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THE INFLUENCE OF 8-WEEKS OF WHEY PROTEIN AND LEUCINE SUPPLEMENTATION ON PHYSICAL AND COGNITIVE PERFORMANCE

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14. ABSTRACT. This study was designed to investigate the ability of 8-weeks of whey protein and leucine supplementation to enhance physical and cognitive performance and body composition. It was further designed to examine the impact of such supplementation on circulating levels of mammalian target of rapamycin (mTOR). Thirty moderately fit subjects completed a modified Air Force fitness test, a PC-based cognition test, and a Dual Energy X-ray Absorptiometry (DEXA) scan for body composition before and after supplementing their daily diet with either whey protein and leucine or a caloric-equivalent placebo (P). The WPL group showed greater increases in strength then the P group over the 8 weeks on bench press and push-ups. Bench press performance increased significantly by 3.9% from week 1 to week 8 in the WPL group, whereas the increase in the P group (1.4%) was not significant. Push-up performance increased significantly by 5.4 push-ups (12.8%) for WPL while P showed a non-significant increase of 3.3 pushups (7.6%). Total mass, fat-free mass, and lean body mass all increased significantly (by 1.0 kg, 0.7 kg and 0.7kg, respectively) in the WPL group but showed no change in the placebo group. No differences were observed within or between groups for crunches, chin-ups, 3-mile run time, or cognition. We were unable to assay mTOR levels of our subjects accurately enough to make firm determinations regarding the influence of whey and leucine on them. We conclude that supplementing with whey protein and leucine may provide an advantage to airmen whose job performance benefits from increased upper body strength and/or lean body mass.

15. SUBJECT TERMS: Whey protein, leucine, mammalian target of rapamycin (mTOR)

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Executive Summary

Purpose:
The purpose of this study was to investigate the ability of 8-weeks of whey protein and leucine supplementation to enhance physical and cognitive performance and body composition. A secondary purpose was to examine the impact of such supplementation on circulating levels of mammalian target of rapamycin (mTOR).

Methods:
Thirty moderately fit subjects completed a modified Air Force fitness test (maximum 1-rep bench press, maximum number of chin-ups, push-ups, and crunches in 1 minute each, and a timed 3-mile run), a PC-based cognition test, and a Dual Energy X-ray Absorptiometry (DEXA) scan for body composition before and after supplementing their daily diet for 8 weeks with either 19.7 g of whey protein and 6.2 g leucine (WPL) or a calorie-equivalent placebo (P).

Results and Conclusions:
The WPL group showed greater increases in strength than the P group over the 8 weeks on bench press and push-ups. Bench press performance increased significantly by 3.9% from week 1 to week 8 in the WPL group, whereas the increase in the P group (1.4%) was not significant. Push-up performance increased significantly by 5.4 push-ups (12.8%) for WPL while P showed a non-significant increase of 3.3 pushups (7.6%). Total mass, fat-free mass, and lean body mass all increased significantly (by 1.0 kg, 0.7 kg and 0.7kg, respectively) in the WPL group but showed no change in the placebo group. No differences were observed within or between groups for crunches, chin-ups, 3-mile run time, or cognition.

We were unable to assay mTOR levels of our subjects accurately enough to make firm determinations regarding the influence of whey and leucine on them.

We conclude that supplementing with whey protein and leucine may provide an advantage to airmen whose job performance benefits from increased upper body strength and/or lean body mass.

INTRODUCTION

Objective

Improvements in strength and cognition likely translate directly into increased operational capability for our Battlefield Airmen, particularly our special operators. The purpose of this study was to investigate the ability of 8-weeks of whey protein and leucine supplementation to enhance physical and cognitive performance and body composition. A secondary purpose was to examine the impact of such supplementation on circulating levels of mammalian target of rapamycin (mTOR).

Background

Military operations require modern warriors to perform at almost super-human levels. Special operations members routinely face high-stress, austere environments, schedules counter to normal circadian physiology, and physically and mentally demanding tasks. Mission completion is so important that some military personnel take medication or dietary supplements of unknown utility and safety in order to accomplish it. In a 2006 survey of Air Force members, 69% of respondents admitted to either currently using or previously using dietary supplements (Greenwood, 2008). However, only 19% had been provided any official guidance or education as to the efficacy and safety of the supplements they were using. These numbers are very similar to previous findings of supplement use in Army soldiers (Bovill, 2003). It would be advantageous to identify those nutritional supplements that could safely and effectively increase military-relevant performance. The dietary combination of whey protein and leucine has promise to be such a supplement.

