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13. ABSTRACT *(Maximum 200*

During this second year we have made significant progress in two areas: 1) We have implemented the study and data analysis protocols. Currently we can acquire and analyze data in both the MRI and the MRS areas of the project. 2) We have completed the majority of studies in the MRI portion of the project, and we have completed a significant number of MRS studies. From the studies that we have completed we can draw some preliminary conclusions. A) Women are capable of completing the task, although men finish about twice as often. B) MRI is capable of detecting gender differences in our lift & carry task, and it directs us to the muscles that are best studied with MRS. C) Liver glycogen is not significantly depleted by the exercise protocol in either gender. D) Quadriceps muscle glycogen is minimally depleted in both genders suggesting that these large muscles are not heavily recruited by our exercise protocol, perhaps because the work is distributed among 25-35 different muscles. This is in agreement with our MRI data. Size may be a better indicator of the ability to complete this task than gender.

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H. B. Poe 12/10/98

PI - Signature Date

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INTRODUCTION

The second year of this project has been focused in two major areas: A. Getting the study protocols set up and running successfully, and B. Making significant progress on the two primary studies that were originally funded (the MRI study and the MRS study). In our first major area we had four goals: 1) Validation of the exercise protocol and its suitability for male and female subjects [see Year 1 report (1)], 2) Implementation of a reliable subject recruitment and screening protocol, 3) Completion of differential muscle recruitment experiments and preparation of a manuscript for publication (see Year 1), and 4) Completion of acquisition and implementation of the hardware necessary for expired gas analysis and MRI data processing. In our second major area we had two goals: 5) Completion of total body MRI experiments (see Year 1) and identification of the muscles that are most consistently recruited in male & female subjects, and 6) Implementation of MRS studies of liver & muscle carbohydrate metabolism associated with our exercise protocol such that we have acquired a significant number of male & female data points.

In our first area of focus (goal 1) we have completed twenty studies in both male and female subjects (14 different subjects). The intention of the exercise protocol was that it be easy enough that at least some of the female subjects would be able to complete the entire task, and that it would be difficult enough that not all male subjects would be able to complete the entire task (i.e.- a significant challenge to both sexes) (see Year 1). This has been successfully accomplished (Figure 1), demonstrating completion of the entire task (3hr of exercise over a 6hr period, or 540 total lifts) by 44% of female subjects and 91% of male subjects. A further goal in this area was that, if female subjects had to work harder to accomplish the task, the difference be measurable. This has also been accomplished demonstrating significantly higher percent of maximum work capacity (Figure 2) in female subjects as compared to male subjects (76±5%, F; 59±2%, M; $p \leq 0.01$). This goal of the project has been accomplished.

We have experienced some success on the second goal; however, recruitment of a large number of subjects has been difficult. Of the subjects interviewed, 46% qualify and are successfully recruited into the study, and 37% actually complete at least one study (i.e.- we lose ~9% of qualified subjects after they are enrolled in the study). The remainder of potential subjects that are interviewed (54%) either decline or fail to qualify. Another area of concern is the racial/ethnic cross section, which is currently almost entirely Caucasian. We feel that we have not been entirely successful in this area, and we are continuing to explore alternatives to increase our subject volume and improve our ethnic representation. The third goal was not funded in this contract; however, it is an essential preamble to the MRI data that will be reported from this contract (see Year 1). Data collection for this study has been completed by both our Yale group (12 studies) and the group at the University of Miami (12 studies), and we are currently writing the manuscript. Because this area is not funded in this contract, we will not give final data in this report. However, based upon our Year 1 report, it is necessary to mention this work in order to complete the discussion.

The fourth goal, purchase of support equipment and implementation protocols, has been completed this year. During the year we purchased an expired gas analyzer (SensorMedics EGA model Vmax 29), two data processing computers (Dell), and software necessary for data analysis and subject classification. The EGA is online and currently in use in the project. The data processing computers are currently being used to process the volume of MRI data and to assess subject exercise & nutritional status. This area of focus has been successfully completed.

The first goal of second major area of focus (goal 5) this past year has been an attempt to complete the MRI portion of the project. In this area we have successfully completed 11 of 12 proposed experiments; however, data analysis is lagging behind. Owing to the large volume of data collected per subject (see Year 1), analysis has proven to be much more time intensive than originally predicted. While we are close to completing the data acquisition portion of this part of the project, it will require more time to complete the analysis. The total data analysis requires some 35-70 hours per subject; however, we have been able to complete some partial analysis on 7 of the 11 subjects who have completed the study. From this, we have seen statistically significant differences between male and female muscle recruitment patterns, which have led to some preliminary conclusions about how men and women perform this exercise protocol. This area of the project has not been fully accomplished; however, the experimental portion will be complete within the next two months and the data analysis will continue into the summer of 1999. We feel that this area of focus, while not complete, has been largely accomplished and is therefore a success.

The final goal (goal 6) during this past year has been to implement the carbohydrate metabolism (MRS) studies that will embody the bulk of the remainder of this project. Our goal, to complete a significant number of male and female studies, has been accomplished with nine MRS studies (5 M, 4 F mid-luteal) complete. Because the data processing is not as volumous in these studies, it is largely complete for all nine studies. We have been able to combine data acquisitions for the most consistently recruited muscles (Vastus Lateralis, Rectus Femoris, and Vastus Intermedius) and the liver by using an interleaved protocol. This streamlining of the original proposal has enabled us to be a little ahead of the original statement of work (SOW) in this area of the project, and preliminary data assessments suggest that some differences between male and female subjects may be ultimately documented. Blood samples, obtained during many of these studies, have not yet been analyzed; however, we have demonstrated that the sample collection protocol works well and does not hinder most subjects from performing the exercise protocol. This part of the project will continue throughout the remaining two years.

This project has successfully moved forward in all areas of focus during the past year. The technical development that was completed in Year 1 has supported the uniform success during Year 2 of this contract. While MRI data analysis is incomplete and the number of MRS studies is not great, early results suggest some preliminary conclusions that are likely to hold true when the project is ultimately completed.

BODY OF REPORT

A. Experimental methods, assumptions, and procedures:

1. Validation of the exercise protocol and its suitability for both male and female subjects - The exercise protocol, described in detail in last year's annual report (see Year 1), was performed in both male and female subjects. In order to evaluate the protocol and determine whether female subjects could complete the task, we first studied a highly trained female subject of average height and weight (27yr, 5ft6in, 157lbs). The

subject completed the protocol with only moderate effort (heart rate $\leq$100 beats per minute throughout the study). All subsequent studies have incorporated the same exercise protocol as employed in this first female study. We have noticed some bruising on the thighs and arms of some subjects, particularly female, and have increased the padding on the corners of the exercise box so as to minimize this. One female subject declined to return for a second study citing bruises on her upper legs as the reason.

2. Implementation of a reliable subject recruitment and screening process - In order to optimize our subject recruitment efficiency a "Subject Recruitment Conversation Checklist" was created (see Appendix I). We use this "checklist" as a "designed conversation" created so that the person who is interviewing a prospective new subject has a guide to follow. It is not intended that subjects see the "checklist"; hence, we have not sought HIC approval of this document. It is simply intended to have all subject recruitment conversations follow a uniform order and to insure that no part of the conversation is forgotten by the interviewer. Kevin Cleary, a personal trainer, was hired as a subject recruiter. Because Mr. Cleary also administers the physical test for qualification into the Connecticut Police Academy, we have been able to draw from a pool of subjects that is well trained and qualify for our study. In addition to Mr. Cleary, we have recently hired a student, Michael Landi, who has improved our subject retention. It is our opinion that subject recruitment and retention is currently the most important component required to insure the ultimate success of this project; hence, this will be a continuing area of focus.

3. Completion of differential muscle recruitment experiments - This study is not discussed in this section.

4. Purchase and implementation of support equipment and protocols - The intention of this project is to gather information that may be directly related to an active military population. We purchased an expired gas analyzer (SensorMedics EGA model Vmax 29), in order to rigorously assess the level of fitness of our civilian male and female subject pool. Prior to participation in the study, subject maximum oxygen uptake volumes (VO_{2MAX}) were measured using a modified Balke exercise testing protocol (see Appendix II). Tests were performed on a treadmill (Max 1 exercise testing system, Marquette Electronics) that articulated with our Vmax 29 EGA. VO_{2MAX} values are not

significantly different between the group of male and female subjects that have been tested to date (61.5±1.5ml/kg-min M, 55.7±2.3ml/kg-min F, n=6). Tracking %VO_{2MAX} during the exercise protocol has proven difficult to date and we are currently evaluating our exercise protocol to determine whether we can include this measurement. However, subject maximum heart rates are also tracked on the Vmax 29. We have been able to track subject heart rates (beats per minute, bpm) during the exercise protocol with a heart rate monitoring system (Polar) that is strapped onto the subject. For subjects who have not yet performed a VO_{2MAX} test, maximum heart rate was estimated from the equation (maximum heart rate = age - 20). All % maximum work capacity data are calculated from heart rate measurements.

The Windows based PC's have been used primarily for MRI data processing; however, subject classification and nutritional data are also processed on these computers. A nutritional analysis & fitness software package (The Food Processor, ESHA Research, Salem, OR) has been purchased to compile and process the daily eating and exercise data that each subject records during the month prior to the study. Nutritional and exercise status, as well as basic subject data, is reported in Appendix III.

5. Completion of total body MRI and identification of the muscles that are working - Six male and five female subjects have completed the exercise protocol that we have previously described (see Year 1). MRI scans were obtained using a General Electric Signa 1.5 Tesla instrument (version 4.8), equipped with resonant gradients for echoplanar imaging (Instascan, Advanced NMR, Wilmington,MA). The MRI protocol previously described in our Year 1 annual report was altered such that the continuously moving patient bed protocol is no longer employed (2). Instead, subjects are positioned at four stations (shoulders, abdomen, pelvis/thighs, and lower legs) at rest and during the first 10min immediately following cessation of each block of exercise. Thirty slices are collected about each position (NEX=4, TE=30ms,60ms, total scans = 240 per station). All other MRI parameters are the unchanged (see Year 1). The advantage of this MRI protocol is that each station can be set up and shimmed individually, thus giving cleaner data. Image reconstruction and T_2 calculations are performed as described in our Year 1 annual report. All T_2 calculations are currently performed in Matlab (The MathWorks, Inc., Natick, MA) using a software program that was developed "in house" specifically

for the processing requirements of this project and designed to enable a more thorough review and evaluation of MR imaging data. The program, which was developed to facilitate selection of regions of interest (ROI's) within muscles for determination of their T_2 values and, therefore, their activation status, is able to run on a Windows-based PC platform. This software takes as its input an image file containing a series of image slice pairs, where each pair consists of images reconstructed from raw MRI data acquired with a long (60msec) and short (30msec) echo time (TE). The reconstruction of these images has only been changed to account for changes in the data acquisition strategy that was adopted to improve the quality of the acquired images and eliminate problems due to subject motion during the imaging sessions. The new software has also eliminated the computation of pixel-wise T_2 maps. T_2 values are now computed directly by our ROI sampling software such that an average pixel intensity is computed for the selected ROI in each image of an image slice pair, and these average values are used to compute a single T_2 value for the ROI. This method produces more robust T_2 values than the T_2 map method previously used (see Year 1). The ROI program computes both a raw T_2 value and a corrected T_2 value that accounts for the decay of T_2 value changes over the period of time immediately following the exercise period. The T_2 correction calculations are implemented with the following equations (3):

$$T_2 = 1/R_2 \qquad (1)$$

Where: $R_2 = 1/(TE_1 - TE_2) * \ln(S_1/S_2)$

Where: TE_1 and TE_2 = the two echo times

and: S_1 and S_2 = the average per pixel signal intensities in the ROI of the image pair slice.

