THE EFFECTS OF GROWTH AND EXERCISE ON PROTEIN TURNOVER AND

SENSITIVITY TO TESTOSTERONE ADMINISTRATION IN SKELETAL MUSCLE OF

PREPUBERTAL RATS

by

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THE EFFECTS OF GROWTH AND EXERCISE ON PROTEIN TURNOVER AND

SENSITIVITY TO TESTOSTERONE ADMINISTRATION IN SKELETAL MUSCLE

OF PREPUBERTAL RATS.

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                (date)

ABSTRACT

The effects of growth, exercise acclimatization (Ac) and
exhaustive exercise (A) on skeletal muscle protein sythesis,
turnover and sensitivity to testosterone administration (T) were
studied in the homogenate (HOM), mitochondrial (MIT) and
sarcoplasmic (SAR) fractions in soleus and gastrocnemius muscles
of prepubescent rats. Growth effects were monitored from five to
six weeks of age in sedentary animals (C5 and C6). Exercised
animals were acclimatized to mild treadmill exercise for five
days after which acclimatized groups (Ac and AcT) rested 72
hours.

In order to study the acute response to exercise, two
groups of animals (A.Ac and A.AcT) were sacrificed 24 hours
following a run to exhaustion. Three groups of animals (C6T, AcT
and A.AcT) received an intraperitoneal injection of testosterone
propionate (10 mg/kg body weight) 24 hours prior to sacrifice.
Protein turnover was assessed in vivo using a double isotope
method and protein synthesis by in vivo uptake of radioactively
labelled leucine.

Protein turnover rates in both muscles fell from five to
six weeks of age. Protein concentration was increased in the HOM
fraction of soleus muscle. Exercise acclimatization resulted in
increased HOM protein concentration and elevated succinate
dehydrogenase (SDH) activity. Reduced protein synthesis and
elevated rates of protein degradation suggest the onset of a
detraining response in these animals. High protein turnover

rates in the young animals may account for the early onset of this response.

Testosterone administration to sedentary rats (C6T) resulted in reduced protein concentration and rates of synthesis. As an opposite effect was observed in both groups AcT and A.AcT it is suggested that the direction of the protein response to testosterone was mediated by prior exposure to exercise. Twenty four hours following severe exercise, mitochondrial and sarcoplasmic protein concentration in both muscles was diminished. This is in direct contrast to usual observations in adult animals. When testosterone was administered immediately following exhaustive exercise, the changes in protein synthesis and content were reversed. Therefore it is hypothesized that testosterone is required for supression of glucocorticoid induced protein catabolism following exhaustive exercise.

DEDICATION

For Tim;

the best friend a girl could ever hope for;

finally! something you can measure on a force platform!

ACKNOWLEDGEMENTS

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I. Introduction

General

Protein turnover in skeletal muscle is a dynamic process in which both structural and enzymatic proteins are continually being restructured. Turnover may be regarded as the physiological process in which cellular components are constantly synthesized and degraded, thereby describing the ongoing "overall renewal process" which occurs in skeletal muscle (Arias et al., 1969; Young, 1970,1974). When the rate of protein synthesis equals that of degradation, the cell is said to be in a 'steady state'. Any perturbation of either synthetic or degradative rate may result in a condition of net anabolism or catabolism.

Factors known to influence skeletal muscle protein turnover rate include nutrition (Trenkle, 1974; Giovanetti and Strothers, 1975; Millward et al., 1976), function and usage (Goldberg et al., 1975; Lemon and Nagle, 1981; Booth et al., 1982), neurotrophic influences (Ionesescu et al., 1978) development (Burleigh, 1974; Millward et al., 1975) and hormones (Rannels et al., 1977; Rosenfeld and Barrieux, 1979). Investigations have primarily been directed towards studying the effects of one of the above parameters on protein turnover. The degree to which

these factors interact _in_ _vivo_ to control protein turnover remains unclear.

The effects of normal muscle growth and development on protein turnover have been well documented (Devi et al., 1963; Winnick and Noble, 1965; Srivastava and Chaudhary, 1969; Howarth and Baldwin, 1971; Edgerton, 1973; Young, 1974; Burleigh, 1974; Giovanetti and Strothers, 1975; Millward et al., 1975, 1978, 1980; Bates and Millward., 1983). In the neonate, skeletal muscle proteins turn over rapidly, and under optimal conditions (adequate nutrition) net protein synthesis, manifest in the rapid accumulation of myofibrillar proteins, occurs (Burleigh, 1974; Young, 1974). During early postnatal development, enhanced rates of both protein synthesis and degradation are observed (Millward et al., 1975, 1978, 1980). While neurotrophic factors appear largely responsible for the differentiation of fibres into predominantly slow or fast fibre types (Close, 1972; Young, 1974; Buresova et al., 1975) usage, endocrine, and nutritional factors likely govern hypertrophy and whole muscle growth (Giovannetti and Strothers, 1975; Goldberg et al. 1975; Millward et al., 1975, 1978). Any attempt to evaluate the effect of exercise on skeletal muscle during growth must distinguish carefully between normal growth processes and the effects of exercise _per_ _se_. While endurance training in young rats has been shown to slow the rate and prolong the phase of nuclei proliferation (Bailey et al., 1973; Hubbard et al., 1974) little is known about the effects of exercise on protein turnover rate.

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The chronic and acute effects of exercise on skeletal muscle protein turnover are less clearly understood. While numerous investigations have addressed this problem, the results are controversial (Booth et al., 1982). Endurance exercise training has been shown to increase (Rogozkin, 1976) decrease (Dahlmann and Reinauer, 1978) and produce no change (Beecher et al., 1979) in the rate of skeletal muscle protein synthesis. These discrepancies may largely be explained by the variety of sampling times employed by investigators following the final exercise bout (Booth et al., 1982). While training appears to have a "protective" effect limiting skeletal muscle degeneration following an acute exercise bout (Salminen et al., 1984), specific changes reported in acid hydrolase and alkaline and myofibrillar proteinase activities following training vary widely according to muscle studied, species and training regime employed (Dahlmann et al., 1981; Salminen and Vihko, 1981; Salminen et al., 1983). Furthermore, the factors which trigger enzymatic changes and the extent to which the observed changes themselves may stimulate and effect skeletal muscle protein degradation patterns is not known.

Factors which have been implicated as regulators in skeletal muscle protein turnover following exercise include muscular tension (Booth et al., 1982), stretch (Goldspink et al., 1983) and intracellular calcium levels (Kamenyama and Etlinger, 1979). Endocrine factors have not been given serious consideration as Goldberg (1967, 1968) found that enhanced

protein synthesis occurs largely independently of endocrine controls in skeletal muscles undergoing compensatory hypertrophy. Given the 'unphysiological' nature of the exercise stimulus, this may be an unfair conclusion.

Basal, exercise and recovery levels of a number of hormones may be altered as a result of physical training (Hartley et al., 1972a, 1972b; Galbo et al., 1977; Winder et al., 1979). Enhanced tissue sensitivity to insulin and testosterone with respect to changes in hormone receptor number and affinity have also been demonstrated following training (Kovisto et al., 1979; Rogozkin, 1979b). While McManus et al. (1975) were unable to detect any changes in testosterone binding to guinea pig gastrocnemius following endurance training, Rogozkin (1979b) has demonstrated a 71% increase in sarcoplasmic testosterone binding capacity following endurance exercise in mature rats, concomitant with a 47% increase in RNA polymerase I activity. Furthermore, in response to a single injection of androgen (methylandrostenolone) in untrained animals, he observed increased ^{14}C leucine incorporation into myofibrillar and sarcoplasmic proteins. Effects of a single androgen injection in trained animals were not determined.

In growing animals, enhanced testosterone-binding capacity following endurance exercise may be of significance. If sarcoplasmic testosterone binding can be enhanced by regular exposure to exercise in young animals, a significantly greater pubertal anabolic response might be anticipated. To date, this

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possibility has not been investigated.

Summary and Statement of Problems

There are still many questions which remain to be answered
concerning the effects of exercise on skeletal muscle growth and
protein turnover. Assessment of skeletal muscle protein turnover
implies the simultaneous measurement of rates of protein
synthesis and degradation. The administration of radioactively
labelled amino acids is commonly employed for assessing protein
turnover, but a number of limitations are inherent in this
technique (Zak et al., 1979). A further complication is the lack
of agreement among investigators studying protein turnover
regarding label, doseage, tracer administration method and
schedule, and analysis and interpretation of results. At
present, the double isotope labelling method (described in the
methodology section "Assessment of Protein Turnover") appears to
be the best available technique for assessing protein turnover
in vivo. To date, it has not been employed in exercise related
muscle protein research. Instead, the literature reports protein
synthesis and degradation as unique phenomena, often studied in
vitro, a situation which simply does not exist in vivo.

Many of the endocrine data collected with respect to
exercise are descriptive or related to the role hormones play in
substrate delivery and metabolism within skeletal muscle .
Results from protein turnover studies involving exogenous

hormone administration alone or in conjunction with a particular surgical or chemical manipulation are difficult to generalize to normal animals. Also, the extent to which exercise may alter skeletal muscle sensitivity to a particular hormone and how that in turn may affect protein synthesis and degradation in skeletal muscle is unknown.

While the general pattern of protein synthesis and degradation during growth and the factors governing these processes are reasonably well documented, the effect of exercise on same is unknown. In adult animals, exercise induced changes in protein turnover are also unclear. This is largely due to inconsistent experimental design, animal models studied and sacrifice schedule chosen. In fact, it is this general lack of methodological consistency which makes drawing any firm conclusions so difficult.

Given the questions and inconsistencies mentioned above, the purposes of this study were:

1. To determine the effects of

    (a) exercise acclimation

    (b) a single exhaustive exercise bout, and

    (c) growth

        on body weight, muscle weight and protein concentrations in whole muscle homogenate, mitochondrial and sarcoplasmic fractions of young rat soleus and gastrocnemius muscles.

2. To determine the effects of

(a) endurance exercise acclimation, and

(b) a single exhaustive exercise bout

on rates of protein synthesis and protein turnover in

three fractions of young rat gastrocnemius and soleus

muscle.

3. To determine the effects of

(a) endurance exercise acclimation, and

(b) a single exhaustive exercise bout

on the sensitivity to testosterone administration as

measured by protein synthesis rate in three fractions of

young rat soleus and gastrocnemius muscle.

Definition of Terminology

Definitions and abbreviations used in this thesis

include:

Endurance Exercise Acclimation A mild endurance exercise running

program designed to accustom young rats to treadmill

running. The ultimate goal was six minutes continuous

running at 30 m/min up a 5% slope.

Counts Per Minute (CPM) The number of disintegrations per minute

of a radioactively decaying element registered in a counting

system.

Disintegrations per Minute (DPM) The absolute number of

disintegrations per minute of a radioactive element in a

component sample equivalent to the CPM corrected for sample

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preparation and recovery, background and the efficiency of the counting system.

Protein Synthesis (^{14}C DPM/mg protein) Protein synthesis will be reported as the uptake of radioactively labelled leucine (^{14}C-DPM) into skeletal muscle in vivo, and expressed relative to protein concentration.

Relative Protein Turnover (^{14}C DPM: ^{3}H DPM) Relative protein turnover will be reported as a ratio of ^{14}C DPM: ^{3}H DPM. This is described in depth in the methodology section "Assessment of Protein Turnover".

Scope and Limitations

1. Inherent in the use of radioactively labelled amino acids to assess protein turnover, is the problem of possible reutilization of the amino acid for the synthesis of new protein. In the present investigation this has been minimized by the choice of administration schedule and the use of internal controls.

2. The double isotope technique provides information about "relative" protein turnover (^{14}C:^{3}H ratio) and protein synthesis(^{14}C DPM per mg protein). Protein degradation rates are not directly measured, but may be inferred from relative protein turnover data given that synthesis rates are known.

3. Different isotopes were used to measure protein synthesis in experiments 1 and 2. Hence, DPM/mg protein expressed as a

percentage of control values was used for comparisons of
protein synthesis from experiments 1 and 2.

4. Every effort was taken to ensure consistent treatment between
experimental and control animals in terms of diet,
availability of food and water, daily handling and caging
arrangements. However, it must be conceded that many factors
such as appetite, sleep and habitual activity patterns
remained beyond the investigator's control.

5. The degree to which conclusions drawn about rat skeletal
muscle protein turnover rates and exercise may be
extrapolated to humans is unknown.

II. Methodology

General

The present study consists of two sets of experiments. In the first, the effects of growth, exercise acclimation and acute exercise on muscle protein content, synthesis, and turnover were investigated. In the second, the effects of exercise acclimation and acute exercise on the muscle's sensitivity to testosterone were studied.

In the following sections it may be assumed that descriptions of experimental techniques and methods apply to both experiments unless otherwise noted.

Animal Care

For experiment 1, 46 male Wistar rats (specific pathogen free, Charles River Farms, Montreal, P.Q.) were obtained at approximately three weeks of age (40-60 g). For experiment 2, 30 male Wistar rats (specific pathogen free, CFN, University of British Columbia) were obtained at approximately five weeks of age (80-100 g). Animals were housed in pairs in suspended cages in an air conditioned room at 22°C and the day-night cycle was reversed (ie. light from 1800 to 0600 hr). The rats were fed a

standard diet of Purina rat chow (23% protein) and given water
ad libitum. Each morning, rats were handled and food and water
replenished. Every second day, the cages were rotated on the
cage rack. The cages were washed and sterilized every week and
the rats were weighed weekly. After ten days of orientation to
the laboratory, the animals were randomly assigned to
experimental groups as indicated in Tables 1 and 2.

Exercise Acclimation and Performance Protocol

The rats were exercised on a precalibrated, motor driven
treadmill (Quinton Instruments) which was divided into 10
compartments with a shock grid at the back of each. Acclimation
to running was performed at approximately 0900 h and 1400 h for
five consecutive days. The acclimation schedule is outlined in
Table 3. This procedure was designed to progressively load the
animals until they were capable of performing work at a speed of
30 m/min and at a grade of 5% for six minutes. Following the
final session, all animals received a 72 hour rest. This was
followed by three consecutive days of maintenance exercise at
the same speed and grade for the same duration as during the
final exercise acclimation bout. The animals in groups Ac and
AcT then rested for the duration of the study. Groups A.Ac and
A.AcT performed one final exercise bout four days later at a
speed of 30 m/min and up a 5% grade until exhaustion. Exhaustion
was defined as the point in time at which animals could no

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Table 1.

Experiment 1: Groups of Animals, Treatment, and Age at Sacrifice

Group	Treatment	N	Age at Sacrifice
C5	Control	12	5 weeks
C6	Control	8	6 weeks
Ac	Acclimated	10	6 weeks
A.Ac	Acclimated Acute run	10	6 weeks

Table 2.

Experiment 2: Groups of Animals, Treatment and Age at Sacrifice

Group	Treatment	N	Age at Sacrifice
C6T	Control Testosterone	8	6 weeks
AcT	Acclimated Testosterone	8	6 weeks
A.AcT	Acclimated Acute run Testosterone	8	6 weeks
SHAM	Control Oil	6	6 weeks

Table 3.

Endurance Exercise Acclimation and Performance Protocol

Day	Time	Speed (m/min)	Grade (%)	Duration (min)
1	a.m.	10	0	3
1	p.m.	15	0	3
2	a.m.	20	5	3
2	p.m.	20	5	3
3	a.m.	20	5	5
3	p.m.	20	5	5
4	a.m.	25	5	5
4	p.m.	30	5	5
5	a.m.	30	5	6
5	p.m.	30	5	6

Maintenance Day	Bout	Speed (m/min)	Grade (%)	Duration (min)
1	a.m.	30	5	6
1	p.m.	30	5	6
2	a.m.	30	5	6
2	p.m.	30	5	6
3	a.m.	30	5	6
3	p.m.	30	5	6

longer run at the specified exercise intensity, were
unresponsive to mild electrical stimulation and were unable to
right themselves when placed on their backs. This type of
exhaustive running has been shown to result in significant
glycogen depletion in both soleus and gastrocnemius muscles
(Wilkinson and Malcolm, 1983). The run to exhaustion was
followed by a 24 hour rest period prior to sacrifice.

Experimental Design: Experiment 1

Four groups of experimental animals were used (Table 1).
Two groups were acclimated to endurance exercise (Ac and A.Ac)
while two remained sedentary (C5, C6). Young, unexercised
controls, groups C5 and C6, were used to provide data on the
normal developmental patterns of skeletal muscle between five
and six weeks of age. Ac and A.Ac groups were used to provide
data on chronic (72 hours post exercise) and acute (24 hours
post exercise) endurance exercise responses respectively, in
growing skeletal muscle at six weeks of age. All groups of
animals (C5, C6, Ac A.Ac) received an intraperitoneal injection
of L[4,5-^{3}H (N)]-leucine (New England Nuclear, Chicago, S.A.
51.6 Ci/mmole, 50 μCi/100 g body weight) seven days prior to
sacrifice and an intraperitoneal injection of L[^{14}C-(U)]leucine
(.328 mCi/mmole, 4 μCi/100 g body weight) 25 minutes prior to
sacrifice. A six hour fast preceeded the final injection of
radioisotope to minimize the differences in absorption of

unlabelled leucine from the gut just prior to sacrifice.
Experimental design is illustrated in Table 4.

Selected parameters of growth, protein synthesis, protein
turnover and training were investigated as indicated below. With
the exception of succinate dehydrogenase (SDH), body weight,
muscle weight, and blood, data were collected for three
fractions (whole muscle homogenate (HOM), mitochondrial (MIT)
and sarcoplasmic (SAR)) of soleus and lateral gastrocnemius
muscles. SDH activities were determined only for the soleus
muscles of groups C6 and Ac.

1. Body weight (grams)

2. Muscle weight (grams wet weight)

3. Protein Concentration (mg protein/gram wet weight tissue)

4. SDH activity (μM/g/min)

5. Protein synthesis (^{14}C DPM/mg protein)

6. Relative protein turnover (^{14}C/^{3}H)

Experimental Design: Experiment 2

Four groups of experimental animals were used (Table 2).
Two groups were acclimated to endurance exercise (AcT and A.AcT)
while two remained sedentary (C6T and SHAM). Animals in groups
C6T, A.AcT and AcT received an intraperitoneal injection of
testosterone propionate (10 mg/kg body weight) in 0.2 ml canola
oil 24 hours prior to sacrifice. Animals in the SHAM-injected
group received 0.2 ml canola oil 24 hours prior to sacrifice.

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Table 4.

Experimental Design: Experiment 1

Group	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
						Age (days)										
C5	^{3}H							^{14}C								
C6									^{3}H							^{14}C
AC		A	A	A	A	A	*	*	^{3}H*	m	m	m	*	*	*	^{14}C
A.AC	A	A	A	A	A	*	*	*	^{3}Hm	m	m	*	*	*	Ex	^{14}C

  A Acclimated
  m Maintenance
  * Rest
 Ex Exhaustive run
^{3}H ^{3}H-leucine injection
^{14}C ^{14}C-leucine injection

C6T animals provided data on the protein synthetic response to testosterone administration in sedentary six week old animals. The degree to which chronic (72 hours post exercise) and acute (24 hours post exercise) exercise modified this response was evaluated using data from AcT and A.AcT groups respectively. Results from experimental groups C6, Ac and A.Ac (Experiment 1) provided control data from non-steroid treated animals. All groups received an intraperitoneal injection of L[4,5-^{3}H (N)]-leucine (New England Nuclear, Chicago, S.A. 51.6 Ci/mmole, 2 μCi/100 g body weight) 25 minutes prior to sacrifice. A six hour fast preceded the final injection of radioisotope to minimize the differences in absorption of unlabelled leucine from the gut just prior to sacrifice. Experimental design is illustrated in Table 5.

Parameters investigated are listed below. With the exception of body and muscle weights and blood, data was collected for three fractions (HOM, MIT, SAR) of soleus and lateral gastrocnemius muscles.

1. Body weight (grams)

2. Muscle weight (grams wet weight)

3. Protein (mg protein/gram wet weight tissue)

4. Protein synthesis (^{3}H DPM/mg protein as a percent of SHAM controls)

5. Serum testosterone concentration (ng/100 ml)

Table 5.

Experimental Design: Experiment 2

Group	Age (days)									
	35	36	37	38	39	40	41	42	43	44
SHAM									O	^{14}C
C6T									T	^{14}C
AcT		A	A	A	A	A	*	*	*T	^{14}C
A.ACT	A	A	A	A	A	*	*	*	ExT	^{14}C

A Acclimation
* Rest
Ex Exhaustive Run
^{14}C ^{14}C-leucine injection
T Testosterone
O Oil

Temporal changes in serum testosterone following testosterone injection

A study was conducted using mature sedentary rats to establish that an intraperitoneal (IP) injection of testosterone propionate (TP) resulted in elevated serum testosterone levels. Three male wistar rats were sacrificed to provide basal testosterone values. Six animals received an IP injection of TP (10 mg/kg body weight in 0.2 ml canola oil). Three animals were sacrificed one hour later and three more after 24 hours. Results are shown in Figure 1.

One hour following testosterone injection, serum testosterone was significantly elevated from basal levels. Twenty-four hours later, serum values were slightly depressed relative to controls. Thus, low serum testosterone values 24 hours following an IP injection of testosterone propionate are indicative of the normal physiological response to the injection and do not imply that the animal did not receive the initial dose of steroid.

Tissue Preparation

All animals were sacrificed via exsanguination 25 minutes following the final injection of radioactively labelled leucine. Rats were anaesthetized with ether and the abdominal cavity opened. Blood samples were extracted from the abdominal aorta.

SERUM TESTOSTERONE: TEMPORAL RESPONSE
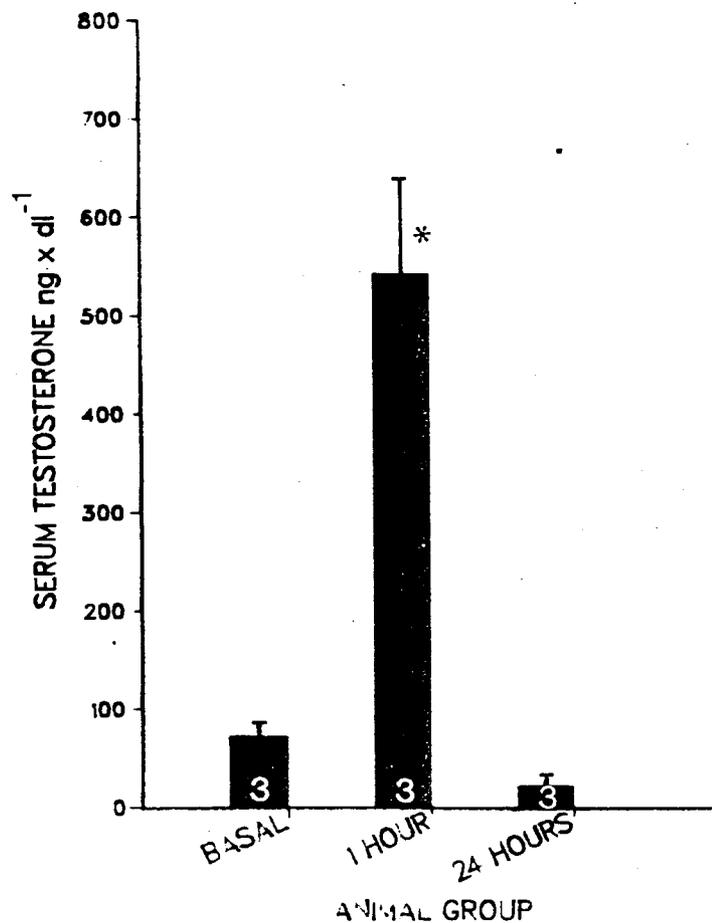
Figure 1. Serum testosterone concentrations
1 and 24 hours following intraperitoneal
injection of testosterone propionate (10 mg /
kg body weight).
* significantly different from basal levels
 (p<0.05)

The right and left soleus and gastrocnemius muscles were isolated, excised, blotted and weighed. A portion of the right soleus (10-20 mg) was removed from the C6 and Ac groups for the biochemical determination of SDH activity. Muscle samples for SDH activity were frozen in liquid nitrogen and stored at -80°C for subsequent analysis. The remaining muscle samples were placed in polycarbonate tubes with 5 ml of an ice cold buffer containing 10 mM leucine and .25 M sucrose, in preparation for muscle fraction separation.

Blood Preparation

Blood samples were allowed to stand at room temperature for 30 minutes and then spun for 30 minutes at 2500 g. Serum was drawn off and stored at -80°C for subsequent analysis.

Biochemical Procedures

Muscle Fractionation

The procedure followed for muscle fractionation into whole muscle homogenate (HOM), mitochondrial (MIT), and sarcoplasmic (SAR) fractions is illustrated in Figure 2. The homogenizing buffer consisted of .25 M sucrose and 10 mM leucine at pH 7.2 (Seraydarian and Mommaerts, 1965). Both the buffer and 15% TCA solutions were ice cold. Muscle samples were homogenized with

MUSCLE SAMPLE
|
+ 5 ml homogenizing buffer (HB)
        Homogenize ─────────┐
                    500 μl for protein
                    500 μl + 2 ml TCA for counts
                                |
                        HOMOGENATE

    Spin, 2 500 g, 15 min.
                |
            Discard pellet
Take supernant to 10 ml with HB
                |
    Spin, 22 000 g, 20 min, 4°C
                |
        Supernant ─────────┐
                    1 ml for protein
                    2 ml + 2 ml TCA for counts
                                |
                        SARCOPLASMIC

    Discard remaining supernant
                |
    Homogenize pellet in 2 ml HB
                |
            1 ml for protein
        1 ml + 2 ml for counts
                |
            MITOCHONDRIAL
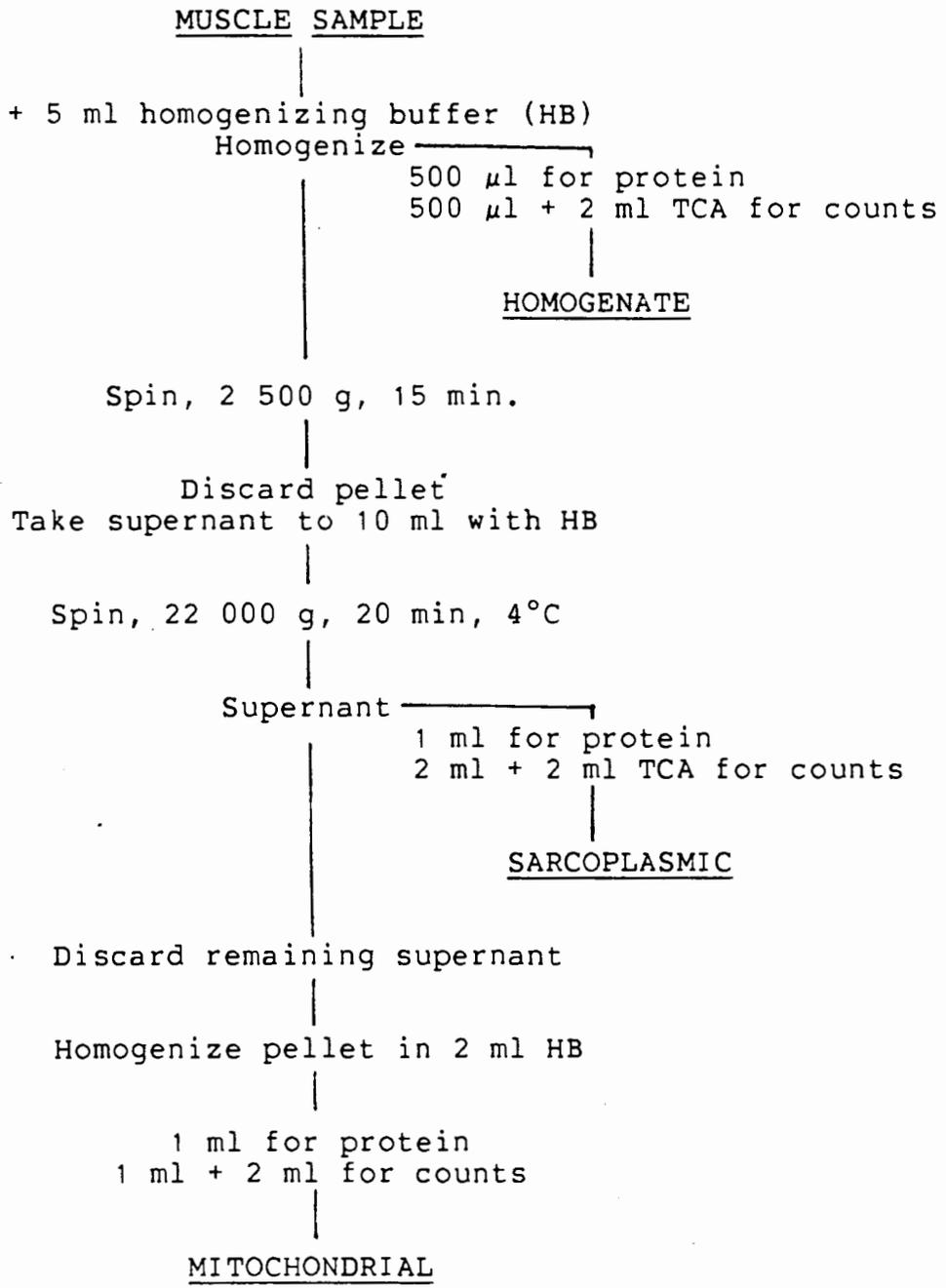
Figure 2. Muscle fraction separation.

two 2.5 second bursts in a Polytron Homogenizer (Brinkman Company).

