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THE STRUCTURE AND FUNCTION OF THE QUADRICEPS MUSCLE
IN
HEALTH AND DISEASE

Submitted to the University of Glasgow
for the degree of M.D.

by

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DECEMBER 1982

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CORE PUBLICATIONS

This thesis is based on the following 7 publications plus some unpublished data:

- I EDWARDS, R.H.T., YOUNG, A., HOSKING, G.P. & JONES, D.A. (1977) Human skeletal muscle function: description of tests and normal values. Clinical Science and Molecular Medicine, 52, 283-290.

- II YOUNG, A., EDWARDS, R.H.T., JONES, D.A. & BRENTON, D.P. (1981) Quadriceps muscle strength and fibre size during the treatment of osteomalacia. In: Mechanical Factors and the Skeleton, pp. 137-145. Ed. Stokes, I.A.F. John Libbey, London.

- III WILES, C.M., YOUNG, A., JONES, D.A. & EDWARDS, R.H.T. (1979) Muscle relaxation rate, fibre-type composition and energy turnover in hyper- and hypo-thyroid patients. Clinical Science, 57, 375-384.

- IV SARGEANT, A.J., DAVIES, C.T.M., EDWARDS, R.H.T., MAUNDER, C. & YOUNG, A. (1977) Functional and structural changes after disuse of human muscle. Clinical Science and Molecular Medicine, 52, 337-342.

- V YOUNG, A., HUGHES, I., RUSSELL, P., PARKER, M.J. & NICHOLS, P.J.R. (1980) Measurement of quadriceps muscle wasting by ultrasonography. Rheumatology and Rehabilitation, 19, 141-148.

- VI YOUNG, A., HUGHES, I., ROUND, J.M. & EDWARDS, R.H.T. (1982) The effect of knee injury on the number of muscle fibres in the human quadriceps. Clinical Science, 62, 227-234.

- VII YOUNG, A., STOKES, M., ROUND, J.M. & EDWARDS, R.H.T. (in press) The effect of high-resistance training on the strength and cross-sectional area of the human quadriceps. European Journal of Clinical Investigation.

Appendix 1 comprises the full texts of Papers I to VII, which are referred to throughout the thesis by their Roman numerals.

Appendix 2 lists further related publications to which I contributed.

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My greatest debt is, as always, to my wife and family for their forbearance and encouragement.

DECLARATION

I declare that this thesis has been composed by myself and that the books and papers cited were all consulted by me personally, except where it is otherwise stated.

The experimental work described in the 'Core Publications' was all carried out by myself or under my direct supervision, except for . Paper III where the responsibility was shared with Professor Edwards and Dr. Wiles. Six of the 'Core Publications' were written by me; Paper III was written in equal partnership with Dr. Wiles. All other studies described in the thesis were carried out by me, except where it is otherwise stated.

SUMMARY

INTRODUCTION

Proximal muscle weakness is a common feature of a wide range of clinical conditions and contributes significantly to disability. There is a need for a scientific approach to the problems of muscle weakness in man and its correction. The principle questions considered in the thesis are:

1. Is it possible to describe the isometric strength and contractility of the normal human quadriceps with objective and repeatable indices?
2. What is the cause of quadriceps muscle weakness in osteomalacia?
3. What is the cause of abnormal quadriceps function in thyroid disease?
4. What is the nature of the quadriceps wasting which follows knee injury and/or immobilisation? What is its effect on the function of the muscle?
5. How should quadriceps atrophy and hypertrophy be measured?
6. What is the relationship between the size and strength of the normal quadriceps? What are the effects of strengthening exercise?
7. Do type I and type II fibres differ in their isometric strength/cross-sectional area?

METHODS

Quadriceps muscle function was studied by isometric dynamometry during both voluntary and electrically-stimulated contractions. The former were used for measurements of maximal strength and the latter were used to determine indices of contractility such as relaxation rate and the relative forces produced by different frequencies of stimulation.