Supplementation with leucine (Crowe, 2006) and whey protein (Burke, 2001) has been shown to improve single bout exercise performance and to chronically increase nitrogen balance and promote anabolism, thereby resulting in greater physical strength. Crowe et al. (2006) observed a 14% increase in exercise time-to-exhaustion and a 12% increase in upper-body power in rowers who were supplemented for 6 weeks with 45 mg $kg^{-1}d^{-1}$ of L-leucine. Work by Koopman et al. (2005) has suggested that the combination of whey protein with leucine may be more powerful than either supplement alone to increase whole body net protein balance. Similarly, Coburn et al. (2006) recently reported that the combination of whey protein with leucine elicited greater strength gains (30%) following 8 weeks of supplementation and unilateral leg extension resistance training than did a carbohydrate placebo (22%).

Protein and branched chain amino acids (BCAA) supplementation may also improve cognitive performance while fatigued. Studies from Blomstrand et al. (1991, 1997) and Hassmen et al. (1994) have observed subjects supplemented with BCAAs scored better on both mood levels and

cognitive tasks following exercise. However, other studies have not supported this thesis (Cheuvront, 2004.)

Leucine supplementation may also be a critical up-regulator of mTOR (Norton, 2006). mTOR is a complex protein integrating signals of the energetic status of the cell and environmental stimuli to control protein synthesis and breakdown, thereby controlling cell growth. Although research as to the cause and effect of increased mTOR levels is very incomplete, it is strongly suspected to positively influence strength, lean body mass, cognition, and learning (Bodine, 2006).

METHODS

Volunteers

Thirty-five volunteers signed an informed consent document and completed a medical screening questionnaire (Appendix A), prior to participating in this protocol. Thirty-three subjects completed the study, thirty male and three female. (One subject suffered an injury unrelated to the protocol and could not participate in post-testing; another moved from the local area prior to post-testing.) The study was open to both genders. However, because only three female participants completed the study and due to the inherent difficulty in comparing macronutrient responses between genders, results for the three female subjects were removed for this report. The mean age of the remaining thirty male participants was 26.9 ± years old, and 24 of the 30 were military members. All participants were recruited from local area military installations and colleges.

The following inclusion criteria were used to determine participation in this study:

1) Meet American College of Sports Medicine definition of "Low Risk"
2) Regular exercise three times per week for the past three months
3) No use of nutritional supplements for 30 days prior to trial start

Experimental Design and Assessment Overview

Materials

The physical tests throughout the study required the use of two bench presses, a chin-up bar, a floor mat for sit-ups and push-ups, and a track for a three-mile timed run. Two of the subjects did not have access to the track; instead they used a measured three-mile course during both pre- and post-testing. For all three-mile runs, the Sprint 8 track timing device was used. Each participant underwent a body composition scan utilizing the GE Lunar Dual Energy X-ray Analysis (DEXA) machine (GE Healthcare, Chalfont St. Giles, United Kingdom). In addition to completing body composition scans, all participants provided samples of blood to be tested for

mTOR content. Each of the participants consumed two packets of either placebo or whey protein and leucine powder daily. Each packet of the protein treatment contained 112 kcals, to include 19.7 g of whey protein and 6.2 g leucine. Placebo doses were 112 kcals of carbohydrate with 0.0 g protein.

Procedures

Upon acceptance into the study, participants completed their informed consent documentation and began the training session for the first of two testing sessions (pre-supplement and post-supplement testing). The first pre-supplement training consisted of a medical screening, a DEXA scan, a blood draw, and approximately one hour of training on cognitive tests.

Two days following training, participants underwent the pre-supplement testing session. Within the testing session participants completed a 1-RM bench press and maximum chin-up, pushup and crunch repetitions completed within one minute. Subjects were given a three minute break between each exercise. Following crunches, subjects received a five minute rest before beginning the timed three-mile run. Subjects were required to complete twelve laps on the track in as short a time as possible. They were also asked to sprint as fast as possible for the last 40 yards. Following the three-mile run, participants took a 10-15 minute break before beginning the cognitive testing. The computer-based cognitive testing took approximately 15-20 minutes and included the Continuous Performance Task, the Sternberg Memory Task, and the Stanford Sleepiness Scale.

