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THE EFFECTS OF HIGH INTENSITY AND ENDURANCE EXERCISE ON HUMAN MYOFIBRILLAR PROTEIN DEGRADATION

by

Catherine Ann Gaul

B.Ed University of New Brunswick 1979

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in the Department

of

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ABSTRACT

The rate of myofibrillar protein degradation was assessed following two separate exercise tests of different intensities. Untrained male subjects (N=13) with a mean maximum oxygen consumption of 45.30 $ml \cdot kg^{-1} \cdot min^{-1}$ performed two exercise protocols: A) a one hour continuous bicycle ergometer exercise (E) at a workrate of 60% maximum and, B) a set of 40 high intensity intervals (HI) at 120% maximum (30 sec work:60 sec rest). All subjects maintained a meat-free diet for 3 days prior to each exercise and throughout a 3 day post-exercise period. 24 hour urine samples were collected before the diet (pre-diet), following 3 days of diet (pre-ex) and for each of three days immediately post-exercise (Post1, Post2, Post3). Urinary 3-Methylhistidine (3MH) was significantly depressed (30%) following 3 days of meat-free diet while urinary nitrogen (N) and creatinine (CR) excretion were not affected by the diet. 3MH excretion was slightly elevated following HI exercise at post2 and returned to pre-ex levels by post3. Following E exercise, 3MH excretion increased 28% and 33.5% post1 and post2 respectively and returned to pre-ex values by post3. HI exercise resulted in 18% and 29% increases in CR excretion at post2 and post3, respectively. Urinary N was significantly elevated following HI exercise compared to E exercise at post3. The ratio 3MH/CR was only slightly elevated at post1 after both exercises. It was concluded that: 1) A meat-free diet is necessary when using 3MH as a measure of myofibrillar protein breakdown. 2)

Endurance work promotes myofibrillar protein catabolism 24 to 48 hours post exercise. 3) the 3MH/CR ratio may not be a valid measure of the fractional rate of muscle protein catabolism. 4) longer duration exercise may be needed to show differences in myofibrillar protein degradation following HI and E exercise.

DEDICATION

To my mother and father whose love, support and encouragement helped make all this possible.

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I. INTRODUCTION

The effects of exercise on skeletal muscle structure and function have been known for many years. As early as 1895, Morpurgo observed that strenuous exercise could cause the cross-sectional area of a muscle to increase with no change in the total number of muscle fibers (Morpurgo, 1895 as cited in Poortmans, 1976). Biochemical investigations have established that muscle hypertrophy results from a large increase in the myofibrillar content of muscle fibers (Helander, 1961). It has also been suggested that myofibrillar growth requires an increase in breakdown of the muscle protein in order for remodelling to occur (Neeninjun and Dubowitz, 1977; Laurent and Millward, 1980). Several exercise studies have documented evidence to support this theory (Mole and Johnson, 1971; Consolazio et al., 1975; Rennie et al., 1980a;1980b;1981).

Skeletal muscle constitutes a major tissue of the body, contributing more than 20% of body weight at birth which increases to 45% in the mature adult (Cahill, 1971; Daniel et al., 1977). By virtue of its large mass, skeletal muscle is the body's predominant reservoir of amino acids and protein. The bulk of this protein is present in the form of actin and myosin which make up the contractile elements of the muscle cell. The turnover of skeletal muscle protein thus plays an important role in the regulation of whole body protein metabolism.

The rate of protein turnover in tissues may be altered by variations in the rates of synthesis, degradation, or a combination of both. Continuous protein turnover provides a means for the redistribution of amino acids into new proteins as they are required. Hence, protein turnover represents an integral part of the continuing adaption of man to his changing environment.

Considerable scientific research has been devoted to both skeletal muscle synthesis and degradation. It is well recognized that both processes are responsive to a variety of stimuli including diet, hormones, disease and exercise. Many of these investigations have focused on the degradative aspect of protein turnover; however, reports on skeletal muscle myofibrillar protein catabolism have been equivocal and often confusing (Swick and Song, 1974; Waterlow et al., 1975; Hegsted et al., 1975). The results from these studies are difficult to interpret due to the pronounced reutilization of amino acids in skeletal muscle and the problems associated with the interpretation of urea nitrogen data.

The use of protein for energy metabolism, while controlled by the endocrine system, is dependent upon the intensity and duration of the exercise, the state of training and the diet (Gontzea et al., 1974, 1975; Astrand, 1977; Lemon and Nagle, 1981). Carbohydrates provide the major energy source for high intensity short duration work (Lemon and Nagle, 1981), whereas fat becomes the prime fuel in longer bouts of exercise due to

the depletion of muscle glycogen (Gontzea et al. ,1974; Lemon and Nagle, 1981). Muscular activity can also cause increased metabolism and oxidation of proteins (Gontzea, 1974), however this change appears to be time dependant. Enhanced protein utilization has only been observed during one hour or more of prolonged exercise (Cerney, 1975; Haralambie and Berg, 1976; Decombaz et al., 1979). One explanation of this may be the existence of a glucose-alanine cycle proposed by Felig and Wahren (1971), where increased amounts of alanine are released from skeletal muscle during exercise. Circulating alanine is then taken up by the liver where it's carbon skeleton is converted to glucose via gluconeogenesis.

During heavy work, plasma epinephrine, norepinephrine, glucagon, growth hormone and cortisol have all been observed to increase with a concomitant decrease in insulin levels (Hartley et al., 1972a; Galbo et al., 1977c; 1979; Tomas et al., 1979). Galbo and co-workers (1979) have suggested that during prolonged exercise the secretion of these hormones may be a response to a glucose privation. These investigators and others have observed that such a decrease in glucose levels significantly enhances cortisol secretion at workrates greater than 60% of maximal oxygen uptake (Davies and Few, 1973; White et al., 1976; Bloom et al., 1976; Bonen, 1976; Galbo et al., 1977c). Since cortisol is known to have a catabolic effect on skeletal muscle, an increase in muscle protein degradation might be expected following high intensity exercise.

Protein degradation during exercise may also be related to an accumulation of lactate in the muscle. Increased blood lactate inhibits mobilization of fatty acids (Fredholm, 1969) which would suppress fat metabolism and perhaps cause a larger demand for gluconeogenesis or protein metabolism. Furthermore, evidence for myofibrillar necrosis and degradation has been observed and documented by means of electron microscopy of muscle biopsies from marathon runners (Apple et al., 1983; Hagerman et al., 1983; Siegal et al., 1983). Fiber abnormalities have been observed to persist for 7 days post-marathon with the most prevalent changes occuring at 24 and 48 hours recovery. Recent observations of elevated lysosomal enzyme activities following endurance exercise also have implications for muscle protein degradation (Pilstrom et al., 1978; Vihko et al., 1978; Dohm et al., 1980). Lysosomal enzymes, particularly Cathepsin D, Cathepsin B and Calcium (CA^{++}) activated protease are capable of degrading myofibrillar proteins (Schwartz and Bird, 1977). Clark and Vignos (1981) have suggested cathepsin D as an active mediator of glucocorticoid induced skeletal muscle catabolism. They found cathepsin D activity to be proportional to the amount of muscle breakdown measured after administration of glucocorticoids to rabbits. This hypothesis may have direct implications for the role of cathepsin D following physical exercise.

Formerly, methods of examining protein synthesis or catabolism following exercise in man have been difficult and

questionable. Urinary nitrogen (N) has, in the past, been generally considered a classic protein catabolism determinant. However, one must be cautious when relating increased N metabolism to increased protein degradation because: 1) excreted N may be a product of oxidation of amino acids from free pools and not of muscle proteins, 2) working muscles may produce N by the deamination of AMP and furthermore, 3) since the sweating mechanism in man is an important mode of urea N excretion during exercise it must be considered along with urine when determining muscle protein catabolism (Lemon and Nagle, 1981). Labelled amino acids have also been used to measure degradation of muscle protein, however such methods may also be confounded by the reutilization of these tracer substances.

Urinary 3-Methlyhistidine (3MH) excretion has recently been recognized as a potentially valuable index of myofibrillar protein degradation in subjects eating a meat-free diet or on a fixed meat intake (Haverberg et al., 1974; 1975a; 1975b; Nishizawa et al., 1977a; 1977b; Long et al., 1975; Young and Munro, 1978; Rennie et al., 1980; Millward et al., 1980a). 3MH originates from the degradation of actin and of myosin heavy chains of white, fast twitch muscle fibers (Young and Monro, 1978). Since it does not charge tRNA it cannot be incorporated into protein after protein catabolism. Thus it is quantitatively excreted unchanged in the urine (Long et al., 1975; Young et al., 1970; 1972; 1978).

In contrast to N excretion, 3MH does not appear to be excreted in the sweat of human subjects (Dohm,1982). Also, 3MH is not oxidized in man as confirmed by the absence of detectable $^{14}CO_2$ in expired air following injection of labelled ^{14}C-3MH (Long et al., 1975).

In order to use 3MH as a index of muscle protein degradation it is necessary to show that skeletal muscle is the major source of this amino acid. Haverberg et al., (1975a) and Nishizawa et al., (1977a; 1977b) have both determined that skeletal muscle is the primary source of 3MH in the rat. Millward et al.,(1980) have suggested the assumption that 3MH originates almost entirely from skeletal muscle is erroneous since their study indicated that skeletal muscle only accounts for 74.5% of the urinary 3MH excretion in rats. On the other hand, Harris (1981) has criticized Millward's methods and calculations which challenged the idea that skeletal muscle may not be the primary source of 3MH.

How relevant these results are to the 3MH studies in humans remains to be determined. The only relevant literature on humans concerns a single paralyzed subject with no detectable skeletal muscle (Afting et al., 1981). It is generally agreed that 90% of 3MH in the human body is found in skeletal muscle (Young et al., 1978; Monro et al., 1978). Therefore, any change in the excretion rate of 3MH should reflect a change in myofibrillar protein degradation. Nevertheless, Rennie (1983) suggests that the key to the interpretation of 3MH excretion rates lies with

the turnover rates of other non-skeletal sources.

There are a limited number of studies on the effects of exercise on 3MH excretion rate. The results of these studies are inconclusive and contradictory. Some investigators have found decreases in urinary 3MH both during and following long duration exercise (Refsum et al., 1979; Rennie et al., 1980a; 1980b; 1981; Millward et al., 1982). while others have seen no change (Decombaz et al., 1979) or an increase (Dohm et al., 1982). A possible explanation for these conflicting results may be the type of diet used, the timing of urine collections and the state of training of the subjects.

In order to quantify the effect of muscle protein catabolism using urinary 3MH it is important to know the total muscle mass of the individual. Urinary creatinine has been used as an index of total muscle mass since it's only precursor, creatine, is found principally in skeletal muscle (Graystone,1968; Tomas et al., 1978; Uauy et al., 1978; Ballard et al., 1979). However, few experiments have examined the effect of exercise on creatinine excretion. Muscle biopsy studies have shown severe depletion of creatine phosphate stores in muscle immediately following exhaustive exercise (Bergstrom, 1967). Decombaz and coworkers (1979) observed a 50% reduction in creatinine clearance in man during a long duration exhaustive run, however pre-run levels were regained within 24 hours. Creatinine, whose clearance is also used as a measure of renal blood flow, is difficult to assess during exercise since renal

flow and glomerular filtration may be decreased. It has been suggested that except for such changes in renal function, exercise has no influence on 24 hour urinary creatinine excretion (Van Pilsum, 1958; Decombaz et al., 1979). However, Dohm, et al. (1982), using male subjects, found significant increases in creatinine excretion during the 24 hour period following a 10-12 mile run. They also observed that 1 hour of weight-lifting did not produce any measurable changes in urinary creatinine. These results imply that creatinine excretion post exercise is related to the type of work performed. However, the actual mechanisms of such a response remain to be clarified.

Since 3MH and creatinine are related specifically to myofibrillar protein and general muscle mass, respectively, the ratio of 3MH/Cr has been used to assess the fractional degradation rate of myofibrillar protein (Tomas et al., 1979b; Ballard et al., 1979; Seashore et al., 1981; Dohm et al., 1982). This method of measurement should make allowances for individual differences in body composition, particularly muscle content. To date, only one exercise study has used the 3MH/Cr ratio as a method of measuring muscle protein breakdown (Dohm et al., 1982). Their study found that both running and weight-lifting produced significant increases in the 3MH/Cr ratio.

It appears, therefore, that exercise does trigger a change in the degradation of myofibrillar protein in man. It is conceivable that the intensity and duration of the exercise plays an important regulatory role in the catabolism of muscle

protein. Hence, the primary purpose of this study was to determine the effects of endurance and high intensity short duration exercise on myofibrillar protein degradation in man. In order to solve this problem, it was necessary to ascertain:

1. If a meat-free diet changes the urinary excretion rates of Creatinine, urea Nitrogen or 3-Methlyhistidine.
2. If exercise alters urinary concentration of creatinine, urea Nitrogen and 3-Methlyhistidine.
3. If the time course of the urinary concentration change in these variables is altered post-exercise,
4. Whether the exercise intensity influences the time course of any concentration change.

II. METHODOLOGY

2.1 Research design

Thirteen untrained male university students (Table 1) were studied under three exercise conditions in a repeated measure design as outlined in Table 2. Initially, as a control procedure, each subject performed a maximum bicycle ergometer ride to determine his maximum oxygen uptake ($\dot{V}O_2$ max), heart rate (HR) and workrate. After the $\dot{V}O_2$ max was determined, each subject completed two experimental exercise protocols: 1) an endurance ergometer test of 1 hour and 2) a set of 40 thirty second ergometer sprints. Both experimental exercise protocols were performed in the Environmental Chamber (Kinesiology,SFU) where room temperature was held constant at 15°C and humidity at 20%.

TABLE 1. SUBJECT CHARACTERISTICS

AGE (yrs)	HEIGHT (cm)	WEIGHT (kg)	RESTING HEART RATE ($B \cdot Min^{-1}$)
24.26 ±1.17	179.79 ±1.75	72.34 ±1.83	73 ±3

Values are means ± Standard errors of the means.
N=13

A controlled dietary period preceeded and continued throughout the periods in which the exercise tests were undertaken. Subjects were randomly assigned to one of two groups: Group 1 performed the endurance work first, followed by the sprint exercise on a subsequent visit while group 2 completed the tests in reverse order. This experimental design was chosen to negate any confounding factors associated with repeated measures. A minimum of seven days rest was allowed between each of the exercise conditions. Subjects collected 24 hour urine samples prior to and following each diet and exercise period (Table 2). Samples were analyzed for concentrations of 3-Methylhistidine, Creatinine and Urea Nitrogen. As an incentive, each subject received thirty dollars upon completion of the study.

TABLE 2. EXPERIMENTAL DESIGN: EXERCISE CONDITIONS

CONDITION	N	PROTOCOL	MEASURED VARIABLES
1.Maximum Oxygen uptake Ergometer Test.	13	Ride to Exhaustion	Heart Rate, $\dot{V}E$, $\dot{V}O2$, Workrate.
2.Endurance Ergometer Test.	13	1 hour at 60% work-rate of $\dot{V}O2$ max.	Heart rate, $\dot{V}E$, $\dot{V}O2$, Pre-Diet, Pre-exercise and Recovery urinary 3MH,Cr,N.(*)
3.Sprint Ergometer Test.	12	40-30 sec. sprints at 120% max.	Same as condition 2.

*For details of urine collection see Table 3.

2.2 Exercise protocols

All participants were examined by a physician prior to any exercise testing. All subjects were asked to refrain from any prolonged physical activity (lasting 30 minutes or more, heart rate > 130 bpm) for two days before each test as well as for the duration of the urine collection period. In order to control for diurnal effects an attempt was made to ensure individual subjects performed exercise tests at the same time of day. In addition, subjects kept a record of their diet and physical activity during each testing period. The consumption of alcohol was prohibited for 24 hours prior to collection of the first urine sample and throughout the testing period until the final, post-exercise collection was completed.

$\dot{V}O_2$ max was measured on a bicycle ergometer by the Douglas bag method described by Astrand and Rodahl (1977) and outlined in Appendix 1. Subjects were given a 6 minute warmup prior to performing the $\dot{V}O_2$ max test. The test involved riding on the bicycle ergometer at a pedal frequency of 60 rpm (Astrand and Rodahl, 1977), with workrate based on the heart rate attained in the final minute of warmup. The workrate was increased by 180 $kpm \cdot min^{-1}$ every minute until the subject reached exhaustion. For purposes of this study, exhaustion was defined as the point at which the subject could no longer continue pedalling at 60 rpm. Heart rates were monitored directly for the last 10 seconds of

each minute of the ride and for three minutes of recovery, from three electrodes placed on the ridge of the scapula and on the right and left of the thorax, at the 5th intercostal space.

The endurance protocol consisted of a one hour continuous bicycle ergometer ride at 60% of the predetermined VO_2 max. Heart rate was monitored for 10 seconds and oxygen consumption for 30 seconds every 5 and 10 minutes respectively as described in Appendix 2. All thirteen subjects completed this exercise regime.

The sprint exercise protocol included 40 thirty second high intensity (HI) ergometer rides at a workrate corresponding to 120% $\dot{V}O_2$ max. Each 'sprint' was followed by a recovery period of sixty seconds. Only twelve subjects participated in this exercise protocol as one subject withdrew from the study following the endurance exercise for personal reasons. All 12 subjects, with the exception of one, completed all 40 efforts at 120%: It was necessary to decrease the workrate for one subject to 100% of the $\dot{V}O_2$ max.for the final 10 sprints. Heart rate was monitored for the last 10 seconds of every minute during the warmup, every eighth minute of the test and for three minutes following cessation of work as described in Appendix 2. As in the endurance protocol, oxygen uptake was measured during the sprint exercise. (Appendix 2).

2.3 Diet

Each subject ate a meat-free diet during a period of 7 days encompassing the period preceding and following the endurance and sprint tests in order to eliminate any dietary contribution to 3MH excretion (Table 3). Initial pilot work indicated that three days on a meat free diet causes a significant depression of urinary 3MH excretion (See Review of Literature, Figure 14).

The importance of maintaining a well balance diet through out the study was emphasized. Diets of all subjects were similar prior to this study; no participant was a vegetarian. All subjects were asked to refrain from alcohol, tea and coffee due to their antidiuretic effects. Subjects were asked to substitute fish and dairy products for muscled meat during the diet period so that protein intake would not be reduced. Individuals who felt that this diet would interfere too much with their normal lifestyle were screened out of the study.

