BIOCHEMICAL MARKERS OF ECCENTRICALLY AEROBIC INDUCED POST EXERCISE MUSCLE SORENESS

by

JAMES EMMETT CARRABRE

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INTRODUCTION

Localized or diffuse muscle soreness as an aftermath of strenuous work is a problem common to both sports medicine and occupational or environmental health. Post exercise muscle soreness (POEMS) is a determinant in many persons' adherence to physical activity programs. POEMS has been shown to be prominent in both the trained and the untrained as well as in all age groups (Brumback, Staton, & Susag, 1981). In the untrained, POEMS could be the end of an overly vigorous and overambitious start to an exercise program, while for the trained, it may be the cause of premature cessation of a career in competitive athletics. For the professional athlete, it could mean countless losses in both training time and salary.

Although there are several theories relating to the cause of POEMS, a mechanism for the production of pain has not been substantiated and cellular damage has only recently been shown in humans (Friden, 1984). Because POEMS is one of the primary reasons why individuals cease programmes of physical activity, it is imperative that the nature and cause of this debilitation be determined.

Recent research on POEMS has focused on muscle tissue trauma and connective tissue trauma. This study will pro-

vide a better understanding of the nature and cause of muscle soreness by investigation as to whether either of these 2 research themes are important in the etiology of POEMS. Hopefully, by knowing the structural and chemical mechanisms underlying POEMS, it will be possible to recommend a means of preventing and treating muscle soreness.

Statement of the problem

There were two purposes to this study:

1. To examine whether concentric and/or eccentric exercise affect muscle fibre/girth measures or known urinary markers of post exercise muscle soreness.
2. To determine whether any of these parameters were related to any post exercise muscle soreness experienced.

Connective tissue components

Urine hydroxyproline (HP), by itself, and standardized to urine creatinine (CR) and lean body mass (LBM) were examined as indicators of connective tissue trauma.

Contractile tissue components

Uric acid (UA), by itself, and standardized to LBM were examined as indicators of muscle protein catabolism. In addition, m. vastus lateralis was examined to determine wheth-

er any muscle fibre area changes are predominant to a particular fibre type.

Hypotheses

1. There will be no significant differences in urine hydroxyproline, hydroxyproline:creatinine or uric acid within the eccentric and concentric exercised groups.
2. There will be no significant difference within the parameters of hypothesis 1 when standardised to lean body mass.
3. There will be no significant differences between the eccentric and concentric exercised groups for the parameters stated in hypothesis 1 & 2.

Assumptions

1. The relative contribution of each of the lower body anti-gravity muscle groups to the acceleration:decceleration of the center of mass were assumed to be the same for each subject. The relative percentage force absorption for the quadriceps muscle group was thus assumed equal for each subject.
2. The relative pathophysiology of muscle damage:repair was assumed equal for all subjects. (i.e. the underlying mechanisms producing soreness occur along the same relative course of time).

Delimitations

1. Due to the great demands placed on the subjects, a large N was an unreasonable expectation.
2. Inferences based on the results of this investigation will pertain to young healthy male and female adults.

Definition of terms

POEMS ..... Post exercise muscle soreness (POEMS) is a phenomena which typically occurs 1 to 3 days after intense physical activity and is generally localized to myotendinous areas. The exercise is usually of a nature and intensity to which the individual is unaccustomed, but typically is an activity involving a great deal of eccentric muscle contractions. POEMS can be qualitatively classified as one of 3 degrees of soreness. These are movement, touch and ache soreness respectively.

LFFS ..... Low frequency fatigue soreness. This term is synonymous with POEMS. It refers to soreness induced after repetitive low frequency nerve stimulation of the muscle.

REVIEW OF RELATED LITERATURE

Introduction

Although POEMS is a concern which has been in existence for many years, little research has actually been accomplished. It has only been within the last half decade that investigators have focused their attention on this important phenomena. This chapter contains an in depth analysis of the current literature pertaining to muscle soreness, presented as follows:

1. Symptoms of muscle soreness.
2. Classes of muscle soreness.
3. Current research focuses with underlying theories.
4. Exercise causing muscle soreness.
5. Means of investigation of muscle soreness.
6. Justification of analysis parameters used in this investigation.
7. Summary of factors relevant to POEMS.

Symptoms of muscle soreness

Timelines for soreness development

The onset of POEMS usually occurs as early as several hours and as late as 1 to 3 days after heavy exercise (Friden, Sjostrom, & Ekblom, 1981; Schlitz & Fitzgerald, 1975; Abraham, 1977), and is more common in those who are unaccustomed to the activity. These occurences tend to be most severe the day following exercise, and gradually fade away over the next 4 to 6 days (Astrand & Rodahl, 1977). Asmussen (1956) assigned an arbitrary classification to POEMS with the most severe soreness on the second post exercise day. Similarily, Talag (1973) found that POEMS was most intense 48 hours after exercise. In a study comparing the subjective sensations of muscular soreness following level and downhill running, Schwane, Johnson, Vandenakker, and Armstrong(1983). Ogilvie, and Schwane (1983) reported soreness at 24, 48 and 72 hours after a downhill run.

Physical symptoms

In a study of exercised individuals, symptoms of POEMS were only found in those subjects who followed a regimen of eccentric exercise (Asmussen, 1956). The major symptoms included hard and swollen muscles which were painful when contracted and sore to the touch (Friden et al, 1981). Because of the extreme pain and tenderness, movement of the involved muscles was restricted. In addition, when the soreness re-

sulted from eccentric contractions, strength was decreased in the affected muscles (Talag, 1973; Friden et al, 1981).

This feeling of weakness and instability is usually experienced for a few hours immediately following exercise in the muscle which had contracted eccentrically (Newham, Mills, Quigley & Edwards, 1983). This sensation is likely an indication of pain to follow and is presumably a reflection of profound low-frequency fatigue with inappropriate forces being generated by the relatively low physiological firing frequency (Clamann, 1970; Milner-Brown, Stein, & Yemm, 1973).

Newham et al (1983) measured the severity and distribution of muscle tenderness in m. quadriceps after eccentric contraction by means of a pressure probe. Their results indicated localized tenderness, primarily at the distal, medial, and lateral parts of m. quadriceps, while the central and proximal regions were relatively spared. Asmussen (1956) noted that POEMS was most evident at the muscle attachments to tendon and fascia. The most commonly reported location of POEMS is therefore at locations where connective tissue is most abundant (eg. the myotendinous junctions) (Komi & Buskirk, 1972; Komi & Rusko, 1974; Newham et al, 1983) At peak intensity of soreness, the distribution of tenderness was more diffuse (Newham et al, 1983). Newham et al (1983) concluded that eccentric contractions result in uneven tension over the myotendinous junction thereby causing mechanical damage and POEMS.

Classes of muscle soreness

Introduction

Much confusion exists in distinguishing POEMS from the extreme pain and fatigue experienced during a vigorous session of exercise. The fact that 2 types of soreness exist in association with exercise was first documented by Hough (1901). These have since been classified as either temporary or residual muscle soreness (Talag, 1973). Current terminology used in muscle physiology has designated them "High Frequency Fatigue Soreness" (HFFS) and "Low Frequency Fatigue Soreness" (LFFS) respectively. These are schematically distinguished in Appendix B.

High frequency fatigue soreness

This class of soreness characteristically occurs near the end of an exhaustive exercise session and usually results in the termination of that exercise session, with an extreme occurance being a cramping of the muscle. Thus, HFFS is more a sensation of total muscle fatigue than inherent soreness in the muscles. The soreness may sometimes last for several hours, but presents no lasting problems. It is accompanied by stiffness and decreased strength, but the soreness is generally moderate and only aggrevated slightly by active movements. The primary causes are thought to be the biochemical end products of metabolism affecting free nerve endings coupled with local tissue edema (Brendstrup, 1962; Hough,

1902; Helwig, 1934). This phenomenon is covered here so as to distinguish it from muscle soreness with a delayed onset (LFFS). This phenomenon is not in question in this study.

Low frequency fatigue soreness

This soreness classification is the subject of the present investigation. Hence LFFS is synonymous with POEMS. Hereafter, the POEMS terminology shall be used. In contrast to HFFS, this soreness type refers to soreness of a delayed onset in nature, occurring as a peak from 24 to 72 hours following severe exercise. The following physiological rationale attempts to account for this phenomenon by presenting known alterations to muscle which occur in parallel to LFFS symptoms under simulated experimental conditions.

Repetitive, fatiguing isometric and isotonic contractions have been shown to produce specific long-lasting alterations in contractile properties of muscle. After severe exercise, a decrease in the twitch size occurs which is long lasting and may be caused by some structural change in the fibre (Edwards, Hill, Jones, & Merton, 1977). Both fast and slow muscles show this effect and it has been shown to occur in situ or in isolated animal preparations. These alterations are such that the force:frequency curve is shifted to the right and electrical stimulation at low frequency (1 to 20 Hz) results in decreased force generation when compared with fresh muscle. However, force generated by high frequency

stimulation was relatively preserved (Edwards et al, 1977). This type of fatigue, termed "low frequency fatigue" has been demonstrated in the quadriceps, adductor pollicis, diaphragm (Moxham, Morris, Spiro, Edwards, & Green, 1980a) and the sternomastoid (Moxham, Wiles, Newham, & Edwards, 1980b). Although the underlying mechanism is not clear, it was assumed that the amount of low frequency fatigue produced in a muscle was related to the work performed by the muscle. Edwards, Mills and Newham (1981) demonstrated that eccentric contractions caused greater low frequency fatigue than concentric contractions. Davies and White (1981) measured tetanic and twitch tension after concentric and eccentric contractions in m. triceps surae. They suggest that prolonged negative work, during which the muscle is repeatedly stretched during its contracted phase, results in soreness and weakness. Moreover, they found that muscles are weaker but not more fatiguable following eccentric exercise. The effect of eccentric and concentric muscle conditioning on muscle tension and the integrated EMG (IEMG) was observed by Komi and Buskirk (1972). In the early stages of conditioning, the subjects in the eccentrically exercised group experienced POEMS with an accompanying decrease in their maximum strength. In a later study, Komi and Viitasalo (1977) found an increased neural activation for a given tension in sore muscles. The delayed recovery of strength after eccentric fatigue was suggested to be due to changes in the muscles other than lowered ATP levels.

Mechanical damage to the sarcoplasmic reticulum, resulting in less calcium release for each excitatory action potential, has been suggested as a cause of POEMS in eccentrically contracting muscles (Jones, 1981). This would justify the decrease in twitch size and right side shift in the force:frequency curve. This is also the most plausible explanation for the noted delay in recovery of strength with POEMS. There is evidence to support this suggestion (Newham et al, 1983). Merton and Morton (1980) were able to show via simultaneous stimulation of the motor cortex with scalp electrodes and the electromyogram (EMG) response that the "pathway of command" was working normally in fatigued muscle. However, direct stimulation of fatigued muscle utilizing electrical stimulation through the overlying skin resulted in its contractility being much reduced (Hill, McDonnell, & Merton, 1982; Merton & Morton, 1980). It was thus found that the muscle fibres must be failing and not the nerves supplying the fibres. This suggests that for fatigue of sustained maximal voluntary contractions (MVC's), there is a reduction in the muscles electrical activation which is not due to central fatigue nor to neuro-muscular block, but due to peripheral inhibition such as with mechanical damage to the sarcoplasmic reticulum.

Eccentric exercise and POEMS

Introduction

POEMS is usually a result of an individual participating in an activity to which he or she is unaccustomed. It is especially noted after sessions of eccentric exercise (Asmussen, 1956;1953) such as running down hill, walking down stairs, jumping exercises and similar modes of exercise. This is not surprising because POEMS has usually been associated with work involving high muscle tension and it is well documented that greater tension per muscle fibre is always generated under eccentric contraction conditions (Katz, 1939; Curtin & Davies, 1973) which is characterized by elongation of the muscle at the same time as contraction.

Eccentric exercise based problems

Studies on POEMS have involved protocols which are usually of high eccentric intensity. At some point in each running stride, the gluteal, hamstring, anterior leg, and posterior leg muscle groups contract eccentrically to deccelerate the body's center of mass in the vertical plane. These contractions occur in level running, but are accentuated in downhill running because of the relatively greater lowering of the center of mass (Margaria, 1968). The highest 'in series' forces during the normal stride cycle in level locomotion are developed in the eccentric contractile phase when active tension is used to deccelerate the center of

mass following foot placement (Walmsley, Hodgson & Burke, 1978). When an animals' mass performs work on the muscle during eccentric contractions, local muscle temperatures may be higher than during equivalent concentric exercise (Nadel, Bergh, & Saltin, 1972). These higher temperatures could contribute to the observed eccentric induced tissue damage (Armstrong et al, 1983). Greater tension per muscle fibre is produced under eccentric contraction conditions than under any other form of in vivo contraction (Katz, 1939; Curtin & Davies, 1973). In this situation, relatively few fibres are recruited, resulting in relatively large forces per fibre. Therefore, the uneven mechanical stresses produced in the muscle and at its attachments are analagous to the weakest link in a chain (Newham et al, 1983).

Newham et al (1983) stated that the high forces generated by few fibres during eccentric contractions are transmitted to the non contractile tissues with resultant mechanical damage, and are not related to the metabolic energy cost of the contractions. It is well established that the metabolic cost (Knuttgen & Klausen, 1971; Davies & Barnes, 1972a; 1972b), ATP hydrolysis (Infante, Klaupik, & Davies, 1964), and the electrical activity required to produce a given tension (Bigland-Ritchie, 1981; Basmajian, 1974; Bigland-Ritchie & Woods, 1976) are less under eccentric than concentric contraction conditions. In addition, fewer motor units are activated for any given load in eccentric work (Bigland & Lippold, 1954).

Current research focuses on POEMS

Current research attributes POEMS to either connective tissue or muscle tissue trauma. A number of theories have been hypothesised as to the cause of POEMS. These areas have been investigated through ergographic, biochemical, electromyographic, rating scale and various exercise techniques (Friden, 1984).

Connective tissue trauma

Introduction

There are 2 major hypotheses relating to connective tissue trauma. These are the torn tissue hypothesis and overstretching of the muscles' elastic components. All literature summarized in this section deals with the non-contractile components of the muscle (hence connective tissue).

Initial speculation about connective tissue damage as a factor in POEMS was published by Hough(1902), who noted that untrained individuals suffered from muscle soreness after heavy resistance exercise. Hough suggested that a possible explanation may be a rupturing of connective tissue transmitting the pull of the muscle fibre to the tendon. Hough also postulated that the soreness could be due to damage to the muscle tissue itself. Research techniques of that time did not permit any further exploration of these two hypotheses.

Torn tissue hypothesis

General evidence.

Collagen decreases in tensile strength both with increasing temperature and decreasing pH (Chvapril, 1967). Thus, it may be that when a level of exercise becomes sufficiently severe, a reduction in the physical strength of the collagen allows it to be damaged (Nadel et al, 1972). This is supported by subjects reporting the greatest localized soreness in the tendinous areas of the muscle (Newham et al, 1983).

Biochemical evidence.

There is biochemical evidence in support of the torn tissue theory as it applies to connective tissue. Abraham (1977) demonstrated an increased urinary HP:CR ratio in the 24 hours prior to and including the muscle soreness. Since HP is known to be a specific breakdown product of connective tissue (Kivirikko, 1970), Abraham (1977) concluded that POEMS could be related to disruption of the connective tissue elements.

Overstretching hypothesis

Asmussen (1956) as well as Komi and Buskirk (1972) suggested that overstretching of the muscles' elastic components (eg. the connective tissue between the fibres and the fibrils), especially during eccentric types of work, results

in POEMS. Abbott and Bigland (1968) agreed with the findings of Asmussen (1956) and Abbott, Bigland and Ritchie (1952) by demonstrating that fewer motor units are needed to produce the same tension during eccentric contraction than during concentric contraction. Based on this information, Asmussen assumed that the elastic tension per active unit would be greater in eccentric work, and the risk of damage to the muscle and tendons ,and hence soreness, would increase.

During negative work, the number of participating fibres is decreased, resulting in a greater pull per muscle fibre. This excessive pull may be traumatic to the connective tissue (Karpovich & Sinning, 1971).

Muscle tissue trauma

Introduction

There are several hypotheses relating to muscle tissue trauma. These are the torn tissue hypothesis; lactic acid hypothesis; muscle spasm hypothesis; and the edema hypothesis.

Torn tissue Hypothesis

Myofilament evidence.

Even though muscle soreness was described early in the twentieth century, with a postulated cause being injury to

the muscle (Hough, 1902), the cause has never been adequately demonstrated in humans. Hough noted that the soreness was accompanied by a loss of contraction strength. He explained the soreness as "Involving actual rupture within the muscle" (Hough, p 91, 1902) This loss of contraction strength was assumed by Hough to be a result of a reduction in the functional cross-sectional area of the muscle. Hettinger (1968) reached a similar conclusion as he believed that the soreness after intense conditioning was due to rupture of the muscle fibres and/or the sarcolemma.

Mechanical damage to the sarcoplasmic reticulum, resulting in less calcium release for each excitatory action potential has been suggested as the cause of low frequency fatigue (Jones, 1981). Gollnick and King (1969) reported no disruption of the ultrastructure after exhaustive dynamic exercise, although they reported that mitochondrial swelling may occur. It has been shown that the mitochondrial disruption with exercise (Nimmo & Snow, 1982) or with skeletal muscle ischemia (Hanzlikova & Schiaffino, 1977) is greater at the sarcolemma than deep within the fibre. Armstrong et al (1983), using a rat model, noted some sarcolemmal damage after downhill treadmill running. After repeated bouts of maximum eccentric contractions, Komi, Viitasalo, Vihko and Rusko (1974) reported no ultrastructural changes in the sarcoplasmic reticulum or in the organization of the contractile material.

Friden et al (1981) observed z-line disruption and streaming in soleus muscle fibres of human subjects who had run down a flight of stairs 10 times. They further suggested that the z-line may be the weakest link in the myofibrillar contractile chain, and that the apparent z-line disruption results form the high forces of eccentric contractions. In 1983, Friden demonstrated further that the Z-band is, morphologically, the most sensitive myofibrillar component with eccentric exercise. He further noted that type II fast twitch fibres were primarily affected. These changes were found to be most extensive 2 to 3 days after exercise, corresponding to times of peak soreness within the investigation.

