Muscle protein metabolism in female swimmers after a combination of resistance and endurance exercise

KEVIN D. TIPTON, ARNY A. FERRANDO, BRADLEY D. WILLIAMS, AND ROBERT R. WOLFE

Departments of Surgery and Anesthesiology, University of Texas Medical Branch, and Metabolism Unit, Shriners Burns Institute, Galveston, Texas 77550

Tipton, Kevin D., Arny A. Ferrando, Bradley D. Williams, and Robert R. Wolfe. Muscle protein metabolism in female swimmers after a combination of resistance and endurance exercise. J. Appl. Physiol. 81(5): 2034–2038, 1996.—There is little known about the responses of muscle protein metabolism in women to exercise. Furthermore, the effect of adding resistance training to an endurance training regimen on net protein anabolism has not been established in either men or women. The purpose of this study was to quantify the acute effects of combined swimming and resistance training on protein metabolism in female swimmers by the direct measurement of muscle protein synthesis and whole body protein degradation. Seven collegiate female swimmers were each studied on four separate occasions with a primed constant infusion of ring-[^13]C_6 phenylalanine (Phe) to measure the fractional synthetic rate (FSR) of the posterior deltoid and whole body protein breakdown. Measurements were made over a 5-h period at rest and after each of three randomly ordered workouts: 1) 4,600 m of intense interval swimming (SW); 2) a whole body resistance-training workout with no swimming on that day (RW); and 3) swimming and resistance training combined (SR). Whole body protein breakdown was similar for all treatments (0.75 ± 0.04, 0.69 ± 0.03, 0.69 ± 0.02, and 0.71 ± 0.04 μmol·min⁻¹·kg⁻¹ for rest, RW, SW, and SR, respectively). The FSR of the posterior deltoid was significantly greater (P < 0.05) after SR (0.082 ± 0.015%/h) than at rest (0.045 ± 0.006%/h). There was no significant difference in the FSR after RW (0.048 ± 0.004%/h) or SW (0.064 ± 0.008%/h) from rest or from SR. These data indicate that the combination of swimming and resistance exercise stimulates net muscle protein synthesis above resting levels in female swimmers.

Competitive swimmers train for 3–4 h/day and up to 10,000 m/day (6, 7, 17), which leads to the cardiovascular and muscle adaptations commonly found in other endurance athletes (12, 14–16). Endurance performance may be improved by the addition of strength training (13). One likely explanation for increased performance as a result of combined resistance and endurance training is increased muscle hypertrophy and strength due to alterations in protein metabolism.

Although several studies have examined the response of whole body protein metabolism to exercise (18–20, 24), no study has been performed on the response of muscle PS in trained women. Furthermore, the effect of adding resistance training to an endurance training regimen on net protein anabolism has not been established in either men or women. Therefore, the purpose of this study was to quantify the acute effects of combined swimming and resistance training on protein metabolism in female swimmers by the direct measurement of muscle PS and whole body protein degradation. We hypothesized that muscle PS and WBPB would 1) be greater after resistance exercise, swimming, and both combined than at rest and 2) be greater after the combination of swimming and resistance exercise than either alone.

METHODS

Study protocol. The subjects for this study were seven collegiate female swimmers (age 20.4 ± 0.5 yr, wt 63.8 ± 1.9 kg). The study design, purpose, and possible risks were carefully explained to each subject before written consent was obtained. The experimental protocol was approved by the Institutional Review Board of the University of Texas Medical Branch (UTMB) at Galveston.

Each subject was studied four times: 1) at rest, 2) after a swimming workout (SW), 3) after a resistance exercise workout with no swimming on that day (RW), and 4) after swimming and resistance exercise combined (SR). All subjects were studied during the early follicular phase (days 1–8) of their menstrual cycle with two exceptions. Due to prior commitments, two subjects were studied 2 days before the beginning of menstrual flow on one occasion each. Serum estradiol values, measured immediately on awakening, were at follicular levels (≤370 pmol/l) for all subjects during each of the study days. Because the subjects were all competitive athletes, it was not possible to control the diets or workouts before the study days. Each subject was asked not to participate in a resistance exercise workout on the day before each study day. Diet records were kept for 3 days before each study day, and no differences were found between treatments for total energy intake, percent protein, percent carbohydrates, or percent fat (data not shown).

The subjects reported to the UTMB General Clinical Research Center (GCRC) the evening before each study. No
food was ingested after 2200. Immediately after the subjects awoke the following morning (approximately 0700), a 5-ml blood sample was obtained for hormone analysis. For the three exercise treatments, the subjects were then taken to the UTMB Field House, and the assigned workouts were performed. Immediately (~30 min) after each workout, the subjects returned to the GCRC for a 5-h infusion protocol to measure PS and protein degradation. The workouts for each of the three experimental days were randomized, similar to those assigned by their coach, and normally performed by the swimmers during the swim season. The volumes and intensities of weight workouts for both RW and SR and of both swim workouts for SW and SR were standardized for all swimmers.

