively).

CLINICAL MEASURES

12-Hour Fasted (PRE versus POST): Changes in 12-hour fasted blood lipids, from PRE to POST, revealed no significant ($p>0.05$) interactions or main effects for time, and no significant between- or within-group changes ($p>0.05$ and $p>0.0125$, respectively) when collapsed across time and group (see **Table 5** in Appendix A). Similarly, group rankings based upon percent of within-group subjects responding favorably to the intervention (histograms not shown) provided no discernable trends other than WPC80+ and WPC80 possibly yielding the most consistent favorable and unfavorable responses, respectively, across all blood lipids. However, no group achieved favorable responder rates of greater or less than 76.9% or 23.1% (WPH for TC and TC:HDL ratio, respectively) for any of the blood lipids measured.

Results for 12-hour fasted, PRE versus POST, changes for blood glucose (GLUCOSE), urea nitrogen (BUN), creatinine, BUN:creatinine ratio, creatine kinase (CK) and total white blood cells (WBC) are presented in **Table 6**, in Appendix A, and **Figure 10**, in Appendix B. No significant ($p>0.05$) interactions or main effects for time were observed for any of the dependent variables assayed, with the exception of a significant interaction effect for BUN ($p=0.027$) and a trend toward significance for BUN:creatinine ($p=0.093$) and WBC ($p=0.065$). Collapsing across time and treatment revealed that all groups significantly decreased creatinine ($p<0.0125$). Also, a significant and non-significant trend for between-group differences was observed for WPH versus WPC80 (BUN: $p<0.05$; BUN:creatinine: $p=0.085$). Similarly, non-significant trends were observed for WBC, both between WPH and WPC80+ ($p=0.075$), and from PRE to POST for WPC80+

(p=0.056).

24-Hour Exercise Response (Week 1 versus Week 8): All groups, except WPH (p=0.093), realized a significant (p<0.0125) decrease in CK from week 1 versus 8 for 24-hour response to the first and final lower-body workout (see **Table 7**, in Appendix A, and **Figure 11** in Appendix B). However, there were no significant (p>0.05) between-group effects observed, despite WPC80+ reducing CK response by over 1.53x the change observed in WPH (p=0.073). Similarly, there were no significant (p>0.05) interactions or main effects for time for WBC; though, collapsing across time revealed a non-significant trend comparing week 1 to 8 for WPC80 (p=0.067).

Repeated 80RM (80RM$_{POST}$ versus 80RM$_{24}$ versus 80RM$_{48}$): Though a positive linear trend for time, across all groups, was observed for WBC and CK for pre- and post-repeated 80RM tests, no significant (p>0.05) between-group differences, and no significant (p>0.05) interaction or main effects for time were observed (see **Table 8**, in Appendix A, and **Figure 12**, in Appendix B). Instead, only a non-significant (p=0.077) difference between PLA and WPH was observed for WBC at timepoint 2. All pre-testing WBC values for 80RM$_{POST}$, 80RM$_{24}$ and 80RM$_{48}$ were significantly different (p<0.0125) from all post-testing values, with the exception of timepoint 2 vs. 5, for WPC80 (p>0.0125). However, there were no significant differences across time (p>0.0125) for pre- or post-testing responses (e.g., pre-80RM$_{POST}$ versus pre-80RM$_{24}$) within any of the four groups; only non-significant trends for timepoints 3 vs. 5 and 1 vs. 5 for WPH (p=0.072) and WPC80 (p=0.105), respectively. Interestingly, for within-group CK

response, timepoints 1 and 2 generally did not differ significantly (p>0.0125) from timepoints 3-6 within any group. A significant effect from timepoint 2 vs. 4 (p<0.0125) and trends from 2 vs. 3 (p=0.080), 2 vs. 5 (p=0.080) , and 4 vs. 5 (p=0.096) were, however, observed for WPC80+. Additionally, PLA realized a significant (p<0.0125) decrease in CK from timepoint 4 vs. 5, and trended toward significant differences for timepoints 3 vs. 5 (p=0.093) and 4 vs. 6 (p=0.060).

DISCUSSION

This is the first study to compare the effects of different forms of a whey protein (WP) on the physiological response to chronic (4 d/wk x 8 wks), heavy resistance exercise. It was hypothesized that the addition of 60 g/d protein (PRO; as one of three forms of WP), versus carbohydrate (PLA), would support greater increases in muscle mass (TBMM) and reductions in fat mass (FM) in previously trained young men. Furthermore, it was postulated that provision of an extensively hydrolyzed 80% whey protein concentrate (WPH) would accentuate gains in TBMM and reductions in FM compared to its native 80% whey protein concentrate (WPC80). This hypothesis was based upon acute data that has previously shown improved exercise recovery and increased insulin response, as well as a trend toward greater muscle protein synthesis arising from the use of extensive hydrolysates versus their native PRO source.(11, 13, 66) A final hypothesis was that a modified WPC80, containing significantly higher concentrations (100x greater than native WPC80) of the antioxidant and immune supporting PRO fraction, lactoferrin (WPC80+) (48, 76), may elicit greater gains in TBMM, as well as improve clinical and training response to repeated anaerobic endurance bouts compared to WPC80. Instead, the current study revealed that, in previously trained, healthy young men (18.89±0.70 %FAT; 21.40±0.36 yrs), eight weeks of heavy resistance training plus twice daily WP, regardless of WP form or molecular weight distribution, was no more effective than PLA at increasing TBMM, lean body mass (LBM), upper- and lower-body strength (1RM BP and 1RM HS, respectively) and anaerobic endurance (80RM BP and 80RM HS, respectively), and response to repeated 80RM bouts. Similarly, no significant between-

group effects were observed for TBMM, LBM, 1RM, 80RM or repeated 80RM for the three WPs under investigation. WPH did, however, result in greater FM loss and reduction of percent body fat (%FAT) versus PLA, and WPH also appeared to improve nitrogen retention and/or metabolic efficiency compared to WPC80 and WPC80+.

The non-significant effects observed between consuming a WP or carbohydrate (CHO) placebo are only surprising if the existing literature is not differentiated between studies involving trained versus untrained subjects. For example, both Willoughby et al. (17) and Andersen et al. (34) have reported that 10-14 weeks of pre- and post-exercise PRO intake (20-25 g/serving/d), combined with ≥ 3 d/wk heavy resistance training in previously untrained males, was significantly more effective than CHO at increasing body mass (BM), fat-free mass, thigh mass, upper- and lower-body 1RM (17), peak power output, muscle cross sectional area (34), myofibrillar protein, and markers of muscle protein synthesis and anabolism.(17) Comparatively, the effects of PRO versus CHO in previously resistance trained males is less positive. For example, Cribb et al. (77) reported that heavy resistance training plus 1.5 g/kg/d x 11 weeks supplemental whey protein isolate (WPI) or CHO provided comparable changes on BM, LBM, FM, %FAT, and muscle hypertrophy (as assessed by muscle biopsies) in previously resistance trained, young men. However, an accentuated ($p<0.05$) response from WPI versus CHO on upper- and lower-body 1RM, as well as vastus lateralis myofibrillar content was observed. Kerksick et al. (18) similarly reported no significant differences ($p>0.05$) for changes in BM, FM, or %FAT between previously resistance trained male subjects consuming an additional 48 g/d CHO or PRO (as a WP and casein blend) while involved

in 4 d/wk x 10 weeks heavy resistance training. Instead, only a significant difference between groups for changes in LBM was observed (PRO = +3.10%; CHO = 0.00%; $p<0.05$). Also, Kerksick et al. (18) reported that both PRO and CHO realized significant improvements over time in upper- and lower-body 1RM and 80RM; however, these changes were not significantly different between groups.

Similar (significant) main effects for time, but no significant group interactions for TBMM, and upper- and lower-body 1RM and 80RM were also observed in the current study, which may be the result of the same linear, periodized training program used in both the Kerksick et al. (18) and the current investigation. Therefore, compared to the results observed by Cribb et al. (77) it could be argued that, 1) the training program selected for the current study did not provide ample total or within-phase time to achieve between-group significance for muscle hypertrophy and strength in previously trained men [Weeks 1-4 (hypertrophy) + Weeks 5-8 (strength)], or 2) the current study did not provide adequate amounts of PRO to achieve some minimum necessary difference in g/kg/d PRO between the PLA and WP treatments [Cribb et al.(77): CHO group = 1.6 g/kg/d vs. PRO group = 3.1 g/kg/d; current investigation: PLA = 1.575 g/kg/d vs. WPH = 1.904 g/kg/d vs. WPC80+ = 1.971 g/kg/d vs. WPC80 = 1.846 g/kg/d; refer to **Table 2**]. In neither argument, though, is the respective affect particularly relevant to assessing the effects of WP source or molecular composition on physiological adaptations to resistance training. It is worth discussing, however, that the second argument posed above may challenge conclusions proposed by Hoffman et al.(63) Specifically, "that protein intakes at or above the recommended levels for strength and power athletes (1.2-1.7 g/kg/d) do

not augment lean body mass, power, or strength gains." Sample size notwithstanding [Cribb et al. (77): CHO group n = 7 vs. PRO group n = 5], it is more unlikely that the significant between-group 1RM differences observed by Cribb et al.(77) was the product of WP type (i.e., WPI) instead of a potential added benefit of doubling subjects' PRO intake, regardless of the fact that all groups were found to consume PRO intakes "at or above recommended levels." If the use of WPI was indeed the significant contributing factor, which is the only other explanation if the conclusion by Hoffman et al.(63) is correct, then at minimal, a trend toward significance for differences between PLA and WPC80 or WPH would have been expected within the current investigation. Thus, it is recommended that future resistance training interventions, in previously trained men, attempt to compare the effects of graded relative doses of PRO versus CHO to identify if (and at what amount) a minimal g/kg/d of PRO difference is required to elicit significant between-group effects on measures of muscle hypertrophy, LBM, and upper- and lower-body strength and muscular endurance. Also, if any benefit exists, at what chronic dose of PRO is a threshold reached by which no additional performance or body composition benefit realized.

Another explanation for the mostly paired effects observed between PLA and each of the WP treatments in the current investigation, may be found within work involving primed constant infusion to assess muscle protein synthesis (MPS) in response to resistance exercise. Collectively, as presented in a recent review paper by Burd et al. (1), MPS appears to peak between 3-4 hours post-exercise in previously trained subjects. In previously untrained muscle, MPS appears to peak between 16 and 28 hours post-

exercise. The implications such findings have on the observed outcomes within the current study are that, aside from the twice daily supplementation guidelines, no additional controls were placed on the subjects' diets. Most importantly, there was no mandatory fasting period in the hours following each exercise session or the non-training day doses.(7) Thus, it is plausible that the suppression of muscle protein catabolism arising from a marked increase in insulin (provided by PLA), if subsequently followed by a protein-containing meal or snack within 3-4 hours post-exercise, may have significantly augmented the corresponding net protein balance response to exercise plus PLA.(78) In other words, it is possible that by attempting to observe "real world" results and not place tight controls on subjects' diets that, instead, the control group (PLA) itself became little more than a fourth PRO treatment group (i.e., CHO + PRO). An underlying assumption to this hypothesis, though, is that WP provided a satiating effect in the hours immediately following its ingestion.(79) If indeed such an affect did occur, there were no between-group effects observed on measures of relative energy intake (kcal/kg/d) that may readily substantiate this hypothesis. In fact, subjects in WPC80+ and WPC80 realized significant increases in energy intake from PRE to POST, while energy intake for both PLA and WPH increased slightly, but non-significantly (refer to **Table 2**). Hoffman et al.(63) did, however, report what could be concluded as a satiating effect in response to PRO timing occurring immediately pre- and post-resistance training as opposed to when PRO is consumed several hours distal to training. Specifically, subjects consuming a 42 g PRO supplement (predominantly composed of hydrolysate) immediately pre- and post-exercise realized a non-significant (p>0.05) reduction in energy intake (-11.5% kcals/kg/d and -10.5% kcals/d), whereas subjects consuming the PRO supplement at times distal to

training had no change in relative or absolute energy consumed over the 10-week intervention. What makes this difference interesting, though, is that the pre/post supplement intervention did not promote a significant increase in PRO intake (+20.0% g/kg/d and +16.6% g/d) from week 0 to week 10. A significant increase in relative and absolute PRO was, however, observed in the other PRO group (+62.9% g/kg/d and +59.0% g/d). Speculatively, it is possible that when adequate amounts of PRO or essential amino acids are not consumed within a specific post-exercise period of time, increased nitrogen intake (or some nitrogen seeking response) may arise as a secondary outcome (or primary trigger) of the previously well-described behavioral compensatory response to exercise-induced increases in energy expenditure.(80) In partial support, even though subjects in PLA were shown to be consuming an adjusted average of 1.452 g/kg/d PRO at baseline, the group's adjusted average intake throughout the 8-week intervention increased to 1.575 g/kg/d (+8.47%; $p>0.0125$). Thus, it is possible the PLA group may have benefited from a combined effect of prior insulin stimulation and added PRO ingestion/seeking within the 3-4 hours post-exercise. Or, the high relative PRO intake across all groups may just provide additional support to the aforementioned conclusion by Hoffman et al.(63) regarding no augmented effects arising between groups consuming adequate amounts of PRO.