In a comparison of uphill vs downhill ran rats, Armstrong et al (1983) reported that injuries of the myofibrillar band pattern occurred immediately after eccentric exercise. Necrotic fibres, macrophages and satelite cells were observed 24 hours after eccentric exercise. The type II fibres were predominantly affected. Armstrong et al (1983) concluded that fibre damage was strongly associated with eccentric contractions.

Myoglobin evidence.

It has been demonstrated that there is an increase in plasma myoglobin levels with POEMS. These changes seen in plasma myoglobin are independent of total muscle mass, sug-

gesting that increased plasma myoglobin concentrations may depend upon the amount of muscle fibre damage (Danneskiold-Samsoe, Christiansen, Land, & Andersen, 1982). Conversely, Abraham (1977) reported myoglobinuria in 88% of his subjects who were experiencing POEMS. Abraham (1977) also noted that 92% of the subjects who performed exercise without experiencing POEMS had myoglobinuria. Abraham thus concluded that myoglobinuria is a manifestation of normal exertion and is not specifically a phenomena associated with POEMS.

Karpovich and Sinning (1971) noted that since the metabolism of negative work is 5 to 7 times smaller than the metabolism of positive work, soreness cannot be explained as a result of excess metabolites.

Energy function enzyme evidence.

Plasma enzymes have been a recent focus of attention in the investigation of POEMS. The possible causes for the elevation in plasma enzyme activities immediately after exercise are particularly elusive. It has been suggested that contractile activity induced changes in membrane permeabilities, short of observable damage, may explain the increased plasma enzyme activities after exercise (Atland & Highman, 1961; Halonen & Konttinen, 1962).

Plasma lactate dehydrogenase (LDH) and white blood cell counts appear unchanged while CR kinase levels in the plasma are reportedly elevated after exercise (Schwane, Johnson,

Vandenakker, & Armstrong, 1981; 1983). Schwane et al (1983) concluded that plasma CR kinase levels may be related to POEMS. Plasma CR kinase activity has been shown to be directly related to the extent of fibre necrosis in muscles injected with toxic enzymes (Steiness, Rasmussen, Svendsen, & Nielsen, 1978). Frequent reports of elevated serum CR kinase levels after exercise with and without subsequent soreness have been documented (Hagberg, Michaelson, & Ortelius, 1982; Maxwell & Bloor, 1981; Schumate, Brooke, Caroll, & Davies, 1979) The elevation of CR kinase has been interpreted to be due to a leakage of the enzyme through the cell walls (Maxwell & Bloor, 1981). Armstrong et al, (1983) demonstrated that plasma levels of CR kinase were 2.9 times greater in rats run on a downhill grade than rats run uphill and two fold greater than level runners. This indicates that the immediate post exercise elevations in plasma enzymes are most probably related to the eccentric component of the exercise when intensity and duration are constant. In the same study, intermittently exercised rats (5 minutes on; 2 minutes off; at a velocity of 16 m min^{-1}) on a downhill grade demonstrated a secondary late phase (1.5 to 2 days) elevation in plasma enzymes (CK and LDH), whereas the uphill runners did not. Thus, the plasma enzyme response was most probably related to the eccentric component of the exercise, both immediately after exercise and during the late phase changes in activity.

Glucose-6-Phosphatase (G-6-Ptase) activity has been shown by a number of investigators to be elevated in skeletal muscles following traumatization of the tissue (Armstrong, Marum, Tullson, & Saubert, 1979; Beaconsfield, 1963; Rifenberick, Koski, & Max, 1974). Armstrong, Garshnek, and Schwane (1980) noted a 248% elevation in this enzyme's activity 48 hours after downhill running in rats in m. triceps brachii. G-6-Ptase activity has been specifically localized in regions of nuclear accumulation in injured muscle. This elevated activity in injured muscle was associated with proliferation of cells involved in degenerative/regenerative processes (Armstrong et al, 1979; Armstrong et al, 1983; Tullson & Armstrong, 1981). The time course of the change in G-6-Ptase activity (Armstrong et al, 1983) was similar to that for changes in lysosomal enzymes in the muscles following prolonged exertion (Vihko, Rantamaki, & Salminen, 1978a).

Tiidus and Ianuzzo (1983) noted that increased intensity and duration of exercise produced increased serum enzyme activities and muscle soreness, with intensity having the more pronounced effect.

Hydrolytic enzyme evidence.

The activation of the lysosomal system is considered as a general sign of sublethal injury to the cell (Arstila, Hirsimaki, & Trump, 1974). The lysosomal system has been found

to be stimulated after exercise (Vihko, Salminen & Rantamaki, 1978b; Salminen & Vihko, 1980). This exercised induced temporary stimulation of the lysosomal system suggests that proteolytic processes contribute to the repair of the reversibly injured muscle fibres and hence an accelerated protein degradation (Ballard, 1977). Animal experiments have demonstrated that sublethal and lethal fibre injuries and an inflammatory response occur after high load or endurance exercise (Salminen & Vihko, 1981; Salminen,Kainulainen, & Vihko, 1982; Vihko, Salminen, & Rantamaki, 1979).

The intermyofibrillar location of the acid hydrolase activity in normal muscle suggests that the enzymes may be associated with the sarcoplasmic reticulum (Canonico & Bird, 1970). Histochemical studies on acid hydrolases indicated that the increase in total acid hydrolase activities originated mainly in muscle fibres and to a lesser extent in inflammatory cells (Vihko et al, 1978a). Vihko et al (1978a) further noted that B-Glucuronidase (B-Gdase) was the acid hydrolase whose activity increased the most. Since B-Gdase activity in skeletal muscle primarily resides in the lysosomes of macrophages and other interstitial cells (Canonico & Bird, 1970), it is reasonable to assume that intracellular disruption could lead to release of these enzymes. High tensions in the fibres could release or activate the enzymes located on the sarcotubular network leading to autolysis of the muscle cell. Activation of these enzymes could bring

about an associated inflammation, resulting in soreness (Tullson & Armstrong, 1981; Armstrong et al, 1980; Schott & Terjung, 1979).

Salminen and Vihko (1983) noted that the acid proteolytic capacities (Cathepsin D and B-Gdase) of exercised rats were considerably increased on the 4th day after exertion and partially decreased by the 10th day. Vihko et al (1978a) found that peaks in the activities of several lysosomal hydrolases occurred in muscles of mice between 3 and 7 days after exhausting level treadmill exercise. The greatest change in activity (as determined from biochemical analyses) occurred in type I fibres (Vihko, Salminen, & Rantamaki, 1978b). Thus, histochemistry verified that the largest increases in lysosomal enzyme activity were in type I fibres (Vihko et al, 1979). The elevations in enzyme activity were isolated both within muscle fibres and in the interstitium, but it was reported that the greatest change occurred in the muscle fibres per se (Vihko et al, 1979; Vihko et al, 1978b).

Lactic acid hypothesis

A disease of muscle termed "myopathia e functione", which seems to be identical with POEMS, was described by Helwig (1934). He believed that the swelling of the muscle was due to some physical or chemical alteration within the muscle and that the soreness was a result of local accumulation of

lactic acid. This latter conclusion was not supported by experimental evidence in Helwigs' study. Hill (1951) discounted the notion of stiffness due to unusual acidity while Watrous, Armstrong, and Schwane (1981) demonstrated that lactic acid was not a causative factor in POEMS. Subjects who ran level on a treadmill and produced no soreness were found to have a mean increase in lactate (over rest) of 208 %. Conversely, subjects who ran downhill on a treadmill had POEMS, with a non-significant elevation in lactate both during and after exercise (mean of 24 % over rest). Current research does not support the notion that lactic acid is a factor related to POEMS.

Muscle spasm hypothesis

Differences between sore and normal muscles have been described by deVries (1961;1966) based upon the observation that surface EMG recordings have a greater resting activity in sore muscles than in controls. The spasm hypothesis (deVries, 1965; 1976; 1980) proposes that POEMS results from tonic spasms in localized motor units, with the severity of pain directly related to the number of motor units involved. This assumes an incomplete relaxation of the muscle and nerve endings (Edington & Edgerton, 1976; Karpovich & Sinning, 1971). deVries (1961;1966) spasm hypothesis implies that exercise above a minimal level will cause ischemia and pain, resulting in a protective, reflexive, muscle contrac-

tion. This concentric contraction will result in localized ischemia of the muscle, completing the cycle. Furthermore, deVries (1961) postulated that POEMS could be prevented by periodic stretching of the affected muscle. These results have not yet been confirmed.

Pain and fatigue were studied by Newham et al (1983) after concentric and eccentric exercise contractions in the quadriceps. Pain and tenderness developed solely in the muscle which had contracted eccentrically. They were not able to detect any evidence of localized muscle spasm during soreness as reported by deVries (1966). Newham et al (1983) further noted that the relative contribution of the rectus femoris, and medial and lateral vasti, to the total muscle electrical activity, did not change significantly during either HFFS or POEMS. This provides no evidence of changes in recruitment patterns with fatigue or inhibition of sore areas. Newham et al (1983) suggested that mechanical stress and trauma could explain the measured reduction in maximal voluntary force, the increase in electrical activation for a given muscle tension, and the extreme POEMS. There is thus no evidence that the spasm theory has a relationship to muscle soreness. trauma.

Edema hypothesis

Brendstrup (1962) reported an increase in the water, chloride content and weight of muscles in rabbits 24 hours after heavy exercise. This edema was found to increase for 1 day and then subside over the next 6 days. Brendstrup stated that the time course of POEMS and the occurrence of edema coincided and therefore concluded that the sensation of pain was due to edema. Talag (1973), using concentric, eccentric and static contractions, in a study on 60 subjects contractions), reported increased limb volumes 24, 48, and 72 hours after eccentric exercise. When the subjects were exercised concentrically or isometrically, the limb volume returned to pre exercised values immediately after exercise. Boyle and Scott (1938) reported no muscle girth increases from pre to post exercise in muscles of subjects with POEMS. The literature thus presents conflicting reports as to whether swelling of a limb is in association with POEMS.

Protocols for the inducement of muscle soreness

There has not been a unified attempt at developing a quantification model for inducement of muscle soreness. Abraham (1977) ran subjects on a downhill grade at 3 mph for 1 hour. Newham et al (1983) used a step test where step height was 46 cm per step with 15 cycles of stepping per minute (1 second step phase). Armstrong et al (1983) ran rats downhill om a 16 degree grade at 16 $m.min^{-1}$ for 90 minutes. Schwane

et al (1983) ran his subjects at 11.7 ± 4.4 km hr^{-1} at grades form 0% to - 10 % for 45 minutes. The common factor for all of these tests is that no justification is given for the use of their protocol. If a quantitative system can be developed whereby certain force levels encountered by muscle groups are sufficient to induce soreness, then the study of POEMS can be facilitated.

Use of hydroxyproline as a marker of skeletal muscle trauma

Introduction

The purpose of this section is to examine the factors relevant to the use of HP and the HP:CR ratio as a marker of skeletal muscle trauma following exercise.

Anatomical location of hydroxyproline

Hydroxyproline is an Amino Acid found only in collagen (Lamport & Northcote, 1960) and in elastin in limits not exceeding 10% of that in collagen (Bently & Hanson, 1969). Collagen is a fibrous protein that provides the framework for salt deposition in calcified tissues and is the skeleton which holds the "soft tissues" together to resist external mechanical forces. It is present in nearly all multicellular animals and constitutes approximately 25% of the total protein within the body (Gould, 1968). On a dry weight basis, collagen represents approximately 60 to 80% of the total weight of tendons (Elliot & Crawford, 1965; Harkness, 1968;

Sjoerdsma, Udenfriend, Keiser, & Leroy, 1965). Since about about one half of the collagen in the body is found in the bones, a large part of the urinary hydroxyproline is probably derived from the catabolism of bone collagen (Dull & Henneman, 1963; Klein, Lafferty, Pearson, & Curtis, 1964)

In normal subjects, urinary free HP, like any free amino acid, has a high tubular maximum, and therefore is reabsorbed almost completely in the renal tubules (Laitenen, Nikkila, & Kivirikko, 1966). Over 95% of HP in the urine is excreted in the peptide bound form, and less than 5% as the free amino acid. Urinary peptide HP represents only about 10% of the HP released during collagen degradation (Kivirikko, 1970).

Chemistry of hydroxyproline

Proline and HP are the 2 amino acids in proteins which contain the pyrrolidine ring. They therefore have the chemical and metabolic properties conferred by a secondary, rather than a primary amino group (Kivirikko, 1970).

The principal isomer of HP in nature is 4-hydroxyl-L-Proline, the form in which the configuration at both carbon 2 (with reference to the C-N bond) and carbon 4 (with reference to the C-O bond) is L (Weast & Astle, 1983:84). This epimer occurs in mammalian collagen at about 80 to 100 residues per thousand residues (Eastoe, 1967).

Collagen also contains small amounts of the position isomer, 3-HP, first demonstrated by Ogle, Arlinghaus, and Logan (1962). The epimer of 3-HP in collagen is 3-erythro-hydroxy-Ls-proline (Irrevere, Morita, Robertson, & Witkop, 1963), configurationally resembling the epimer of 4-HP found in collagen. Other isomers of 4-HP and 3-HP are known to occur only rarely in nature (Adams, 1970), (Appendix J).

Problems in measurement of urinary hydroxyproline

Introduction.

Since nearly all of the HP of the body is found in collagen, the urinary excretion of this amino acid is an important index of collagen metabolism (Ziff, Kibrick, Dresner, & Gubetz, 1956; Sjoerdsma, Davidson, Udenfriend, & Mitoma, 1958). If urinary hydroxyproline arises primarily from body collagen, then at least 3 factors may be expected to influence the amount of HP excreted, and thus measured:

1. The rate of collagen breakdown (Prockop & Kivirikko, 1967;1968). This will determine the balance of HP and HP containing peptides in plasma.
2. The rate at which HP peptides are metabolized to carbon dioxide and urea. This determines what percentage of total HP is left in plasma for clearance by the kidneys.

3. The plasma clearance of HP by the kidneys. This determines the Kidneys' ability to clean the plasma of HP and HP containing peptides.

Thus, the release into plasma, the stability within plasma and the rate of removal from plasma by the kidneys are all important factors for HP determination in urine. These 3 factors shall now be examined.

Release of hydroxyproline into plasma.

Although peptides are the major products from the degradation of other proteins, several observations suggest that the degradation of collagen HP is not complete. About 90% of the HP in collagen breakdown products are liberated as free HP, while about 10% are in the peptide form. (Kivirikko, 1970).

The hydroxylation of ^{14}C-Proline into ^{14}C-HP is a useful procedure to study collagen metabolism. This is because any ^{14}C-Proline measured will give an accurate measure of the rate of ^{14}C-HP formation from ^{14}C-proline and degradation from collagen without interference from exogenous sources. Studies on ^{14}C-Proline indicate that plasma HP is derived both from recently synthesized collagen molecules, which form the so-called soluble collagen of the tissues, and from catabolism of mature insoluble collagen fibres (Lindstedt & Prockop, 1961; Prockop, 1964). Plasma HP peptides do not originate in significant amounts from direct hydroxylation

of free proline, protly tRNA, or metabolic pathways unrelated to the metabolism of collagen. They are derived exclusively form the breakdown of polypeptide chains of collagen (Kivirikko, 1970). The isotopic studies of Stetten (1949) in rats indirectly suggested that most of the free and peptide HP in the body arises from the breakdown of collagen. It has been shown that only minimal amounts of peptides are absorbed during protein digestion (Crane & Neuberger, 1960).

Stability of hydroxyproline in plasma.

One of the problems of using urinary HP as an indicator of collagen metabolism is the rapid degradation of HP in the body. Approximately 90% of the THP released during collagen breakdown is metabolized (Kivirikko, 1970). Ziff et al (1956) stated that the age differences in HP degradation may be related to the decrease of the various soluble collagens in old animals (Kao & McGavrick, 1959) suggesting that urinary HP directly reflects the proportion of body collagen in a soluble form. Free HP and HP in peptides are not used for the synthesis of new collagen (Kivirikko, 1970).

The results of the above studies generally conclude that the daily excretion of HP is largely independent of factors that influence the excretion of other amino acids, and they support the suggestion that urinary HP arises from the breakdown of body collagen.

Plasma clearance of hydroxyproline.

In normal subjects, urinary free HP, like any free amino acid, has a high tubular maximum, and therefore is reabsorbed almost completely in the renal tubules (Laitenen et al, 1966). Over 95% of HP in the urine is excreted in the peptide bound form, and less than 5% as the free amino acid. Urinary peptide HP represents only about 10% of the HP released during collagen degradation (Kivirikko, 1970). These facts suggest that even though greater alterations in the serum HP level may be occurring, the rapid metabolism of the HP and efficient renal handling may be masking these changes. The urine THP depends on the age and the sex of the individual (Laitinen et al, 1966).

Urinary measurement techniques

Since only free HP reacts in assay procedures, urine samples must be initially hydrolyzed to convert peptide bound HP to free HP. Free HP can be assayed directly, and the difference between this and the value obtained after hydrolysis corresponds to the peptide bound HP (Kivirikko, 1970).

Most of the methods used for the determination of THP in urine are based on the methods of Neumann and Logan (1950) or Stegemann (1958). These methods, however, suffer from at least 7 analysis problems (Goidanich, Lenzi, & Silva, 1965; Koevoet, 1965; Neumann & Logan, 1950; Hutterer & Singer,

1960; Borel, Caranjoet, & Jayle, 1967; Firschein & Schill, 1966; Bergman & Loxley, 1970).

There have been a variety of techniques employed to increase the accuracy of measurement of urinary total hydroxyproline. The major problem with these procedures, however, is that they require tedious procedures covering a time span of approximately 48 hours. One procedure is supposed to be rapid, but its results fall, on average, 19% below the actual values (Firschein & Schill, 1966).

Parekh and Jung (1970) developed an improved method for determining urinary THP which incorporates the best features of some of these existing methods. These include acid hydrolysis in sealed tubes; decolorization of hydrolyzate and evaporation of hydrochloric acid; oxidation by Chloramine-T; color reaction with p-dimethyl-aminobenzaldehyde (p-DMAB). This method is valuable in that it is rapid (1 day) and does not sacrifice analytical accuracy.

Effect of diet on urinary hydroxyproline

Diet and free hydroxyproline.