The weight of each participant was recorded during each phase of the study. Very little fluctuation in individual weight was observed (less than 0.8kg). It was felt that the lack of weight change confirmed that the diets were well balanced.

2.4 Urine Collection

For the first exercise program, whether endurance or sprint, each subject collected five 24 hour urine samples as explained in Table 3. The first collection, labelled 'pre-diet', was not included in the second exercise program due to contraints on the availability of the Amino Acid Analyser used in this study. Subjects were required to void at the time chosen to begin the first 24 hour collection period, and then collected all urine passed up to and including that voided at the same time the next day.

TABLE 3. DIET AND URINE COLLECTION

DAY 1	Pre-diet 24 hour urine collection
DAY 2-4	Meat-free diet begins.
DAY 5	Pre-exercise 24 hour urine collection (diet continues)
DAY 6	Exercise in a.m.;Post 1, 24 hour collection (diet continues)
DAY 7	Post 2, 24 hour urine collection (diet continues)
DAY 8	Post 3, 24 hour urine collection (diet continues)

Urine was collected in plastic 4 liter containers (Coast Plastics, Pitt Meadows, BC) containing 1 ml toluene as a preservative (Tomas et al., 1979). At the end of each 24 hour collection, the volume of urine excreted was measured and an 8

ml aliquot saved. The sample aliquot was deproteinized with 30% sulfosalicyclic acid (2:1 urine:acid ratio, Long et al.,1975; Tomas et al., 1979; Afting et al., 1981), passed through a double Millipore filter system (mesh size 45μ) and stored at -20° C until the time of analysis. All analyses were performed with each sample labelled with a random number which could later be identified with a particular exercise protocol and condition. Due to laboratory constraints, only one analysis of each collection was conducted for concentration of 3MH. However, double blind duplicate analyses were performed for nitrogen and creatinine concentrations.

2.5 3-Methylhistidine Determination

3MH was determined in a two column 11 hour physiological fluid analysis system on a Beckman Amino Acid Analyzer (model 119, Bioscience Dept., SFU.). Standard methodology for the analysis of physiological fluids requires a long column for the separation of the acid and neutral amino acids and a short column for separation of the basic amino acids such as 3MH. Columns were packed with Beckman custom spherical resins (Palo Alta, Calif.) and sodium citrate (Sigma, St.Louis) was used as a buffer. Important parameters for this analysis are summarized in Appendix 3. Constituents of the urine samples separated in the columns react with ninhydrin in order to render them visible for colorimetry at 570 nanometers. Peak elution time and

concentration were determined in two ways using 3MH standards (Sigma, St.Louis): 1. Through analysis of chromatographs taken from the amino acid analyzer, and 2. Via computer analysis (Nova Computer System, SFU). The concentration of 3MH in each urine sample was also analysed in this same manner (Appendix 3).

2.6 Determination of Creatinine

Creatinine concentrations were determined using a Beckman DU-8 Spectrophotometer according to the alkaline creatine picrate method (Jaffe reaction). Urinary creatinine reacts with picric acid under alkaline conditions to form a yellow-orange complex. This color is developed not only by creatinine but also by certain non-specific substances present in the sample (Cook, 1975). Upon the addition of acid, the color specific to creatinine is removed, while that contributed by non-specific substances remains. Therefore, the difference in the optical density of the sample when measured at 500nm prior to and following the addition of acid is directly proportional to the concentration of creatinine present. (Refer to Appendix 4 for reagents and procedures used for determination of creatinine.)

2.7 Urea Nitrogen Determination

Urinary nitrogen (N) was determined colorimetrically using the urease method described by Bauer (1974) on a Beckman DU-8 spectrophotometer. This reaction is specific for urea, however it also includes any preformed ammonia in the urine. Unlike blood plasma, urine may contain a substantial amount of preformed ammonia (NH_4^+). Therefore it is essential to measure the preformed NH_4^+ in the urine sample being analyzed and subtract it from the total N measured. Preformed NH_4^+ may be determined by omitting incubation with urease and substituting water for the urease solution. The optical density of the blue color product obtained after incubation of all samples was measured at 540nm. Reagents and procedures used for the measurement of urea N are described in Appendix 5.

2.8 Determination of Myofibrillar Protein Degradation

Using urine samples and muscle biopsies, Tomas et al (1979b) were able to determine 3MH content in human muscle to be 3.59 μM per gram myofibrillar protein. The average degradation rate of muscle protein (g/day) may therefore be obtained by dividing the 3MH excretion per day by the 3MH content of muscle protein:

Amount of Myofibrillar Protein Degraded per Day=

$$\frac{\text{3MH } (\mu M \text{ / 24 hours})}{\text{Amount of 3MH found in Myofibrillar protein}}$$

Where,
3.59 μM/g myofibrillar protein is the denominator.

2.9 Statistical Analysis

Group means +/- the standard errors of the mean (SEM) were calculated for all variables. A two way analysis of variance (SPSS MANOVA) was performed for each variable between conditions (PreDiet, PreEx, Post1, Post2, Post3) and exercise protocols (Endurance and High Intensity). When the F ratio indicated significance at the $P < 0.05$ level, one way analyses of variance tests (SPSS Anova) were conducted for the specific variable: 1) condition by protocol and 2) protocol by condition. This statistical procedure was followed by Post Hoc Student Neuman Keuls analysis in order to locate the significant differences. Pearson correlation coefficients were determined between each of the following variables: weight, urine volume, creatinine, nitrogen and 3-Methylhistidine. When the r value indicated a relationship ($r > 0.07$), analysis of covariance (SPSS) was performed. Thus, total nitrogen and urea nitrogen were adjusted for urinary volume using urinary volume as the covariate. Adjusted means and standard errors were determined through a

BMDP covariate statistical package (BMDP1V). Varying subject numbers in the various conditions arose from missing data due to methodological as well as subject error.

III. RESULTS

3.1 Maximal Exercise Response

The total group response to the maximal exercise, as described by means ± SEM for $\dot{V}O_2$, $\dot{V}_E$STPD, maximum heart rate and maximum workrate is shown in Table 4. Mean oxygen consumption was 45.30 (± 1.41) $ml \cdot kg^{-1} \cdot min^{-1}$.

TABLE 4. MAXIMAL EXERCISE RESPONSE

VO2 ($l \cdot min^{-1}$)	VE(BTPS) ($l \cdot min^{-1}$)	MAX. H.R. ($b \cdot min^{-1}$)	MAX. WORKRATE ($kpm \cdot min^{-1}$)
3.25 ±0.12	131.24 ±5.65	191.30 ±2.65	1495.30 ±47.30

Values are means ± Standard errors of the means.
N=13

3.2 Submaximal Exercise Response

Table 5 describes the effects of the two exercise protocols as a percentage of the maximal response. Average $\dot{V}O_2$ during the endurance exercise (E; 60% max) was 30.21 $ml \cdot kg^{-1} \cdot min^{-1}$ while the short, high intensity (HI) work (119% max) resulted in a mean $\dot{V}O_2$ of 32.31 $ml \cdot kg^{-1} \cdot min^{-1}$. Concomittanly, HI work caused heart rate to remain significantly higher (161.1 $b \cdot min^{-1}$) than the E ride (146.0 $b \cdot min^{-1}$). This relationship is clearly demonstrated in Figure 1.

TABLE 5. SUBMAXIMAL EXERCISE RESPONSE

PROTOCOL	VO2 %	VE(BTPS) %	H.R. %
Endurance (13)	66.6	26.45	76.9
High Intensity (12)	71.00	28.49	85.5

Values are presented as percentage of means of groups.
Subject numbers are in parenthesis.

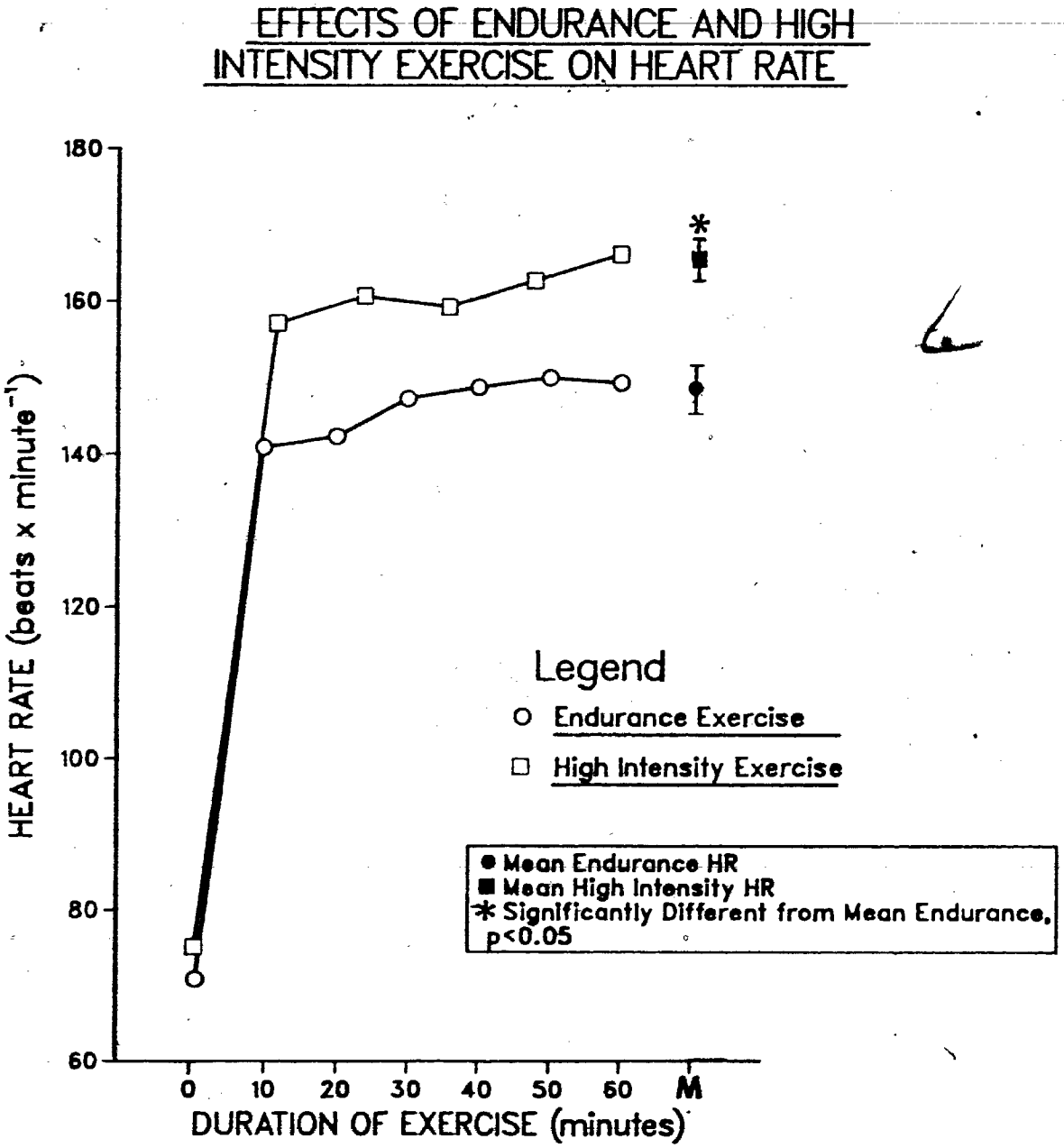


Figure 1. Heart rate response to endurance and high intensity exercise. Values are means of groups at 8 and 10 minute intervals for high intensity and endurance respectively.

3.3 Correlations

Pearson correlation coefficients were determined between each of variables measured. No relationship was achieve between creatinine or 3MH and weight or urine volume, nor between nitrogen or 3MH and creatinine. In addition nitrogen showed no relationship to weight. However, both total nitrogen and urea nitrogen were found to be highly related ($r > 0.91$) to urine volume.

Table 6. Pearson Correlation Coefficients

Variable	Weight (kg)	Volume (ml)	Creatinine (g)
Creatinine (g)	0.0757 (115)	0.3834 (117)	
Total Nitrogen ($mM \cdot kg^{-1}$)	0.0486 (128)	0.9346* (130)	
Urea Nitrogen ($mM \cdot kg^{-1}$)	0.0028 (128)	0.9179* (130)	0.2915 (117)
3-Methylhistidine ($uM \cdot kg^{-1}$)	0.3540 (115)	0.2246 (115)	0.1268 (115)

Values are Pearson correlation coefficient results.
Numbers in parenthesis are N on which 'r' was computed.
* significantly correlated ($p < 0.000$).

3.4 Urinary Volumes

Figure 2 demonstrates the considerable intersubject variability in urinary output during the experimental periods. A significant difference in urinary excretion volume was found between the post3 collections, where HI mean values were 30% greater than those collected at the same time post E.

3.5 Creatinine

The prescribed diet had no significant effect on creatinine excretion corrected for body weight (Figure 3). Similarly, the endurance work did not produce any variation in urinary creatinine levels. However, high intensity exercise was marked by a 19% and 30.6% increase in the rate of creatinine excretion at 48 and 72 hours post-HI exercise respectively. At 72 hours post exercise, a significant difference ($p < 0.05$) was observed between the E creatinine value (36.5 $mg \cdot kg^{-1} \cdot day^{-1}$) and the HI value (52.9 $mg \cdot kg^{-1} \cdot day^{-1}$).

3.6 Urinary Nitrogen

Endurance cycling caused a slight increase in total urinary N (adjusted for urine volume) for the first two days post exercise, which returned to pre-exercise levels within 72 hours

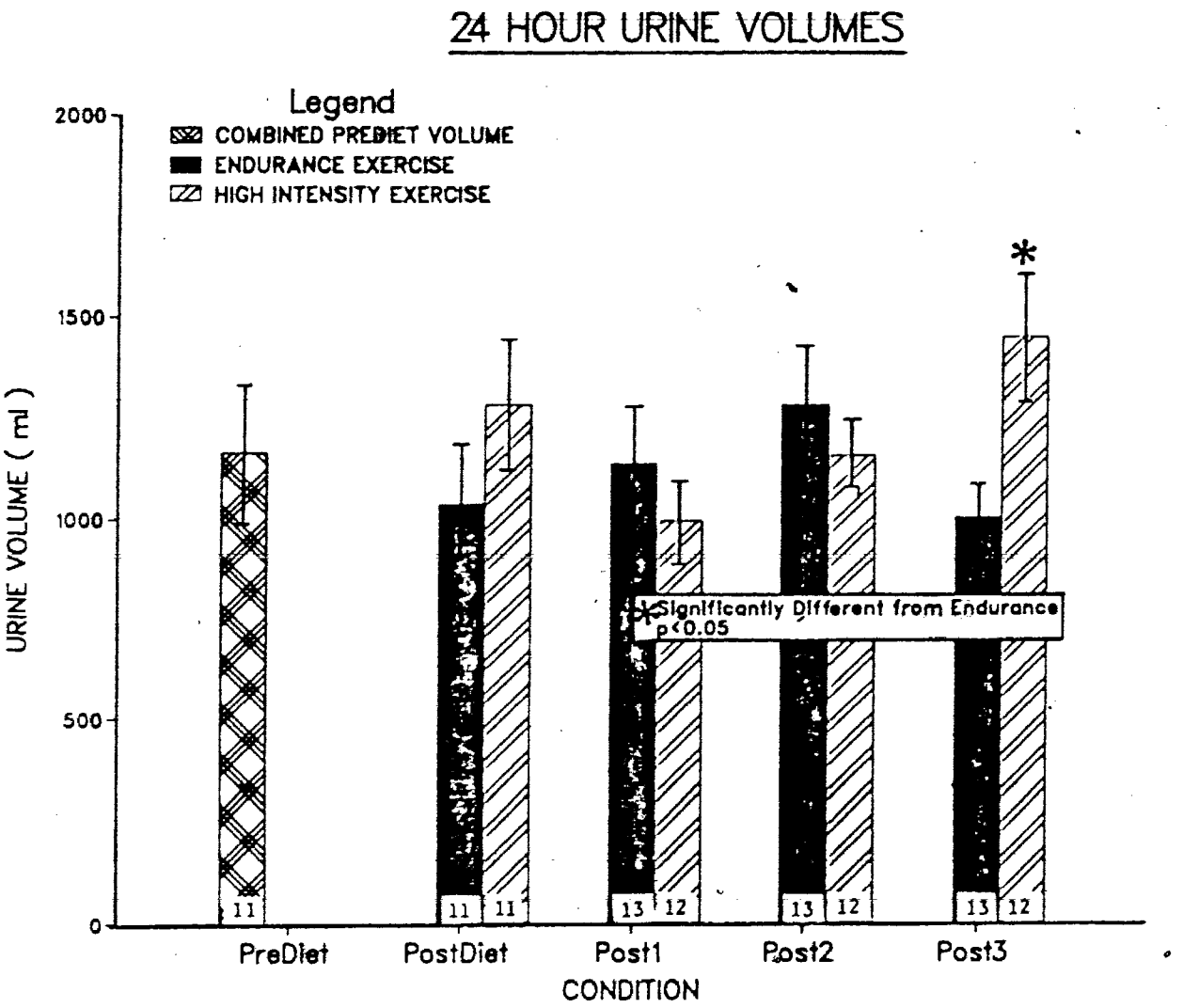


Figure 2. Daily urine volumes. Values are means ± SEM. Pre Diet values are the means of both groups (endurance and high intensity) prior to their respective exercises. N's are given in the bars.