Regardless of daily PRO intake or nutritional variables that were not adequately controlled, acute data (11, 13, 66) would seem to support the hypothesis that WP form or molecular distribution may affect adaptations to chronic resistance training. For example, Buckley et al. (66) reported that, compared to native WPI, 25 g of an

extensively hydrolyzed WPI, consumed 3x within a 24-hour period following a maximal eccentric exercise bout, significantly improved recovery time on measures of peak isometric torque in previously sedentary males. Contrary to those findings, no significant differences in repetitions or blood measures were observed in the current investigation involving previously trained subjects. A time effect was, however, observed for WPC80+ between days 1 and 2 (+15.56% repetitions on day 2 vs. day 1; $p<0.0125$), though subject effort as opposed to any direct benefit specific to the WP itself may have caused this lone difference within- or between-groups for repeated 80RM tests. The observed, significant increase in post-exercise blood creatine kinase, from day 1 vs. 2 for WPC80+, would seem to support this conclusion (refer to **Table 8** and **Figure 12**). Interestingly, comparison of groups does seem to indicate supplementation with WPH may have elicited less variability between repeated 80RM bouts (see **Figure 6c.**). This observational trend was not, however, represented by a correspondingly significant increase in training volume over the 8-week intervention, as may have been expected to occur if non-significant improvements in recovery culminated over time. Notably, no significant differences were observed between PLA or any of the WP groups for repeated 80RM bouts, which is in disagreement with the results observed by Hoffman et al.(64) There are two plausible explanations for these differences: 1) subjects in the Hoffman et al. (64) study were intercollegiate football players or competitive powerlifters, and therefore less likely than recreational weight lifters to deviate from putting forth maximal effort on every exercise attempt, and 2) the repeated exercise protocol used by Hoffman et al.(64) was of a multi-set design, and thus may be a more valid model to replicate in future studies than the single exhaustive set design as was used in the current

investigation.

Acute data from Koopman et al. (13) recently reported 25-50% greater total plasma amino acid concentrations ($p<0.01$) and a trend ($p=0.10$) toward a greater incorporation rate of amino acids into skeletal muscle arising from the ingestion of 35 g of casein hydrolysate versus native casein in healthy, but elderly men (64±1 yrs). In fact, similarly related acute data provides some evidence that, versus native WP, extensively hydrolyzed WP, or WP explicitly manufactured to be high in specific fractions may offer improved effects on body composition response to resistance training.(10-12, 29, 30, 35, 81-84) For example, Power et al. (11) presented data that revealed a 43% greater 3-hour area under the curve and 28% greater peak insulin response in healthy male volunteers (22.4±0.48 yrs) after consuming 45 g of a WPH (similar to the WPH used in the current investigation) versus WPI. Therefore, it is not unreasonable to postulate that if greater plasma amino acid concentrations and insulin responses occur acutely in response to WPH versus native WP, then any summating of differences on net protein balance may be able to be observed grossly over time. In the present investigation, however, no significant differences for either TBMM or LBM were observed between WPH and WPC80. However, 12-hour fasted blood data does offer some evidence that WPH may have provided higher nitrogen retention than WPC80 (or WPC80+).(37, 85) Specifically, fasting blood urea nitrogen (BUN) was significantly reduced in WPH and increased in WPC80 [WPH: -2.760 mg/dL (-18.064%) vs. WPC80: +2.128 mg/dL (+16.908%); $p<0.05$], and a corresponding trend was observed between groups for BUN:creatinine ratios from PRE to POST [WPH: -0.920 (-6.195%) vs. WPC80: +2.886 (+23.346%);

p=0.085]. Furthermore, though all groups significantly reduced PRE to POST fasted blood creatinine, POST values differed significantly between WPH and WPC80+ (WPH: 0.911±0.030 mg/dL vs. WPC80+: 1.062±0.027 mg/dL; $p<0.05$), and WPH provided the greatest absolute reduction relative to the other WP groups though no between-group differences reached significance (WPH - WPC80 = -51.24%; WPH - WPC80+ = -55.37%; see **Table 6**). However, since post-prandial BUN and creatinine response to PRO feeding was not assessed in this investigation, it is unwarranted to draw any specific conclusions from the observed differences noted above.

Regardless of a possible improvement in nitrogen retention from WPH, based upon fasted blood data, there were no real differences observed between WP groups for TBMM. However, it would be premature to assume that since no significant differences were observed (for TBMM) in this trial, that similar effects can be expected from combined resistance training and WP of any source or molecular distribution. One limitation to this assumption is the lack of dietary controls applied to this investigation. For example, a significant increase in total energy intake was observed in both WPC80 [+6.030 kcal/kg/d (+23.325%); $p<0.0125$] and WPC80+ [+4.086 kcal/kg/d (+14.793%); $p<0.0125$], versus WPH [+0.996 kcal/kg/d (+3.415%); $p>0.0125$]. At an adjusted baseline BM of 83.471±1.511 kg for WPC80 and 82.261±1.572 kg for WPH, and a 6.030 and 0.996 kcal/kg/d average increase in energy intake during the 8-week intervention for WPC80 versus WPH, respectively, a total 8-week caloric surplus of 23,598.30 kcals for subjects in WPC80 versus WPH would have occurred to support changes in TBMM. The significant PRE to POST increase in BM that was observed for both WPC80 and

WPC80+, but not WPH, may provide evidence to support this hypothesis. [NOTE: Including energy intake as a fourth covariate was assessed and deemed unnecessary; in fact, its inclusion increased the ANCOVA model sum of squared errors and thus was not included as a covariate for analyses.] Similarly, the PRO intervention amongst the three WP groups resulted in a 28.92% and 14.63% greater increase in g/kg/d PRO for WPC80 and WPC80+ versus WPH. It is therefore recommended that future research in this area include tighter dietary controls to decrease the influence potentially confounding variables may have on more accurately assessing the research question.

Though no significant effects were observed between WP groups on measures of TBMM or exercise performance, WPH did appear to significantly affect body fatness. Specifically, WPH reduced FM and %FAT by -5.42% and -1.601%, respectively (p<0.0125), which resulted in FM and %FAT losses for WPH being approximately 4x and 1.4x greater than was observed for WPC80, and 13.8x and 3.2x greater than WPC80+ (WPC80: -1.325% and -0.672%; WPC80+: -0.474% and -0.379%; p>0.0125). Though these changes were not significantly different between WP groups (p>0.05), the effects on FM and %FAT between WPH versus PLA did achieve significance (p<0.05). Since there was neither a significant difference in TBMM (e.g., hyperaminoacidemia-induced MPS) or energy intake (e.g., satiety-induced deficit) between WPH and PLA, there is little supporting evidence to readily explain this effect. It is possible that, as has been observed in rodents, the high β-lactoglobulin concentration present in the WPH may have influenced the loss of fat tissue.(35) Similarly, leucine has recently been shown to increase mitochondrial mass, oxygen consumption, and gene expression in human

myocytes and adipocytes, and could then theoretically have an impact on lipid metabolism that elicit chronic changes in total fat mass.(86) However, if indeed β-lactoglobulin or leucine concentrations were significant contributing factors, then similar fat loss would have been expected from all WP groups. That is, unless there is a specific peptide fragment within β-lactoglobulin that is responsible for promoting fat loss and happened to be more readily available from WPH, and/or if a greater leucine response was elicited as a result of WPH delivery, respectively. Similarly, glucagon-like peptide-1 (GLP-1) and glucagon have each been shown to increase, and the insulin:glucagon ratio to decrease significantly in response to WP, but to date there is no data to show a marked response difference between native WP and its hydrolysate.(10, 29, 83) For example, Aziz et al. (83) reported that when WP or WPH was provided to rats, following induced GLP-1 agonism, plasma amino acid removal and free fatty acid presence increased significantly by 30 and 60 minutes post-prandial. These results led the researchers to conclude that peptides arising from digestion (or delivered as hydrolysates) may significantly affect metabolic regulation. In fact, recent evidence suggests that gastrointestinal peptides such as GLP-1 and glucose-dependent insulinotropic polipeptide (GIP) may have increased metabolic processes that could have contributed to the FM and %FAT differences observed between WPH and PLA.(87, 88) If correct, greater metabolic efficiency involving an increase in amino acid removal from circulation (or increased nitrogen retention) would explain the reduced BUN and other observed changes in fasted blood data presented earlier. Further evidence of WP improving metabolic response has also recently provided by Hackney et al.(89) These researchers reported that consuming WP immediately after a heavy resistance training bout

significantly lowered the non-protein respiratory exchange ratio (RER) and significantly increased resting energy expenditure (REE) in the 24- and 48-hours following exercise. In fact, the REE effect 24-hours after exercise was found to be significantly different from the effect observed after consuming an isocalorically matched CHO. Another possible explanation for the observed difference in fat loss may simply be the WPH group's dietary changes. Covariate adjusted nutritional analysis (**Table 2**) revealed that the WPH group significantly reduced relative lipid intake (% of total kcals), which cannot be ruled-out as a contributing factor affecting fat loss. (90).

CONCLUSION

Unique to the results observed from this investigation, versus previous studies that have assessed the chronic effects of heavy resistance training in combination with supplemental whey protein (WP) versus carbohydrate (CHO), is the present trial's use of ANCOVA analysis to address the impact selected covariates may have had on the primary dependent variables of interest – body composition, strength, muscular endurance, and various blood measures indicative of adaptation. Thus, hopefully providing more confidence in the conclusions drawn from the observed differences within and between groups. Specifically, baseline (PRE) upper-body strength-to-body mass status (1RM BP/BM), and total relative training volume (kg/min) and average relative protein intake per day (g/kg/d) for the 8-week intervention were pre-selected covariates used across all analyses.

In summary, the current data provides evidence to support the hypothesis that WP source and molecular distribution affects the physiological response to an 8-week heavy resistance training program in previously trained, healthy adult men (18-35 yrs). Most notably, an extensively hydrolyzed 80% whey protein concentrate (WPH), providing greater than 80% of the contained protein fractions as weighing $\leq$ 1 kD in molecular weight, appeared to provide a superior body composition and fasted blood analyte response versus its native 80% whey protein concentrate source (WPC80) or a high lactoferrin-containing 80% whey protein concentrate (WPC80+) provided by different supplier. Specifically, the WPH significantly reduced fat mass and percent body fat, and

appeared to improve nitrogen retention (or uptake), while achieving a statistically similar increase in lean body mass and muscle mass as provided by the other WP supplements or a CHO placebo (PLA). However, WP form or molecular distribution provide no added or differential effect on changes in upper- and lower-body muscular strength or endurance, compared to CHO or amongst WP groups.

Whether the difference in fat loss occurred due to factors arising from WPH possibly providing a faster rise in, or total amino acid response, an improved insulin:glucagon ratio, upregulation of mitochondrial gene expression within myocytes and adipocytes, an increased effect on gastrointestinally regulated incretin hormone response or increased regulatory peptide availability arising from the WPH, or some other effect unrelated to the protein (e.g., dietary factors not being controlled) remains to be elucidated. However, as the first study to assess the chronic effects of WP form and molecular distribution on the physiological adaptations to heavy resistance training in a group of likely sports nutrition target consumers, this study offers both practical consumer application and future research direction that should be further explored.

Therefore, it could be concluded that an extensively hydrolyzed 80% WP may be most beneficial to reduce body fat and percent body fat, while simultaneously increasing muscle mass, strength and muscular endurance within a relatively short period of time (eight weeks). However, if an increase in body mass, in addition to increasing muscle mass, strength and muscular endurance, is more important than a significant reduction in body fat or percent body fat within the same short period of time, then it appears any 80%

whey protein concentrate will be more effective than an extensive hydrolysate. It is recommended, however, that future studies on this topic utilize tighter dietary controls to minimize both PRE to POST within- and between-group differences on dietary intake, as well as minimize potentially additive effects on net protein balance that are indirectly related to the WP intervention. Additionally, the effects of WPH on fat mass warrant future studies within overweight and obese populations to determine if a similar such effect arises in response to *ad libitum*, controlled and energy-restricted dieting. Lastly, it is recommended that the effects of WP source or molecular distribution be observed within 25-45 year-old, previously trained adults to assess if the effects observed within the current investigation (involving 94.7% college undergraduates), may be accentuated within the context of a group of adults adhering to a more consistent lifestyle and better overall dietary habits.