The structure and chemistry of the quadriceps were examined in samples of tissue taken by the needle biopsy technique. Biochemical analysis concentrated on the availability of 'fuel', viz. adenosine triphosphate (ATP), phosphorylcreatine, and glycogen. The microscopic studies concentrated on estimating the amount of muscle tissue present, seeking evidence of muscle cell destruction, regeneration, atrophy or hypertrophy. In particular, how big a change did these features imply in the effective size of the whole muscle? Also, if present, were these processes selectively affecting one or other histochemically-defined 'type' of fibre?

It became apparent in the earlier studies that there was a need for a means of measuring changes in the size of the whole quadriceps. Extrapolation from measurements of myofibre size was not good enough and limb anthropometry underestimated changes localised to the quadriceps. A technique was therefore devised for measuring the cross-sectional area (CSA) of the quadriceps from tranverse ultrasound B-scans.

CONCLUSIONS

1. The voluntary isometric strength of the quadriceps femoris is a repeatable and valid measure. Other contractile characteristics can be measured, reproducably, by dynamometry during transcutaneous electrical stimulation. They are influenced by the fibre-type composition of the muscle.
2. Muscle atrophy is a major, immediate cause of weakness in osteomalacia. The recovery of strength during treatment with vitamin D is associated with growth of muscle fibres, is a slow process, and may be incomplete.
3. Quadriceps weakness in thyrotoxicosis is associated with myofibre atrophy. Subjective weakness in submaximal contractions is probably also due to the higher stimulus frequency required to achieve tetanic fusion. In hypothyroidism, the rate of ATP turnover and the production of lactate during isometric

contractions held to fatigue is slow but there is no evidence of impaired ATP generation. There are abnormalities of the quadriceps' fibre-type composition in both hyper- and hypothyroidism but they do not fully explain the changes in the muscle's relaxation rate.

4. The severity of quadriceps wasting following knee injury can be explained entirely by atrophy; there is no evidence of a change in the total number of fibres in the muscle. It is not known what particular clinical features determine whether an individual patient's quadriceps will show type I atrophy, type II atrophy or combined atrophy of both fibre types following knee injury and/or immobilisation; all 3 patterns of atrophy were observed. In the absence of pain or an effusion, the loss of quadriceps strength is similar to the degree of atrophy.
5. Quadriceps CSA may be measured by ultrasound B-scanning. The severity of the quadriceps wasting which follows knee injury is seriously underestimated by measurements of thigh circumference, even when they are supplemented by caliper measurements of subcutaneous fat. The tape measure also underestimates the increase in muscle mass which occurs with strengthening exercise. Studies of muscle hypertrophy or atrophy must include a direct measurement of the size of the individual muscle or muscle group being considered.
6. Preliminary studies of normal subjects show a close correlation between the quadriceps' strength and its CSA but high-resistance training may increase the isometric strength of the normal quadriceps by more than it increases its total CSA.
7. In isometric contractions of the human quadriceps, type II fibres may be about twice as strong, for their cross-sectional area, as type I fibres.

ABBREVIATIONS

ATP	adenosine triphosphate
C	mid-thigh circumference
CK	creatine kinase (EC 2.7.3.2)
CSA	cross-sectional area, of a whole muscle or muscle group
%CSA II	percentage contribution of type II muscle fibres to the cross-sectional area of a muscle
CV	coefficient of variation
EMG	electromyogram
FFTV	fat-free thigh volume
LA	lactate
MFA	mean fibre area, i.e. mean cross-sectional area of muscle fibres, irrespective of fibre type
MFA I	mean cross-sectional area of type I muscle fibres
MFA II	mean cross-sectional area of type II muscle fibres
MFA II/MFA I	ratio of mean cross-sectional area of type II muscle fibres to that of the type I fibres
mid-thigh	halfway between greater trochanter and lateral joint line of knee
MRC scale	system of scoring muscle strength (Medical Research Council, 1943)
MRR	maximal relaxation rate, i.e. maximum percentage of plateau force lost per 10ms following a tetanic contraction
MVC	force of a maximal voluntary isometric contraction
MVT	torque about the knee in a maximal voluntary isometric contraction
NS	not statistically significant
PC	phosphorylcreatine
SF ₉₅	time (ms) elapsing, at the end of an electrically stimulated tetanic contraction, between the last stimulus and the decay of force to 95% of its plateau value