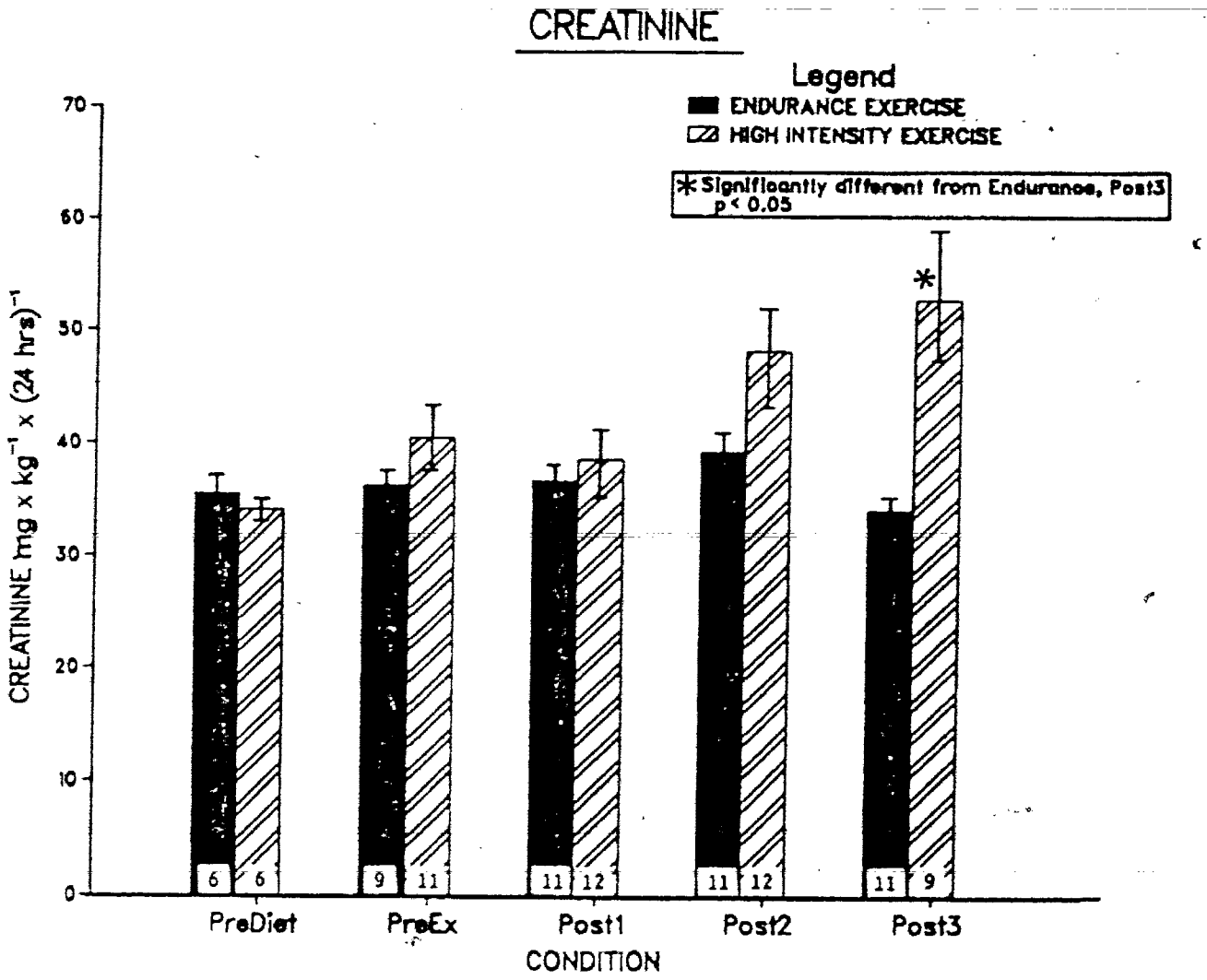


Figure 3. Daily urinary creatinine excretion. Values are means of groups corrected for body weight (Kg) ± SEM. N's are given in the bars.

of recovery. However, this trend was not significant. In addition, HI exercise caused no change in total urinary N excretion.

The urinary N data corrected for preformed NH_4^+ is shown in Figure 5. These results are similar to the uncorrected N excretion are adjusted for urine volume. It appears that the meat free diet might have caused a small increase in the excretion of urea N. However, no significant alterations in urea N were observed following either one of the exercises.

3.7 3-Methylhistidine

Since the method of 3MH analysis was identical, the results of both the current study and a pilot study (N=5) were pooled to demonstrate the effects of diet on urinary 3MH. The 72 hour meat-free diet caused a significant ($p < 0.01$) depression (40%) of 3MH excretion as shown in Figure 6. Endurance exercise resulted in an 18% and 24% increase one and two days post exercise, respectively. However, no statistical significance was achieved (Figure 7). The HI exercise protocol resulted in small insignificant increases in urinary 3MH post1 and post2. These changes were independent of urinary volume and returned to normal 72 hours post-exercise.

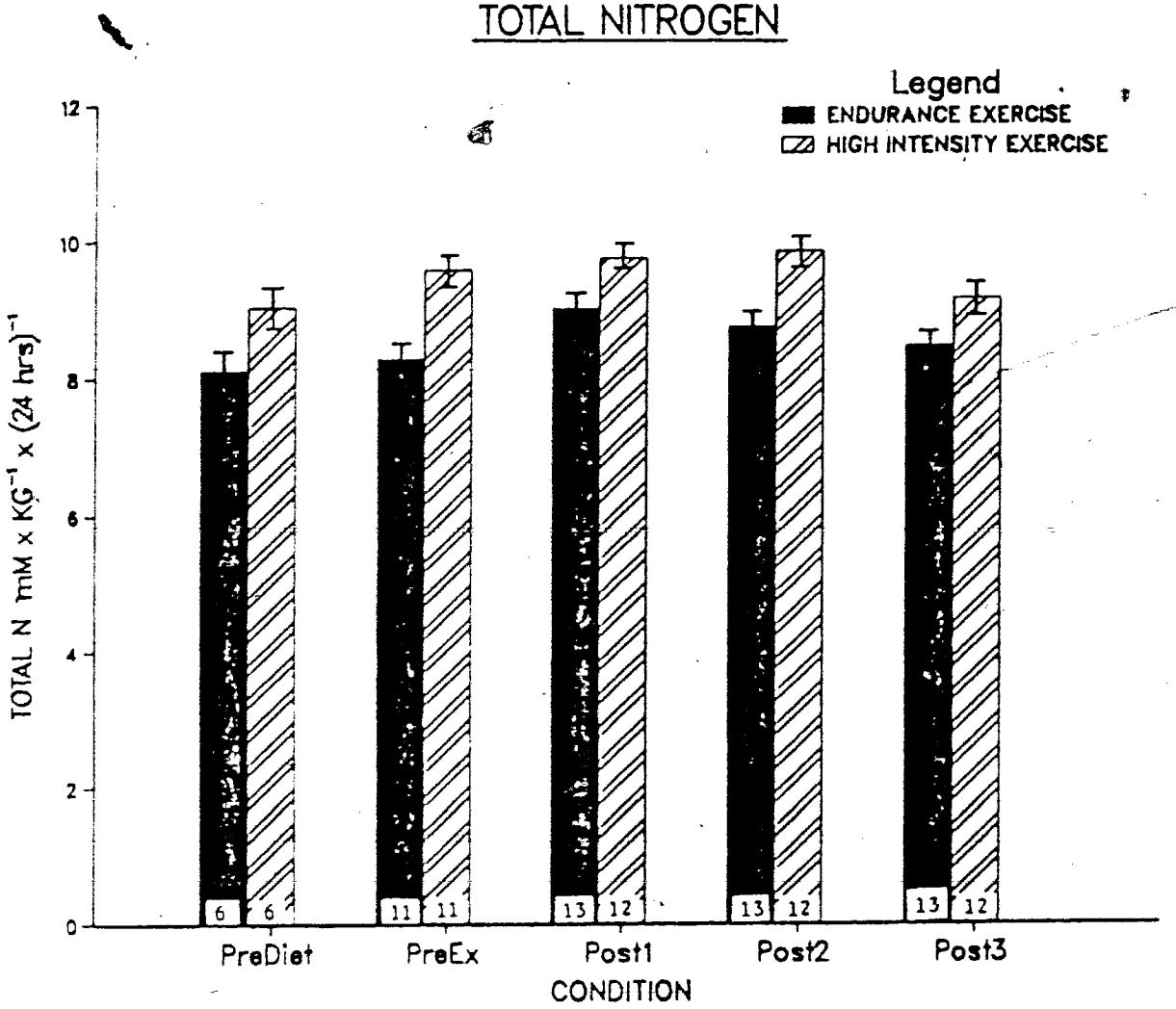


Figure 4. Total urinary nitrogen uncorrected for preformed ammonia. Values are means of groups corrected for body weight (Kg) and adjusted for urine volume. N's are given in the bars.

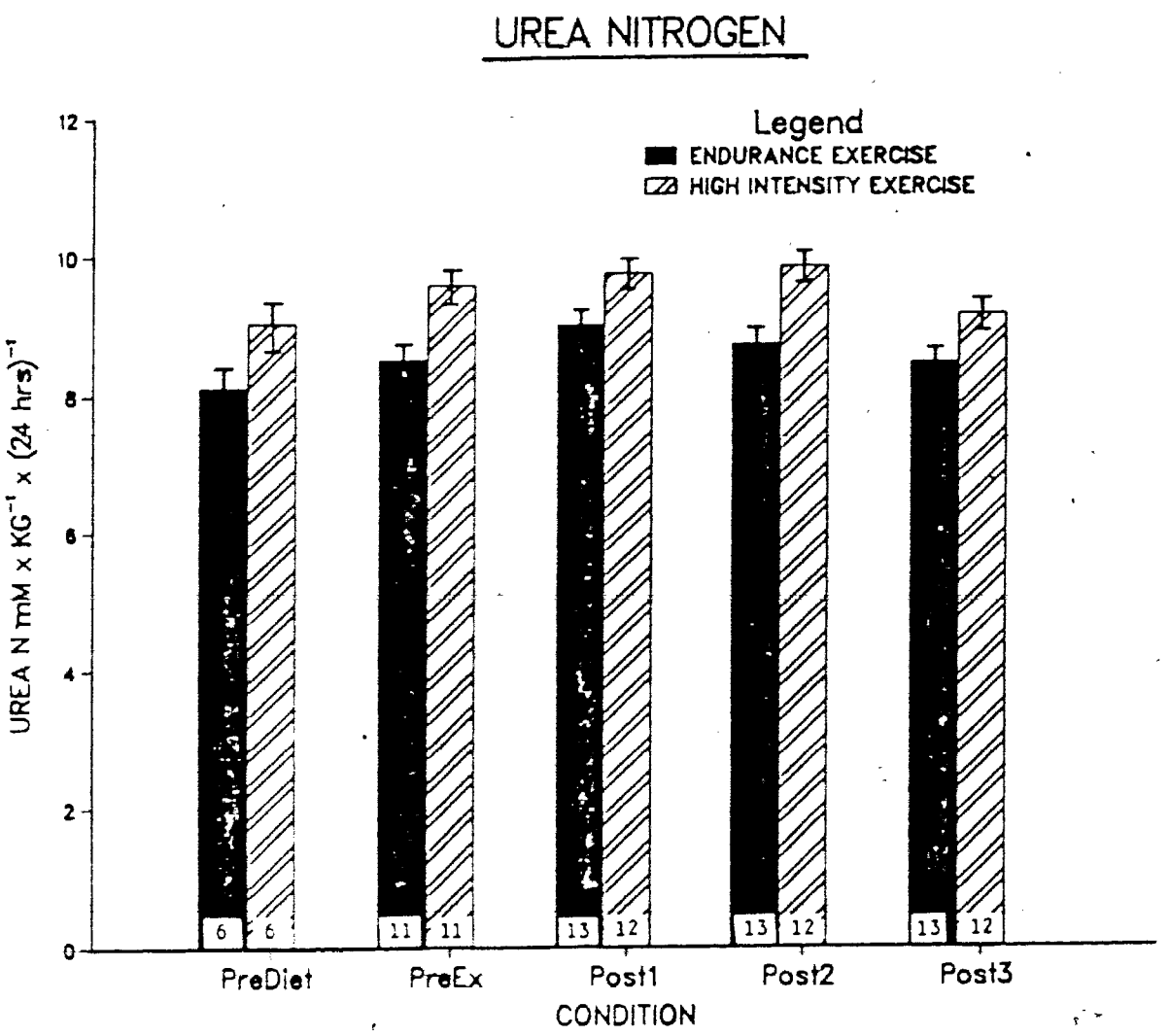


Figure 5. Urinary urea nitrogen excretion corrected for any preformed ammonia found in the urine. Values are means of groups adjusted for urine volume, corrected for body weight (Kg) $\pm$ SEM. N's are given in the bars.

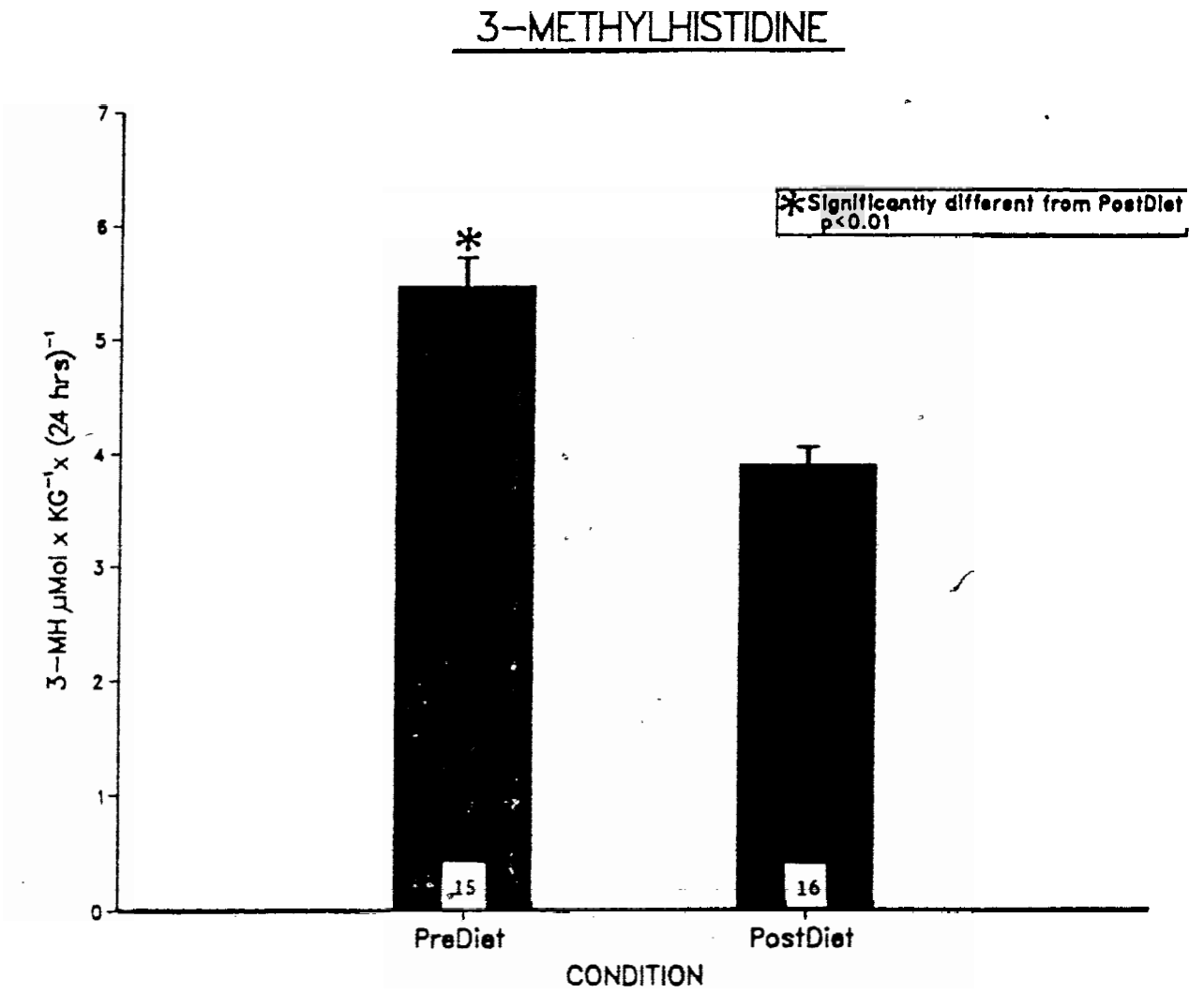


Figure 6. Effects of a three day meat-free diet on the excretion of 3-Methylhistidine. Values include data from pilot work and are group means ± SEM. N's are given in the bars.

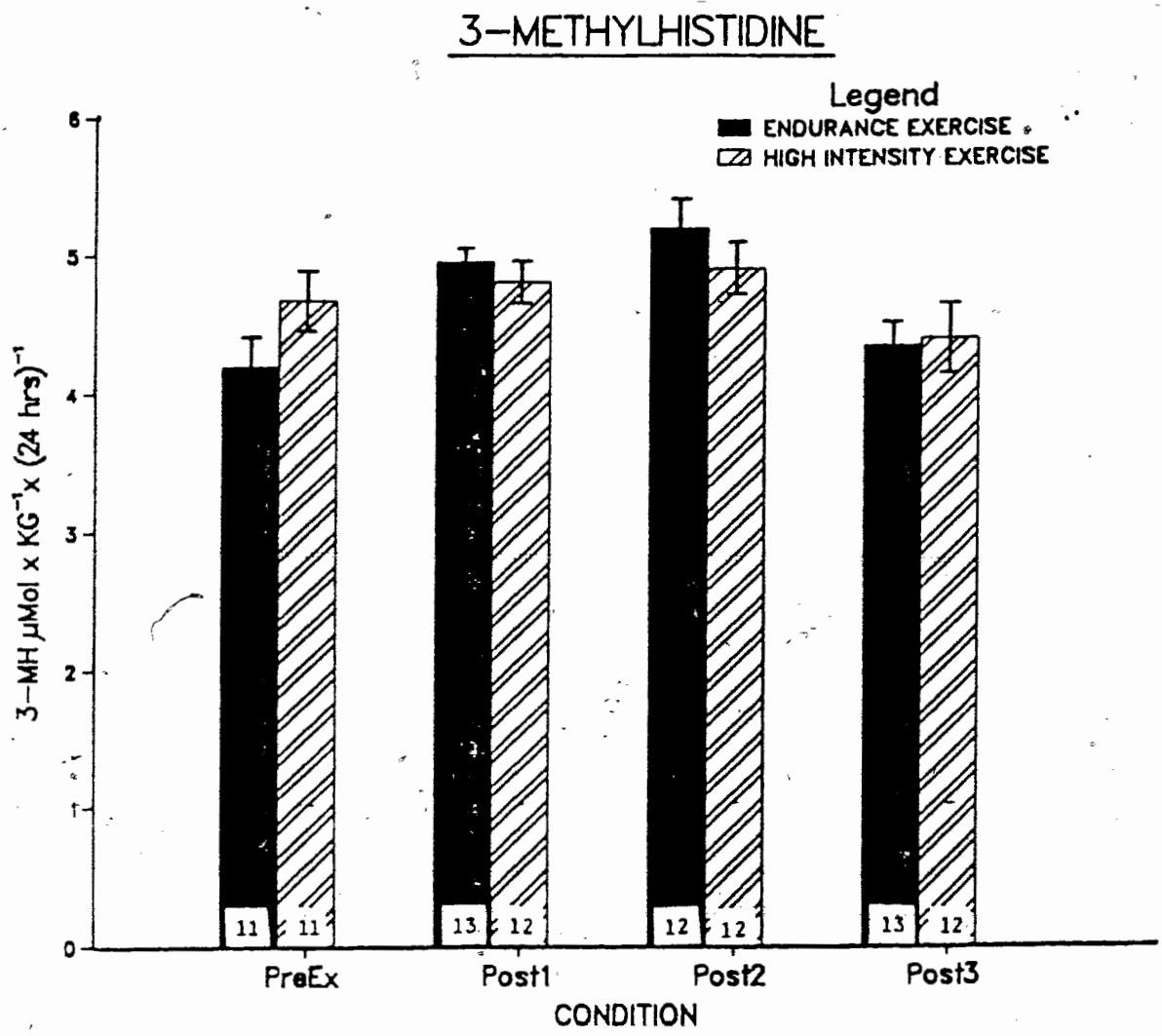


Figure 7. Daily urinary 3-Methlyhistidine excretion following exercise. Values are means of groups corrected for body weight (Kg) ± SEM. N's are given in the bars.