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APPENDIX A

TABLE 1. Descriptive statistics at baseline (Unadjusted MEAN±SEM)

		N	MEAN	SEM	SKEWNESS STATISTIC	KURTOSIS STATISTIC	*p* -value
AGE (yrs)	PLA	15	20.93	0.41	-0.141	-1.479	
	WPH	13	21.55	0.90	1.829	3.951	
	WPC80+	15	21.85	0.89	2.513	7.523	0.825
	WPC80	13	21.27	0.66	1.727	3.784	
	TOTAL	**56**	**21.40**	**0.36**	**2.213**	**6.538**	
HEIGHT (cm)	PLA	15	178.63	1.68	0.188	-1.190	
	WPH	13	177.56	1.19	-0.950	-0.224	
	WPC80+	15	177.83	1.10	0.813	-0.280	0.449
	WPC80	13	180.42	1.15	0.103	-1.056	
	TOTAL	**56**	**178.59**	**0.66**	**0.115**	**-0.586**	
TRAINING STATUS	PLA	15	1.21	0.06	0.149	-0.758	
	WPH	13	1.23	0.05	-0.763	1.441	
	WPC80+	15	1.24	0.04	0.325	-0.494	0.801
	WPC80	13	1.29	0.07	-0.292	0.153	
	TOTAL	**56**	**1.24**	**0.03**	**-0.074**	**-0.093**	
BM (Kg)	PLA	15	76.21	2.18	0.167	-1.415	
	WPH	13	79.57	1.77	-0.523	-0.100	
	WPC80+	15	78.85	2.24	0.222	-0.875	0.079[†]
	WPC80	13	83.78	1.62	-0.191	-0.306	
	TOTAL	**56**	**79.46**	**1.04**	**-0.172**	**-0.858**	
% FAT	PLA	15	17.34	1.39	0.628	0.833	
	WPH	13	21.49	1.05	-0.606	0.269	
	WPC80+	15	17.43	1.62	0.177	-1.050	0.111
	WPC80	13	19.75	1.23	-0.240	-0.503	
	TOTAL	**56**	**18.89**	**0.70**	**-0.133**	**-0.645**	
CALS (kcal/kg/d)	PLA	15	33.17	2.59	-0.049	-0.548	
	WPH	12	31.75	2.50	0.384	0.760	
	WPC80+	15	31.03	3.01	1.433	2.341	0.434
	WPC80	13	27.43	1.40	0.859	0.905	
	TOTAL	**55**	**30.92**	**1.26**	**0.926**	**1.190**	
PRO (g/kg/d)	PLA	15	1.33	0.14	1.101	0.699	
	WPH	12	1.37	0.12	0.689	-0.507	
	WPC80+	15	1.35	0.18	1.913	3.790	0.742
	WPC80	13	1.16	0.12	2.082	6.068	
	TOTAL	**55**	**1.31**	**0.07**	**1.613**	**2.993**	
1RM BP (Kg)	PLA	15	92.68	5.35	0.288	-0.132	
	WPH	13	97.87	4.86	-0.097	0.419	
	WPC80+	15	97.22	3.60	0.049	1.676	0.193
	WPC80	13	108.69	6.96	0.016	0.706	
	TOTAL	**56**	**98.82**	**2.66**	**0.306**	**0.631**	
1RM HS (Kg)	PLA	15	161.48	11.75	0.529	-0.651	
	WPH	13	164.34	7.94	-0.049	-1.004	
	WPC80+	15	169.34	7.43	0.314	-0.301	0.681
	WPC80	13	151.78	13.04	1.198	0.786	
	TOTAL	**56**	**162.00**	**5.07**	**0.485**	**-0.284**	

PLA = PLACEBO; WPH = WHEY PROTEIN HYDROLYSATE; WPC80+ = HIGH LACTOFERRIN 80% WHEY PROTEIN CONCENTRATE; WPC80 = 80% WHEY PROTEIN CONCENTRATE. No significant differences ($p > 0.05$) were observed between groups for baseline measures of Age, Height, Training Status [1RM Bench Press (Kg) / Body Mass (Kg)], Body Mass (BM), Percent Body Fat (%FAT), Relative Energy per day (CALS kcals/kg/d), Relative Protein per day (PRO g/kg/d), One-Rep Max Bench Press (1RM BP), and One-Rep Max Hack Squat (1RM HS). [†]WPC80 - PLA ($p = 0.050$).

TABLE 2. Changes in dietary intake from baseline (Adjusted MEAN±SEM)

ENERGY (kcal/kg/d)

Time (p =0.170, ES=0.039, 1-β=0.277); Time*Group (p =0.330, ES=0.068, 1-β=0.295)

	BASELINE		AVG WK 1-8		Δ	%Δ	
	MEAN	±SEM	MEAN	±SEM			
PLA	40.018	2.593	41.576	1.579	1.558	3.893	
WPH	29.162	2.401	30.158	1.462	0.996	3.415	
WPC80+	27.622	2.230	31.708	1.358	4.086	14.793	*
WPC80	25.852	2.268	31.882	1.381	6.030	23.325	*

CARBOHYDRATE (g/kg/d)

Time (p =0.061, ES=0.071, 1-β=0.469); Time*Group (p =0.953, ES=0.007, 1-β=0.069)

	BASELINE		AVG WK 1-8		Δ	%Δ	
	MEAN	±SEM	MEAN	±SEM			
PLA	5.070	0.385	5.151	0.260	0.081	1.598	
WPH	3.334	0.357	3.255	0.241	-0.079	-2.370	
WPC80+	3.337	0.331	3.290	0.224	-0.047	-1.408	
WPC80	3.240	0.337	3.370	0.228	0.130	4.012	

PROTEIN (g/kg/d)[¥]

Time (p =0.413, ES=0.014, 1-β=0.128); Time*Group (p =0.068, ES=0.136, 1-β=0.589)

	BASELINE		AVG WK 1-8		Δ	%Δ	
	MEAN	±SEM	MEAN	±SEM			
PLA	1.452	0.199	1.575	0.126	0.123	8.471	
WPH	1.330	0.166	1.904	0.105	0.574	43.158	*
WPC80+	1.313	0.148	1.971	0.093	0.658	50.114	*
WPC80	1.106	0.154	1.846	0.097	0.740	66.908	*

CARBOHYDRATE (% of kcals/d)

Time (p =0.259, ES=0.026, 1-β=0.201); Time*Group (p =0.086, ES=0.127, 1-β=0.551)

	BASELINE		AVG WK 1-8		Δ	%Δ	
	MEAN	±SEM	MEAN	±SEM			
PLA	51.156	2.591	50.507	1.806	-0.649	-1.269	
WPH	45.838	2.399	43.444	1.672	-2.394	-5.223	
WPC80+	47.998	2.228	41.721	1.553	-6.277	-13.078	*
WPC80	49.460	2.266	42.313	1.579	-7.147	-14.450	*

PROTEIN (% of kcals/d)

Time (p =0.433, ES=0.013, 1-β=0.121); Time*Group (p =0.001, ES=0.277, 1-β=0.947)

	BASELINE		AVG WK 1-8		Δ	%Δ	
	MEAN	±SEM	MEAN	±SEM			
PLA	17.323	1.271	16.817	1.052	-0.506	-2.921	
WPH	17.232	1.177	24.152	0.974	6.920	40.158	*†
WPC80+	16.883	1.093	23.471	0.905	6.588	39.022	*†
WPC80	16.343	1.112	23.461	0.920	7.118	43.554	*†

FIBER (g/d)

Time (p =0.577, ES=0.007, 1-β=0.085); Time*Group (p =0.960, ES=0.006, 1-β=0.067)

	BASELINE		AVG WK 1-8		Δ	%Δ	
	MEAN	±SEM	MEAN	±SEM			
PLA	25.727	3.162	24.427	1.928	-1.300	-5.053	
WPH	21.721	2.928	20.694	1.785	-1.027	-4.728	
WPC80+	17.231	2.720	15.138	1.658	-2.093	-12.147	
WPC80	20.900	2.766	18.167	1.686	-2.733	-13.077	

FAT (g/kg/d)

Time (p =0.769, ES=0.002, 1-β=0.060); Time*Group (p =0.096, ES=0.123, 1-β=0.531)

	BASELINE		AVG WK 1-8		Δ	%Δ	
	MEAN	±SEM	MEAN	±SEM			
PLA	1.400	0.132	1.387	0.079	-0.013	-0.929	
WPH	1.198	0.123	1.011	0.074	-0.187	-15.609	
WPC80+	1.068	0.114	1.136	0.068	0.068	6.367	
WPC80	0.918	0.116	1.107	0.069	0.189	20.588	*

VITAMIN D (IU/d)

Time (p =0.365, ES=0.017, 1-β=0.146); Time*Group (p =0.527, ES=0.045, 1-β=0.199)

	BASELINE		AVG WK 1-8		Δ	%Δ	
	MEAN	±SEM	MEAN	±SEM			
PLA	115.840	49.934	105.613	45.557	-10.227	-8.829	
WPH	140.254	46.234	124.528	42.182	-15.726	-11.213	
WPC80+	111.747	42.943	75.884	39.179	-35.863	-32.093	
WPC80	114.663	43.672	119.829	39.845	5.166	4.505	

FAT (% of kcals/d)

Time (p =0.218, ES=0.031, 1-β=0.231); Time*Group (p =0.131, ES=0.110, 1-β=0.477)

	BASELINE		AVG WK 1-8		Δ	%Δ	
	MEAN	±SEM	MEAN	±SEM			
PLA	32.099	2.285	30.388	1.554	-1.711	-5.330	
WPH	35.918	2.116	30.000	1.439	-5.918	-16.476	*
WPC80+	34.349	1.965	32.405	1.336	-1.944	-5.660	
WPC80	31.847	1.998	30.777	1.359	-1.070	-3.360	

CALCIUM (mg/d)

Time (p =0.049, ES=0.079, 1-β=0.509); Time*Group (p =0.027, ES=0.173, 1-β=0.724)

	BASELINE		AVG WK 1-8		Δ	%Δ	
	MEAN	±SEM	MEAN	±SEM			
PLA	1244.984	138.085	1152.699	93.065	-92.285	-7.413	
WPH	983.481	127.855	1223.523	86.170	240.042	24.407	
WPC80+	824.985	118.754	1221.807	80.036	396.822	48.101	*†
WPC80	924.003	120.771	1163.923	81.396	239.920	25.965	*

PLA = PLACEBO; WPH = WHEY PROTEIN HYDROLYSATE; WPC80+ = HIGH LACTOFERRIN 80% WHEY PROTEIN CONCENTRATE; WPC80 = 80% WHEY PROTEIN CONCENTRATE. Time = Main Effect by Time; Time*Group = Interaction Main Effect; ES = Effect Size; 1-β = Power. Estimated average means adjusted for covariates: Training Status Ratio (PRE), Total 8-wk Relative Training Volume (kg/min), and Average 8-wk Relative Protein Intake (g/kg/d). [¥]AVG WK 1-8 PROTEIN (% of kcals/d) used as covariate to assess PROTEIN (g/kg/d). Main effects set at $p \leq 0.05$. [*]Different from PRE ($p \leq 0.0125$); [†]Different from PLA ($p \leq 0.05$); [‡]Different from WPC80+ ($p \leq 0.05$); [§]Different from WPC80 ($p \leq 0.05$).

TABLE 3. Changes in strength and anerobic endurance from PRE to POST and for repeated 80RM (Adjusted MEAN±SEM)

1RM BENCH PRESS (Kg)

Time (p =0.005, ES=0.154, 1-β=0.816); Time*Group (p =0.731, ES=0.027, 1-β=0.130)

	PRE		POST		POST-PRE		
	MEAN	SEM	MEAN	SEM	Δ	%Δ	
PLA	87.81	2.23	94.79	2.61	6.98	7.95	*
WPH	100.69	2.22	105.43	2.60	4.74	4.71	*
WPC80+	100.98	1.91	107.18	2.25	6.20	6.14	*
WPC80	103.80	1.96	109.88	2.30	6.08	5.86	*

80RM BENCH PRESS (reps)

Time (p =0.002, ES=0.179, 1-β=0.879); Time*Group (p =0.800, ES=0.021, 1-β=0.110)

	PRE		POST		POST-PRE		
	MEAN	SEM	MEAN	SEM	Δ	%Δ	
PLA	7.81	0.51	11.04	0.86	3.23	41.32	*
WPH	6.70	0.51	8.81	0.85	2.11	31.55	*
WPC80+	7.86	0.44	10.53	0.74	2.68	34.05	*
WPC80	7.25	0.45	10.04	0.76	2.78	38.38	*

1RM HACK SQUAT (Kg)

Time (p =0.001, ES=0.208, 1-β=0.936); Time*Group (p =0.853, ES=0.016, 1-β=0.096)

	PRE		POST		POST-PRE		
	MEAN	SEM	MEAN	SEM	Δ	%Δ	
PLA	166.52	8.72	210.85	8.29	44.34	26.63	*
WPH	164.17	8.26	203.51	7.86	39.34	23.96	*
WPC80+	170.91	7.50	215.72	7.13	44.80	26.21	*
WPC80	141.25	7.68	190.50	7.31	49.25	34.87	*

80RM HACK SQUAT (reps)