In contrast to the N^{15} studies of Stetten (1949), it has been observed that an increase in excretion of HP will occur when added to the diet of human subjects in the form of gelatin (Ziff et al, 1956; Prockop and Sjoerdsma, 1961). Prockop and Sjoerdsma (1961) found that the excretion of

free HP was potentiated by simultaneous feeding of proline or glutamic acid. Kaminute and Handler (1951) reported similar results with the infusion of other amino acids in dogs. These results are probably due to competition for the same reabsorption mechanism in the renal tubules. These results suggest that under normal conditions, much of the free urinary HP may originate from dietary proteins. However, the large amounts (up to 28 gm) of amino acids necessary to demonstrate the effect make it unlikely that this mechanism normally has much influence on free urinary HP.

Prockop and Kivirikko (1968) noted that although ingestion of large amounts of gelatin was found to increase free urinary HP, its urinary excretion did not decrease when HP was eliminated from the normal diet, or even if subjects were placed on a protein free diet. They further noted that no diurnal variation in free urinary HP existed and also that hydration or dehydration did not alter excretion. When they observed the ingestion of large amounts of HP as free amino acids, they recorded an increase of free, but not of bound HP, the peptide form which accounts for over 95% of the urinary HP (Kivirikko, 1970).

Diet and bound hydroxyproline.

Since less than 0.1% of the HP incorporated into collagen is from the diet (Stetten & Schoenheimer, 1944), the increase of bound HP excretion after ingestion of gelatin

raises the possibility that all the HP peptides absorbed were excreted into the urine. These exogenous peptides may be altered before excretion (Mechanic,Skupp, Safier, & Kilbrick, 1960), suggesting that they are metabolized slowly, if at all.

Diet and total hydroxyproline.

Ziff et al (1956) found that adult patients on diets containing less than 100 mg of alpha-HP excreted 20 to 40 mg of THP per day. This is identical to normal values reported for adult subjects (Laitenen et al, 1966). Similarly, Prockop and Sjoerdsma (1961) found no significant decrease in THP as subjects were changed from a normal to a low HP diet, and then finally to a HP free diet. These results suggest that normal dietary HP is not an important source of THP. However, daily variations in HP excretion tend to be minimized with a gelatin free diet. Consequently, it has been suggested that gelatin free diets should be used in all exact studies on THP (Kivirikko, 1970)

Effect of exercise on urinary hydroxyproline

Documentation on the effects of exercise on the urinary excretion of HP is scarce. Exercise has been shown to accelerate the turnover of collagen and other proteins (Heikkinen & Vuori, 1972). Booth and Tipton (1970) demonstrated that a single bout of exercise had no significant influence on the

hydroxylation of proline in isolated tendons. Abraham (1977) found that the mean HP excretion for all subjects developing muscle soreness did not change significantly from the control day to the second post exercise day. Abraham, however, found a significant rise between the first and second post exercise day. No significant difference was demonstrated in the HP:CR ratio between the control day and either of the post exercise days. The THP:CR and THP were, unfortunately, not measured using a concentric control for an eccentric test group. Abraham (1977) could thus not conclude whether the increase found was due to the exercise itself or due to some manifestation of POEMS. In addition, the muscle groups used to invoke POEMS (m. biceps brachii) were a small percentage of the total muscle mass.

Creatinine determination for hydroxyproline standardization

CR is a waste product of creatine and creatine phosphate, which are almost found solely within muscle. Cr is formed from these compounds at a rate of about 2% per day (Lehninger, p831, 1980). CR is then removed from plasma by glomerular filtration and is excreted into the urine without any reabsorption from the filtrate (Tortora & Anagnostakos, 1978). Hence, all CR filtered from the plasma is excreted in the urine. It is evident that urinary CR values would be representative of total body muscle mass by indicating the degree of catabolism of muscle tissue.

The ratio of HP:CR corrects at least partially for differences in body size between individuals, yielding a narrower range of values than the range of HP alone. This ratio follows a definite pattern: increasing from birth to one month of age, decreasing from 6 months to about 5 years of age, remaining constant to puberty, decreasing again to about age twenty and subsequently remaining constant through age 70. In addition, the HP:CR ratio is essentially the same for both sexes for a given age. (Allison, Walker, & Smith, 1966).

Significance of uric acid in exercise myopathy

Hyperuricemia during exercise and transient overproduction of UA for several days thereafter is derived from UA precursors liberated from skeletal muscle. In 1905, Burian demonstrated that exercise was associated with the appearance of a purine compound in the muscle. Intense exercise was followed by increased production of UA. In 1908, Cathcart, Kennaway and Leathes demonstrated that although UA excretion fell during a single bout of exhausting exercise, it rose to values far in excess of normal for 2 or 3 days afterward.

Knochel, Dotin, and Hamburger (1974), in a study of military recruits, reported a significant rise in UA excretion 4 days after the beginning of training. There was a further increase in UA excretion eleven days post exercise. At this

time, there was also a rise in urinary CR, suggesting skeletal muscle injury. These changes also coincided with muscle pain, tenderness and swelling.

Adenosine monophosphate deaminase (AMP-deaminase) is an ubiquitous allosteric enzyme which catalyzes the irreversible deamination of AMP to IMP and NH_3. It's importance in muscle metabolism is not fully understood, although it is present in higher concentrations in skeletal muscle than in any other tissue (Ronca-Testoni, Raggi, & Ronca, 1970; Lowenstein, 1972). Recent observations suggest that absence of AMP-deaminase may be one cause of POEMS (Fishbein, Armbrustmacher, & Griffin, 1978; DiMauro, Miranda, Hays, Franck, Hoffman, Schoenfeldt, & Singh, 1980).

Although not studied in human muscle, decreased cellular concentration of ATP in the liver activates AMP-deaminase and 5'-nucleotides (Woods, Eggleston, & Krebs, 1970), leading to the formation of inosinic acid and inosine monophosphate (IMP). These substances, in turn, are rapidly converted to hypoxanthine, xanthine and UA. Fast twitch muscle, which is more dependent upon anaerobic glycolysis for its energy needs, contains much higher levels of AMP-deaminase than slow twitch muscle (Winder, Terjung, Baldwin, & Holloszy, 1974) and during tetanus shows a rapid and substantial rise in both IMP and ammonia (NH_3) (Meyer & Terjung, 1979).

The high NH_3 levels generated during vigorous contraction would tend to stimulate glycolysis by activating the rate limiting enzyme phosphofructokinase (Passoneau & Lowry, 1962). Increased levels of IMP may also be sufficient to increase glycogenolysis through non-covalent action of phosphorylase-b (Griffiths & Rahim, 1978; Rahim, Perrett, Lutaya, & Griffiths, 1980). These findings are meaningful since it was contraction which initially led to the high NH_3 levels and the low cellular ATP levels which eventually led to the formation of IMP.

Liberation of adenine nucleotides from injured skeletal muscle could well provide substrate for increased urate production in the liver. That these mechanisms might be responsible for hyperuricemia and increased UA excretion is supported by observations that hyperuricemia is apparent immediately upon completion of exercise Schrier (1970) and to such an extent that neither simple inhibition of urate excretion nor a concentrative change due to extracellular fluid volume contraction would seem to provide a plausible explanation (Knochel et al, 1974).

Summary of factors relevant to POEMS

POEMS is a phenomena which typically occurs 1 to 3 days after intense physical exercise and is localized to myotendinous areas. The exercise is usually of a nature and intensity to which the individual is unaccustomed, but typically

is an exercise involving a great deal of eccentric muscle contractions.

Current research indicates that the torn tissue hypothesis is the most plausible explanation. This has been examined from 2 viewpoints; connective tissue damage and muscle tissue damage.

In regards to connective tissue damage, evidence is primarily biochemical. Research indicates that there is an increased rate of turnover of connective tissue with POEMS, as exemplified by increased urinary HP:CR values. Since HP values standardized to CR are representative of connective tissue catabolism relative to total muscle tissue, they are most representative of what is actually occuring physiologically.

In regards to muscle tissue damage, most evidence is biochemical, although some morphological evidence exists. Morphological examination of muscle tissue with POEMS has indicated disruptions of the Z-band and sarcolemma. Primary evidence lies in the release of UA from skeletal muscle, which is indicative of muscle trauma and myofibrillar degradation respectively. These values are usually in association with CR, indicating the degree of muscle protein catabolism. There is a great deal of literature supporting hyperuricemia following intense exercise sessions. This is attributed to a release of adenine nucleotides from injured skeletal muscle.

Associated energy pathway and hydrolytic enzyme studies provide evidence that some muscle contractile tissue trauma is associated with POEMS. Morphologically, studies provide evidence of myofibrillar band disruption in type II fibres. This is contrary to reports of highest hydrolytic enzyme activity levels in association with type I fibres with POEMS.

Current research does not provide conclusive evidence for either the muscle or connective tissue theories. The common ties are that the torn tissue hypothesis is most probable and that both fibre types are affected, but in a different fashion.

METHODS AND PROCEDURES

Subjects

Overview

Thirty-one normal healthy volunteer subjects, from whom informed consent had been obtained (Appendix A) were used in this study. Fifteen subjects withdrew voluntarily after the first biopsy, leaving a final experimental group of 16 subjects. Twelve of these 16 were male and 4 were female. For the experimental design of the study, all subjects were randomly assigned to either concentric (group C) or eccentric (group E) exercise groups. Of the final total of 16 subjects, 7 were group C and 9 were group E. This inequality of group assignment was due to assignment prior to attrition. All raw descriptive data on the subjects is presented in Appendix L-5.

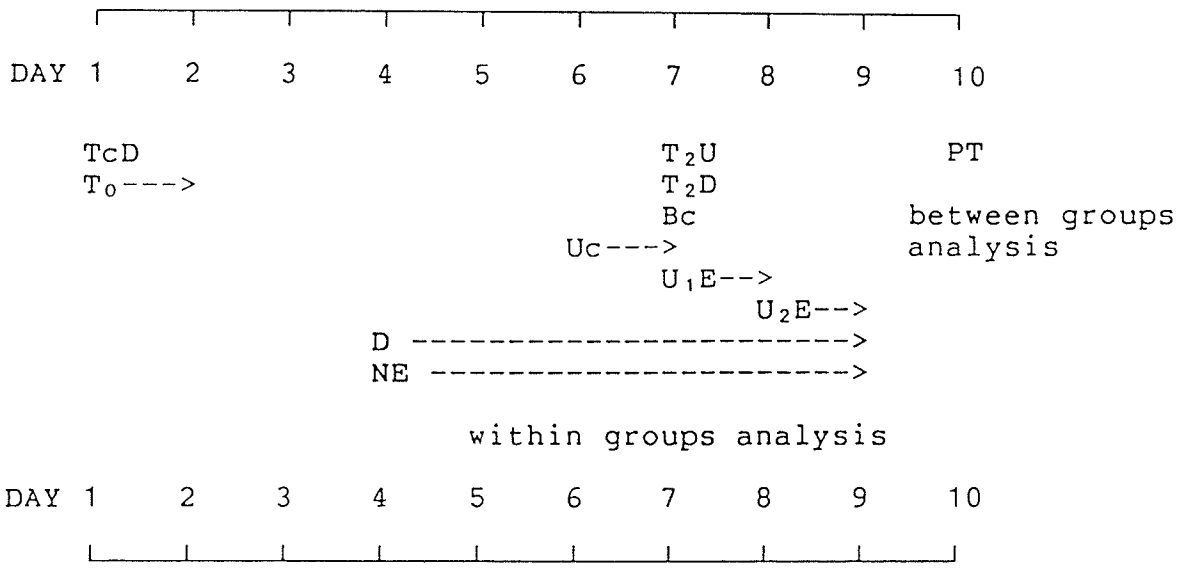
All procedures used in this study conformed to the ethical standards of the University of Manitoba Committee on Research Involving Human Subjects (Appendix K). All subjects signed an informed consent form prior to participating in the study.

Chronology of test occurrence

Introduction

Figure 1 depicts the order of test occurrence. The time lines for test occurrence are depicted in days on the horizontal axis. The following paragraphs examine the sequential order of tests as depicted in Figure 1. A detailed explanation of each variable and its results are discussed elsewhere in this thesis. The purpose of this synopsis is to briefly review the order of test occurrence in the design. The experimental design focuses around the urine collection periods Uc to U_2E. This is indicated on Figure 1 by the within and between group analysis brackets. The remainder of Figure 1 delineates the chronology of events leading up to and after the experimental portions of the thesis.

CHRONOLOGY OF TEST OCCURENCE



LEGEND OF SYMBOLS FOR THE ORDER OF TEST OCCURRENCE

CONTROL MEASURES	EXPERIMENTAL MEASURES
TcD - Eccentric control treadmill test T_0 - Time to peak soreness Uc - Baseline urine sample D - Controlled diet NE - Controlled exercise	T_2U - Experimental (Grp C) treadmill test T_2D - Experimental (Grp E) treadmill test PT - Maximal treadmill (progressive) U_1E - Experimental urine 1 U_2E - Experimental urine 2 Bc - Body composition

Figure 1: Chronology of test occurrence

Control measures

There were a total of 4 control procedures utilized in this design. All subjects performed a baseline downhill treadmill test (TcD) to determine the time frame for the development of peak POEMS (T_0). At least 48 hours following time to peak soreness (T_0), the 2nd and 3rd control measures were implemented for all subjects. These were a special diet and a period of restricted exercise respectively. Each of these were identical in length, beginning 48 hrs prior to the first urine collection phase and ending at the end of the 3rd urine collection phase for an average length of 153 hours.

For the diet restriction, all 16 subjects were required to maintain a HP intake of less than 100 mg per day. All subjects were requested not to consume any jams, jellies, jell-o, soft candy, ice cream or red meat.

For the exercise restriction, all 16 subjects were requested to abstain from participating in any activity which they were not well accustomed to (ie at least 3 times per week).

The 4th control procedure was the baseline urine collection period, (Uc) equivalent in time to T_0 or an average of 35 ± 14 hrs (as were all urine collection phases). This is the only control procedure which falls in the parameters of the experimental design.

Experimental measures

There were a total of 6 experimental procedures utilized in this design. For the experimental measures, the total subject sample was randomly divided into concentric (group C) and eccentric (group E) exercise groups. The experimental measures began immediately following the completion of the baseline urine sample (Uc). All experimental tests were common to all subjects, except for the experimental treadmill test (T_2). Group C subjects performed an uphill test (T_2U), while group E subjects performed a downhill test (T_2D). Both groups exercised for a work time and equivalent HR to that in TcD. Every subject had their body composition (Bc) determined immediately following T_2.

The first experimental urine collection period (U_1E) began when T_2 and Bc were completed and extended for a time period equal to T_0. The second experimental urine collection period (U_2E) commenced at the completion of U_1E and also extended for a time period equal to each individuals T_0.

The 6th and final experimental procedure was the progressive maximal treadmill test (PT), which was performed no sooner than 24 hrs following U_2E.

Data collection

Eccentric control treadmill test

The purpose of this procedure was to determine the time lines for development of POEMS as well as the heart rates at which exercise ceased. POEMS was experimentally induced in the anti gravity musculature of the lower body (in all 16 subjects) by running on a negative grade at a slope of 20%. The speed was 6 mph and all 16 subjects were required to complete a maximum of 1 hour running. All subjects were provided access to fluids. In-test information was collected on heart rates (HR). Post test information was collected up to 4 days post-test on the perceived rating of 3 degrees of muscle soreness (Appendix B).

Consequently, for each subject, the onset and time course of development of the symptoms of POEMS was documented.

Experimental treadmill test

Seven group C subjects (the control group) were required to run uphill. The uphill grade was initially 5% at 3 mph. If this did not produce the desired steady state HR, the grade was increased 1% per minute until the HR was within 10 beats of the desired steady state HR. The grade was then increased by 1% every 5 minutes until the HR equaled the steady state HR from the eccentric control treadmill test. This was to facilitate reaching the desired HR as rapidly as

possible, yet allowing the subject to adjust to the increasing workloads. They worked at this HR for a time not exceeding that exercised in the eccentric control treadmill test.

Nine group E subjects repeated the eccentric control treadmill test. Information was monitored on steady state HR values for both groups C and E to ensure adherence to protocol guidelines. Steady state Vo_2 values were calculated for both groups using a Beckman MMC Horizon cart.

Progressive maximal treadmill test

All 16 subjects completed a final maximum treadmill test for the determination of Vo_2max as described in Appendix C. This value would then be used to calculate the Vo_2, as a percentage of maximum, where subjects ceased working in the experimental treadmill test.

Soreness rating

Following the eccentric control treadmill test, each subject was requested to rate the degree of muscle soreness experienced in their quadriceps muscle. The soreness rating was subjectively based on a 10 point scale (Appendix B) for each of the 3 defined degrees of muscle soreness.

Oxygen uptake determination

Vo_2 was calculated from direct gas analysis during a progressive maximal treadmill test (Appendix C). Analysis was performed on expired gases using a Sensormedics Metabolic Measurement Cart. The Vo_2 was calculated acording to the deviation of each subjects' machine analysed breath to known percentages of O_2 (16.47) and CO_2 (3.48) in the 2 calibrating gases used and the commonly accepted laboratory room air concentrations of CO_2 and O_2 (Appendix D). Breath samples were collected for a minimum of 5 minutes at the end of the experimental treadmill test. For treadmill test 2, the Sensormedics MMC was also programmed to give an analysis summary for every 60 seconds of breath collection. For the maximal treadmill test, breath collection began 2.5 minutes into each exercise stage and lasted until the end of these 4 minute stages for a total breath collection time of 1.5 minutes per stage. The Sensormedics MMC was programmed to give a final summary of the exercise test for every 30 seconds of breath collection.

The Sensormedics MMC system was calibrated with the aforementioned calibrating gases prior to and following each of the tests if the calibration gas check indicated that gas calibration was necessary.

Body composition

All 16 subjects had their percent body fat measured immediately following the experimental treadmill test, as this was a mean time in relation to the urine measurements. This was performed by volumetric weighing according to a technique described by Sloan (1962) and calculated using a modification of the body density formulae derived by Brozek, Grande, Andersen, and Keys (1963) (Appendix E).

Urine collection

Each subject was to collect, measure and save all urine samples for a time period corresponding to T_0, on the following 3 occasions:

1. Starting a mean of 35 hours prior to the second treadmill test. This is the baseline urine sample and is designated Uc;
2. Immediately following the baseline urine sample. This sample is the first experimental sample, reflecting changes in urine parameters for the first 35 hours following the experimental treadmill test. This has been designated U_1E.
3. Immediately following U_1E. This is the second experimental urine sample, reflecting changes in urine parameters for the time between 35 to 70 hours past the experimental exercise session. This has been designated U_2E.