3.8 3-Methylhistidine/Creatinine Ratio

When the 3MH results are expressed as the ratio of 3MH to creatinine, the previously observed effect of diet on 3MH disappears (Figure 8). 3MH/Cr tends to increase in the first 24 hours following both types of work although this increase is not significant (Figure 9). This transient change is followed by a gradual return to normal pre-exercise values by the third day of recovery.

3.9 Urea Nitrogen/Creatinine Ratio

Figure 10 shows that no changes in the ratio of Urea N/CR were observed between exercises or conditions. Pre-diet measures are slightly varied depending upon the exercise protocol as were post 3 results, however these results were not significant.

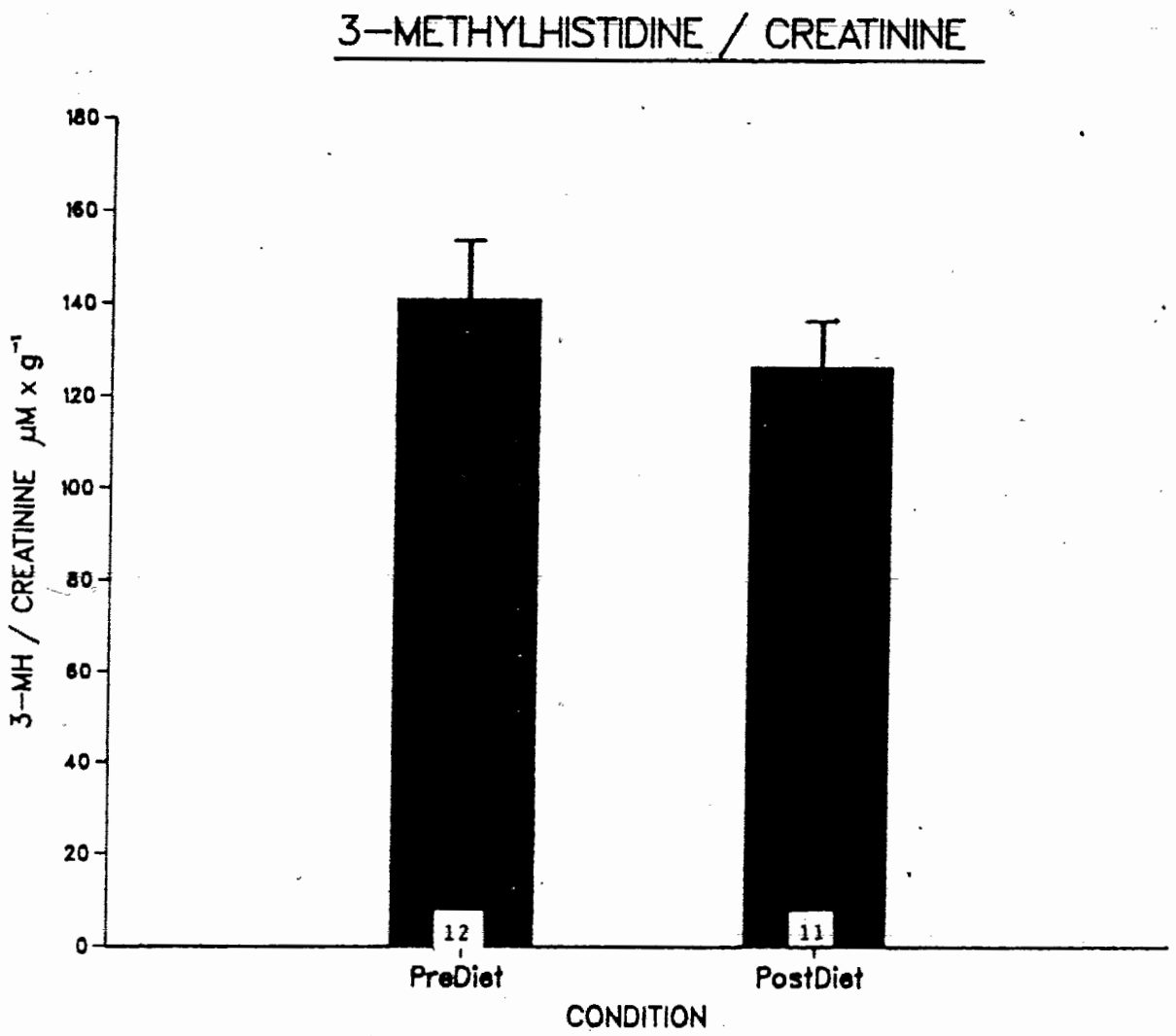


Figure 8. Effects of a three day meat-free diet on the 3-Methylhistidine to Creatinine ratio. Values are means of groups ± SEM. N's are given in the bars.

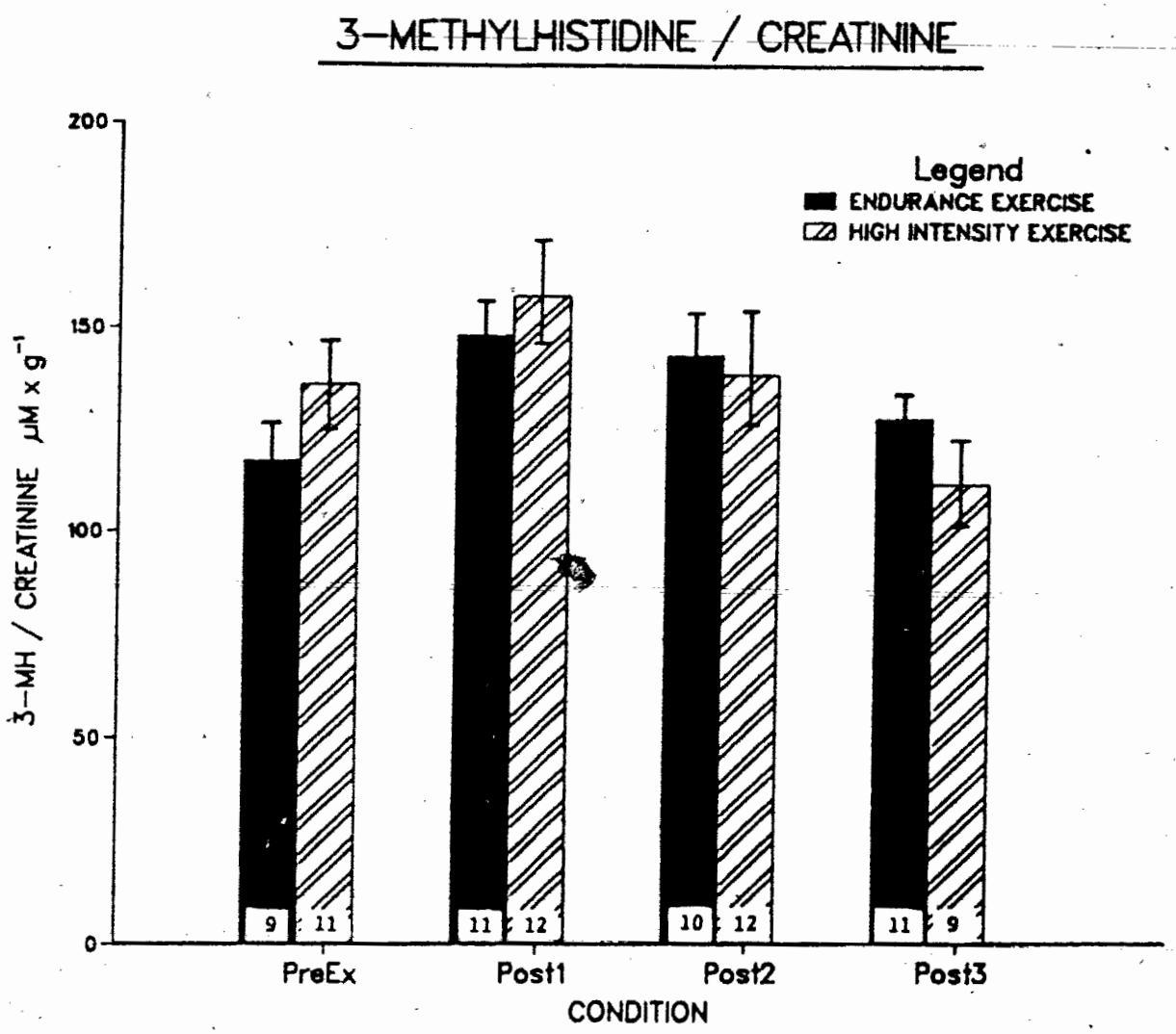


Figure 9. 3-Methlyhistidine to Creatinine ratio following exercise. Values are means of groups ± SEM. N's are given in the bars.

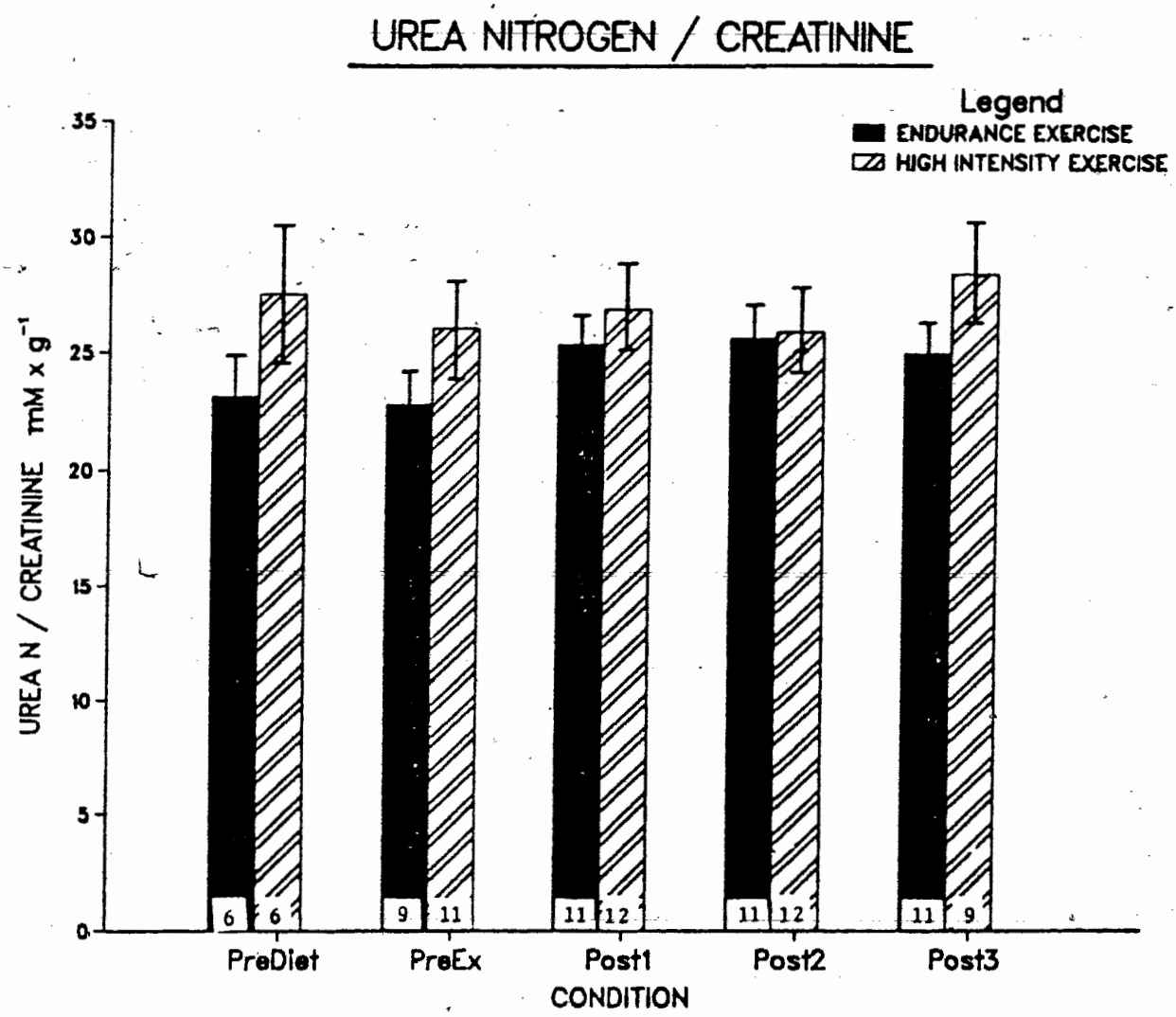


Figure 10. Urinary urea Nitrogen to Creatinine ratio. Determined from 24 hour urinary urea nitrogen levels corrected for daily urinary creatinine excretion. Values are means of groups ± SEM. N's are given in the bars.

3.10 Myofibrillar Protein Degradation

Myofibrillar protein catabolism reached a mean peak value of 104.57 grams of protein per 24 hours following 2 days of recovery from endurance exercise (Table 7). A similar trend was found during the first 48 hours post-HI work with values of 96.72 $g \cdot day^{-1}$ and 98.73 $g \cdot day^{-1}$, Post1 and Post2 respectively. As described earlier, these elevated measures returned to normal pre-ex values within 72 hours of either exercise protocol.

TABLE 7. MYOFIBRILLAR PROTEIN CATABOLISM ($g \cdot day^{-1}$)

PROTOCOL	PREEX	POST1	POST2	POST3
Endurance	84.47 ±3.47 (11)	99.74 ±2.01 (13)	104.57 ±4.23 (12)	87.45 ±3.22 (12)
High Intensity	94.08 ±3.85 (11)	96.72 ±3.02 (12)	98.73 ±3.62 (12)	88.45 ±5.03 (12)

Values are means ± Standard errors of the means.
Subject numbers are in parenthesis.

IV. DISCUSSION

4.1 Exercise Response

4.1.1 Maximal Exercise Response.

The group mean maximal oxygen uptake was 3.25 $l \cdot min^{-1}$ (45.30 $ml \cdot kg^{-1} \cdot min^{-1}$), characterizing the group as untrained, according to White et al., (1976) and Astrand (1977). These authors have suggested that subjects with a $\dot{V}O_2$ max. of above 50 $ml \cdot kg^{-1} \cdot min^{-1}$ should be considered as relatively trained individuals. The mean maximal minute ventilation, ($\dot{V}_E$ BTPS), (131.24 $l \cdot min^{-1}$ ± 5.79) and maximal heart rate (191 $b \cdot min^{-1}$ ± 2.65) appeared somewhat low in the present study, however they are acceptable results for bicycle ergometer exercise. Maximal exercise response from bicycle ergometer work is 10-15% lower than work performed on a treadmill (Astrand and Rodahl, 1977). The specific muscle groups being used on the bicycle result in greater local muscle fatigue. In addition, the size of the muscle group exercised while on a bicycle ergometer is smaller than that used when running on a treadmill. Thus the demand for and consumption of oxygen will be lower during bicycle exercise than during treadmill running.

4.1.2 Submaximal Exercise Response.

Endurance training results in improved efficiency of the cardiorespiratory system as well as of skeletal muscle energy metabolism (Ivy et al., 1981). Since the purpose of this experiment was to study the effects of two different types of exercise, it was felt that any changes occuring due to exercise would be more easily observed in untrained subjects.

During submaximal exercise, heart rate varies linearly with both workrate and oxygen uptake (Astrand and Rodahl, 1977)). As expected, mean heart rates were significantly higher ($p < 0.001$) throughout the high intensity exercise than during the endurance exercise (Figure 3). During exercise heart rate and cardiac output increase in order to supply oxygenated blood to the working muscles (Fox and Mathews, 1981). The rise in heart rate is a response to an increased sympathetic drive and elevated epinephrine levels, which are both related to the severity of the exercise performed (Terjung, 1979).

Circulating norepinephrine and epinephrine levels increase with increasing workrates (Hartley et al., 1972a). A reciprocal response has been reported to occur as a function of exercise duration (Hartley et al., 1972b; Kinderman et al., 1982). Kinderman and co-workers (1982) found that short term, high intensity exercise elicited a greater elevation of catacholamine levels than did long duration steady state exercise. These

observations support the findings of the present study where mean heart rates were higher during HI exercise than during exercise.

Endurance exercise, calculated to be 60% max., produced a mean $\dot{V}O_2$ which reached 66% of $\dot{V}O_2$ max. during a period of one hour. While the HI exercise (120% max) resulted in an oxygen uptake of 71% $\dot{V}O_2$ max. However, it must be realized that the HI $\dot{V}O_2$ results are not representative of true steady state work. Toward the end of each 30 second work interval, subjects had a tendancy to perform a valsalva maneouver which resulted in a lower than expected $\dot{V}O_2$. Furthermore, in high intensity, short duration exercise, energy production is obtained predominantly from ATP and CP stores and anaerobic glycolysis. During the high intensity protocol there was not adequate time for the cardiorespiratory system to become equilibrated with the workrate.

4.2 Dietary Contributions to Urinary Metabolites

4.2.1 Dietary Contributions to Urinary Creatinine Excretion.

Creatinine is a waste product of creatine phosphate metabolism and is closely related to total muscle mass (Ballard, et al., 1979). Fat free weight, as determined by densitometry (Greystone, 1968) and by ^{40}K radiotracer studies (Forbes and Bruining, 1976) has been shown to correlate with 24 hour urinary

creatinine levels. Therefore, urinary creatinine excretion (UCE) has been generally accepted as a valid determinant of total lean body mass. However, this relationship may not always hold true. It has been suggested that the rate of creatinine excretion is affected by diet (Peters, 1973; Wood, et al., 1976). The meat-free diet used in this study did not appear to cause any appreciable change in UCE. However Crim et al., (1975), observed a gradual decline in creatinine excretion after subjects changed from diets containing meat to creatine-free formula diets. Since creatine phosphate (CP) stores are found predominatly in the muscle, a diet including meat, could conceivably cause an elevation in UCE. While some investigators have observed definite diet-induced elevations in creatinine excretion (Crim et al., 1975; Forbes and Pruning, 1976), others have reported only slight changes in urinary creatinine with prolonged intake of a meatless diet (Garza et al., 1977). In order for diet to contribute to creatinine excretion in the urine injested creatine would have to be absorbed across the gastrointestinal lining, for subsequent hepatic metabolism of creatine. The hepatic formation of creatinine would then increase UCE. Since it could also be argued that fish muscle contains CP, elimination of all meat products, regardless of their source, from the diet may be necessary to demonstrate a change in creatinine excretion.