Time (p =0.014, ES=0.120, 1-β=0.710); Time*Group (p =0.439, ES=0.054, 1-β=0.236)

	PRE		POST		POST-PRE		
	MEAN	SEM	MEAN	SEM	Δ	%Δ	
PLA	9.97	1.52	25.56	2.82	15.58	156.27	*
WPH	8.60	1.44	21.59	2.67	12.99	151.08	*
WPC80+	10.91	1.30	20.57	2.43	9.66	88.52	*
WPC80	8.12	1.34	21.46	2.49	13.34	164.36	*

REPEATED 80RM HACK SQUAT (reps)

Time (p=0.837, ES=0.003, 1-β=0.067); Time*Group (p=0.438, ES=0.060, 1-β=0.322)

	POST		24HR		48HR		24HR-POST		48HR-POST		48HR-24HR		
	MEAN	SEM	MEAN	SEM	MEAN	SEM	Δ	%Δ	Δ	%Δ	Δ	%Δ	
PLA	25.92	2.93	28.17	2.99	25.62	2.64	2.26	8.71	-0.30	-1.14	-2.55	-9.06	
WPH	21.40	2.70	20.29	2.75	20.91	2.43	-1.11	-5.20	-0.49	-2.31	0.62	3.05	
WPC80+	20.31	2.46	23.47	2.51	21.49	2.21	3.16	15.56	1.18	5.82	-1.98	-8.43	¶
WPC80	22.15	2.60	23.93	2.65	21.18	2.34	1.78	8.04	-0.97	-4.38	-2.75	-11.50	

PLA = PLACEBO; WPH = WHEY PROTEIN HYDROLYSATE; WPC80+ = HIGH LACTOFERRIN 80% WHEY PROTEIN CONCENTRATE; WPC80 = 80% WHEY PROTEIN CONCENTRATE. 1RM = One-Repition Maximum; 80RM = Maximum Repitions to Failure at 80% of 1RM; REPEATED 80RM = Repeated 80RM Hack Squat tests occurring 24 and 48 Hours after $80RM_{POST}$ Hack Squat. Time = Main Effect by Time; Time*Group = Interaction Main Effect; ES = Effect Size; 1-β = Power. Estimated average means adjusted for covariates: Training Status Ratio (PRE), Total 8-wk Relative Training Volume (kg/min), and Average 8-wk Relative Protein Intake (g/kg/d). Main effects set at $p \leq 0.05$. * Different from PRE ($p \leq 0.0125$); † Different from PLA ($p \leq 0.05$); ‡ Different from WPC80+ ($p \leq 0.05$); § Different from WPC80 ($p \leq 0.05$); ¶ 24HR Different from POST ($p \leq 0.0125$).

TABLE 4. Changes in body composition from PRE to POST (Adjusted MEAN±SEM)

BODY MASS (Kg)

Time (p =0.845, ES=0.001, 1-β=0.054); Time*Group (p =0.272, ES=0.076, 1-β=0.335)

	PRE		POST		Δ	%Δ	
	MEAN	±SEM	MEAN	±SEM			
PLA	70.777	1.729	73.126	1.699	2.349	3.319	*
WPH	82.261	1.572	82.902	1.545	0.641	0.779	
WPC80+	82.225	1.477	83.662	1.451	1.437	1.748	*
WPC80	83.471	1.511	85.036	1.484	1.565	1.875	*

FAT MASS (Kg)

Time (p =0.093, ES=0.057, 1-β=0.391); Time*Group (p =0.048, ES=0.148, 1-β=0.645)

	PRE		POST		Δ	%Δ	
	MEAN	±SEM	MEAN	±SEM			
PLA	9.462	1.236	10.323	1.193	0.861	9.100	
WPH	18.949	1.124	17.823	1.084	-1.126	-5.942	*†
WPC80+	16.048	1.056	15.972	1.019	-0.076	-0.474	
WPC80	17.060	1.080	16.834	1.042	-0.226	-1.325	

LEAN BODY MASS (Kg)

Time (p =0.086, ES=0.059, 1-β=0.405); Time*Group (p =0.919, ES=0.010, 1-β=0.079)

	PRE		POST		Δ	%Δ	
	MEAN	±SEM	MEAN	±SEM			
PLA	59.100	1.541	60.551	1.512	1.451	2.455	*
WPH	60.511	1.401	62.396	1.374	1.885	3.115	*
WPC80+	63.538	1.316	65.113	1.291	1.575	2.479	*
WPC80	63.639	1.346	65.500	1.320	1.861	2.924	*

% FAT

Time (p =0.037, ES=0.086, 1-β=0.558); Time*Group (p =0.032, ES=0.162, 1-β=0.699)

	PRE		POST		Δ	%Δ	
	MEAN	±SEM	MEAN	±SEM			
PLA	13.688	1.376	14.328	1.316	0.640	4.676	
WPH	23.092	1.251	21.491	1.196	-1.601	-6.933	*†
WPC80+	19.145	1.175	18.766	1.124	-0.379	-1.980	
WPC80	20.374	1.202	19.702	1.149	-0.672	-3.298	

TOTAL BODY SKELETAL MUSCLE MASS (Kg)

Time (p =0.009, ES=0.132, 1-β=0.763); Time*Group (p =0.818, ES=0.019, 1-β=0.106)

	PRE		POST		Δ	%Δ	
	MEAN	±SEM	MEAN	±SEM			
PLA	34.241	1.099	35.311	1.086	1.070	3.125	*
WPH	35.276	0.999	36.581	0.987	1.305	3.699	*
WPC80+	36.991	0.938	38.440	0.927	1.449	3.917	*
WPC80	37.186	0.960	38.707	0.948	1.521	4.090	*

PLA = PLACEBO; WPH = WHEY PROTEIN HYDROLYSATE; WPC80+ = HIGH LACTOFERRIN 80% WHEY PROTEIN CONCENTRATE; WPC80 = 80% WHEY PROTEIN CONCENTRATE. %FAT = Percent Body Fat. Time = Main Effect by Time; Time*Group = Interaction Main Effect; ES = Effect Size; 1-β = Power. Estimated average means adjusted for covariates: Training Status Ratio (PRE), Total 8-wk Relative Training Volume (kg/min), and Average 8-wk Relative Protein Intake (g/kg/d). Main effects set at $p \leq 0.05$. *Different from PRE ($p \leq 0.0125$); †Different from PLA ($p \leq 0.05$); ‡Different from WPC80+ ($p \leq 0.05$); §Different from WPC80 ($p \leq 0.05$).

TABLE 5. Changes in blood lipids from PRE to POST (Adjusted MEAN±SEM)

TOTAL CHOLESTEROL (mg/dL)

Time (p =0.271, ES=0.025, 1-β=0.193); Time*Group (p =0.386, ES=0.059, 1-β=0.263)

	PRE		POST		Δ	%Δ
	MEAN	±SEM	MEAN	±SEM		
PLA	152.273	9.301	155.010	8.410	2.737	1.797
WPH	161.689	8.456	154.356	7.646	-7.333	-4.535
WPC80+	150.768	7.943	154.335	7.182	3.567	2.366
WPC80	162.725	8.124	163.246	7.345	0.521	0.320

HIGH DENSITY LIPOPROTEINS (mg/dL)

Time (p =0.432, ES=0.013, 1-β=0.121); Time*Group (p =0.196, ES=0.090, 1-β=0.400)

	PRE		POST		Δ	%Δ
	MEAN	±SEM	MEAN	±SEM		
PLA	48.076	4.299	52.368	3.664	4.292	8.928
WPH	58.500	3.909	55.163	3.331	-3.337	-5.704
WPC80+	55.039	3.672	55.779	3.129	0.740	1.345
WPC80	57.828	3.755	55.129	3.200	-2.699	-4.667

TRIGLYCERIDES (mg/dL)

Time (p =0.360, ES=0.017, 1-β=0.148); Time*Group (p =0.559, ES=0.041, 1-β=0.187)

	PRE		POST		Δ	%Δ
	MEAN	±SEM	MEAN	±SEM		
PLA	87.606	12.362	101.322	10.419	13.716	15.656
WPH	86.134	11.239	86.750	9.473	0.616	0.715
WPC80+	94.214	10.557	88.339	8.898	-5.875	-6.236
WPC80	100.073	10.797	95.411	9.101	-4.662	-4.659

LOW DENSITY LIPOPROTEINS (mg/dL)

Time (p =0.632, ES=0.005, 1-β=0.076); Time*Group (p =0.264, ES=0.077, 1-β=0.340)

	PRE		POST		Δ	%Δ
	MEAN	±SEM	MEAN	±SEM		
PLA	86.732	7.441	82.362	6.819	-4.370	-5.039
WPH	85.857	6.766	81.825	6.200	-4.032	-4.696
WPC80+	76.876	6.355	80.994	5.824	4.118	5.357
WPC80	84.981	6.500	88.995	5.956	4.014	4.723

TC:HDL

Time (p =0.818, ES=0.001, 1-β=0.056); Time*Group (p =0.777, ES=0.022, 1-β=0.117)

	PRE		POST		Δ	%Δ
	MEAN	±SEM	MEAN	±SEM		
PLA	3.140	0.197	3.012	0.158	-0.128	-4.076
WPH	2.809	0.179	2.833	0.144	0.024	0.854
WPC80+	2.914	0.168	2.853	0.135	-0.061	-2.093
WPC80	2.967	0.172	3.008	0.138	0.041	1.382

PLA = PLACEBO; WPH = WHEY PROTEIN HYDROLYSATE; WPC80+ = HIGH LACTOFERRIN 80% WHEY PROTEIN CONCENTRATE; WPC80 = 80% WHEY PROTEIN CONCENTRATE. TC:HDL = Total Cholesterol-to-High Density Lipoprotein ratio. Time = Main Effect by Time; Time*Group = Interaction Main Effect; ES = Effect Size; 1-β = Power. Estimated average means adjusted for covariates: Training Status Ratio (PRE), Total 8-wk Relative Training Volume (kg/min), Average 8-wk Relative Protein Intake (g/kg/d), and Blood Collection Time. Main effects set at $p \leq 0.05$. [*]Different from PRE ($p \leq 0.0125$); [†]Different from PLA ($p \leq 0.05$); [‡]Different from WPC80+ ($p \leq 0.05$); [§]Different from WPC80 ($p \leq 0.05$).

TABLE 6. Changes in select blood measures from PRE to POST (Adjusted MEAN±SEM)

GLUCOSE (mg/dL)

Time (p =0.395, ES=0.015, 1-β=0.134); Time*Group (p =0.992, ES=0.002, 1-β=0.056)

	PRE		POST		Δ	%Δ	
	MEAN	±SEM	MEAN	±SEM			
PLA	88.660	1.664	88.672	1.792	0.012	0.014	
WPH	86.342	1.577	87.305	1.698	0.963	1.115	
WPC80+	89.351	1.414	89.768	1.524	0.417	0.467	
WPC80	88.441	1.443	89.057	1.554	0.616	0.697	

UREA NITROGEN (mg/dL)

Time (p =0.771, ES=0.002, 1-β=0.059); Time*Group (p =0.027, ES=0.176, 1-β=0.724)

	PRE		POST		Δ	%Δ	
	MEAN	±SEM	MEAN	±SEM			
PLA	14.898	0.990	14.726	0.947	-0.172	-1.155	
WPH	15.279	0.938	12.519	0.898	-2.760	-18.064	*§
WPC80+	15.238	0.841	15.039	0.805	-0.199	-1.306	
WPC80	12.586	0.858	14.714	0.821	2.128	16.908	*

CREATININE (mg/dL)

Time (p =0.327, ES=0.020, 1-β=0.163); Time*Group (p =0.157, ES=0.104, 1-β=0.442)

	PRE		POST		Δ	%Δ	
	MEAN	±SEM	MEAN	±SEM			
PLA	1.029	0.041	0.961	0.032	-0.068	-6.608	*
WPH	1.032	0.039	0.911	0.030	-0.121	-11.725	*‡POST
WPC80+	1.116	0.035	1.062	0.027	-0.054	-4.839	*
WPC80	1.041	0.035	0.982	0.028	-0.059	-5.668	*

BUN:CREATININE (ratio)

Time (p =0.539, ES=0.008, 1-β=0.093); Time*Group (p =0.093, ES=0.126, 1-β=0.537)

	PRE		POST		Δ	%Δ	
	MEAN	±SEM	MEAN	±SEM			
PLA	14.410	0.953	15.308	1.061	0.898	6.232	
WPH	14.851	0.903	13.931	1.005	-0.920	-6.195	
WPC80+	13.795	0.810	14.239	0.902	0.444	3.219	
WPC80	12.362	0.826	15.248	0.920	2.886	23.346	*

WHITE BLOOD CELL COUNT (1000/μL)

Time (p =0.223, ES=0.031, 1-β=0.227); Time*Group (p =0.065, ES=0.139, 1-β=0.598)

	PRE		POST		Δ	%Δ	
	MEAN	±SEM	MEAN	±SEM			
PLA	6.585	0.540	6.653	0.452	0.068	1.033	
WPH	5.867	0.490	5.186	0.410	-0.681	-11.607	
WPC80+	5.333	0.461	5.979	0.385	0.646	12.113	
WPC80	5.597	0.470	6.061	0.393	0.464	8.290	

CREATINE KINASE (U/L)

Time (p =0.910, ES=0.000, 1-β=0.051); Time*Group (p =0.440, ES=0.055, 1-β=0.236)

	PRE		POST		Δ	%Δ	
	MEAN	±SEM	MEAN	±SEM			
PLA	246.572	41.643	212.613	39.104	-33.959	-13.772	
WPH	192.281	39.275	195.987	36.881	3.706	1.927	
WPC80+	285.247	35.757	191.820	33.577	-93.427	-32.753	§PRE
WPC80	140.026	36.315	125.897	34.101	-14.129	-10.090	

PLA = PLACEBO; WPH = WHEY PROTEIN HYDROLYSATE; WPC80+ = HIGH LACTOFERRIN 80% WHEY PROTEIN CONCENTRATE; WPC80 = 80% WHEY PROTEIN CONCENTRATE.
BUN:CREATININE = Urea Nitrogen-to-Creatinine ratio. Time = Main Effect by Time; Time*Group = Interaction Main Effect; ES = Effect Size; 1-β = Power. Estimated average means adjusted for covariates: Training Status Ratio (PRE), Total 8-wk Relative Training Volume (kg/min), Average 8-wk Relative Protein Intake (g/kg/d), and Blood Collection Time. All blood draws under confirmed euhydrated state and after a 12-hour fast (water only). Main effects set at $p \leq 0.05$.*Different from PRE ($p \leq 0.0125$); †Different from PLA ($p \leq 0.05$); ‡Different from WPC80+ ($p \leq 0.05$); §Different from WPC80 ($p \leq 0.05$).