And:

$$T_{2CORR} = T_2 / [1 - \text{correction factor} * (\text{acquisition delay} - 1) * T_2] \qquad (2)$$

Where: correction factor = 0.42msec/min (T_2 decay with time after 1min post-ex)

and: acquisition delay = [(the time after cessation of exercise) - 1min]

A complete data set (i.e.- the subject completes the exercise protocol) consists of 13 blocks of data (1 at rest and 1 after each of the 12 15min bouts of exercise, 3 lifts per min = 45 lifts per bout). Each block of data contains four components that correspond to each of the 4 stations imaged. Four transients and two echo's per slice for

30 slices per station at each of 4 stations yields 960 images per block for 13 blocks, or 12,480 images per study (~1 GB). Data are condensed by averaging the 4 transients together yielding 60 images per station (2 echo's per slice) or 120 total slices to be assayed per block of exercise (~200MB of data per study). For each block of data, processing is done slice-by-slice for 50 different muscles (25 each, right and left side of body). Each slice has 3-10 different muscles to assess for T_2, and regions of interest (ROI's) must be selected so as to avoid vessels, fascia, and MRI artifacts (4). In order to accumulate a statistically significant number of ROI's for each muscle in a given block of data, ~10 slices per muscle are assessed. This translates to 500 individual muscle slices per block of data, or 6500 per complete study. Figuring 20-40sec per individual muscle slice, total MRI data processing will likely require 36-72 manhours per subject, or 432-864 total manhours. It is anticipated, however, that some of the data processing time may be eliminated by reducing the number of individual muscle slices analyzed in obviously non-recruited and heavily recruited muscles.

In each muscle, the significance of the change in T_2 value at each time point during the exercise session is tested (t-tests) by comparison of the mean T_2 time obtained for n slices of a given muscle at rest (before beginning the exercise protocol) with the uncorrected mean T_2 time (raw T_2) of n slices of the same muscle obtained at time t after cessation a specific block of exercise. By employing this method of testing for significance, we ran the risk of missing a small increase in T_2 value. However, if the correction factor had been applied before a test of significance was performed, we would have risked introducing an erroneous significant exercise induced T_2 increase. Exercise induced T_2 increases are transient and decay over the initial time period following cessation of exercise (3,4). In order to optimize the chances for identifying real T_2 increases, we positioned subjects and began MR scans as quickly as possible following each bout of exercise. In addition, so that any one MRI station would not consistently be imaged following the longest period of time after cessation of exercise, we alternated the order of MRI stations for which we began acquiring images. On average, all images were completed within 10min of cessation of exercise. By acquiring MRI data in this way, we have obtained consistent T_2 data over the MRI studies that have been completed and analyzed thus far.

6. Implementation of MRS studies of muscle and liver carbohydrate metabolism - This goal of the project will constitute the bulk of our research during the remainder of this project. We have been able to combine muscle and liver measurements because, according to our MRI data, the most consistently recruited muscle group in both male and female subjects if the quadriceps group. Because we measure both the liver and the quadriceps with the same radiofrequency (RF) coil, we are able to alternate MR spectral acquisitions between the two during the course of the exercise protocol. While this technique does not give the same time resolution as was proposed in the original protocol, the time resolution is sufficient to measure muscle and liver glycogen depletion patterns and track potential differences between groups; hence, doubling the number of studies to obtain better time resolution would not be an efficient use of time and resources.

The exercise protocol is the same as was used in the MRI study (see Year 1), with the only difference being what we are measuring. On the day of the study subjects are allowed to eat immediately after they rise (liquid meal) and no further meals are allowed until the exercise session is complete. Upon arriving at the Yale Medical Complex, subjects have a Teflon catheter inserted into the antecubital vein for blood sampling. Before beginning exercise, baseline MR spectra are obtained from the liver and quadriceps muscle group (rectus femoris, vastus lateralis, and vastus intermedius), and immediately before exercising, baseline blood samples are drawn. Subjects then exercise in 15min blocks (see Year 1) and MR spectra are obtained after each exercise block (alternating liver and quadriceps). Blood samples are drawn every other time subjects complete a block of exercise (every 30min of exercise). On the morning following the exercise study some subjects returned for a final MR spectrum of the quadriceps so that recovery could be assessed.

MR spectra were obtained on a 2.1 tesla 1m bore NMR-spectrometer (Bruker Biospec, Billerica, MA), and both ^{13}C and ^{31}P MR spectra were obtained in 10min blocks. ^{13}C muscle spectra consisted of 3200 proton decoupled transients using a 90° pulse at 1 coil radius away from the 9cm circular radiofrequency (RF) coil and a repetition time of 120msec. Proton decoupling was accomplished with an 11cm X 11cm series butterfly RF coil. For liver glycogen measurements a modified ISIS protocol was employed to optimize fat suppression. ^{31}P muscle spectra consisted of 192 transients. Subjects were positioned with an image-guided localization MRI protocol (Gradient Echo).

Glycogen (GLY) concentrations were determined by comparison with an external standard solution (150mM glycogen + 50mM KCl) that loaded the RF coil the same as the subject (5,6). ^{13}C spectra were processed by methods that have been described in detail in several of our earlier studies (5-7). Briefly, gaussian broadened spectra (30Hz) were baseline corrected ±300Hz on either side of the 1-^{13}C glycogen resonance of both subject spectra and sample spectra. Areas were then assessed ±100Hz about the resonance and compared.

Concentrations of muscle inorganic phosphate (Pi) and creatine phosphate (PCr) were also calculated by comparison with β-ATP (7). Values of pH were calculated according to the chemical shift difference between the Pi peak and the PCr peak using the equation:

$$pH = 6.77 + \log [(\Delta\delta - 3.29) / (5.68 - \Delta\delta)]$$

where: $\Delta\delta$ = the chemical shift difference between Pi and PCr

Corrections were made when exercise resulted in swelling of the muscle that could alter the NMR sensitive volume and make the NMR peaks appear smaller. In the processing mode of the spectrometer the post-exercise ^{31}P total spectral intensity was corrected to equal the resting spectrum. When the total spectrum area correction factor was applied there were no significant differences in the β-ATP resonances following exercise. Time domain data were apodized and zero-filled using a 10-Hz exponential function. Intramuscular G6P was quantified by comparison with the β-ATP resonance as an internal reference standard (7). A constant concentration of 5.5mM was assumed for resting muscle ATP (8). This quantification of muscle G6P differs from the method which has been described by Rothman, et al. (9) in that the chemical shift of G6P was determined relative to Pi rather than PCr. Pan, et al. have shown that over the pH range of 6.60-7.05 exercise induced changes in the chemical shift of Pi paralleled those of G6P (10). We measured the chemical shifts of the major constituents of the phosphomonoester

(PME) region at pH 6.60-7.05 relative to Pi at 0.00 ppm and found them to remain constant [G6P, 2.29 ppm; α-glycerol phosphate (α-GP), 2.04 ppm; inosine monophosphate (IMP), 1.61 ppm]. The basal G6P concentration was determined by integrating over the chemical shift range of 2.61-2.29 ppm and multiplying the area by 2 in order to minimize any contribution from upfield (lower ppm) PME resonances (9). When difference spectra were obtained by subtracting resting spectra from spectra obtained after exercise, the increase in G6P following exercise was cleanly resolved at 2.29 ppm . Measurement of G6P by ^{31}P NMR has been validated in an animal model by comparison with chemical assay of G6P done on rat muscle frozen *in situ* (11).

Venous blood samples were assayed for glucose, lactate, insulin, epinephrine, norepinephrine, free fatty acids (FFA) and glucagon. Glucose and lactate were assayed by enzymatic methods (12,13). FFA's were assayed by a microfluorometric method (14). Glucagon, insulin, epinephrine and norepinephrine were determined by radioimmunoassay methods (15).

B. Results:

1. Validation of the exercise protocol – These results have been described in some detail in previous sections. Briefly, both male and female subjects are challenged by the exercise protocol, and 91% of male subjects complete 3hr of exercise during the 6hr period, while 44% of female subjects complete the task. The ability to complete the protocol was correlated with gender, height, and weight. We observed a significant correlation with all three; however, the correlation with gender was least significant of the three. The P values (two-tailed, for 19 pairs) were 1) 0.0374 for gender versus total exercise time, 2) 0.0191 for height versus total exercise time, and 3) 0.0030 for weight versus total exercise time. Because women are generally smaller than men gender correlates with the ability to complete our exercise protocol. However, as data are gathered from more subjects it is possible that we will find that the best predictor of whether a given subject will complete this task is his or her size. We reported in a previous section that females worked at a significantly greater ($p \leq 0.0001$) % of their maximum work capacity (76±5%) than males (59±2%) (Figure 2). However, when the

data are grouped as subjects who completed the task versus those who did not, the % of maximum work capacity was similar between male and female subjects who completed (60±2% M vs 63±7% F). Among those who failed to complete, the lone male subject worked at 53% of his maximum capacity; however, the female subjects who failed to complete worked significantly harder (85±4%, $p \leq 0.005$) than their female counterparts who completed the task.

2. Subject recruitment – These results have been presented in previous sections of this report. The information here is given in a little more detail: 1) 59 [39 male (66%), 20 female (34%)] prospective subjects were interviewed, 2) 27 [17 male (44%), 10 female (50%)] have been enrolled into the study (14 have completed at least 1 study, 3 are currently scheduled and are tracking diet & exercise, 2 are enrolled but not yet scheduled, 3 are in the screening process, and 5 have been scheduled and then decided to drop out of the study). This represents 46% overall recruitment efficiency and 37% overall retention efficiency.

3. Differential recruitment study – This result is not reported here.

4. Support equipment – Because the recently purchased support equipment was used to organize subject data, the historical, nutritional, and exercise information are given in this section. This information is presented in its entirety in Appendix IV.