4.2.2 Dietary Effects on Urinary Nitrogen Excretion.

The meat-free diet did not cause any change in urinary nitrogen excretion. Any alteration of the excretion of nitrogen (N) represents a change in nitrogen balance. Although subjects removed meat from their diets, they did not decrease their protein intake. This lack of change in urinary N following three days of the diet attests to the well balanced diet the subjects followed during the period of the experiment.

4.2.3 Dietary Contributions to Urinary 3-Methylhistidine.

While the subjects in this study were not vegetarian, most did not consume large amounts of meat in their regular diets. This may partially explain why the mean values of urinary 3MH observed prior to the meat free diet were slightly lower than previous reports (Tomas, et al., 1979b). A 40% reduction in urinary 3MH occurred following the diet in this study, which is similar to other reports (Tomas, et al., 1979b; Bilmazes, et al., 1978; Rennie, et al., 1981; Dohm, et al., 1982; Decombaz, et al., 1979). Any substantial intake of this particular amino acid in the normal diet in which meat is a common component presents a problem to the use of 3MH as an index of myofibrillar protein catabolism. Exogenous sources of 3MH (ie. diet) will result in an increase in 3MH excretion and a subsequent overestimation of myofibrillar protein degradation. Haverberg et

al., (1975), determined that there was virtually no 3MH in foods other than meat. Tomas and co-workers (1979b) observed that by replacing meat with other sources of protein such as fish and dairy products, 3MH excretion decreased to a substantially lower level within 3 days in human subjects. Similar results were observed during pilot work preceding this study (See Review of Literature, Fig.14). Thus, 3MH excreted following a meat free diet is considered here to be from an endogenous skeletal muscle source.

4.3 Effects of Exercise

4.3.1 Urinary Volumes Following Exercise

During exercise, sympathetic vasoconstriction results in reduced renal blood flow with a concomittant reduction in urine production (Castenfors, 1967; Peart, 1978). This reduction in renal function will vary with the intensity and duration of the work being performed (Kachadorian and Johnson, 1970; Epstein and Zambraski, 1979). No significant changes in urine volumes were observed following either the HI and E exercise compared to pre-exercise volumes in this study. The lack of a significant alteration in urine production post-exercise was likely due, at least in part, to large inter- and intra subject variation.

Exercise elicits an endocrine response which reduces water and sodium loss; thereby conserving extracellular fluid. Renin

is secreted from the kidney in response to sympathetic vasoconstriction and decreased renal blood flow as well as sodium depletion and reduced plasma volume. Renin activates angiotensin which in turn stimulates the release of aldosterone from the adrenal cortex. The maintenance of body sodium content is controlled by aldosterone which increases sodium reabsorbtion from the kidney. The renin-angiotensin-aldosterone system provides for the regulation and maintenance of plasma volume as well as blood osmolarity, both of which are compromised during exercise. Furthermore, elevated plasma antidiuretic hormone (ADH) has been reported even after short term exercise (Baroga and Ciplea, 1978) which would result in lowered urine production. In this study, all exercise protocols were performed in a controlled environmental chamber set at 15°C in order to minimize the effects of temperature on performance. Even so, it was observed that in some subjects sweat loss was quite heavy. One would suspect that, in such cases, there would be a response by aldosterone and ADH in order to maintain homeostatic control.

Elevated renin and ADH during exercise has been found to persist through recovery (Francis and MacGreggor, 1978). In addition, the return to normal levels can take up to 6 hours (Costill, et al., 1976). Any reduction in urine flow will result in alterations in metabolic clearance such that excretion of certain metabolites may be delayed until after termination of endocrine response post-exercise (Refsum et al., 1974). Such a possibility must be considered when taking urine samples

immediately post-exercise. A continuation of renal hormonal response following exercise may account for the trend towards the lowered urine volumes observed postl HI. However, the endocrine response may not be an appropriate explanation for the significantly greater volume excreted at post3 following endurance as opposed to HI exercise. This difference may simply reflect a change in fluid intake during day 3 of recovery, since any re-hydration response to exercise would be expected to have taked place within 24 hours post-exercise. There is no reason to suspect that the increased urinary volume post3 HI and the return to normal post3 E were in response to the exercise protocols.

4.3.2 Changes in Urinary Creatinine Following Exercise.

High intensity work produced a delayed, but significant 30.6% rise in the urinary creatinine excretion (UCE) 72 hours post-exercise. There was no change in UCE following the endurance exercise protocol. There have been few investigations of exercise effects on UCE. Decombaz et al., (1979) and Refsum et al., (1975), observed a 40% increase in UCE immediately following extreme long duration exercise, decreasing again towards normal within 24 hours of the race. This acute elevation in creatinine post-exercise is in agreement with Dohm et al., (1982) who found UCE elevated 24 hours after a 1.5 hour run. However these same investigators also observed that creatinine

excretion was not significantly altered by 1 hour of weightlifting. The results of this study appear to conflict with the above reports as no change occurred post E exercise whereas HI work caused a gradual rise in UCE. A possible explanation for this discrepancy may be the trained state of the subjects used in these studies cited. Untrained subjects, such as the ones used in this thesis work, will not adapt to the workrate as quickly or as efficiently as a trained subject. Another major difference between the present study and these former investigations is that both Refsum and Stromme (1975) and Decombaz et al., (1979), collected urine immediatly post-exercise (8-10 minutes). Thus the discrepancy between their results and those of this study are almost inevitably due to the timing of urine collections. It is possible that a 24 hour collection could dilute and thereby mask any immediate, yet short-lived, elevation in creatinine following exercise. This is an effect which the studies of Refsum et al.,(1975) and Decombaz and co-workers (1979) appeared to favour. Nonetheless, the lack of change in UCE 24 hours post-exercise reported by Dohm et al., (1982), may in fact be an actual delay in creatinine excretion, comparable to that observed post HI. Had these investigators extended their urine collection periods to 48 or 72 hours they may have discovered a latent increase in urinary creatinine.

In addition, the intensity of the exercise being performed may play an important role in the rate of UCE. None of the described exercise studies where UCE was measured actually

quantified the work being performed. Thus the interpretation of their results becomes difficult. Furthermore, as the duration of work performed in the studies of Refsum et al., (1975) and Decombaz et al., (1979) was much greater (5-8 hours) than that of the HI or E protocols of this study, extension of their results to interpretation of the results of this study may not be appropriate.

There is evidence of elevated creatinine excretion during fever and injury (Wannamacher, et al., 1975; Long et al., 1977). These reports may have important implications for exercise since very intense or prolonged work may cause some minor tissue damage (Tomas et al., 1979; Hagerman et al., 1983).

In a recent study, Armstrong and workers (1983) observed a large increase in serum creatine kinase (CK) activity immediatly post-exercise in rats. The concentration of CK returned to normal levels within 12 hours of exercise termination. CK catalizes the reaction whereby CP donates its phophate group to ADP to form ATP and free creatinine. Armstrong et al., (1983), also demonstrated a secondary elevation in serum CK activity 36-48 hours post-exercise. Although the exercise was downhill running (eccentric contraction) the timing of events may be useful in discussing the UCE results in the current study. Any increase in blood CK activity could indirectly result in similar elevations in creatinine excretion. Thus the increased UCE at post2 HI exercise may will be representative of a rise in CK activity.

An increased serum CK activity following exercise has been shown to be directly related to necrosis of muscle fibers following physical activity. It has been suggested that contractile activity induces alterations in muscle membrane permeability (Armstrong et al., 1983) which may result in increased enzyme leakage across the muscle membrane.

An increase in CK leakage may be accompanied by an increase in creatine leakage from the muscle as well. There is some evidence for an elevated serum creatine following acute short-term exercise (Bohmer, 1973). This elevation supports the suggestion that CK leakage from the muscle could be accompanied by an increased flow of creatine across the muscle membrane. If this hypothesis is correct, then urinary creatinine levels would overestimate muscle mass. Since Armstrong et al., (1983), did report that serum CK activity returned to control levels 72 hours following exercise it may not be appropriate to try and explain the continued elevation of UCE post3 HI as a residual effect of elevated CK activity.

The elevation in UCE post3 HI remains to be explained. Initially, it may appear that UCE is proportional to urine volume, as volume post3 HI is also elevated. However, correlation analysis does not support this observation. Therefore some other unidentified factor must be involved with urinary creatinine excretion. Should this hypothesis be correct, the use of creatinine as an index of whole body muscle mass may not be valid, as previously suggested.

4.3.3 Urinary Nitrogen Alterations Following Exercise.

In the current study, urea N excretion did not change following endurance exercise or high intensity exercise. Lemon and Mullin (1980) observed decreased urinary N levels 1 and 4 hours after 1 hour of cycling at 60% of $\dot{V}O_2$ max. However, other studies have shown urinary N to increase within 24 hours of prolonged endurance exercise with a trend to continue increasing after one day of recovery (Refsum and Stromme, 1974; Dohm et al., 1982). The effects of reduced urine production and excretion post-exercise may prevent urinary N alterations from being observed immediately following exercise. Indeed, the results of other research suggest that urinary N increases following endurance type exercise of one hour or more. (Refsum et al., 1974; Dohm et al., 1982). Thus, these latter investigators may not have demonstrated any changes in urinary N simply because of the timing of urine collections. A common methodological problem in all studies cited is the small subject populations tested. Statistical differences are difficult to achieve with small subject numbers. Perhaps significant changes would have been observed had the number of subjects used been greater.

When the urea N results in the present study were expressed simply in terms of body weight, there appeared to be a gradual increase in excretion rate for 48 hours post endurance exercise. In addition, HI exercise produced an initial depression 24 hours

following termination of work, followed by a gradual increase above pre-exercise values with 72 hours of recovery. However, correlation analysis demonstrated a highly significant relationship between urine volume and urinary urea N excretion. By using volume as a covariate in the statistical analysis of urinary urea N, all changes initially observed following exercise disappear. These findings would suggest that prior to interpretation of urea N results, an adjustmemt for urine volume must be made. It is possible, therefore, that the exercise induced changes in urea N reported by Refsum and Stromme (1974) and others, may not have been directly related to the exercise performed but of the production and excretion of urine following the work.

In the past, it was commonly acknowledged that protein was not used as a source of fuel to any appreciable extent during exercise (Astrand and Rodahl, 1977)). Recently however, reports of several experiments have shown elevated urea N levels post-exercise, implying that protein catabolism and amino acid oxidation is increased as a result of muscular work (Gontzea et al., 1974; 1975; Refsum and Stromme, 1974; Dohm et al., 1977; Askew, 1979; Decombaz et al., 1979).

Evidence has been documented demonstrating exercise induced gluconeogenesis (Felig and Wahren, 1971; Huston et al., 1975; Dohm et al., 1982). Nitrogen and gluconeogenic carbon skeletons from the muscle are conveyed to the liver via alanine where the C skeletons from alanine are subsequently reconverted to glucose

(Felig and Wahren, 1971).Amino groups for alanine synthesis are provided by oxidative deamination and transamination of branched chain amino acid. Additional sources of amino groups may also be derived by utilization of ammonia liberated from the purine nucleotide cycle.

There is strong evidence for increased glucocorticoid (GC) levels and inturn gluconeogenesis, during and immediatly following exercise (Davies and Few, 1973; White et al., 1976). However, GC levels will not remain elevated much beyond 6 hours of recovery. If GC are responsible for the induction of protein catabolism in muscle several days post-exercise, the effects appear to be delayed by some intermediate mechanism.

The results of the current study demonstrate that the exercise duration of both protocols did not warrant substantial amino acid oxidation. Urea N content in the sweat of the exercising subjects was not measured in this study. Lemon and Mullin (1980), found that sweat urea N levels dramatically increase with exercise. In order to obtain an accurate description of amino acid oxidation through nitrogen excretion it would be necessary to include sweat N results with urinary N excretion.

Although nitrogen excretion is regarded as a classical determinant of muscle protein catabolism, this assumption is not totally valid. The contribution of N from the body's N pools and liver protein catabolism cannot be ruled out as potential sources of urinary N. Furthermore, an elevation in the

production and/or excretion of N may be a result of the deamination of AMP (Lowenstein, 1972). These metabolic occurances, could lead to an overestimate of the actual quantity of muscle protein degraded.

Of the three types of muscle fibers, fast glycolytic (FG), fast oxidative glycolytic (FOG) and slow oxidative (SO), fast twitch fibers are predominantly overloaded during short, high intensity exercise, such as that performed during the HI exercise protocol. During long-term endurance activity, SO fibers are the first to be recruited (Gollnick et al., 1973a, 1973b). This, coupled with greater AMP deamination in FG fibers than in SO fibers (Meyer and Terjung, 1979), might exaggerate the rise in N excretion. Such a hypothesis may be extended to explain the rise in urinary N levels following HI work.

4.3.4 Effects of Exercise on Urinary 3-Methylhistidine Excretion.

While the endurance exercise protocol resulted in a slight increase in urinary 3MH concentration during the first 48 hours following the cessation of work, no changes occured post HI exercise. Dohm et al.,(1982) made similar observations following 1.5 hours of running. Unfortunately, urine collections in their study were only taken immediately post-exercise and 24 hours later. In contrast, Decombaz et al., (1979), reported no change in 3MH output during heavy work, while Rennie et al., (1981) and

Millward et al., (1982) reported decreases. However, one must be cautious when comparing these results with the present study since 1) urinary volumes were not reported, 2) the state of dehydration of the subjects is unknown, 3) the timing of post-exercise urine collection varied, and finally, 4) the intensity of exercise performed were not quantified and varied from study to study.

Unlike nitrogen, 3MH is not found in any measurable quantity in the sweat (Dohm et al., 1982). Since the 3MH excreted in the urine presumably comes from skeletal muscle, then the amount of protein degraded may be calculated. There was a 33% increase in protein degraded over pre-exercise levels 48 hours following endurance exercise, which represents 104.5 grams of muscle protein per day. A similar, yet smaller increase (16%) was noted following HI exercise as well. However, this increase in the amount of myofibrillar protein breakdown does not necessarily represent a net increase in the degradation of muscle protein. While the rate of degradation has been elevated, it is quite possible that a similar response has been elicited from protein synthesis resulting in a rise in total protein turnover.

Fuel homeostasis and metabolism varies with the intensity and duration of the exercise being performed. As previously discussed, urine flux is depressed during exercise and may not return to normal immediately post-exercise. Hence, the timing of urine sample collection may be crucial. It is conceivable that

the 24 hour urine collections made in the present study disguised any actual increases that may have occured. Shorter collection periods may in fact give a better indication of what is actually happening in the muscle following exercise.

Several proteinases which can degrade myofibrillar proteins have been described in muscle. Lysosomal enzymes, with their acid pH optima no doubt participate in the autolysis of muscle protein and, thus, have definite implications for exercise (Schwartz and Bird, 1977; Pilstrom, et al., 1978; Clark and Vignos, 1981). Work by Vihko et al. (1978) and others (Pilstrom, et al., 1978; Dohm, et al., 1982) using animal models, have brought forth interesting evidence of increased lysosomal activity with exercise. A single bout of exhaustive exercise has been observed to increase the activity of Cathepsin D, Cathepsin B and several other lysosomal enzymes in mouse muscle (Pilstrom, et al., 1978; Vihko, et al., 1978; Clark, et al., 1981). Interstingly, Vihko, et al. (1978) found that lysosomal enzyme activity peaked 3 to 4 days following one single exhaustive bout of exercise.

The timing of these proteolytic events may help to explain reports of myofibrillar necrosis and selective degradation in human muscle following marathon running (Hagerman, et al., 1983; Seigal, et al., 1983; Apple, et al., 1983). According to Hagerman and co-workers (1983), the most prominent muscle fiber alteration occurs between day 1 and day 3 post-exercise. This observation is similar to the present study where myofibrillar

degradation, calculated from urinary 3MH, reached a peak 48 hours post endurance exercise. In view of these reports, one may speculate that had the duration of the exercise protocols in the present study been prolonged, more dramatic changes in myofibrillar protein degradation might have taken place.

4.3.5 Rate of Muscle catabolism

Expressing 3MH in terms of body weight may not be very accurate due to changes in body weight and state of hydration of subjects, particularly in exercise studies. Furthermore, since 3MH is derived from the catabolism of myofibrillar protein, calculations per unit muscle mass are more appropriate than body weight corrections. The 3MH/CR ratio has been used as a method of expressing the rate of skeletal muscle being degraded per day since CR excretion is used to estimate total body muscle mass (Tomas, et al., 1979; Ballard, et al., 1979). The amount of whole body muscle mass degraded following both exercise protocols was calculated and expressed as the ratio 3MH/Creatinine (3MH/CR). The 3MH/CR ratio is elevated, although not significantly, 24 hours following the termination of both endurance and HI exercise protocols. These results are comparable to those documented by Dohm et al., (1982), and Millward et al., (1982). Similar observations were also made using the ratio of Urea N/Cr: no significant alterations in protein degradation were demonstrated following either exercise.

These observations are supported by the results of Dohm et al., (1982), who found urea N/CR ratio to remain unchanged following both running and weightlifting exercise bouts. The calculation of the 3MH/CR and urea N/CR ratios make the assumption that creatinine is an accurate measure of lean body mass and therefore is at stable levels in the urine. Furthermore, they also assume that both urinary 3MH and urea N are from endogenous skeletal muscle sources. In the present study, the excretion rate of creatinine was altered significantly following exercise. Therefore the ratios of 3MH/CR and urea N/CR do not appear to be an accurate method of expressing the rate of skeletal muscle protein breakdown, following exercise.

LIMITATIONS OF THIS STUDY

A) The rate of myofibrillar protein degradation was determined by measuring urinary 3MH excretion using the assumption that skeletal muscle is the major source of 3MH. There is a possibility that a portion of urinary 3MH was derived from the turnover of other body tissues, namely the gastrointestinal tract (GI). While at rest the GI tract contribution to urinary 3MH is small; an exercise induced increase in the turnover of non-skeletal muscle protein would increase 3MH excretion.