During the Continuous Performance Task subjects were asked to monitor a randomized sequence of numbers. The numbers were presented one at a time in the center of the screen. While continuously monitoring the numbers, the subjects pressed a specified key indicating whether or not the current number on the screen matched the number that was presented two numbers prior. Outcome measures for analysis consisted of Accuracy and Mean Reaction Time for Correct Responses (MRTC).

The Sternberg Memory Task utilizes a set of letters displayed horizontally in the center of the monitor, known as the memory set. The subjects viewed the list and attempted to memorize it within a specified time period. The list was then removed from the screen and letters were presented one at a time in the middle of the screen. Subjects were required to determine if the number they were currently looking at was a member of the initial memory set. Over the course of the task, more numbers were added to the initial memory set and the task increased in difficulty, but the process remained the same. Outcome measures were Accuracy and MRTC.

The Stanford Sleepiness Scale presents a Likert-like scale using fatigue descriptors ranging from 1 (feeling active and vital) to 7 (almost in reverie; sleep onset soon; struggle to remain awake).

The scale correlates with standard measures of performance and usually reflects the effects of sleep loss. This scale was included to determine the level of fatigue post physical testing.

Following the pre-supplement testing, subjects were assigned to either the protein group or the placebo group in a random, but balanced, double-blind manner. At final count, there were 18 males in the protein group and 12 males in the placebo group. (The imbalance between groups was due to randomization with the initial goal of 40 subjects, the two subjects who started but did not complete the protocol and the exclusion of the three female subjects' data from the final results.) Subjects consumed the protein or placebo daily for 8 weeks. During non-exercise days, the participants consumed both packets in the morning. On days they exercised, participants consumed one packet of powder 30-45 minutes before exercising and the second packet 30-45 minutes after exercising. Subjects were required to maintain US Air Force standards of physical training, meaning that at least three days a week they engaged in endurance ("aerobic") training along with push-ups and crunches. If subjects had been exercising at volumes and/or intensities above the minimum requirements prior to the start of the study, they were allowed to continue doing so. They were instructed to not exceed Air Force minimum physical training guidelines during the study if they had not done so prior to study start. Each day participants recorded their exercise to include activity, duration, and intensity. Upon study completion, we categorized the subjects into three groups: Those who participated in at least 2 hours of resistance training per week were categorized as High-RT, those completing 1-2 hours per week as Medium-RT, and those completing less than 1 hour per week as Low-RT. We also recorded "packet compliance" by calculating the percent of required packets that were actually consumed by each participant over the duration of the study.

Subjects were also asked to record their daily food consumption for three days total, once near the beginning of the 8-week period and again near the end of the 8 weeks. The food logs were utilized to ensure that subjects had not made substantial changes in their dietary habits during the 8 weeks of the study.

At the end of the first four weeks subjects returned to the laboratory. Compliance was measured and a medical screening accomplished, but no testing was conducted at that time. After the final four weeks of consuming the supplement or placebo, subjects completed post-supplement training and testing. The post-supplement training and testing procedures were identical to the pre-supplement training and testing procedures.

Figure 1: 1-RM Bench Press Testing.

Figure 2: Chin-Up Testing

Data Analysis

Initially, a repeated measures analysis of variance (ANOVA) with one within-subjects factor (week) and one between-subjects factor (treatment group) was performed on each outcome measure. Two covariates (level of resistance exercise during the study: high, medium, low; and packet compliance: % of packets taken) were included in the analysis to adjust for potential bias within the groups. After reviewing the outcomes of these initial analyses (details to be discussed in "results"), we reanalyzed the data by performing Student's paired t-tests, for each group, separately, to determine whether there were significant changes from week 1 to week 8, and we performed Student's independent t-tests to compare the week 1 to 8 change in the supplement group with the change in the placebo group. (This test is identically equivalent to the ANOVA group by week interaction test but unadjusted for the covariates). Finally, viewing the data from a different perspective, we calculated, for each outcome measure, the percentage of subjects in each group who showed at least a 5% improvement, and compared these percentages using a chi-square test. The purpose of this approach was to test the hypothesis that, if the supplement proved not to be beneficial to all subjects, it might at least show large beneficial effects on a greater subset of the subjects than would be found in the placebo group. All testing was performed at the 0.05 level of significance.