(see Figure 1)

All samples were collected daily and analysed immediately for UA and CR as per the procedures in Appendices G and H respectively. Representative urine samples were frozen for subsequent analysis of HP (Appendix I). This data was stored on urine collection data sheets (Appendix F).

Data analysis

Introduction

The control and experimental variables for this experiment are outlined in Figure 1. The level of significance was set at 0.05 for all statistical procedures. All graphical procedures were derived from a Hewlett Packard 9122 computer and 7475A plotter system.

Determination of normality of data samples

The SAS procedure PROC UNIVARIATE was used in this analysis. The paired comparison t-test was observed in this procedure for all variables. If the value of prob<W was significant (ie. a significance of $p<0.05$), then the population was assumed to not be normal. If not significant (ie. a significance >0.05), then the population was assumed to be normal.

Within groups analysis

This analysis was to seek any intra group differences for each of the two groups of this study (see Figure 1). Within each of the two groups, if the samples were normal, then the 2-way ANOVA test was used as the basis for sample comparison using the SAS PROC NPAR1WAY procedure. Non-normal samples were initially analysed using the non-parametric Kruska-Wallis test to determine differences as a whole. From these, specific inter sample differences were determined using the Wilcoxin 2-sample test.

Between groups procedure

This procedure was used to determine any differences between the 2 groups of this study (see Figure 1). All non-normal values were analysed non-parametrically using the Kruska-Wallis test to determine any differences as a unit. Specific inter group differences were then isolated using the Wilcoxin 2-sample test. Differences were assumed to be significant for Z_0 values >1.96 or < 1.96 with prob> or $<Z_0$ less than 0.05.

The normal variables were compared between groups using the 2-way Anova test.

Measurement validity

Hydroxyproline

Since no external lab validity check could be obtained on the hydroxyproline samples, a check was performed by plotting the absorbance of random samples of: the urine vs blank; blank vs water; and a 1.0 mg standard vs blank; over wavelengths of 420 to 700 nm. Validity of analysis was assumed if the following was observed:

1. Zero absorption of the blank around 580 nm.
2. Peak absorbance of urine sample around 580 nm.
3. Peak absorbance of the standard in conjunction with the sample.

One random sample per experimental procedure was analysed in this manner. All analysed samples adhered to the above 3 criteria. A typical curve from this procedure is depicted in Figure 2.

Creatinine and uric acid

Validity checks were performed for UA and CR by having 2 random urine samples analysed through Unicity lab services of Winnipeg. The values obtained for CR by Unicity labs (21.3 mmol/l & 23.9 mmol/l) corresponded within 4.5% of those obtained by the experimental analysis used in this experiment (21.0 mmol/l & 22.1 mmol/l). The first urine UA

sample analysed in this lab was invalid as the improper cuvet type was utilized in the analysis. After correcting for cuvet type, the second sample validity check for UA by Unicity labs (4.1 mmol/l) corresponded within 1% to those obtained by procedures used in this experiment (4.07 mmol/l).

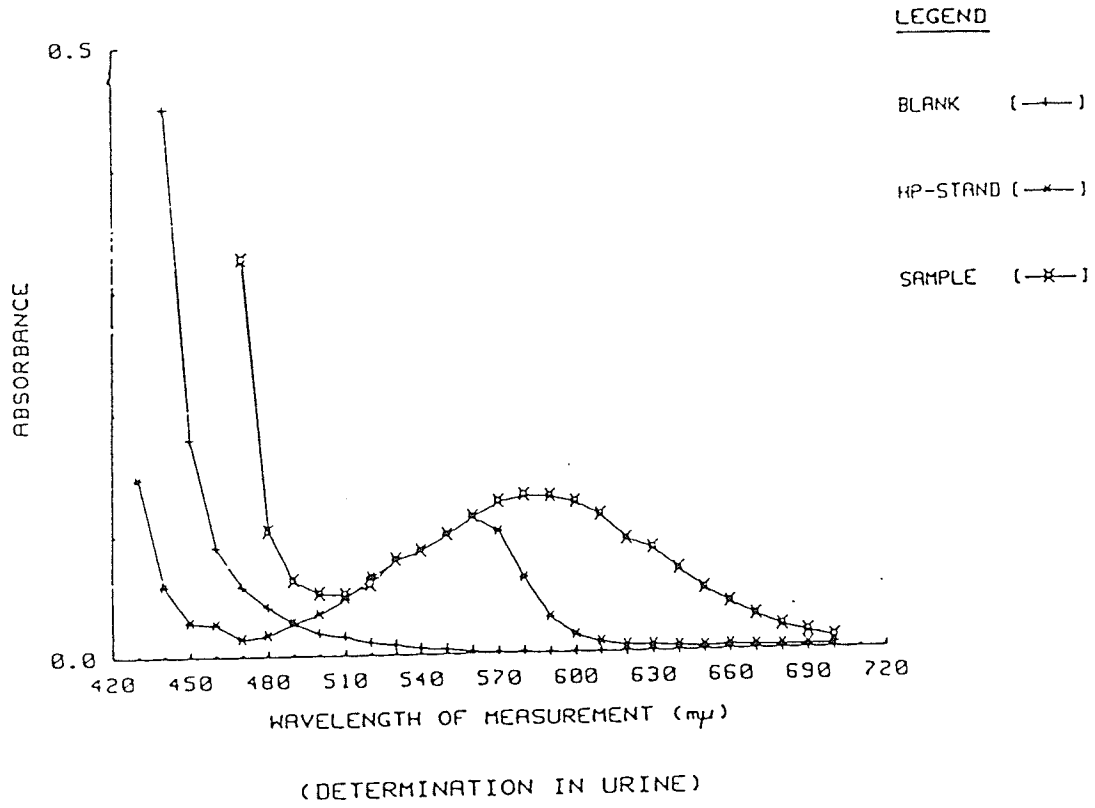


Figure 2: Standard colorimetric response of hydroxyproline

RESULTS AND DISCUSSION

Subjects

Physical characteristics

A physical description of each subject, by group, for age and weight, is presented in Table 1. There were no significant differences between group C and group E in age. Males and females differed significantly ($p<0.05$) in weight (84.6 ± 13.0 kg vs 55.3 ± 3.0 kg). All subjects ranged in age from 20 to 32 years of age.

None of the subjects were accustomed to the eccentric exercise demands of downhill treadmill running. Sex was not considered a limiting factor since the measured variables are expressed by two methods which normalize for the percentage of LBM. These methods are a ratio of urine HP and UA to total LBM and to CR (an indicator of LBM).

TABLE 1

Physical descriptors of all subject groups

Variable	Group	Mean	S.D.
Age (yrs)			
	Concentric	24.9	4.6
	Eccentric	24.3	1.8
	All Ss	24.6	3.2
Weight (kg)			
	Concentric	76.7	23.4
	Eccentric	77.3	12.1
	All Ss	77.3	17.3
% Fat			
	Concentric	15.9	3.6
	Eccentric	16.6	5.9
	All Ss	16.3	4.9
Lean mass (kg)			
	Concentric	64.3	18.9
	Eccentric	63.9	6.8
	All Ss	64.1	12.9
Lbm/weight (%)			
	Concentric	84.1	3.5
	Eccentric	83.0	6.7
	All Ss	83.5	5.4

Eccentric control treadmill test

Introduction

There were 2 purposes to this test. One purpose was to determine the individual time frames for the development of POEMS. The other purpose was to determine HR and time lines of exercise for repetition in the experimental treadmill test. The protocol for this test was to run subjects on a negative treadmill grade of 20% at 6mph for a maximum of 1 hour (hr) All data for this test is outlined in Appendix L. All data for this test is summarized in Tables 2, 3, and 4.

Results

Work time.

All data on work time for the eccentric control treadmill test is presented in Appendix N-2a and is summarized in Table 2.

There were no significant differences between groups C and E in relation to work time. The only study on POEMS to use a similar protocol was Abraham (1977), who used a protocol of 3 mph at -20% on 1 subject who did not develop soreness using an initial step test protocol. His test speed was such that the subject could walk the whole test. The protocol utilized in the present investigation was more severe as the subjects were exercised at 6 mph. This severity of ex-

ercise is related to most subjects not completing the 1 hr exercise period.

TABLE 2

Heart rate and work time for maximal eccentric aerobic work

Variable	Group	Mean	S.D.
Work time (min:sec)			
	Concentric	46:31	12:03
	Eccentric	39:26	13:27
	All Ss	42:34	21:39
Heart rate (bpm)			
	Concentric	144.8	14.2
	Eccentric	148.7	23.6
	All Ss	146.4	19.5

Using a similar protocol, Schwane et al (1983) ran subjects intermittently for a total exercise time of 45 minutes. Each subject performed 9 bouts of 5 minute runs interspaced by 2 minute rest periods at -10%. The average treadmill speed was given as 7.31 ± 0.25 mph. No range of treadmill speeds was given. Although Schwanes' protocol involved greater speed and 10% less negative grade than the current investigation, all subjects completed the required work loads.

One advantage of the current protocol over the two previously cited ones is that subjects were worked to total eccentric fatigue. This is reflected in that only 4 of the 16 subjects completed the maximum work time of 1 hr (Appendix L-2a). If the protocol enabled all subjects to complete the entire session, it would be likely that the protocol was not severe enough to produce the desired level of POEMS. No investigations to date have utilized an identical protocol. Therefore, it is not possible to state whether the obtained work times are normal.

Heart rates.

There were no significant differences between group C and E in relation to steady state submaximal HR (Appendix L-2b). No differences between the two groups were expected as they both were subjected to the same exercise protocol.

Soreness development.

All data on soreness rating is presented in Appendix L-1 and is summarized in Table 3.

All subjects rated the degree of soreness experienced following the eccentric control treadmill test in relation to 3 degrees of soreness (Appendix B), where degree I is soreness on movement; degree II is soreness to touch, and degree III is ache soreness. A level of soreness was assumed if the subjects had ratings greater than 5. The raw data with times for the ratings are presented in Appendix L-1.

In all but 2 cases, the subjects had identical order of ranking for the degree of soreness (ie. degree I 24 hrs; degree II 30 hrs; degree III no ranking). Note that the mean time for soreness degree III had no observations with a ranking of 5 or

better

TABLE 3

Effect of maximal eccentric aerobic work on degree of POEMS

Soreness Degree	Soretime(hours) (x ± S.D.)	n
Movement (degree I)	35 ± 14	16
Touch (degree II)	40 ± 19	16
Ache (degree III)	*****	16

***** no significant soreness rating.

Soreness upon movement (degree I POEMS) occurred for all subjects from 20 to 63 hrs following eccentric exercise with a mean of 35 ± 14 hrs. Group C subjects ranged in peak soreness from 20 to 60 hrs with a mean of 37 ± 15 hrs. Group E subjects ranged in peak degree I POEMS from 20 to 63 hrs with a mean of 33 ± 14 hrs. These ratings reflect the timelines of soreness experienced after TcD.

Soreness in response to touch was also documented in this investigation. For all subjects it ranged from 10 hrs to 72 hrs with a mean of 40 ± 19 hrs. Group C subjects ranged in peak touch soreness from 21 to 72 hrs with a mean of 47 ± 17 hrs. Group E subjects ranged in peak touch soreness from 10 to 72 hrs with a mean of 35 ± 20 hrs. There was no documentation of degree III POEMS in this investigation.

There were no significant differences between group C and E for either degree I or degree II POEMS. Degree I and degree II mean soretimes were not significantly different from each other when expressed as a total sample or as by individual groups. This absense of a difference between groups was expected as both groups were subjected to the same exercise protocol.

Schwane et al (1983) using an intermittent negative treadmill exercise protocol, found maximal soreness in the quadriceps muscle groups 24 hrs post exercise. This maximal soreness rating was for a classification similar to the de-

gree I soreness of this investigation. It was also similar in time of development for that reported here for degree I soreness. Although the classification scheme is similar, the exercise protocol was one which may not have induced maximal eccentric contractions. This could explain the earlier reported maximal soretimes in those investigations.

In a study using a similar protocol to that used in the present investigation, Abraham, (1977) reported maximal POEMS in the gastrocnemia 48 hrs following exercise cessation. The time of maximal soreness reported by Abraham is similar to the results recorded here for degree I POEMS (35 ± 14 hrs) and degree II POEMS (40 ± 19 hrs) for all subjects. However, the degree of POEMS in Abrahams' subjects was not reported. Hence, no comparison can be made of maximal soreness related to degree of soreness.

Although no other studies have attached time lines for development of POEMS with the degree of soreness, some studies have reported similar time lines for "general" POEMS at 48 hrs post exercise (Newham et al, 1983; Tiidus & Ianuzzo, 1983). Newham et al (1983) induced POEMS using a step test for 15 or 20 min on a 46 cm step at a rate of 15 cycles/min. Tiidus and Ianuzzo (1983) induced POEMS using concentric and eccentric muscle contractions with a dynamic leg extension apparatus. As with the other studies cited, these protocols did not elicit maximal eccentric contractions and hence the

values reported by them are not representative of the values reported for this investigation.

Since all subjects developed POEMS after test 1, and since the level of eccentric exercise was considered maximal, the HR and Vo_2 values in test 2 can be considered representative of a threshold of work necessary for the development of POEMS. This is, of course, assuming that an earlier cessation of exercise (and hence lower HR and Vo_2 values) would not have resulted in the same degree of POEMS.

Experimental treadmill test

Introduction

There were two protocols for this test. The protocol for group E was essentially the protocol for the eccentric control treadmill test. The protocol for group C involved walking uphill from an initial grade of 5% and 3 mph to a final grade which would elicit the same steady state HR as in the eccentric control treadmill test. Each group performed this test for the identical time performed in the eccentric control treadmill test. All data for this test is listed in Appendix L-3.

Results

Work time. By nature of design, the time each subject worked was identical to that of the eccentric control treadmill test. The work times between groups C and E were not significantly different.

Heart rates.

By nature of design, the heart rates elicited by each subject were identical to those of the eccentric control treadmill test. Hence, there were no significant differences between groups C and E for heart rates.

When expressed as a percentage of maximal (progressive maximal treadmill test) HR (Appendix L-4b), there were no significant differences between groups C and E. These results are summarized in Table 4. These results were expected since, as already discussed, they are representative of the percent of maximal HR where POEMS will be elicited. This is supported by Schwane et al (1983) who noted a HR(%max) for a protocol inducing POEMS of 77 ± 2%. This is virtually identical to the results found here (77.1 ± 10.5 %).

TABLE 4

Physiological reponse to maximal eccentric aerobic exercise

Variable	Group	Mean	S.D.
Heart rate (%max)			
	Concentric	77.0	9.5
	Eccentric	77.1	11.7
	All Ss	77.1	10.5
Vo_2 (ml kg^{-1} min^{-1})			
	Concentric	29.9	7.0
	Eccentric	26.4	5.9
	All Ss	27.9	6.5
Vo_2 (%max)			
	Concentric	57.9	12.3
	Eccentric	51.8	15.8
	All Ss	55.3	14.6

Soreness development.

Time for soreness development was not documented for this test. However, all subjects were verbally questionned as to their sensation of POEMS following this test in relation to the eccentric control treadmill test. All group C subjects reported no evidence of POEMS. All group E subjects reported a development and severity of POEMS along similar time lines as in the eccentric control treadmill test.

Oxygen uptake.

Oxygen uptake (Vo_2) was measured in this test to calculate the percentage of maximal oxygen consumption (Vo_2max) where exercise ceased with documentation of POEMS. All data is presented in Appendix L-3a and is summarized in Table 4. There were no significant differences between groups C and E for Vo_2.

Since Vo_2 typically increases linearly with HR (Mathews and Fox, 1976), it is not surprising that the Vo_2 values obtained in this investigation are similar. This is because the protocols were based on the HR from the eccentric control treadmill test. Therefore, these values (29.9 ± 7.0 ml kg^{-1} min^{-1} for group C; 26.4 ± 5.9 ml kg^{-1} min^{-1} for group E) are representative of the Vo_2 required to induce POEMS. This is substantiated in that the total subject mean value of 27.9 ± 6.5 ml kg^{-1} min^{-1} is quite similar to the eccen-

tric submaximal Vo_2 (to induce POEMS) reported at 31.1 ± 1.6 ml kg^{-1} min^{-1} by Schwane et al (1983).

Abraham utilized a protocol similar to the present investigation but unfortunately did not report submaximal Vo_2 values from eccentric exercise. Watrous et al (1983) reported submaximal Vo_2 (%max), but did not list the actual submaximal Vo_2 values found. Therefore, no comparison can be made with the present study.

Even though eccentric exercise is thought to cost less metabolically for a given level of work (Knuttgen & Klausen, 1971; Davies & Barnes, 1972b; 1972b) it does not appear to differ metabolically from concentric exercise in this investigation when exercise is matched for HR. This is demonstrated through the non significance between the Vo_2 of groups C an E when matched for HR.

The Vo_2 from the experimental treadmill test was also expressed as a fraction of Vo_2max, (progressive maximal treadmill test). These values are presented in appendix L-4c and are summarized in Table 4. There were no significant differences between groups C and E for Vo_2(%max). This was expected since, as previously discussed, these values are representative of the Vo_2 (%max) where POEMS will be elicited. This is supported by investigations such as Schwane et al(1983) who reported a Vo_2 (%max) of 57 ± 2%. This is similar to the total group mean of this investigation of 55.3 ±

14.6 and almost identical to the group E mean of 57.9 ± 12.3%.

Treadmill grade of group C.

Test 2 involved variations in grade from 5% (starting grade) to a maximum of 20 % with a mean of 12 ± 5% grade. These variations in grade are due to both the natural variations in fitness levels and the differences in steady state heart rates from the eccentric control treadmill test. Hence, different grades produce different HR and Vo_2 responses amongst the subjects at the set speed of 3.0 mph. This variation was expected since each person had different steady state heart rates from the eccentric control test.

Progressive maximal treadmill test

Introduction

This test was performed so as to ascertain each individuals aerobic level of fitness. It was also performed so as to enable calculation of VO_2 (%max) and HR (%max) which subjects were working at during the experimental treadmill test. The protocol for this test is outlined in appendix C. All data for this test are tabulated in appendix L-4.

Results

Work time.

Treadmill work time for all subjects are presented in appendix L-4a and are summarized in Table 5. There were no significant differences between group C and E for total work time (appendix L-4a). No norm values on work time for the current exercise protocol exist in the literature.