B) There are a number of problems associated with the use of human subjects in an experimental design such as the one used in this study:

i. It was the responsibility of each subject to remain on the meat free diet throughout the experiment. It is possible that some meat was consumed and not reported. In addition, while alcohol consumption was limited during the tests, fluids were not restricted or measured.

ii. It was necessary for subjects to collect all urine excreted during the testing period. Any urine excretion missed and not reported would cause great

alterations in the results.

iii. Large biological variations were demonstrated in urine production as well as urea N and creatinine. This coupled with a small subject population may have contributed to the lack of statistically significant observations.

C) As all participants were untrained males, caution is advised when relating the results of this study to female subjects or trained individuals.

D) In this study, only the degradative phase of myofibrillar protein turnover was measured. In order to appreciate the effects of exercise on muscle metabolism, protein synthesis must also be assessed. An increase in skeletal muscle protein breakdown may not actually define a net loss of protein, but may in fact reflect an increase in myofibrillar turnover.

CONCLUSIONS

Within the limitations of this study, the following conclusion were drawn:

1. High intensity (120% max) interval exercise produces a greater elevation in heart rate than endurance exercise (60% max) in men.

2. Three days of a well balanced meat free diet results in a significant depression in the excretion rate of 3MH, however no alterations in creatinine or urea nitrogen occur.

3. No significant changes in daily urine production occur following one hour of either high intensity or endurance exercise.

4. No statistical differences in the urinary excretion of 3MH are observed between one hour of high intensity interval work or one hour of steady state endurance exercise.

5. Urinary urea nitrogen excretion is effected by urine volume.

6. High intensity exercise causes an elevation in urinary creatinine while no changes in UCE are observed following prolonged steady state work.

7. The ratio of 3MH/CR may not be an accurate index of fractional myofibrillar protein degradation post exercise due to the changes in creatinine excretion following work.

APPENDIX 1. EXERCISE PROTOCOL

All exercise tests were performed on a manually braked Monarche bicycle ergometer. All exercises were conducted at a pedal frequency of 60 rpm, with the exception of the high intensity test where a pedal rate of 90 rpm was used. Pedal frequency was monitored by an electric metronome and a revolution counter attached to the front wheel of the ergometer. Each test was preceeded by a 6 minute warmup. All subjects began the warmup at 360 $kpm \cdot min^{-1}$ (1kp), with workrate increasing by 1 kp at minute 3 and again at minute 5. The final increase in workrate was dictated by the individual's heart rate at the end of minute 4. Warmup was followed by a five minute rest while subjects were aquainted with the procedures of the test to be performed.

Maximum Oxygen Uptake Test.

The starting workrate for the maximum test was set upon the basis of heart rate (Saltin and Astrand, 1967). The initial workrate was performed for 2 minutes followed by an increase of 180 kpm/min (0.5 kp) at the end of every minute until the subject could no longer continue pedalling at the prescribed frequency.

Heart rates were monitored on a Nihon Kohden electrocardiograph (cardiofax ECG 2101) using 3 Meditrace

silver-silver chloride disposable ECG electrodes (Graphic Controls Ltd, Quebec) set in a CM5 position. Heart rates were taken for the last 10 seconds of every minute during warmup, exercise test and for 3 minutes post exercise.

Head gear was placed on each exercising subject to hold a two-way, low resistance respiratory valve in their mouth. All expired air passed from this valve, through 70cm of Collins uncorrugated tubing, past a two-way stop-cock controlled valve and into meteological balloons. Each bag contained one full minute of expired air. Expired gas was collected during minutes 2,4 and 6 of the warmup and for every minute or part of minute of the test until the subject stopped. All meteological balloons were tested for leakage prior to use.

Samples of gas were drawn from each balloon into a Beckman LB-2 Carbon dioxide analyzer and an Applied electrochemistry Oxygen analyzer in order to determine the fraction of expired oxygen (F_EO_2) and CO_2 (F_ECO_2). The volume of expired gas ($\dot{V}_E$) was obtained by evacuating the contents of each balloon through a Parkinson-Cowan ventilation meter by means of a small .25hp vacuum pump attached to the exhaust side of the meter. Gas temperature was recorded as the air traveled through the meter. Barometric pressure was also recorded in the morning and afternoon of each day of testing.

Oxygen consumption ($\dot{V}O_2$) was determined by the equation:

$$\dot{V}O_2 = \dot{V}_IO_2 - \dot{V}_EO_2$$

where $\dot{V}_EO_2$, being the volume of O_2 expired

$$= \dot{V}_E(STPD) \times \dot{F}_EO_2 /100$$

and $\dot{V}_IO_2$, the volume of $\dot{O}_2$ inspired

$$= (\dot{V}_I \times \dot{F}_IO_2)/100$$

when

$$\dot{V}_I = \dot{V}_E(STPD) \times (100-(\dot{F}_EO_2 + \dot{F}_ECO_2))/79.04\%$$

Known gases, analyzed by the micro-scholander technique (1945), were used to calibrate the gas analyzers prior to each ventilation analysis.

It was the intention of this study to use untrained subjects. Final choice of subjects was made on the basis of their $\dot{V}O_2max$. test. Any subject with a $\dot{V}O_2max$ of greater than 50 $ml \cdot Kg^{-1} \cdot min^{-1}$ (White et al.,1976; Astrand and Rodahl, 1977) was not accepted into the study.

APPENDIX 2. EXERCISE PROTOCOL

Endurance Exercise

Subjects exercised on the bicycle ergometer at a pedal frequency of 60 rpm for one hour at 60% of their predetermined maximum $\dot{V}O_2$. A six minute warmup (as described in Appendix 1) was conducted prior to the test. Heart rate was monitored, as previously described (appendix 1), during the last ten seconds of each minute of warmup, every fifth minute of the exercise test and for a three minute recovery period. A thirty second collection of expired gas was made every 10 minutes of the endurance test for determination of oxygen consumption.

High Intensity Exercise

Subjects rode the bicycle ergometer at a pedal rate of 90 rpm for a set of 40 thirty second sprints at 120% of their predetermined maximum $\dot{V}O_2$. Each effort was followed by a recovery period of 60 seconds. Participants were requested to remain seated at all times throughout the sprint work, but were allowed to stand during the intervals between sprints. Heart rates were monitored for the last 10 seconds of every minute of warmup, for every eighth sprint and for the three minutes of recovery. Using separate meteological balloons, oxygen consumption was measured during every eighth sprint (30 sec) and

for the subsequent 30 sec of recovery. Methods for measurement of heart rate and $\dot{V}O_2$ have been described in detail in Appendix 1.

APPENDIX 3. 3-METHYLHISTIDINE DETERMINATION

REAGENTS

Sodium Citrate: at concentrations of 0.2N, 0.35N and 0.38N. Buffer solution pH adjusted with 50% NaO_2H or concentrated HCl.

Resin: Resin slurry was prepared at a 2:1 resin to distilled water solution (Resins:PA-28, PA-35, Beckman Co., Palo Alta, Calif.)

Sodium Acetate Buffer: (used for ninhydrin solution). 4N Na acetate buffer formed by adding 2730g Na acetate with 500ml concentrated acetic acid in 5 liters distilled H_2O.

Ninhydrin: 80g ninhydrin disolved in 1 liter of 4N Sodium acetate buffer and 3 liters Methyl Cellosolve (Beckman Co., Palo Alta, Calif.). Add 1.6g Stannous Chloride ($SnCl_4H_2O$).

Regeneration Reagent: 0.2N NaOH.

Protein Precipitation: 30g sulfosalicylic acid diluted into 100ml distilled H_2O = 30% SSA.

Urine constituents were separated by the analytical columns and then reacted with ninhydrin in order to become visible for colorimetry. As the colored compound passed through a cuvette in the analyzer, light from a tungsten light source also passes through and falls on a photoelectric cell which generates a voltage proportional to the transmittance of the material in the

cuvette. The record of the optical density forms a gaussian curve. 3MH will react with ninhydrin at 570 nanometers. Peak areas are determined in terms of absorbance multiplied by units of time, or height x width. The area of the peak represents the quantity of the original constituent. Calculation of this area and comparison with peak areas from calibration standards are a quantitation of 3MH (Figure 11).

In addition, a Nova Digital Computer was connected to the Beckman Amino Acid Analyzer. This integrator automatically added the absorbance values at small time increments and printed out the total when the proper baseline return was achieved (Figure 11).

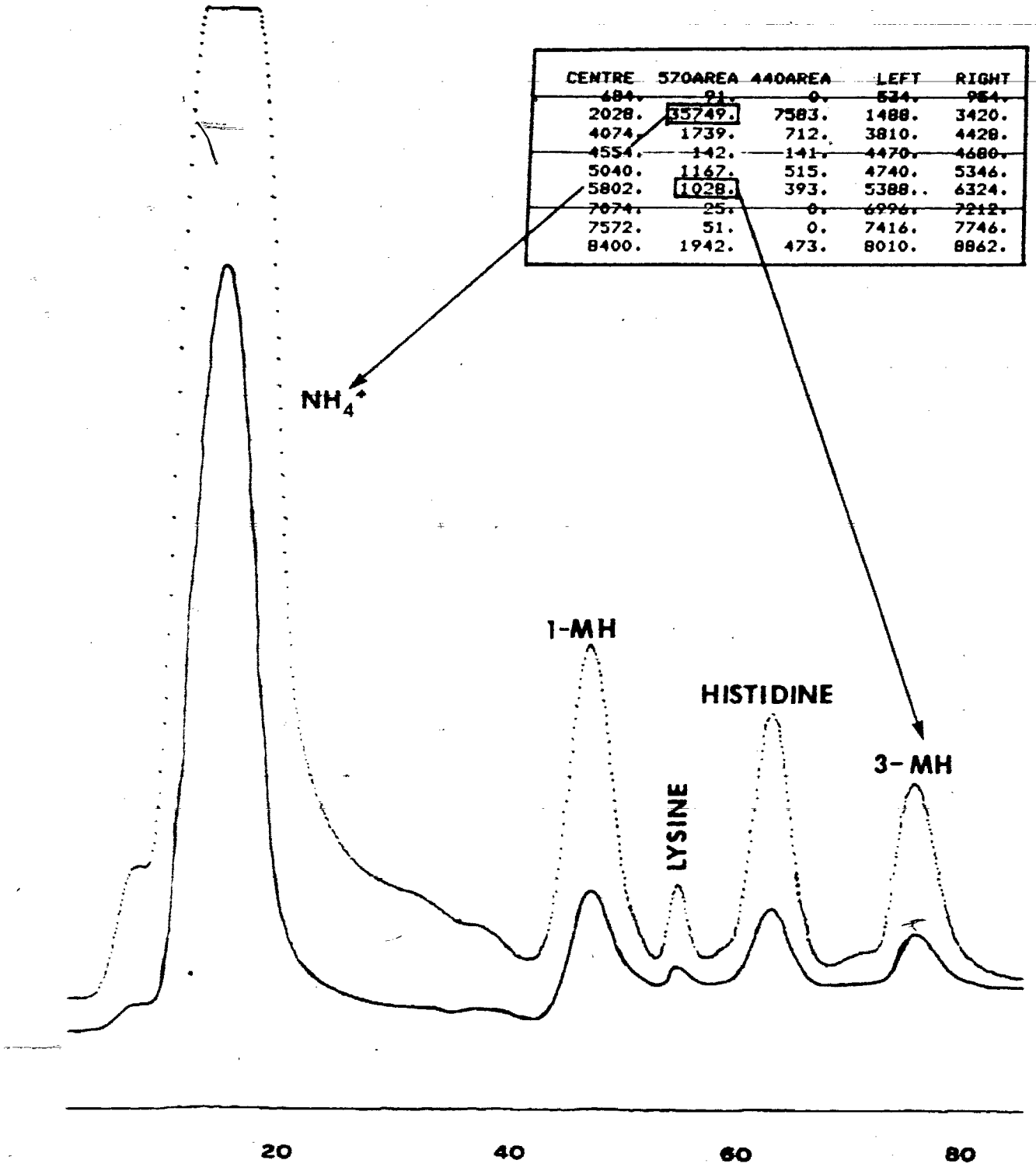


Figure 11. Chromatograph and Nova Computer printout from one 24 hour urine sample. Peaks represent concentration. Printout values represent area under each peak.

APPENDIX 4. CREATININE DETERMINATION

Reagents

1. Sodium Hydroxide: 1.0 N
2. Creatinine Color Reagent: Contains Picric Acid, Approx.0.6%; Sodium Borate and Surfactant. (Sigma, St.Louis, #555-1)
3. Alkaline Picrate Solution: Prepare by adding 150 ml creatinine color reagent with 30 ml sodium hydroxide solution (ratio 5:1). Mixture is stable for 1 week in dark at room temperature.
4. Acid Reagent: Contains a mixture fo sulfuric Acid and Acetic Acid (Sigma, St.Louis, #555-2).
5. Creatinine Standards: Contain creatinine 3.0 mg/100 ml (0.26 mmol/l) and 15 mg/100 ml (1.32 mmol/l) respectively in 0.02 N HCL solution (Sigma, St.Louis, #925-3,925-15).

Procedure

1. Dilute 1 ml of each urine sample by 10 fold using deionized H_2O (dH_2O).

2. Set up directly in cuvets:

   Reagent Blank: 3.0 ml dH_2O

Standard 1: 3.0 ml (STD 1)

Standard 2: 3.0 ml (STD 2)

Unknown: 3.0 ml Diluted urine.

3. To all cuvets add 3.0 ml Alkaline Picrate Solution. Mix well and let stand for 10 minutes +/- 2 minutes at room temperature.
4. Read initial Absorbance of all cuvets, using blank as reference, at 500 nm.
5. To all cuvets, add 0.1 ml Acid Peagent. Mix immediately and allow to stand 5 minutes at room temperature.
6. Read final absorbance of all cuvets against the blank at 500 nm.

Each standard was analysed for every batch of urine samples run (8 unknown samples). Calculation of the concentration of creatinine in each sample was determined using the slope of the standard curve produced per batch of samples analysed.

APPENDIX 5. URINARY NITROGEN DETERMINATION

Reagents

1. Buffered Urease Disolve 5gm disodium salt of EDTA in 200ml glycerin and 250ml dH_2O. Adjust to pH 6.5 with 4% NaOH (approx 10 ml required). Dilute to 500ml. Then disolve 30mg urease (type III, Sigma St. Louis) in 100ml of this buffer. Stable in refrigerator for 1 month.
2. Phenol Reagent Disolve 5gm phenol and 25gm Na Nitroprusside in dH_2O to make 100ml. Stable for several months in amber bottle in refrigerator.
3. Alkaline Hypochlorite Disolve 12.5gm NaOH in 400ml H_2O. Cool, add 20ml Na Hypochlorite (Javex, Proctor and Gamble: 5.25% chlorine) and dilute to 500ml. Store in glass container in refrigerator.
4. Benzoic Acid (0.016M) Disolve 2gm benzoic Acid in 1 liter H_2O. Add 0.8ml concentrated sulfuric Acid and mix.
5. Urea Standard 1 Disolve 0.644gm urea in benzoic Acid solution and dilute to 500ml. Contains 60mg/100ml urea (46.42% Nitrogen).
6. Urea Standard 2 Contains 30 mg/100 ml Urea Nitrogen (Sigma, St.Louis).

Procedure

1.Dilute urine 1:10

2.Set up in test tubes:

Blank: 0.5ml buffered urease

Standard: 0.5ml Buffered urease + 0.02ml urea standard.

Unknown: 0.5ml Buffered urease + 0.02ml diluted urine.

Ammonia: 0.5ml Water + 0.02ml of same sample as unknown.

3. Incubate in water bath at 37°C for 15 minutes (excluding Ammonia sample).

4. To all samples:

Add 1ml phenol to each tube, mix.

Add 1ml alkaline hypochlorite and mix immediately.

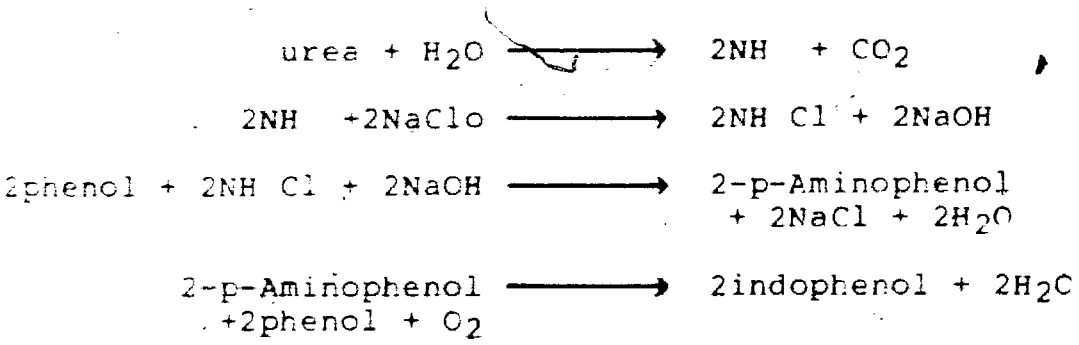
MUST be mixed in this order.

5. Incubate in water bath at 37°C for 15 minutes.

6. Add 5 ml dH_2O, mix, place into cuvets and read samples and standards against blank at 540 nm.

Each standard was analysed with every batch of urine samples run (10 unknown samples). Calculation of the concentration of urea nitrogen in each sample was determined using the slope of the standard curve produced per batch of samples analysed.

Principle:



(Bergmeyer, Vol 4., 1974)

APPENDIX 6. REVIEW OF THE LITERATURE

The theory that protein was the essential fuel for exercise began as early as 1850 with the work of Von Leibig. This hypothesis was dispelled in the early 1900's by Zuntz, Von Petterkofer and Voil who showed that protein was not metabolized in significant quantities during muscular exercise of well nourished men (as cited in Consolazio et al., 1975). It is now understood that carbohydrate and fatty acids are the major energy sources for muscular work in the isocaloric state (Astrand 1967; Essen et al., 1977; Lemon and Naigle, 1981).

In recent years, there has been an accumulation of evidence in support of active protein utilization particularly during long duration exercise (Cerney, 1975; Gontzea et al., 1975; Refsum and Stromme, 1974; Haralambie and Berg, 1976; Decombaz et al., 1979; Dohm et al., 1977; 1978; 1980; Lemon and Mullin, 1980). Furthermore, it would appear that a human requirement for protein is to provide nitrogen and essential amino acids for the synthesis of body protein necessary for growth and repair (Neerunjen and Dubowitz, 1977).