5. Total body muscle recruitment study with MRI – The data from this goal have been partially analyzed. For reasons described earlier in this document, we have been unable to obtain a complete set of processed data; however, enough data has been processed to yield some basic preliminary results. Baseline images and images obtained following the initial block of exercise have been processed for 7 subjects (4 female, 3 male). Data sets from 2 subjects (1 female, 1 male) have been processed completely. From the two completely processed data sets it appears as if muscles recruited during the initial block of exercise continue to be recruited throughout the protocol, and no new muscles become significantly recruited as the exercise sessions near completion. However, with only a single subject of each sex processed, it is too early to draw any conclusions. Of the two subjects completely processed, both completed the task and neither was significantly challenged. The male subject consistently worked at 68% of his maximum work capacity, while the female subject worked at only 44% of her maximum

capacity, the lowest of any subject (both sexes). Hence, as more data are analyzed muscle recruitment pattern changes during the latter stages of the protocol may become detectable.

During the course of each study subjects are asked to report when they can begin to feel specific muscles working. Subjects are not told the regions where they should be feeling the work, we wait until they mention the region, or specific muscle where they are feeling work and record the name of the muscle and the time that it was reported. This is then tracked throughout the 3hr of exercise. Figure 3 is a plot (for men and women) of the number of different specific muscles (or regions) reported at each time point during the exercise session. On average, the female subjects reported feeling a greater number of muscles working earlier in the exercise session than did the male subjects. The total number of muscles reported by female subjects was not significantly different from that of the male subjects (14±2 muscles, M; 21±3 muscles, F). Of the muscles that were reported, 58% of the muscles detected by MRI in the male group as significantly recruited were reported and 58% of those detected in the female group were reported. This suggests that MRI is a more accurate means of detecting muscle recruitment patterns in complicated exercise protocols than asking the subject what muscles he or she feels working.

The results of muscle recruitment during the first block of exercise demonstrate significant differences in muscle recruitment between men and women. Total body MRI reveals that there were a significantly greater number of muscles recruited by female subjects than by male subjects (31±1 muscles F; 19±2 muscles M, $p \leq 0.002$) (Figure 4). The mean degree of significance of all muscles tested is given in Appendix III. The number of significantly recruited muscles is greater in this representation because, in order for a muscle to be considered recruited, the majority of subjects, not all subjects, had to demonstrate significant recruitment of each muscle tested. Figure 5a and 5b demonstrates muscle recruitment patterns in male (5a) and female (5b) subjects. The color is coded to represent the change in T_2 values for the muscles drawn in the anatomical maps. Muscles that are outlined (in red) with no color present indicate that the muscle was tested for significance and determined to be not significantly recruited. The Δ T_2 values presented in this figure have been corrected to account for the time elapsed between cessation of exercise and acquisition of the image. These anatomical maps clearly indicate greater upper body muscle recruitment in the female group. When male versus female ΔT_2 values were compared muscle-by-muscle, significant differences were detected in the right deltoid ($p \leq 0.0458$), the right biceps ($p \leq 0.0104$), and the right trapezius ($p \leq 0.0087$). These preliminary results suggest that when all data are analyzed there will be additional differences in muscle recruitment patterns between the men and women who perform this exercise protocol.

6. Carbohydrate metabolism MRS - This section of the project was successfully initiated during the past year, generating data that supports the MRI portion of the study. ^{13}C MR spectra of the liver and the quadriceps region were acquired over the time course of exercise by 5 male and 4 female (mid-luteal) subjects. In Figure 6 glycogen concentrations are plotted over the 6hr experimental protocol for both genders. While there is a trend toward lower glycogen levels in the female group, Figure 6 suggests that the net liver glycogen depletion rate during the experimental protocol is not significantly different between genders. Liver glycogen depletion rates are given in Figure 7. In Figure 8 quadriceps glycogen concentrations are plotted over three total hours of exercise for the male and female groups. These early data suggest that the male group depletes more quadriceps glycogen than the female group during exercise. However, with such a small number of studies complete thus far, it may be a bit early to draw any conclusions. The total amount of glycogen depleted in both groups, does support the conclusion that the quadriceps muscles are working at a low-intensity workload in both male and female subjects, and that the workload is similar in both genders. This agrees with the exercise induced changes seen with MRI during the same exercise. The muscle glycogen depletion rate looks as if it could be greater in the male group; however, there is a great deal of variability due to such a small n (Figure 9). This result would be significant if it held true over the remainder of the study. Some subjects have returned the following morning to verify recovery of resting quadriceps glycogen levels. In all cases quadriceps glycogen was fully recovered.

Some ^{31}P MR spectra have been obtained and analyzed; however, not enough data is fully analyzed to give a thorough data set in this year's report. The preliminary ^{31}P data suggest that there is a small but insignificant drop in intramuscular quadriceps pH with exercise and small rise in glucose-6-phosphate immediately after exercise (1.4-fold) that could possibly become significant with more studies. Blood samples have not yet been analyzed. The MRS portion of this project will continue throughout the next 2yrs, and female mid-follicular data collection will be starting in Jan. 99. In addition, we are currently assessing biceps glycogen depletion patterns in male and female subjects. This is just getting underway, however, and there are no data available for this report. If the MRI data are a reliable indication, there could be significant gender differences in this muscle.

C. Discussion:

During this last year we have focused our attention on two major areas of this research contract: 1) Getting the remainder if the study protocols set up and running, and 2) Making major progress on the two primary studies that were originally funded. In the first major area we have completed the four goals that were previously described. We have successfully demonstrated that our exercise protocol works and that it is a viable test for comparing male and female test subjects. We are currently examining the possibility of bringing some of the female subjects back for a second MRI study where the mass of the box is lowered so that the women will be working at the same workload as the men. These studies would be expected to produce muscle recruitment patterns in the women that are not significantly different from those found in the men. This information would add support to the viability of MRI technology for assessing unknown muscle recruitment patterns in a variety of different tasks.

The subject screening and recruitment percentages are high (46%) and the subject retention is good (only a 9% loss). However, this may be the result of our recruiting within a select group of potential subjects that are well trained and highly committed to their overall fitness. We would like to increase the overall number of interviews that we are conducting, and we feel that we will be able to accomplish this if we can get the recruitment posters out into a greater number of locations. We are currently tracking where potential subjects heard about our study; however, the data are not available as of this report.

The lower leg muscle recruitment study has been completed and the manuscript is currently being written. This study is a necessary preamble to the MRI study of muscle recruitment during our lifting and carrying exercise because it demonstrates that we can rely of MRI data to give us muscle recruitment patterns. We intentionally altered a muscle recruitment pattern by changing the orientation of the knee, and we were able to see recruitment changes on MR images as well as on the known "gold standard" for tracking muscle recruitment, electromyography (EMG). The results were similar with both techniques, demonstrating that, if EMG data are reliable, MRI data are also reliable. This study, while not funded in the current contract, "opens the door" for MRI data obtained in this project to be reported powerfully in the literature. Finally, the support equipment that we have implemented during this past year allows us to more carefully evaluate each subject's level of fitness before, during, and after his or her participation in an exercise session.

During year 2 of this project we have made major progress on both the MRI and the MRS portions of this contract. The MRI study has demonstrated a reproducible muscle recruitment pattern in both male and female subjects that perform the lift and carry exercise protocol. From these recruitment patterns we have been able to conclude that women must recruit their upper bodies more than men in order to accomplish the task. We have established that in the female group the right biceps, the right deltoid, and the right trapezius produce significantly higher post-exercise T_2 increases when compared with the male group. This increased upper body recruitment in the female group is in agreement with the higher percent of maximum work capacity that is also seen in this group. If more muscles were significantly recruited, it would reasonably follow that the heart would need to pump more blood to the working muscles. We have seen that a large number of muscles are recruited in order to accomplish the task, and that the number of muscles significantly recruited is greater in the female group. We would point out that the failure to produce a significant T_2 increase does not necessarily mean that the muscle is not at all recruited. It is possible that many of the muscles that are not significantly recruited in the male group are working at low intensity. From these MRI studies we have been able to identify the muscles that are consistently and significantly recruited in both genders. Since T_2 increases are thought to reflect increased metabolic activity, the muscles that have been identified with MRI to be most consistently recruited by our exercise protocol have been also studied with MRS.

Our MRS studies have tracked liver and quadriceps glycogen changes during exercise in 5 male and 4 female subjects. We have not seen an increase in liver glycogen depletion in either group. It is possible that, in fact, liver glycogen rates may be slower during this exercise protocol than they would be if the subject were lying at rest. This seems to be true in both groups. It is, however, too early to take this result seriously at this time. When exercising glycogen depletion rates for both male and female subjects in our study were compared with the depletion rates of an age and weight matched group at rest, the net glycogen depletion rates were significantly slower during the lift and carry task. However, these results are not given in this report because the resting subjects had fasted overnight and were studied >11hr after their last meal. The subjects in this study were studied 4-11hr after their last meal, and this difference could have resulted in the different rates that were observed. The possibility that these early liver results might be correct dictates that we recall the subjects that have participated thus far and rescan them at rest during the same time period after they have eaten. We have completed these resting liver measurements on one male subject and found that his resting net liver glycogen depletion rate was greater (-0.18mmol/kg-min during exercise, and -0.32mmol/kg-min at rest); however, more data are required in order for us to draw a conclusion. If real, this reduced net liver glycogen depletion rate with exercise would suggest that all of the muscles recruited by this exercise protocol may be increasing the pool of gluconeogenic precursors in the blood stream thereby generating substrate for increased liver gluconeogenesis (i.e.- more liver glycogen is being synthesized than is being depleted). While the technology exists to track liver gluconeogenesis, the lifting and carrying exercise protocol used in this project are not conducive to those measurements.

Quadriceps glycogen depletion patterns are not significantly different between our two groups, perhaps because of the low number of studies that we have completed to date. The MRI study demonstrates that both men and women recruit these muscles consistently to a similar degree, and the failure to detect metabolic differences between the two groups with MRS appears to support the MRI results. This early data does, however, suggest a trend toward greater glycogen use in males. Another way of looking at this is that, at the light workload placed upon the quadriceps of both male and female subjects, the women are burning fat more efficiently than the men, as suggested in the literature. We currently have no data on the biceps muscles; however, based upon the MRI data, we predict that the biceps muscles present the best chance that we have of being able to detect metabolic gender differences for our lift and carry task. It is also possible that, since all female subjects studied with MRS thus far have been in the mid-luteal phase of their menstrual cycle, we may discover metabolic gender differences when the same subjects repeat the exercise protocol during their mid-follicular phase. The normal hormonal levels in the follicular phase may be more conducive to observing metabolic gender differences with our exercise protocol. Data from phosphorus MRS and blood samples have not yet been fully analyzed, and are not presented in this report. However, the blood data may shed some light on whether or not there is an increase in gluconeogenic precursors with exercise, and the phosphorus data is expected to yield some information about whether or not our exercise protocol induces a significant amount of glucose transport into the recruited muscles.