RESULTS

Influence of Uncontrolled Factors

There were two uncontrolled factors (covariates) that we felt might bias the tests of our primary hypothesis that week 1 to 8 changes would differ between the protein and placebo groups: the amount of resistance training that the individuals were routinely performing, and compliance in taking the supplement/placebo packets. In the protein group, four subjects were classified as "low" resistance trainers, five as "medium," six as "high," and information was not available for the remaining three. Their packet compliance ranged from 63% to 100%, with only one subject below 80%. In the placebo group, six were "low" resistance trainers, one was "medium," three were "high," and information was unavailable for two. Their packet compliance ranged from 77% to 100%, with only two subjects below 80%.

For each outcome measure of the study, we performed a repeated measures analysis of variance (ANOVA) with treatment group as a between-subjects factor and week as a within-subjects factor, and we included the two covariates described above. We found no statistical evidence, for any of the outcome measures, that either of the covariates might be biasing our primary tests (i.e., there were no significant treatment group by week by packet compliance interactions, and no significant treatment group by week by resistance training interactions). Because of these

findings, we decided to reanalyze the data, ignoring the covariates. This allowed us to increase the sample size, and consequently the power, for the primary tests of interest (recall that there were 5 subjects for whom we did not have covariate information, and our initial tests were therefore based on a reduced number of subjects). The results of the final statistical tests are discussed below, and are summarized in Tables 1 (physical performance), 2 (body composition), and 3 (cognitive performance). Some data was missing from the final data sets due to three cognitive test computer files being corrupted and due to non-study related injuries to two subjects that limited their ability to complete all the physical post-tests. This is reflected in the sample sizes shown in the tables.

Physical Performance

Bench press performance increased significantly by 3.5 Kg (a 3.9% improvement) from week 1 to week 8 in the protein group, whereas the increase in the placebo group was not significant (1.3 Kg for a 1.4% improvement). However, the difference between these two changes was not significant. On the other hand, from the perspective of "large" changes, we found that 55.6% (10 of 18) of the protein subjects showed a 5% or greater improvement compared to only 16.7% (2 of 12) of the placebo subjects. These percentages were significantly different (chi sq (1df) = 4.54, p = .033).

Push-up performance increased significantly by 5.4 push-ups (12.8% improvement) in the protein group while the placebo group showed a non-significant increase of 3.3 push-ups (7.6% improvement). The difference between these two changes was not significant. The percentage of subjects who showed "large" improvement (i.e., 5% or more) in the protein group was somewhat higher than in the placebo group (72.2% vs. 50%), but these two percentages did not differ statistically.

Crunch performance in the protein group increased by 3.2 crunches (7.2% improvement) compared to a 1.6 crunch increase (3.4% improvement) in the placebo group. Neither of these increases was significant, and they did not differ statistically from each other. In addition, the percentage of subjects who showed 5% or greater improvement was comparable for the protein and placebo groups (55.6% vs. 58.3%, respectively).

Chin-ups improved by 0.6 (10.1%) and 0.2 (1.7%) for the protein and placebo groups, respectively. Even though the 10.1% improvement looks impressive for the protein group, note that, on average, the protein group only did about half as many chin-ups as the placebo group at week 1 (6 chin-ups vs. 12). Thus, even small changes in the protein group result in fairly large percent changes. Neither of the changes from week 1 to 8 was significant, and they did not differ significantly from each other. The percentage of subjects who showed increases of 5% or more were 60.0% and 41.7% for the protein and placebo groups, respectively, and they did not differ statistically.

For the 3-mile run, there was no significant difference observed between groups or over time (decreases of 0.9 min (3%) vs. 0.4 min (1%)). The percentage of subjects in the placebo group who improved by at least 5% was 45.5% (5 of 11) compared to 18.8% (3 of 16) in the protein group. These percentages also did not differ statistically.

For the sprint, both groups showed a decrease of 0.3s (4.3% and 4.8% improvement for protein and placebo, respectively). These changes were not significant, and did not differ significantly from each other.

Table 1: Physical Performance Means, Standard Deviations, and Test Results.