The rate of protein turnover is altered by variations in synthesis, degradation or a combination of both. By virtue of its large mass, skeletal muscle is the body's predominant resevoir of amino acids and protein; the bulk of this protein being in the form of the contractile elements, actin and myosin. The turnover of muscle proteins is not as rapid as some of the other body tissues. However, due to the enormous mass of this

tissue, making up above 45% of body weight, it may be assumed that the turnover of skeletal muscle protein plays a major role in the regulation of whole body protein metabolism (Poortmans, 1976). As demonstrated in Figure 12, changes in the dynamic state of muscle protein and amino acid metabolism will be brought about by a number of stimuli including diet, hormones and exercise (Young et al., 1981).

It is well undestood that exercise results in adaptations of muscle metabolism and function. The processes involved include changes in both protein synthesis and breakdown as the muscle composition and size are altered. This review attempts to relate intensity and duration of exercise with alterations in the degradation of myofibrillar protein.

1.1 Amino Acid Metabolism

In order for proteins to contribute to energy supply they must first be degraded into their component amino acids. Should these amino amino acids be subsequently deaminated, energy will be produced by the oxidation of the resulting carbon skeletons. This oxidation is normally measured in one of two ways: 1) CO_2 production from protein is quantified through the use of radioactive label tracers and/or, 2) by the measurement of products from the degradation of protein . This latter method has, in the past been commonly used in human studies, by quantifying urea nitrogen excretion.

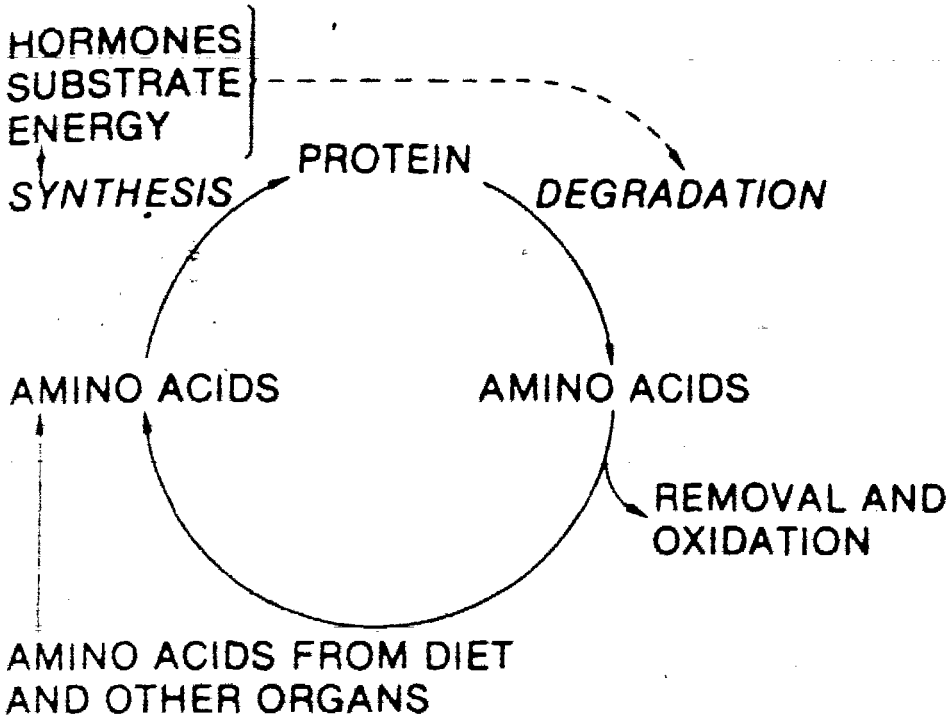


Figure 12. Muscle protein content is determined by the rates of protein synthesis and degradation and the balance between them. (From V.R. Young, 1981)

It has been clearly documented that strenuous exercise particularly of prolonged duration is accompanied by enhanced protein catabolism (Refsum and Stromme, 1974; Cerny, 1975; Haralambie and Berg, 1976). Studies of exercise metabolism on activity durations of less than one hour strongly indicate that the energy requirement can easily be met by glycogen and fatty

acid breakdown. More recently, reports have indicated a small, but significant role of amino acid metabolism during short term work (Felig and Wahren, 1971; Johnson, 1971) with increasing involvement as the duration of work increases (Keuls et al., 1972; Mole, Baldwin, Terjung and Hollozy, 1973).

Several reports have demonstrated elevated urea N levels following exercise. An increase in the excretion of N in the form of urea would imply that protein utilization and therefore amino acid oxidation is indeed elevated with physical activity (Gontzea et al., 1974; 1975; Refsum and Stromme, 1974; 1975; Dohm et al., 1977; Decombaz et al., 1979).

The magnitude of protein involvement during exercise may be determined by the duration of the activity being performed. Cerny (1975) demonstrated a 20% elevation in serum urea during exercise, but not until after one hour of bicycle exercise at 60-65% $\dot{V}O_2$ max, while urinary urea excretion was depressed 1.5 hours post exercise. This decrease probably is a reflection of an exercise induced depression of kidney function and urine production. Haralambie and Berg (1976), in a study of 6 groups of subjects exercising at various intensities and durations, observed that serum urea increased quite linearly with exercise duration beginning after 70 minutes of work. Of interest, is the simultaneous fall in serum amino N. As the authors explain, these observations occur at a time when liver glycogen is considerably decreased (Hultman and Nilsson, 1973) and muscle glycogen severely depleted (Bergstrom et al., 1967; Costill et

al., 1971).

More recently, Dohm and co-workers (1982) demonstrated that 1.5 hours of running significantly increases urinary urea excretion during the 24 hours post exercise. In the same study, these researchers found no significant elevation in excreted urea following approximately one hour of a weightlifting routine.

Results of increased urea N production following severe long duration exercise have been documented. Decombaz and co-workers (1979) observed that a 100 km running race produced an immediate elevation in plasma urea levels though an increase in urinary urea was observed only after 24 hours of recovery from the exercise. These results support the observations of Refsum and Stromme (1974; 1975) who observed a 60% elevation in plasma urea immediately following 70 and 90 km cross country ski racing. These investigators also found elevated urinary urea levels although no significance was achieved until the day following the events. The reason for immediate elevations in plasma urea levels and delayed changes in urinary urea may be explained as a result of kidney function during exercise. It is generally accepted that urinary urea elimination rate is impaired, to some extent, by exercise (Haralambie and Berg, 1976). In fact, a depression in urea clearance has been reported during a 70 km ski race (Refsum and Stromme, 1974). However, this lower elimination by the kidney may be compensated for by the excretion of urea through the process of sweating (Lemon and

Mullin, 1980). Lemon and Mullin (1980) determined that by combining both methods of urea N excretion (urine and sweat), the observed exercise depression in kidney excretion was completely compensated for by sweat urea N. They proposed that the increased serum urea N levels found with exercise do in fact reflect an increased protein catabolism.

Increases in nitrogen production reflect an increase in amino acid catabolism. This may occur due to an increase in gluconeogenesis during exercise as amino acids may be used as gluconeogenic precursors. Alanine is quantitatively the most important gluconeogenic amino acid as it represents a means of conveying nitrogen groups from muscle to liver (Felig, Owen and Wahren, 1969). Because of the predominance of alanine in the amino acid output from muscle and in view of the fact that alanine is largely derived from pyruvate, Felig (1973) has described a glucose-alanine cycle involving both muscle and liver. The carbon skeletons of alanine may originate from other amino acids, thus posing the possibility that alanine transfer between muscle and liver may not only represent the recycling of carbon skeletons but also 'de novo' synthesis of glucose (Goldstein and Newsholm, 1976). While amino acids could be utilized in this fashion as a source of carbon for energy production, their role would most likely be minimal. An elevation in protein metabolism probably occurs to provide an amino acid source for pyruvate removal (Mole and Johnson, 1971; Wahren et al., 1973) via the glucose alanine cycle as well as

for controlling levels of tricarboxilic acid cycle (TCA) intermediates (Lowenstein, 1972). A variety of conditions result in alterations in TCA cycle intermediates, including exercise. The suggestion that exercise induces an increased leakage of intermediates from the mitochondria (Cerny, 1975) would imply the need for increased participatin of amino acids for the replacement of the intermediates during exercise metabolism.

Nitrogen excretion has been traditionally used as a measure of muscle protein catabolism. However, caution is advised when using this method of determining myofibrillar degradation. Interpretation of results from studies using amino acid tracers are made difficult since most amino acids released during intracellular protein catabolism can be extensively reutilized for protein synthesis within the cell (intracellular recycling) or may be transported to other organs where they may enter pathways of protein anabolism (intercellular recycling) (Young and Monro, 1978). While an increase in skeletal muscle degradation will result in a subsequent increase in N production and excretion, an elevation in N levels may not necessarily reflect a proportional rise in muscle protein breakdown. The contribution of N from the body's N pools and from the degradation of protein in liver (Gontzea et al., 1975) must not be ignored as sources of urinary N. Moreover, it has long been recognized that exercising muscles have the ability to produce N by the deamination of AMP (Lowenstein, 1972).

Of the three types of muscle fibers, fast glycolytic (FG), fast oxidative glycolytic (FOG), and slow oxidative (SO), AMP deamination is greater in FG fibers than in slow twitch fibers (Meyer and Terjung, 1979). In view of these facts and the evidence of greater recruitment of fast twitch motor units as exercise intensity increases (Gollnick et al., 1973(a); 1973(b)), there is a definate possibility that N excretion values may overestimate the actual quantity of myofibriller protein being degraded with high intensity exercise.

1.1.1 Endocrine Response to Exercise

The role played by individual hormone repsonses of muscle protein metabolism is difficult to evaluate. Exercise in human subjects causes many changes in the circulating levels of hormones. The degree of change depends upon the the nutritional state of the subject (Ahlborg and Felig, 1977), the extent of training (Hartley et al., 1972 b; Sutton et al., 1978) and the intensity of the exercise (Hartley et al., 1972 a; White et al., 1976; Davies and Few, 1973). Furthermore, evidence of endocrine response to exercise demonstrate that these hormonal changes are temporary and occur during, as opposed to following work (Hartely et al., 1972 b; Keul et al., 1981).

Mild exercise enhances blood glucose uptake into skeletal muscle (Wahren et al., 1971). Maintenance of blood glucose homeostasis is made possible via endocrine changes which

preserve blood glucose for use by the CNS and brain (Pruett, 1971). Hormonal responses to hypoglycemia include increases in epinephrine, growth hormone, glucogon and cortisol as well as a depression of insulin levels, all of which are likely mediated by blood glucose levels as well as from sympathetic discharge from the autonomic nervous system. The elevation of glucocorticoids, are of particular interest in this review. The principle functions of glucocorticoids are concerned with the metabolism of carbohydrates, fatty acids and protein. These hormones stimulate gluconeogenesis resulting in an increase in the hepatic conversion of amino acids into glucose. Cortisol stimulates protein catabolism in muscle and subsequent release of amino acids into the circulation (Terjung, 1979).

The response of glucocorticoid release with exercise appears to be dependant upon the intensity of work involved in relation to the subject's maximal work output rather than the absolute amount of work being performed (Hartely et al., 1972 a; Davies and Few, 1973; White, Ismail and Bottoms, 1976). Davies and Few (1973) have suggested that a workload of greater than 60% of $\dot{V}O_2max.$ is necessary to elicit a rise in cortisol levels.

1.2 Urinary 3-Methylhistidine Excretion

There is, accordingly, a need for a non-invasive method of measuring the absolute rate of skeletal muscle protein catabolism. Urinary 3-Methylhistidine (3MH) has recently been

exploited as a quantitative index of myofibrillar protein turnover in humans. 3MH was first identified as a component of urine by Talen (1954). It was later indentified as a constituent of both actin and myosin. (Assatoor and Armstrong, 1967; Johnson et al., 1967). 3MH is present in the globular head of the myosin heavy chain (MHC) and constitutes the 73rd residue of the actin polypeptide chain (Elzinga et al., 1973). This derived amino acid is produced by methylation of a histidine residue and is present in actin of all cells and myosin of FG fiber types (Young et al., 1970; Johnson et al., 1967). It has been demonstrated that there is 1 mole of 3MH per mole MHC in FG myosin however, it is absent in the myosin of fetal muscle, cardiac muscle and both oxidative fiber types (Keuhl, 1970). 3MH does not charge tRNA, therefore does not undergo metabolism or reutilization for protein synthesis and consequently is quantitatively excreted in an identifiable form in the urine (Figure 13). It is released at the same time as other amino acids during myofibrillar protein catabolism (Monro and Young, 1978). Studies have demonstrated that 3MH is excreted as the unchanged amino acid in man (Young et al., 1972; Haverberg et al., 1977) rather than as it's N-acetyl derivative in rat (Haverberg et al., 1975(a); 1975(b); Long et al., 1975). In addition, Long et al., (1975), confirmed the quantitative excretion of 3MH by man by demonstrating the absence of detectable $^{14}CO_2$ in the expired air following administration of (^{14}C)3MH.

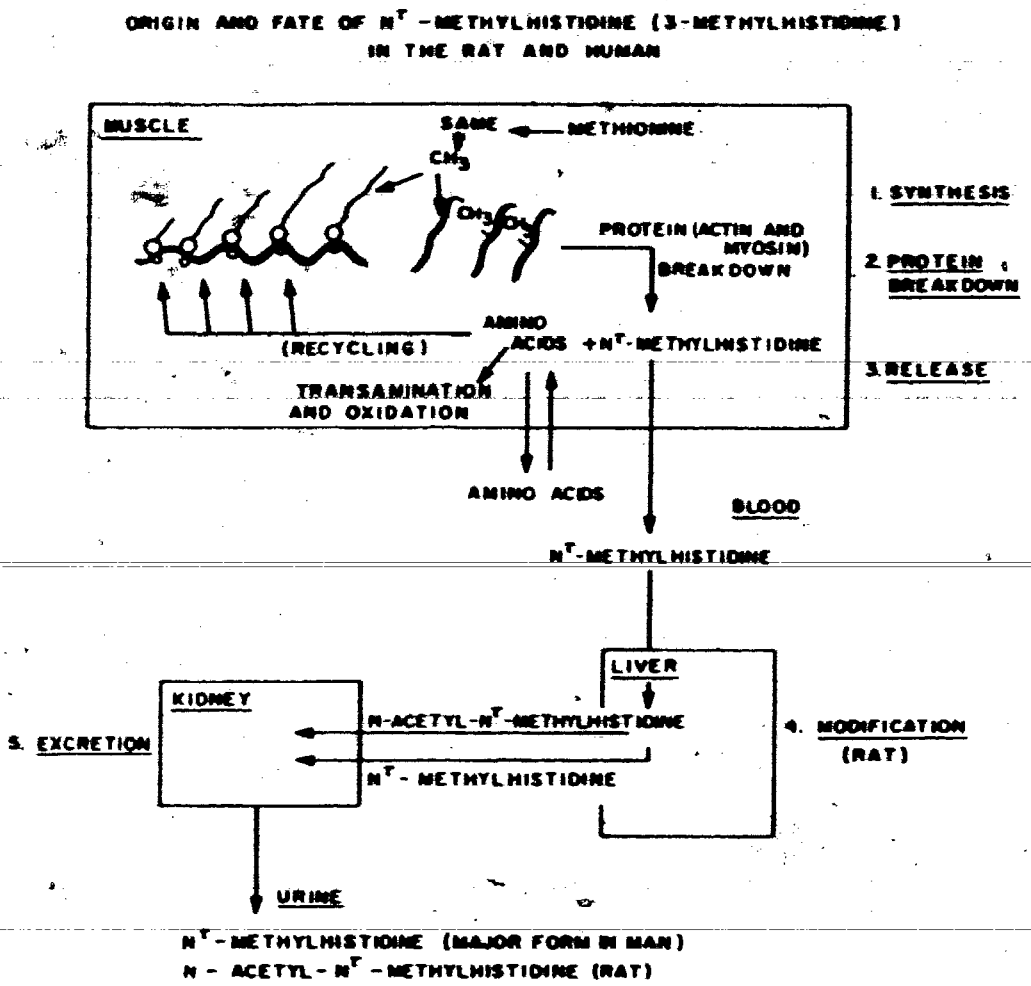


Figure 13. Outline of metabolism of 3-Methylhistidine, in relation to the skeletal musculature. (From Young and Munro, 1978).

In order to use 3MH to estimate the actual quantity of myofibrillar protein degraded in human subject it is necessary to know the concentration of protein bound 3MH in skeletal muscle. Tomas, Ballard and Pope (1979(b)) have determined the 3MH content of human muscle to average 3.59 ±0.06 umol·g^{-1} for subjects between 4 and 65 yrs (Table 8).

TABLE 8. 3-METHYLHISTIDINE CONTENT IN HUMAN MUSCLE

Age of subject	Protein in wet muscle (mg·g^{-1})	3MH in muscle protein (umol·g^{-1})
2 days	169	2.49
21 days	195	2.61
4.0 yrs	172	3.41
5.6 yrs	199	3.62
9.2 yrs	198	3.54
9.5 yrs	198	3.89
13.2 yrs	188	3.41
13.3 yrs	247	3.68
19.0 yrs	213	3.84
65.0 yrs	220	3.64

Values are for left vastus lateralis muscle except for the subjects aged 19.0 and 65.0 yrs, from whom samples were obtained at autopsy. (From Tomas et al, 1979(b)).

The use of 3MH as a measure of the rate of muscle protein degradation assumes that the majority of urinary 3MH is release from skeletal muscle, with only small amounts coming from other organs and tissues. The analysis of the major tissue of the rat by Haverberg and co-workers (1975) suggests that more than 90%

of protein bound 3MH in the body is located in the actin and myosin of skeletal muscle. A report by Nishizawa et al., (1977) demonstrated skin and gastrointestinal tract (GI) smooth muscle to be additional sources of endogenous 3MH. Although the 3MH content of these tissues is low, their contribution to total 3MH excretion was estimated to be approximately 16%. However, in their discussion, Nishizawa et al., (1977), suggest that due to segments of skeletal muscle that remained on the bones of their animals, their assessment of skeletal muscle contribution of 3MH was probably underestimated. In view of this problem they estimated that the actual contribution should be 90%, a value similar to that shown by others (Haverberg et al., 1975). Millward, Bates, Grimble and Brown, (1980(c)), using a single injection of (methly-^{14}C)methionine, to determine that up to 75% of urinary 3MH was derived from non-skeletal muscle sources. While in a more recent study, using a procedure incorporating labelled histidine in rats, Bates and Millward (1981), provide evidence that approximately half of the 3MH excreted is derived from skeletal muscle. Harris (1981) has questioned the validity of both studies, critisizing the fact that the 3MH pool size was taken from the Nishizawa study (1977) which miscalculated the turnover rates of skeletal muscle. On the other hand, the results of Wassner and Li, (1982), would appear to support those of Bates and Millward (1981). In their recent study, (Wassner and Li, 1982), they report that skeletal muscle contains approximately 90% of total body 3MH of young rats, whereas GI

tract and skin contain 3.8 and 6.4% respectively. These authors observed that GI tract actomyosin turns over 17 times faster than skeletal muscle actomyosin. The large fractional catabolic rate of GI muscle resulted in the production of 41% of 3MH produced.