D. Relationship to Statement of Work (SOW) outlined in the proposal:

Currently this project is almost on track with the original SOW. This is due, in large part the combination of liver and muscle experiments into a single study. We have not completed the number of studies that we had originally predicted; however, the data has been very good. The small variability of our data suggests that we may be able to draw significant conclusions from a smaller number of studies. If this holds true, we should be able to complete the project in accordance to our original SOW, should we choose to. It is also possible that our early results will lead to some alteration of the original SOW, so as to place our efforts in a more productive direction.

E. Negative results:

The only problem that has been encountered during this past year is a difficulty with our 4.7T small bore MR spectrometer. The system is currently down, and this has necessitated that we collect our phosphorus data on the large bore 2.1T machine that is not equipped to perform simultaneous interleaved carbon/phosphorus acquisitions. This is the reason why we have less phosphorus data, and why the existing data is not as thoroughly analyzed. We are currently acquiring back-to-back spectra on the 2.1T system.

F. Problems in accomplishing tasks:

There are 3 problems that have been persistent over the past year: 1) Suitable subjects have been difficult to find for this study, particularly those of black and Hispanic ethnic origin. Female subjects are also difficult to find. We are addressing this problem by posting advertisements in a greater variety of different locations. 2) It has been difficult to get some subjects to pick up meals and follow their diet during the week prior to the study. This may be due to the distance that some of the subjects drive to participate (70-100 miles) in the study. We are addressing this problem by looking at a way to design the diet so that subjects need only pickup their food one time rather than picking food up each day, as is currently done. 3) There have been some scheduling problems with our General Clinical Research Center (GCRC) in getting blood samples drawn and blood samples processed. In addition, some of the female subjects have had such small veins that we have been unable to insert a venous catheter thus excluding their blood samples from the study. We are currently in negotiation with our GCRC in an effort to reduce scheduling difficulties.

CONCLUSIONS

From this second year of our project we conclude that women recruit their upper bodies more than men in order to accomplish our lift and carry task. Women are capable of completing the task; however, the men complete about 2X as often as the women do. MRI is a viable tool to detect gender differences in muscle recruitment, and it is a viable predictive tool to specify where MRS studies should be focused. Liver glycogen is not significantly depleted by our exercise protocol in either gender. Muscle glycogen depletion in the quadriceps group is minimal in both genders indicating that these large muscles are not working hard, perhaps because the work is distributed among a large number of muscles. Size may matter more than gender in determining whether a subject can complete this protocol and how much metabolic effect the protocol might be expected to have on the subject.

REFERENCES

1. Price,T.B. Year 1 annual report for Contract Number: DAMD17-96-C-6097.
2. Johnson, K.M.R.,et al. *Radiology.* 202: 262-267, 1997.
3. Kennan,R.P., et al. *Mag.Res.Imagimg.* 13(7): 935-941, 1995.
4. Price,T.B., et al. *Med.Sci.Spor.Ex.* 27(10): 1421-1429, 1995.
5. Price,T.B., et al. *J.Appl.Physiol.* 76: 104-111, 1994.
6. Price,T.B., et al. *Proc.Natl.Acad.Sci. USA.* 93: 5329-5334, 1996.
7. Rothman,D.L., et al. *Proc.Natl.Acad.Sci. USA.* 92: 983-987, 1995.
8. Harris,R.C., et al. *Scand.J.Clin.Lab.Invest.* 33: 109-120, 1974.
9. Rothman,D.L., et al. *J.Clin.Invest.* 89: 1069-1075, 1992.
10. Pan,J.W., et al. *Soc.Mag.Res.Abstr.* 8: 541, 1989.
11. Bloch,G., et al. *Mag.Res.Med.* 30: 347-350, 1993.
12. Clark,L.C. *U.S.Patent 3,979,274.* 1980.
13. Kadish,A.H., and J.C.Sternberg. *Diabetes.* 18: 467-470, 1969.
14. Miles,J., et al. *J.Lipid Res.* 24: 96-99, 1983.
15. Starr,J.I. and A.H.Rubenstein. *Methods of Hormone Radioimmunoassay.* (Academic. New York), 289-315, 1974.

FIGURE LEGEND

Figure 1: Completion of the exercise task by male and female subjects.

Figure 2: Percentage of maximum work capacity [maximum heart rate (bpm)] during exercise by male and female subjects. $*p \leq 0.01$

Figure 3: Male and female subjects reporting that they can feel muscles working during performance of the exercise protocol. The number of muscles reported during each block of exercise, over the time course of the protocol.

Figure 4: Total number of muscles identified as significantly recruited by MRI analysis of both genders. $* p \leq 0.002$

Figure 5a: Total body map of the T_2 increase observed in the different muscles of male subjects (n=3).

Figure 5b: Total body map of the T_2 increase observed in the different muscles of female subjects (n=4).

Figure 6: Time course of liver glycogen concentration [mmol/kg] at selected times of the 6hr period of the study in male (n=5) and female, mid-luteal (n=4) subjects.

Figure 7: Net liver glycogen depletion rates [mmol/kg-min] in male and female subjects.

Figure 8: Time course of quadriceps glycogen concentration [mmol/kg] at selected times of the 3hr of exercise during the 6hr study in male (n=5) and female, mid-luteal (n=4) subjects. $* p \leq 0.05$ versus resting concentration.

Figure 9: Net quadriceps glycogen depletion rates [mmol/kg-min] in male and female subjects.

Figure 1:

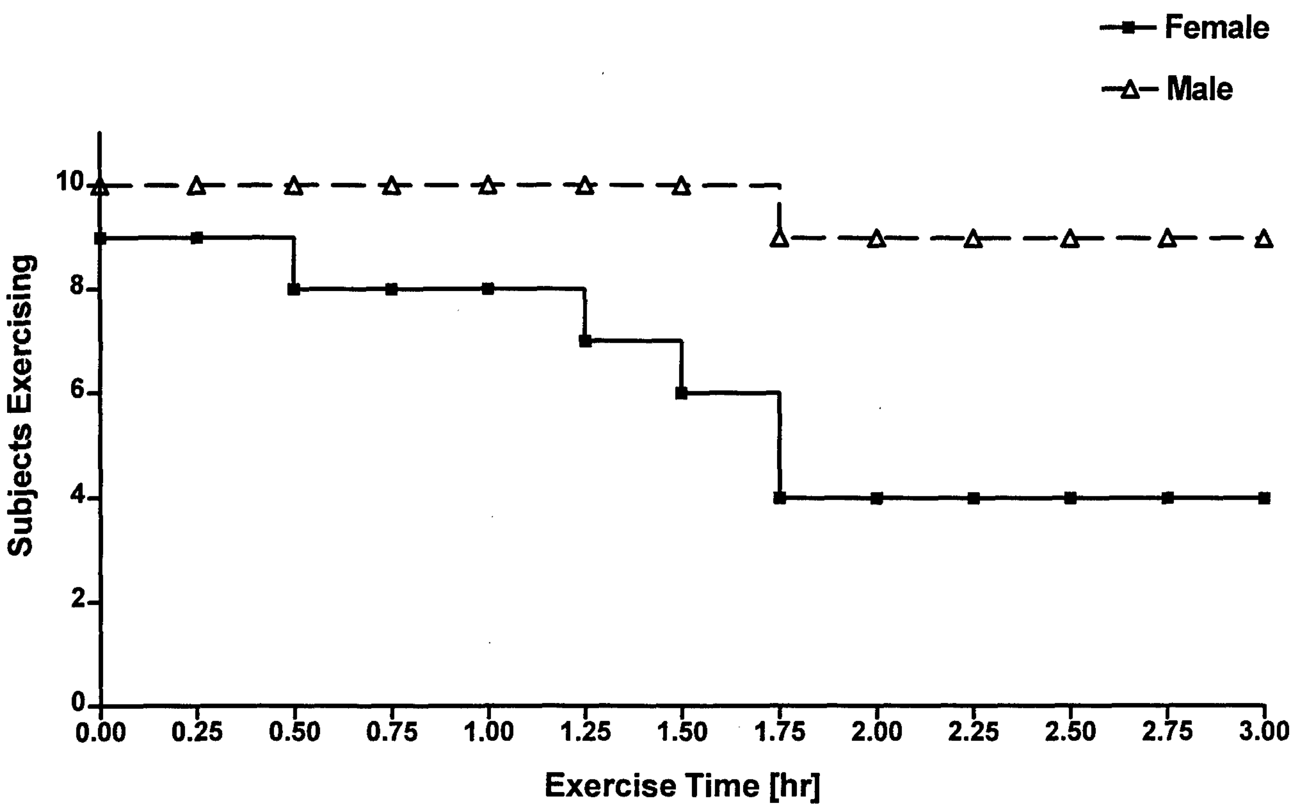

Female
Male
Subjects Exercising
0
2
4
6
8
10
0.00
0.25
0.50
0.75
1.00
1.25
1.50
1.75
2.00
2.25
2.50
2.75
3.00
Exercise Time [hr]

Figure 2:

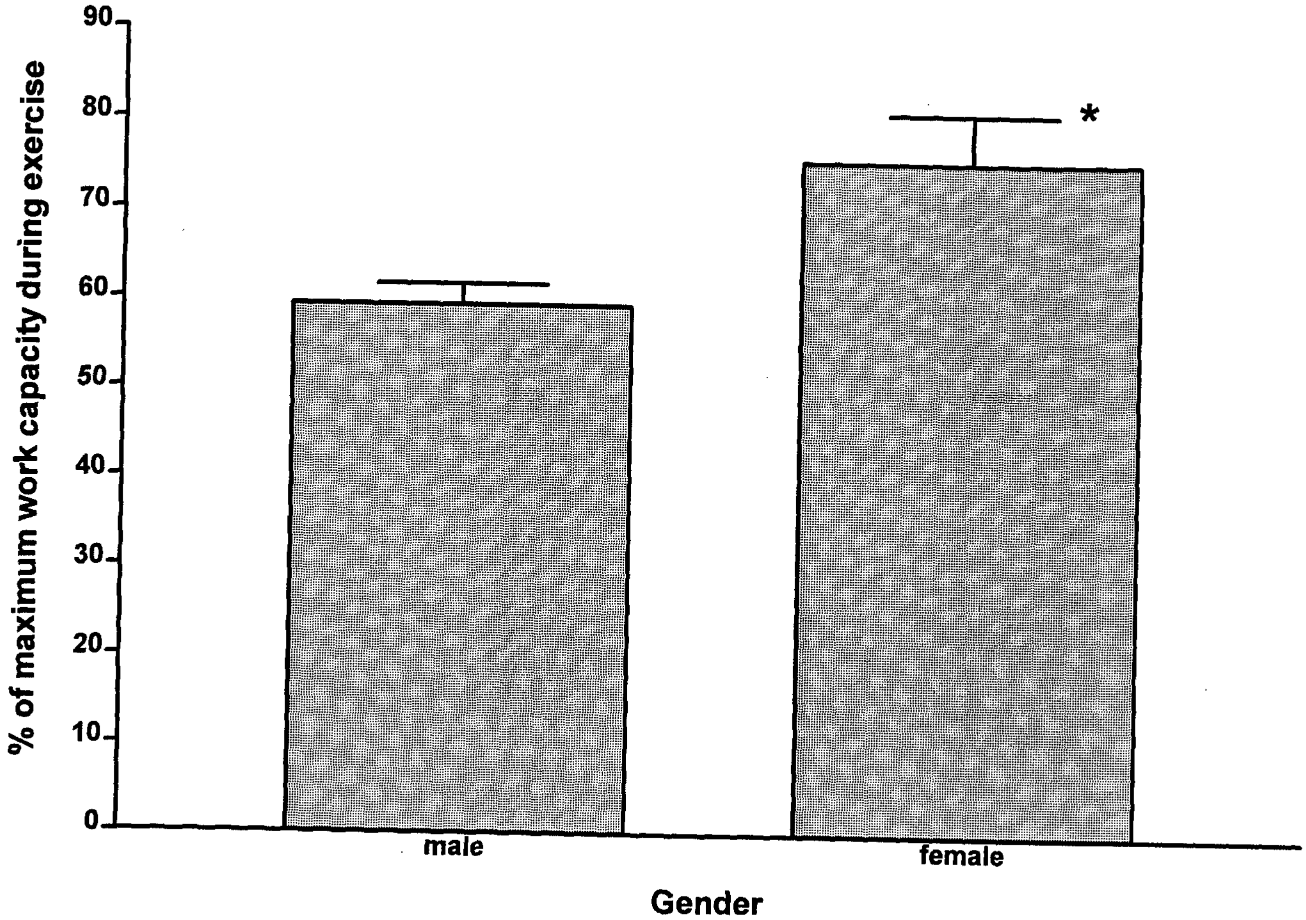

% of maximum work capacity during exercise
90
80
70
60
50
40
30
20
10
0
*
male
female
Gender

Figure 3:

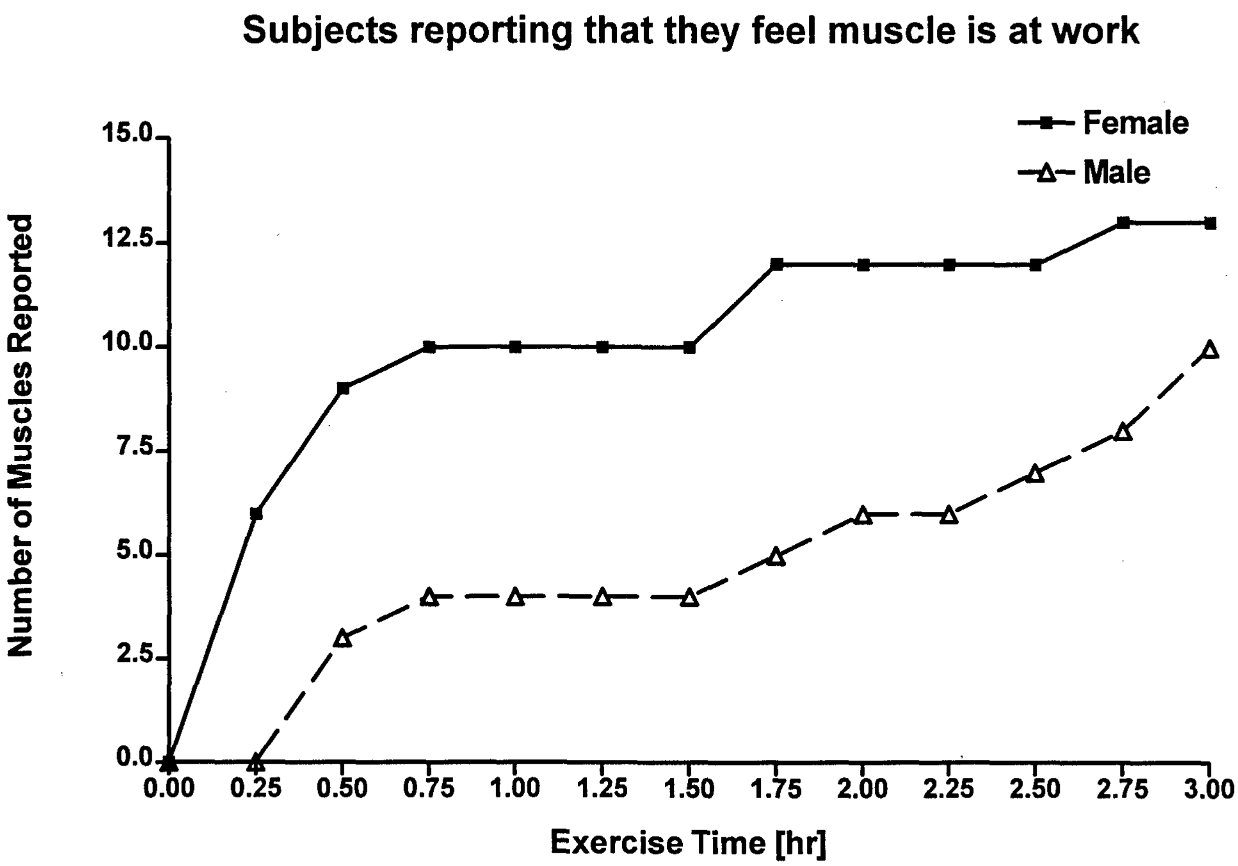

Subjects reporting that they feel muscle is at work
Female
Male
15.0
12.5
10.0
7.5
5.0
2.5
0.0
Number of Muscles Reported
0.00
0.25
0.50
0.75
1.00
1.25
1.50
1.75
2.00
2.25
2.50
2.75
3.00
Exercise Time [hr]

Figure 4:

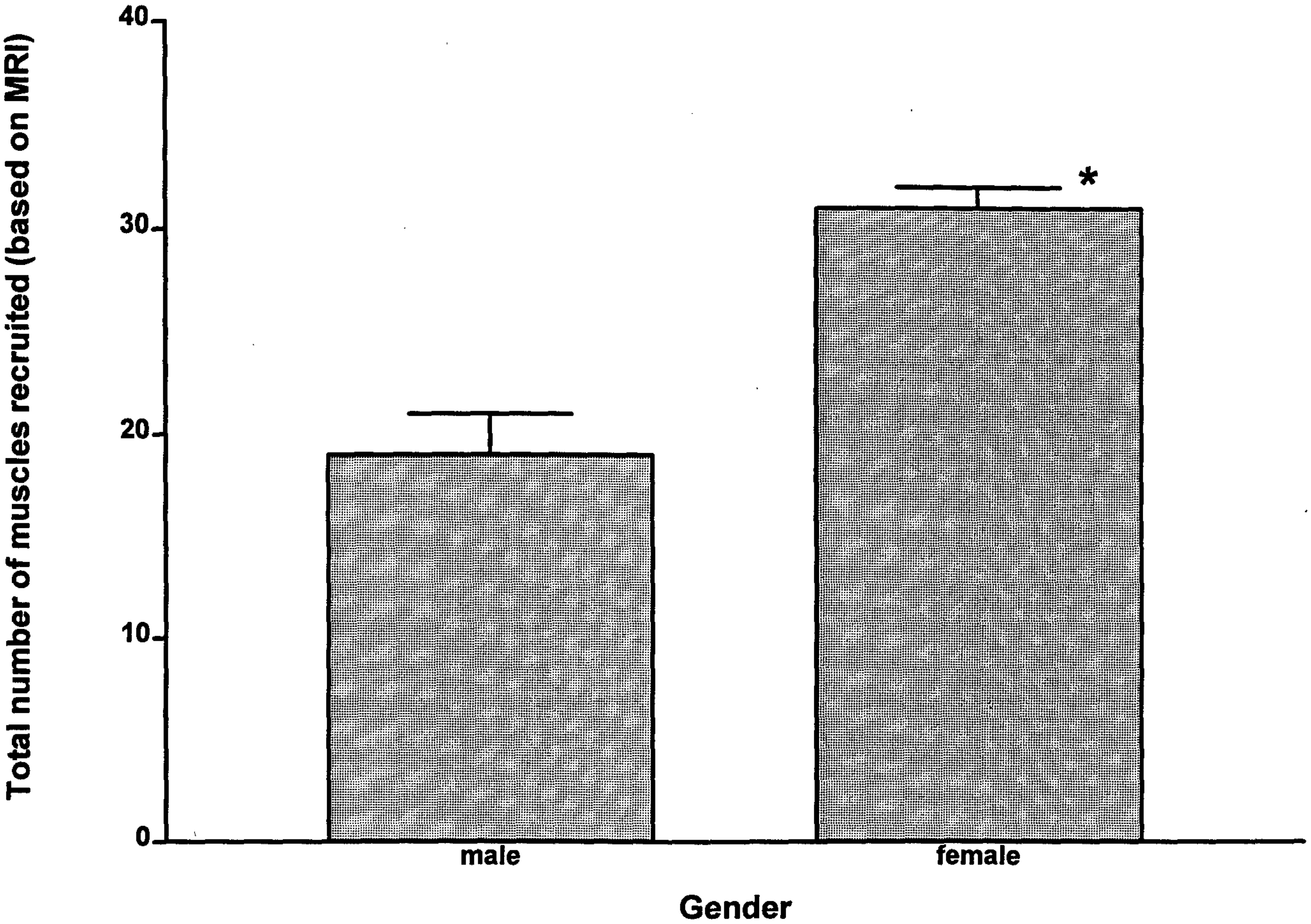

Total number of muscles recruited (based on MRI)
40
30
20
10
0
*
male
female
Gender

Figure 5a:

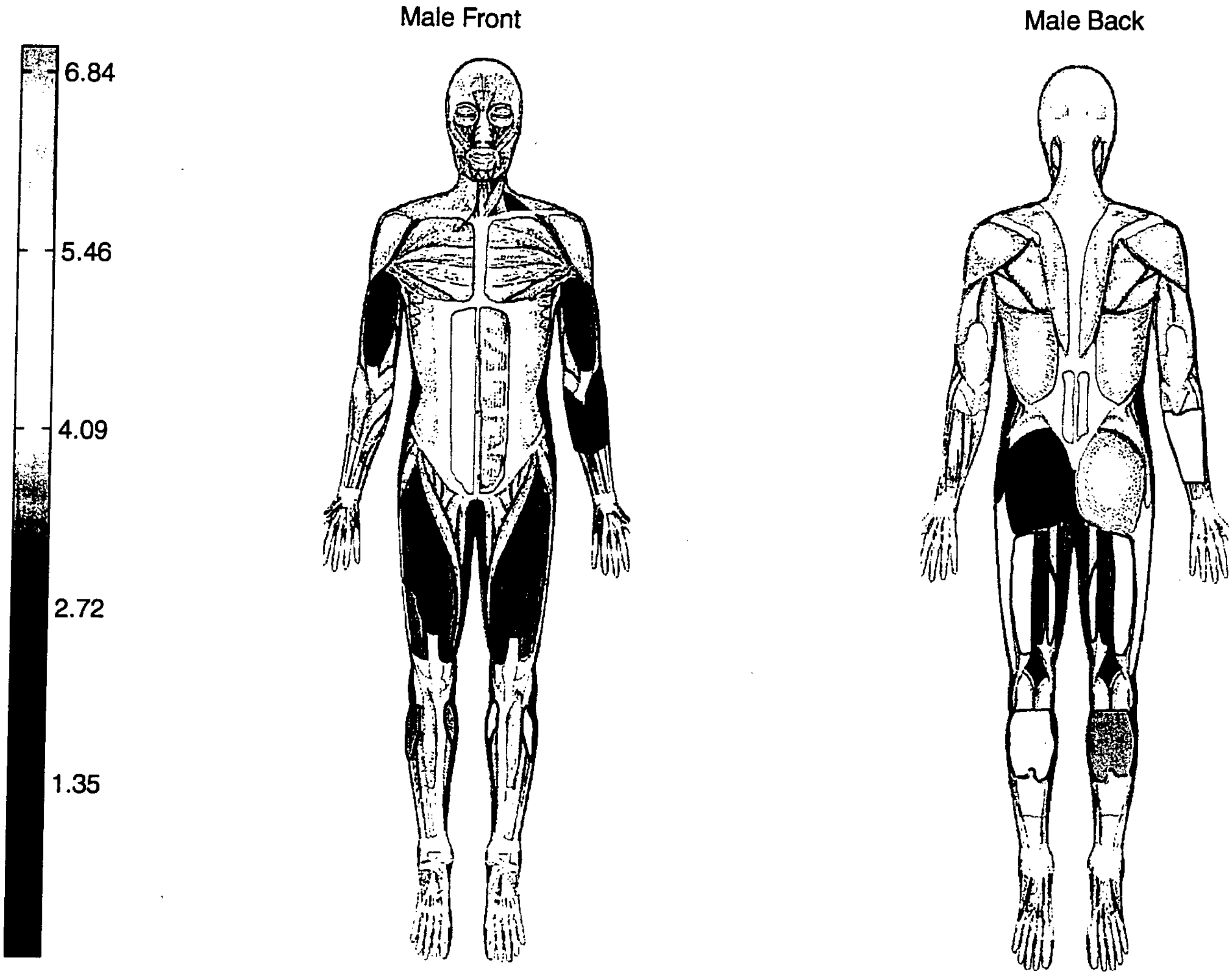


Figure 5b:

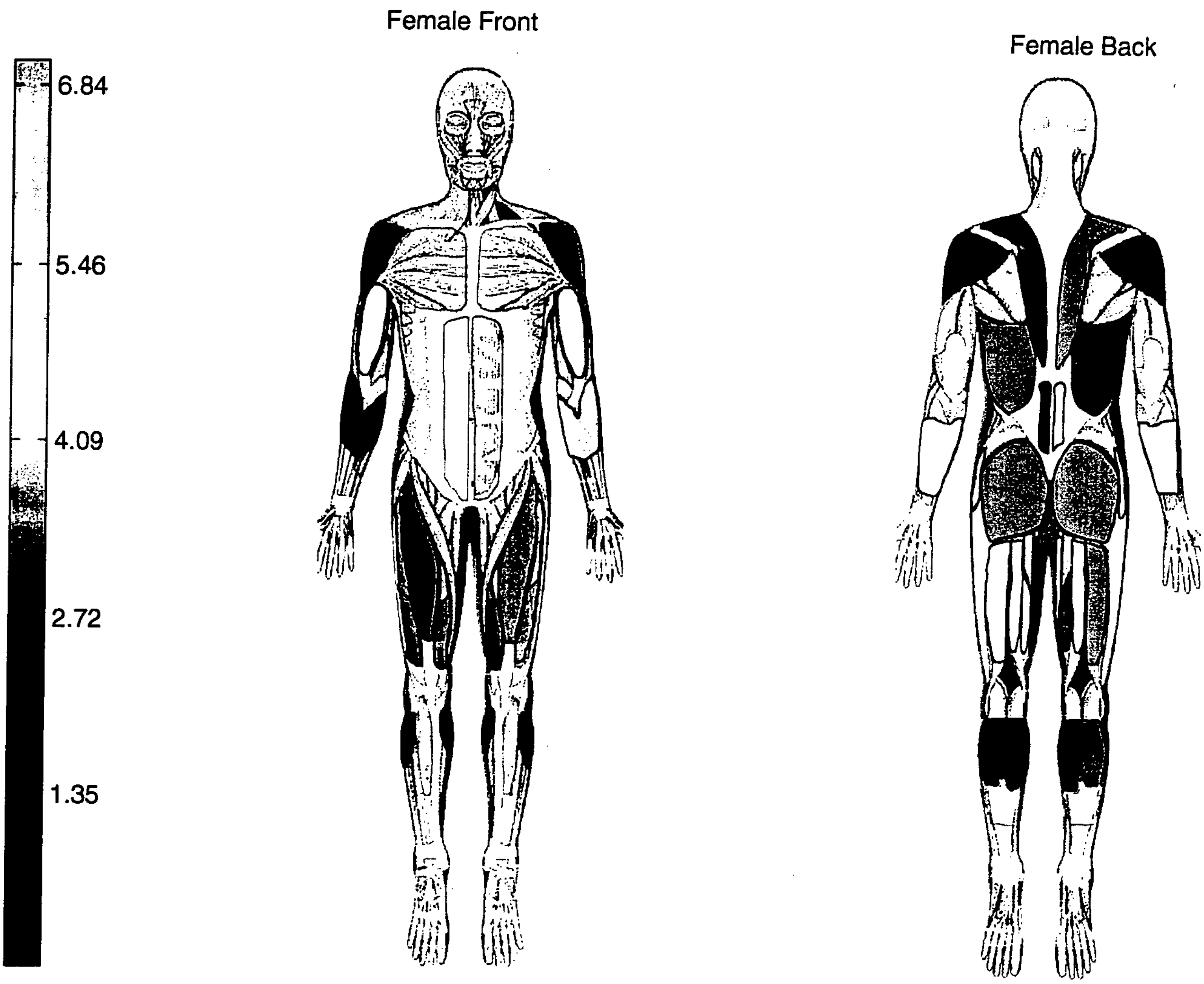


Figure 6:

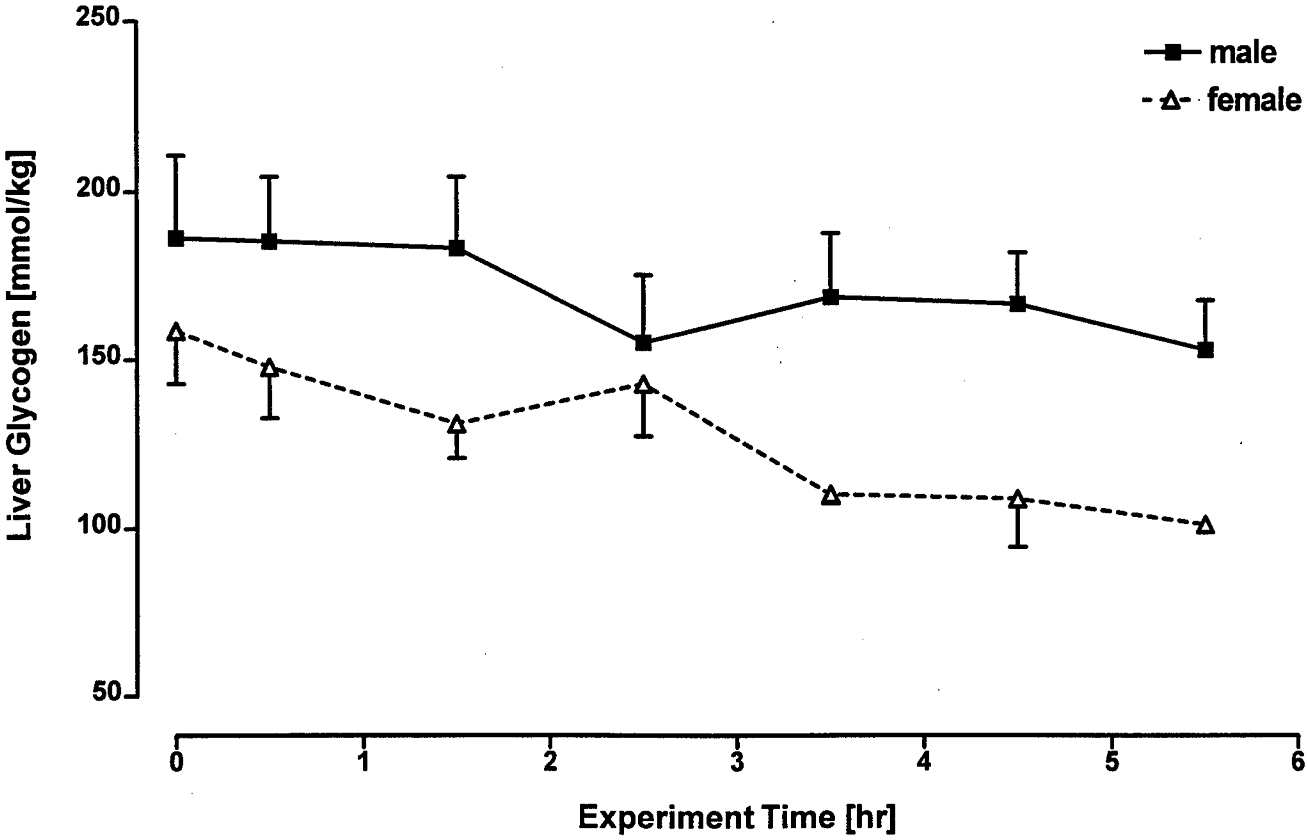
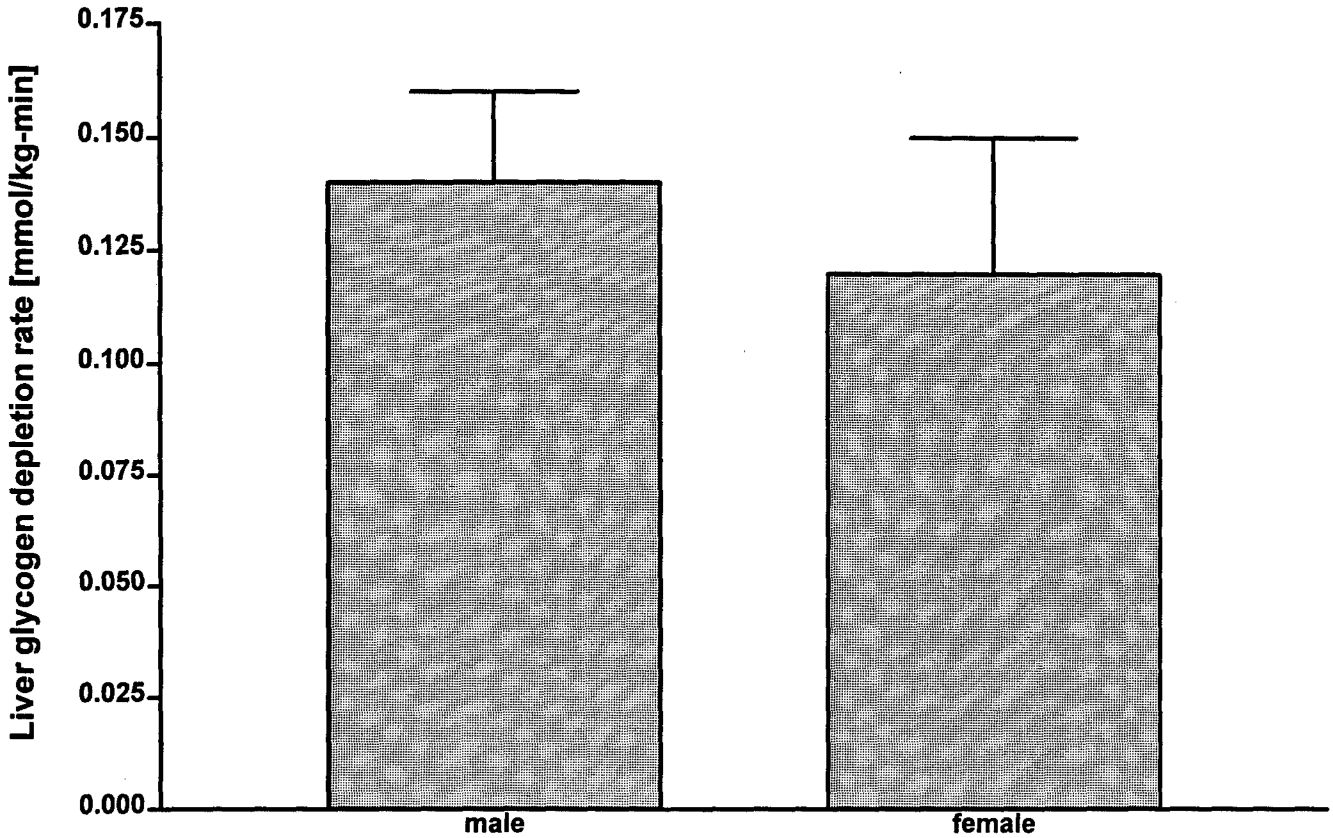