TABLE 7. Changes in 24-hour WBC and CK response to lower-body training from Week 1 to Week 8 (Adjusted MEAN±SEM)

WHITE BLOOD CELL COUNT (1000/μL)

Time (p =0.715, ES=0.003, 1-β=0.065); Time*Group (p =0.119, ES=0.114, 1-β=0.494)

	WEEK 1		WEEK 8		Δ	%Δ	
	MEAN	±SEM	MEAN	±SEM			
PLA	7.020	0.556	7.301	0.478	0.281	4.003	
WPH	6.162	0.497	6.327	0.427	0.165	2.678	
WPC80+	6.442	0.471	6.008	0.405	-0.434	-6.737	
WPC80	7.097	0.474	5.947	0.407	-1.150	-16.204	

CREATINE KINASE (U/L)

Time (p =0.024, ES=0.101, 1-β=0.626); Time*Group (p =0.105, ES=0.119, 1-β=0.516)

	WEEK 1		WEEK 8		Δ	%Δ	
	MEAN	±SEM	MEAN	±SEM			
PLA	1276.910	346.884	357.812	69.501	-919.098	-71.978	*
WPH	1044.593	310.067	414.418	62.125	-630.175	-60.327	
WPC80+	1950.151	294.051	353.969	58.916	-1596.182	-81.849	*
WPC80	1458.952	295.618	226.758	59.230	-1232.194	-84.457	*

PLA = PLACEBO; WPH = WHEY PROTEIN HYDROLYSATE; WPC80+ = HIGH LACTOFERRIN 80% WHEY PROTEIN CONCENTRATE; WPC80 = 80% WHEY PROTEIN CONCENTRATE. Time = Main Effect by Time; Time*Group = Interaction Main Effect; ES = Effect Size; 1-β = Power. Estimated average means adjusted for covariates: Training Status Ratio (PRE), Total 8-wk Relative Training Volume (kg/min), Average 8-wk Relative Protein Intake (g/kg/d), and Blood Collection Time. Main effects set at $p \leq 0.05$. *Different from Week 1 ($p \leq 0.0125$); †Different from PLA ($p \leq 0.05$); ‡Different from WPC80+ ($p \leq 0.05$); §Different from WPC80 ($p \leq 0.05$).

TABLE 8. Changes in WBC and CK in response to repeated 80RM bouts (Adjusted MEAN±SEM)

WHITE BLOOD CELL COUNT (1000/μL)

Time (p =0.517, ES=0.017, 1-β=0.225); Time*Group (p =0.220, ES=0.081, 1-β=0.660)

	$80RM_{POST}$				$80RM_{24}$				$80RM_{48}$			
	$DAY\ 1_{PRE}$		$DAY\ 1_{POST}$		$DAY\ 2_{PRE}$		$DAY\ 2_{POST}$		$DAY\ 3_{PRE}$		$DAY\ 3_{POST}$	
	MEAN	±SEM	MEAN	±SEM	MEAN	±SEM	MEAN	±SEM	MEAN	±SEM	MEAN	±SEM
PLA	6.597	0.480	10.478	0.701 [1]	6.778	0.444 [2]	9.199	0.628 [1,3]	7.500	0.627 [2,4]	10.244	0.734 [1,3,5]
WPH	5.238	0.414	7.852	0.604 [1]	5.663	0.383 [2]	8.020	0.541 [1,3]	6.091	0.540 [2,4]	8.581	0.632 [1,3,5]
WPC80+	6.017	0.404	8.196	0.590 [1]	5.821	0.374 [2]	8.293	0.529 [1,3]	6.002	0.528 [2,4]	8.106	0.617 [1,3,5]
WPC80	6.001	0.415	8.533	0.605 [1]	6.765	0.384 [2]	8.845	0.542 [1,3]	6.898	0.541 [4]	9.236	0.633 [1,3,5]

CREATINE KINASE (U/L)

Time (p =0.341, ES=0.022, 1-β=0.211); Time*Group (p =0.607, ES=0.045, 1-β=0.247)

	$80RM_{POST}$				$80RM_{24}$				$80RM_{48}$			
	$DAY\ 1_{PRE}$		$DAY\ 1_{POST}$		$DAY\ 2_{PRE}$		$DAY\ 2_{POST}$		$DAY\ 3_{PRE}$		$DAY\ 3_{POST}$	
	MEAN	±SEM	MEAN	±SEM	MEAN	±SEM	MEAN	±SEM	MEAN	±SEM	MEAN	±SEM
PLA	191.339	39.849	157.276	25.452	235.266	59.417	258.851	63.801 [3]	182.309	45.194 [4]	201.649	45.627 [5]
WPH	202.999	34.519	197.211	22.048	262.242	51.469	287.195	55.267 [3]	232.851	39.149	249.180	39.524 [5]
WPC80+	189.585	32.537	166.547	20.782	299.100	48.514	323.930	52.094 [2,3]	260.987	36.901	273.996	37.255 [5]
WPC80	133.208	34.655	170.266	22.134	204.385	51.672	218.383	55.485 [3]	180.817	39.303	199.052	39.680 [5]

PLA = PLACEBO; WPH = WHEY PROTEIN HYDROLYSATE; WPC80+ = HIGH LACTOFERRIN 80% WHEY PROTEIN CONCENTRATE; WPC80 = 80% WHEY PROTEIN CONCENTRATE. $80RM_{POST}$ = Day 1 of repeated 80RM testing; $80RM_{24}$ = Day 2 of repeated 80RM testing; $80RM_{48}$ = Day 3 of repeated 80RM testing; $DAY\ 1_{PRE}$ = Pre-$80RM_{POST}$ blood draw (T1); $DAY\ 1_{POST}$ = Post-$80RM_{POST}$ blood draw (T2); $DAY\ 2_{PRE}$ = Pre-$80RM_{24}$ blood draw (T3); $DAY\ 2_{POST}$ = Post-$80RM_{24}$ blood draw (T4); $DAY\ 3_{PRE}$ = Pre-$80RM_{48}$ blood draw (T5); $DAY\ 3_{POST}$ = Post-$80RM_{48}$ blood draw (T6); Time = Main Effect by Time; Time*Group = Interaction Main Effect; ES = Effect Size; 1-β = Power. Estimated average means adjusted for covariates: Training Status Ratio (PRE), Total 8-wk Relative Training Volume (kg/min), Average 8-wk Relative Protein Intake (g/kg/d), and Blood Collection Time. Main effects set at $p \leq 0.05$. [1]Different from T1 ($p \leq 0.0125$); [2]Different from T2 ($p \leq 0.0125$); [3]Different from T3 ($p \leq 0.0125$); [4]Different from T4 ($p \leq 0.0125$); [5]Different from T5 ($p \leq 0.0125$); †Different from PLA ($p \leq 0.05$); ‡Different from WPC80+ ($p \leq 0.05$); §Different from WPC80 ($p \leq 0.05$).

APPENDIX B

Figure 1.

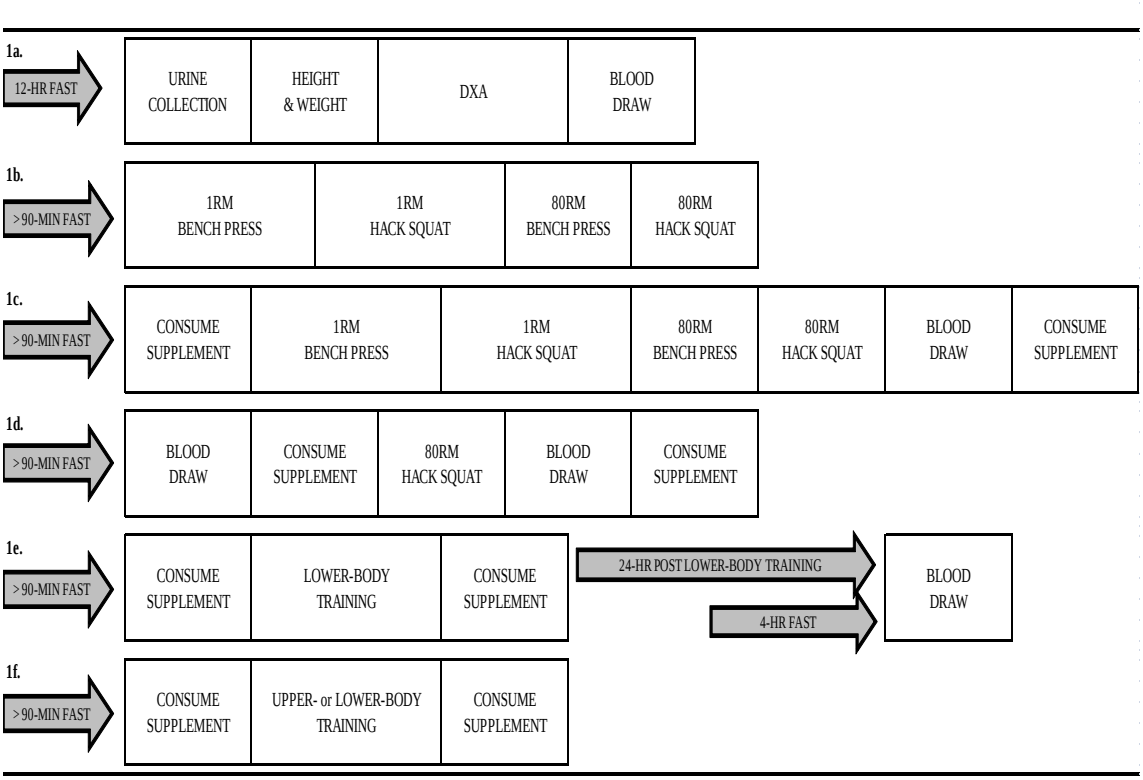

FIGURE 1. *Testing and training day schematics*. **1a** = Body composition testing (PRE and POST); **1b** = Strength and anaerobic endurance testing (PRE); **1c** = Strength and anaerobic endurance testing (POST); **1d** = Repeated 80RM testing (POST); **1e** = Blood draws to assess 24-hour response to lower-body training (Week 1 and Week 8); **1f** = Resistance training days (Weeks 1-8). DXA = Dual-energy X-ray absorptiometry.

Figure 2.