Workouts. The RW workout included bench press (three sets of six repetitions at 80% of one repetition maximum (1 RM, the maximum weight that could be lifted in one attempt); military press, side laterals, latissimus pulldowns, bicep curls, and triceps pushdowns (3 sets of 10 at 65% of 1 RM); leg press, leg extension, leg curl, hip adduction, and hip abduction (3 sets of 10 at 65% of 1 RM); and abdominal crunches (2 sets of 30). There was a 60- to 90-s rest between each set. The RW workout was completed in ~1 h. Determinations of 1 RM were made at least 1 wk before participation in the study.

The SW workout was a 4,600 m workout of primarily high-intensity intermittent swimming. The workout consisted of 1) warm-up (500-m freestyle, 200-m kick, 200-m pull, and 200-m technique drills (4 × 50 m at increasing intensity)); 2) main set (10 × 200 m at a pace that resulted in an intensity of 85–90% of maximum age-predicted heart rate); 3) kick set (4 × 100 m); 4) pull sets (2 × 100-m (4 × 25-m × 2 sets) alternating freestyle and nonfreestyle); and 5) 200-m cool down. The SW lasted ~1.5 h. The SR treatment was a combination of the SW workout followed by the RW workout with a 15- to 20-min rest between the two. SR was completed in ~2.75 h.

Infusion protocol. Immediately (~30 min) after each workout, the swimmers returned to the GCRC for the infusion protocol. A 20-gauge polyethylene catheter was placed into a forearm vein for infusion of labeled amino acids. A background blood sample (3 ml) was obtained, and a primed constant infusion of ring-[13C6]phenylalanine (Phe; Cambridge Isotope Laboratories, Woburn MA) was started (prime = 2.0 µmol/kg, infusion rate = 0.05 µmol·kg⁻¹·min⁻¹). Tracer infusion was maintained throughout the experiment by a Harvard Apparatus 22 infusion pump (South Natick, MA). A wrist vein was cannulated in a retrograde manner with a 20-gauge polyethylene catheter and maintained at ~65°C. Three milliliters of arterialized blood were drawn 60, 240, 260, 280 and 300 min after the infusion began. The blood was immediately placed into tubes containing 3 ml of 15% sulfosalicylic acid for precipitation of proteins, shaken vigorously, and centrifuged. Deproteinized plasma from these samples was stored at -20°C until processing and analysis for Phe enrichment.

Two muscle biopsies were taken from the subject's posterior deltoid muscle under sterile conditions with a 4-mm Bergström needle. Approximately 10-40 mg of muscle tissue were sampled with each biopsy. The first biopsy was taken after 1 h of infusion at isotopic equilibrium (2). The second biopsy was taken at the end of the infusion period (~300 min) to measure the incorporation of tracer into muscle protein. Blood, visible fat, and connective tissue were quickly removed from the sample, and the tissue was immediately frozen in liquid nitrogen and stored at ~70°C for later processing and analysis.

Analysis. Phe was partially purified from plasma samples by cation-exchange chromatography and prepared as described previously (2, 28). The t-butyldimethylsilyl derivative of Phe was prepared for each dried amino acid sample. Isotopic enrichment of Phe in the derivatized samples was determined by gas chromatography-mass spectrometry (GC-MS), A Hewlett-Packard (HP) model 5971A fitted with an HP 5890-II gas chromatograph and using a 30-m fused-silica capillary column (Supelco, Belafonte, PA), electron impact ionization, and selected-ion monitoring at m/z 234 and 240 was utilized for GC-MS analysis. Enrichment data are expressed as the tracer-to-tracee ratio (t/T). The t/T is essentially equal to the isotopic abundance of the sample minus the isotopic abundance of the background and is analogous to the specific activity term used in radioactive tracer studies (27, 28).

Muscle biopsy tissue samples were analyzed for protein-bound Phe enrichment as described previously (2, 28). Briefly, each sample was weighed, and the muscle protein was precipitated with 0.5 ml of 10% trichloracetic acid. The tissue was then homogenized and centrifuged. The supernatant was collected, and this procedure was repeated twice more. The remaining pellet of muscle tissue was washed in double-distilled H2O and three times in absolute ethanol and then placed in a 50°C oven overnight. The oven-dried pellet was placed in 6 N HCl and hydrolyzed for 24 h at 110°C. The protein hydrolysate was then passed through columns of acid-washed celite to remove carbon particles. The purified samples were dried with a speed vacuum. Phe was isolated from the amino acid mixture and purified by high-performance liquid chromatography (Pharmacia LKB Biotechnology, Uppsala, Sweden) as described previously (2). The samples containing pure Phe were combusted at 700°C in a vacuum with a carbon-nitrogen analyzer (Nitrogen Analyzer 1500, Carlo Erba, Serono, Italy). The resulting CO2 gas was automatically injected into an isotope-ratio mass spectrometer (IRMS; VG Isogas, VG Instruments, Middlewich UK) for determination of the 13C-to-12C isotope ratio (13C/12C) in protein-bound Phe. Each sample was checked against a reference gas (2).