Variable	Group	N	Week 1	Week 8	Change	Test Result [#]
Bench Press (Kg)	WPL	18	89.4 ± 24.0	93.0 ± 24.0	3.5* ± 5.2	t(28)=1.21
	Placebo	12	91.1 ± 15.6	92.4 ± 17.3	1.3 ± 4.4	p=.235
Chin Ups	WPL	17	5.9 ± 4.7	6.5 ± 4.5	0.6 ± 1.8	t(27)=0.67
	Placebo	12	12.1 ± 5.0	12.2 ± 5.4	0.2 ± 2.1	p=.508
Crunches	WPL	18	44.4 ± 14.3	47.6 ± 14.9	3.2 ± 7.3	t(28)=0.53
	Placebo	12	45.2 ± 10.0	46.8 ± 10.1	1.6 ± 9.5	p=.597
Push Ups	WPL	18	42.2 ± 14.6	47.6 ±15.3	5.4* ± 6.8	t(28)=0.84
	Placebo	12	41.9 ±11.4	45.2 ± 9.1	3.2 ± 6.8	p=.407
Sprint (seconds)	WPL	15	6.7 ± 1.1	6.4 ±0.8	-0.3 ±0 .7	t(25)=0.04
	Placebo	12	6.2 ± 1.0	5.9 ± 1.2	-0.3 ± 0.7	p=.965
3 Mile Run (minutes)	WPL	16	28.2 ± 5.0	27.8 ± 4.2	-0.4 ± 1.4	t(25)=0.54
	Placebo	11	27.1 ± 2.5	26.2 ± 3.3	-0.9 ± 3.3	p=.596

[#] Student's t-test comparing the protein group change with the placebo group change.
* Significant change from week 1 to week 8 (paired t-test, $p<.05$).

Body Composition

Average body weight for the protein group increased significantly by 1.0 Kg while weight decreased non-significantly by 0.8 Kg for the placebo group. Total fat-free mass and lean body mass both increased significantly (0.7 Kg) in the protein group, and both showed no change in the placebo group. There were no statistical differences between the changes of the two groups for either fat-free mass or lean body mass. Finally, for both total fat and percent fat, there were no significant findings of any kind.

Table 2: Body Composition Means, Standard Deviations, and Test Results.

Variable	Group	N	Week 1	Week 8	Change	Test Result*
Body Weight (Kg)	WPL	18	86.8 ± 16.4	87.8 ± 17.2	1.0* ± 1.8	t(28)=2.42 p=**.022**
	Placebo	12	83.0 ± 7.7	82.3 ± 7.0	-0.8 ± 2.0	
Fat (Kg)	WPL	18	23.1 ± 9.9	23.4 ± 10.1	0.3 ± 1.7	t(28)=1.56 p=.129
	Placebo	12	15.9 ± 7.8	15.1 ± 7.6	-0.8 ± 1.9	
Percent Fat	WPL	18	26.8 ± 6.7	26.8 ± 6.5	0.0 ± 1.5	t(28)=1.22 p=.231
	Placebo	12	19.7 ± 8.4	18.9 ± 8.4	-0.7 ± 1.9	
Fat Free Mass (Kg)	WPL	18	63.7 ± 8.4	64.4 ± 8.7	0.7* ± 1.2	t(28)=1.65 p=.111
	Placebo	12	67.1 ± 6.6	67.1 ± 6.4	-0.0 ± 0.9	
Lean (Kg)	WPL	18	60.4 ± 7.9	61.0 ± 8.2	0.7* ± 1.3	t(28)=1.52 p=.139
	Placebo	12	63.3 ± 6.3	63.3 ± 6.1	0.0 ± 0.9	

[#] Student's t-test comparing the protein group change with the placebo group change. * Significant change from week 1 to week 8 (paired t-test, $p<.05$).

mTOR

Although we collected pre- and post- blood samples from all subjects we were unable to successfully assay mTOR from our samples. We have been unable to accurately measure mTOR in samples that have been frozen rather than immediately processed. We suspect this is related to S6 kinase phosphorylation stability, or rather lack thereof, when frozen. Although we do not report mTOR values here, we included it to be complete in our description of methods (blood draws) and purpose.

Cognitive Performance

Accuracy for the CPT and Sternberg tests remained relatively constant from week 1 to week 8 for both groups. No significant changes were seen for any of the tests for either group. Furthermore, no significant differences were seen between the groups with respect to the changes in accuracy.