		SETS	REPS *(WKS 1-4 / WKS 5-8)*
UPPER-BODY	**Barbell Flat Bench Press**	3	10-12 / 5-8
	Standing Cable Flye	3	10-12 / 5-8
	Bent-Over Barbell Row	3	10-12 / 5-8
	Wide-Grip Front Lat Pulldown	3	10-12 / 5-8
	Seated Front Military Press	3	10-12 / 5-8
	Barbell Shrug	3	10-12 / 5-8
	Barbell Biceps Curl	3	10-12 / 5-8
	Lying E-Z Bar Triceps Extension	3	10-12 / 5-8
LOWER-BODY	**Incline Hack Squat**	3	10-12 / 5-8
	Barbell Romanian Deadlift	3	10-12 / 5-8
	Barbell Lunge	3	10-12 / 5-8
	Seated Leg Extension	3	10-12 / 5-8
	Lying Leg Curl	3	10-12 / 5-8
	Seated Calf Raise	3	10-12 / 5-8
	Supine Abdominal Crunch	3	20-25

FIGURE 2. *Upper- and lower-body resistance training program* . Subjects performed a split-body, linear periodized resistance training program 4x/wk x 8wks, following a 2-on/1-rest/2-on/2-rest training days per week regimine (e.g., Monday-UPPER, Tuesday-LOWER, Wednesday-REST, Thursday-UPPER, Friday-LOWER, Saturday-REST, Sundary-REST). A 5-min moderate intensity warm-up preceeded each workout. Barbell Flat Bench Press and Incline Hack Squat preceeded all other resistance training exercises on Upper- and Lower-Body training days, respectively. Subjects performed 3 sets x 10-12 and 6-8 repetitions (REPS) during Weeks 1-4 and 5-8, respectively. All sets were instructed to be taken to voluntary muscle failure within the specified number of repetitions. Subjects were provided 1- and 2-min rest periods between sets and exercises, respectively. All subjects were provided a stopwatch to ensure accuracy of rest period duration and to track total workout duration. All subjects recorded resistance per set, successfully completed reps, and total training time and sets during each training session.

Figure 3.

	WPH*	WPC80+**	WPC80*
Degree of Hydrolysis (%)	32.0 ± 2	N/A	N/A
Molecular Weight Profile (%)			
>10 kD	4	~80	82
5-10 kD	1	~20	11
2-5 kD	4	<1	7
1-2 kD	9	<1	0
0.5-1 kD	17	<1	0
<0.5 kD	65	<1	0
Average Molecular Weight	1.569 kD	>10 kD	>10 kD

FIGURE 3. *Molecular weight distributions/profiles of whey-containing supplements, by group* . WPH = WHEY PROTEIN HYDROLYSATE; WPC80+ = HIGH LACTOFERRIN 80% WHEY PROTEIN CONCENTRATE; WPC80 = 80% WHEY PROTEIN CONCENTRATE. *Molecular weight as determined by size exclusion chromatography and reported by the raw material supplier. **Molecular weight of WPC as reported by Perea et al. [*Enzyme Microb Technol* 1993;15(5):418-23].

Figure 4.

	PLA	WPH	WPC80+	WPC80
ENERGY (kcal)	176.430	166.244	162.581	156.591
FAT (g)	4.848	3.038	3.312	3.188
SATURATED FAT (g)	0.493	2.201	2.427	2.336
UNSATURATED FAT (g)	4.319	0.836	0.884	0.851
TRANS-FAT (g)	0.048	0.000	0.000	0.000
CHOLESTEROL (mg)	0.987	60.000	70.130	67.500
CARBOHYDRATE (g)	32.124	4.770	3.065	2.979
SUGARS (g)	31.442	4.510	2.996	2.910
FIBER (g)	0.630	0.000	0.000	0.000
PROTEIN (g)	0.597	30.053	30.001	30.001
CALCIUM (mg)	23.060	187.700	194.903	187.598
SODIUM (mg)	80.723	113.429	58.882	56.690
POTASSIUM (mg)	0.005	562.523	155.849	150.005
MAGNESIUM (mg)	0.004	22.516	19.485	18.754
PHOSPHOROUS (mg)	28.555	243.750	136.364	131.250
CHLORIDE (mg)	0.000	18.750	38.961	37.500
IRON (mg)	0.076	0.092	0.060	0.060
VITAMIN A (IU)	5.019	0.115	0.077	0.077
VITAMIN C (mg)	0.011	0.006	0.004	0.004

FIGURE 4. *Nutritional comparison of supplements, by group* (units per single serving). PLA = PLACEBO; WPH = WHEY PROTEIN HYDROLYSATE; WPC80+ = HIGH LACTOFERRIN 80% WHEY PROTEIN CONCENTRATE; WPC80 = 80% WHEY PROTEIN CONCENTRATE. All subjects consumed two servings per day; one serving immediately pre- and post-exercise on training days (4d/wk), and two divided doses between meals on non-training days (3d/wk). All supplements were blinded for packaging, flavor, texture and appearance. Supplements were mixed with 8-10 fl ozs of water and consumed on an empty stomach.

Figure 5.

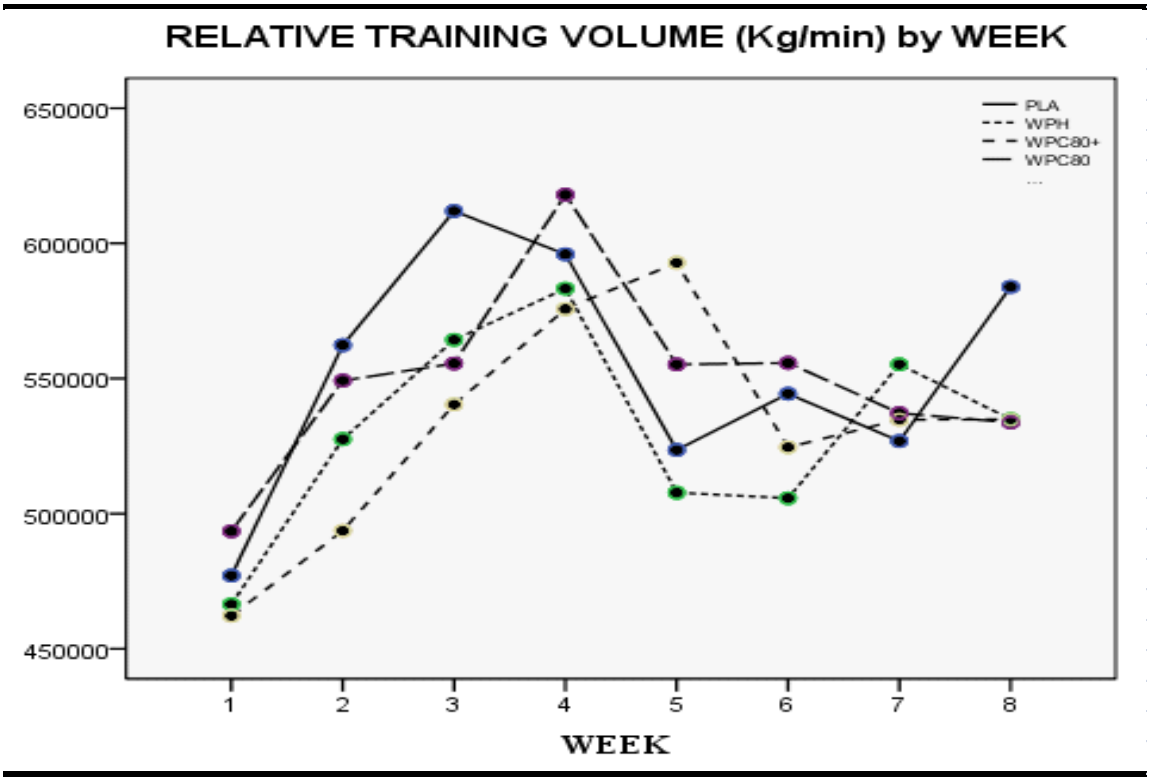

FIGURE 5. *Relative training volume, by week* . PLA = PLACEBO; WPH = WHEY PROTEIN HYDROLYSATE; WPC80+ = HIGH LACTOFERRIN 80% WHEY PROTEIN CONCENTRATE; WPC80 = 80% WHEY PROTEIN CONCENTRATE. Estimated average means adjusted for covariates: Training Status Ratio (PRE) and Average 8-wk Relative Protein Intake (g/kg/d). Main effects set at $p \leq 0.05$. Relative training volume calculated as: [load (Kg) x reps x sets] / time (mins). No significant differences ($p > 0.05$) were observed between groups for total or weekly volume.

Figure 6.

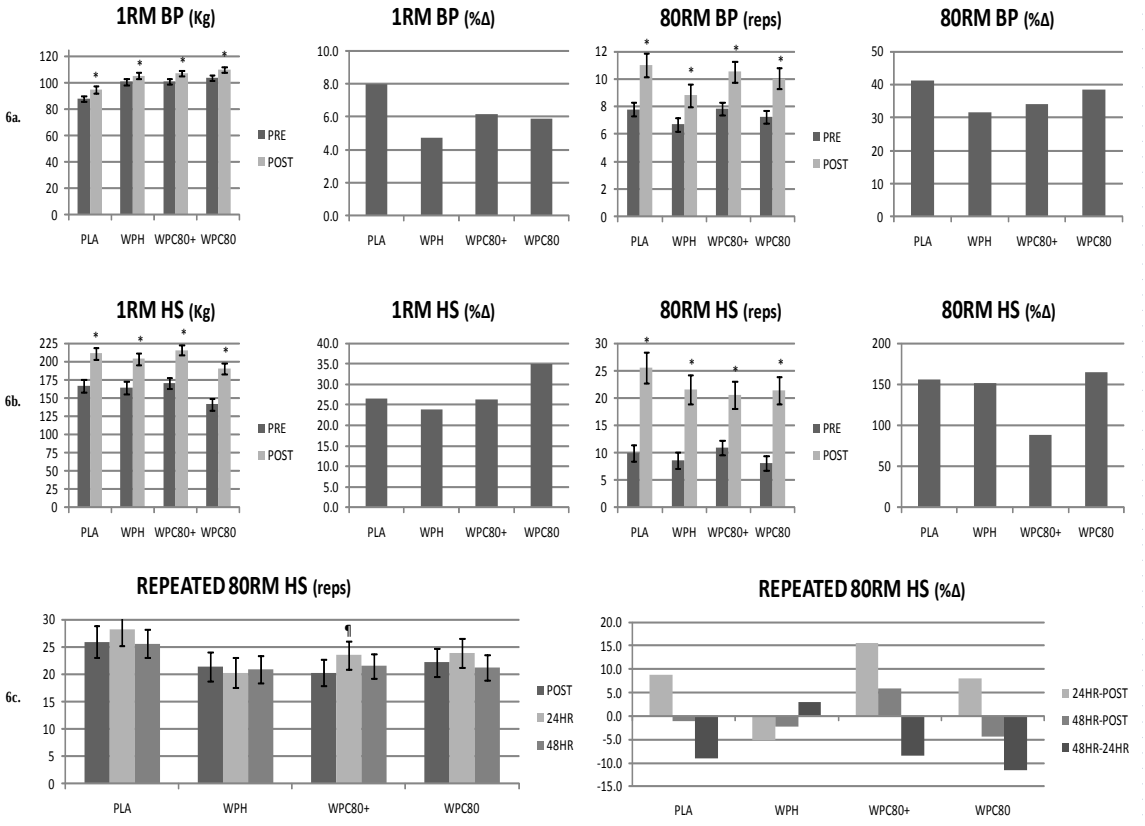

FIGURE 6. *Absolute and relative strength and anaerobic endurance changes from PRE to POST and for repeated 80RM* . PLA = PLACEBO; WPH = WHEY PROTEIN HYDROLYSATE; WPC80+ = HIGH LACTOFERRIN 80% WHEY PROTEIN CONCENTRATE; WPC80 = 80% WHEY PROTEIN CONCENTRATE. **6a** = Effects on Bench Press 1RM and 80RM; **6b** = Effects on Hack Squat 1RM and 80RM; **6c** = Effects on Repeated Hack Squat 80RM. 1RM BP = One-Repition Maximum Bench Press; 1RM HS = One-Repition Maximum Hack Squat; 80RM BP = Maximum Repitions to Failure at 80% of 1RM BP; 80RM HS = Maximum Repitions to Failure at 80% of 1RM HS; REPEATED 80RM HS = Repeated 80RM Hack Squat tests occurring 24 and 48 Hours after $80RM_{POST}$ Hack Squat. Estimated average means adjusted for covariates: Training Status Ratio (PRE), Total 8-wk Relative Training Volume (kg/min), and Average 8-wk Relative Protein Intake (g/kg/d). Main effects set at $p \leq 0.05$. *Different from PRE ($p \leq 0.0125$); †Different from PLA ($p \leq 0.05$); ‡Different from WPC80+ ($p \leq 0.05$); §Different from WPC80 ($p \leq 0.05$); ¶24HR Different from POST ($p \leq 0.0125$).

Figure 7.