Preparation of Samples for Protein Determination

Following sample fractionation (Figure 2), all protein samples were washed in 2 ml of ice cold 15% TCA. Samples were spun at 2500 g for 10 minutes and the supernatant discarded. This was repeated once more. Protein pellets were then solubilized in 1, 0.5 or 2 ml of 1N NaOH and allowed to stand for 30 minutes at 50°C in a water bath for determination of homogenate, mitochondrial and sarcoplasmic protein respectively. Four hundred μl aliquots were taken from each sample for subsequent protein analysis. Two hundred μl was placed in each of two test tubes for duplicate protein determinations.

Preparation for Radionuclide Counting

Following fraction separation, muscle samples were washed in 2 ml ice cold 15% TCA and spun at 2500 g for 10 minutes. The supernatant was discarded. Protein pellets were washed and spun two more times in 5 ml of ice cold 15% TCA plus 10 mM leucine. The final protein pellets were then dissolved in either 2 ml of NCS solubilizer (Amersham) or in 1 ml of Soluene (Packard) at 50°C for two hours for experiments 1 and 2 respectively. Once

the pellets were evenly dissolved, the samples were transferred
into 2 scintillation vials and mixed with 20 ml of
Liquiflour-toluene scintillation cocktail (New England Nuclear).

Blood Sample Preparation for Counting

In order to establish that each animal successfully
received the injections of radioactively labelled leucine, serum
radioactivity was measured.

Two hundred μl aliquots were used to determine
radioactivity. Serum was washed with 400 μl 15% TCA and spun for
10 minutes at 2500 g. The supernatant was then transfered to a
clean tube and the pellet discarded. This procedure was
repeated. Finally, a 500 μl sample of protein-free serum and an
equal volume of distilled water were transferred into a
scintillation vial and mixed with 20 ml of Aquasol II (New
England Nuclear). All counting was done in 22.0 ml polycarbonate
disposable scintillation vials in a Beckman L.250 liquid
scintillation counter (LSC). Counting efficiency (ie. quench
correction) was provided by this LSC using the External Standard
Ratio.

Serum Endocrine Determinations

The remaining serum samples in experiment 2 were used for
radioimmunoassay determinations of testosterone (Testosterone,
Dihydrotestosterone RIA Kit, Amersham).

Muscle SDH and Protein Determinations

Succinate dehydrogenase was measured in samples of soleus
muscle using a modification of the method described by
Cooperstein (1950) (Appendix B). Protein was measured according
to the technique of Lowry et al. (1951) (Appendix C).

Assessment of Protein Turnover

Figure 3 illustrates the precursor product relationship
following a single injection of radioactively labelled amino
acid. Initially, a rapid rise in precursor specific
radioactivity (F*) is observed. Its subsequent rapid decline is
caused by mixing of amino acids in the blood with those in the
extracellular and intracellular compartments of various organs
(Zak et al., 1979). A peak in precursor specific radioactivity
is observed 10 minutes following an intraperitoneal injection of
labelled amino acid (Airhart et al., 1974).

Precursor-product relationship after a single injection of label. Schematic drawing shows specific radioactivities of precursor (free amino acid, F) and product (amino acid in protein, P) as a function of time.
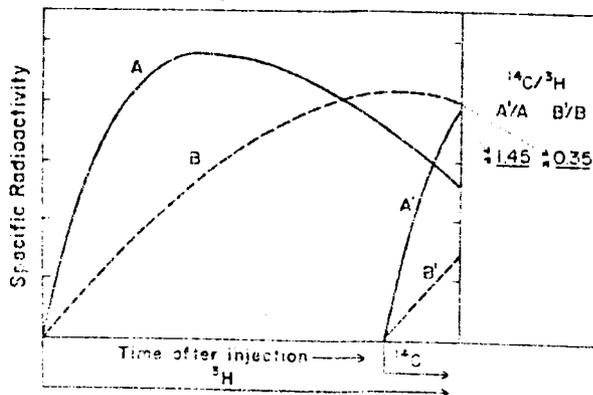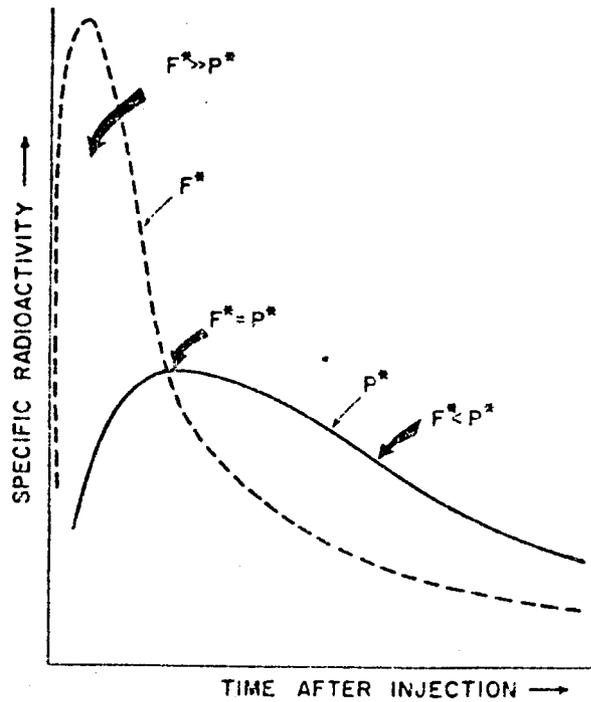
Principle of double-isotope method. Injection of 1 radioisomere of a given amino acid (^{3}H-labeled in scheme above) is followed by administration of a 2nd radioisomere at some later interval. Experiment is terminated shortly afterward and the ratio of the 2 radioisomeres is determined in 2 isolated proteins, A and B.

Figure 3. Double isotope technique of protein turnover. (from Zak et al. 1979).

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Due to the asynchrony of protein synthesis, radioactivity appears in a pool of protein molecules immediately after the tracer amino acid enters the cell. Protein radioactivity (P*) increases most rapidly during the early post injection interval since there is a large difference between specific radioactivities of precursor and product. Label accumulation continues at an increasing rate until specific radioactivities of F* and P* have become equal. This time is referred to as the crossover point. Thereafter, the radioactivity of the product always exceeds that of the precursor until finally the specific radioactivity of the protein molecule declines as some labelled amino acids are replaced by unlabelled ones (Zak et al., 1979).

In the present experiment, relative rates of protein turnover were measured by a modification of the double isotope technique first described by Arias et al. (1969). In this procedure, two isotopically labelled forms of an amino acid, ^{3}H- and ^{14}C-leucine are used to establish two time points on the curve describing degradation and synthesis of isolated proteins (Dice and Walker, 1978).

The rationale of the experimental protocol of the double isotope method is shown schematically in Figure 3. Tracer administration is chosen so that the cells or tissues are exposed to the first isotope (^{3}H) over the time necessary for the protein specific radioactivity to pass the crossover point. Thus, ^{3}H reflects the descending limb of the protein specific radioactivity. The second isotope (^{14}C) is administered just

27

before the experiment is terminated, thereby reflecting the ascending limb of protein specific radioactivity (Zak et al., 1979). Proteins with rapid turnover rates, that is, those synthesized rapidly (high ^{14}C) and degraded rapidly (low ^3H), will have higher relative $^{14}C/^3H$ ratios (Glass and Doyle, 1972; Dice and Walker, 1978).

The double isotope method is therefore very convenient, as it is not necessary to measure the precursor specific radioactivity or the rate of either loss or accumulation of label. For comparison of several proteins or protein pools, it is only necessary to determine the ratio of the two isotopes (Zak et al., 1979).

Sensitivity of the method depends on the careful selection of appropriate injection schedules for the radioisomers. The length of exposure to the first label (^3H) should be selected so that all the proteins being compared are in the phase of declining specific radioactivity. This should correspond to no more than one to three half lives of the protein with the most rapid turnover in a group that is to be compared (Zak et al., 1979).

In the present investigation, where heterogeneous skeletal muscle proteins were being observed, administration of the ^3H-leucine seven days prior to sacrifice fulfilled this criterion since the half life of myosin in young animals is 5.4 days (Millward et al., 1980) and that of sarcoplasmic proteins is approximately two to three days (Zak, personal communication,

1980).

The optimal labelling period for the second tracer (^{14}C) is between 20 and 30 minutes. If exposure exceeds 30 minutes, the sensitivity of the method is progressively reduced and eventually lost altogether (Poole, 1971; Zak, 1977). Thus, a 25 minute exposure to ^{14}C was employed in this study.

Unfortunately, the double isotope method for assessing protein turnover is not without limitations. Reutilization of radioactive label is a common problem encountered in this area of research, and leucine, the radiolabelled amino acid used in the present investigation is reutilized. The brief exposure (25 minutes) to the second label (^{14}C-leucine) attempts to minimize the confounding effect of reutilization. As protein pools being compared statistically are relatively homogenous, it may be assumed that reutilization of ^3H-leucine will vary according to the changes induced by the experimental treatment or overall protein turnover (degradation) rates.

Another limitation concerns the nonsteady state. In the present investigation, the animals were undergoing a period of rapid growth, thus the labelled amino acid may be expected both to accumulate in the protein molecules for a period beyond the crossover point and to be incorporated at a higher rate than is observed during the steady state. However, Zak et al. (1979) have demonstrated that this distortion of observed ratios during growth is rather small. The relative data observed between experimental groups should therefore provide a good index of

protein turnover while absolute rates of synthesis and degradation may be slightly modified.

Statistical Analysis

Group means and standard errors of the means were calculated for all the dependent variables listed. One way analyses of variance (SPSS 9 ANOVA Programme) were performed between groups for protein content, protein synthesis, relative protein turnover, body weight, muscle weight and serum testosterone. When the F ratio indicated significance ($p<0.05$), post hoc Student Neuman-Keuls analyses were used to locate significant group differences. A two way analysis of variance was performed in experiment 2 for comparison of muscle protein content and synthesis. Main effects were calculated for testosterone treatment and exercise effects. Varying Ns for the dependent variables in some groups may arise from methodological error in laboratory work. In some cases, protein synthesis or relative protein turnover data was eliminated on the basis of questionable or very low blood leucine specific radioactivity.

III. Results

In Figures 4 to 34 in this chapter, values shown are group means. Error bars indicate the standard error of the mean. The number of animals in each group is indicated in white numbering at the bottom of each bar.

Results of Experiment 1

Muscle Weights, Body Weights, Run Time to Exhaustion and SDH Activity

Group means for body weight and muscle weights and run time to exhaustion (Group A.Ac) are shown in Table 6. SDH activity for groups C6 and Ac are also included. In all parameters, a significant ($p<0.05$) growth effect from the fifth to sixth week was observed. Body and muscle weights were significantly greater in six week old animals. When muscle weights were expressed relative to body weight, this significance remained, indicating an independent growth effect in soleus and gastrocnemius muscles. Body and muscle weights of exercised animals (Ac and A.Ac) were significantly greater than controls. Similarily, acutely exercised animals displayed significantly greater absolute body and muscle weights than acclimated rats. When

Table 6.

Experiment 1: Body Weights, Muscle Weights, SDH activity and Run Time to Exhaustion

	Body Weight (g)	Soleus Weight (mg)	Gastroc Weight (mg)	Soleus % B.W.	Gastroc % B.W.	SDH(μM /g/min)	Run Time (min)
C5	127.3* (2.4)	79.9* (1.6)	498.5* (10.0)	.063* (.002)	.392* (.008)		
C6	177.5 (4.1)	126.0 (2.9)	795.7 (13.1)	.071 (.001)	.447 (.009)	2.64 (0.3)	
Ac	202.7* (9.2)	153.4* (5.9)	1012.7* (16.2)	.076 (.003)	.50* (.007)	4.04* (0.4)	
A.Ac	215.1** (4.4)*	172.6** (7.7)*	1047.0* (16.2)	0.080* (.003)	.487* (.010)		68.5 (10.8)

Means (± standard errors) for five and six week old controls (C5 and C6), acclimated (Ac) and acutely run (A.Ac) rats.

 * Different from C6 (p<0.05)
** Ac Different from A.Ac (p<0.05)

muscle weights were expressed as a percentage of body weight, no significant difference was found between C6 controls and acclimated rats with respect to soleus muscle weight. Relative Ac gastrocnemius weight, however, remained significantly elevated. Relative weights of both soleus and gastrocnemius muscles in acutely run animals (A.Ac) were higher than those observed in controls (C6) ($p < 0.05$). No difference was found between exercised groups when relative muscle weights were compared.

SDH activity ($\mu M/g/min$) was significantly greater in the soleus muscle of exercise acclimated rats compared with controls.

Protein Concentration: Experiment 1

Protein concentration values are summarized in Figures 4 to 9. No significant growth effects were observed between 5 and 6 week old animals in the homogenate fraction of either soleus or gastrocnemius muscles (Figures 4 and 7). While mitochondrial protein concentration increased from five to six weeks in soleus muscle (Figure 5), a decrease was observed in C6 gastrocnemius muscle (Figure 8). Similarly, gastrocnemius sarcoplasmic protein concentration fell from five to six weeks of age (Figure 9). No differences were observed between groups C5 and C6 in soleus sarcoplasmic protein concentration (Figure 6).

Endurance exercise acclimated animals (Ac) displayed significantly elevated homogenate protein concentration in the soleus muscle compared with sedentary controls (Figure 4). No differences were found between groups Ac and C6 in gastrocnemius homogenate protein concentration (Figure 7). While exercise acclimation did not appear to affect mitochondrial protein concentration (Figures 5 and 8), diminished sarcoplasmic protein was found in both soleus and gastrocnemius muscles of Ac animals (Figures 6 and 9).

No changes were found in the homogenate protein concentration twenty four hours following exhaustive exercise (A.Ac) in either soleus or gastrocnemius muscle (Figure 4 and 7). Soleus mitochondrial protein concentration was less in group A.Ac compared with sedentary controls (Figure 5). Group A.Ac gastrocnemius mitochondrial protein concentration was unchanged from control values (Figure 8). In both muscles, exhaustive exercise resulted in diminished sarcoplasmic protein concentration (Figures 6 and 9) and in the soleus muscle, sarcoplasmic protein was significantly less than that observed in acclimated animals.

SOLEUS HOMOGENATE
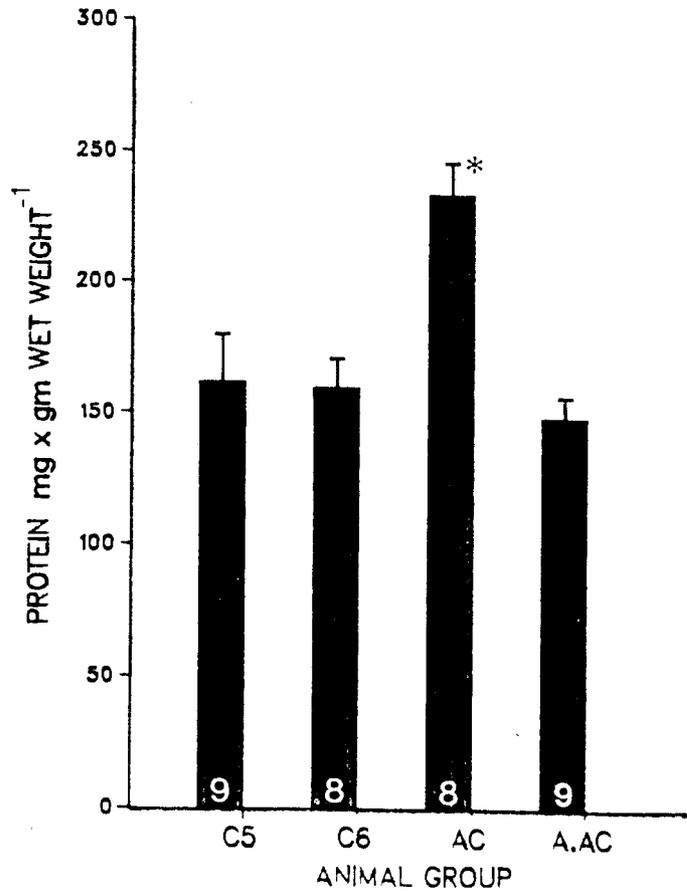
Figure 4. Soleus homogenate protein
concentration in 5 and 6 week old controls
(C5 and C6), acclimated (AC) and acutely
run rats (A.Ac) 24 hours post exercise.
* significantly different from C6 (p<0.05).

SOLEUS MITOCHONDRIAL
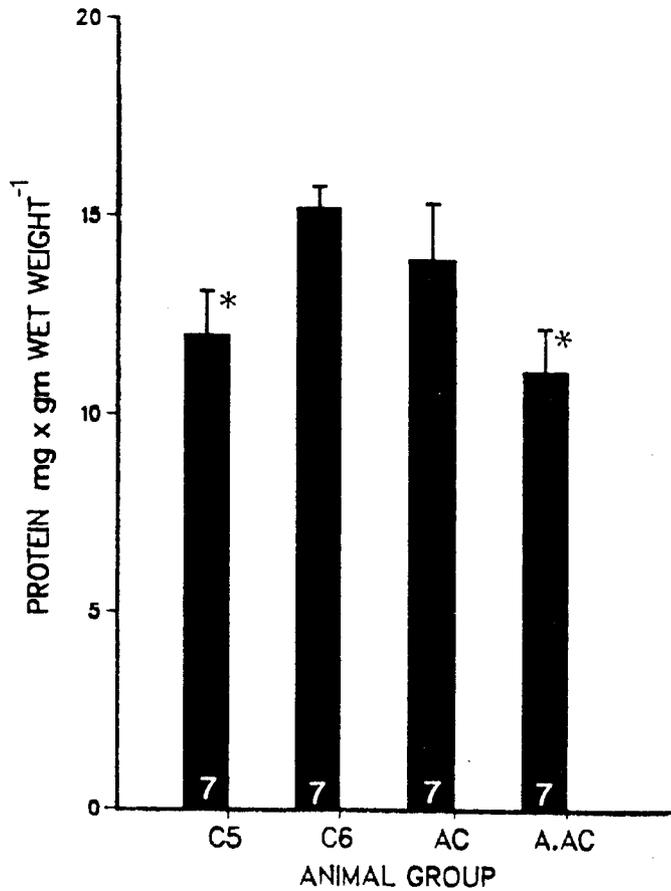
Figure 5.   Soleus mitochondrial protein
concentration with groups as described
in Fig 4.
* significantly different from C6 (P<0.05)

SOLEUS SARCOPLASMIC
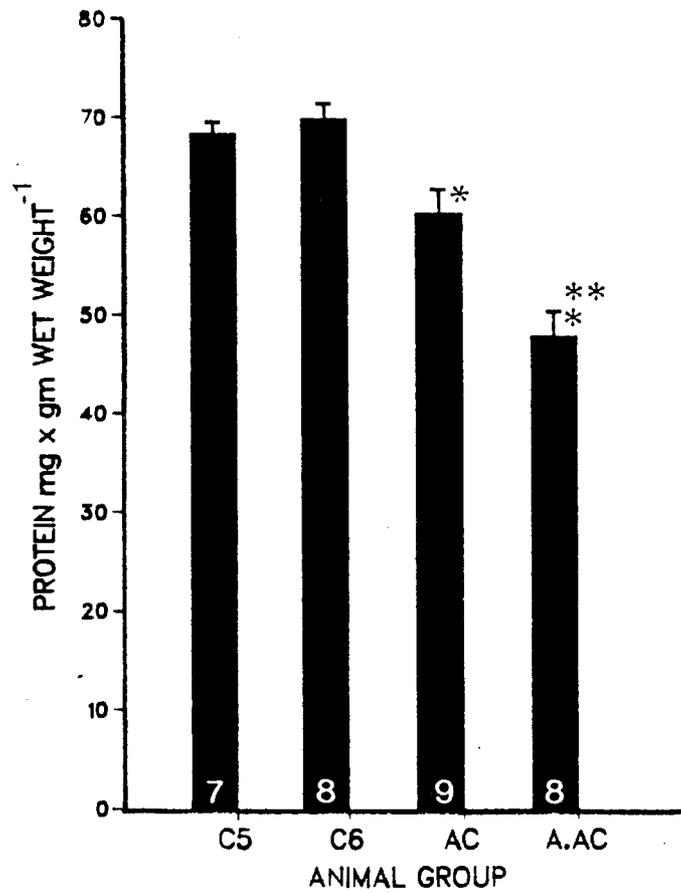
Figure 6.   Soleus sarcoplasmic protein
concentration with groups as described
in Fig 4.
* significantly different from C6 (P<0.05)
** significantly different from AC (P<0.05)

36a

GASTROCNEMIUS HOMOGENATE
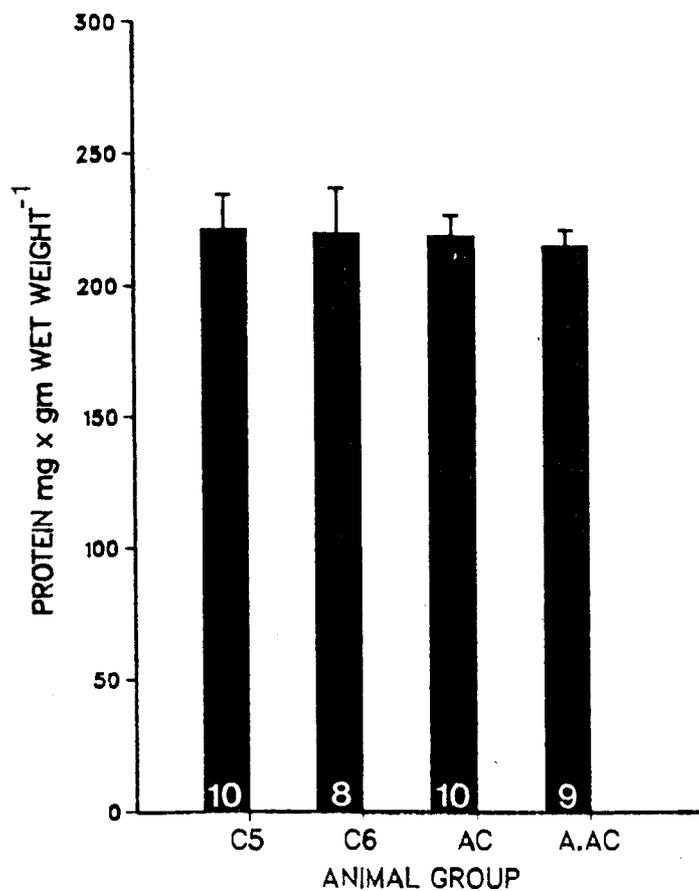
Figure 7. Gastrocnemius homogenate protein concentration with groups as described in Fig 4.

GASTROCNEMIUS MITOCHONDRIAL

Figure 8. Gastrocnemius mitochondrial
protein concentrations with groups as
described in Fig 4.
* significantly different from C6 (P<0.05)

GASTROCNEMIUS SARCOPLASMIC

Figure 9. Gastrocnemius sarcoplasmic
protein concentrations with groups as
described in Fig 4.
* significantly different from C6 (P<0.05)

Protein synthesis and relative protein turnover: Soleus Muscle

Soleus data for protein synthesis and relative protein turnover are shown in Figures 10 to 15.

In all fractions of soleus muscle, protein synthesis was unchanged between five and six week old animals (Figures 10, 12 and 14). The significantly elevated protein turnover values observed in the younger animals (Figures 11, 13 and 15) therefore denote an increased rate of protein degradation.

In all soleus muscle fractions, exercise acclimation resulted in significantly depressed protein synthesis (Figures 10, 12 and 14). In the homogenate fraction, no difference in relative protein turnover was observed between exercised and control animals (Figure 11). Significantly elevated protein turnover values in mitochondrial and sarcoplasmic fractions of Ac animals (Figures 13 and 15) implies increased protein degradation in these fractions.

Twenty four hours following exhaustive exercise, protein synthesis was significantly decreased in soleus homogenate and mitochondrial fractions (Figures 10 and 12). Sarcoplasmic protein synthesis was unchanged in group A.Ac animals (Figure 14). Significantly elevated protein turnover values were observed in all fractions of A.Ac soleus muscle (Figures 11, 13 and 15) compared with both sedentary controls (C6) and acclimated animals (Ac). This suggests higher rates of protein

degradation in acutely run rats.