SF_{50}	time (ms) elapsing, at the end of an electrically stimulated tetanic contraction, between the last stimulus and the decay of force to 50% of its plateau value
SR	sarcoplasmic reticulum
II %	percentage frequency of type II fibres, irrespective of fibre size
TC	total creatine, i.e. phosphorylcreatine plus free creatine
$\dot{V}O_{2max}$	maximal rate of oxygen uptake during voluntary physical activity
\hat{Y}	the estimated value of Y calculated for a given value of X (as distinct from the observed value Y in any actual case) (Sokal & Rohlf, 1969, p. 413)

VARIABILITY OF MEASUREMENTS

	Coefficient of variation	Thesis page
<hr/>		
<u>Quadriceps chemistry</u>	3-6%	29
 <u>Quadriceps micromorphometry</u>		
MFA (same section)	($\leq 5\%$ ($\leq 4\%$	30 31
MFA)	16%	31
II %) (same skin incision)	17%	31
%CSA II)	16%	31
 <u>Quadriceps dynamometry</u>		
MVC	4-8%	39
SF ₉₅ , SF ₅₀	$\leq 4\%$	46
MRR (calculated from SF ₉₅ & SF ₅₀)	$\leq 4\%$	46
 <u>Thigh anthropometry</u>		
C ²	$\leq 2\%$	85
C ² _{left} /C ² _{right}	$\leq 2\%$	85
FFTV	$\leq 2\%$	85
FFTV _{left} /FFTV _{right}	$\leq 2\%$	85
 <u>Quadriceps ultrasonography</u>		
CSA (mean of 4 scans)	(4% ($\leq 2\%$	86 110

CHAPTER 1

INTRODUCTION

MUSCLE FUNCTION IN CLINICAL MEDICINE AND REHABILITATION

Central importance

Need for objective evaluation

SCOPE OF INVESTIGATIONS

Principal questions considered

Choice of techniques

Choice of muscle

Choice of pathology

- (i) *Reversible "myopathies"*
- (ii) *"Disuse"*
- (iii) *Fibre-type composition*

Normal relationship between muscle size and strength

CHAPTER 1

INTRODUCTION

MUSCLE FUNCTION IN CLINICAL MEDICINE AND REHABILITATION

Central importance

Muscle weakness is a common feature of a wide range of clinical conditions and contributes significantly to disability.

"The importance of muscular contraction to us can be stated by saying that all man can do is to move things, and his muscular contraction is his sole means thereto."
(Sherrington, 1937/38)

Rehabilitation is the attempt to restore a person to the maximal possible level of function in the presence of disease or residual impairment; much of rehabilitation practice therefore comprises measures to restore muscle strength or to compensate for its loss. In particular, much of a physiotherapist's time is spent in attempts to restore muscle strength.

Need for objective evaluation

It is high time that these efforts were properly evaluated. Yet most clinicians and physiotherapists seem strangely reluctant to measure muscle strength, preferring instead to judge it subjectively or from all-or-none performance tests (e.g. the ability to rise from the chair). Clinical dynamometry is not a new idea (vd. Chapter 4, "Introduction"), but at the time this work was started there was not even an adequate, objective, cross-sectional record of the pattern of progression of the commonest form of muscular dystrophy (Duchenne muscular dystrophy). Even the effects of immobilization, corticosteroids, vitamin D, or thyroid hormone on muscle strength had never been quantified in man. At a microscopic level, little was known of the nature of the histochemical and morphological changes

which accompanied muscle weakness in orthopaedic practice or in endocrinopathies. Still less was known of the relevance of such changes to the patient's disability and its correction. Changes in muscle mass, too, are rarely measured reliably. At best, they are estimated from limb circumference measurements, a technique of poor repeatability (Kirwan, Byron, Winfield, Altman & Gumpel, 1979) and, as this thesis will show, one which is of very limited relevance when wasting is secondary to joint injury and/or immobilization.