Each of these experiments raises doubts about the assumption that skeletal muscle is the source of 3MH in the urine of the rodents. How relevant these studies are to the human body remains to be determined. The only evidence in the literature concerns a single paralyzed subject with motor degradation and no detectable skeletal muscle (Afting et al., 1981). It was found that skeletal muscle tissue is the major contributor of 3MH in the urine of man, forming approximately 75% to that excreted in the urine. One may speculate that in a healthy human body, the skeletal muscle contribution to 3MH might be even greater due to the large mass and its high content relative to other tissues (Elia et al., 1977).

A major problem in the use of urinary 3MH arises from the demonstration that ingestion of protein containing 3MH (meat) will alter 3MH excretion in a dose-related fashion (Marliss et al., 1979). Nonetheless, there is strong evidence that three days of a meatless diet will dramatically reduce the excretion of 3MH, thereby reducing the dietary contribution of the amino acid and allowing for the measurement of endogenous 3MH (Elia et al., 1980; Tomas et al., 1979). Results from pilot work in this laboratory support these findings (Figure 14).

The use of this derived amino acid has an advantage over other procedures for use in human studies inasmuch as it is non-invasive and since all skeletal muscle contribute to the output of 3MH, not only a select few as observed in other methods.

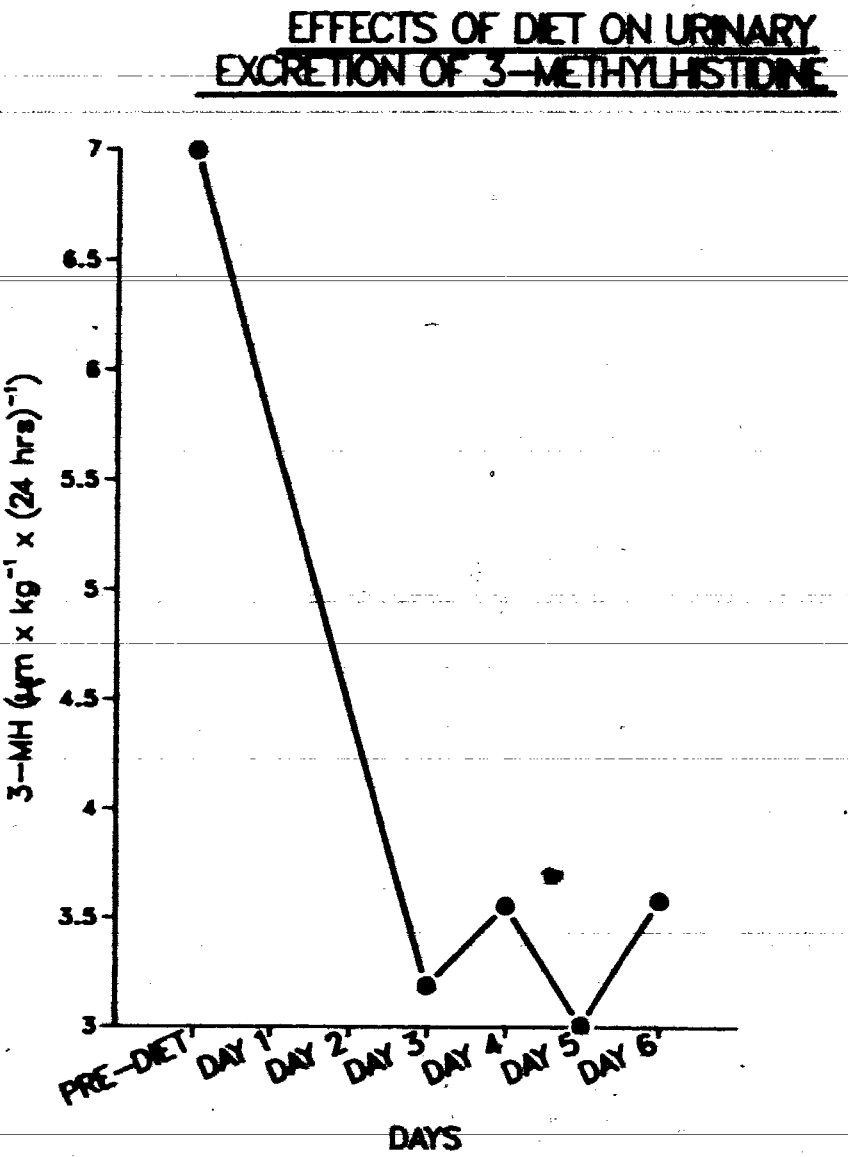


Figure 14. Daily urinary excretion of 3-Methylhistidine for five adult male subjects prior to and during 6 days of a meat free diet. Values are means of 24 hour collections. (Unpublished observations)

1.3 3-Methlyhistidine Excretion with Exercise

With these limitations in mind, 3MH has been used as an *in vivo* label of the rate of myofibrillar protein degradation in man with exercise (Decombaz *et al.*, 1979; Rennie *et al.*, 1981; Millward *et al.*, 1982; Dohm *et al.*, 1982). Unfortunately, results to date have been conflicting. Decombaz *et al.* 1979, in a study described previously, observed a 48% depression in urinary 3MH excretion during the final half of a 100 km run. This returned to slightly above pre exercise levels during a post exercise recovery period. Interpretation of these results are difficult as the timing of the urine collection post exercise varied from 18-25 hours (although discussed as '24 hours'). Similar decreases in the excretion rates of 3MH were observed by Rennie *et al.* (1981) and Millward *et al.* (1982). Rennie and co-workers also observed a return of urinary 3MH to just above normal pre-exercise values within 5 hours of recovery from 3.75 hours of treadmill running at 50% $\dot{V}O_2$ max. Contrary to this, Dohm *et al.*, (1982), using rats observed an elevation in 3MH excretion that continued for 48 hours following 3.5 hours of treadmill running to exhaustion. In the same study, but wih human subjects, steady state submaximal running (1.5 hours) resulted in an increase in urinary 3MH during the 24 hours period following the exercise bout, while one hour of circuit weight training resulted in a slight, yet insignificant,

elevation during the same urine collection period.

It must be noted that the experiments in which depressed 3MH excretion has been reported all vary in post-exercise urine collection timing, none of which are a full 24 hours. In addition, the study displaying the greatest depression in 3MH excretion involved an extremely strenuous prolonged single bout of exercise (Decombaz et al., 1979).

1.4 Urinary Creatinine Excretion

The expression of 3MH excretion corrected for body weight takes no account of the proportion of muscle tissue in the body and may not be very accurate due to subject variations in body composition as well as fluid shifts, especially during exercise.

Creatinine is considered to be a relatively 'constant' component of urine, grossly associated with body composition (Folin, 1905). Because of the apparently large precursor source, creatine, in the muscle tissue (Brosook et al., 1947) and the direct proportionality of body creatine to UCE demonstrated in a number of isotopic dilution (Hoberman et al., 1976; Forbes and Bruining, 1976) and body density studies (Greystone, 1968), it has been generally acknowledged that the amount of urinary creatinine excreted in the urine is related to muscle and fat free weight. According to Greystone's report (1968), 1 mg of creatinine excreted is equivalent to 20 grams of muscle.

Creatinine is formed by the hydrolysis of free creatine liberated during the dephosphorylation of creatine phosphate and is subsequently excreted by the kidney (Harper, 1979). However, it is recognized that the rate of creatinine excretion is not precisely constant and may be effected by diet (Peters, 1973; Crim et al., 1975; Wood et al., 1976; Forbes and Bruining, 1976) as well as exercise (Refsum and Stromme, 1974; 1975; Decombaz, 1979).

Exogenous dietary creatine and creatinine sources will definately influence UCE variability (Crim et al., 1975). High protein foods, such as meat and fish also have high creatine and creatinine content which when ingested may be excreted unchanged in the urine (Crim et al., 1975; Forbes and Bruining, 1976).

Muscle biopsy studies have demonstrated severe depletion of creatine phosphate stores in skeletal muscle with exhaustive exercise (Bergstrom, 1967). It thus may be hypothesised that accelerated creatinine production will occur during exercise. Results of studies on the effects of exercise on UCE are confounded by exercise induced depression of renal flow and glomerular filtration since creatinine clearance is a measure of renal plasma flow.

While creatinine excretion is depressed during exercise (Refsum and Stromme, 1974; Decombaz et al., 1979) it is significantly elevated following termination of endurance exercise of greater than one hour duration (Srivastava et al., 1967; Refsum and Stromme, 1974; 1975; Decombaz et al., 1979;

(Dohm et al., 1982). While creatinine excretion was reduced by 50% during a 100 Km road race, it rose significantly above pre exercise levels within 18-25 hours of recovery (Decombaz et al., 1979). These observations are in agreement with Refsum and Stromme (1974) who found marked increases in creatinine production during the 24 hour period following prolonged exercise (5-8 hours). Similar results were reported after 1.5 hours of submaximal running (Dohm et al., 1982). The only known study of UCE following short duration work, Dohm and co-workers (1982) revealed no significant alteration in creatinine excretion after an acute exercise bout by weight lifters.

In view of the fact that urinary creatinine may be an index of lean body mass, many investigators have begun to express urinary 3MH in terms of total muscle mass, or creatinine excretion (Tomas et al., 1979; Ballard et al., 1979; Seashore et al., 1981; Dohm et al., 1982) as this method makes an allowance for differences in muscle content.

The fraction of contractile proteins degraded may be calculated from the expression:

$$\% \text{ Protein degraded per day} = \frac{\mu\text{mol of 3MH excreted per day} * 100}{\mu\text{mol of 3MH in muscle protein.}}$$

Where,
the total amount of 3MH in muscle protein is equivalent to the product of 3MH content in muscle protein (3.59) and the amount of muscle protein.

and,

the amount of muscle protein in the body is calculated from the UCE rate (1 mg CR = 20gm muscle) and the protein content of muscle (20%).

$$= \frac{\text{3MH excreted per day} * 100}{(3.59)((CR * 20)0.2)}$$

(From Ballard et al., 1979)

While many have reported 3MH/CR ratio, only one exercise study in the literature has utilized this method to describe myofibrillar protein degradation. Dohm et al., (1982) corrected urinary 3MH for UCE and reported that as prolonged running increased 3MH excretion more dramatically than creatinine excretion resulting in an elevated 3MH/CR ratio. This would suggest a rise in the fractional rate of myofibrillar protein catabolim. However caution must be taken when interpreting this ratio measure since changes in creatinine would suggest an alteration in the muscle mass of the subjects. Since this study utilized only one acute exercise bout, it is doubtful that lean muscle mass changed. It may be suggested that while the 3MH/CR ratio will be an accurate index of the fraction of skeletal muscle protein degradation during rest, any situation leading to increases in creatinine would cause an underestimation of myofibrillar breakdown.

1.5 Lysosomal Enzyme Activity

Recent investigations of lysosomal enzyme activity and exercise point toward a possible delayed response to exercise. There is direct evidence of increased lysosomal enzyme activities, particularly Cathepsin B, Cathepsin D (Cat B and Cat D, respectively), and Calcium Activated Protease (CAF), during periods of physical exertion (Vihko et al., 1978(a); 1978(b)). It has been clearly demonstrated that intense exhaustive (Vihko, 1978) and prolonged submaximal (Salminen and Vihko, 1980) exercises both induce necrotic lesions in skeletal muscle. exhaustive running also stimulates the lysosomal system of surviving fibers, especially of red oxidative fibers (Vihko, 1978a); 1979; Salminen and Vihko, 1980). The highest activities of acid hydrolase, particulary Cat D, has been demonstrated by Vihko et al., (1978a), to be measured 3-5 days post exhaustive exercise. These findings have been substantiated by more recent work (Salminen and Vihko, 1980) which demonstrated major necrotic lesions 5 days following prolonged running in mice concomitant with elevated acid protease activity. Schott and Terjung (1979) in a study using trained animals, observed no alterations in any lysosomal activity at 0 hr and 24 hrs of recovery from a single acute exhautive exercise bout. There are many possible explanations for the apparent discrepancy between these results and those of others: the animals in their study (Schott and Terjung, 1979) performed the acute work immediately

following 2-4 weeks of training. It has recently been reported that training results in lower activities of lysosomal enzymes (Salminen and Vihko, 1981). In addition, while no changes were observed immediatly following the exercise, it is quite conceivable that there was a delayed effect much like that described by others (Vihko et al., 1978; Salminen and Vihko, 1980).

Both Cat D and Cat B are capable of degrading purified actin and myosin heavy chains (Weinstock and Iodice, 1969; Hardy et al., 1977; Bird, 1978). In addition, a Ca^{++}-activated factor (CAF) has been purified from skeletal muscle tissue and found to cause complete removal of Z-bands (Busch et al., 1978) as well as M-lines from intact myofibrils (Smith, 1978). Dayton et al., 1979, and others (Martin et al., 1974; Reddy et al., 1975) have purified CAF and determined it to be a very potent proteinase capable of selective degradation of certain myofibrilar proteins. When CAF is incubated with intact myofibrils, a-actinin is released from the Z-lines with a resulting degradation of troponin, tropomyosin C-protein and M-protein (Bird and Carter, 1980).

These observations have definate implications for exercise. Friden, Sjostrom and Ekbloom (1981) have provided evidence of structural changes following eccentric exercise, involving broadening and occasional disruption of Z-bands in muscle fibers. Schwane et al., (1983), determined that running down an incline resulted in greater, but delayed, muscle soreness than

did level running. The greater soreness following eccentric exercise is believed to result from greater stress on connective tissue in addition to higher tension per working muscle fiber as opposed to concentric contraction (Walmsley, 1978). Friden and workers have put forth the hyopthesis that disruption of the Z-band in muscle may lead to the degradation of protein, the release of protein bound ions and thus the ensuing soreness. The disturbance of myofibrillar protein during exercise may present the structural components to hydrolysis by the lysosomal acid proteases. Furthermore, as Armstrong et al. (1982) suggest, intermyofibrillar acid hydrolases may conceivably be released or stimulated as a result of high tensions in the fibers.

While it is well documented that these lysosomal acid hydrolases indeed degrade skeletal muscle protein and are activated following prolonged exercise, no direct measurements of protein degradation have been made in conjunction with hydrolase studies. Moreover, all present investigations of lysosomal activity have been performed using animal models. Therefore the physiological significance of acid hydrolases in human skeletal muscle following exercise remains to be determined.

The mechanisms involved in elevated skeletal muscle lysosomal enzyme activities are, as yet, unclear. Protease activity appears to be inversely related to pH. The pH optima of Cat D has been estimated at 4.0 (Bird et al., 1978) with that of Cat B being pH 5.2 (Barrett, 1978; Bird et al., 1978). While it

may be suggested that the acid pH required for complete activation of these enzymes is unphysiological, both Cat B and D have been shown to have sufficient activity at higher pH to be significantly involved in degradative processes (Barrett, 1978). In addition, Dayton et al., (1974), has demonstrated that CAF is optimally active at pH 7.0.

During maximal exercise of short duration, large changes occur in the acid-base metabolism, primarily due to the production of lactic acid which results in a decrease in blood and muscle pH (Hermanson, 1971). Intermittent work can result in a blood pH of 6.8 and slightly lower muscle pH (Osnes et al., 1972; Sahlin et al., 1976). This decrease in muscle and blood pH following high intensity exercise may be an activating mechanism for lysosomal enzymes and subsequent turnover of myofibrillar proteins.

Another possible mechanism for elevated protease activity post-exercise may be endocrine mediated. Mayer et al., (1976), reported a two-fold increase in the activity of an unidentified myofibrillar proteinase following a glucocorticoid treatment in rats, similar to that of Tomas et al., (1979) and Santidirian and co-workers (1981). These latter research groups demonstrated that administration of glucocorticoids similar to levels found with high intensity exercise, resulted in an acceleration of myofibrillar protein catabolism as indicated by 3MH excretion in adult rats. These preliminary findings indicate a need for greater investigation of endocrine control of lysosomal

activity. Indeed, further research of the mechanisms controlling protease activity is required for a better understanding of the role they play in exercise induced myofibrillar protein degradation.

1.6 Skeletal Muscle Protein Metabolism

While it has been demonstrated that exercise does elicit an elevation in the degradation of myofibrillar protein, the magnitude of this change is dependent upon the intensity and, to a greater extent, duration of the work performed. As degradation is only a part of protein turnover, exercise effects on protein synthesis must also be considered prior to discussing skeletal muscle protein turnover. While the rate of protein synthesis post-exercise was not determined in this thesis, this phase of turnover must be considered when interpreting an increase in protein degradation.

Exercise studies have demonstrated that skeletal muscle protein synthesis is initially depressed by exercise (Bates et al., 1980; Rennie et al., 1980) while others have found an increase above normal levels 2-24 hours following termination of exercise (Goldberg, 1968b; Rogers et al., 1979; Rennie et al., 1980; Dohm et al., 1982).

Elevated myofibrillar degradation following exercise may in fact be related to an increase in protein synthesis. It is unlikely that exercise results in a net loss of myofibrillar protein. Indeed, muscle hypertrophy has been observed in trained

athletes along with concomitant increases in myofibrillar degradation (Consolazio et al., 1975). This would, at first, appear contradictory, however a net protein synthesis (hypertrophy) could occur in the muscle even though overall protein turnover has increased.

The physiological significance of increased myofibrillar protein degradation following exercise remains to be clarified. However several explanations could be extended. Post-exercise protein catabolism may serve: 1) as a method of providing amino acid precursors for gluconeogenesis and maintenance of blood glucose levels, 2) as a mechanism for redistribution of amino acids for growth and remodelling of skeletal muscle tissue, 3) as a trigger mechanism for net protein synthesis.

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