SOLEUS HOMOGENATE
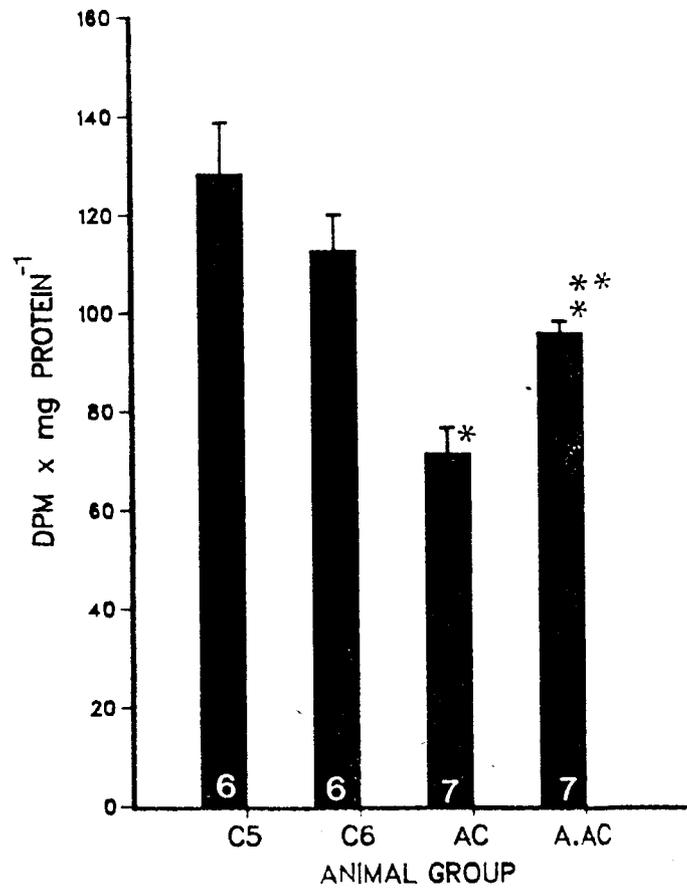
Figure 10.   Soleus homogenate protein synthesis measured by ^{14}C leucine uptake into protein in 25 minutes. The groups are as described in Fig 4.
*   significantly different from C6 (P<0.05)
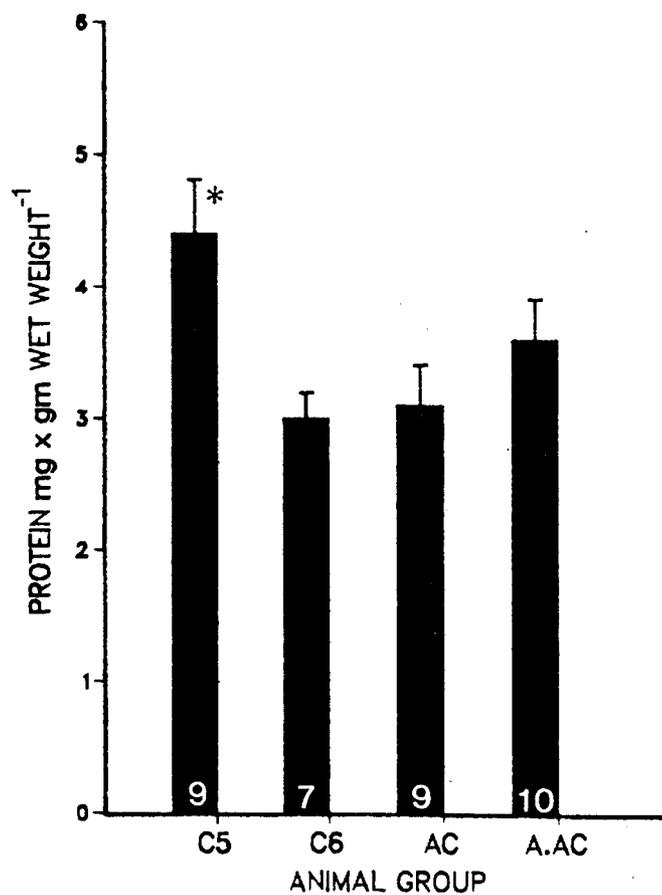** significantly different from AC (P<0.05)

SOLEUS HOMOGENATE
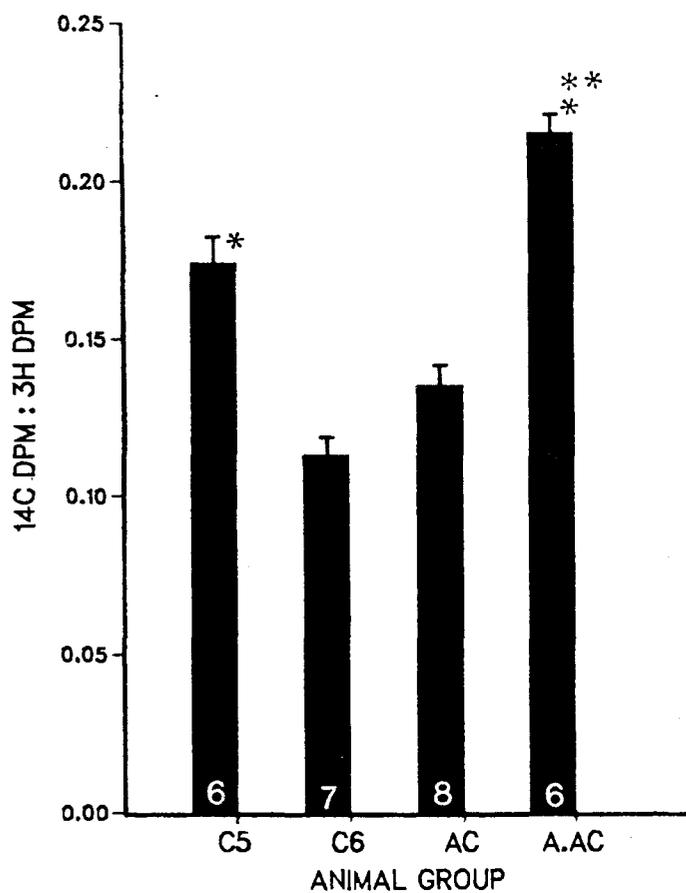
Figure 11. Soleus homogenate relative
protein turnover as measured by the ratio
of ^{14}C to ^{3}H leucine uptake into protein
The groups are as described in Fig 4.
* significantly different from C6 (P<0.05)
** significantly different from AC (P<0.05)

SOLEUS MITOCHONDRIAL

Figure 12. Soleus mitochondrial protein
synthesis with groups as described in
Fig 4.
* significantly different from C6 (P<0.05)

SOLEUS MITOCHONDRIAL

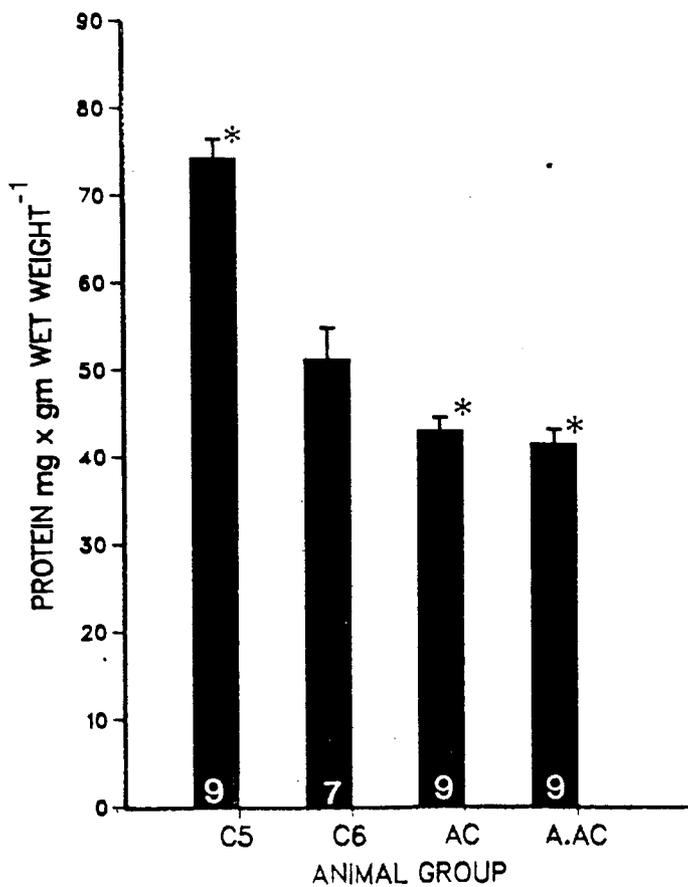Figure 13.   Soleus mitochondrial protein
turnover with groups as described in Fig
4.
* significantly different from C6 (P<0.05)
** significantly different from AC (P<0.05)

SOLEUS SARCOPLASMIC

Figure 14. Soleus sarcoplasmic protein
synthesis with groups as described in
Fig 4.
* significantly different from C6 (P<0.05)
** significantly different from AC (P<0.05)

SOLEUS SARCOPLASMIC

Figure 15.   Soleus sarcoplasmic protein
turnover with groups as described in
Fig 4.
*   significantly different from C6 (P<0.05)
** significantly different from AC (P<0.05)

42b

Protein synthesis and relative protein turnover: Gastrocnemius Muscle

Gastrocnemius data for protein synthesis and relative protein turnover are shown in Figures 16 to 21.

Protein synthesis was significantly elevated in the whole muscle homogenate (Figure 16) and mitochondrial (Figure 18) fractions of gastrocnemius muscle in five week old animals. While enhanced protein synthesis may account for the significantly elevated protein turnover values observed in these fractions (Figures 17 and 19), the possibility of concomitantly high degradation rates cannot be excluded. Only in the sarcoplasmic fraction was protein synthesis significantly greater in the more mature animals (Figure 20). Here, however, significantly greater sarcoplasmic protein turnover in the C5 animals (Figure 21) strongly supports higher rates of protein degradation in younger rats.

In endurance exercise acclimatised animals, no change in gastrocnemius homogenate protein synthesis was observed when compared with controls (Figure 16). Protein synthesis was, however, significantly depressed in mitochondrial and sarcoplasmic fractions of Ac animals (Figures 18 and 20). In all muscle fractions, significantly elevated protein turnover values were observed in acclimated animals which indicated higher rates of protein degradation than in sedentary controls (Figures 17,

19 and 21).

In exhaustively exercised animals (A.Ac) no change was found in protein synthesis in either the homogenate (Figure 16) or sarcoplasmic (Figure 20) fraction. Protein synthesis was however severely depressed in group A.Ac mitochondrial fraction (Figure 18) compared with sedentary controls. Significantly elevated rates of relative protein turnover in all fractions of acutely run animals (Figures 17, 19 and 21) again suggest higher rates of protein degradation.

GASTROCNEMIUS HOMOGENATE
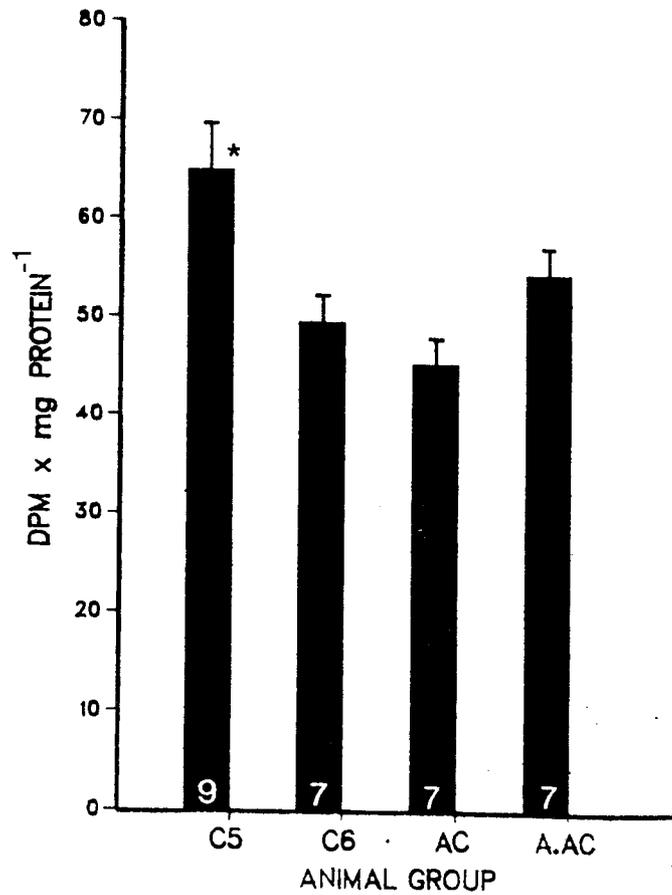
Figure 16. Gastrocnemius homogenate
protein synthesis with groups as described
in Fig 4.
* significantly different from C6 (P<0.05)

GASTROCNEMIUS HOMOGENATE
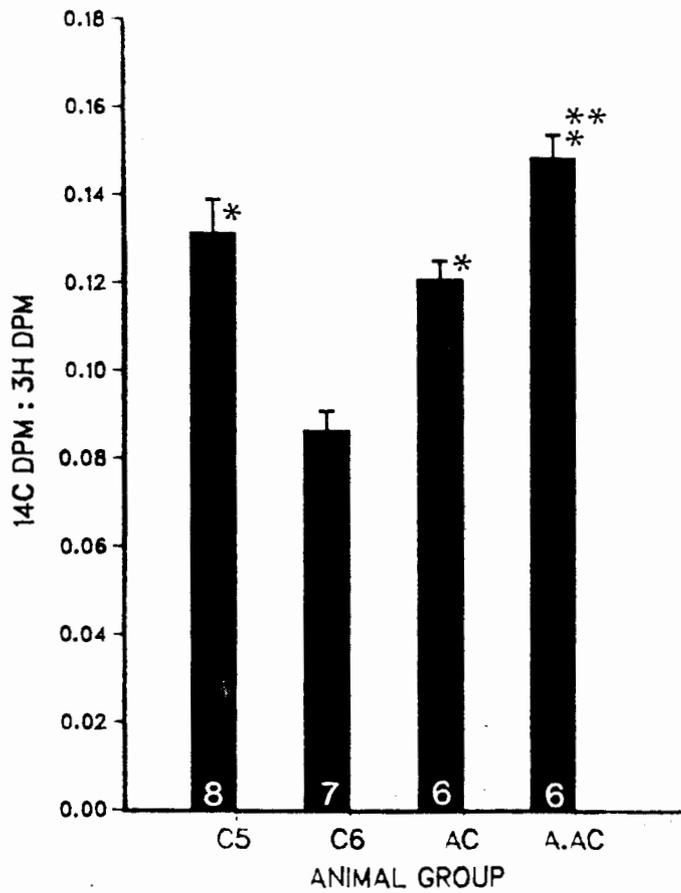
Figure 17.   Gastrocnemius homogenate
protein turnover with groups as described
in Fig 4.
*   significantly fifferent from C6 (P<0.05)
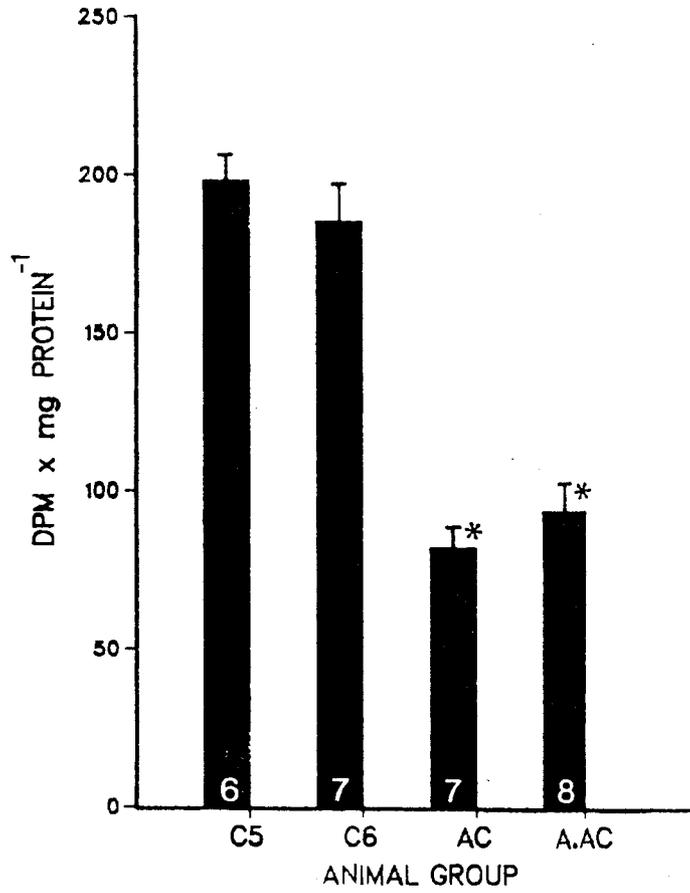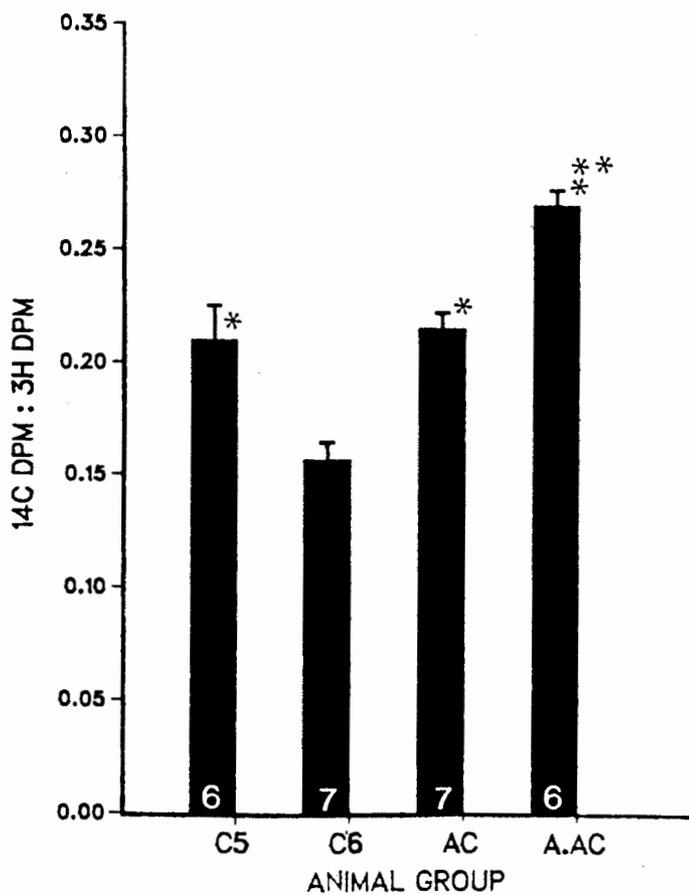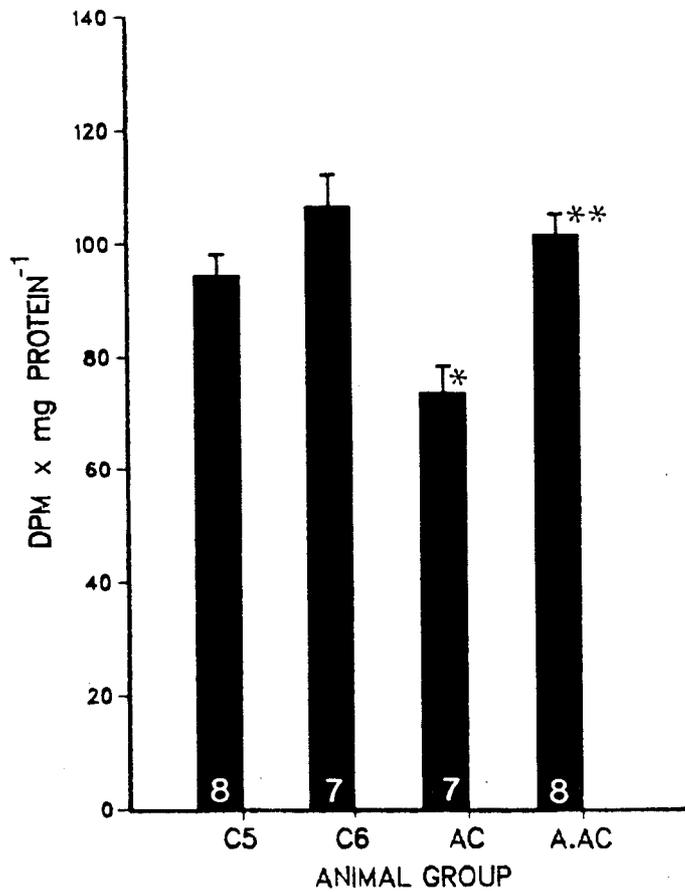** significantly different from AC (P<0.05)

GASTROCNEMIUS MITOCHONDRIAL
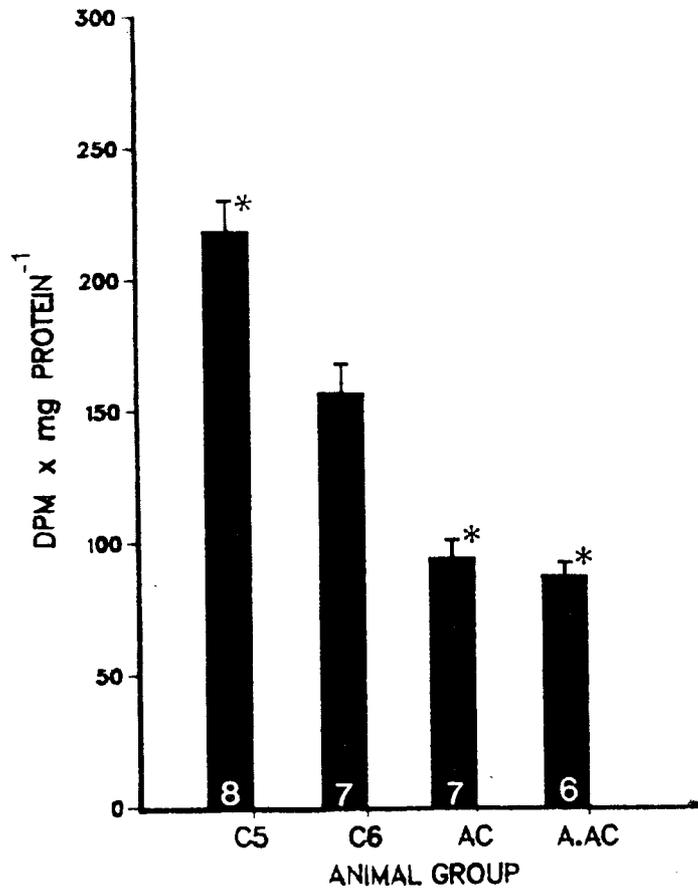
Figure 18. Gastrocnemius mitochondrial
protein synthesis with groups as described
in Fig 4.
* significantly different from C6 (P<0.05)

GASTROCNEMIUS MITOCHONDRIAL
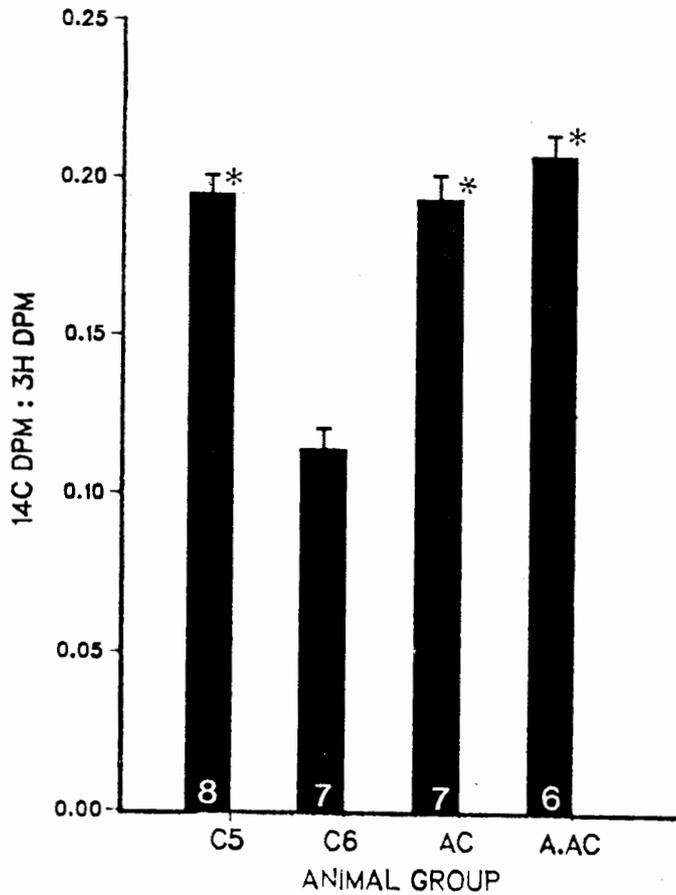
Figure 19.   Gastrocnemius mitochondrial
protein turnover with groups as described
in Fig 4.
* significantly different from C6 (P<0.05)

GASTROCNEMIUS SARCOPLASMIC
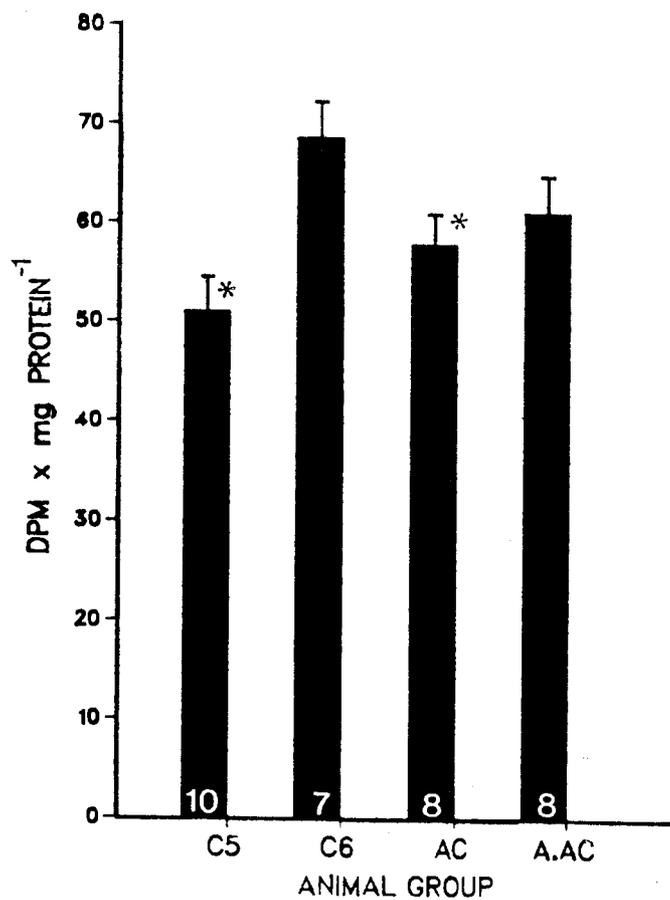
Figure 20. Gastrocnemius sarcoplasmic
protein synthesis with groups as described
in Fig 4.
* significantly different from C6 (P<0.05)
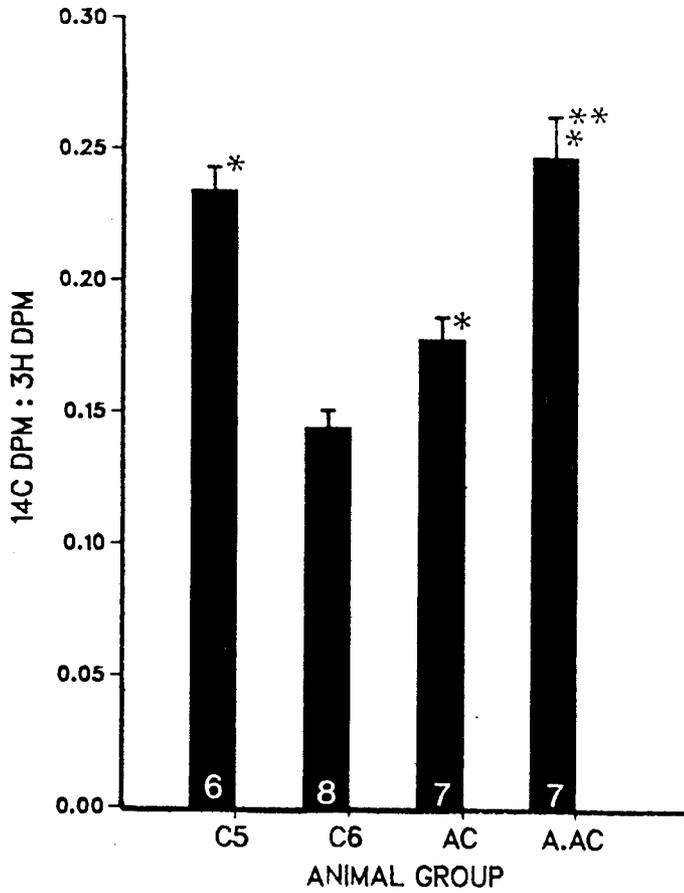
GASTROCNEMIUS SARCOPLASMIC
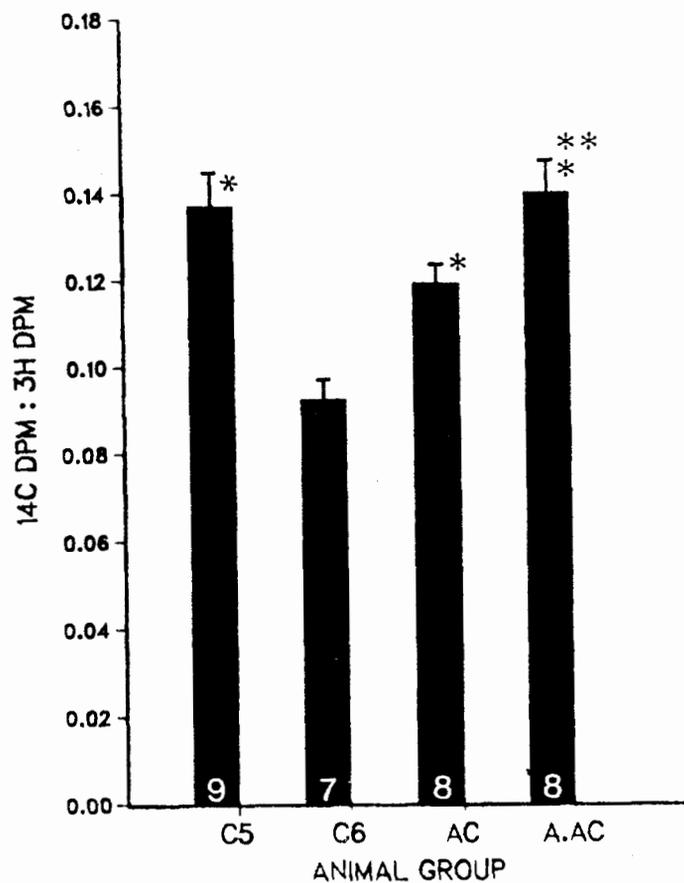
Figure 21. Gastrocnemius sarcoplasmic
protein turnover with animal groups as
described in Fig 4.
*   significantly different from C6 (P<0.05)
** significantly different from AC (P<0.05)

Results of Experiment 2

Muscle Weight, Body Weight and Run Time to Exhaustion

Group means for muscle and body weights and run time to exhaustion are listed in Table 7. No significant differences were observed between groups in any of these parameters.

Serum Testosterone: Experimental Results

Twenty four hours following injection of testosterone propionate, serum testosterone levels were signficatly depressed in groups C6T and A.AcT compared with oil injected controls (SHAM). Results are illustrated in Figure 22.

Table 7.