There is a need for a scientific approach to the problems of muscle weakness and its correction. This thesis is a compilation of steps in this direction. Attempts to analyse the cause of "weakness" - failure of a muscle to produce adequate force - followed the conceptual scheme outlined by Edwards (1978) (Fig. 1.1).

SCOPE OF THE INVESTIGATIONS

Principal questions considered

The principal questions considered in this thesis are:

1. Is it possible to describe the isometric strength and contractility of the normal human quadriceps with objective and repeatable indices? (Chapters 4 and 5)
2. What is the cause of quadriceps muscle weakness in osteomalacia? (Chapter 6)
3. What is the cause of abnormal quadriceps function in thyroid disease? (Chapter 7)
4. What is the nature of the quadriceps wasting which follows knee injury and/or immobilization? What is its effect on the function of the muscle? (Chapter 8)
5. How should quadriceps atrophy and hypertrophy be measured? (Chapters 8 and 9)
6. What is the relationship between the size and strength of the normal quadriceps? What are the effects of strengthening exercise? (Chapter 9)
7. Do type I and type II fibres differ in their isometric strength/cross-sectional area? (Chapter 10)

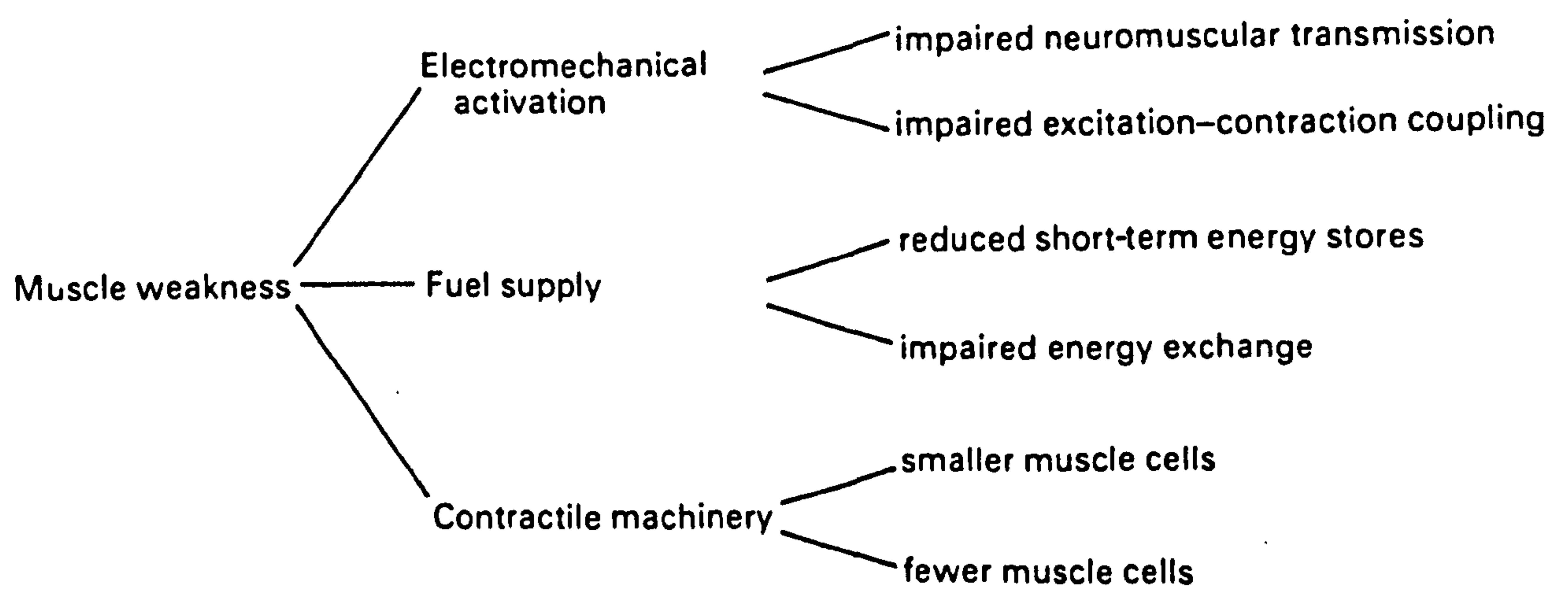


Fig. 1.1 Practical scheme for analysis of muscle weakness (Edwards, 1978).