male
female
Liver Glycogen [mmol/kg]
250
200
150
100
50
0
1
2
3
4
5
6
Experiment Time [hr]

Figure 7:



0.175
0.150
0.125
0.100
0.075
0.050
0.025
0.000
Liver glycogen depletion rate [mmol/kg-min]
male
female

Figure 8:

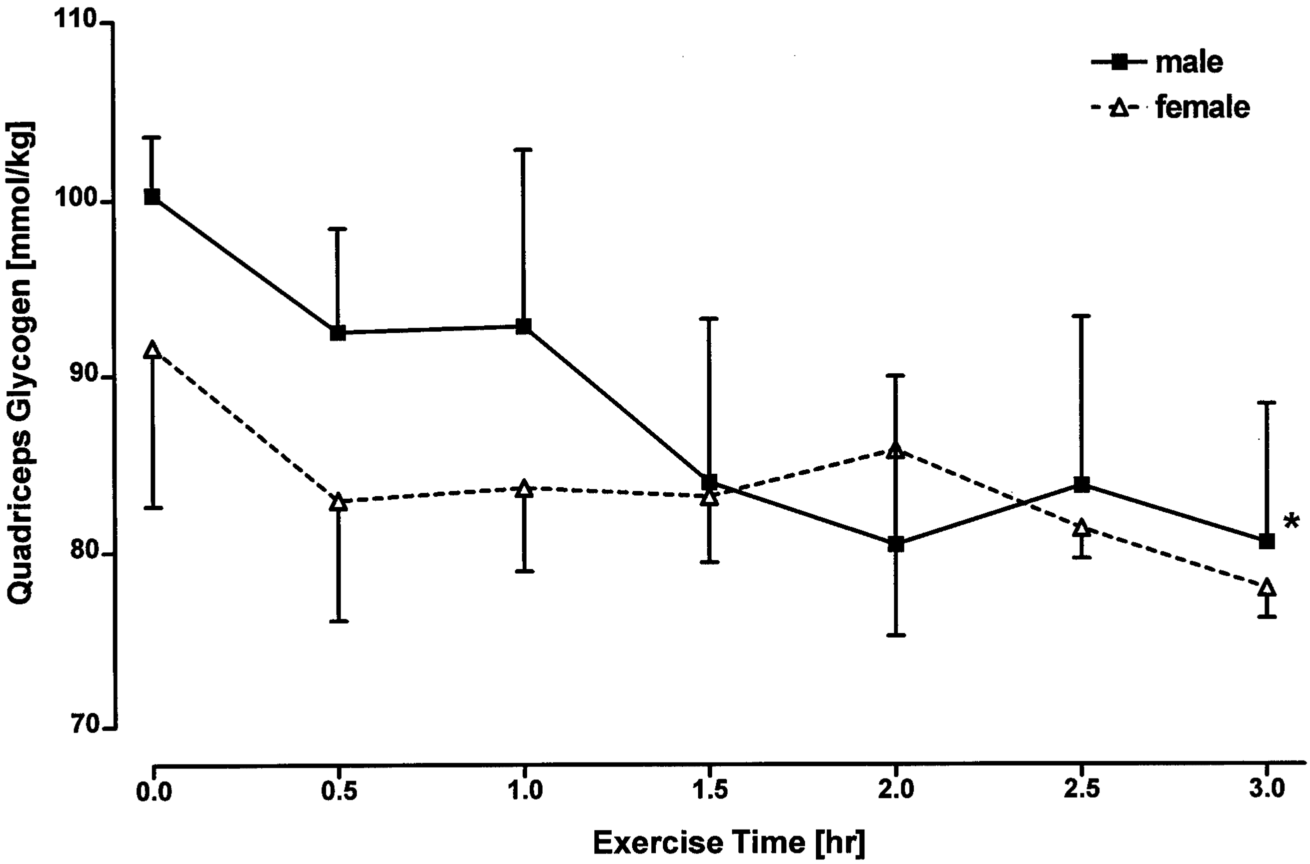
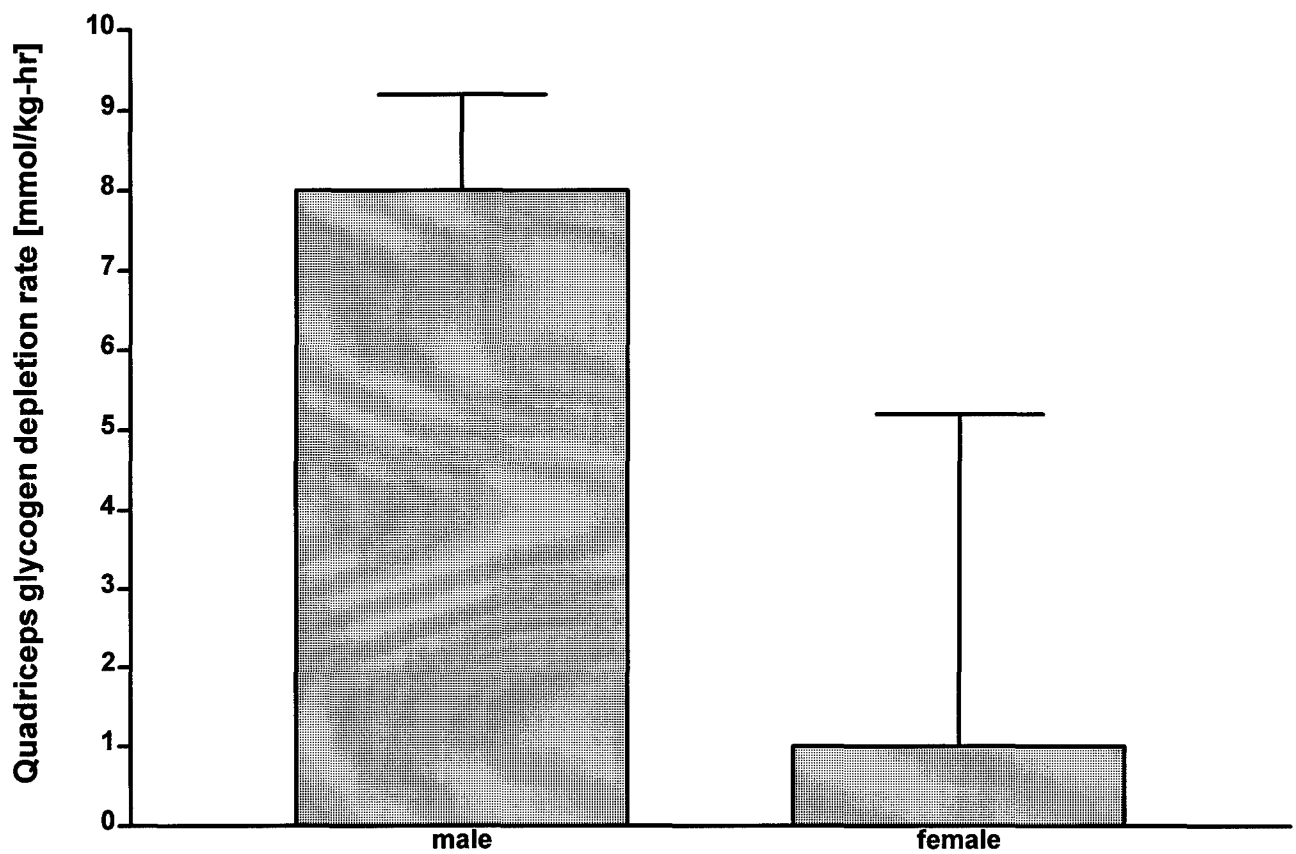

110
100
90
80
70
Quadriceps Glycogen [mmol/kg]
male
female
*
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Exercise Time [hr]

Figure 9:


Quadriceps glycogen depletion rate [mmol/kg-hr]
10
9
8
7
6
5
4
3
2
1
0
male
female

APPENDIX I:
SUBJECT RECRUITMENT CONVERSATION CHECKLIST

☐ 1. The potential for contribution to a topic of national interest & importance:

- September, 1997 USA Today articles:

  Marines train men & women apart because: Freedom to concentrate without worries about harassment, highest % of combat jobs; therefore, lowest % of jobs open to women (62%) **BECAUSE** by law women are barred from combat positions (Air Force = 99% of jobs open to women), bars on obstacle courses are set lower than men's to compensate for height, more time to run 3mi. (31min for women/ 28min for men).