Body Weight, Muscle Weights and Run Time to Exhaustion

	Body Weight (g)	Soleus Weight (mg)	Gastroc. Weight (mg)	Run Time to Exhaustion (min)
C6T	191.6 (9.7)	149.9 (5.6)	778.0 (36.3)	
AcT	179.7 (8.3)	141.1 (6.8)	780.9 (55.3)	
A.AcT	184.2 (7.2)	148.3 (5.8)	765.8 (26.0)	55.8 (8.3)
SHAM	165.5 (1.5)	155.0 (3.8)	700.6 (18.7)	

Means (± standard errors) for SHAM-injected controls (SHAM), six week old testosterone treated controls (C6T) and testosterone treated acclimated (AcT) and acutely run (A.AcT) rats.

SERUM TESTOSTERONE
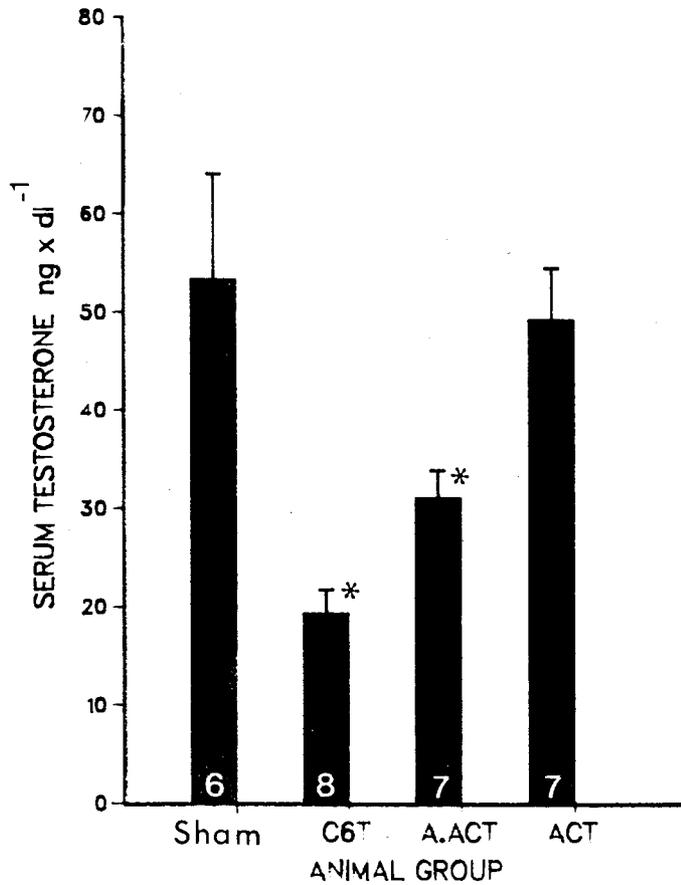
Figure 22. Serum testosterone concentration 24
hours following injection of testosterone
propionate in 6 week old controls (C6T),
acclimated (ACT) and acutely run rats (A.AcT).
Sham control animals (SHAM) received 0.2 ml
oil vehicle.
* significantly different from SHAM group,
(p<0.05).

Protein Concentration: Experiment 2

In six week old sedentary animals, testosterone administration had no effect on homogenate protein concentration in either soleus or gastrocnemius muscles (Figures 23 and 26). While testosterone administration resulted in significantly elevated gastrocnemius mitochondrial protein concentration (Figure 27), severely depressed mitochondrial protein content was found in the soleus muscles of testosterone treated rats (Figure 24). Sarcoplasmic protein concentration was decreased ($p < 0.05$) in the soleus muscle of group C6T (Figure 25) while no change was observed in gastrocnemius sarcoplasmic protein following testosterone injection (Figure 28).

In exercise acclimated animals, testosterone treatment produced contrasting results in the homogenate fraction of the two muscles studied. In soleus muscle, homogenate protein was diminished ($p < 0.05$) after hormone treatment (Figure 23) whereas significantly elevated homogenate protein concentrations were observed in gastrocnemius muscles of group AcT rats (Figure 26). In both muscles, mitochondrial protein concentration was unaffected by testosterone administration (Figures 24 and 27). Elevated sarcoplasmic protein concentrations ($p < 0.05$) were observed in both soleus and gastrocnemius muscles following testosterone injection (Figures 25 and 28).

The combination of acute exercise and testosterone administration had no effect on soleus homogenate protein concentration (Figure 23). Gastrocnemius homogenate protein concentration, however, was significantly increased in exhausted, testosterone treated animals (Figure 26). In both muscles of group A.AcT rats, mitochondrial (Figures 24 and 27) and sarcoplasmic (Figures 25 and 28) protein concentrations were elevated following testosterone administration.

SOLEUS HOMOGENATE
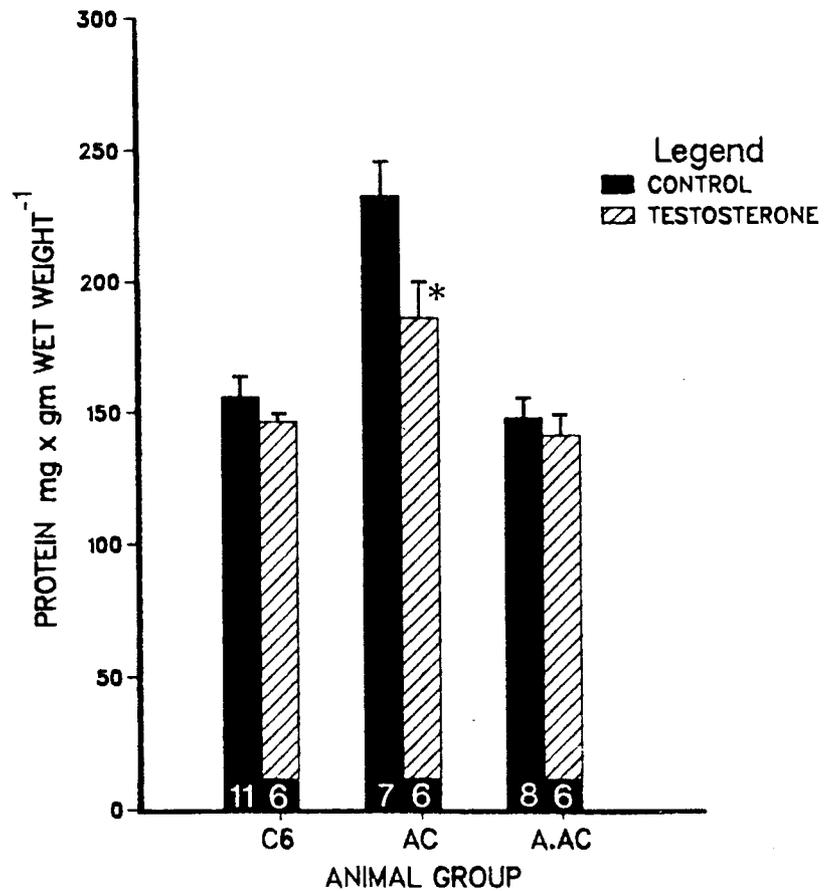
Figure 23. Soleus homogenate protein concentration in control (C6), acclimated (AC) and acutely run rats (A.AC). * significantly different from control animals (p<0.05)

SOLEUS MITOCHONDRIAL
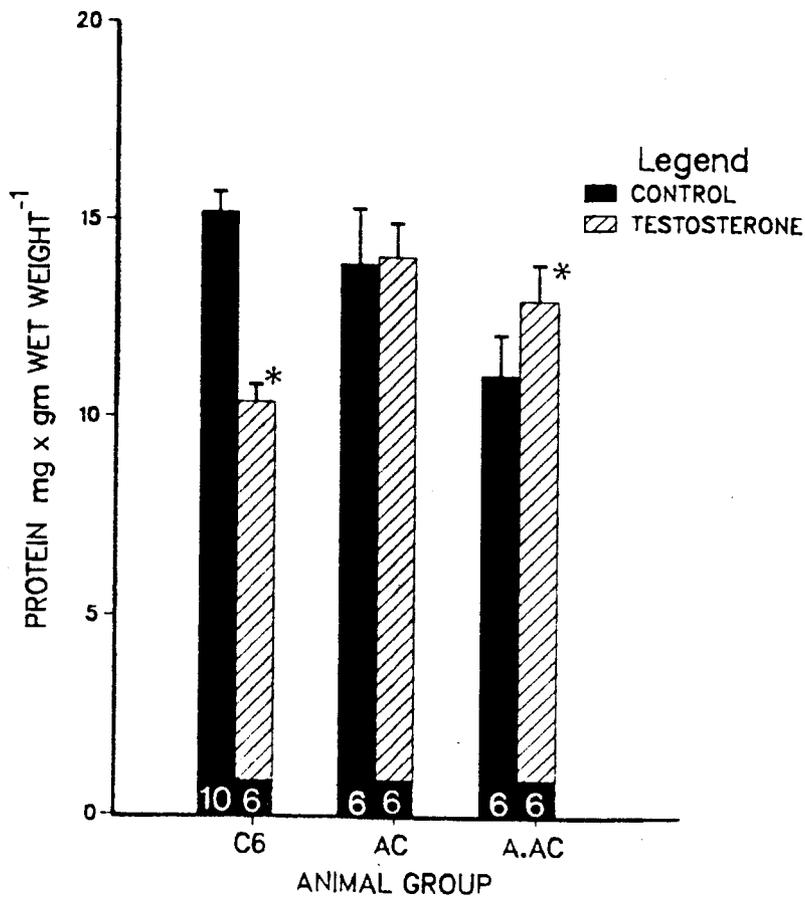
Figure 24. Soleus mitochondrial protein concentration with groups as described in Fig 23.
* significantly different from control animals ($p < 0.05$)

SOLEUS SARCOPLASMIC
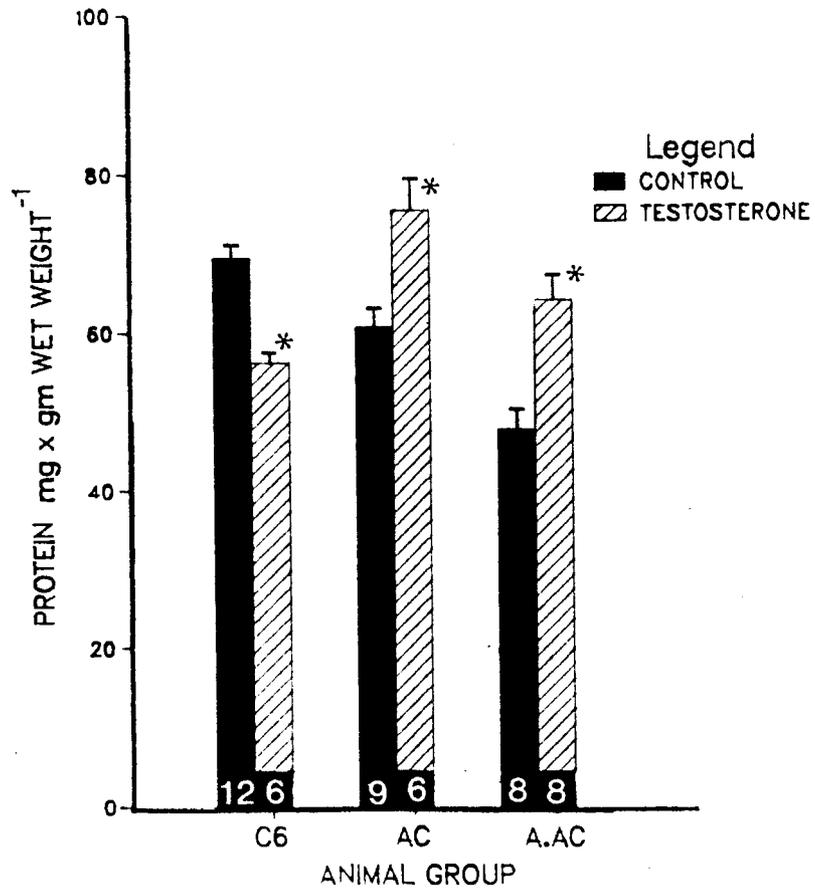
Figure 25. Soleus sarcoplasmic protein concentration with groups as described in Fig 23.
* significantly different from control animals (p<0.05)

GASTROCNEMIUS HOMOGENATE
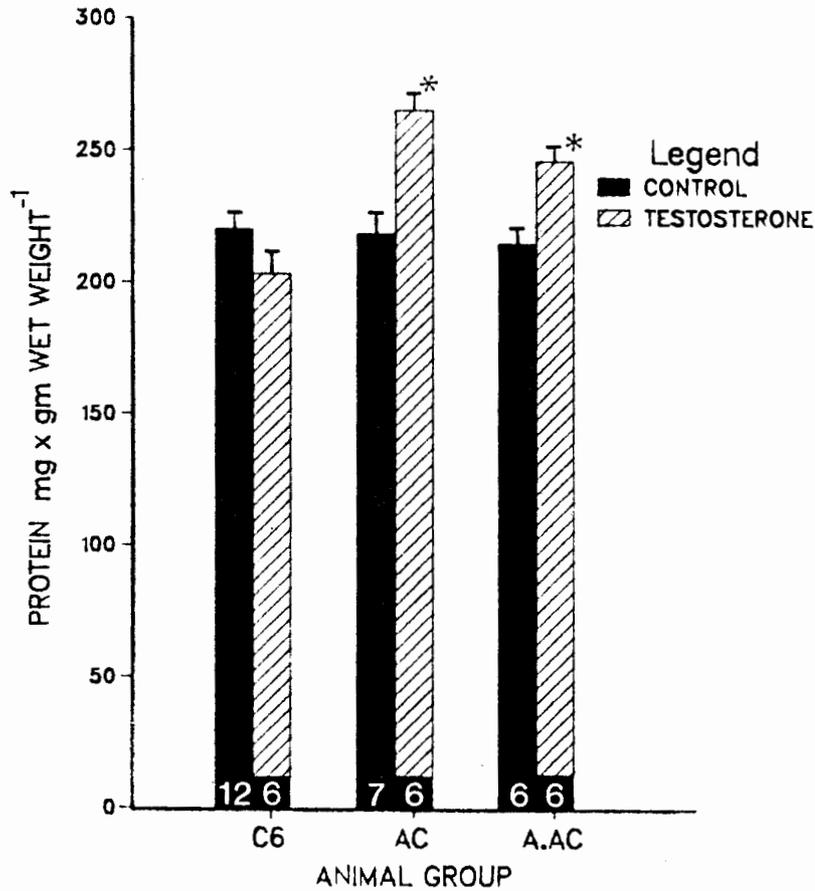
Figure 26. Gastrocnemius homogenate protein concentration with groups as described in Fig 23.
* significantly different from control animals (p<0.05)

54b

GASTROCNEMIUS MITOCHONDRIAL
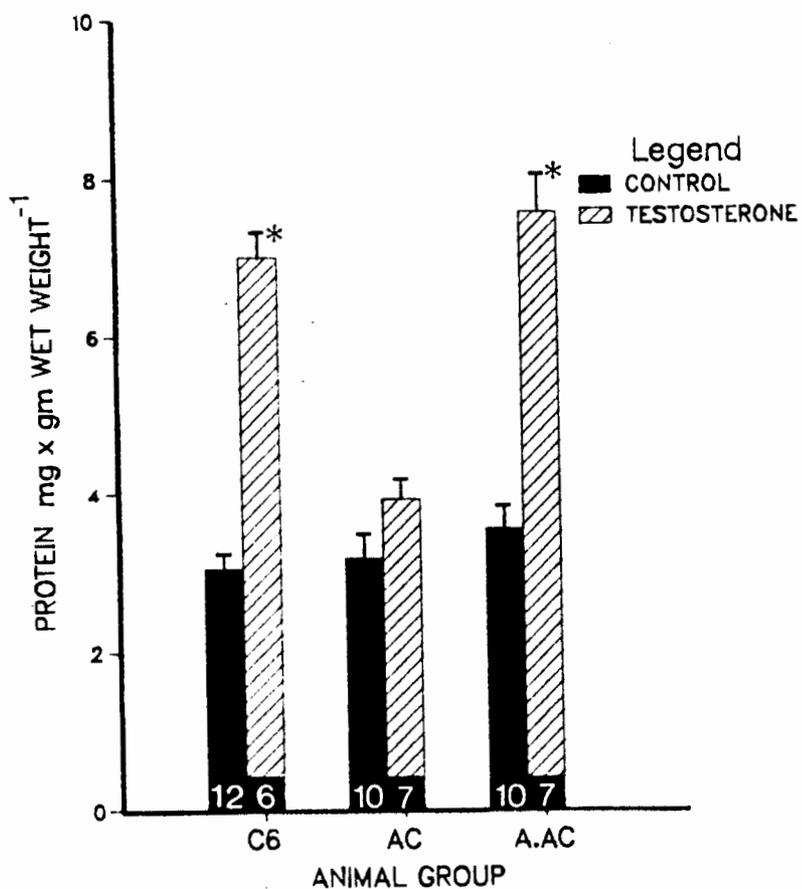
Figure 27. Gastrocnemius mitochondrial protein concentration with groups as described in Fig 23.
* significantly different from control animals (p<0.05)

GASTROCNEMIUS SARCOPLASMIC

Figure 28. Gastrocnemius sarcoplasmic protein concentration with groups as described in Fig 23.
* significantly different from control animals (p<0.05)

Protein synthesis: Soleus muscle

In six week old sedentary control animals, testosterone administration had no effect on soleus homogenate protein synthesis (Figure 29). Mitochondrial protein synthesis was significantly decreased in testosterone treated animals (Figure 30), while no change was observed in the sarcoplasmic fraction (Figure 31).

In all fractions of soleus muscle, testosterone administration resulted in significant increases in protein synthesis in endurance exercise acclimated rats (Figures 29, 30 and 31).

Testosterone administration immediately following exhaustive exercise resulted in significant increases in soleus homogenate (Figure 29) and mitochondrial (Figure 30) protein synthesis. Protein synthesis in the sarcoplasmic fraction of group A.AcT animals was unaffected by testosterone treatment (Figure 31).

SOLEUS HOMOGENATE
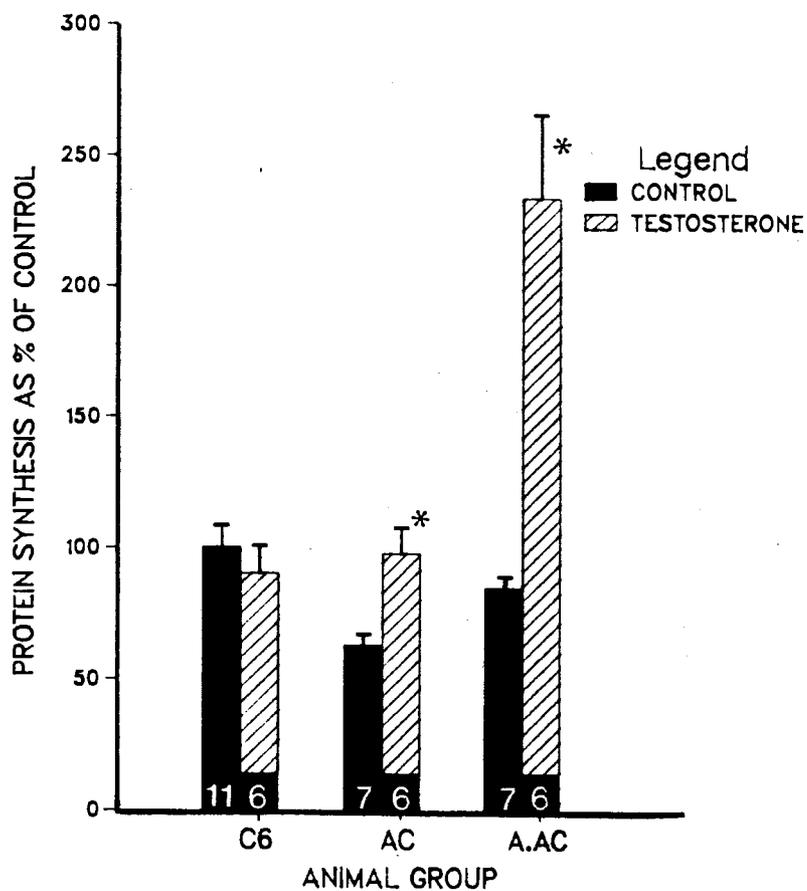
Figure 29. Soleus homogenate protein synthesis expressed as a percentage of 6 week old control values with groups as described in Fig 23.
* significantly different from control animals (p<0.05)

SOLEUS MITOCHONDRIAL
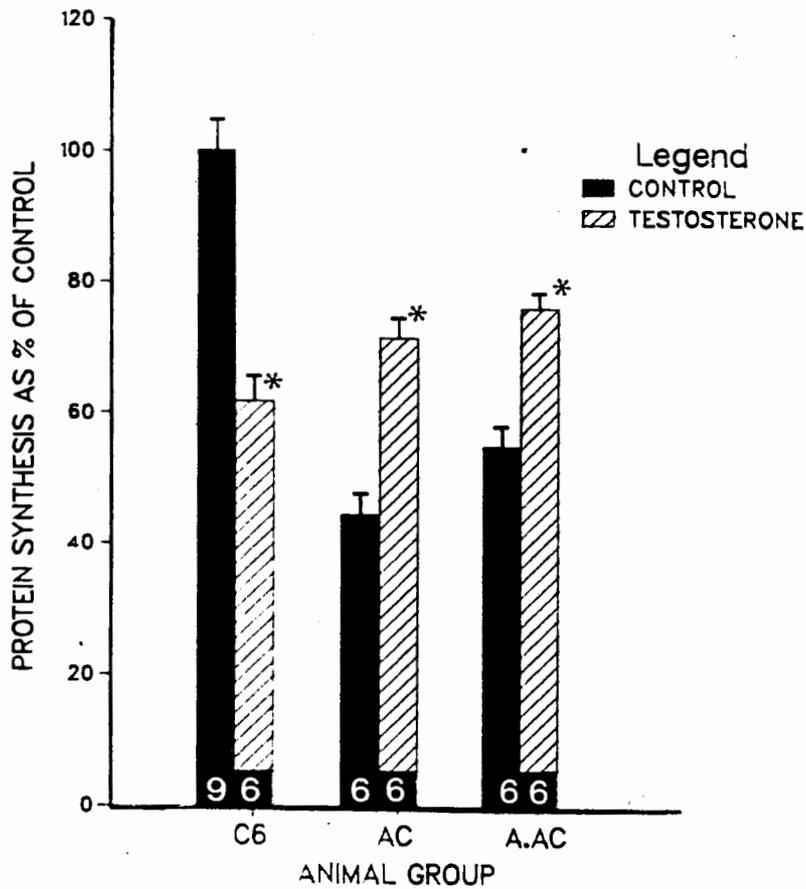
Figure 30. Soleus mitochondrial protein synthesis expressed as a percentage of 6 week old control values with groups as described in Figure 23.
* significantly different from control animals (p<0.05).

SOLEUS SARCOPLASMIC
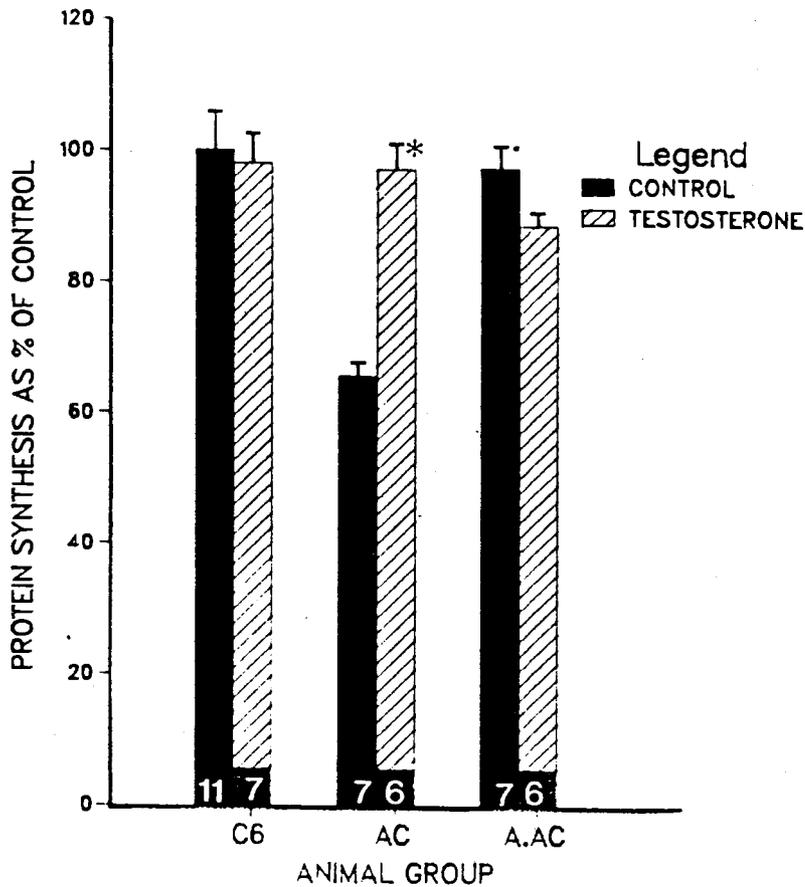
Figure 31. Soleus sarcoplasmic protein
synthesis expressed as a percentage of
6 week old control values with groups
as described in Figure 23.
* significantly different from control
  animals (p<0.05).

Protein synthesis: Gastrocnemius Muscle

In all fractions of the gastrocnemius muscle, testosterone administration resulted in significantly depressed rates of protein synthesis in sedentary, six week old animals (Figures 32, 33 and 34).

In animals acclimated to endurance exercise, testosterone administration did not affect protein synthesis in the homogenate fraction of gastrocnemius muscle (Figure 32). Mitochondrial protein synthesis was increased ($p < 0.05$) following testosterone treatment (Figure 33) while significantly depressed rates of protein synthesis were observed in the sarcoplasmic fraction of group AcT (Figure 34).

Testosterone administration immediately following exhaustive exercise had no effect on protein synthesis in any fraction of the gastrocnemius muscle (Figures 32, 33 and 34).

GASTROCNEMIUS HOMOGENATE
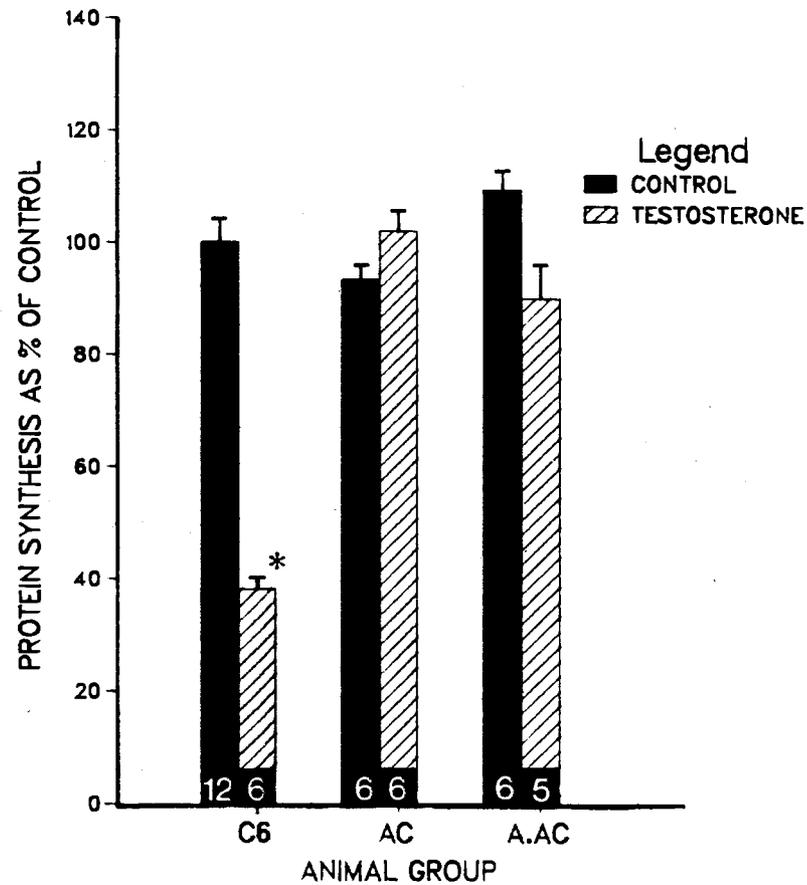
Figure 32. Gastrocnemius homogenate protein
synthesis expressed as a percentage of 6
week old control values with groups as
described in Figure 23.
* significantly different from control
  animals (p<0.05).