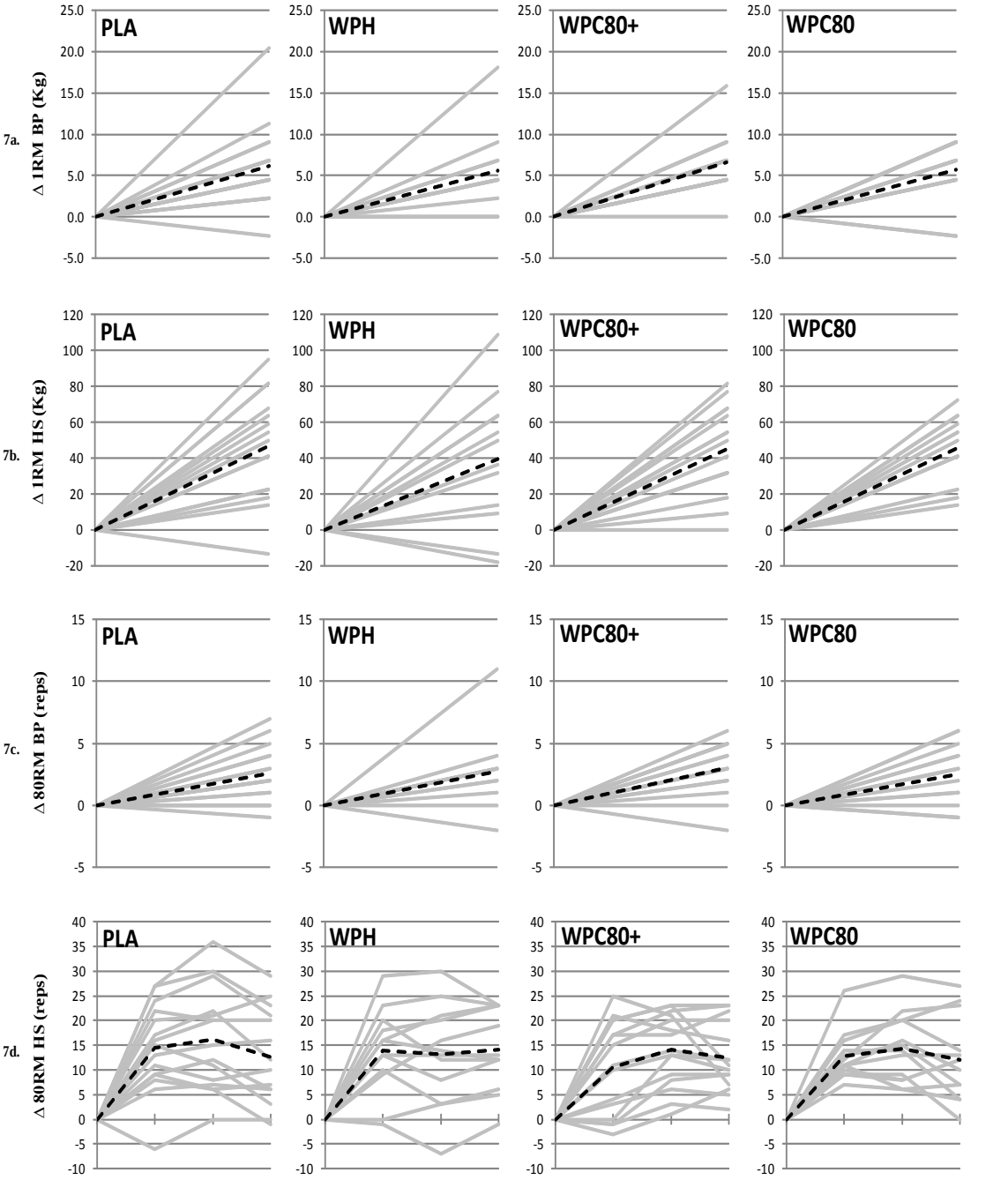

FIGURE 7. *Individual responses for strength and anaerobic endurance changes from PRE to POST and for repeated 80RM* . PLA = PLACEBO; WPH = WHEY PROTEIN HYDROLYSATE; WPC80+ = HIGH LACTOFERRIN 80% WHEY PROTEIN CONCENTRATE; WPC80 = 80% WHEY PROTEIN CONCENTRATE. **7a** = Individual Responses on 1RM BP for PRE v POST; **7b** = Individual Responses on 1RM HS for PRE v POST; **7c** = Individual Responses on 80RM BP for PRE v POST; **7d** = Individual Responses on 80RM HS for PRE v POST v 24HR v 48HR. 1RM BP = One-Repition Maximum Bench Press; 1RM HS = One-Repition Maximum Hack Squat; 80RM BP = Maximum Repitions to Failure at 80% of 1RM BP; 80RM HS = Maximum Repitions to Failure at 80% of 1RM HS. Unadjusted subject responses used for individual response analyses, by group. Dashed line represents the unadjusted group mean change from PRE to POST.

Figure 8.

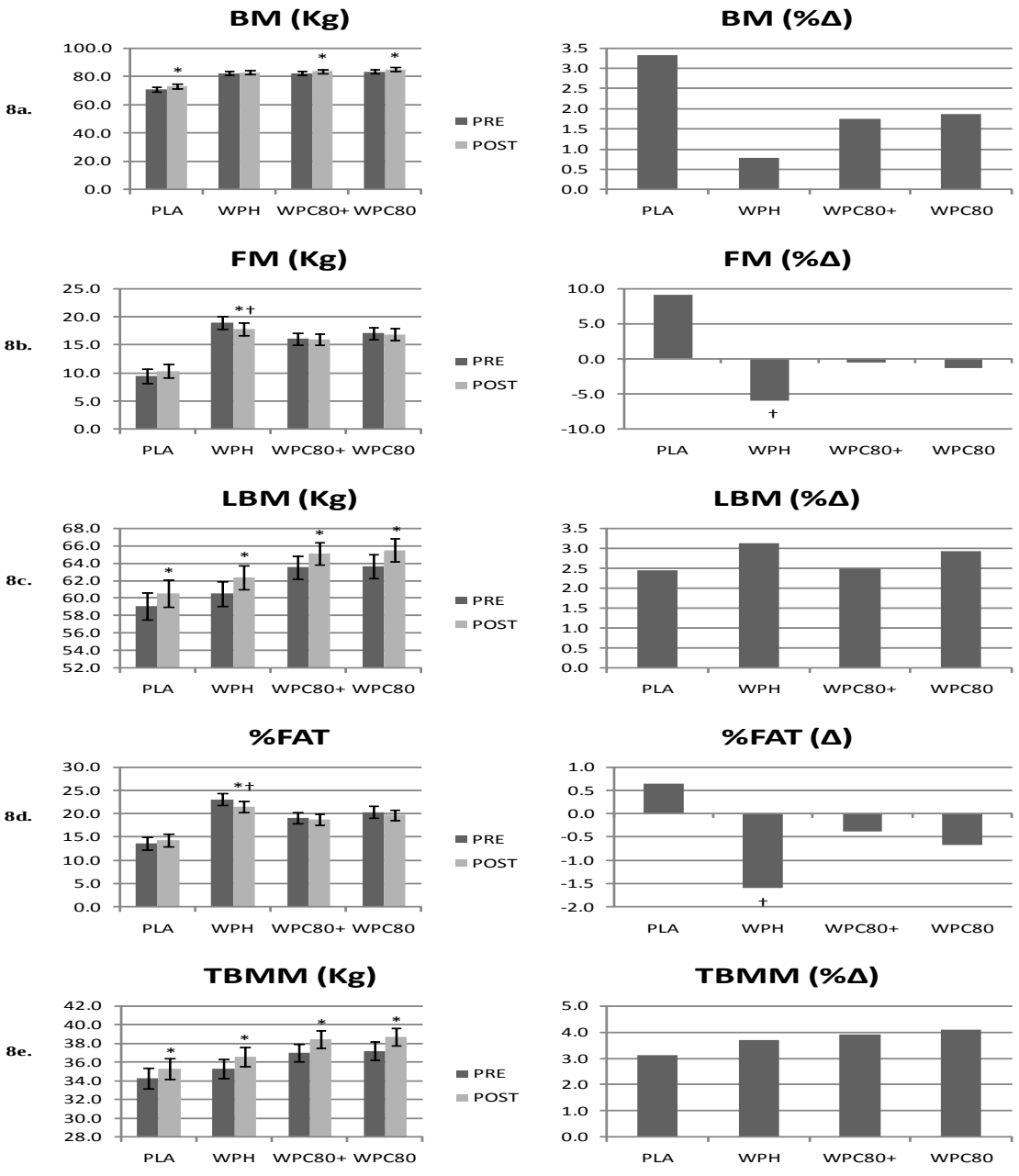

FIGURE 8. *Absolute and relative body composition changes from PRE to POST* . PLA = PLACEBO; WPH = WHEY PROTEIN HYDROLYSATE; WPC80+ = HIGH LACTOFERRIN 80% WHEY PROTEIN CONCENTRATE; WPC80 = 80% WHEY PROTEIN CONCENTRATE. BM = Body Mass; FM = Fat Mass; LBM = Lean Body Mass; %FAT = Percent Body Fat; TBMM = Total Body Muscle Mass. **8a** = Effects on Body Mass; **8b** = Effects on Fat Mass; **8c** = Effects on Lean Body Mass; **8d** = Effects on Percent Body Fat; **8e** = Effects on Total Body Muscle Mass. Estimated average means adjusted for covariates: Training Status Ratio (PRE), Total 8-wk Relative Training Volume (kg/min), and Average 8-wk Relative Protein Intake (g/kg/d). Main effects set at $p \leq 0.05$. *Different from PRE ($p \leq 0.0125$); †Different from PLA ($p \leq 0.05$); ‡Different from WPC80+ ($p \leq 0.05$); §Different from WPC80 ($p \leq 0.05$).

Figure 9.

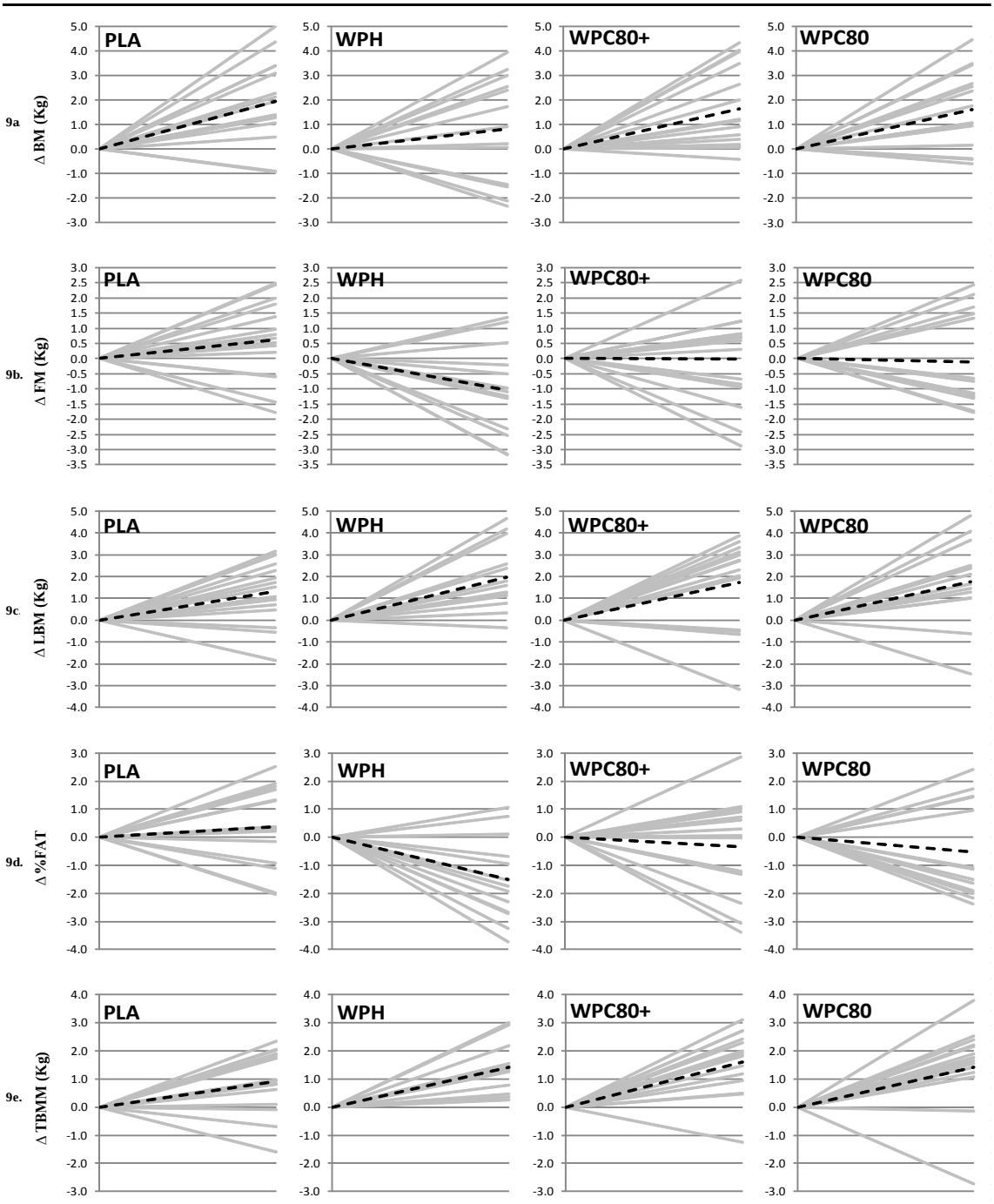

FIGURE 9. *Individual responses for body composition changes from PRE to POST*. PLA = PLACEBO; WPH = WHEY PROTEIN HYDROLYSATE; WPC80+ = HIGH LACTOFERRIN 80% WHEY PROTEIN CONCENTRATE; WPC80 = 80% WHEY PROTEIN CONCENTRATE. **9a** = Individual Responses on BM for PRE v POST; **9b** = Individual Responses on FM for PRE v POST; **9c** = Individual Responses on LBM for PRE v POST; **9d** = Individual Responses on %FAT for PRE v POST; **9e** = Individual Responses on TBMM for PRE v POST. BM = Body Mass; FM = Fat Mass; LBM = Lean Body Mass; %FAT = Percent Body Fat; TBMM = Total Body Muscle Mass. Unadjusted subject responses used for individual response analyses, by group. Dashed line represents the unadjusted group mean change from PRE to POST.