Choice of techniques

"The essential service of muscle to life is, quickly and reversibly, to shorten so as to pull."
(Sherrington, 1937/38)

The most fundamental feature of any logical assessment of a muscle must be measurement of the force with which it pulls, i.e. dynamometry. The first question asked in this thesis is whether the strength of a large and functionally important human muscle can be reliably measured in normal subjects (Chapter 4).

Techniques giving a measure of changes in total body muscle have only a small place in the thesis. (Nitrogen balance studies are referred to in Chapters 6 and 10.) Such techniques may be most valuable in other circumstances (e.g. Edwards, Isenberg, Wiles, Young & Snaith, 1981) but are of limited relevance where the objective is to relate changes in strength to changes in the structure, chemistry or size of the same individual muscle or muscle group.

The structure and chemistry of an individual muscle can be examined in samples of tissue taken from that muscle by the needle biopsy technique (Chapter 3). Moreover, the technique is sufficiently nearly atraumatic that further samples can be taken during a course of treatment while changes in strength are also being recorded. Biochemical analysis of biopsies concentrated on the availability of 'fuel' for the contractile 'machinery', viz. adenosine triphosphate, phosphorylcreatine, and glycogen and their breakdown products. The microscopic studies concentrated on the amount of muscle tissue present, seeking evidence of muscle cell destruction, regeneration, atrophy or hypertrophy. How big a change did they imply in the effective size of the whole muscle? If present, were these processes selectively affecting one or other, histochemically-defined 'type' of fibre?

It became apparent that there was a need for a means of measuring changes in the size of the whole quadriceps. Extrapolation from measurements of myofibre size was not good enough and limb anthropometry would underestimate changes localized to the

quadriceps. It was therefore only at this stage that significant effort was devoted to finding a way to obtain a satisfactory image of a transverse section through the whole quadriceps and so measure its cross-sectional area (Paper V). In retrospect, it is unfortunate that the full importance of measuring the whole muscle cross-sectional area was not appreciated sooner. It is clear, however, that any future studies of muscle atrophy or hypertrophy must include some such measure.

Choice of muscle

The quadriceps femoris is a large, proximal muscle of major, functional importance. It also seems to be selectively weakened in many conditions.

From a practical, investigative point of view, it has a number of features which make it particularly suitable for study. Most of the muscle acts across only one joint (the knee) and it is a simple matter to immobilise the other joint (the hip) while examining the muscle's mechanical characteristics through the lever system provided by the knee and lower leg. Large parts of the muscle are free of major nerves or blood vessels, making it possible to take repeated needle biopsy specimens in safety. A proximal tourniquet will effectively isolate the limb, facilitating study of the quadriceps' energy metabolism during anaerobic exercise.

The quadriceps proved to have a few disadvantages from an investigative point of view. It was not possible to produce electrically-stimulated contractions of the whole muscle without undue hazard. The heads of the quadriceps may differ in their fibre-type composition and possibly even in the ease with which they atrophy or hypertrophy. The quadriceps' muscle fibres do not run the full length of the muscle; changes in fibre thickness inevitably mean changes in their packing and their lines of pull.

Choice of pathology

(i) Reversible 'myopathies'

Clinical observation had indicated that the quadriceps is severely affected by the muscle weakness which accompanies several endocrine disorders. Patients with weakness due to osteomalacia or thyrotoxicosis are not uncommon in hospital practice. In addition, patients with hypothyroidism are known to have abnormally slow muscle relaxation and may sometimes be weak.