Heart rate data.

The heart rates for the total sample are presented in appendix L-4b and are summarized in Table 5. There were no significant differences between groups C and E for maximal HR. These results are similar to what one would predict based on theoretical maximum HR (220 minus age). Since the mean age of all subjects is 24.3 yrs, the mean theoretical maximal HR should be 196 bpm. This discrepancy between theoretical maximum and actual HR can be attributed to the greater Vo_2max and hence aerobic fitness level of these subjects. It is known that a decrease in maximal HR occurs in trained individuals (Saltin & Astrand, 1967).

TABLE 5

Physiological response to progressive maximal work

Variable	Group	Mean	S.D.
Work time (min:sec)			
	Concentric	20:49	4:39
	Eccentric	21:01	5:07
	All Ss	20:56	4:44
Heart rate (bpm)			
	Concentric	188.1	8.9
	Eccentric	191.3	6.3
	All Ss	189.9	7.5
Vo_2 max (ml kg^{-1} min^{-1})			
	Concentric	50.4	9.4
	Eccentric	52.7	9.5
	All Ss	51.7	9.2

Maximal oxygen uptake.

Individual Vo_2max values, expressed relative to body weight, are presented in appendix L-4c and are summarized in Table 5. There were no significant differences between group C and group E in terms of either absolute Vo_2max or Vo_2 (%max) The current Vo_2 values are higher than those reported in other investigations for persons in similar age categories.

Body composition

Introduction

The purpose of this test was to determine percent body fat and lean body mass (lbm). Lbm was subsequently used in standardizing urine values, eliminating any sex related differences due to body composition. All calculations used for percent body fat and lbm determination are listed in appendix E. All individual results for this section are listed in appendix L-5b and are summarized in Table 1.

Results

Percent body fat.

All body fat results are listed in appendix L-5b and are summarized in Table 1. These values are similar to 15% body fat reported for non-athlete college males (Fox, 1979).

There were no significant differences between groups C and E for percent body fat. There were no significant differences between males and females for body fat (16.9 ± 5.2 vs 14.6 ± 3.8 % respectively).

Lean body mass.

All LBM results are listed in appendix L-5b and are summarized in Table 1. Fox(1979) reports the average fat free weight of college males as about 85% of their total weight. In this investigation, 85% corresponds to 65.2 kg for group C and 65.7 kg for group E subjects. These values agree with the lbm values suggested as normal.

There were no significant differences between groups C and E for lbm. Male and female subjects differed significantly ($P<0.05$) in lbm (69.8 ± 9.3 kg vs 47.3 ± 3.8 kg respectively). However, when lbm was expressed as a percentage of total body mass, there were no significant differences between males and females (82.9 ± 5.8 vs 85.5 ± 3.7 %) respectively.

Urine measurements

Introduction

The purpose of this test was to examine several urine parameters and note any relationship between them and the time of degree I POEMS. Urine CR, HP and UA values were calculat-

ed from each of three urine sample taken from each subject. All data is recorded in appendix L-6.

Normality of urine parameters

Table 6 lists the non-normal and normal variables. It also lists the statistical extent to which the non-normal variables are non-normal.

TABLE 6

Experimental variables and their degree of normality

VARIABLE	NON NORMAL	NORMAL
total mg HP Uc	**	
total mg HP U_1E	*	
total mg HP U_2E		X
total mg UA Uc	*	
total mg UA U_1E	*	
total mg UA U_2E		X
total mg CR Uc	**	
total mg CR U_1E	*	
total mg CR U_2E	*	
mg % HP:CR Uc		X
mg % HP:CR U_1E	**	
mg % HP:CR U_2E	**	
total mg HP:LBM Uc	**	
total mg HP:LBM U_1E	*	
total mg HP:LBM U_2E	*	
total mg UA:LBM Uc		X
total mg UA:LBM U_1E	**	
total mg UA:LBM U_2E	**	
Urine volume Uc		X
Urine volume U_1E	**	
Urine volume U_2E	**	

* $p<0.01$ level of significance.

** $p<0.05$ level of significance.

X normal populations

Urine volumes

The descriptive statistics on urine volumes for all 3 urine samples are presented in Table 7. There were significant differences within Group E for urine volumes 1 and 3 ($p<0.05$). There were no significant differences in urine volume within group C.

Normal urine excretion is in the range of 1 l/day (Ganong, 1973). Since the time for each urine collection period is, on average, 35 hrs (appendix N-6a), the values in this investigation were expected to be slightly higher than normal. It appears that urine volume 3 is a larger than normal volume. This likely is due to the small sample size and resultant large skewness of the data. It undoubtedly counts for the difference within Group E for urine samples 1 and 3 noted above.

TABLE 7

Urine biochemical response to concentric & eccentric exercise

Group	Urine sample					
	Control		Experimental 1		Experimental 2	
	X	S.D.	X	S.D	X	S.D.
Urine volume (l)						
Concentric	1.23	0.69	1.13	0.49	1.33	0.53
Eccentric	1.00*	0.36	1.39	0.57	1.64*	0.57
Hydroxyproline (total mg)						
Concentric	26.7	16.0	28.5	26.9	20.6	14.8
Eccentric	19.8	19.7	22.5	10.9	28.3	15.9
Creatinine (total mg)						
Concentric	15.50	6.25	14.16	5.36	16.46	7.73
Eccentric	15.28	12.36	23.90	17.53	25.81	19.55
Uric acid (total mg)						
Concentric	700.8	252.2x	689.1	253.4	860.1	586.9
Eccentric *	363.3**	188.7x	899.8**	551.7	*1058.5	615.3
Hydroxyproline:creatinine (mg%)						
Concentric *	.016*	.013	.016*	.012	*.014	.008
Eccentric	.013	.008	.011	.007	.014	.012

Within groups difference *p<0.05
**p<0.01
Between groups difference xp<0.05

Note: Within group relationships are indicated according to which side of the sample mean the indicators are located. Two samples with indicators to the right of the mean have the indicated relationship and conversely to the left.

Concentric group analysis

Introduction.

This section deals with the analysis of the individual parameters from each urine sample. All descriptive data on group C urine samples are presented in appendix L-6 and summarized in Table 7.

The degree of normality of a particular urine parameter determines which subsequent statistical procedure was to be followed for within (and between) group analysis. Since the values listed in Table 7 all follow a normal approximation, the 2-way Anova test was used for analysis as it has as a basic assumption of normality for accurate analysis.

Hydroxyproline and creatinine.

All raw CR data is presented in appendix L-6c and is summarized in table 7. The values in Table 7 are similar to 24 hr norm values reported by DiGiorgio (1974) at 9.7 to 24.7 mmoles/24hrs.

All raw HP data is presented in appendix L-6b and is summarized in table 7. Laitinen et al (1966) suggest that the 24 hr normal HP for a person aged 22 to 40 yrs as 15.1 to 42.4 mg. The values in the present investigation all are within this range and can thus be considered normal.

The HP/CR desriptive data are presented in Table 7 and are summarized in appendix L-6e. The only study to provide norm values for HP:CR were Allison et al (1966). They state that a normal mg% ratio of HP:CR from 20 to 76 yrs of age is .026 ± .001 mg%. All the group C values of this investigation (within their respective deviations), agree with this value. For persons ranging in age from 18 to 45, Allison et al (1966) reported values ranging from .018 to .034 mg%. The total sample used in the study by Allison et al consisted of 21 persons aged 18 or greater. Hence, the small sample used may have resulted in a skewness of the obtained results. The same problem exists in this study. Due to the small sample size, a large skewness is likely present which may not allow a true representation of findings.

It is interesting to note that the relative mg% ratio of HP to CR is significantly different between Uc and U_2E as well as samples U_1E and U_2E, but not samples Uc and U_1E. (for group C). This indicates that significant changes are occurring, but not until a mean of 37 to 74 hours following cessation of exercise. The major changes thus occur immediately following peak soreness.

These results are in definite contradiction to the one past investigation on HP and CR as related to POEMS. Abraham (1977) noted an increased HP:CR ratio following exercise in a time equivalent to T_0 In Abraham's investigation, this

time period was about 24 hours and was, by definition, the time from the exercise cessation to the time of peak soreness. In this investigation, and for the sample group C in question here, this time to peak degree I POEMS (determined from the eccentric control treadmill test) ranged from 20 to 60 hours with a mean and standard deviation of 37 ± 15 hours. In this investigation, no such increase in HP:CR was noted in this time period. Of note however, is that the subject group with significant HP:CR values was group C and, by design, did not experience muscle soreness at any time following the activity of the experimental treadmill test. In addition, the HP:CR ratio increased from both Uc to U_2E, and from U_1E to U_2E, but not significantly between Uc and U_1E. The major urinary changes, therefore, were not immediately apparent following exercise. Consequently, the delayed onset nature of the urinary HP:CR increase may just be a normal response to muscle working concentrically.

There are a few plausable explanations as to why the other investigation of this nature (Abraham, 1977) found significant elevated HP:CR values in eccentrically exercised subjects and this investigation did not.

Firstly, in Abrahams' investigations, several exercise protocols were used, including downhill treadmill walking; bench presses; arm curls; and a step test. In all protocols but the treadmill walking, subjects worked both eccentrical-

ly and concentrically. Consequently, it is not evident how Abraham could conclude the HP:CR increase was related to POEMS, when the values could be attributed to either the concentric or eccentric portions of the exercise.

Secondly, Abrahams' study utilized a subject pool with a age span of 22 years vs one of 12 years in the current investigation (appendix L-5a). Since urine HP values depend on the age of the subject (Laitenen et al, 1966), a small age span would be most beneficial to reduce the chance of differences due to age alone. This is especially so when there is such a small sample involved.

The inconsistency of the experimental protocol in Abraham's study leads one to doubt the accuracy of his results. No mention was made of his subjects history of eccentric exercise or as to whether their exercise levels were controlled during the experiment. The present investigation has controlled for these factors by the use of 1 protocol, distinguishing concentric from eccentric exercise, and utilizing large body musculature. In addition, this investigations activity history indicated no training adaptation of a nature similar to the exercise protocol used to induce soreness. The subjects activity throughout the testing period was also controlled for in the present investigation.

In this investigation, the nature of the exercise protocol (downhill treadmill running) utilized greater than 50 %

of the total body musculature. All group C subjects reported soreness in the anti-gravity musculature of the lower body. This total use of the lower body antigravity musculature ensured that any changes in urinary values could be reflected with less trauma and/or metabolic change (theoretically) of the muscles in question than would be necessary with a small muscle group such as biceps brachii or gastrocnemius, hence facilitating an ethical protocol. The nature of the exercise enabled the investigator to be certain that these anti-gravity muscles were working almost entirely eccentrically.

Since HP is found only in collagen (Lamport & Northcote, 1960) and since approximately one half of the collagen in the body is found in the bones (Dull & Henneman, 1963; Lafferty et al, 1964) it seems reasonable that the exercise stimulus on the bones would be responsible for a significant level of the urinary HP. There is, however, not yet any documentation on the effect of exercise on HP metabolism in bone.

It is possible that the lower level of myosin:actin interaction during eccentric contractions may not have been great enough to initiate the metabolic machinery necessary to result in an increased rate of turnover of HP. In addition, it is possible that increased HP:CR values just reflect increased collagen synthesis. This is possible as Goldberg, Etlinger, Goldspink and Jablecki (1975) demon-

strated that rat muscle can produce a 50% greater collagen content in 6 days. Perhaps this phenomena of an increased urinary HP:CR ratio is not so much an indicator of muscular trauma per se, but is a normal response of a concentrically working muscle that has been subjected to a limit of aerobic work (such as 55% Vo_2max or 77% max HR).

Uric acid.

The group C data on urine UA are summarized in Table 7 and are presented in appendix L-6d. Normal urine UA values for 24 hrs have been reported at 250 to 750 mg (Fiereck, 1970). The mean values found here can all be considered normal except for U_2E. However, the longer urine collection period of this investigation (x = 37 hrs) would likely account for this discrepancy. It is apparent, however, that increased urine UA is associated with various forms of concentric exercise (Burian, 1905; Cathcart et al, 1908) and usually severe exercise (Levine, 1924). Since this protocol was not severe (77% of max HR) and 50.4 % of Vo_2max is not taxing, significant urine UA changes pre/post exercise were not expected. Since there were no significant findings for UA for group C subjects, the results here were to be expected.

Eccentric group analysis

Introduction.

The significant differences for samples within Group E are presented in Table 7. One subject failed to collect U_2E. As a result, all calculations using U_2E were made with n-1 (8) subjects from group E. The statistical analysis format used was the same as that for group C.

Hydroxyproline and creatinine.

The results of HP in group E urine samples are summarized in Table 7 and are presented in appendix L-6b. These mean values all fall within the normal range of urine HP values suggested by Laitinen et al (1966).

The data on Group E urine CR results are summarized in Table 7 and are presented in appendix L-6c. These values are similar to those suggested as normal by DiGiorgio (1974). U_2E had CR values slightly higher than those considered normal (25.81 vs 24.70). This is likely due to the longer period of urine collection for group E (x = 37hrs) in relation to the 24 hrs from which DiGiorgios' value is derived.

The data on all group E HP/CR urine results are presented in Table 7 and are summarized in appendix L-6e. These values are slightly lower than those presented by Allison et al

(1966) and as already discussed. Due to the small sample size of both Allisons' and this current study, the resultant skewness may present misleading results.

No significant differences could be found between samples for either HP, CR, or HP/CR. As per the group C discussion on HP, CR and HP/CR, these results are opposite those found by other research of this nature (Abraham, 1977). The possible reasons for this difference have already been discussed.

Uric acid.

The data on urine UA for group E subjects are summarized in Table 7 and are presented in appendix L-6d. It is evident that sample 2 and 3 represent non-normal concentrations of UA for their time period. This is in relation to the normal values of 250 to 750 mg/24 hr presented by Fiereck (1970). Some of this difference may be due to the longer urine collection period of this investigation. However, it is unlikely that such a discrepancy would be due to the time period alone.

Table 7 presents the significant differences between urine samples analysed in group E. For this analysis, it is evident that all significant changes between samples are related to UA. Urine UA values changed significantly from sample 1 to sample 2 as well as from sample 1 to sample 3. Since there was no significant change from sample 2 to sam-

ple 3, it is evident that the significant change from sample 1 to sample 3 is primarily attributable to the change between sample 1 and sample 2. By observing this in relation to what "normal" urine 24 hr UA values are, it becomes evident that Uc is a normal sample. In contrast, U_1E and U_2E are non-normal samples. These findings indicate that with a maximal eccentric running protocol such as that used here, UA values increase at betweeen 37 and 74 as well as between 74 and 111 hours post exercise. Samples were not taken past this time. Consequently, further rises in UA excretion could not be noted. Knochel et al (1974) reported maximal UA excretion at 4 and 11 days post exercise.

In the study by Knochel et al (1974), UA excretion per 24 hrs was reported greatest at 11 days post exercise, with a value of 1031.0 mg. This is similar to the value presented here, except that this investigation found the mean excretion of 1058.5 mg/35 hrs between 74 and 111 hrs post exercise. Urine UA values are usually associated with severe exercise (Levine et al, 1924). These findings of hyperuricemia may be attributable to muscle trauma from the eccentric exercise demands of this experimental group. Thus, liberation of adenine nucleotides from traumatized skeletal muscle provides the substrate for increased UA production in the liver with a concomitant observation of acute hyperuricemia. Since eccentric exercise is well known to be traumatic to muscle tissue (Curtin & Davies, 1973), it is not surprising to find increased UA levels with this protocol.

Concentric:eccentric relationships

Introduction.

This analysis was performed to see if the response of the urine parameters in question differed according to the type of exercise. The Kruska-Wallis test was initially performed to acknowledge any general between group differences. These results are presented in Table 7.

Hydroxyproline and creatinine.

No significant differences between HP and CR could be found between groups C and E for any sampling period. This is when expressed individually or as a ratio of HP:CR. To date, no investigations have differentiated findings of HP:CR to eccentric/concentric exercise differences. These findings support the notion that urine HP:CR values are not significantly different after either concentric or eccentric exercise.

Uric acid.

Uric acid values between groups C and E are significantly different.

As Table 7 illustrates, the main difference between the concentric (group C) and eccentric (Group E) exercised groups is in the degree of hyperuricemia. By referring to the values of UA in the discussion on group E, it is evident

that hyperuricemia is most prevalent after the peak degree I POEMS has passed. Consequently, the differences between Uc and U_1E are due to differences between a normal Uc and an experimentally derived sample. These results confirm previous findings of post exercise hyperuricemia (Schrier, 1970; Knochel et al, 1974) but demonstrate 2 new findings. These are firstly that hyperuricemia is more prevalent amongst eccentrically exercised subjects than in concentrically exercised subjects at a given metabolic exercise load. Secondly, with an eccentric exercise bout of great enough intensity to induce POEMS, the post exercise hyperuricemia is most prevalent beginning at the time for peak degree I muscle soreness, and increasing in severity for at least 70 hrs following this time period from exercise cessation to occurrance of degree I muscular soreness.

SUMMARY AND CONCLUSIONS

The urine chemical response of 16 subjects subjected to POEMS has been studied here. Nine subjects acted as controls. The experimental and control groups were designated according to respective eccentric and concentric exercise protocols. All exercise protocols were conducted using a negative/positive adaptable treadmill. Urine samples for both experimental groups were collected in 3 equivalent time periods. Each time period was established individually as the time for each subjects development of maximal POEMS following an initial negative treadmill test.

Within the limitations of the experimental protocol, the following conclusions appear to be justified:

1. POEMS (or LFFS) can now be classified in order of 3 degrees, each degree increasing in severity and being time related.
2. The metabolic cost of eccentric and concentric treadmill exericise does not differ when exercise protocols are matched for HR.
3. Hyperuricemia is more prevalent amongst eccentrically exercised subjects than concentrically exercised subjects at a given metabolic exercise level.

4. With an eccentric exercise bout of great enough intensity to induce POEMS, the post exercise hyperuricemia is prevalent from the occurance of degree I POEMS and increases for at least 3 days after.
5. The phenomena of an increased urinary HP:CR ratio is not an indicator of POEMS, but is a normal response of a concentrically working muscle.
6. There is no difference in urinary HP:CR values between concentric and eccentric exercise when matched for metabolic load.
7. Eccentric exercise is not enough of a stimulus to metabolism of human skeletal muscle to result in significant urinary levels of HP and CR.
8. POEMS is apparently induced when working levels of eccentric exercise reach a mean steady state Vo_2 of 55 % of maximum.
9. POEMS is elicited when eccentrically induced steady state HR reach a mean value of 77% of maximum HR.