GASTROCNEMIUS MITOCHONDRIAL
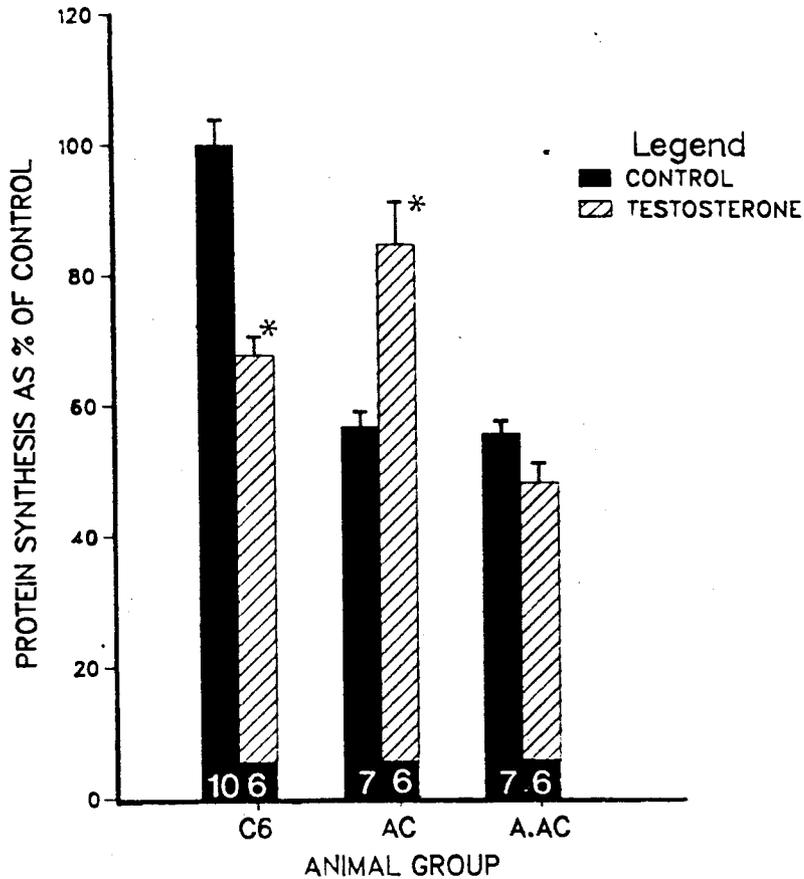
Figure 33. Gastrocnemius mitochondrial protein
synthesis expressed as a percentage of 6 week
old control values with groups as described
in Figure 23.
* significantly different from control
  animals (p<0.05).

GASTROCNEMIUS SARCOPLASMIC

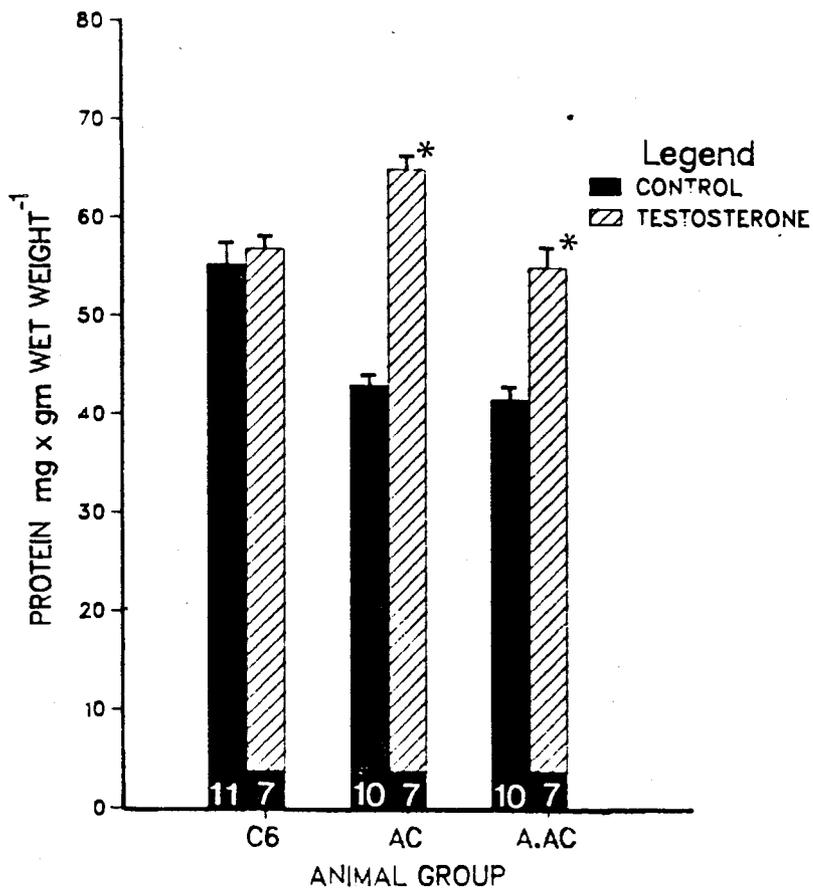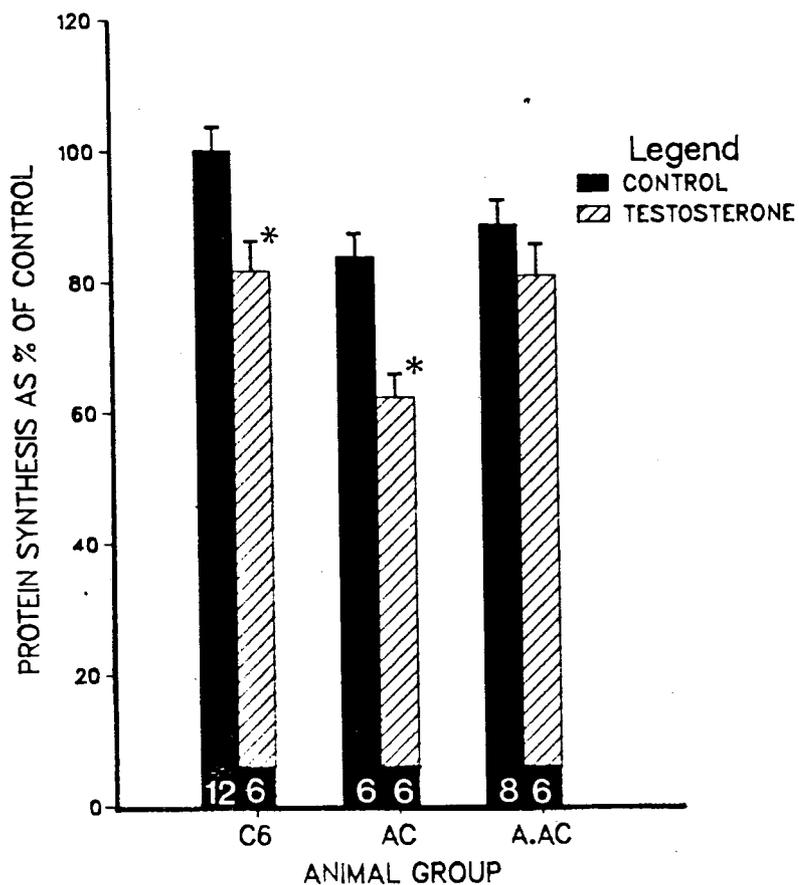Figure 34. Gastrocnemius sarcoplasmic protein synthesis expressed as a percentage of 6 week old control values with groups as described in Figure 23.
* significantly different from control animals (p<0.05).

Summary of Data from Experiments 1 and 2

Comparisons of treatment (testosterone or no testosterone) and group (control, acclimated, acute) effects using combined protein concentration and protein synthesis data from Experiments 1 and 2 were analysed using a two way analysis of variance. A high degree of significance prompted further one way analyses of variance and post hoc comparisons using Student Newman-Keuls tests. Dependent variables were compared by group or treatment. The combinations of animal groups used in these analyses and group means are shown in Tables 8, 9 and 10.

In the soleus homogenate fraction, exercise acclimation resulted in significantly elevated homogenate protein concentrations (Table 8). A significant loss of protein was observed in the sarcoplasmic fraction of acutely run animals.

Similar findings were observed in gastrocnemius protein concentration in the homogenate fraction. However, in the mitochondrial fraction of gastrocnemius, muscle protein concentration was significantly depressed compared with acutely run animals.

Protein synthesis was elevated (p<0.05) in the homogenate fraction of soleus muscle of accutely run animals (Table 9). Depressed rates of protein synthesis (p<0.05) were observed in the soleus mitochondrial fraction of both groups of exercised animals. The rate of protein synthesis in soleus sarcoplasmic

fraction was not affected by either exercise treatment. In the homogenate fraction of the gastrocnemius muscle, exercise did not affect protein synthesis. Protein synthesis was significantly reduced in the mitochondrial fraction of both groups Ac and A.Ac, with acutely run animals demonstrating significantly lower protein synthesis values than those observed in the acclimated groups. In the gastrocnemius sarcoplasmic fraction, protein synthesis was reduced (p<0.05) in acclimated animals.

Whereas testosterone had no apparent effect on soleus muscle protein concentration, all fractions of gastrocnemius muscle from animals receiving testosterone showed significantly elevated protein concentrations (Table 10).

Testosterone treatment resulted in significantly increased protein synthesis in the homogenate fraction of soleus muscle (Table 10). Protein synthesis in mitochondrial and sarcoplasmic fractions of soleus was unaffected by treatment with the hormone. In the gastrocnemius muscle, protein synthesis was significantly reduced in homogenate and sarcoplasmic fractions of testosterone treated animals.

Table 8.

Protein Concentration: Experiments 1 and 2

	Control	Ac	A.Ac
Soleus Homogenate	152.5 (4.3)	211.2 * (10.7) **	144.9 (4.8)
Soleus Mitochondrial	11.4 (0.4)	13.2 (0.7)	11.9 (0.6)
Soleus Sarcoplasmic	64.8 (1.7)	66.8 ** (2.9)	56.0 * (2.8)
Gastrocnemius Homogenate	213.5 (4.7)	240.3 * (8.4)	230.1 (5.8)
Gastrocnemius Mitochondrial	4.2 (0.4)	3.5 ** (0.2)	5.2 (0.5)
Gastrocnemius Sarcoplasmic	54.9 (1.6)	51.8 (2.6)	47.3 (2.0)

Means (± standard errors) in milligrams per gram wet weight.
Control includes data from groups C6, C6T, and SHAM.
Ac includes data from groups Ac and AcT.
A.Ac includes data from groups A.Ac and A.AcT.

 * Significant difference from control ($p < 0.05$)
** Ac significantly different from A.Ac ($p < 0.05$)

Table 9.

Protein Synthesis: Experiments 1 and 2

	C6	Ac	A.Ac
Soleus Homogenate	94.1 (6.0)	81.1 ** (6.8)	146.9 * (5.3)
Soleus Mitochondrial	84.1 (5.9)	57.3 * (5.1)	64.7 * (3.7)
Soleus Sarcoplasmic	97.1 (4.3)	82.0 (6.3)	89.7 (3.0)
Gastrocnemius Homogenate	81.8 (7.1)	98.3 (2.5)	101.2 (4.1)
Gastrocnemius Mitocondrial	84.9 (4.9)	71.0 ** (5.3) *	52.5 * (1.8)
Gastrocnemius Sarcoplasmic	94.1 (3.2)	71.9 ** (4.2) *	85.3 (3.0)

Means (± standard errors) expressed as percent of control
Control includes data from groups C6, C6T, and SHAM.
Ac includes data from groups Ac and AcT.
A.Ac includes data from groups A.Ac and A.AcT.

 * significant difference from control (p<0.05)
** Ac significantly different from A.Ac(p<0.05)

Table 10.

Protein Concentration and Protein Synthesis:
Experiments 1 and 2

	Protein Concentration		Protein Synthesis	
	No Test.	Test.	No Test.	Test.
Soleus Homogenate	174.1 (8.5)	157.8 (6.5)	85.2 (4.6)	135.2 * (18.6)
Soleus Mitochondrial	11.7 (0.5)	12.4 (0.5)	71.4 (6.0)	69.9 (2.2)
Soleus Sarcoplasmic	60.7 (2.0)	65.2 (2.3)	90.5 (4.1)	92.1 (3.2)
Gastrocnemius Homogenate	217.6 (3.0)	237.6 * (7.2)	100.6 (2.4)	78.3 * (7.4)
Gastrocnemius Mitochondrial	3.4 (0.1)	6.2 * (0.4)	73.3 (4.8)	68.0 (4.4)
Gastrocnemius Sarcoplasmic	46.9 (1.4)	58.1 * 1.2)	92.8 (2.4)	74.5 * (3.2)

Group means(±Standard errors) for protein concentration expressed as mg protein per g wet weight and protein synthesis expressed as a percentage of sedentary, non-testosterone treated controls.
No Test. groups include data from C6, SHAM, Ac and A.Ac.
Test. groups include data from C6T, AcT and A.AcT.

* Test. significantly different from No Test. (p<0.05).

IV. Discussion

Experiment 1

Body and muscle weights of six week old exercised animals
were greater than those of sedentary controls. This was
particularly true of acutely exercised animals. These results
are in contrast to those from previous studies where exercise in
growing animals resulted in lower body and muscle weights than
sedentary controls (Hubbard et al., 1974, Reed et al., 1974).
Since the acclimation period to running in the present study was
brief (five days) and the exercise stress was mild, it is
conceivable that the combination of these factors provided a
transient stimulus to growth. Stevenson et al. (1966) report
that prolonged exercise reduced food intake in male rats. While
this may account for diminished body weight in young rats
following a strenuous, prolonged training program, it is
improbable that this occured in the present study given the mild
and brief nature of the exercise stimulus. As food intake was
not monitored, this remains as speculation.

In the soleus mitochondrial fraction of C6 animals,
significantly elevated protein concentrations were observed.
While research from other laboratories has demonstrated
significant increments in protein concentration in all fractions

of skeletal muscle with age (Srivastava and Chaudhary, 1969;
Beecher, 1974; Giovanetti and Strothers, 1975), data in these
studies were collected at approximately four week intervals. It
is possible that the growth period employed in the present study
may have been too short to significantly alter protein
concentrations even though significant changes in body weight
were observed.

Relative skeletal muscle protein turnover rate declined
from five to six weeks of age in all fractions of both soleus
and gastrocnemius muscles. Protein synthesis was depressed in
gastrocnemius homogenate and mitochondrial fractions of C6 rats
compared with C5 animals. These results are in agreement with
others who report a decrement in protein synthesis over the
first 60 days of life (Srivastava and Chaudhary, 1969) and a
decline in overall muscle protein turnover rate from birth to
puberty (Bates and Millward, 1983; Lewis et al., 1984). The
observed consistently higher rate of protein turnover in the
slow soleus muscles relative to the gastrocnemius, a
predominantly fast muscle, is consistent with previous research
findings (Kelly et al., 1984; Lewis et al., 1984). The reduction
in protein synthesis with age has been attributed to reduced RNA
synthesis and RNA capacity (rate of translation) in skeletal
muscle (Giovanetti and Strothers, 1975; Lewis et al., 1984). The
accompanying high rate of protein degradation observed during
growth, however, is less clearly understood and has been
implicated in myofibrillar remodelling and differentiation

(Millward et al., 1980). Increased protein degradation during growth is "anabolic" in nature and is not accompanied by altered lysosomal activity as is observed in "catabolic" conditions of increased muscle protein degradation such as starvation (Millward et al., 1978). Although Stewart (1982) has suggested that normal regulation of muscle growth (ie. high protein turnover rate) is achieved through stretch imposed on muscles by bone growth, other factors such as high levels of growth hormone and somatomedin or neural input should not be overlooked.

In acutely exercised, acclimated, mature animals, protein synthesis generally increases approximately 24 hours following endurance exercise (Wenger et al., 1981). Subsequent alterations in protein concentration occur sometime later, depending upon the particular pool being studied (Dallaire, 1980; Wenger et al., 1981). The magnitude of the response appears to be related to exercise intensity (Dohm et al., 1980, 1982b). In endurance trained animals, slow oxidative and fast oxidative glycolytic muscle fibres would primarily be recruited (Burke and Edgerton, 1975) and corresponding changes in protein content might be anticipated in the mitochondrial fraction of the muscle (Holloszy, 1975). While previous studies are inconclusive, protein degradation appears to be depressed in mature animals during exercise and the early phase of recovery, whereafter enhanced degradation occurs (Booth et al., 1982).

The elevated rates of protein degradation and turnover in all fractions of both soleus and gastrocnemius muscles 24 hours following exhaustive exercise found in the present study are similar to observations in older animals (Booth et al., 1982). However, a significantly depressed rate of protein synthesis in the sarcoplasmic fraction of gastrocnemius and in both sarcoplasmic and mitochondrial fractions of soleus muscle observed in the present study suggests that a catabolic increase in protein degradation is occuring. These results are in contrast to observations in mature, acclimated animals where protein synthesis is elevated 24 hours following acute endurance exercise (Dallaire, 1980; Wenger et al., 1981). Clearly, the muscles of young animals respond differently to acute exhaustive exercise compared to mature animals. Possible factors regulating the diminished acute protein synthetic response to exercise must be considered.

In the present study, young animals were run to exhaustion following an initial period of acclimation. As food intake was not restricted, it may be assumed that rats were adequately nourished at the time of exhaustive exercise. All experimental groups, however, were fasted six hours prior to sacrifice to minimize variation in absorbtion of radioactively labelled leucine from the gut. Millward et al. (1976, 1978) have found that two days of starvation resulted in significant reductions in rates of both protein synthesis and degredation. By the third day of the fast, the protein degradation rate had begun to

increase and by the fourth day was higher than that observed in fed, sedentary controls. In the present study, the six hour fast may have resulted in a slight reduction in overall protein turnover rate. Given the exhausted, and presumably carbohydrate depleted state of the exhausted group, A.Ac, this effect (ie. reduced protein turnover) may have been amplified. The opposite result, however, of significantly elevated protein turonver, was observed.

Since animals performed maximally during the exhaustive run, depressed insulin levels and elevated catecholamines, growth hormone, glucagon and glucocorticoids immediately following exercise would be anticipated (Hartley et al., 1972a, 1972b; Terjung, 1979). In adult animals, elevated androgen levels might also be anticipated following a high intensity, exhaustive run (Sutton et al., 1973; Aldercruetz et al., 1976; Galbo et al., 1977b; Kindermann et al., 1982). While elevated androstenedione levels during intense exercise are probably due to an increase in adrenal activity (Aldercruetz et al., 1976), testosterone appears to be of testicular origin (Guezennec et al., 1982). Given the undeveloped gonadal status of prepubescent rats, it is unlikely that an increase in testosterone comparable in magnitude to that observed in adult animals would be observed in sexually immature rats following exhaustive exercise.

Immediately following exercise, animals were returned to their cages and offered food and water ad libitum. Both feeding (Millward et al., 1983) and decreased muscle usage (Hartley et

al., 1972a, 1972b) are known to restore plasma insulin levels to normal very rapidly. In starved animals, refeeding has been observed to restore glucocorticoids to normal levels within 40 minutes (Millward et al., 1983) and in exercising man, cortisol levels generally return to control values within the first two hours of recovery (Bonen, 1976).

Corticosterone in the rat has been shown to depress muscle protein synthesis even when insulin levels are very high (Odera and Millward, 1982; Odera et al., 1982, 1983). Thus, high glucocorticoid levels during the early phase of recovery from exhaustive exercise may have contributed to depressed protein concentrations in soleus mitochondrial and sarcoplasmic fractions and gastrocnemius sarcoplasmic fractions, as well as to the reduced rate of protein synthesis observed in soleus homogenate and the mitochondrial fractions of both muscles. This glucocorticoid effect may have been potentiated in young animals by reduced endogenous testosterone levels as testosterone is known to both compete for glucocorticoid receptors (Mayer and Rosen, 1977) and accelerate dissociation of previously bound glucocorticoid hormone (Jones and Bell, 1983). Support for this hypothesis comes from results in Experiment 2 where testosterone injection immediately following exercise appeared to suppress the acute catabolic response to exhaustive exercise. This is indicated by a significant increase in protein content of testosterone treated rats 24 hours following exhaustive exercise in the mitochondrial and sarcoplasmic fractions of both muscles

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and by an increase in protein synthesis in soleus homogenate and mitochondrial fractions of group A.AcT. Thus, data from the young, testosterone treated animals resembles more closely the protein synthetic profile reported in adult animals (Wenger et al., 1981).

In summary, it is suggested that skeletal muscles of prepubescent rats respond to acute, exhaustive exercise differently from those of adult animals. While increased protein synthesis is observed in adult skeletal muscle 24 hours following acute exercise, (Wenger et al., 1981; Booth et al., 1982), protein synthesis was depressed in the skeletal muscle of young rats. It is speculated that this difference may be related to low endogenous levels of testosterone in the young animals following exhaustive exercise, which would act indirectly through their inability to modify or diminish the catabolic actions of glucocorticoids on skeletal muscle.

In young animals sacrificed three days following a five day mild exercise acclimation program, protein synthesis was reduced in all fractions of soleus muscle while degradation and turnover were elevated. The increased protein content of soleus homogenate fraction points to a ·preceding rise in protein synthesis. Significantly elevated SDH activity in muscles of exercised animals suggests that the acclimation protocol stimulated synthesis of mitochondrial enzymes.

With the exception of gastrocnemius homogenate fraction, protein synthesis was diminished in all fractions of both

muscles of acclimated animals 72 hours following the final exercise session. It is suggested that removal of the exercise stimulus, or decreased use may account for this observation. Elevated relative protein turnover and degradation rates in both muscles of acclimated animals would lend support to this theory by contributing to a return of protein content towards that seen in controls. It is unlikely that glucocorticoids played a significant role in mediating depressed protein synthesis as these animals were never subjected to stressful, exhaustive exercise. A second explanation for increased protein degradation following exercise acclimation comes from Vihko et al. (1978b, 1979) and Pilstrom et al. (1978) who found that very prolonged endurance exercise increases the activities of some acid hydrolases (notably B-glucouronidase and Cathepsin D), with a peak in activity occuring between three and five days post exercise. As protein degradation rates were not directly measured by these investigators, the degree to which enhanced lysosomal activity actually affected protein degradation is unknown. Furthermore, the extent to which the exercise acclimation in the present study might have stimulated lysosomal enzyme activity in young animals in unknown.

In the gastrocnemius muscle, protein content was unchanged from controls with the exception of the sarcoplasmic fraction where reduced levels were observed. In mature animals the gastrocnemius muscle is generally composed of "fast" muscle fibres (Ariano, 1973) and is increasingly recruited during high

intensity exercise (Burke and Edgerton, 1975). In the rat, most muscles are uniformly slow at birth and changes in metabolic and dynamic properties of the muscles with growth have been attributed in part to the influence of their motor neurones (Close, 1972; Hubbard et al., 1974). Wilkinson et al. (1978) found that chronic exercise (both aerobic and anaerobic) in young rats (5 to 15 weeks) resulted in maintenance of the fast oxidative glycolytic (FOG) fibre type population in soleus and plantaris muscles rather than in overt shifts in fibre types as observed by 15 weeks in sedentary controls. Hubbard et al. (1974) report that endurance exercise in young animals slows the rate and prolongs the phase of nuclear proliferation. Together, these reports suggest that exercise in young animals may slow the rate of normal developmental shifts in protein accretion and fibre type differentiation. In the present study, the observed lower gastrocnemius sarcoplasmic protein concentration in acclimated animals may reflect a delay or disturbance in the normal differentiation process of this muscle from FOG to fast glycolytic (FG).

In the present study, when comparisons are made between groups C5 and Ac, similar rates of relative protein turnover are observed. While it is tempting to speculate that the high turnover rates observed in acclimated animals reflect a delay in normal reductions observed with growth, the concomitantly reduced rates of protein synthesis in homogenate and mitochondrial fractions of both muscles make this doubtful. The

high protein turnover rates associated with growth are anabolic in nature, whereas those observed in both soleus and gastrocnemius muscles of acclimated animals appear to be catabolic. While the acclimation protocol appeared to provide an adequate training stimulus as indicated by elevated SDH activity and increased soleus homogenate protein concentration, subsequent disuse appears to have resulted in diminished protein synthesis and increased protein degradation.

Factors regulating these changes in protein synthesis and turnover remain obscure. Although it is tempting to ascribe the changes observed 72 hours post exercise to decreased useage, this alone does not accurately account for the observed shifts in protein metabolism. The factors which underlie and regulate protein changes due to "useage" must first be isolated and studied. Included among these are increased muscle tension, stretch, intracellular free calcium levels and endocrine influences. The extent to which each or a combination of these act to mediate changes in protein metabolism following exercise is unknown.

Experiment 2

In mature animals, exogenous testosterone administration was shown to result in an initial (one hour) rise in serum testosterone concentration followed by significantly reduced levels 24 hours post injection. It may be hypothesized that the

reduction observed in testosterone following injection resulted from suppression of endogenous gonadotrophin release due to high testosterone levels immediately following the injection. Smith et al. (1977) have found that injections of androgenic steroids in prepubertal animals are even more effective in suppressing gonadotrophins. While serum testosterone values were reduced in experimental animals 24 hours following injection, values from Group AcT were not significantly different from SHAM injected controls. The manner in which exercise acclimation might modify testosterone metabolism and/or clearance to account for this observation is unknown.

In the present study, testosterone administration produced different effects in sedentary and exercised animals. In the sedentary animals (C6T), testosterone administration was generally followed by both reduced protein content and rates of protein synthesis in both the soleus and gastrocnemius muscles. Similar results were observed by Grigsby et al. (1976) who found depressed protein synthesis in the sarcoplasmic fraction of growing rabbits following steroid treatment. A single exception in the present study was increased mitochondrial protein content in the gastrocnemius muscle. While one might be tempted to relate reduced protein synthesis to concomitantly low testosterone levels observed 24 hours following testosterone injection, this is doubtful given the significant anabolic effect observed in muscles of exercised animals with comparable serum testosterone levels. Furthermore, in mature rats,

Kochakian (1976) reports that the typical anabolic response to a single testosterone proprionate injection consists of an almost immediate rise in protein synthesis, perhaps significant by 24 hours, plateauing three days later. In fact, it was based on this observation that the selection of 24 hours between injection and sacrifice was made in the present investigation.

Krieg (1976) has observed a significantly higher cytosolic testosterone receptor content in skeletal muscle of prepubertal rats compared to adult animals. Therefore, a lack of receptors in young animals can not account for the observed effects in sedentary control animals. Perhaps a more plausible explanation comes from Wright (1980) who states that the response following a single injection of anabolic steroid is dose dependent up to a point beyond which a suppression of myotrophic activity, nitrogen balance and normal growth may occur. In the present experiment, animals received approximately 2 mg of testosterone proprionate based on a dosage of 10 mg per kilogram of body weight. This represents a pharmaclolgical dose which is approximately 100 times the adult serum levels. However, such a dosage has been employed by Dahlmann et al. (1980, 1981) in mature animals and was found to reduce alkaline proteolytic activity in skeletal muscle of rats exposed to prolonged activity. While it is conceivable that in young animals with very low levels of endogenous testosterone this dosage might have exceeded the "upper anabolic limit", it is curious that the same dosage would in fact elicit an anabolic response in both

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exercised groups of rats.

In trained, mature rats, Rogozkin and Feldkoren (1979) have observed an increase in cytoplasmic testosterone binding capacity. If, in fact, exercise training results in increased skeletal muscle protein turnover, this result would tend to support Krieg's (1976) speculation that testosterone receptor level is partially dependent on the growth activity of the muscle. Given the high content of testosterone receptors in the muscle of young animals and the lack of effect of testosterone administration on sedentary animals, the anabolic advantage of additional receptors in trained young rats is questionable. Rogozkin (1979) reports enhanced protein synthesis in muscles from trained animals receiving anabolic steroids. Unfortunately, the reader is not informed of either the time elapsed between exercise and sacrifice, or the effects of the steroids on sedentary animals.

In the present study, testosterone administration to acutely run animals immediately following exercise generally resulted in enhanced rates of protein synthesis in the soleus muscle and elevated protein content in the gastrocnemius muscle. In experiment 1, it was suggested that the severe reduction observed in protein concentration and protein synthesis following exhaustive exercise in prepubertal rats was due to low endogenous testosterone levels. The results of experiment 2 support this hypothesis. The proposed mechanisms of action of testosterone are twofold: through competition for glucocorticoid

receptor sites, indirectly reducing the rate of protein degradation, and by stimulating protein synthesis directly. In the gastrocnemius muscle, the observation of no change in protein synthesis along with elevated protein content would lend support to the former mechanism of action. This is particularly convincing given the greater sensitivity of white muscle to the actions of glucocorticoids (Kelly and Goldspink, 1982). In the soleus muscle, enhanced protein synthesis following steroid injection with elevations observed only in sarcoplasmic protein content may have resulted from either a direct action of the hormone or by means of competition for glucocorticoid receptors. While a direct anabolic effect of testosterone in gastrocnemius muscle to produce the elevated protein concentrations observed cannot be overlooked, it is unlikely that this process would precede that observed in the soleus muscle given the higher protein turnover rates known to exist in the soleus. It is suggested that the primary effect of testosterone in the soleus muscle was directly anabolic, resulting in the observed enhanced rates of protein synthesis. This is supported by Kelly and Goldspink (1982) who report little effect of glucocorticoids on protein turnover in soleus muscle.