Calculations. The fractional synthetic rate (FSR) of muscle protein was calculated according to the equation: FSR (in %) = [(E₁₁ - Eₜₜ) × 1.5(Eₜₜ/E₀)] 100, where E₀ is the enrichment (13C/12C) in the protein-bound Phe tracer from the first biopsy at t₀ (isotopic equilibrium at 1 h), E₁₁ is the enrichment (13C/12C) of the protein-bound Phe tracer from the second biopsy at t₁ (at 5 h), t is the incorporation time (t₀ – t₀), and FSR is the average plasma Phe enrichment during the time period for determination of protein incorporation (average from the blood samples taken at isotopic equilibrium and over the last hour of infusion). The numerator is multiplied by 1.5 to normalize the IRMS with the enrichment of the precursor determined by GC-MS. Only six of nine carbons in the Phe tracer are enriched so a t/T of 0.01 determined by GC-MS would yield a ratio of 0.0067 if measured by IRMS. E₀ is the t/T skew corrected (multiplied) by a factor of 0.9376 to account for the different isotopomer distributions of the tracer and the naturally occurring Phe (28).

Phe was chosen as the tracer because it is not oxidized in muscle or synthesized in the body. Therefore, the new appearance of Phe results entirely from protein breakdown (PB). Whole body Phe appearance was calculated according to the equation: Rₜ = F (in µmol·kg⁻¹·h⁻¹)E₀, where Rₜ is the rate of appearance of Phe and F is the infusion rate of ring-[13C₆]Phe.

Statistical analysis. This study was designed as a randomized block design, where each “block” is a subject and the four treatments are RW, SW, SR, and rest. The average values of FSR and whole body Phe Rₜ were compared by one-way
repeated-measures analyses of variance with SigmaStat software (Jandel Scientific Software, San Rafael, CA). Any significant differences were then tested with a Student-Newman-Keuls multiple comparison post hoc test. Statistical significance was set at the $P < 0.05$ level. Values are means ± SE.

RESULTS

Isotopic steady state was reached after 1 h of infusion. An enrichment vs. time curve for each treatment for a representative subject is shown in Fig. 1.

Figure 2 illustrates the whole body Phe $R_a$ (in $\mu$mol·min$^{-1}$·kg$^{-1}$) calculated over the 60-min period from 240–300 min after the beginning of the tracer infusion. Whole body Phe $R_a$ is representative of WBPB. WBPB values were similar after each of the workouts and at rest.

The FSR in the posterior deltoid at rest and after each of the workouts is illustrated in Fig. 3. The SR workout significantly increased the FSR by 81% over resting levels ($P < 0.05$). Although the mean FSR values after SW were 35 and 41% greater than those after RW and at rest, respectively, there were no significant differences between the means. FSR after SR was 30% greater than after SW and 73% greater than after RW, but the differences did not reach statistical significance.

DISCUSSION

The purpose of this study was to examine the acute response of muscle PS in female swimmers after exercise by measuring the incorporation of labeled Phe (FSR). Although there was no significant effect of either swimming alone or resistance exercise alone on FSR, the combination of swimming and resistance exercise significantly stimulated the FSR above the resting value. There were no increases over rest in WBPB 3 h after any of the workouts. Thus it appears that the combination of swimming and resistance exercise workout provided a greater stimulatory effect on PS than either swimming or resistance exercise alone.

Before the present study, muscle PS after the combination of endurance and resistance exercise had not been examined in human subjects. However, several studies have reported the response of muscle PS to resistance exercise or endurance exercise alone. Previously, Biolo et al. (3) found that muscle PS in the vastus lateralis 4 h postexercise was increased by 133% in untrained men. Chesley et al. (5) reported a 50% increase in the FSR in the biceps immediately after weight lifting in trained men. Furthermore, Carraro et al. (4) reported a qualitatively similar significant increase in FSR after the completion of 4 h of treadmill walking at 40% of the maximal $O_2$ uptake in six untrained men. In the present study, FSR was increased by 80% after the combination of swimming plus resistance exercise.