Based on the experience of this investigation, a number of recommendations can be made for further research into the phenomenon of POEMS.

1. An investigation should be conducted into the effects of exercise on the metabolism of HP in bone and the possible relationship/confusion of bone "ache" and POEMS.

2. A study should be conducted to compare the results of POEMS induced by aerobic activity vs POEMS induced by anaerobic activity. This applies to all parameters of sorenesas investigation, including morphology and metabolic excretory products.

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Appendix A

INFORMED CONSENT FORM

Purpose of the study

There are 3 major purposes to this study. These are:

1. To morphologically examine human skeletal muscle in which soreness has been experimentally induced. The tissue will be examined to determine the nature as well as the possible mechanisms of injury.
2. To qualitatively analyse human urine under conditions of experimentally induced muscle soreness to determine the content of hydroxyproline, uric acid, and creatinine, all markers of cellular stress in muscle.
3. To determine any relation between the qualitative aspects of this investigation (2) and the amount of lean body mass.

Explanation of the tests

Introduction

To most easily understand the following procedures, it is recommended that you follow along on the attached Experimental Design Time-Line Chart and its associated legend.

Muscle soreness test

Control treadmill test.

You will be asked to run downhill on a treadmill at a grade of 20% and a speed of 6 mph (10 minute miles). You can anticipate this test lasting from 30 minutes to one hour. Total fatigue does not usually occur in this test. Like all tests in this investigation, you are free to stop at any time you wish. You will be provided with free access to apple juice and water throughout this test.

Approximately 24 hours following this test, you will likely experience soreness in both of your legs. You will be provided with a rating sheet which asks you to rate the degree of soreness experienced and the time of this rating following your treadmill test. This is to determine the time of peak soreness following your exercise session. This is important in that it establishes the time lines for the urine collection and biopsies associated with the second treadmill test. A recording of the soreness on the scale every 1 to 2 hours (except sleep) would be ideal.

Experimental treadmill test.

This test will be performed at least 3 days after the soreness (if present) has subsided from the first test. For this test, you will either be doing the same test as treadmill test 1 or an uphill treadmill test at a workload which gives you a heartrate identical to that in treadmill test 1. Whether you do the uphill or the downhill test will be randomly determined.

If chosen for the uphill test, the protocol will use a grade of 5% and 3 mph. The grade will be increased gradually until your working heart rate equals the steady state heart rate from treadmill test 1. You will then work at this heart rate for the same period of time (as a maximum) as you did in test 1.

Maximal treadmill test (progressive)

This will be the last test that you will perform. This test will measure the maximal amount of oxygen that can be utilized while walking or running. You will be provided with full instructions in the proper method of walking or running on the treadmill. You will also be given instructions as to the proper method of breathing into the gas collecting chamber. Practise will be allowed. Immediately prior to the actual test, 3 electrodes will be attached to your chest for the purpose of measurement of your heart rate. The treadmill

will be set at a constant speed of 3.5 mph, and the inclination will will be increased 5% every 4 minutes from an initial grade of 0%. You will be asked to keep walking or running until you are unable to continue. At the point of exhaustion, you merely have to grasp the handrail and step off of the treadmill. In addition, your tester has an automatic stop switch which can be activated at any point in the test. It is expected that your heart rate and oxygen consumption will reach maximal levels for your age. Total fatigue may be experienced following the test, lasting from several minutes to an hour or more. The total test time should not exceed 30 minutes. You will be provided with free access to apple juice and water during this test.

Two muscle biopsies

This technique is to be performed by a qualified licensed physician. On 2 separate occassions, a muscle biopsy will be taken from your left thigh. The first biopsy will be taken at least one day before your first treadmill test. The second biopsy will be taken after the second treadmill test (either uphill or downhill), at either 0, 20, 40, 60, 80, or 100% of the time following completion of the second treadmill test and the time of peak soreness (as determined from treadmill test 1), with 0% being immediately after the test and 100 % being the previously determined time of peak soreness.

The skin over your mid-lateral right thigh will be swabbed with 70% alcohol and iodine. Approximately 1 cc of 2% Xylocaine will be injected to anaesthetize the area. After ensuring that the area is properly anaesthetized, a small incision (less than 1 cm) will be made 15 to 20 cm below the hip. The sterile biopsy needle will then be introduced into the muscle via the incision and a small sample of muscle will be obtained. The incision will be closed with an adhesive strip dressing.

Urine samples

There are 3 equal time periods here, all in sequence with one another in which you will be asked to collect urine samples. These are :

1. Knowing the time of maximal soreness after treadmill test 1, you will be asked to collect all of your urine samples in this same time period before your second downhill treadmill test (control urine).
2. In this same time period after the second treadmill test. (eg. presumably up until the time of peak soreness occurs - experimental 1).
3. In an equal time period after the time of maximal soreness (experimental 2).

Two thigh girth measures

At the times of both biopsies, your right thigh girth will be measured at distances of 40, 60, and 80% of the distance between the top of your knee cap and the front bony portion of your pelvis.

Body composition

This test is performed to measure your percentage of body fat. You will require a swim suit. The test itself is performed by having you immerse yourself in a specially constructed body fat immersion tank. This test will be performed immediately prior to the experimental treadmill test. It will take approximately 5 minutes to complete.

Diet

You will be requested to maintain a hydroxyproline free diet for the period beginning 2 days prior to the start of the urine collection period and extending to the end of the urine collection period (approximately 5 days). This involves avoiding the following foods: jams, jellies, jujubes, soft jellied candies of any type, ice cream, and red meats.

Exercise

You are requested to refrain from all excessive forms of exercise for the period of time extending from 2 days prior to the urine collection period to the end of the urine collection periods (a period of about 5 days).

Risks and discomforts

Control and experimental treadmill tests

The risks and discomforts are identical for those in the maximal treadmill test. However, due to the nature of the exercise, you will likely experience painful thigh and calve muscles for a period of to 5 days following the test. There will be no permanent damage or long lasting effects.

Maximal treadmill test (progressive)

It is possible that certain changes may occur in this test. These include: abnormal blood pressure; fainting; disorders of the heart; and in rare cases, heart attacks. If you are of normal health and have a genetic history of good health, you stand little chance of these risks occurring to you. In the unlikely event of a stumble or a fall, the treadmill will be stopped immediately.

Muscle biopsy

Some minor discomfort may be experienced from the anaesthetic injection. As the biopsy is being taken, there will be a sensation of deep pressure. This pressure subsides immediately once the biopsy needle is withdrawn. In a few instances, subjects have reported a slight ache in the muscle for 1 or 2 days. There may also be a slight discoloration or bruising of the skin around the incision. No serious complications have been documented from the muscle biopsy procedure. Many subjects are able to perform activities such as running, cycling or swimming within a few hours following the biopsy.

Girth measures

There are no risks or discomforts associated with this test.

Urine samples

The only discomfort to be experienced in this test is the necessity to collect your urine samples in the time frame requested.

Body composition

There will be little discomfort here. The water temperature in the tank is adjusted to body temperature or higher. Total body immersion is necessary and you will be totally wet upon leaving the tank. This may make you feel chilled.

Special diet

There are no risks associated with this test. There may be some discomfort if you happen to frequent any of the selected foods.

No exercise period

There are no risks associated with this request. You may, however, feel uncomfortable if you do not exercise for 5 days. This is very important as it is a controlling factor in this study.

Inquiries

You are freely encouraged to ask questions about the procedures to be used in any of these tests. If you have any doubt as to what is expected of you, please ask for a further explanation.

Freedom of consent

Your agreement to participate in this study is voluntary. You are free to deny consent if you so desire. You are free to withdraw from the study at any time with no prejudice whatsoever. You will be granted full access to your personal data at any stage of the study. You also consent to the use, by the investigator, of your data being published in part or in whole as part of this study.

I, _____ have read the foregoing description of the procedures and do hereby give my consent to participate in this study.

Date: _____.

Signature: _____.

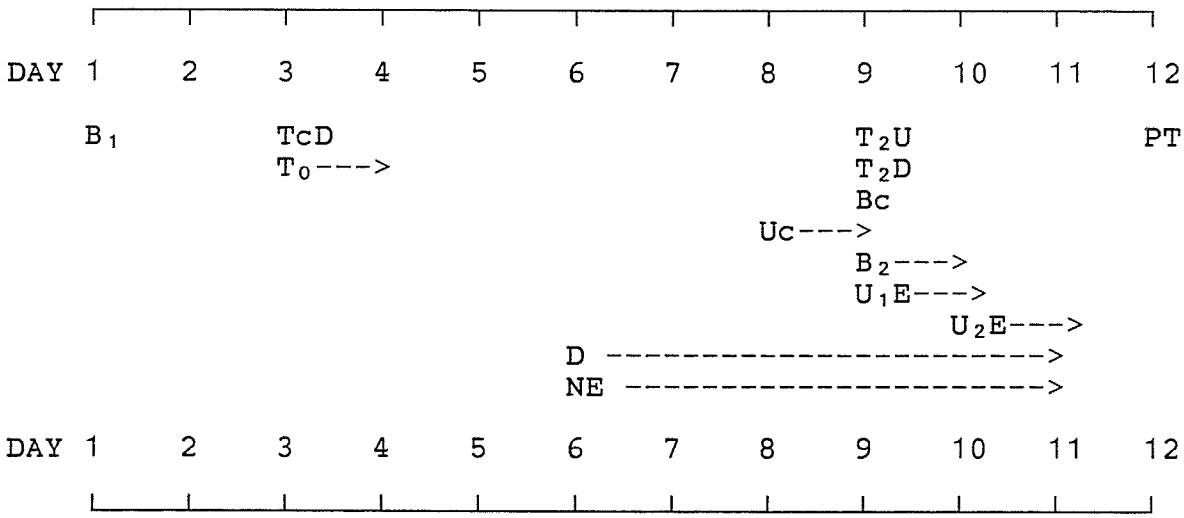
Order of test occurrance

1. Biopsy 1.
2. Eccentric control treadmill test.
3. Diet and exercise control periods begin (2 days before urine collection 1).
4. Urine collection 1 begins.
5. Urine collection 1 ends; Urine collection 2 begins; Experimental treadmill test occurs; Biopsy 2 occurs.
6. Urine collection 2 ends; Urine collection 3 begins.
7. Urine collection 3 ends; Diet and exercise control periods end.
8. Maximal treadmill test (progressive) occurs.

Checklist of items to bring to the tests

1. Shorts and t-shirt: treadmill tests & biopsy tests.
2. Bathing suit and towel: body composition test.
3. Signed consent form: For the first biopsy (your first test).

EXPERIMENTAL DESIGN TIME-LINES



LEGEND OF SYMBOLS FOR THE EXPERIMENTAL DESIGN

CONTROL MEASURES	EXPERIMENTAL MEASURES
B_1 - biopsy 1	B_2 - biopsy 2
TcD - Control treadmill	T_2U - Experimental treadmill
T_0 - Time to peak soreness	PT - maximal treadmill
Uc - Control urine	U_1E - Experimental urine 1
D - Diet control period	Bc - Body composition
NE - Exercise control	U_2E - Experimental urine 2

Appendix B

SORENESS RATING SCALE AND RECORD SHEET

SORENESS RATING SCALE

This scale is designed to help you rate the degree of muscle soreness that you are experiencing in your quadriceps muscle groups. Please refer to your informed consent sheet for test and rating scale instructions (treadmill tests 1 and 2).

1.0 <----------> 5.0 <----------> 10.0

no detection of soreness at all — moderate discomfort — excruciating pain.

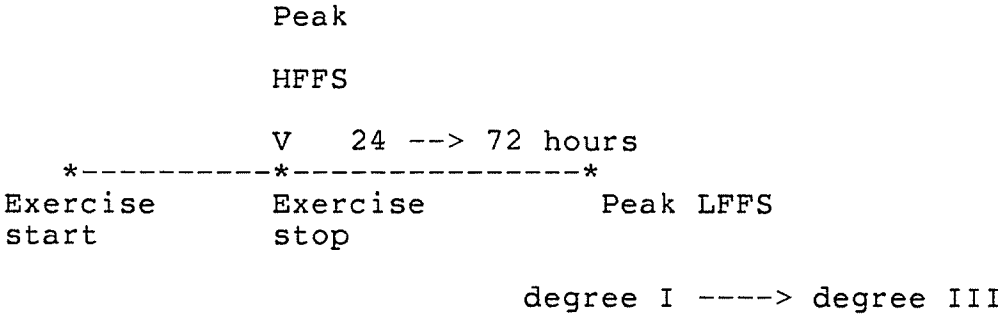
It would be desireable if you could use this rating with respect to the DEGREE of soreness experienced in your quadriceps muscle groups. Please provide periodic (ie. every hour) ratings for all of the following classes of muscle soreness:

1. DEGREE I: SORENESS TO MOVE. Here, your muscles will be sore just through normal movement.

2. DEGREE II: SORENESS TO TOUCH. Here, your muscles will be painful to any external pressures (touch, brushing against an object, etc.).

3. DEGREE III: ACHE SORENESS. Here, your muscles will have an "aching" soreness when you are neither touching them nor applying any external pressures.

Schematic division of the classes of muscle soreness



Soreness scale record sheet

Name:_____. Total time:_____.

Date:_____. Heart rate:_____.

Time Frame :_____.

Time	Degree 1	Degree 2	Degree 3

Appendix C

PROGRESSIVE MAXIMAL TREADMILL TEST PROTOCOL

Throughout this test, the speed of the treadmill was set at a constant rate of 3.5 miles per hour. The elevation was increased by 5% every 4 minutes beginning at a grade of 0 %. This elevation increase, as related to each stage of exercise, is listed below. Gas was collected at 2 1/2 minutes into every stage for a total time of one and one-half minutes.

STAGE	ELEVATION
1	0
2	5
3	10
4	15
5	20
6	25
7	30
8	35
9	40

Appendix D

FORMULAE FOR CALCULATION OF TREADMILL DATA

Oxygen analysis

The MMC uses a polarographic oxygen analyser consisting of a gold cathode and a silver anode immersed in potassium chloride gel. Both these electrodes are situated behind a semi-permeable membrane, allowing the diffusion of oxygen and hence enabling measurement. The polarizing voltage between the 2 electrodes and the resultant current flow are directly proportional to the partial pressure of oxygen to which the sensor is exposed.

Carbon dioxide analysis

The MMC uses a nondispersive infrared analysis technique for carbon dioxide measurement. This is a highly specific technique in that gas molecules absorb energy from different portions of the infrared spectrum. Consequently, the aborbance in relation to wavelength will give a unique pattern and accurate measurement analysis of the carbon dioxide present.

Calculation of Vo_2

$$Vo_2 \ (ml.\ kg^{-1}.\ min^{-1}) = \frac{VO_2 \ (l\ min^{-1}) \ x\ 1000}{Kg.}$$

Appendix E

CALCULATION OF PERCENT BODY FAT

$$\text{Body Density} = \frac{Ma}{V} = \frac{Ma}{\frac{Ma - Mw}{Dw} - [Rv + .1]} = Db$$

where Ma = Body weight in air (Kg.).
Mw = Body weight in water (Kg.).
Dw = Density of water ($Kg.l^{-1}$)
(= .994 $kg.l^{-1}$ at 35 ± 1 °C)
** Rv = Residual lung volume
(via vital capacity).
V = Body volume.
Db = Body density.
.1 = Correction for gastrointestinal gas.

** Vital Capacity Correction (l.)	Age (yrs.)
.25 x VC	16 - 34
.305 x VC	35 - 49
.445 x VC	50 - 69

$$\% \text{ FAT} = \frac{4.57}{Db} - 4.142 \times 100$$

$$\text{FAT WEIGHT} = \frac{\% \text{ FAT}}{100} \times Ma$$

LEAN BODY MASS = Ma - FAT WEIGHT

This procedure is approximately 1% accurate for % fat values from 4 to 30%. (Brozek et al, 1963).

Appendix F

URINE COLLECTION DATA FORM

Name:_____

For this segment of the study to be effective, please complete the following procedures for each void.

1. Collect each urine sample for the indicated time period. A missed sample could throw off the final result.
2. Store the amount in the container provided.
3. Store the sample in a refrigerator until it is collected daily by the investigator.

The urine sample will be collected for analyses after each of the time segments is completed. This will be arranged with each of you individually.

Urine Collection Data Sheet

Name: _____

Date: _____

Time Segment: _____

Begins at _____ on _____, 1984

Ends at _____ on _____, 1984

Void	Time	Comment	Void	Time	Comment
1			16		
2			17		
3			18		
4			19		
5			20		
6			21		
7			22		
8			23		
9			24		
10			25		
11			26		
12			27		
13			28		
14			29		
15			30		

Appendix G

URIC ACID ANALYTICAL PROCEDURE

Principle

UA undergoes oxidation to Allontoin through the action of uricase. The decrease in absorbance at 292 nm (A-292-UV) is proportional to the UA concentration.

$$\underset{\text{(High A-292-UV)}}{\text{Uric acid}} + 2\,H_2O + [O] \xrightarrow[\text{pH 9.4}]{\text{uricase}} \underset{\text{(Low A-292-UV)}}{\text{Allantoin}} + CO + H_2O$$

The procedure was standardized in terms of the molar extinction coefficient of UA taken as 12,300 at 292 nm using a 1 cm lightpath cuvet. Performance characteristics of the spectrophotometer were frequently monitored to assure reliable readings. The lowest and highest practical limits of measurements are 1 mg/100ml and 20 mg/100 ml respectively.

Reagents and their preparation

Glycine buffer solution (sigma stock 292-6)

1. 0.7 mol/l, pH 9.4 at 25°C.
2. Stored in the refrigerator at 0 to 5°C.

Uricase enzyme (sigma stock 292-8)

1. This enzyme (from porcine liver) was suspended in ammonium sulfate solution. It had an activity of 0.2 to 0.4 units/ml (when prepared).

2. It was stored in the refrigerator at 0 to 5°C.
3. To negate the effects of settling during storage, the bottle was inverted twice prior to each useage.

Note: 1 unit of uricase activity will convert 1 umole of UA to Allontoin per minute at pH 8.5.