Reaction time (MRTC) for the CPT and Sternberg tests generally decreased (i.e., improved) from week 1 to week 8 for both groups. The greatest improvement occurred when taking the most difficult (Sternberg 6) test, with reaction time decreasing from baseline by 10.7% and 15.0% for the protein and placebo groups, respectively. These improvements suggest that our subjects were likely not trained to asymptote before beginning the study, and therefore showed improvement with repetition of the tests. The only statistically significant improvements were seen for the placebo group during the Sternberg 2 and Sternberg 6 tests and for the protein group during the Sternberg 4 test. However, in no case was there a significant difference between the protein group change and the placebo group change. In addition, the percentage of subjects who showed large (5% or greater) improvement was comparable for the protein and placebo groups

(58% vs. 46% for CPT, 36% vs. 46% for Sternberg 2, 50% vs. 46% for Sternberg 4, and 57% vs. 73% for Sternberg 6), and in no case were they significantly different.

Finally, scores on the Stanford Sleepiness Scale remained essentially unchanged from week 1 to week 8 in both groups, with no significant results found.

Table 3: Cognitive Performance Means, Standard Deviations, and Test Results.

Variable	Group	N	Week 1	Week 8	Change	Test Result*
CPT Accuracy	WPL	12	87.9 ± 15.2	88.8 ± 14.1	0.8 ± 7.6	t(21)=0.43
	Placebo	11	90.7 ± 5.0	92.7 ± 4.3	2.0 ± 5.3	p=.675
CPT MRTC	WPL	12	455.5 ± 80.3	424.0 ± 74.1	-31.5 ± 65.8	t(21)=0.43
	Placebo	11	456.0 ± 55.8	435.3 ± 69.1	-20.8 ± 53.8	p=.674
Sternberg 2 Accuracy	WPL	14	96.0 ± 4.2	94.4 ± 5.1	-1.6 ± 4.0	t(23)=1.98
	Placebo	11	92.6 ± 8.4	94.9 ± 4.9	2.4 ± 5.9	p=.059
Sternberg 2 MRTC	WPL	14	448.3 ± 80.5	429.4 ± 70.7	-18.8 ± 65.0	t(23)=0.38
	Placebo	11	417.7 ± 29.3	390.6 ± 28.6	-27.1* ± 33.3	p=.705
Sternberg 4 Accuracy	WPL	14	94.4 ± 5.2	95.4 ± 4.6	1.0 ± 3.7	t(23)=1.62
	Placebo	11	95.5 ± 4.2	93.4 ± 7.7	-2.0 ± 5.6	p=.119
Sternberg 4 MRTC	WPL	14	506.1 ± 98.7	468.4 ± 88.0	-37.7 * ± 56.2	t(23)=0.96
	Placebo	11	471.7 ± 49.0	455.3 ± 60.3	-16.4 ± 53.7	p=.347
Sternberg 6 Accuracy	WPL	14	91.9 ± 11.9	93.3 ± 4.7	1.4 ± 11.5	t(23)=0.17
	Placebo	11	93.4 ± 5.9	94.2 ± 5.8	0.8 ± 5.8	p=.870
Sternberg 6 MRTC	WPL	14	619.7 ± 153.8	553.5 ± 117.6	-66.2 ± 124.9	t(23)=0.54
	Placebo	11	603.1 ± 142.4	512.6 ± 66.1	-90.5* ± 92.7	p=.596
Stanford Sleepiness	WPL	12	2.0 ± 1.0	2.0 ± 1.5	0.0 ± 1.9	t(20)=0.70
	Placebo	10	2.6 ± 1.5	2.1 ± 0.9	-0.5 ± 1.4	p=.495

[#] Student's t-test comparing the protein group change with the placebo group change.
* Significant change from week 1 to week 8 (paired t-test, $p<.05$).

DISCUSSION

The primary findings of this investigation were that 8 weeks of supplemental whey protein with leucine resulted in increases in muscular strength and lean body mass (LBM), but did not promote increases in endurance performance or cognitive performance. However, the increases in strength and LBM were not as great as hypothesized, nor as large as was demonstrated in two previous investigations (Willoughby, 2007; Cribb, 2007).