A further important point was that the muscle weakness of osteomalacia and of thyroid disease are both relieved by treatment of the underlying disorder. This made it possible to study the structural, chemical and contractile correlates of the weakness, not only cross-sectionally but also longitudinally.

(ii) 'Disuse'

As will be apparent in Chapters 6 and 7, it seemed likely that a major factor in the weakness of osteomalacia and that of thyrotoxicosis was atrophy of the muscle fibres. In osteomalacia this was sometimes particularly pronounced in the type II muscle fibres. This had been generally believed to be the result of disuse (Dubowitz & Brooke, 1973); it was presumed that patients with osteomalacia would only rarely activate their type II (high-threshold) muscle fibres since bone pain would discourage them from making strong or rapid muscle contractions. In order to test the suggestion that type II atrophy was simply the result of disuse the investigations were extended to include patients with unilateral thigh muscle wasting following knee immobilization for tibial fracture (Paper IV and Chapter 8). Experimentally this had the advantage that each wasted thigh was matched by a relatively normal thigh for comparison.

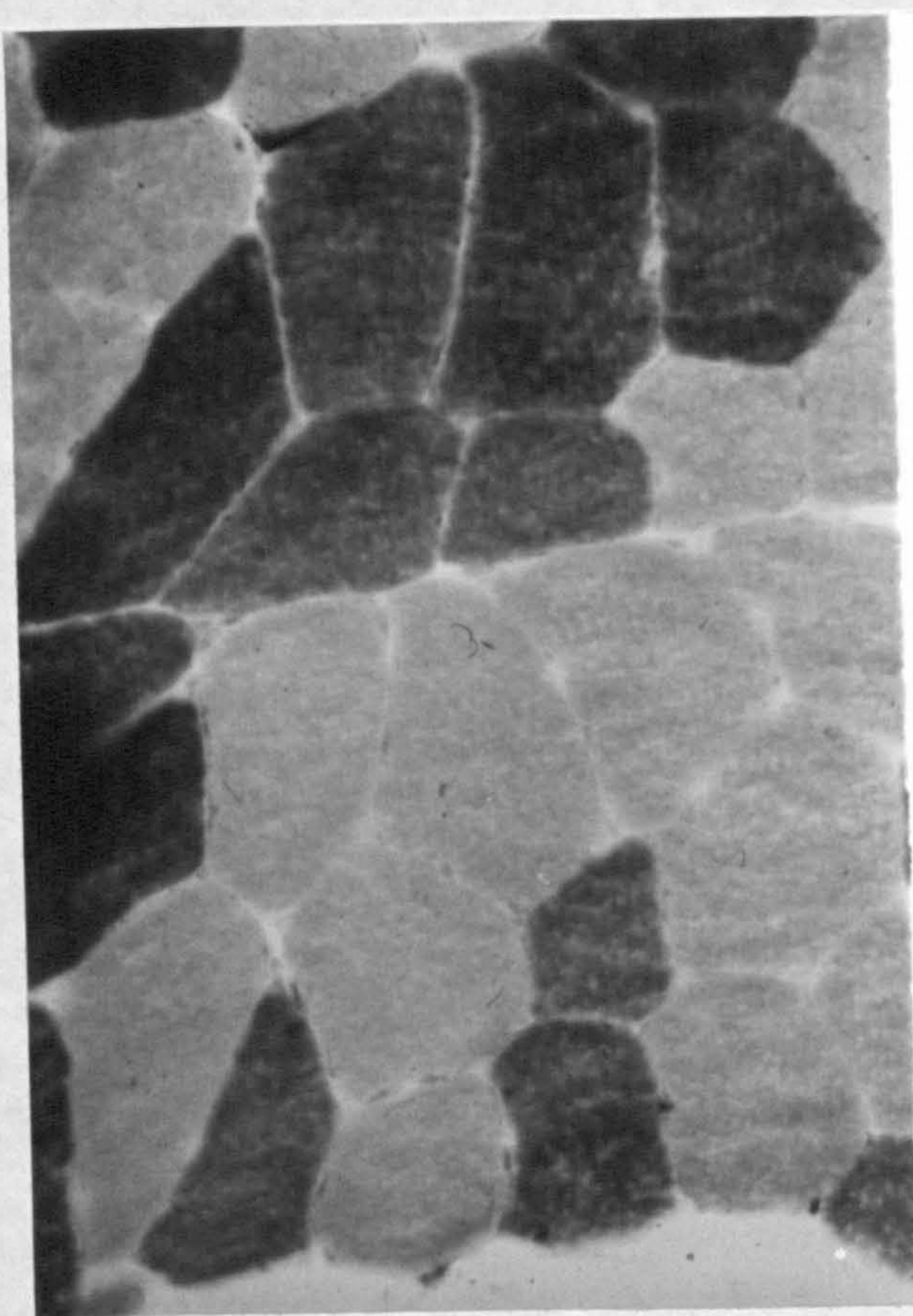
Contrary to expectation, these patients proved to have greater atrophy of their type I fibres than of their type II fibres. This prompted further study of patients with thigh muscle wasting secondary to knee injury and/or immobilization, particularly as there appeared to be important implications for the therapeutic exercise prescribed for such patients (Chapter 8, 'Discussion').