  Other branches train together: In Army men only when training for infantry, artillery, and armored units, men required to do more push-ups (greater upper body strength), men required to run 2mi faster, **Question of standards being lowered to accommodate women**, same sex buddy systems in Army, Quote from Sec. of Army about sexual harassment " Far more of concern to our women in uniform is their perception of discrimination in their jobs based on their sex." **Women are 14% of the military.**

☐ 2. What is available to you as a subject:

- You will have access to physical data about yourself that could be helpful in planning your ongoing physical fitness habits and will allow you to compare your own level of fitness with what is considered normal for an athlete.
- You will be paid for your participation ($10 per hour on the day of the study + a flat rate ($70) for recording your diet & exercise habits and submitting to preliminary testing during the month before the study).
- You will receive a weight maintenance diet during the week prior to the study.

☐ 3. The information that will be provided by this study includes:

- Muscle recruitment profiles of male versus female participants performing identical exercise protocols in a head-to-head manner.
- Muscle and liver glycogen depletion patterns during exercise so as to determine the systemic carbohydrate balance during exercise and recovery in men versus women.

- The effect of four consecutive days of exercise upon the body's energy stores. For example, if someone works four consecutive days at such a high level, will the body's energy stores completely recover overnight or will energy reserves be gradually depleted over the four day period? Is there a difference between men and women over the four day period? You will be paid $10 per hour each day that you participate.
- The effect of a carbohydrate supplement upon depletion of energy stores. Does a carbohydrate supplement taken in conjunction with our exercise protocol cause the body's energy stores to be spared?

☐ 4. <u>What is expected of you:</u>

- The time commitment for this study involves a month of preparation, during which you will come in to the center for testing on two occassions (about 1-1.5hr each time). During the week before the study you will need to come by the metabolic kitchen to pick-up your meals. You can work out that schedule with the staff nutritionist. On the day of the study you will be in the center for 7-9hr. If you choose to participate in the consecutive days study, you will be in the center for 7-9hr on four consecutive days.
- This study requires that you be in similar physical condition to a soldier who has completed basic training. You will be required to pass the U.S.Army Physical Readiness Test (PRT) the same as if you were a soldier.
- During the month prior to the study you will be given diet & exercise forms to fill out on a daily basis. This information will allow us to track your normal diet & exercise habits so that we can compare you with the rest of the participants. There is a minimal time commitment with this part of the study and we ask that you simply maintain your normal diet & exercise habits unless told otherwise.
- During the week prior to the study you will receive a mixed diet designed to maintain your body weight. This is included in the protocol so that we can have all participants at the same nutritional status on the day of the study. Three hours before you begin to exercise you will be given a liquid nutritional supplement.
- On the day of the study, you will perform 3hr exercise during a 6hr period. You will be asked to lift a weighted box from floor level, carry it approximately ten feet, and place it on a rack that is 52in tall. These lifts will be done three times per minute during a 15min period, then you will be allowed 15min rest while we

make measurements. There may be measurements for some time following exercise.

- Some studies will involve blood sampling. If you participate in such a study, you will be asked to arrive at the Yale General Clinical Research Center prior to the study and a catheter will be inserted into your arm. We will draw 8-10 blood samples of less than 3 tablespoons each from the catheter over thcourse of the study.
- We will conduct several interviews with you in preparation for this study, and you will be monitored carefully during exercise with questions designed to give us information about your perceived level of exertion.
- During the month of preparation menstrutation cycle will be monitored in female subjects so that we may account for its effect upon performance. You may be asked to record your temperature each day upon waking.

☐ 5. <u>What is the pay & how will you be paid:</u>

- As mentioned earlier, the pay during the month of preparation is $70 and the pay on the day of the study is $10 per hour.
- You will fill out a subject pay form that will be submitted to Yale Disbursements, and you will receive your pay through the mail.

☐ 6. <u>Informed consent to participate:</u>

- You will be given a subject consent form to read thoroughly and sign. This form has been reviewed and approved by the Yale University Human Investigations Committee and by the National Human Use Committee of the U.S.Army. It gives detailed information about procedures and responsibility in the event of accident or injury. We request that you read this form carefully and be completely satisfied that all questions have been answered before you sign.

☐ 7. When will you be available:

- If you intend to participate in this study, please look at your calender and determine when you will be available to participate.
- When you have determined what is your availability, we request that you put this time into your calender so that we may rely on your participation according to the time schedule on which we agree.

☐ 8. **If you have chosen not to participate, thank you for taking the time to look at this study. If you have chosen to participate, thanks for your participation, and welcome to the study.**

BALKE (modified) Exercise Testing Protocol for Treadmill

	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6	Stage 7	Stage 8	Stage 9	Stage 10	Stage 11	Stage 12	Stage 13	Stage 14
Time (min)	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Speed (mph)	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.0	7.0	7.5	7.5
Grade (°)	0	0	0	2	4	6	8	10	12	14	16	18	18	18

STEEP test (Standardized Exponentiel Exercise Protocol)

(from : Northbridge, D.B. et al. *Br.Heart J.* 64:313-316, 1990)

Advantages: Performed at a lower speed without simultaneous increases in speed and grade thereby accomodating a wider range of individuals
Disadvantages: Less suited to very fit individuals.

	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6	Stage 7	Stage 8	Stage 9	Stage 10	Stage 11	Stage 12	Stage 13	Stage 14	Stage 15
Time (min)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Speed (mph)	1.5	2.0	2.0	2.0	2.5	2.5	2.5	3.0	3.0	3.0	3.5	3.5	3.5	4.2	5.0
Grade (°)	0	0	1.5	3	3	5	7	7	9	11	11	13	16	16	16

MUSCLE	FEMALE LEFT	FEMALE RIGHT	MALE LEFT	MALE RIGHT
DELTOIDS	0.0055	0.0114	----	----
TRICEPS	----	----	----	----
BICEPS	0.0008	0.0003	0.0096	0.0112
SUPERIOR FOREARM	0.0073	0.001	----	0.0055
INFERIOR FOREARM	0.017	0.017	----	0.0025
TRAPEZIUS	0.0003	0.0005	----	----
PECTORALIS MAJOR	----	----	----	----
PECTORALIS MINOR	----	----	----	----
LATISSIMUS DORSI	0.01	0.05	----	----
RECTUS ABDOMINUS	----	----	----	----
LOWER BACK	0.0043	0.0005	XX	XX
GLUTEUS MAXIMUS	0.025	0.0005	0.001	----
GLUTEUS MEDIUS	0.0046	0.0001	0.001	----
GLUTEUS MINIMUS	0.018	0.0027	----	----
RECTUS FEMORIS	0.0005	0.0028	0.011	0.0074
VASTUS LATERALIS	0.001	0.007	0.0068	0.0045
VASTUS INTERMEDIUS	0.005	0.017	0.0237	0.0015
VASTUS MEDALIS	0.025	0.0251	0.0306	0.0087
GRACILIS	0.0251	0.0186	0.0092	0.0233
BICEPS FEMORIS	0.0008	0.0321	0.0007	0.0047
SEMIMEMBRANOSUS	0.0007	0.0006	0.0399	0.021
SEMITENDINOSUS	0.017	0.0101	0.0007	0.0077
GASTROCNEMIUS	0.02	0.025	0.0007	0.0051
SOLEUS	----	----	----	----
ANTERIOR COMPARTMENT	----	----	----	----

APPENDIX III: Mean degree of significance of each of the 50 muscles tested for female and male subjects in the total body MRI protocol. This data is for the time point following the first 15min block of exercise by 4 female and 3 male subjects. The remainder of MRI data remains to be analyzed.

APPENDIX IV:

1. BASIC INFORMATION:

	# MALE	# FEMALE
Marital Status	8 single	6 single / 2 married
Ethnic Origin	7 caucasian / 1 other	7 caucasian / 1 other
Birth Control	--------	6 no / 2 yes (oral)
Smoker	8 no	8 no
Alcohol	5 no / 2 yes / 1 no ans.	5 yes / 3 no
Allergies	4 no / 3 yes / 1 no ans.	5 no / 3 yes
Medication	8 no	5 no / 3 yes
Ever Had Surgery?	6 no / 2 yes	4 no / 4 yes
Medical Condition	7 no / 1 yes	7 no / 1 yes
Diabetes in Family	4 no / 3 yes / 1 no ans.	4 no / 4 yes
Food Allergies	7 no / 1 yes	8 no
On a Diet	6 no / 2 yes	7 no / 1 yes
Vegetarian	8 no	6 no / 2 yes

APPENDIX IV:

2. EXERCISE HABITS:

	# MALE	# FEMALE
Type of exercise:		
Running	8 yes	7 yes / 1 no
Cycling	6 yes / 2 no	2 yes / 6 no
Weight Lifting	7 yes / 1 no	7 yes / 1 no
Walking	3 yes / 5 no	5 yes / 3 no
Aerobics	1 yes / 7 no	4 yes / 4 no
Calesthenics	4 yes / 4 no	3 yes / 5 no
Machines (stair climber, etc.)	1 yes / 7 no	3 yes / 5 no
Team Sports & Other Activities	6 yes / 2 no	6 yes / 2 no
> 9 months on this schedule	7 yes / 1 no	7 yes / 1 no
Moderate-to-high intensity	6 yes / 2 no ans.	7 yes / 1 no ans.
Work both upper & lower body (weight lifting)	6 yes / 1 no / 1 no ans.	4 yes / 2 no / 2 no ans.
Warm-up before exercising	6 yes / 2 no	6 yes / 2 no
Cool-down after exercising	7 yes / 1 no	5 yes / 3 no
Total Hours per Week	17.4±3.0 hrs/week	13.0±1.9 hrs/week

APPENDIX IV:

3. NUTRITIONAL STATUS:

	MALE	FEMALE
Calories per day	2453±268	1712±111 *(p≤0.001)
% Carbohydrate	51±3%	46±2%
% Fat	24±3%	32±3%
% Protein	25±4%	21±2%
Cal. burned/da. in directed exercise	687±186	479±112
% Total cal burned in exercise	28±3%	28±5%

4. VITAL STATISTICS:

	MALE	FEMALE
Age	25±3 years	27±3 years
Weight	186±6 lbs.	133±7 lbs. *(p≤0.001)
Height	71±1 in.	65±1 in. *(p≤0.005)

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MARYLAND 21702-5012

REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y) 28 July 03

MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for this Command. Request the limited distribution statement for the enclosed accession numbers be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Kristin Morrow at DSN 343-7327 or by e-mail at Kristin.Morrow@det.amedd.army.mil.

FOR THE COMMANDER:

Encl

PHYLLIS M. RINEHART
Deputy Chief of Staff for
Information Management

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