It therefore appears that a dose of testosterone which resulted in protein catabolism in sedentary animals was also capable of stimulating protein synthesis in acutely exercised animals. This observation is supported by results from groups Ac and AcT, where again testosterone administration resulted in

enhanced protein synthesis in soleus muscle and elevated protein concentration in gastrocnemius muscle and soleus sarcoplasmic fraction. As glucocorticoids were probably not elevated in acclimated animals, it is probable that testosterone acted directly to stimulate protein synthesis.

Clearly, in young animals, exposure to exercise modifies the response to a given dose of testosterone. The manner in which this occurs is unknown. As was mentioned before, an increase in number of receptors in trained animals would simply potentiate the anabolic response, not reverse it. Given the limited scope of the present experiment and the paucity of other research in this area, this problem cannot be solved at present.

Overview of Experiments 1 and 2

In order to analyze the effects of exercise treatment and testosterone administration independently, data from groups were pooled accordingly and group means compared. Results have been discussed earlier. (See Tables 8,9 and 10)

Testosterone administration resulted in significantly elevated protein in all fractions of the gastrocnemius muscle while no changes were observed in the soleus muscle. To date, relative sensitivity of fast and slow muscles to anabolic steroids has not been investigated. While it is known that glucocorticoids exert a greater effect in predominantly fast muscles (Kelly and Goldspink, 1982), the effects of testosterone

on different motor unit and muscle types is not known. The present results, however, suggest that predominantly fast muscles are more sensitive to anabolic steroids. Despite elevated protein content in the gastrocnemius muscle of testosterone treated animals, significantly depressed rates of protein synthesis were observed in homogenate and sarcoplasmic fractions. It is possible that these values represent a rebound effect from initially elevated rates of protein synthesis. As data from all exercise treatment groups were pooled for this analysis, it is unlikely that values from any given exercise group substantially influenced this finding. In the mitochondrial fraction of gastrocnemius muscle, no changes in protein synthesis were observed. Koenig et al. (1980) have presented evidence supporting an independent anabolic effect of testosterone on mitochondrial DNA and RNA synthesis and activity. Perhaps the conflicting testosterone response observed in the mitochondrial fraction in the present study is due to this independent action of the steroid on mitochondrial protein turnover.

In the soleus muscle, testosterone administration did not alter protein content. Enhanced protein synthesis was observed only in the homogenate fraction. Although Florini (1970) was unable to detect any quantitative changes in muscle protein synthesis following testosterone administration in mature rats despite an overwhelming general anabolic response, the present results suggest a specific steroid effect on myofibrillar

proteins in soleus muscle.

In young animals acclimated to exercise, significantly elevated whole muscle homogenate protein was observed in both soleus and gastrocnemius muscles. Given the moderate nature of the exercise, these results are surprising as in mature animals, any changes occuring would have been anticipated in the mitochondrial fraction of the muscles (Holloszy, 1975). While substantially higher mitochondrial protein was observed in the soleus muscle of acclimated animals, depressed rates of protein synthesis may be indicative of decreased usage. It therefore appears that the exercise acclimation protocol employed in the present study may have stimulated a transient increase in muscle growth. Mechanisms underlying growth may include increased muscle stretch, tension and elevated intracellular calcium levels which would occur during exercise sessions. Also, mild tissue hypoxia during exercise may have stimulated mitochondrial protein synthesis in the muscles of young acclimated rats. Mechanisms underlying the acute (24 hour) anabolic response to exercise in mature animals are unknown. Young animals, however, displayed a predominantly catabolic protein profile 24 hours following exhaustive exercise. Factors which have been suggested as mediators of increased protein synthesis in adult animals following acute, exhaustive exercise include increased muscle stretch (Goldspink et al., 1983), muscular tension (Booth et al, 1982) and intracellular calcium levels (Kamenyama and Etlinger, 1979). It is unlikely that age modified the stimuli of muscle

stretch or tension during the acute exercise bout. In young

animal skeletal muscle, differentiation is not yet complete. It

may be suggested, therefore, that intracellular calcium levels

in young animals responded differently to acute exercise when

compared with adult animals. Specific changes in the

sarcoplasmic reticulum of young, exercised animals have not been

investigated. Thus, the direction and degree to which

differences in intracellular calcium may have contributed to the

observed difference in protein synthesis between adult and young

rats is unknown.

In mature animals, endocrine factors have not been given

serious consideration in mediating protein synthesis following

acute exercise as Goldberg (1967, 1968) found that enhanced

protein synthesis occurs largely independent of endocrine

controls in skeletal muscles undergoing compensatory

hypertrophy. Results from the present study suggest that

endocrine factors in fact may play a significant role in

modifying the acute response to exercise.

V. Summary

In the young animal, skeletal muscle proteins turn over rapidly. Protein turnover rates fall from a high rate at birth to a lower muscle specific rate some time after the attainment of puberty. The response of muscle proteins to mild and exhaustive exercise in prepubertal rats differs significantly from that observed in mature animals.

In six week old animals, a very mild endurance exercise programme appears sufficient to elicit increases in mitochondrial oxidative enzyme activity and myofibrillar protein content in both soleus and gastrocnemius muscles. A significant decrease in protein synthesis 72 hours following the last exercise session may be indicative of a decreased muscle use.

Twenty four hours following a run to exhaustion, reduced protein concentration and rates of protein synthesis were observed in skeletal muscle of young animals. This is in direct contrast to changes observed in mature skeletal muscle. Low levels of endogenous testosterone following the exhaustive exercise bout are implicated in the acute response to exhaustive exercise in six week old animals. When testosterone was administered to young rats immediately following exhaustive exercise, the changes in protein synthesis and content more closely resembled those observed in adult animals. It is speculated that testosterone is required for supression of

glucocorticoid induced protein catabolism following exhaustive exercise.

Finally, in six week old rats, prior exposure to exercise reversed the catabolic response observed in sedentary rats to an injection of testosterone propionate. While it is suggested that the unexpected catabolic response observed in sedentary animals may have been dose related, factors regulating this exercise effect are unknown.

REFERENCES

Airhart,J., A.Vidrich, E.A.Khairallah. Compartmentation of free
      amino acids for protein synthesis in rat liver. Biochem.J.
      140:539-548, 1974.

Aldercreutz, M.Harkonen, K.Kuoppasalmi, K.Kosunen, H.Naveri,
      S.R|hunen. Physical activity and hormones. Adv.Cardiol.
      18:144-157, 1976.

Ariano,M.A., R.B. Armstrong, V.R.Edgerton. Hindlimb muscle fibre
      populations of five animals. J.Histochem.Cytochem.
      21:51-55, 1973.

Arias,I.M., D.Doyle, R.T.Schimke. Studies on the synthesis and
      degradation of proteins of the endoplasmic reticulum of
      rat liver. J.Biol.Chem. 244:3303-3315, 1969.

Armstrong,R.B., C.W.Saubert, W.L.Sembrowich, R.E.Shepherd,
      P.D.Gollnick. Glycogen depletion in rat skeletal muscle
      fibres at different intensities and durations of exercise.
      Pflugers Arch. 352:243-256, 1974.

Armstrong,R.B., C.W.Saubert, W.L.Sembrowich, R.E.Shepherd,
      P.D.Gollnick. Glycogen depletion in rat skeletal muscle
      fibres during exercise. In Metabolic Adaptation to
      Prolonged Physical Exercise: Proceedings of the Second
      International Symposium of Biochemistry of Exercise.
      Kirkhauser, ed. Magglingen, Switzerland, 1975.

Bailey,D.A., R.D.Bell, R.E.Howarth. The effect of exercise on
      DNA and protein synthesis in skeletal muscle of growing
      rats. Growth 37:323-331, 1973.

Ballard,F.J., G.L.Francis. Effects of anabolic agents on protein
      breakdown in L6 myoblasts. Biochem.J. 210:243-249, 1983.

Barnard,R.J., R.V.Edgerton, J.B.Peter. Effect of exercise on
      skeletal muscle. 1. Biochemical and histochemical
      properties. J.Appl.Physiol. 28:762-766, 1970.

Barnard,R.J., R.V.Edgerton, T.Furukawa, J.B.Peter. Histochemical
      biochemical, and contractile properties of red, white and
      intermediate fibres. Am.J.Physiol. 220:410-414, 1971.

Bates,P.C., D.J.Millward. Myofibrillar protein turnover.
      Biochem.J. 214:587-592, 1983.

Beecher,G.R. Some practical applications of protein synthesis
      and muscle growth. J.Animal Sci. 38:1071-1078, 1974.

Beecher,G.R., F.R.Puente, B.L.Dohm. Amino acid uptake and
    levels: Influence of endurance training. Biochem.Med.
    21:196-201, 1979.

Bird,J.W.C., A.N.Spanier, W.M.Schwartz. Cathepsins B and D:
    Proteolytic activity and ultrastructural localization in
    skeletal muscle. In Protein Turnover and Lysosome
    Function. H.J.Segal and D.J.Doyle, eds. Academic Press,
    New York, 1978.

Bird,J.W.C., J.H.Carter. Proteolytic enzymes in striated and
    non-striated muscle. In Degredative Processes in Heart and
    Skeletal Muscle. K.Wildenthal, ed. Elsevier/North-Holland
    Biomedical Press, Amsterdam, 1980.

Bonen,A. Effects of exercise on excretion rates of urinary free
    cortisol. J.Appl.Physiol. 40:155-158, 1976.

Bonen,A., S.A.Malcolm, R.D.Kilgour, K.P.McIntyre, A.N.Belcastro.
    Glucose injestion before and following intense exercise.
    J.Appl.Physiol. 50:766-771, 1981.

Booth,F.W., W.F.Nicholson, P.A.Watson. Influence of muscle use
    on protein synthesis and degradation. Ex.Sport Sci.Rev.
    10:27-48, 1982.

Brisson,G.R., M.A.Volle,M.Desharnais,M.Dion,M.Tanako.
    Pituitary-gonadal axis in exercising man. Med.Sci.Sport
    9:47, 1977.

Buchanan,T.A.S., J.J.Pritchard. DNA content of tibialis anterior
    of male and female white rats measured from birth to 50
    weeks. J.Anat. 107:185-190, 1970.

Burke, R.V., R.V.Edgerton. Motor unit properties and selective
    involvement in movement. Exercise and Sport Sci. Rev.
    3:31-81, 1975.

Burleigh,I.G. On the cellular regulation of growth and
    development in skeletal muscle. Biol.Reviews 49:267-320,
    1974.

Buresova,M., V.Hanzlikova, E.Gutmann. Changes in ribosomal
    capacity for protein synthesis, contraction and
    histochemical properties of muscle after implantation of
    fast nerve into denervated and into self-reinervated slow
    soleus muscle of the rat. Pflugers Arch. 360:95-108, 1975.

Burstein,P.J., B.Draznin, C.J.Johnson, D.S.Schalch. The effect
    of hypothyroidism on growth, serum growth hormone, the
    growth hormone-dependent somatomedin, insulin-like growth
    factor and its carrier protein in rats. Endocrinology
    104:1107-1111, 1979.

Caralis,D.G., L.Edwards, P.J.Adams. Serum total and free
        thyroxine and triiodothyronine during dynamic muscular
        exercise in man. Am.J.Physiol. 233:E115-E118, 1977.

Cheek,D.B., D.E.Hill. Muscle and liver cell growth: role of
        hormones and nutritional factors. Fed.Proc. 29:1509, 1970.

Clark,M.G., C.J.Beinlich, E.E.McKee, J.A.Lins, H.E.Morgan.
        Relationship between alkaline proteolytic activity and
        protein degradation in rat heart. Fed.Proc. 39:26-30,
        1980.

Close,R.I. Dynamic properties of mammalian skeletal muscle.
        Physiol.Rev. 52:129-197, 1972.

Cooperstein,S.J. Succinate dehydrogenase assay. J.Biol.Chem.
        186:129-130, 1950.

Dahlmann,B., H.Reinauer. Purification and some properties of an
        alkaline proteinase from rat skeletal muscle. Biochem.J.
        171:803-810, 1978.

Dahlmann,B., C.Schroeter, L.Herbertz, H.Rheinauer. Myofibrillar
        protein degradation and muscle proteinases in normal and
        diabetic rats. Biochem.Med. 21:33-39, 1979.

Dahlmann,B., B.Mai, H.Reinauer. The influence of testosterone on
        the alkaline proteolytic activity in rat skeletal muscle.
        Biochim.Biophys.Acta 641:479-486, 1980.

Dahlmann,B., A.Widjaja, H.Reinauer. Antagonistic effects of
        endurance training and testosterone on alkaline
        proteolytic activity in rat skeletal muscles.
        Eur.J.Appl.Physiol. 46:229-235, 1981.

Dallaire,J.A. The protein synthesis response in rat skeletal
        muscle following acute sprint or endurance running as
        measured by the incorporation of L-(4,5-3H)-leucine in
        protein and leucyl-tRNA. Ph.D. Thesis, University of
        Alberta, 1980.

Daughaday,W.H., A.C.Herrington, L.S.Phillips. The regulation of
        growth by endocrines. Ann.Rev.Physiol. 37:211-244, 1975.

Davies,C.T.M., J.D.Few. Effects of exercise on adrenocortical
        function. J.Appl.Physiol 35:887-891, 1973.

Dean,R.T. Regulation and mechanisms of degradation of endogenous
        proteins by mammalian cells: general considerations. In
        Degredative Processes in Heart and Skeletal Muscle.
        K.Wildenthal, ed. Elsevier/North-Holland Biomedical Press,
        Amsterdam, 1980.

DeMartino,G.M., A.L.Goldberg. Thyroid hormones control lysosomal enzyme activities in liver and skeletal muscle. Proc.Natl.Acad.Sci.U.S.A. 75:1369-1373, 1978.

Desai,I.D. Regulation of lysosomal enzymes. I. Adaptive changes in enzyme activities during starvation and refeeding. Can.J.Biochem. 47:785-790, 1969.

Devi,A., M.A.Mukandan, U.M.A.Srivastava, N.K.Sarkar. The effect of age on the variations of DNA, RNA and total nucleotides in liver, brain, and muscle of rat. Exp.Cell Res. 32:242-250, 1963.

Dice,F.J., C.C.Walker. The general characteristics of intracellular protein degradation in diabetes and starvation. In Protein Turnover and Lysosomal Function. H.J.Segal and D.J.Doyle eds. Academic Press, New York, 1978.

Dohm,G.L., G.R.Beecher, A.L.Hecker, F.R.Puente, G.E.Klain, E.W.Askew. Changes in protein synthesis in rats in response to endurance running. Life Sci. 21:189-197, 1977a.

Dohm,G.L., A.L.Hecker, W.E.Brown, G.J.Klain, F.R.Puente, E.W.Askew, G.R.Beecher. Adaptation of protein metabolism to endurance training. Increased amino acid oxidation in' response to training. Biochem.J. 164:705-708, 1977b.

Dohm,G.L., F.R.Puente, C.P.Smith, A.Edge. Changes in tissue protein levels as a result of endurance exercise. Life Sci. 23:845-850, 1978.

Dohm,G.L., G.J.Kasperek, E.B.Tapscott, G.R.Beecher. Effect of exercise on synthesis and degradation of muscle protein. Biochem.J. 188:255-262, 1980.

Dohm, G.L., R.T.Williams, G.J.Kasperek, A.M.VanRij. Increased excretion of urea and N-methylhistidine by rats and humans after a bout of exercise. J.Appl.Physiol.: Respirat.Environ.Exercise Physiol. 52: 27-33, 1982a.

Dohm,G.L., E.B.Tapscott, H.A.Barakat, G.J.Kasperek. Measurement of in vivo protein synthesis in rats during an exercise bout. Biochem.Med. 27:367-373, 1982b.

Edgerton,V.R. Exercise and the growth and development of muscle tissue. In Physical Activity: Human Growth and Development. L.Rarick, ed. Academic Press, New York, 1973.

Fahmy,L.H., D.P.Leader. The effect of diabetes and insulin on the polyadenylic acid-containing RNA of rat skeletal muscle. Biochem.Biophys.Acta 608:344-357, 1980.

Fitts,R.H., F.J.Nagle, R.G.Cassens. The adaptation of myoglobin with age and training and its relationship to the three fibre types of skeletal muscle in miniature pig. Eur.J.Appl.Physiol. 33:275-284, 1974.

Fitts,R.H., W.W.Winder, M.H.Brooke, K.K.Kaiser, J.O.Holloszy. Contractile, biochemical and histochemical properties of thyrotoxic rat soleus muscle. Am.J.Physiol. 238:C15-C20, 1980.

Flaim,K.E., J.B.Li, L.S.Jefferson. Effects of thyroxine on protein turnover in rat skeletal muscle. Am.J.Physiol. 235:E231-E236, 1978.

Florini,J.R. Effects of testosterone on qualitative pattern of protein synthesis in skeletal muscle. Biochem. 9:909-912, 1970.

Fulks,R., J.B.Li, A.L.Goldberg. Effects of insulin, glucose and amino acids on protein turnover in rat diaphragm. J.Biol.Chem. 250:290-298, 1975.

Galbo,H., E.A.Richter, J.J.Holst, N.J.Christensen. Diminished hormonal responses to exercise in trained rats. J.Appl.Physiol. 43:953-958, 1977a.

Galbo,H., L.Hummer, E.B.Petersen, N.J.Christensen, N.Bie. Thyroid and testicular hormone responses to graded and prolonged exercise in man. Eur.J.Appl.Physiol. 36:101-106, 1977b.

Gardiner,P.F., B.R.Botterman, E.Eldred, D.R.Simpson, V.R.Edgerton. Metabolic and contractile changes in fast and slow muscles of the cat after glucocorticoid-induced atrophy. Exp.Neurology 62:241-255, 1978.

Gardiner,P.F., V.R.Edgerton. Contractile responses of rat fast-twitch and slow-twitch muscles to glucocorticoid treatment. Muscle and Nerve 2:274-281, 1979.

Ginsberg,B.H. The insulin receptor: Properties and regulation. Biochem.Action Horm. 4:313-349, 1977.

Giovannetti,P.M., S.C.Strothers. Influence of diet and age on ribonucleic acid, protein, and free amino acid levels of rat skeletal muscle. Growth 39:1-16, 1975.

Glass,R.D., D.Doyle. On the measurement of protein turnover in animal cells. J.Biol.Chem. 247:5234-5242, 1972.

Goldberg,A.L. Protein synthesis in tonic and phasic skeletal muscles. Nature 216:1219-1220, 1967.

Goldberg,A.L. Protein synthesis during work induced growth of skeletal muscle. J.Cell.Biol. 36:653-658, 1968.

Goldberg,A.L., J.D.Etlinger, D.F.Goldspink, C.Jablecki. Mechanism of work-induced hypertrophy of skeletal muscle. Med.Sci.Sport 5:248-261, 1975.

Goldberg,A.L., G.E.Griffin. Hormonal control of protein synthesis and degradation in rat skeletal muscle. J.Physiol. 270:52P-53P, 1977.

Goldberg,A.L. The regulation of protein turnover by endocrine and nutritional factors. In Plasticity of Muscle. D.Pette, ed. Walter de Cruyter and Co., New York, 1980.

Goldspink,D.F., P.J.Garlick, M.A.McNurlan. Protein turnover measured in vivo and in vitro in muscles undergoing compensatory growth and subsequent denervation atrophy. Biochem.J. 210:89-98, 1983.

Goll,D.E., A.Okitani, W.R.Dayton, W.J.Reville. A Ca2+-activated muscle protease in myofibrillar protein turnover. In Protein Turnover and Lysosome Function . H.J.Segal and D.Doyle, eds. Academic Press: New York, 1978.

Gordon,E.E., K.Kowalski, M.Fritts. Muscle proteins and DNA in rat quadriceps during growth. Am.J.Physiol. 210:1033-1040, 1966.

Grigsby,J.S., W.G.Bergen, R.A.Merkel. The effect of testosterone on skeletal muscle development and protein synthesis in rabbits. Growth 40:303-316, 1976.

Guezennec, P.Ferre, B.Serrurier, D.Merino, P.C.Pesquies. Effects of prolonged physical exercise and fasting upon plasma testosterone level in rats. Eur.J.Appl.Physiol. 49:159-168, 1982.

Guttmann,E., J.A.Melichna. Contractile and histochemical properties of denervated and reinnervated fast and slow skeletal muscles of new-born and adult guinea pigs. Physiologica Bohemoslovaca 28:35-42, 1979.

Harper,R.S. Review of Physiological Chemistry. Lange Medical, California, 1977.

Hartley,L.J., J.W.Mason, R.P.Hogan, L.G.Jones, T.A.Kotchen, E.H.Mougey, F.E.Wherry, L.L.Pennington, P.T.Ricketts. Multiple hormonal responses to graded exercise in relation to physical training. J.Appl.Physiol. 33:602-606, 1972a.

Hartley,L.J., J.W.Mason, R.P.Hogan, L.G.Jones, T.A.Kotchen, E.H.Mougey, F.E.Wherry, L.L.Pennington, P.T.Ricketts.

Multiple hormonal responses to prolonged exercise in relation to physical training. J.Appl.Physiol. 33:607-610, 1972b.

Henneman,E., C.B.Olsen. Relations between structure and function in the design of skeletal muscle. J.Neurophysiol. 28:581-598, 1965.

Holloszy,J.O. Biochemical adaptations to exercise: aerobic metabolism. Ex.Sport.Sci.Rev. 1:46-73, 1973.

Holloszy, J.O., P.Mole, K.Baldwin, R.Terjung. Exercise induced enzymatic adaptations in muscle. In Limiting Factors of Physical Performance. J.Keul, ed. Georg Thieme, Stuttgart, 1973.

Holloszy,J.O., F.Booth, W.Winder, R.Fitts. Biochemical adaptation of skeletal muscle to prol nged physical exercise. In Metabolic Adaptation to Prolonged Physical Exercise. H.Howald, J.R.Poortmans, eds. Birkhauser Verlag, Basel, 1975.

Holloszy,J.O. Adaptation of skeletal muscle to endurance exercise. Med.Sci.Sport 7:155-164, 1975.

Howarth,R.E., R.L.Baldwin. Synthesis and accumulation of protein and nucleic acid in rat gastrocnemius muscles during normal growth, restricted growth and recovery from restricted growth. J.Nutrit. 101:477-484, 1971.

Howarth,R.E. Influence of dietary protein on rat skeletal muscle growth. J.Nutrit. 102:37-44, 1972.

Hubbard,R.W., J.A.Smoake, W.T.Matthew, J.D.Linduska, W.D.Bowers. The effects of growth and endurance training on the protein and DNA content of rat soleus, plantaris and gastrocnemius muscles. Growth 38:171-185, 1974.

Ingwall,J.S. Metabolism of proteins and nucleic acids during muscle differentiation: regulation of gene expression. In Degredative Processes in Skeletal Muscle. K.Wildenthal, ed. Elsevier/North-Holland Biomedical Press, Amsterdam, 1980.

Ionesescu,V., R.Lewis, B.Schottelius. Neurogenic control of muscle ribosomal protein synthesis. Acta Neurol.Scand. 51:253-267, 1978.

Jones,T.R., P.A.Bell. Glucocorticoid-receptor interactions: Studies of the negative co-operativity induced by steroid interactions with a secondary, hydrophobic, binding site. Biochem.J. 188:237-245, 1983.

Kamenyama,T., Etlinger,J.D. Calcium-dependent regulation of
    protein synthesis and degradation in muscle. Nature
    279:344-346, 1979.

Kelly,F.J., D.F.Goldspink. The differing responses of four
    muscle types to dexamethasone treatment in the rat.
    Biochem.J. 208:147-151, 1982.

Kelly,F.J., S.E.M.Lewis, P.Anderson, D.F.Goldspink. Pre- and
    postnatal growth and protein turnover in four muscles of
    the rat. Muscle and Nerve 7:235-242, 1984.

Kindermann,W., A.Schnabel, W.M.Schmitt, G.Biro, J.Cassens,
    F.Weber. Catecholamines, growth hormone, cortisol,
    insulin, and sex hormones in anaerobic and aerobic
    exercise. Eur.J.Appl.Physiol. 49:389-399, 1982.

Kirnos,M.D., B.F.Vanushin. Isolation of mitochondrial DNA free
    of nuclear DNA from animal tissue. Translated from
    Biokhimiya 41:68-74, 1976.

Kochakian,C.D. Metabolic effects of |anabolic steroids. In
    Anabolic Androgenic Steroids. C.D.Kochakian, ed. Springer
    Verlag, New York, 1976.

Koenig,H., A.Goldstone, C.Y.Lu. Androgens regulate mitochondrial
    cytochrome C oxidase and lysosomal hydrolases in mouse
    skeletal muscle. Biochem.J. 192:349-353, 1980.

Kovisto,V.A., V.Soman, P.Conrad, R.Hendler, E.Nadel, P.Felig.
    Insulin binding to monocytes in trained athletes. Changes
    in the resting state and after exercise. J.Clin.Invest.
    64:1011-1015, 1979.

Kreig,M. Characterization of the androgen receptor in the
    skeletal muscle of the rat. Steroids 28:261-272, 1976.

Kuipers,H., J.Drukker,P.M.Frederik, P.Geurten, G.V.Kranenburg.
    Muscle degeneration after exercise in rats. Int.J.Sports
    Med. 4:45-51, 1983.

Lamb, D.R. Androgens and exercise. Med.Sci.Sport 7:1-5, 1975.

Laurent,G.J., D.J.Millward. Protein turnover during skeletal
    muscle hypertrophy. Fed.Proc. 39:42-47, 1980.

Lemon,P.W.R., F.J.Nagle. Effects of exercise on protein and
    amino acid metabolism. Med.Sci.Sport 13:141-149, 1981.

Lewis,E.M., F.J.Kelly, D.F.Goldspink. Pre- and postnatal growth
    and protein turnover in smooth muscle, heart and slow- and
    fast-twitch skeletal muscles of the rat. Biochem.J.
    217:517-526, 1984.

Li,J.B., A.L.Goldberg. Effects of food deprevation on protein synthesis and degradation in rat muscle. Am.J.Physiol. 231:441-448, 1976.

Li,J.B. Protein synthesis and degradation in skeletal muscle of normal and dystrophic hamsters. Am.J.Physiol. 239:E401-E406, 1980.

Liao,S. Molecular actions of androgens. In Biochemical Actions of Hormones Vol. 4. G.Litwack, ed. Academic Press, New York, 1977.

Libby,P, A.L.Goldberg. The control and mechanism of protein breakdown in striated muscle: studies with selective inhibitors. In Degredative Processes in Heart and Skeletal Muscle. K.Wildenthal, ed. Elsevier/North-Holland Biomedical Press, Amsterdam, 1980.

Lowry,O.H., N.J.Rosebrough, L.A.Farr, R.J.Randall. Protein measurement with the Folin phenol reagent. J.Biol.Chem. 193:265-275, 1951.

Manchester,K.L. Sites of hormonal regulation of protein synthesis. In Mammalian Protein Metabolism. Vol. A. H.N.Munroe, ed. Academic Press, New York, 1970.

Markelonis,G.J., T.H.Oh. A protein fraction from peripheral nerve having neurotrophic effects on skeletal muscle cells in culture. Exp.Neurology 58:285-295, 1978.

Matsukura,V., A.Okitani, H.Kato, M.Fujimaki. Purification and some properties of a myofibrillar protein degrading protease, Cathepsin L, from rabbit skeletal muscle. J.Biochem. 87:1133-1143, 1980.

Mayer,M., R.Amin, E.Shatir. Rat myofibrillar protease: enzyme properties and adaptive changes in conditions of muscle protein degradation. Arch.Biochem.Biophys. 161:20-25, 1974.

Mayer,M. F.Rosen. Interactions of glucocorticoids and androgens with skeletal muscle. Metabolism 26:937-962, 1977.

McGrath,J.A., D.F.Goldspink. Glucocorticoid action on protein synthesis and protein breakdown in isolated skeletal muscles. Biochem.J. 206:641-645, 1982.