Specimen collection and preparation

Urine UA levels have been reported stable for about 3 days at room temperature (Liddle, Seegmiller, & Laster, 1959). Urine diluted 10 fold, as prescribed for this procedure, may be stored at 0 to 5°C for a week or frozen for several weeks. Refrigeration was recommended only after the urine was diluted, otherwise the UA may precipitate. Urine samples were collected as previously described, diluted, and analysed within one week of collection.

Procedure

1. The X-hour urine volume (ml) was measured and recorded. The urine was mixed well before proceeding further. If the urine was not clear, a small volume (3 ml) was centrifuged.
2. 1 ml of clear urine was diluted with 9 ml of water. This 10 fold dilution was stored at 0 to 5°C for 1 week.
3. The following was pipetted into a test tube and mixed well.
   a) 0.3 ml of the dilution from the 10 fold dilution prepared above.
   b) 1.0 ml Glycine buffer solution, Sigma stock 292-6.
   c) 6.0 ml of distilled water.
4. 3.0 ml of the mixture prepared in step 1 was pipetted into each of 2 test tubes. 1 of these was labeled BLANK and the other TEST.
5. The following was then performed on each of these 2 tubes.
   a) Into the BLANK tube, 0.05 ml of water was pipetted and mixed well.
   b) Into the TEST tube, 0.05 ml of Uricase enzyme (292-8) was pipetted and mixed well.
   c) Both TEST and BLANK were allowed to stand at room temperature for approximately 15 minutes.

6. The mixtures from step 3a and 3b were transferred into cuvets. The following procedure was then followed:
   a) The spectrophotometer was set to an ultraviolet setting of 292 nm
   b) With the BLANK in the lightpath, the instrument was adjusted to read 0.400 absorbance.
   c) The TEST was placed in the lightpath in order to read and record its absorbance.
7. After a wait of approximately 5 minutes:
   a) The spectrophotometer was again adjusted to read 0.400 with the blank in the lightpath.
   b) The Absorbance of the TEST was read and recorded again as in step 6c. If the absorbance had decreased, the readings were repeated at 5 minute intervals until they were constant. This reading was recorded as the final absorbance.

Calculations

This procedure uses ultraviolet spectrophotometry and was esentially that of the Sigma technical bulletin 292-UV. The data for the urine UA values were calculated as follows:

$$\text{Delta Absorbance} = (0.400 - \text{final Absorbance}) + 0.005$$
$$= 0.405 - \text{final absorbance}$$

$$\text{UA (mg/X hr)} = [\text{ Delta Abs }] \times \left[\frac{50 \times V}{10}\right]$$
$$= \text{Delta Abs} \times [\ 5 \times V\ ]$$

Where:

0.005 = Absorbance contributed by the uricase enzyme, Sigma stock 292-8.

V = Urine Volume (X hr)

10 = factor to convert UA in diluted urine (mg/100 ml) to UA (mg/l) in undiluted urine.

The total UA can also be expressed as mmol/l by using the following conversion:

$$\frac{0.23 \text{ mmol/l}}{3.8 \text{ mg/dl}} \times [\text{UA (mg/dl)}] = \text{Total UA (mmol/l)}$$

Note: If the final absorbance was less than zero, the spectrophotometer was reset to read an absorbance of 0.600 with BLANK in the lightpath. The final absorbance of TEST was read and recorded when it remained constant. Under these circumstances,

delta abs = (0.600 - final abs) + 0.005

Appendix H

URINE CREATININE ANALYTICAL PROCEDURE

Principle

Most methods for CR measurement are based on the Jaffe reaction, where yellow/orange color forms when the metabolite is treated with alkaline picrate solution (Jaffe, 1886). Unfortunately, the Jaffe reaction is not specific and a number of substances (including proteins) in body fluids will interfere (DiGiorgio, 1974).

A method with improved specificity was developed by Slot (1965) who noted that under acid conditions the creatinine-picrate color faded faster than the interfering chromogens. Heinegard and Tiderstrom (1973) further simplified the procedure by eliminating the need for protein precipitation. The work of these investigators serves as the basis for the creatinine analysis used in this investigation. With these procedures, color derived from creatinine is destroyed at acid pH. The difference in color intensity measured at 500 nm before and after acidification is proportional to creatinine concentrations.

Reagents and their preparation

NaOH solution

This was the Sigma solution 930-65. It's concentration was 1.0 N and was stored at 18 to 26°C.

Acid reagent

This was the Sigma solution 555-2. It was a mixture of sulfuric and acetic acids. It was stored at 18 to 26°C.

Creatinine color reagent

This was the Sigma solution 555-1. It was a mixture of picric acid, approximately 0.6% sodium Borate and surfactant. It was stored at 18 to 26°C.

Alkaline picrate solution

This was prepared by mixing 5 volumes of creatinine color reagent with 1 volume of NaOH solution (i.e. 50 ml creatinine color reagent with 10 ml NaOH solution).

Creatinine standard I

This was the Sigma solution 925-3. It was a concentration of 0.26 mmol/l. It consisted of 3.0 mg/dl Creatinine in 0.02 N HCL. This was kept at 2 to 6°C in a refrigerator.

Creatinine standard II

This was the Sigma solution 925-15. It was in a concentration of 1.32 mmol/l. This consisted of 1.5 mg/dl creatinine in 0.02N HCL. It was kept at 2 to 6°C in a refrigerator.

Procedure

The following procedure was for a 3.4 ml reaction volume, requiring a 10 to 12 mm cuvet.

1. A 15 fold dilution of urine was used for this assay. The results were multiplied by the dilution factor (15).
   a) 0.3 ml water were added to a cuvet labelled BLANK.
   b) 0.3 ml of sample were added to a cuvet labelled TEST.
   c) 0.3 ml CR standard (Sigma 925-3) were added to a cuvet labelled STANDARD.
2. To all 3 cuvets, 3.0 ml of alkaline picrate solution was added. This solution was then mixed and allowed to stand at room temperature for 12 minutes.
3. The absorbance (A) of STANDARD and TEST were read and recorded at 500 nm using BLANK as a reference. This was INITIAL A.
4. To all cuvets, exactly 0.1 ml acid reagent (Sigma 555-2) was added. This was immediately and thoroughly mixed and allowed to stand for 5 minutes at room temperature. A precipitate often formed upon addition of the acid reagent, but it dissolved after mixing.
5. The absorbance (A) of STANDARD and TEST were read and recorded at 500 nm using BLANK as a reference. This was FINAL A.

Calculations

The procedure for data was essentially that of the Sigma technical bulletin 500.

$$\text{Creatinine (mg/dl)} = \frac{\text{Initial A(test)} - \text{Final A(test)}}{\text{Initial A(stand.)} - \text{Final A(stand.)}} \times 3^{*}$$

* Concentration (mg/dl) of CR standard (925-3).

Concentrations of unknowns were calculated from the absorbance of a 3 mg/dl CR standard included with each series of tests.

The total Creatinine can also be expressed as mmol/l by using the following conversion:

Total Creatinine (mmol/l)

$$= \frac{.22 \text{ mmol/l}}{2.5 \text{ mg/dl}} \times [\text{Creatinine}] \text{ (mg/dl)}$$

However, the limit of linearity for the instrument-cuvet system was determined as follows:

Into labelled cuvets, the solutions indicated in columns 2 & 3 were pipetted.

Cuvet	Creatinine Standard (925-15,ml.)	Water (ml)	Recorded Absorbance	Creatinine (mg/dl)	Creatinine (mmol/l)
1	0	0.30	0.000	0	0
2	0.05	0.25	0.211	2.5	0.22
3	0.10	0.20	0.416	5.0	0.44
4	0.15	0.15	0.623	7.5	0.66
5	0.20	0.10	0.850	10.0	0.88
6	0.25	0.05	1.064	12.5	1.10

1. 3.0 ml of alkaline picrate solution was added to all cuvets. This was mixed and allowed to stand for at least 10 minutes, but no longer than 15 minutes at room temperature.
2. Using cuvet 1 as a reference,thed absorbance of cuvets 2 through 5 was read at 500 nm. The addition of acid reagent was not necessary for preparation of the calibration curve.
3. The absorbance values (column 4) were plotted in relation to corresponding CR concentrations concentrations (column 5) on Figure 3.
4. Normal CR values are as follows:

SEX	NORMAL VALUES [g/24 hr]	[mmol/24 hr]
Men	1.1-2.8	9.7-24.7
Women	0.9-1.6	7.9-14.1

reference: (Young, Pestaner, & Gibberman, 1975; Doolan, Alpen & Theil, 1962)

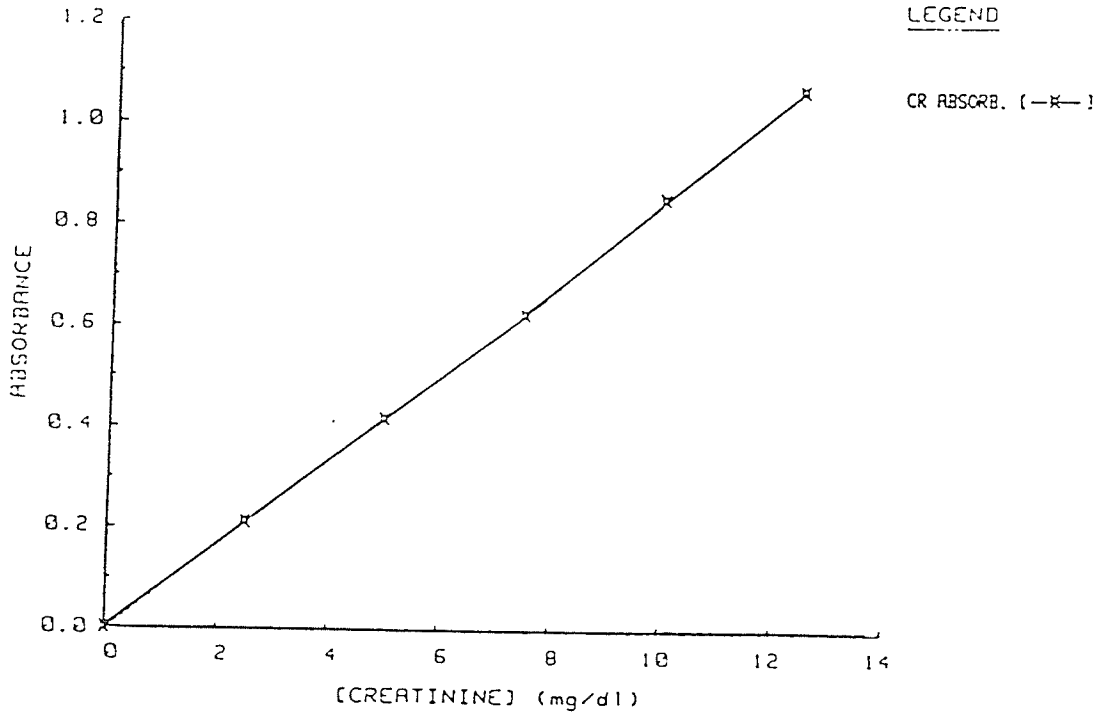


Figure 3: Linearity limit for the spectronic 21/cuvet system

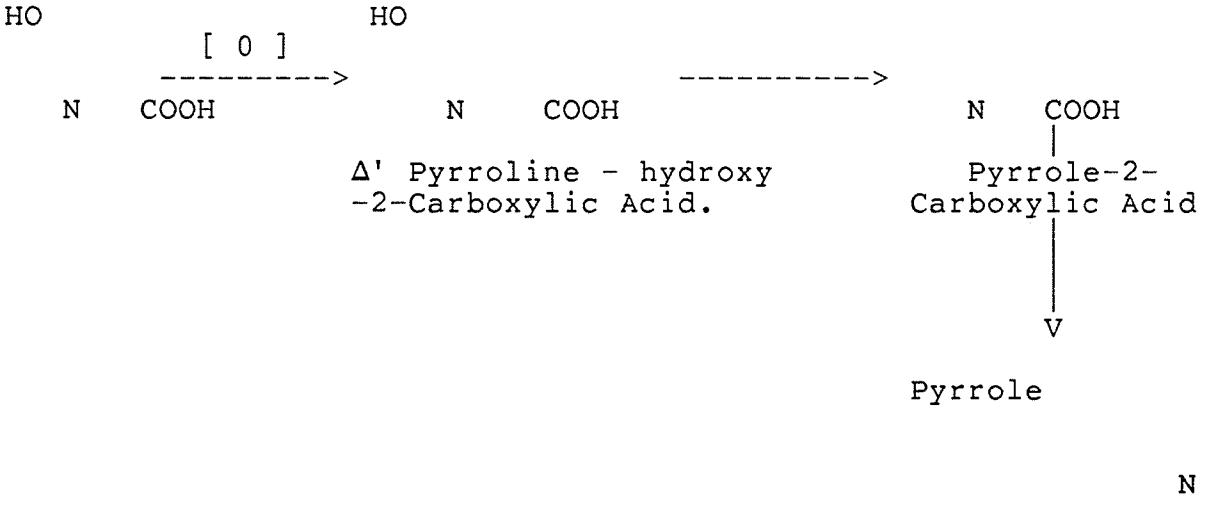
Appendix I

URINE HYDROXYPROLINE ANALYTICAL PROCEDURE

Principle

Most methods for the quantitative assay of HP are based on the oxidation of HP to pyrrole, pyrrole-2-carboxylic acid, or some other oxidation product, which was then converted to a chromophore with p-dimethylaminobenzaldehyde (Kivirikko, 1970).

Since only free HP reacts in assay procedures, the samples must be hydrolyzed beforehand. Free HP can be assayed directly, and the difference between this and the value obtained after hydrolysis corresponds to the peptide bound HP (Kivirikko, 1970).



(Prockop & Udenfriend, 1960)

Reagents and their preparation

Acetate-citrate buffer

This was produced by dissolving 57.0 gm of Sodium Acetate ($NaC2H_20$ - 3 H_20) and 37.5 gm of Sodium Citrate ($NaC2H_20$ - 2 H_20) in 385 ml of isopropanol and 300 ml water mixture. The pH was adjusted to 6.0 with acetic acid and made up to 1 litre with water.

Oxidizing reagent

This was prepared immediately prior to use by mixing 1 vol of freshly prepared 7% Chloramine-T Sodium (Toluene Sulfonchloramide) in water with 4 vol of Acetate Citrate buffer.

Ehrlich's reagent

This was prepared immediately prior to use by mixing 3 vol of p-DMAB solution (1 gm of p-DMAB in 3 ml of 60% perchloric acid) with 13 vol of isopropanol.

Hydroxyproline standards

In a 100 ml volumetric flask, 20 mg HP (Sigma lot H-6002) was dissolved and diluted to 100 ml with 0.001 N HCl. A 5 mg/100 ml working standard was made from this stock solution by dilution. Both the standard solution and the working standards were stored in the refrigerator for subsequent use.

Procedure

Urine collection

The urine was collected as per the procedures listed in the chapter on Methods and Procedures.

Hydrolysis

1. After thawing, 2 ml of the urine sample was delivered into each of 3 18 x 150 mm borosilicate test tubes.
2. 2 ml of concentrated HCl was then added to each tube. The tubes were then sealed using a propane torch.
3. For the standard, 2 ml of 5 mg/100 ml HP solution was used in place of the urine sample.
4. The sealed tubes were then placed in an oven with a constant temperature of 124°C for 2 hours.

Decolorization and evaporation

1. The tip of each tube was fractured and about 3 ml of the hydrolyzate was transferred into a similar tube containing about 50 mg of Norit-A.
2. This mixture was then shaken well and centrifuged for 5 minutes or until a clear supernatant was present.
3. 0.5 ml of the clear supernatant fluid was transferred to a 10 ml beaker which was placed on a shallow aluminum pan (about 3x5x5 cm) filled with water up to a level of approximately 1 cm.

4. The aluminum pan was then placed on a hot plate until the supernatant fluid had evaporated to dryness.
5. The residue of the HP was reconstituted with 0.5 ml of 0.001 N HCl and then mixed well.

Total hydroxyproline assay

1. Into 2 1.0 x 7.5 cm cuvets, (T) for test and (S) for standard, 0.1 ml of the reconstituted HP solution was delivered from the corresponding 10 ml beaker.
2. A blank cuvet (B) was prepared with 0.1 ml water.
3. 0.1 ml of the oxidizing reagent was added to all of the cuvets.
4. This solution was mixed well (by inversion) and 1.3 ml of Ehrlich's reagent was added.
5. The cuvets were then placed in a 60°C water bath for 20 minutes.
6. The cuvets were then placed in an ice bath for approximately 2 minutes.
7. Absorbance values were then immediately measured in a Bausch & Lomb Spectronic 21 Spectrophotometer at 558 mu against the blank.

Calculations

The procedure was essentially that of Parekh & Jung (1970). The amount of HP per time frame frame shall be calculated as follows.

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 5 * = \text{HP sample [ ] (mg/100 ml)}$$

* Concentration of standard in mg/100 ml.

(mg/100 ml) x (total volume in l) x 1/10 = mg THP/Time frame.