There are several possible explanations for the lack of a significant effect of either resistance exercise or swimming on muscle FSR in the present study. Gender differences in the response of protein metabolism may have contributed to the apparent discrepancies between the present results and the previously published papers (3–5). Phillips et al. (19) showed that the leucine oxidation during endurance exercise was different in trained men compared with trained women, although there was no difference in nonoxidative leucine dis...
posal, an indicator of whole body PS. Yarasheski et al. (29) reported that FSR increased over rest in each of four young untrained women immediately after a resistance exercise bout, but no statistics were reported separately on the female and male subjects. Interpretation is further complicated by the fact that the resting measurements were made before 2 wk of resistance training. Ours is the first study to report the acute response of muscle FSR to exercise specifically in trained women. Accordingly, there are no data available on the comparison of the muscle protein synthetic response after either endurance or resistance exercise in trained men and women.

Alternatively, the lack of an increase in FSR with either resistance or endurance exercise over resting levels may be due to the muscles sampled. Previous studies measured incorporation of amino acids in the vastus lateralis (3, 4) and biceps brachii (5), whereas we studied the posterior deltoid. It is possible that the response of the FSR to resistance exercise differs according to muscle fiber type composition. PS and PB respond differently in fast-twitch and slow-twitch muscles (11, 26). Even though the deltoid of trained swimmers has been shown to vary between 62 and 75% slow-twitch fibers (7, 12, 16), the vastus lateralis in untrained men has a relatively even fiber type distribution (22), and the biceps in trained male weight lifters seems to be mostly (60%) fast-twitch fibers (1).

In the present study, the FSR was not increased by resistance exercise. This finding is in contrast to previous studies that found that the FSR increases on the order of 100% in both trained male weight lifters (5) and untrained male volunteers (3). The work performed by the selected muscle in the previous studies may have been greater than that performed by the posterior deltoid of the swimmers in the present study. Using the biceps muscles, the trained subjects in the study by Chesley et al. (5) performed 12 sets of 6–12 repetitions at 80% of 1 RM. Similarly, Biolo et al. (3) sampled the vastus lateralis of untrained subjects after the subjects performed 13 sets of 8–10 repetitions at 10–12 RM. The posterior deltoid of the swimmers in the present study was involved in only 9 sets of 6–10 repetitions at 65–80% of 1 RM. Although the subjects in the previous studies participated only in exercises targeting the muscles to be biopsied, the swimmers in the present study participated in 24 sets of other exercises in which the deltoid was not involved. The work performed by the deltoid during SR was greater than that during either RW or SW, and this may explain why there was a significant increase in FSR after SR.

Postexercise blood flow and amino acid delivery to the muscle have been positively related to muscle PS (3). It is possible that, compared with the exercising muscles in the other studies (3, 5), blood flow to the deltoid was not as high in the present study due to the lower intensity of the resistance exercise and/or the inclusion of exercises involving other muscle groups. It is likely that blood flow was diverted to other exercising muscles and the flow to the deltoid was reduced. Although blood flow could not be measured in the present study, the fact that the RW FSR was similar to resting levels is consistent with a lower level of stress on the deltoid of the swimmers. Even though a more intense weight-lifting routine might have elicited a greater response in the FSR, the aim of the study was to determine the response to the workouts that swimmers typically perform.

In the present study, there were no differences in whole body $R_\alpha$ of Phe 3 h after any of the exercise routines or at rest. Using a variety of methods, others have shown increased (3, 10, 21) and unchanged (4, 9, 25) WBPB after exercise. Although WBPB did not increase due to any of the workouts, as measured with Phe $R_\alpha$, it is reasonable to assume that muscle PB did increase. Muscle PB measured directly (3) and by 3-methylhistidine release (10) has been reported to increase after exercise, but WBPB may not match muscle PB (3). The failure of Phe $R_\alpha$ to increase could reflect either a concomitant increase in muscle PB and decrease in PB from other tissues or simply the insensitivity of the measurement of whole body $R_\alpha$. Previously, Biolo et al. (3) showed that increased muscle PS after exercise is accompanied by a 50% increase in muscle PB, whereas WBPB was only slightly increased.

During exercise, the majority of any increase in WBPB that might occur comes from the splanchnic bed (27). Because there is no evidence that different types of exercise would cause variable responses in splanchnic or muscle PB, it is reasonable to assume that muscle PB was not different among the different workouts in the present study. The combination of swimming and resistance exercise was the only workout that resulted in a significant increase in FSR, and there were no differences in WBPB among any of the workouts. Therefore, the combination workout provided the greatest increase in net muscle PS. It is possible that the stimulatory effect of the combination workout on the FSR was primarily due to the increased work performed during this exercise bout. It is possible that simply adding additional swimming would have increased the FSR to the same extent as adding the resistance exercise to the swimming. However, the purpose of this study was to investigate the practical implications of adding resistance exercise to swimming workouts in trained swimmers. Given the limitations of the study, we can conclude that adding a resistance exercise bout to a swimming workout increases muscle PS in trained female swimmers.

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