McManus,B.M., D.R.Lamb, J.J.Judis, J.Scala. Skeletal muscle leucine incorporation and testosterone uptake in exercised guinea pig muscle. Eur.J.Appl.Physiol. 34:149-156, 1975.

Millward,D.J., P.J.Garlick, W.P.T.James, D.O.Nnanyelugo, J.S.Ryatt. Relationship between protein synthesis and RNA

content in skeletal muscle. Nature 241:204-205, 1973.

Millward,D.J., P.J.Garlick, R.C.Stewart, D.O.Nnanyelugo,
J.C.Waterlow. Skeletal muscle growth and protein turnover.
Biochem.J. 150:235-243, 1975.

Millward,D.J., P.J.Garlick, D.O.Nnanyelugo, J.C.Waterloo. The
relative importance of muscle protein synthesis and
breakdown in the regulation of muscle mass.
Biochem.J.156:185-188, 1976.

Millward,D.J., P.C.Bates, G.J.Laurent, C.C.Lo. Factors affecting
protein breakdown in skeletal muscle. In Protein Turnover
and Lysosome Function. H.L.Segal, D.Doyle, eds. Academic
Press, New York, 1978.

Millward,D.J., P.C.Bates, J.G.Brown, S.R.Rosochacki, M.J.Rennie.
Protein degradation and the regulation of protein balance
in muscle. Exerpta Medica 75:307-329, 1980.

Millward,D.J., Protein tunrnover in skeletal and cardiac muscle
during normal growth and hypertrophy. In Degredative
Processes in Heart and Skeletal Muscle. K.Wildenthal, ed.
Elsevier/North-Holland Biomedical Press, Amsterdam, 1980.

Millward,D.J., B.Odedra, P.C.Bates. The role of insulin,
corticosterone and other factors in the acute recovery of
muscle protein synthesis on refeeding food-deprived rats.
Biochem.J. 216:583-587, 1983.

Nutting,D.F., L.J.Coats. Hormonal alterations of the sensitivity
of amino acid transport to growth hormone in muscle of
young rats. Proc.Soc.Exptl.Biol.Med. 156:446-451, 1977.

Nwoye,L, W.F.H.M.Mommaerts, D.R.Simpson, K.Seraydarian,
M.Marusich. Evidence for a direct action of thyroid
hormone in specifying muscle properties. Am.J.Physiol.
242:R401-R408, 1982.

Odera,B.R., D.J.Millward. Effect of cortisone treatment on
muscle protein turnover in adrenalectomized rats and
diabetic rats maintained on insulin. Biochem.J.
204:663-672, 1982.

Odera,B.R., S.Dalal, D.J.Millward. Muscle protein synthesis in
the streptozotocin-diabetic rat. Biochem.J. 202:363-368,
1982.

Odera,B.R., P.C.Bates, D.J.Millward. Time course of the effect
of catabolic doses of corticosterone on protein turnover
in rat skeletal muscle and liver. Biochem.J. 214:617-627,
1983.

Pilstrom,L., V.Vihko, E.Astrom, A.U.Arstila. Activity of acid
hydrolases in skeletal muscle of untrained, trained and
detrained mice of different ages. Acta.Physiol.Scand.
104:217-224, 1978.

Poole,B. Kinetics of disappearance of labelled leucine from the
free leucine pool of rat liver and its effect on the
apparent turnover of catalase and other hepatic proteins.
J.Biol.Chem. 246:6587-6591, 1971.

Rannels,D., E.E.McKee, H.E.Morgan. Regulation of protein
synthesis and degradation in heart and skeletal muscle.
Biochemical Actions of Hormones. Vol. 4. G.Litwalk, ed.
Academic Press, New York, 1977.

Rannels,S.R., D.E.Rannels, A.E.Pegg, L.S.Jefferson.
Glucocorticoid effects on peptide-chain initiation in
skeletal muscle and heart. Am.J.Physiol. 235:E134-E139,
1978.

Rannels,D.E., A.E.Pegg, S.R.Rannels, L.S.Jefferson. Effect of
starvation on initiation of protein synthesis in skeletal
muscle and heart. Am.J.Physiol. 235:E126-E133, 1978.

Rannels,S.R., L.S.Jefferson. Effects of glucocorticoids on
muscle protein turnover in perfused rat hemicorpus.
Am.J.Physiol. 238:E564-572, 1980.

Rannels,D.E., S.R.Rannels, J.B.Li, A.E.Pegg, H.E.Morgan,
L.S.Jefferson. Effects of glucocorticoids on peptide chain
initiation in heart and skeletal muscle. In Advances in
Myocardiology Vol. 1. M.Tajuddin, P.K.Das, M.Tariq,
N.S.Dhalla, eds. University Park Press, Baltimore, 1980.

Reed,A.T., W.W.Heusner, R.E.Carrow, W.D.VanHuss. Effects of
prepubertal forced exercise upon postpubertal organ and
body weights of female rats. Res.Quart. 44:470-482, 1974.

Refsum,H.E., S.B.Stromme. Serum thyroxine and thyroid
stimulating hormone after prolonged heavy exercise.
Scand.J.Clin.Lab.Invest. 39:455-459, 1979.

Remes,K., K.Kuoppasalmi, H.Aldercreutz. Effect of long-term
physical training on plasma testosterone,
anderostenedione, luteinizing hormone and
sex-hormone-binding globulin capacity.
Scand.J.Clin.Lab.Invest. 39:743-749, 1979.

Rennie,M.J., R.H.T.Edwards, S.Krywawych, C.T.M.Davies,
D.Halliday, J.C.Waterlow, D.J.Millward. Effect of exercise
on protein turnover in man. Clin.Sci. 61:627-639, 1981.

Rogers,P.A., G.H.Jones, J.A.Faulkner. Protein synthesis in

skeletal muscle following acute exhaustive exercise.
Muscle and Nerve 2:250-256, 1979.

Rogozkin,V.A. The role of low molecular weight compounds in the
regulation of skleletal muscle genome activity during
exercise. Med.Sci.Sport 8:1-4, 1976.

Rogozkin,V.A. Anabolic steroid metabolism in skeletal muscle.
J.Steroid Biochem. 11:923-926, 1979a.

Rogozkin,V.A. Metabolic effects of anabolic steroid on skeletal
muscle. Med.Sci.Sport 11:160-163, 1979b.

Rogozkin,V.A., F.Feldkoren. The effect of retabolil and training
on activity of RNA polymerase in skeletal muscles.
Med.Sci.Sport 11:345-347, 1979.

Rosenfeld,M.G., A.Barrieux. Regulation of protein synthesis by
polypeptide hormones and cyclic AMP. In Advances in Cyclic
Nucleotide Research. Vol 11. P.Greengard, G.A.Robinson
eds. Raven Press, New York, 1979.

Rothig,H.J., N.Stiller, B.Dahlmann, H.Reinauer. Insulin effect
on proteolytic activities in rat skeletal muscle.
Horm.Metab.Res. 10:101-104, 1978.

Salminen,A., V.Vihko. Effects of age and prolonged running on
proteolytic capacity in mouse cardiac and skeletal
muscles. Acta Physiol.Scand. 112:89-95, 1981.

Salminen,A., J.Komulainen, E.Ahomaki, H.Kainulainen, T.Takala,
V.Vihko. Effects of endurance training on alkaline
protease activities in rat skeletal muscles. Acta
Physiol.Scand. 119:261-265, 1983.

Salminen,A., M.Kihlstrom, H.Kainulainen, T.Takala, V.Vihko.
Endurance training decreases the alkaline proteolytic
activity in mouse skeletal muscle. Eur.J.Appl.Physiol.
52:287-290, 1984.

Saltin,B. Metabolic fundamentals in exercise. Med.Sci.Sport
5:137-146, 1973.

Schott,L.H., R.L.Terjung. The influence of exercise on muscle
lysosomal enzymes. Eur.J.Appl.Physiol. 42:175-182, 1979.

Schwartz,J. Rapid modulation of protein synthesis in normal rats
by specific neutralization and replacement of growth
hormone. Endocrinology 111:2087-2090, 1982.

Seene,T., A.Viru. The catabolic effect of glucocorticoids on
different types of skeletal muscle fibres and its
dependence upon muscle activity and interaction with

anabolic steroids. J.Steroid Biochem. 16:349-352, 1982.

Seraydarian,K., W.F.H.Mommaerts. Density gradient separation of
        sarcotubular vesicles and other particulate constituents
        of rabbit muscle. J.Cell.Biol. 26:641-656, 1965.

Shoji,S., R.J.T.Pennington. The effect of cortisone on protein
        breakdown and synthesis in rat skeletal muscle.
        Molec.Cell.Endocrinol. 6:159-169, 1977.

Smith,E.R., D.A.Damassa, J.M.Davidson. Feedback regulation and
        male puberty: testosterone-leutenizing hormone
        realtionships in the developing rat. Endocrinology
        101:173-179, 1977.

Sparrow,M.P., C.A.Earl, G.L.Laurent, A.W.Everett. Turnover rates
        of muscle proteins in cardiac, skeletal, and smooth
        muscle: turnover rate related to muscle function. In
        Recent Advances in Studies on Cardiac Structure and
        Metabolism Vol. 12. T.Kobayashi, Y.Ito, G.Rona eds.
        University Park Press, Baltimore, 1978.

Srivastava,U., K.D.Chaudhary. Effect of age on protein and
        ribonucleic acid metabolism in mouse skeletal muscle.
        Can.J.Biochem. 47:231-235, 1969.

Stevenson,J.A., B.M.Box, V.Feleki. Bouts of exercise and food
        intake in the rat. J.Appl.Physiol. 21:118-122, 1966.

Stewart,D.M. In Regulation of Organ and Tissue Growth. R.J.Goss
        ed. Academic Press, New York, 1982.

Sutton,J.R., M.J.Coleman, J.Casey, L.Lazarus. Androgen responses
        during physical exercise. Brit.Med.J. 1:520-522, 1973.

Sutton,J.R. Hormonal and metabolic responses to exercise in
        subjects of high and low work capacities. Med.Sci.Sport
        10:1-6, 1978.

Tapscott,E.B., G.J.Kasperek, G.L.Dohm. Effect of training on
        muscle protein turnover in male and female rats.
        Biochem.Med. 27:254-259, 1982.

Terjung,R. Endocrine response to exercise. Ex.Sport
        Sci.Rev.7:153-180, 1979.

Thompson,W.C., S.M.Heywood. Basic aspects of protein synthesis
        in muscle. J.Animal Sci. 38:1050-1053, 1974.

Trenkle,A. Hormonal and nutritional interrelationships and their
        effects on skeletal muscle. J.Animal Sci. 38:1142-1149.
        1974.

Vihko,V., A.Salminen, J.Rantamake. Acid hydrolase activity in red and white skeletal muscle during a two week period following exhaustive exercise. Pflugers Archiv. 378:99-106, 1978a.

Vihko,V., Y.Hirsimaki, A.O.Ajiduah. B-glucuronidase activity in trained red and white skeletal muscle of mice. Eur.J.Appl.Physiol. 39:255-261, 1978b.

Vihko,V., A.Salminen, J.Rantamaki. Oxidative and lysosomal capacity in skeletal muscle of mice after endurance training of different intensities. Acta Physiol.Scand. 104:74-81, 1978c.

Vihko,V., Salminen, J.Rantamaki. Exhaustive exercise, endurance training, and acid hydrolase activity in skeletal muscle. J.Appl.Physiol. 47:43-50, 1979.

Viru,A., P.Korge. Role of anabolic steroids in the hormonal regulation of skeletal muscle adaptation. J.Steroid Biochem. 11:931-932, 1979.

Wenger,H.A., J.G.Wilkinson, J.Dallaire, T.Nihei. Uptake of 3H-leucine into different fractions of rat skeletal muscle following acute endurance and sprint exercise. Eur.J.Appl.Physiol. 47:83-92, 1981.

Wilkinson,J.G., J.Jobin, F.D.Reardon, R.B.J.Macnab, H.A.Wenger. Aerobic and anaerobic training: effect on developmental patterns in muscle fibre types. Can.J.Appl.Sport Sci. 3:163-167, 1978.

Wilkinson,J.G., S.A.Malcolm. Mitochondrial and sarcoplasmic protein synthesis following endurance exercise. Med.Sci.Sport 15:177, 1983.

Winnick,M., A.Noble. Quantitative changes in DNA, RNA and protein during prenatal and postnatal growth in the rat. Devel.Biol. 12:451-466, 1965.

Winder,W.W., R.C.Hickson, J.M.Hagberg, A.Aehsani, J.A.McLane. Training-induced changes in hormonal and metabolic responses to submaximal exercise. J.Appl.Physiol.: Respirat.Environ.Exercise Physiol. 46:766-771, 1979.

Wright,J.E. Anabolic steroids and athletics. Ex.Sport Sci. Rev. 8:149-202, 1980.

Young,V.R. The role of skeletal and cardiac muscle in regulation of protein metabolism. In Mamalian Protein Metabolism.Vol 4. H.N.Munro ed. Academic Press, New York, 4:586-674, 1970.

Young,V.R. Regulation of protein synthesis and skeletal muscle growth. J.Animal Sci. 38:1054-1069, 1974.

Young,R.B., R.E.Allen. Transitions in gene activity during development of muscle fibres. J.Animal Sci. 48:837-850, 1979.

Zak,R. Comparison of turnover of several myofibrillar proteins and critical evaluation of the double isotope method. J.Biol.Chem. 252:3430-3435, 1977.

Zak,R., A.F.Martin, R.Blough. Assessment of protein turnover by use of radioisotopic tracers. Physiological Reviews 59:407-447, 1979.

APPENDIX A: REVIEW OF LITERATURE

General

Skeletal muscle proteins are continuously being degraded
and replaced. Physiological conditions and experimental
manipulations which affect protein synthesis or degradation will
in turn influence the overall protein turnover rate and
ultimately result in changes in total muscle protein
concentration.

The requirements for protein synthesis are dependent upon a
number of factors. These have been summarized in Figure 35.

The pathways of protein degradation in skeletal muscle are
poorly understood. In the most general terms, the rate of
breakdown is determined by the capacity or activity of the
degrading system, and by the nature of the protein as substrate.
While lysosomal proteases (Bird et al., 1978; Matsukura et al.,
1980), calcium activated proteases (Goll et al., 1978)
extra-lysosomal proteases (Rothig et al., 1978; Dahlman et al.,
1979) and nucleases (Harper, 1977), have all been implicated in
skeletal muscle proteolysis, the relative distributions of these
enzymes between muscle cells and interstitial cells is uncertain
(Bird and Carter, 1980). It remains possible that much of the
proteolytic activity measured in muscle tissue is non-myocytic.
Furthermore, proteolytic activity may not be the rate limiting
determinant of the breakdown rate. Instead, some preliminary
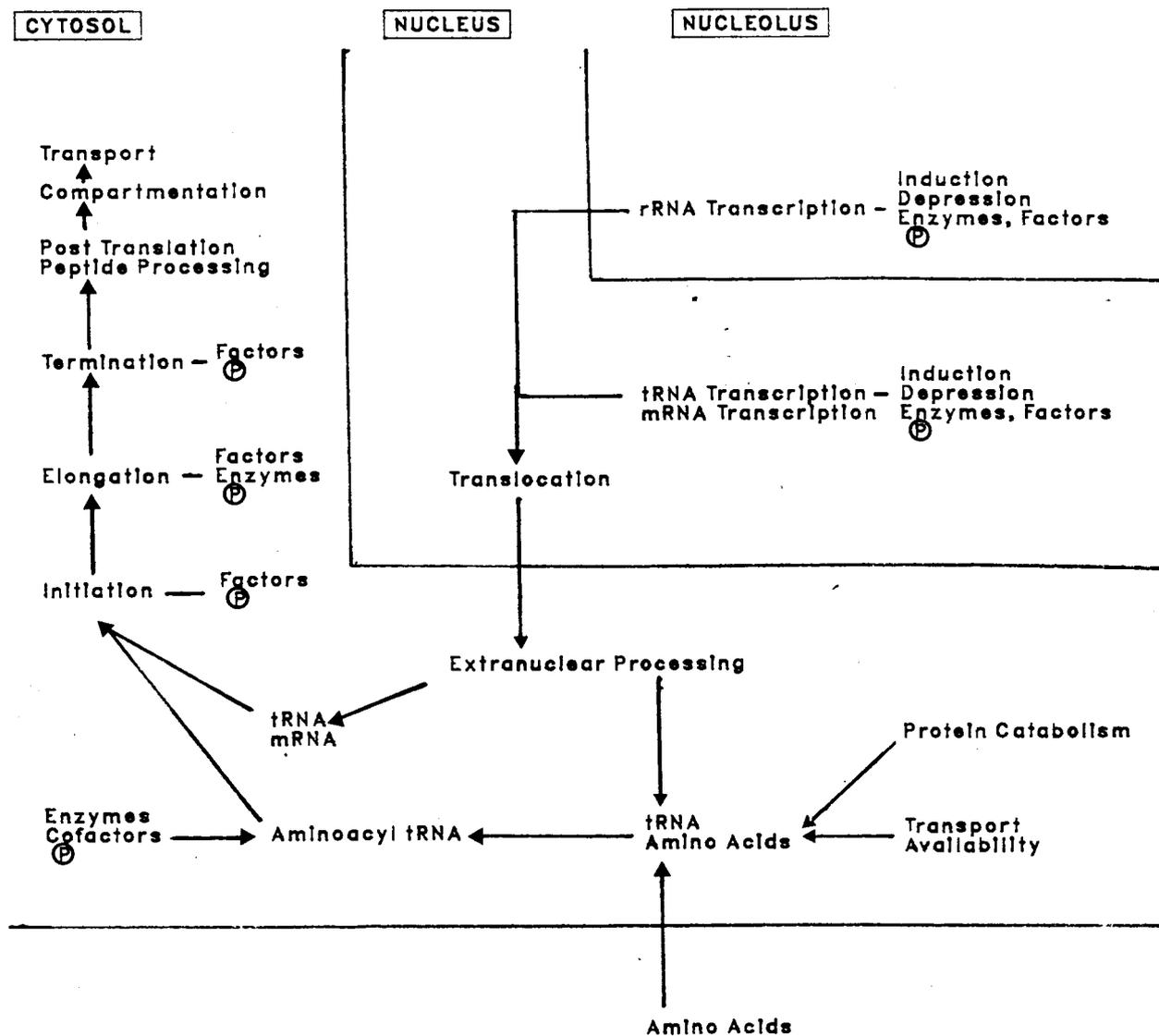
Figure 35. Requirements for protein synthesis.
(from: Thompson and Heywood, 1974; Young and
Allen, 1979).

step such as the disassembly of the contractile proteins, protein inactivation or denaturation, or penetration into the lysosomal compartment may be more important (Dean, 1980; Millward, 1980). Clark et al. (1980) have suggested a model of muscle protein degradation whereby alkaline proteinases process proteins for subsequent complete degradation by the lysosomal proteinases. This model has yet to be verified.

Factors known to influence skeletal muscle protein turnover at one or more of the synthetic and/or degredative stages include growth (Bates and Millward, 1983), endocrines (Rosenfeld and Barrieux, 1979), function (Goldberg, 1975) and nutrition (Giovanetti and Strothers, 1975). These will be further reviewed in light of their contributions to protein adaptations observed during growth and in response to exercise.

Growth

Growth: General

The effect of normal muscle growth and development on protein synthesis and turnover has been extensively researched (Devi et al., 1963; Winnick and Noble, 1965; Srivastava and Chaudhary, 1969; Cheek and Hill, 1970; Young, 1974: Burleigh, 1974; Millward, 1980; Lewis et al., 1984).

In contrast to the traditionally held view that postnatal growth of skeletal muscle results from hypertrophy alone, Cheek

and Hill (1970) have demonstrated an increase in DNA in normally growing muscle. These new myoneucleii are believed to originate from the mitosis of mast cells (satellite cells) which lie between the plasma and basement membranes of the muscle fibre. As postnatal growth continues, the rate of synthesis of new myofibrillar proteins exceeds that of DNA and results in a steady increase in DNA-unit size (protein/DNA ratio) (Millward, 1980). The final DNA-unit varies according to the muscle type with muscles containing predominantly slow oxidative fibres having the lowest values (Millward, 1980). While it is known that during terminal differentiation, skeletal muscle cells withdraw irreversibly from the cell cycle and cease DNA synthesis, the mechanisms involved in suppressing these processes are unknown (Ingwall, 1980).

During postnatal growth and development, there are marked changes in the turnover rates of skeletal muscle proteins. Protein turnover rates fall from a high rate at birth to the lower muscle specific rate at some time after the attainment of sexual maturity (Bates and Millward, 1983). Under optimal physiological conditions, net protein syntheisis occurs during early development, manifest in the rapid accumulation of myofibrillar proteins (Burleigh, 1974). The accompanying high rate of protein degradation observed during growth has been implicated in myofibrillar remodelling and differentiation (Millward et al., 1980). Stewart et al. (1982) suggest that under optimal conditions, regulation of muscle growth is

achieved through stretch imposed on muscles by bone growth. While these developmental changes occur in all muscle fibre types, the pattern of change appears to vary between muscles with protein turnover being fastest in red muscle (Kelly et al., 1984; Lewis et al., 1984). This is in agreement with observations in adult skeletal muscles (Sparrow et al., 1978). The decline in ratios of protein synthesis observed with aging has been related to diminished ribosomal capacity within the muscle (Lewis et al., 1984).

Nutrition, endocrines, and usage patterns play a crucial role in maintaining high protein turnover rates during development. These will be discussed further.

Growth: Nutrition

Dietary manipulation during the neonatal growth period has been shown to influence skeletal muscle protein turnover. Optimum growth of skeletal muscle requires an adequate supply of balanced nutrients (Trenkle, 1974). Feeding either a low energy or low protein diet during the early stages of development results in reduced growth of muscle as well as reduced DNA, RNA and protein content (Horwarth, 1972; Trenkle, 1974). While undernutrition results in muscles with near normal ratio of muscle mass per nucleus (DNA unit size) but fewer nucleii per muscle, a protein-free diet severely reduces DNA unit size due to the significantly greater loss of protein (Trenkle, 1974).

Millward et al. (1978) have found that the feeding of a protein-free diet results in severely reduced protein synthesis and degradation in young animals. As the period of dietary restriction was prolonged from nine to 30 days, protein synthesis diminished to an even greater extent whereas degredative rates began to rise toward control values. It would therefore appear that the loss of protein observed following protein-free or restricted diets arises primarily from diminished rates of protein synthesis.

During periods of caloric restriction and starvation, the rate of both protein synthesis and degradation in skeletal muscle fall (Li and Goldberg, 1976; Millward et al., 1976, 1978, 1980). However, by the third (Li and Goldberg, 1976) or fourth (Millward et al., 1978) day of starvation, protein degradation rates begin to increase above values observed in control animals. Enhanced activity of both lysosomal proteases (Desai, 1969) and myofibrillar proteases (Mayer et al., 1974) have been observed during starvation and may be responsible for the increased rate of protein breakdown observed as the fast becomes prolonged. Millward et al. (1980) have found that the myofibrillar fraction of skeletal muscle is the most sensitive to changes in protein and caloric supply. This may account for Li and Goldberg's findings (1976) that white muscle was the first to respond to fasting and showed the greatest catabolic response in terms of decreased rate of protein synthesis, loss of RNA, and enhanced protein degradation.

Factors thought to mediate the changes in protein turnover rates observed during fasting include reduced circulating levels of insulin and increased concentrations of glucocorticoids.

Refeeding promptly restores protein synthesis to control values and results in a marked drop in the rates of protein breakdown (Millward et al., 1978)

Growth: Endocrine

Skeletal muscle protein turnover is sensitive to a number of hormones. During growth, endocrine influences play a particularly significant role (Young, 1974). It should, however, be emphasized at the outset that no single hormone has absolute control of skeletal muscle growth, but rather several act in concert, often synergistically, to regulate overall protein metabolism.

Insulin plays a central role in regulating protein balance in skeletal muscle (Manchester, 1970; Libby and Goldberg, 1980; Millward et al., 1983). In diabetic animals, decreased rates of both skeletal muscle protein synthesis and degradation are observed (Millward et al., 1976; Li, 1980; Libby and Goldberg, 1980). Insulin is also known to be obligatory for optimal muscle protein synthesis in vitro (Millward et al., 1983).

The effects of insulin on protein turnover occur independent of any effect of the hormone on glucose transport into the muscle (Fulks et al., 1975). Insulin is thought to

stimulate protein synthesis by its actions of enhancing amino acid uptake into the cell, by its maintenance action on peptide chain initiation and by generally stimulating the rate of translation of mRNA (Rannels et al., 1977; Fahmy and Leader, 1980; Li, 1980).

Insulin's action on rates of protein breakdown are less clearly understood. Rothig et al. (1978) have observed enhanced alkaline protease activity in diabetic animals and report an increased latency of lysosomal proteases in diabetic animals following insulin administration. If one assumes a causal relationship between enhanced proteolytic rates and increased protein degradation, then the results of these studies would suggest enhanced protein degradation in diabetic animals. The opposite is in fact observed.

Growth hormone (GH) is required for normal postnatal growth and development. It is thought to act on protein synthesis by increasing synthesis of rRNA and mRNA as well as by facilitating initiation of translation (Edgerton, 1973). Insulin is required for the full anabolic expression of growth hormone (Daughaday et al., 1975). In hypophysectomized animals, decreased rates of both protein synthesis and degradation in skeletal muscle are observed (Millward et al., 1976; Libby and Goldberg, 1980). Schwartz (1982) reports that while physiological levels of GH are essential for maintaining normal rates of muscle protein synthesis in young rats, elevated GH levels have no further effect.

The lower rate of protein breakdown in hypophysectomized animals appears to result from the lack of thyroid hormone (ie. due to decreased TSH levels) (Goldberg and Griffin, 1977). Similar results are observed following thyroidectomy (Flaim et al., 1978). Treatment of hyphophysectomized and thyroidectomized rats with thyroxine resulted in marked improvements in protein synthesis and increased protein degradation (Flaim et al., 1978). This action of thyroid hormone on protein degradation may account for the loss of muscle mass observed in hyperthyroidism. Lysosomal protease activity has been shown to increase (DeMartino and Goldberg, 1978) or exhibit no change (Flaim et al., 1978) following thyroid hormone treatment of thyroidectomized animals. Therefore, the precise mechanism underlying the enhanced skeletal muscle protein degradation associated with thyroid hormones remains unknown.

Alterations in levels of thyroid hormones have recently been shown to be capable of interconversions of contractile and metabolic properties of fast and slow muscle comparable to those produced by neural factors (Nwoye et al., 1982). The effects of thyroid hormones are not neurally mediated. Mechanical properties, myosin phenotype and ATPase, and calcium uptake by sarcoplasmic reticulum have been shown to change in the direction of fast to slow in skeletal muscle of hypothyroid humans and animals (Nwoye et al. 1982). Fitts et al. (1980) demonstrated that contractile and biochemical properties of muscle fibres did not correspond to the histochemical profile of

fibres in thyrotoxic rats. Furthermore, slow to fast conversions have been reported in muscles in hyperthyroidism (Fitts et al., 1980). The underlying mechanisms are unknown.

The anabolic effects of androgens are well documented (Kochakian, 1976). Androgens can stimulate RNA polymerase (Rogozkin, 1979), nucleolar RNA synthesis, DNA polymerase and DNA synthesis in target organs (Edgerton, 1973). Liao (1977) suggests that androgens may act by specifying the DNA sites where initiation of RNA synthesis is to occur or by providing specificity for certain post-transcriptional processes. Florini (1970), however, was unable to detect any qualitative differences in proteins synthesized in soleus muscle following testosterone treatment despite a 60% rise in rates of RNA and protein synthesis.

The effects of androgens on skeletal muscle protein degradation are less clearly understood. Perhaps their most important role in reducing protein degradation occurs as a result of competitive binding for glucocorticoid receptors in the cytosol (Mayer and Rosen, 1977; Viru and Korge, 1979). Steroids have been shown to compete with glucocorticoids for receptors under equilibrium conditions and to accelerate the rate of dissociation of previously bound hormone (Jones and Bell, 1983), while reduced testosterone levels have also been implicated in transient rises in skeletal muscle alkaline proteolytic activity (Dahlmann et al., 1981). Koenig et al. (1980) have demonstrated an increase in the activities of

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several lysosomal enzymes in orchiectomized rats and in rats following testosterone treatment. The latter however occured in concert with an accretion of protein and RNA, suggesting that increased hydrolase activity is an inappropriate indicator of protein degradation. Using rat L6 myoblasts, Ballard and Francis (1983) were unable to detect any effect of testosterone on protein degradation.