Despite a number of investigations there is not yet a clear consensus on the influence of supplemental whey protein and/or leucine on strength performance as reflected by 1-RM BP. Kersick et al. (2006) supplemented subjects with whey and casein (WC), whey and BCAAs (WBC), or placebo (P) over 10 weeks of resistance training (RT). They observed a significant increase in 1-RM BP in all 3 groups with no differences between the groups, although the WC group trended slightly higher. Similarly, Mielke et al. (2009) found that a whey/leucine group, a CHO group, and a control group all increased their 1-RM BP significantly over 8 weeks with no differences between groups. In contrast, Cribb et al. (2007) reported that subjects supplemented

with whey protein over 11 weeks of RT significantly increased their 1-RM BP over their week 0 baseline, and that the change in the whey protein group was significantly greater than that of a carbohydrate-supplemented group. Burke et al. (2001) and Willoughby et al. (2007) observed that both a protein and a placebo group experienced significant increases in strength as reflected by 1-RM BP over a 10-week period with the increases for the protein group being greater than those of the placebo group. Our results lend support, albeit mild, to those of Willoughby et al. (2007) and Cribb et al. (2007) as we observed a significant 1-RM BP increase of 3.54 kg from week 1 to week 8 in the WPL group and a non-significant 1.32 kg increase in the CHO group.

One notable difference between the current study and most of those that have observed significant physiological and performance gains is the length of the trials. The current study was 8 weeks long whereas the studies showing the greatest gains from the use of whey protein and/or leucine were 10 weeks (Burke, 2001; Willoughby, 2007) or 11 weeks (Cribb, 2007) in duration. Another important distinction is that all of these aforementioned studies incorporated a standardized RT program for subjects in all groups, whereas the current study did not. This study simply insisted subjects maintain the USAF minimums for physical training, which did not include substantial RT. We performed a more detailed retrospective inspection of the bench press data and found that the percentage of subjects who routinely performed low levels of RT for the duration of the study but who showed large (5% or greater) improvement in the bench press were about the same in the protein and placebo groups (25.0% vs. 16.7%, respectively). However, for subjects who performed medium to high levels of RT, a higher percentage showed large improvements in the protein group than in the placebo group (54.4% vs. 25.0%). These numbers, while not statistically significant, suggest that an individual who routinely follows a rigorous RT program may benefit from the supplemental whey and leucine to a greater degree than one who does not follow such a program.

We also observed a significant increase in push-ups (5.4) by the WPL group whereas the placebo group showed a non-significant increase of 3.3 pushups. In the other muscular endurance parameters we measured (crunches, chin-ups), none of the changes from week 0 to week 8 were significant, nor were there observed difference between groups, although the scores of the WPL group did trend slightly higher. Push-ups and crunches are an integral part of the USAF PT program and nearly all subjects performed them regularly during the study. Chin-ups are not a standard USAF exercise. Most previous studies that have examined the influence of supplemental protein on physical performance have not examined its influence on muscular endurance. However, Kersick et al. (2006) reported no significant differences over 10 weeks in number of BP repetitions at 80% 1-RM with no differences between groups. Similarly, Mielke et al. (2009) did not observe significant differences in the number of BP and leg extension repetitions between a whey/leucine group and a control group after 8 weeks of supplementation.

No differences in cardio-respiratory endurance were demonstrated by either group in their 3-mile run times over the 8-week test period. This is in contrast to Crowe et al. (2006) who observed

rowers supplemented with leucine for 6 weeks improved their 70-75% VO_2peak rowing time-to-exhaustion by over 10 minutes while a placebo group did not improve. The disparity may be due to moderate intensity rowing potentially placing a greater demand on strength characteristics than moderate intensity running. In the current study high intensity running performance was not influenced by supplementation. However, as the 40-m sprint was done at the end of a 3-mile run it is unlikely that our sprint test was a true test of power.

The WPL group experienced significant increases from week 0 to week 8 in total body weight and lean body mass while the placebo group did not. Body composition did not change significantly over time for either group nor was there a difference between groups. The gain in lean body mass we observed mirrors gains observed in previous studies (Willoughby, 2007; Burke, 2001; Kersick, 2006; Cribb, 2006; Cribb, 2007). Koopman et al. (2005, 2006) has demonstrated that ingestion of supplemental whey protein with leucine significantly increases nitrogen balance. Such an increase over an 8-week period would explain the increase in lean body mass that we observed.