(iii) Fibre-type composition

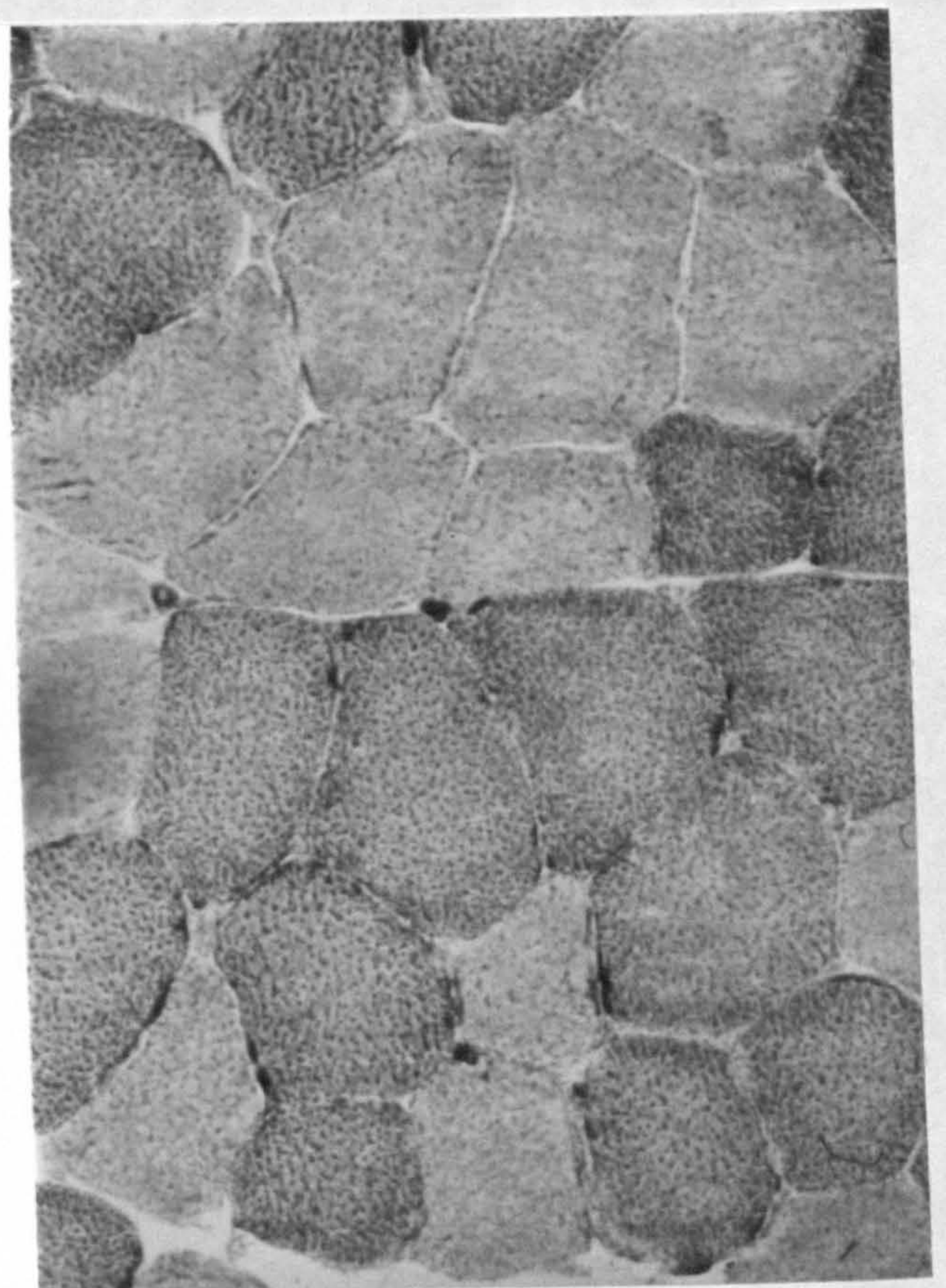
Human skeletal-muscle fibres may be classified histochemically into type I and type II fibres, according to their reaction for the enzyme myosin adenosine triphosphatase (Fig. 1.2). The classification and characteristics of human type I and type II fibres are discussed and tabulated in detail elsewhere (Edwards, Young & Wiles, 1980). Specific features will be discussed in the appropriate chapters of the thesis: only an outline is given here.