Treatment of growing animals with glucocorticoids results in decreased rates of protein synthesis and is generally observed to enhance protein degradation rates in skeletal muscle (Millward et al., 1976; Libby and Goldberg, 1980). The role of glucocorticoids in muscle protein metabolism is difficult to evaluate in these studies as the administration of glucocorticoids to well nourished rats induces severe hyperinsulinemia (Millward et al., 1976). Hence, the situation arises in vivo where potentially anabolic and catabolic hormones are present simultaneously. The reduction in protein synthesis in skeletal muscle observed after administration of glucocorticoid was accounted for by both a loss of tissue RNA and development of a block in peptide chain initiation (Rannels et al., 1980; McGrath and Goldspink, 1982). Glucocorticoids may also reduce synthesis indirectly by supressing growth hormone release (Daughaday, 1975).

Although generally assumed to promote proteolysis, the effects of glucocorticoids on protein breakdown in muscle remain controversial. McGrath and Goldspink (1982) observed reduced

protein degradation in soleus muscle in vitro after treatment
with glucocorticoids. They suggest a stabilization of lysosomal
membranes or stimulation of an endogenous proteolytic inhibitor
as the mechanism of action of glucocorticoids in reducing
protein breakdown. In contrast, Seene and Viru (1982) found
elevated myofibrillar protease activity with a concomitant
reduction in rat skeletal muscle tissue following dexamethasone
treatment.

The diversity of reports concerning glucocorticoid actions
in protein degradation may arise from differences in the initial
nutritional status of the animals being evaluated. In the fed
state, muscles from normal and adrenalectomized rats show
similar rates of protein degradation (Goldberg, 1980). However
in fasting animals, adrenalectomy abolished the increased
protein degradation observed in normal muscle associated with
increased release of gluconeogenic precursors. When the fasting,
adrenalectomized animals were treated for two days with
cortisol, rates of muscle proteolysis increased compared to
those observed in fasting, normal animals (Goldberg, 1980). A
second possible explanation for the variety of reports
concerning glucocorticoid action on protein turnover can be
related to the selection of muscle for study. Kelly and
Goldspink (1982) report different responses of soleus and
tibialis anterior to dexamethasone treatment. In the slow twitch
soleus muscle, growth was merely slowed, whereas in the same
animals, a pronounced atrophy of the fast twitch tibialis

anterior was observed. This was due to a marked inhibition of protein synthesis with no change in degradation rate. Similar observations of greater sensitivity of white muscle to glucocorticoid come from Rannels and Jefferson (1980) using an in vitro preparation.

Endocrine factors play a critical role in regulating skeletal muscle protein turnover during growth. It should, however, be emphasized that the actions of hormones on skeletal muscle are often dependent not only upon one another, but are also sensitive to neural influences, nutritional states and the degree of muscle usage.

Growth: Exercise

Any attempt to evaluate the effect of exercise on skeletal muscle during growth must distinguish carefully between normal growth processes and the effect of exercise per se.

It is known that DNA accumulates in muscle cells post-natally (Cheek and Hill, 1970). Endurance exercise during prepubertal growth has been shown to further stimulate nuclear proliferation and DNA accumulation (Bailey et al., 1973; Buchanan and Pritchard, 1970). Hubbard et al. (1974) reported that endurance training in young rats may slow the rate and prolong the phase of nuclear proliferation. Hence, the increased DNA content of the exercised muscles would seem to indicate that exercise is a significant factor influencing the rise in DNA

content during growth and may possibly determine adult DNA content in skeletal muscle. However, it is not clear whether nuclear proliferation occurs in satellite cells or in muscle fibres (Edgerton, 1973).

Wilkinson et al. (1978) have studied the effects of endurance and anaerobic training in young rats on developmental patterns in skeletal muscle. Results were compared with normal developmental patterns observed in specific muscles rather than with values observed in age matched controls. Chronic exercise training in young animals was primarily reflected in a maintenance of the percent fast oxidative glycolytic (FOG) fibre population rather than an overt shift from one fibre type to another. Perhaps the most consistent finding among researchers of exercise effects on growth is the reduced body weight gain in exercised male rats when compared to sedentary, free eating controls (Reed et al., 1974; Wilkinson et al., 1978). This is probably due to both a decreased caloric intake and increased energy expenditure. However the changes observed in whole body weight following endurance training are not reflected in changes in muscle weight or lean body mass. Adipocyte cellularity and size are responsible for the gain in body weight seen in controls.

The effect of exercise in growing animals on rates of protein synthesis and degradation in skeletal muscle has not yet been studied. This may be largely due to the difficulties inherent in assessing turnover rates in a "dynamic" state.

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Exercise

Exercise: General

Cellular adaptations to endurance exercise training in
mature animals and man are well documented (Holloszy, 1973,
1975). The precise cellular mechanisms which control these
changes are unknown.

Armstrong et al. (1974, 1975) have demonstrated a selective
depletion of glycogen from slow oxidative (SO) and fast
oxidative glycolytic (FOG) skeletal muscle fibres following
prolonged endurance exercise. This observation supports the size
principle postulated by Henneman and Olsen (1965), where at low
to moderate stimulation frequencies, those fibres with the
smallest motor neuron size (SO and FOG) appear to be
predominantly recruited, while those fibres with the largest
motor neuron size (fast glycolytic, FG) are called into play at
higher stimulation intensities. Cellular adaptations to the
specific physiological demands (overload) imposed during chronic
endurance exercise may therefore be anticipated in those muscle
fibres which are predominantly recruited (SO and FOG) and in
those which contain the metabolic machinery capable of
regenerating energy (ATP) aerobically. This is precisely what is
observed (Edgerton, 1973). Enhanced oxidative enzyme activities
in muscle fibres following endurance exercise training are well

documented (Barnard et al., 1970, 1971; Holloszy et al. 1973, 1975; Fitts et al., 1974), and result in the enhanced capacity for aerobic energy regeneration (Saltin, 1973; Holloszy et al., 1975).

While the relative proportions and absolute amounts of skeletal muscle proteins are known to change following exercise training, the concomitant changes in protein turnover rates remain unclear. This is due to inconsistencies in experimental design, animal models studied and the variety of methods selected for assessing protein turnover. Evaluation of experimental results is further complicated by the varied periods of exposure to exercise, time elapsed between the final exercise bout and sacrifice, and unfortunately, also to inadequate reporting by investigators who fail to report the latter.

In reviewing the literature, it soon becomes apparent that the overwhelming majority of investigations have been devoted to the study of protein turnover changes following exercise induced (compensatory) hypertrophy, immobilization, denervation and electrical stimulation. While these studies no doubt provide useful information specific to the model studied, the degree to which results may be generalized to in vivo exercise models is questionable. In the following sections, reports of changes in protein synthesis and degradation resulting from exercise will be reviewed.

Chronic responses to endurance exercise (greater than 72 hours elapsed time between exercise and sacrifice) and acute responses (less than 72 hours) will be treated as unique phenomena. A limitation to this choice of terminology arises because it is based upon the chronic and acute protein synthetic responses to exercise, which have been found to peak approximately 24 hours post exercise (Dallaire, 1980). Vihko et al. (1978a), however, demonstrated a peak catabolic protein response, as measured by increased proteolytic activity, in mouse skeletal muscle, five days post exercise.

Clearly, it is difficult to agree upon a precise definition of the terms acute and chronic given the variable time frame of anabolic and catabolic responses.

Exercise: Acute and chronic responses to endurance exercise training

Whether training and exercise induced alterations in skeletal muscle protein result from changes in protein synthesis and/or degradation remains equivocal. Protein synthetic and degredative responses to exercise will be reviewed. It must, however, always be remembered that it is the interaction of the two processes that determines the final response to exercise.

Protein Synthesis

In a recent review on the influence of muscle use on protein turnover, Booth et al. (1982) have concluded that duration of the exercise is important in determining changes in protein synthesis. In all experiments reporting a decrease in protein synthesis, measurements were made between 30 and 90 minutes of exercise. Increased protein synthesis was observed after the fourth hour of exercise. Measurements made between the first and fourth hour of exercise revealed no change in rates of protein synthesis. If protein synthesis rates are altered during exercise it follows that those rates might again be altered when exercise ceases.

Immediately following either a one hour swim or a run to exhaustion, Dohm et al. (1980) have observed reduced protein synthesis in rat skeletal muscle. The reduction in uptake of radioactively labelled amino acids by isolated polyribosomes compared to control animals was greatest in the animals run to exhaustion. Protein synthesis values were depressed by 30% and 70% one hour following a one hour run and a run to exhaustion respectively (Dohm et al. 1982b). Based on the results from these studies, the investigators suggest that exercise severity determines the magnitude of the early acute catabolic response to exercise. In an earlier study, Dohm et al. (1978) observed decreased protein synthesis in the stromal fraction of rat gastrocnemius muscle with no change in sarcoplasmic and

myofibrillar fractions. The reader, however, was not informed of the time elapsed since the last exercise session. In contrast to the above studies which report reduced protein synthesis shortly after termination of exercise, Rogers et al. (1979), using untrained guinea pigs run to exhaustion, found that 3H-leucine uptake by isolated polyribosomes one hour after exercise was significantly increased 50%) compared to sedentary controls. In endurance trained guinea pigs, McManus et al. (1975) demonstrated an elevation of in vivo incorporation of 3H-leucine into myofibrillar and sarcoplasmic protein 18 hours post exercise. In rats, following a single bout of swimming exercise, a decrease in RNA polymerase activity was observed immediately after exercise. However, by the third hour of recovery, RNA polymerase activity had risen above values observed in controls (Rogozkin, 1976). Tapscott et al. (1982) were unable to detect any difference in protein synthesis rate between sedentary controls and trained, endurance exercised rats 24 hours after exercise.

These studies indicate a biphasic response of skeletal muscle protein synthesis following exercise, whereby an initial reduction in synthesis is followed one to two hours later by increased rates of protein synthesis.

Serial measurements by two investigators support this theory. Wenger et al. (1981) report enhanced in vitro uptake of 3H-leucine into white vastus muscle mitochondrial and microsomal fractions 18 and 24 hours after sprint or enduance exercise

respectively. Significantly reduced protein synthesis was observed immediately post exercise. In red vastus muscle, incorporation of the isotope was significantly elevated in mitochondrial and soluble fractions 2 and 18 hours post exercise respectively. Dallaire (1980) studied protein synthesis in rat plantaris and soleus muscle up to 72 hours post exercise in sprint and endurance trained animals. He found that the protein synthesis response to sprint exercise was delayed with respect to that of endurance exercise (ie. endurance response 12-36 hours; sprint response 24-48 hours) and speculated that the delay may be intensity related.

The factors regulating the observed biphasic response of protein synthesis to exercise are unclear. The stimuli of increased muscle stretch, tension, and increased intracelular calcium levels have all been implicated (Booth et al., 1982). However, these stimuli are all present throughout the exercise performance and might be anticipated to act immediately on muscle protein metabolism. The mechanisms by which these factors act to enhance protein translation remain as speculation.

Protein Degradation

The results from studies investigating protein degradation in skeletal muscle following exercise are controversial (Lemon and Nagle, 1981). Immediately following an acute exercise bout, increased (Dohm et al., 1978, 1980, 1982), no change (Schott and

Terjung, 1979) and decreased (Millward et al., 1980; Rennie et al. 1981) rates of protein degradation have been observed. These discrepancies may be explained by the inconsistent techniques employed by investigators to measure protein degradation rates. Results from studies using urinary urea and 3-methylhistidine (3-MeHis) levels as indicators of protein catabolism suggest that protein degradation is elevated up to 48 hours following exercise (Dohm et al., 1982). A cautionary note, however, should be added concerning use of 3-MeHis as an indicator of myofibrillar protein turnover. While non-muscular sources of 3 MeHis are small, much faster turnover rates in intestine and skin have been found to account for up to half of its excretion in the urine (Millward et al., 1980).

A more common approach in the study of protein degradation is to observe the activities of skeletal muscle proteases following acute exercise and training. Unfortunately, most investigators have assumed that protein catabolism was increased and did not make direct measurements of protein degradation. As has been mentioned earlier, any attempt to infer a direct cause and effect relationship between protein degradation rate and proteolytic enzyme activities should be viewed with caution.

Vihko et al. (1978a, 1979) have observed dramatic changes in skeletal muscle acid hydrolase activity and cell morphology over a period of weeks following a single, acute exhaustive exercise bout in untrained mice. In all fibre types, and particularly in red muscles, an acute exercise bout resulted in

fibre necrosis and marked increases in the activities of several acid hydrolases approximately three to five days later. It is interesting to note that concomitant changes in protein content are not always observed. Immediately following a run to exhaustion, Dohm et al. (1980) demonstrated elevated activity of "free" unbound cathepsin D in skeletal muscle from untrained rats.

Kuipers et al. (1983) have observed serial morphological changes in untrained rat skeletal muscle following a one hour treadmill run. While minor signs of muscle degeneration were observed immediately after exercise, by two to three hours, marked degeneration was apparent. The most pronounced changes were observed 24-48 hours post exercise, whereafter, regeneration occurred.

In trained animals, the proteolytic response to exercise appears to vary according to the method of exercise employed. In mice and rats accustomed to treadmill running, alkaline protease activity is reduced and acid hydrolase activity increased 24 hours after the last exercise session (Dohm et al., 1982; Salminen et al., 1983, 1984). Tapscott et al. (1982) however, report increased activity of alkaline proteases 24 hours following treadmill running in trained rats with no change in acid hydrolase activity. Twenty four hours following swimming exercise, no change in acid hydrolase activity and increased alkaline proteolytic activity has been reported (Dahlmann et al., 1981; Salminen et al., 1983).

Precise factors responsible for inducing such changes are unknown. Potential alterations in the acute exercise condition capable of producing such changes in the proteolytic environment and possibly also in altering the nature of the protein substrate have been outlined in Figure 36.

In trained animals, increased degradation during recovery suggested by direct measurements and by acid hydrolase and alkaline protease activity may, and most probably does, reflect an increased rate of intracellular protein turnover. This would be similar to the "anabolic" condition of protein degradation observed during growth. The need for enhanced protein degradation rates during growth, hypertrophy and adaptation to endurance exercise is unknown. Laurent and Millward (1980) speculate that it might reflect a "wastage" associated with growth (ie. not all increased synthesis results in growth). An alternative explanation proposes an inherent inefficiency in the hypertrophy process (ie. higher proportions of proteins are turned over as growth is accelerated). Even less well understood are the mechanisms governing the enhanced capacity for protein degradation observed in trained muscle. If it is true that muscle growth necessitates protein breakdown (Millward et al., 1978), then perhaps one is simply observing a secondary response to an accelerated rate of synthesis. The factors regulating this condition are also obscure.
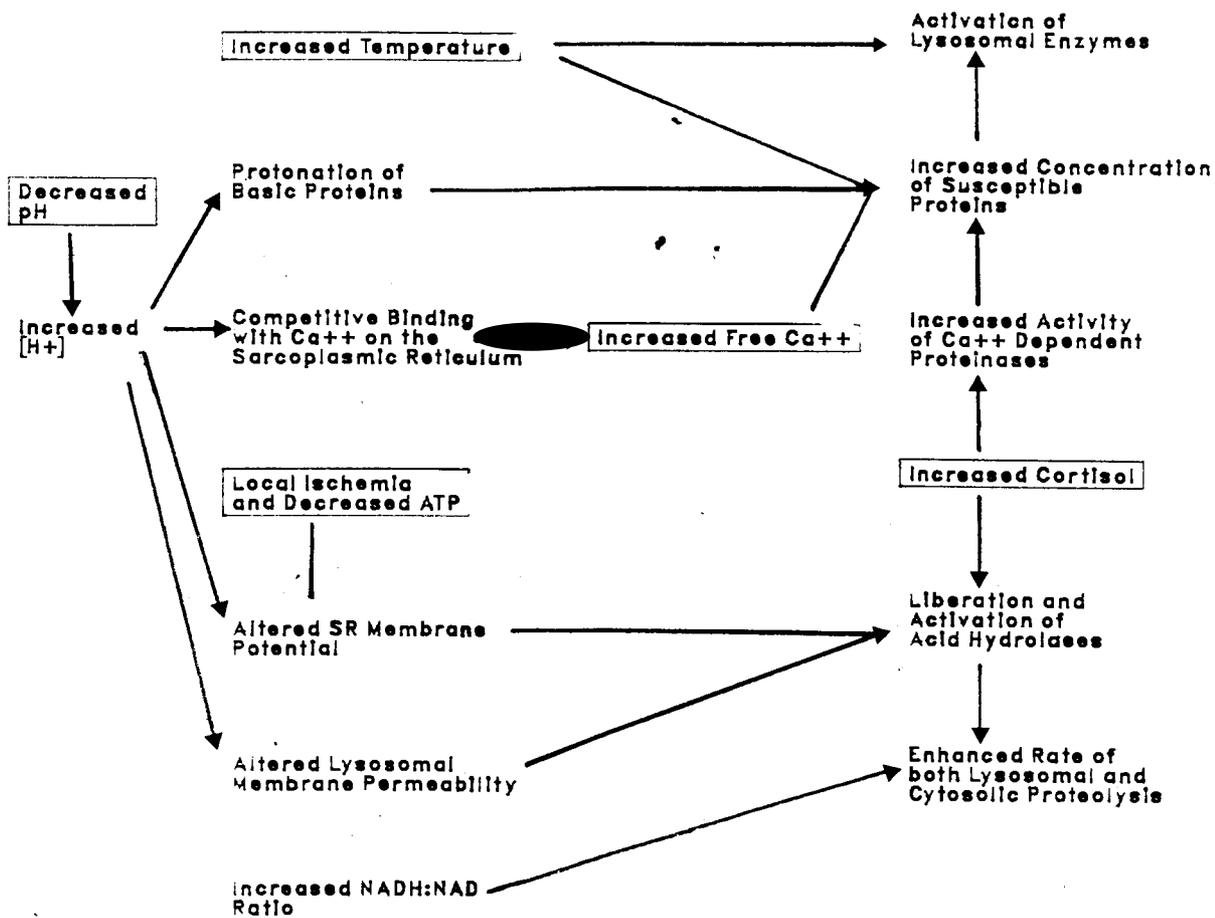
Figure 36. A proposed model of intracellular
events occuring during acute exercise which
may account for enhanced protein degradation
in skeletal muscle.

Exercise: Endocrine Influences

The precise role of endocrines in mediating exercise
induced changes in skeletal muscle protein turnover has not yet
been formally approached. Due to the numerous potential sites
for endocrine regulation, interactions of hormones and the often
transient nature of exercise induced hormone changes, the task
becomes somewhat onerous.

Blood hormone levels undergo marked changes during exercise
and recovery which necessarily modify substrate delivery to and
metabolism in the working muscle (Hartley et al., 1972a, 1972b;
Sutton, 1978; Refsum and Stromme, 1979). Both basal and exercise
levels of a number of hormones may be altered as a result of
physical training (Galbo et al. 1977; Terjung, 1979; Winder et
al., 1979) A number of these alterations will be summarized
below along with implications for potential regulation of
protein turnover.

A decrease in plasma insulin levels during exercise is well
documented (Galbo et al., 1977a). The fall in plasma insulin
levels is reduced in trained individuals who also display an
increased glucose tolerance at rest (Terjung, 1979). An enhanced
tissue sensitivity to insulin has been observed in trained
individuals (Kovisto et al., 1979).

The temporal sequence of shifts observed in plasma insulin
during exercise and recovery closely parallel those of protein
synthesis. Thus, falls in plasma insulin during exercise may

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partially account for diminished protein synthesis with elevations during recovery allowing synthesis to proceed. Enhanced tissue sensitivity to insulin in trained animals may potentiate an increased protein synthesis during recovery. This has not yet been investigated.

As was reviewed earlier, diminished insulin during exercise may act by blocking peptide chain initiation and by reducing amino acid uptake into skeletal muscle. These effects cannot however account for the delayed response of proteolytic enzyme activities to exercise.

High levels of circulating glucocorticoids are generally associated with increased skeletal muscle protein breakdown in fasting subjects. During exercise, the threshold of the glucocorticoid response appears to be related to the exercise intensity relative to maximal aerobic capacity (Davies and Few, 1973). This rise may reflect the point in exercise when the need for gluconeogenic glucose production arises. Bonen et al. (1981) have shown that glycogen depletion prior to high intensity exercise accelerates and enhances the cortisol response to exercise. Thus, glucocorticoids may indeed be active during intense exercise in enhancing protein degradation to provide gluconeogenic precursors. Their potential long term effect on myofibrillar turnover and induction of alkaline lysosomal enzymes is unknown.

During prolonged submaximal exercise, Growth Hormone (GH) levels show a delayed rise and may be seen to return towards

control values as the exercise continues (Terjung, 1979). Apart from the hypothesized effect of sensitizing the muscle to the effects of normal levels of androgens (Lamb, 1975) it is unlikely that GH exerts an important effect during exercise. Furthermore, Schwartz (1982) has shown that elevated GH levels are ineffective in enhancing protein synthesis. As was mentioned earlier, the rates of protein synthesis during exercise are initially depressed and are observed to increase only after four hours of exercise (Booth et al., 1982). This is in direct contrast to shifts in GH which are observed to fall as exercise becomes prolonged. The elevated levels of glucocorticoids observed during prolonged exercise may be responsible for the reduced plasma GH as glucocorticoids are known to supress GH release (Daughday et al., 1975)

While total plasma levels do not change during exercise, increases in plasma free thyroxine (ie. metabolically active thyroxine) have been reported (Caralis et al., 1977). In trained individuals, enhanced turnover and subsequent production of the thyroid hormones has been reported (Terjung, 1979). While an increased turnover of thyroxine (T4) normally corresponds to an excessive hormonal action leading to hyperthyroidism, trained individuals and animals do not show any of the typical clinical symptoms of hyperthyroidism.

These chronic adaptations in thyroid hormone metabolism may be implicated in enhanced rates of skeletal muscle protein turnover in trained individuals as thyroid hormones have been

shown to increase both protein synthesis and degradation (Flaim et al., 1978). The mechanism by which thyroid hormones regulate protein degradation is not clear, although one study has demonstrated elevated levels of lysosomal proteases following thyroxine administration (DeMartino and Goldberg, 1978). This may be related to the chronically elevated acid hydrolase activity observed in trained animals (Salminen et al., 1984).

The response of androgens to acute exercise is dependent upon exercise intensity and duration. During intense exercse, androgen levels increase (Sutton et al., 1973; Brisson et al., 1977) whereas significantly depressed levels (Dahlmann et al., 1981) or no change (Sutton et al., 1973) following prolonged submaximal work have been observed.

Following adherence to a six month endurance training programme, Remes et al. (1979) found significantly higher levels (21%) of plasma testosterone in human subjects at rest. This finding represents an increase in the level of 'free' circulating hormone (ie. metabolically active). Values of testosterone bound to sex hormone binding globulin following training were unchanged. In swim trained animals, Dahlmann et al. (1981) have demonstrated that testosterone administration immediately following prolonged exercise (ie. when endogenous levels are diminished) prevented the rise in alkaline proteolytic activity observed 24 hours after exercise. Similar results were observed with chronic administration of testosterone during training (Dahlmann et al., 1981). As

alkaline proteolytic activities are unaffected (Salminen et al.,
1983) or reduced (Salminen and Vihko, 1981) following treadmill
exercise, the degree to which these actions of testosterone may
be generalized is confounded. It is unlikely that testosterone
actions on proteolytic activity are exercise mode specific. The
rise in testosterone observed following intense exercise may be
associated with enhanced protein synthesis. Also, the
competitive binding of androgens for glucocorticoid receptors
(Mayer and Rosen, 1977; Vihko and Korge, 1979) might diminish
the catabolic response of cortisol during recovery.

Rogozkin (1979) administered anabolic steroids to rats 11
hours prior to a 15 minute swim. While he reports a significant
increase in muscle protein synthesis following exercise, he
fails to report both the time elapsed between exercise and
sacrifice and corresponding data from steroid treated sedentary
controls. Thus, the relative degree to which exercise and/or
steroid treatment contribute to this rise in protein synthesis
is unknown.

In rats trained by swimming, a significant increase in
cytoplasmic testosterone binding capacity has been observed
(Rogozkin and Feldkoren, 1979). Administration of anabolic
steroids to the animals throughout training had an adverse
effect on testosterone binding capacity. Despite this
observation, significantly greater increases in RNA polymerase
activity were observed in trained rats receiving anabolic
steroids. This suggests that steroids may exert their action by

modifying receptor activity and that cytoplasmic testosterone binding capacity may not provide a good measure of the anabolic effects of the hormone.

In summary, changes in blood thyroxine levels and increased thyroid hormone turnover during exercise, recovery, and following training may be important in mediating the observed changes in skeletal muscle protein turnover. During intense exercise, glucocorticoids may be important in enhancing protein degradation; however, it is unlikely that they mediate chronic changes in proteolytic acitivity. Alterations observed in plasma insulin during exercise recovery suggest an important role for this hormone in regulation of protein synthesis.

Principle:

Succinate -------------------------▼-------------> Fumarate

FAD <===> FADH2

Cytochrome C Fe++ ----------------> Cytochrome C Fe+++

                 <------XXXX------

                      CN
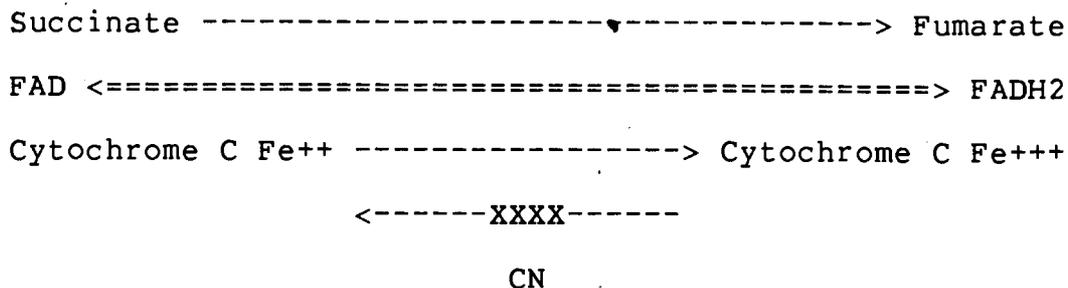
Reagents:

1. Sodium Succinate   0.5 mM   6.754 g/50ml

2. KCN   0.01 mM   32.6 mg/50ml

3. Cytochrome C   0.1 mM   24.8 mg/20ml

   Note: Reagents 1, 2, and 3 are made with phosphate

       buffer.

4. Phophate buffer   0.17 mM   pH 7.4

                     K2HPO4   38.799 g/L (796 ml)

                     KH2PO4   23.135 g/L (200 ml)

Cocktail:

Probe   0.030 ml

Succinate   0.150 ml   A. Mix and leave for 2 min.

KCN   0.150 ml   B. Mix well and leave for 6 min.

Cytochrome C 2.70 ml   C. Leave for 1 min. and record

                            extinction at 550 nm for 3min.

Calculations:

$$\text{Activity} = \frac{1000 \times 3.03 \times 500 \times E}{18.8 \times 30 \times 12.8}$$

$$= E \times 209.85 \text{ uM/g min. with a 30 ul probe}$$

APPENDIX C. MODIFIED LOWRY PROTEIN PROCEDURE

(Lowry et al., 1951)

Reagents:

0.5% CuSO4.H2O in distilled water (D.H2O)

1.0% NaKC4H4O6 (Sodium potassium tartrate)

2.0% Na2CO3 in D.H2O to pH 12.5 with 10 N NaOH

2 N Folin Reagent 1:1 with D.H2O

Lowry C Solution

  61.7% Na2CO3 solution above

  35.7% D.H2O

   1.3% CuSO4 solution above

   1.3% NaKC4H4O6 solution above

  100.0% Lowry C Solution

Reaction Mixture:

0.1 ml sample

5.0 ml Lowry C

0.3 ml Folin Reagent

Procedure:

1. Add 0.1 ml sample to a 15 ml test tube.

2. Add 0.5 ml Lowry C Solution and let stand at least 10 min.

3. Add 0.3 ml Folin Reagent while mixing on Vortex and let stand 2 hours to 2 days.

4. Read spectrophotometrically at 750 nm.

Blank:

Substitute 0.1 ml of buffer for sample

Standards:

Use 0.1 ml of Bovine Serum Albumin (dissolved in the same buffer instead of the samples. Cut a stock protein solution to the appropriate values.