Figure 10.

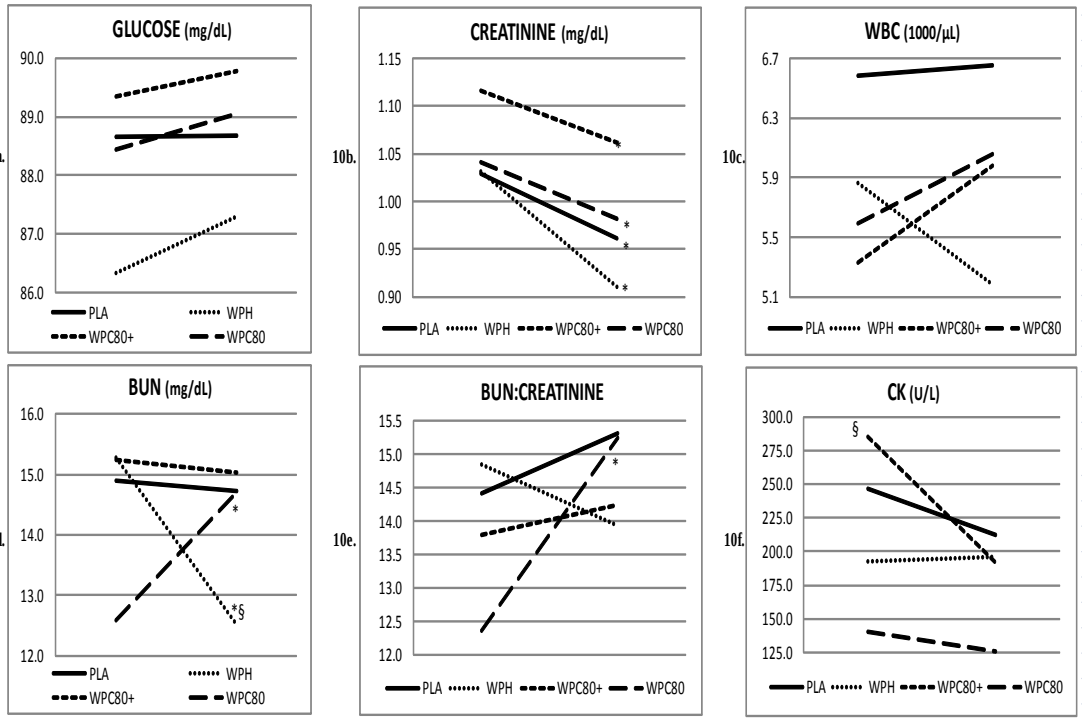

FIGURE 10. *Changes in select blood measures from PRE to POST* . PLA = PLACEBO; WPH = WHEY PROTEIN HYDROLYSATE; WPC80+ = HIGH LACTOFERRIN 80% WHEY PROTEIN CONCENTRATE; WPC80 = 80% WHEY PROTEIN CONCENTRATE. WBC = White Blood Cell count; BUN = Urea Nitrogen; BUN:CREATININE = Urea Nitrogen-to-Creatinine ratio; CK = Creatine Kinase. **10a** = Effect on blood glucose from PRE to POST; **10b** = Effect on creatinine from PRE to POST; **10c** = Effect on white blood cell count from PRE to POST; **10d** = Effect on urea nitrogen from PRE to POST; **10e** = Effect on BUN:Creatinine ratio from PRE to POST; **10f** = Effect on creatine kinase from PRE to POST. All blood draws under confirmed euhydrated state and after a 12-hour fast (water only). Estimated average means adjusted for covariates: Training Status Ratio (PRE), Total 8-wk Relative Training Volume (kg/min), Average 8-wk Relative Protein Intake (g/kg/d), and Blood Collection Time. Main effects set at $p \leq 0.05$. *Different from PRE ($p \leq 0.0125$); †Different from PLA ($p \leq 0.05$); ‡Different from WPC80+ ($p \leq 0.05$); §Different from WPC80 ($p \leq 0.05$).

Figure 11.

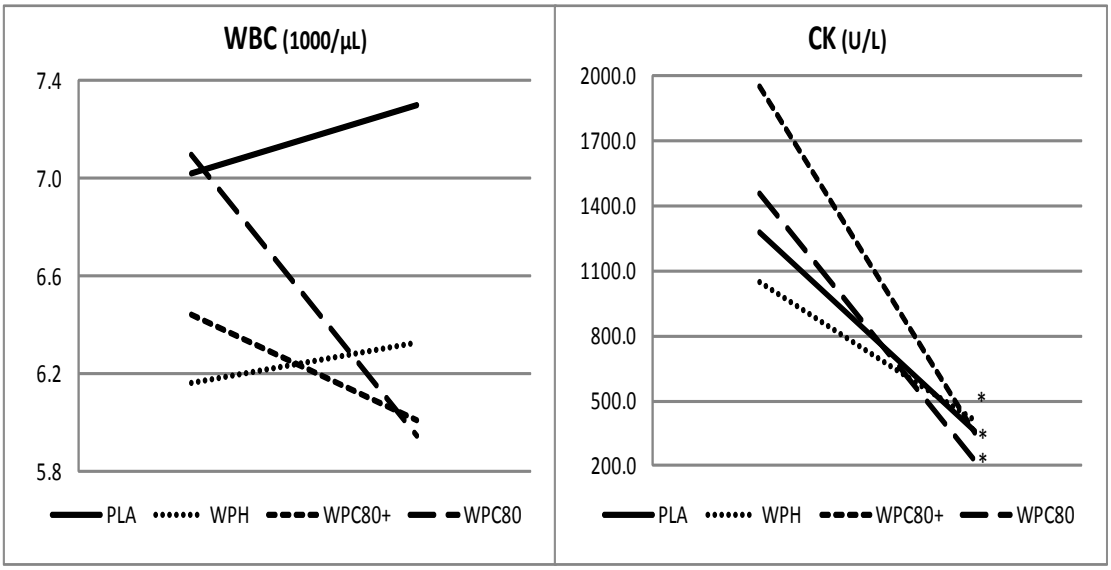

FIGURE 11. *Changes in 24-hour WBC and CK response to lower-body training from Week 1 to Week 8* . PLA = PLACEBO; WPH = WHEY PROTEIN HYDROLYSATE; WPC80+ = HIGH LACTOFERRIN 80% WHEY PROTEIN CONCENTRATE; WPC80 = 80% WHEY PROTEIN CONCENTRATE. WBC = White Blood Cell count; CK = Creatine Kinase. All blood draws took place after a 4-hour fast (water only). Estimated average means adjusted for covariates: Training Status Ratio (PRE), Total 8-wk Relative Training Volume (kg/min), Average 8-wk Relative Protein Intake (g/kg/d), and Blood Collection Time. Main effects set at $p \leq 0.05$. [*]Different from PRE ($p \leq 0.0125$); [†]Different from PLA ($p \leq 0.05$); [‡]Different from WPC80+ ($p \leq 0.05$); [§]Different from WPC80 ($p \leq 0.05$).

Figure 12.

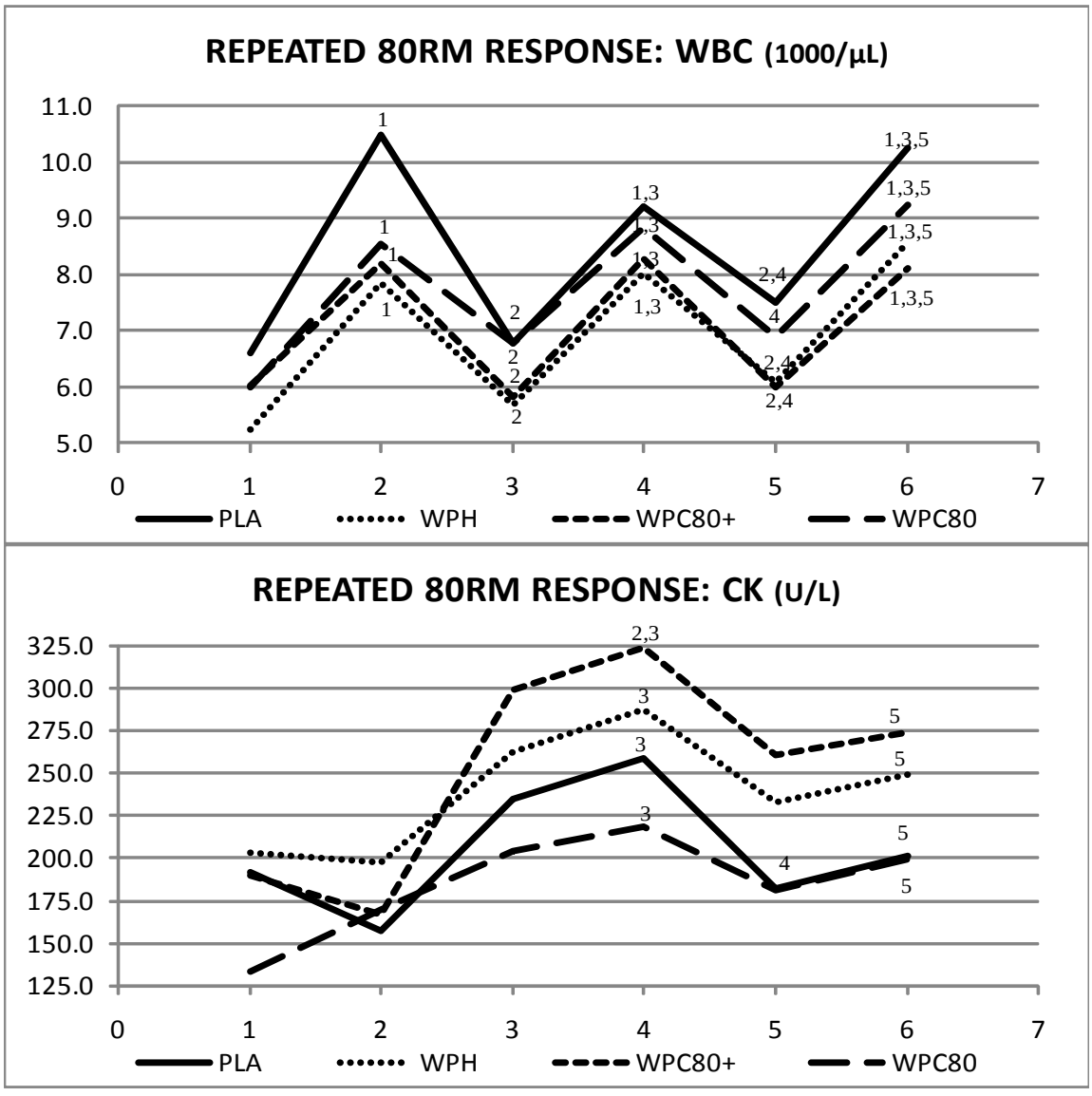

FIGURE 12. *Changes in WBC and CK in response to repeated 80RM bouts* . PLA = PLACEBO; WPH = WHEY PROTEIN HYDROLYSATE; WPC80+ = HIGH LACTOFERRIN 80% WHEY PROTEIN CONCENTRATE; WPC80 = 80% WHEY PROTEIN CONCENTRATE. WBC = White Blood Cell count; CK = Creatine Kinase. 1 = DAY 1 Pre-80RM blood draw (T1); 2 = DAY 1 Post-80RM blood draw (T2); 3 = DAY 2 Pre-80RM blood draw (T3); 4 = DAY 2 Post-80RM blood draw (T4); 5 = DAY 3 Pre-80RM blood draw (T5); 6 = DAY 3 Post-80RM blood draw (T6). Estimated average means adjusted for covariates: Training Status Ratio (PRE), Total 8-wk Relative Training Volume (kg/min), Average 8-wk Relative Protein Intake (g/kg/d), and Blood Collection Time. Main effects set at $p \leq 0.05$. [1]Different from T1 ($p \leq 0.0125$); [2]Different from T2 ($p \leq 0.0125$); [3]Different from T3 ($p \leq 0.0125$); [4]Different from T4 ($p \leq 0.0125$); [5]Different from T5 ($p \leq 0.0125$); [†]Different from PLA ($p \leq 0.05$); [‡]Different from WPC80+ ($p \leq 0.05$); [§]Different from WPC80 ($p \leq 0.05$).