No significant differences in cognitive performance were observed over the 8-week test period. Cognitive test measures were generally unchanged from week 0 to week 8 and between groups. It was hypothesized that supplemental WPL could enhance cognitive performance when physically fatigued by staving off central fatigue. Central fatigue implicates serotonin (5-HT) accumulation as a primary cause of decreased physical and cognitive performance (Romanowski & Grabiec, 1974). Tryptophan (TRP) is an amino acid precursor to 5-HT which normally circulates in the blood bound to albumin. The TRP that is not bound to albumin is transported across the blood brain barrier and ultimately into the brain (Chaouloff, 1985). The TRP that enters the central nervous system (CNS) increases production of 5-HT potentially producing central fatigue, which may decrease cognitive performance. The mechanism that is responsible for transporting TRP into the CNS is also the same system that transports BCAAs like leucine into the CNS (Chaouloff, 1989). BCAAs are available in low concentrations from normal dietary consumption and are primarily taken up from the blood and oxidized for energy in contracting muscle during exercise. Thus the ratio of BCAAs to unbound TRP in the blood stream is normally low. This favors transport of TRP across the blood brain barrier and 5-HT production. However, if BCAA concentration is increased and the ratio of BCAAs to unbound TRP is increased, the BCAAs compete with unbound TRP for entrance into the CNS. This leads to less 5-HT production, staving off central fatigue and a hypothesized enhancement or maintenance of performance.

Some protocols have indeed reported a positive effect of BCAA supplementation on cognitive performance as compared with water (Blomstrand, 1997; Struder, 1998), or carbohydrate ingestion (Hassmen, 1994). However, none of these studies reported providing an isocaloric control condition. Portier et al. (2008) did provide an isocaloric control. Their subjects ate a "standard" diet or isocaloric BCAA-supplemented diet during a 32-hr sailing competition.

Although they did not report differences in physical performance or in other cognitive performance tests between groups they did observe that the standard diet group suffered a significant decrease in short-term memory performance over the event while the BCAA-supplemented group did not. Chevrount et al. (2004) also provided an isocaloric placebo, but failed to observe any influence of BCAA supplementation on cognition in hypohydrated subjects before or after a strenuous cycling bout in the heat. Contrary to the current study, none of these studies administered whey protein with leucine; instead they used various combinations of valine, leucine and isoleucine. Additionally, previous studies examining the effect of BCAA supplementation on cognition used single or short term doses. The current protocol appears to be the first to examine the effect of chronic amino acid supplementation on cognition after exercise. The results of this study did not show any evidence of a positive effect of protein and leucine supplementation on cognition. However, the exercise stress our subjects experienced may not have been severe enough to engender substantial central fatigue.

CONCLUSIONS

Based on the result of this investigation, we conclude that 8 weeks of WPL supplementation while adhering to standard USAF PT guidelines is mildly effective at increasing lean body mass and upper body muscular strength. However, such a brief supplementation regimen appears to be ineffective at influencing endurance performance or cognitive performance. As such we suggest that WPL supplementation could be a useful tool for AF members whose jobs depend highly on strength, such as combat controllers, pararescuemen, bomb loaders, refuelers and civil engineers. It may also benefit fighter and trainer pilots who need considerable strength to effectively perform an anti-G straining maneuver.

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Appendix A. Medical Screening Form.

Volunteer Number _____ Date:_____

Initial Medical History Screening

Please answer Y or N for the following health history questions:

- Father or brother suffered a heart attack before age 55:	Y	N
- Mother or sister suffered a heart attack before age 65:	Y	N
- Have you smoked tobacco within the past 12 months?	Y	N
- Have you been diagnosed with any of the following?		
-- High Cholesterol (>200 mg/dL)	Y	N
-- High Blood Pressure	Y	N
-- Diabetes	Y	N
- Are you currently taking any medications?	Y	N
- Do you have a medical condition that restricts your ability to perform actions such as running/jumping/cycling?	Y	N
- Are you pregnant?	Y	N
- Do you suffer any chronic joint or muscle pain?	Y	N

Volunteers who answer "Y" to any questions will be referred to the medical monitor for further determination of their eligibility to be a volunteer. Signature of Investigator or Medical Monitor below indicates that this volunteer is medically cleared.

_____ _____

Signature of Investigator Signature of Medical Monitor (if applicable)