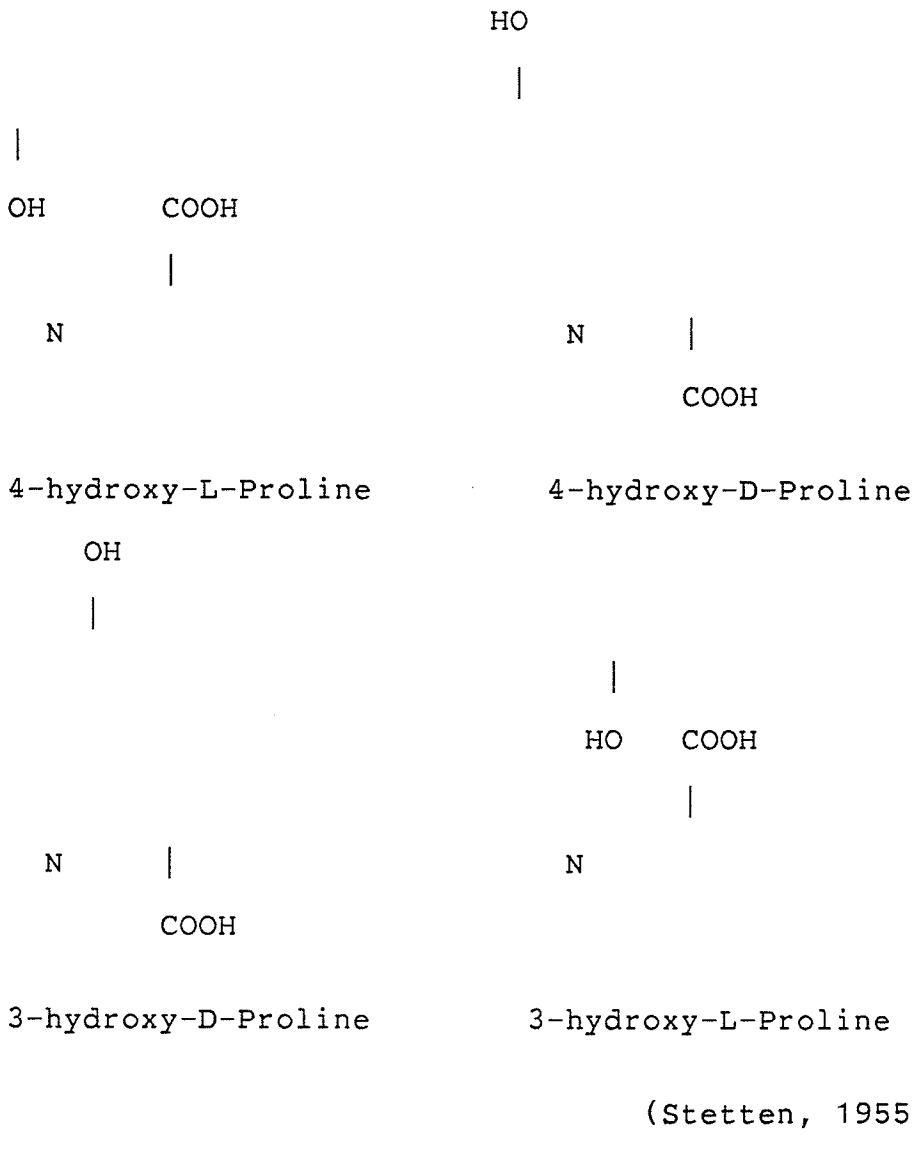
The HP can also be expressed as an amount relative to each of the urine collection time periods. This is accomplished as follows:

$$\frac{\{\text{ml/time frame (hr)}\} \times \{\text{mg THP/100 ml}\}}{100} = \text{mg THP/time frame}$$

Appendix J

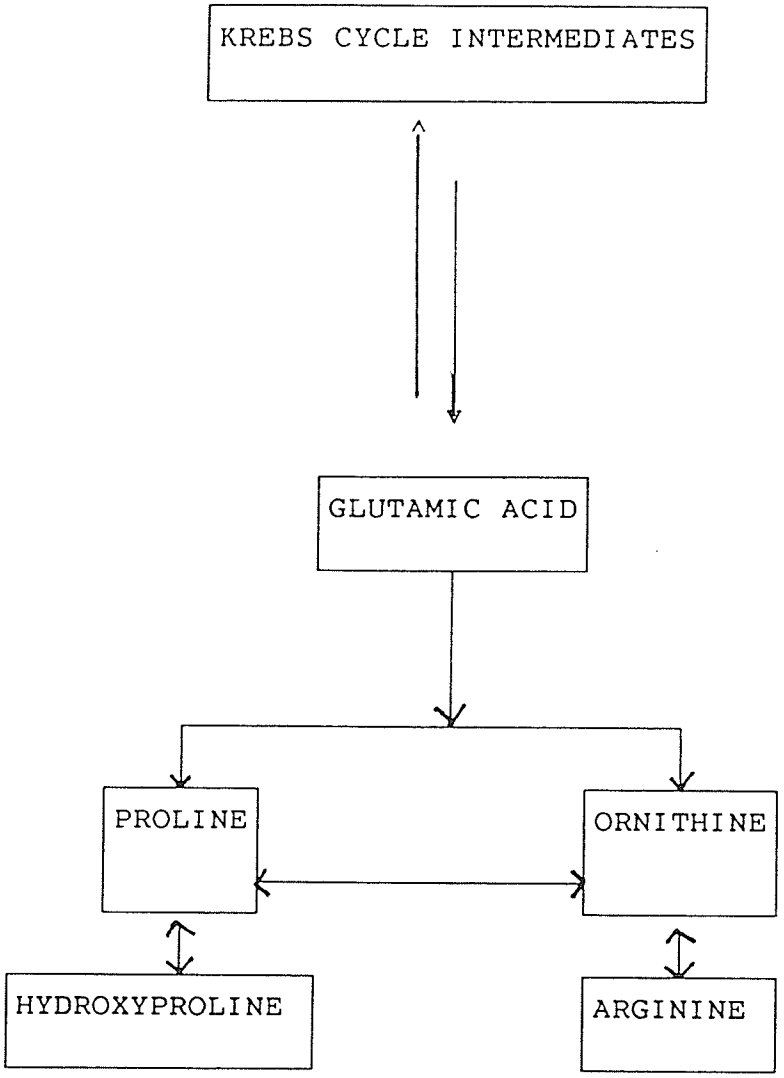
CHEMISTRY OF HYDROXYPROLINE

Isomeric forms of hydroxyproline



(Stetten, 1955)

Hydroxyproline in the metabolic pathways



Appendix K

GUIDELINES FOR HUMAN RESEARCH

Guidelines for Human Research

The following points were proposed by the Committee on Research Involving Human Subjects, A standing committee of the Research Board; approved by the research board as amended on January 15, 1976; approved by Senate as amended on April 6, 1976; approved by the Board of Governors, April 22, 1976.

1. Any research must be justifiable in that it must have scientific value.
2. The benefit of the proposed research to science and the subject must significantly outweigh any risks to the subject. In general, any significant risk to the subject should be offset by the expectation of benefit, such as a therapeutic effect.
3. The research must be conducted under a sound design and protocol and be carried out according to the protocol. The design and protocol may be modified only with the approval of the committee which had originally approved it.
4. No subject should be used in research unless he has given his free consent after being fully informed (except as in Clause 8). It is essential to make a candid explanation of the research and its risks and to ensure that the subject understands them before his consent is taken. When a person is powerless to

determine his own participation because of incapacity due to illness, unconciousness or age, proxy consent of his parent, spouse, other next of kin, or other person responsible must be sought. Such proxy consent is acceptable only when there is no significant risk or discomfort to the subject or when any signififcant risk or discomfort is outweighed by the probability and degree of benefit to the subject.

5. The subject or proxy must appreciate that he is free to withdraw at any time without penalty.
6. Care must be taken throughout to see that the subject will not be harassed and the research should be terminated if risk or harm, physical or emotional, is apparent.
7. Notwithstanding Clause 5, when it is necessary in psychological or social research that the subject be less than fully aware of the purpose of the research, it is sometimes permissable to disguise the purpose of the research, but only if:
   a) This procedure is absolutely necessary to the research design.
   b) The research is not of such a nature that, if the subject realized what was under study, his refusal to participate could be forseen.
   c) The subject is to be informed of the true nature of the study immediately upon the completion of the study.

The subject should be as fully informed as possible about what he will be asked to do and what will be done to him. The subject may have been misinformed, but he must never feel he has been exploited, tricked, or put in a situation where he has acted in a way he regrets.

8. Results of research that gathers information of a personal, such as answers to questionnaires, must be kept confidential and anonymity must be afforded to the subject of the research unless he freely consents to the contrary.
9. Approval of the appropriate Ethics Review Sub-Committee must be acquired before research involving human subjects can proceed.

Appendix L

RAW DATA FOR ALL TESTS

Appendix	Parameter
L-1)	Sore time raw data
L-2)	Eccentric control treadmill test raw data
	a.) Work time
	b.) Heart rates
L-3)	Experimental treadmill test
	a.) Vo_2/kg
	b.) Treadmill grade of group C
L-4)	Progressive maximal treadmill test
	a.) Work time
	b.) Heart rate
	c.) Vo_2/kg
L-5)	Descriptive data on all subjects.
	a.) Age, weight, and sex of subjects.
	b.) Body fat and lean body mass.
L-6)	Urine Raw data
	a.) Urine volume (l)
	b.) Hydroxyproline concentrations (mg/dl)
	c.) Creatinine concentrations (mmole/l)
	d.) Uric acid concentrations (mg/dl)
	e.) Hydroxyproline:creatinine ratios (mg%)

RAW DATA VALUES

APPENDIX L-1

** The data presented herein is expressed in hours.

the "-" symbol for degree III soreness indicates no ranking above 5 on the soreness scale.

GROUP	SORENESS DEGREE		
	Degree I	Degree II	Degree III
A_1	24	40	-
A_2	44	72	-
A_3	20	21	-
A_4	46	48	-
A_5	60	63	-
A_6	40	45	-
A_7	24	42	-
B_1	21	24	-
B_2	44	46	-
B_3	63	72	-
B_4	24	30	-
B_5	24	10	-
B_6	20	10	-
B_7	24	28	-
B_8	36	44	-
B_9	40	48	-

APPENDIX L-2

EXPERIMENTAL TREADMILL TEST RAW DATA

a.) Work time

b.) Heart rate

APPENDIX L-2A

WORK TIME RAW DATA - ECCENTRIC CONTROL TREADMILL TEST

Subject Group	Work Time (min:sec)
A_1	40:14
A_2	30:00
A_3	60:00
A_4	35:00
A_5	60:00
A_6	45:00
A_7	54:00
B_1	60:00
B_2	32:58
B_3	60:00
B_4	40:00
B_5	27:18
B_6	41:49
B_7	27:10
B_8	40:00
B_9	25:00

APPENDIX L-2B

ECCENTRIC CONTROL TREADMILL TEST HEART RATES

Subject Group	Heart Rates (bpm)
A_1	156
A_2	171
A_3	139
A_4	137
A_5	128
A_6	143
A_7	140
B_1	134
B_2	158
B_3	105
B_4	157
B_5	170
B_6	170
B_7	120
B_8	145
B_9	170

APPENDIX L-3

EXPERIMENTAL TREADMILL TEST RAW DATA

a.) Vo_2/kg

b.) Treadmill grade data (group C)

APPENDIX L-3A

EXPERIMENTAL TREADMILL TEST - VO_2/KG DATA

Subject Group	Vo_2/kg (ml kg^{-1} min^{-1})
A_1	36.6
A_2	41.9
A_3	23.5
A_4	30.1
A_5	27.3
A_6	27.1
A_7	22.7
B_1	16.9
B_2	37.7
B_3	25.9
B_4	31.3
B_5	23.8
B_6	28.2
B_7	22.2
B_8	22.9
B_9	28.7

APPENDIX L-3B

EXPERIMENTAL TREADMILL TEST GRADE - GROUP C

Subject Group	Treadmill grade (+%)
A_1	12
A_2	15
A_3	10
A_4	13
A_5	20
A_6	10
A_7	5

APPENDIX L-4

PROGRESSIVE MAXIMAL TREADMILL TEST RAW DATA

4a.) Work time

4b.) Heart rate

4c.) Vo_2/kg

APPENDIX L-4A

PROGRESSIVE MAXIMAL TREADMILL TEST WORK TIME

Subject Group	Work time (min:sec)
A_1	18:54
A_2	22:36
A_3	17:36
A_4	22:19
A_5	30:10
A_6	18:15
A_7	17:00
B_1	23:14
B_2	21:10
B_3	30:29
B_4	17:17
B_5	15:37
B_6	22:14
B_7	23:03
B_8	23:41
B_9	13:46

APPENDIX L-4B

PROGRESSIVE MAXIMAL TREADMILL TEST HEART RATES

Subject Group	maximal HR (bpm)	HR (%max)
A_1	177	88.1
A_2	185	92.4
A_3	206	67.5
A_4	187	73.3
A_5	185	69.2
A_6	186	75.3
A_7	191	73.3
B_1	198	67.7
B_2	193	81.9
B_3	185	56.8
B_4	184	85.3
B_5	198	85.9
B_6	201	84.6
B_7	189	63.4
B_8	187	77.5
B_9	187	90.9

APPENDIX L-4C

MAXIMAL OXYGEN UPTAKE (VO_2MAX)

Subject Group	Vo_2max (ml kg^{-1} min^{-1})	Vo_2 (%max)
A_1	49.3	74.2
A_2	58.1	72.1
A_3	44.9	52.3
A_4	46.4	64.9
A_5	68.0	40.2
A_6	41.7	64.9
A_7	44.7	50.8
B_1	51.6	32.8
B_2	53.9	69.9
B_3	69.5	37.3
B_4	43.7	71.6
B_5	42.3	56.3
B_6	57.5	49.0
B_7	61.5	36.1
B_8	53.8	42.6
B_9	40.7	70.5

APPENDIX L-5

DESCRIPTIVE DATA ON ALL SUBJECTS

5a) Age, weight, and sex of subjects

5b) Body fat and lean body mass

APPENDIX L-5A

BASIC PHYSICAL CHARACTERISTICS

GROUP	CHARACTERISTIC		
	age (yrs)	weight (kg)	sex
A_1	32	86.5	m
A_2	20	54.6	f
A_3	20	102.8	m
A_4	26	51.3	f
A_5	25	75.9	m
A_6	29	57.5	f
A_7	22	108.6	m
B_1	22	72.0	m
B_2	22	78.3	m
B_3	26	75.0	m
B_4	24	80.3	m
B_5	25	103.6	m
B_6	24	80.3	m
B_7	27	57.7	f
B_8	26	80.7	m
B_9	23	71.3	m

APPENDIX L-5B

BODY FAT AND LEAN BODY MASS DATA

Group	% fat	lbm (kg)	lbm/mass (%)
A_1	15.4	73.2	84.6
A_2	12.1	47.9	87.7
A_3	21.3	80.9	78.7
A_4	17.6	42.3	82.5
A_5	10.9	67.6	89.1
A_6	18.0	47.2	82.1
A_7	15.9	91.3	84.1
B_1	13.7	62.1	86.3
B_2	13.7	67.6	86.3
B_3	10.9	67.6	90.1
B_4	19.4	64.7	80.6
B_5	28.5	74.1	71.5
B_6	22.1	59.0	73.5
B_7	10.5	51.6	89.4
B_8	13.4	69.9	86.6
B_9	17.2	59.0	82.8

APPENDIX L-6

URINE RAW DATA

6a) Urine volume (l)

6b) Hydroxyproline concentrations (mg/dl)

6c) Creatinine concentrations (mmole/l)

6d) Uric acid concentrations (mg/dl)

6e) Hydroxyproline:creatinine ratios (mg%)

APPENDIX L-6A

URINE VOLUMES FOR ALL SUBJECTS (L)

GROUP	URINE VOLUMES (l)		
	Vol 1	Vol 2	Vol 3
A_1	0.7	0.9	1.1
A_2	0.7	0.6	0.6
A_3	1.0	0.9	1.9
A_4	0.8	1.2	1.3
A_5	2.5	1.7	1.7
A_6	1.9	1.9	1.9
A_7	1.0	0.7	0.8
B_1	1.1	1.3	1.0
B_2	1.1	2.0	2.0
B_3	1.5	2.0	2.7
B_4	0.8	0.9	1.1
B_5	1.3	0.5	*
B_6	1.1	0.9	1.6
B_7	0.5	2.0	2.0
B_8	1.2	1.1	1.3
B_9	0.4	0.9	1.4

* Missing sample

APPENDIX L-6B

HP CONCENTRATION IN URINE (MG/DL)

GROUP	SAMPLE NUMBER		
	sample 1	sample 2	sample 3
A_1	1.25	2.33	1.86
A_2	3.76	2.72	0.47
A_3	1.34	1.22	1.29
A_4	1.56	2.15	0.99
A_5	2.06	1.73	1.03
A_6	1.89	4.59	2.65
A_7	3.87	1.26	1.96
B_1	0.76	0.85	1.32
B_2	0.49	1.53	1.18
B_3	3.93	1.18	0.85
B_4	1.04	4.07	4.25
B_5	2.00	5.09	*
B_6	1.09	0.34	3.56
B_7	1.52	1.54	1.19
B_8	3.81	2.49	2.23
B_9	1.56	1.49	0.74

* Missing sample
Note: for CR, 1mmol = .011mg
1 mg = 88.3mmol

APPENDIX L-6C

CR CONCENTRATION IN URINE (MMOL/L)

GROUP	SAMPLE NUMBER		
	sample 1	sample 2	sample 3
A_1	16.59	21.32	10.54
A_2	16.59	23.09	11.08
A_3	20.59	04.74	13.67
A_4	11.48	12.91	07.14
A_5	04.26	07.85	13.94
A_6	13.83	10.91	12.09
A_7	17.99	17.54	18.85
B_1	13.47	15.28	17.91
B_2	28.73	27.63	15.64
B_3	26.42	26.09	26.24
B_4	14.84	19.67	24.17
B_5	08.03	24.85	*
B_6	05.84	07.74	07.92
B_7	08.33	06.85	04.45
B_8	12.03	12.79	13.65
B_9	10.34	25.52	14.99

* Missing sample

APPENDIX L-6D

UA CONCENTRATION IN URINE (MG/DL)

GROUP	SAMPLE NUMBER		
	sample 1	sample 2	sample 3
A_1	79.0	53.5	42.0
A_2	82.5	89.0	39.5
A_3	126.0	67.0	88.0
A_4	72.5	57.5	31.5
A_5	23.5	50.0	64.5
A_6	37.5	62.0	80.0
A_7	63.5	67.0	99.0
B_1	54.5	67.0	99.0
B_2	37.0	84.5	46.0
B_3	42.5	39.0	24.0
B_4	75.5	74.0	158.0
B_5	27.0	67.0	*
B_6	24.5	42.0	53.0
B_7	50.5	29.5	31.5
B_8	17.5	57.5	43.0
B_9	119.5	130.5	162.5

* Missing sample
Note: for UA, 1 mg = .006 mmol
1mmol = 165.2 mg

APPENDIX L-6E
HP:CR CONCENTRATION IN URINE (MG%)

GROUP	SAMPLE LUMBER		
	sample 1	sample 2	sample 3
A_1	.007	.009	.016
A_2	.010	.004	.028
A_3	.006	.023	.008
A_4	.012	.015	.012
A_5	.043	.019	.007
A_6	.012	.037	.019
A_7	.019	.006	.009
B_1	.005	.005	.006
B_2	.002	.005	.007
B_3	.013	.004	.003
B_4	.006	.018	.015
B_5	.022	.018	*
B_6	.016	.004	.039
B_7	.016	.019	.023
B_8	.028	.017	.014
B_9	.013	.005	.004

* Missing sample