In general, type I fibres have slower, less fatiguable contractile characteristics and are well equipped for aerobic metabolism, containing numerous mitochondria and staining densely for the activity of oxidative enzymes (Fig. 1.2). Type II fibres have faster, more fatiguable contractile characteristics and (in normal, untrained men) less oxidative capacity, but are better suited for anaerobic glycogenolysis than are the type I fibres. Type II fibres can be further subdivided according to variations in the pH sensitivity of their ATPase. Type IIA fibres have higher oxidative enzyme activities than type IIB fibres.

There is a hierarchy of motor unit recruitment with increasing force of voluntary, muscle contraction. Units comprising type I fibres are utilised at low forces, whereas those made up of type II fibres are recruited only at high forces (Piehl, 1974; Gollnick, Karlsson, Piehl & Saltin, 1974). Similarly, in dynamic exercise of an intensity less than 90% of a subject's maximum aerobic power, the type I fibres are always first to become depleted of glycogen, implying that they have been used more heavily (Piehl, 1974; Gollnick, Piehl & Saltin, 1974).



a



b

Fig. 1.2 Serial transverse sections of a needle-biopsy specimen of normal human quadriceps, showing (a) myosin ATPase activity (type I fibres pale-staining), and (b) succinic dehydrogenase activity (type I fibres darker-staining).

In each of the conditions studied, and in normal subjects, particular attention was paid to the fibre-type composition of the quadriceps. It was believed that selective changes in the size or frequency of one or other 'type' of fibre might have important consequences for muscle function and/or implications for management.

Normal relationship between muscle size and strength

The thesis started with the general problem of the measurement of muscle weakness and the objective evaluation of its treatment. Potential techniques were then tested for their practical value in the analysis of muscle involvement in reversible, endocrine "myopathies". This in turn led to study of arthrogenous muscle wasting in the belief that it exemplified what appeared to be the major cause of weakness in the endocrinopathies, viz. atrophy. This required accurate measurements of whole muscle cross-sectional area. The result was that the experiments described in the thesis end by examining the more fundamental question of the relationship between the strength and the cross-sectional area of the normal human quadriceps. These studies led to important conclusions regarding the relative strength per cross-sectional area of type I and type II muscle fibres.

CHAPTER 2

NORMAL SUBJECTS, STATISTICS AND ETHICS

NORMAL SUBJECTS

STATISTICS

ETHICS

CHAPTER 2

NORMAL SUBJECTS, STATISTICS AND ETHICS

NORMAL SUBJECTS

The normal subjects were all healthy volunteers. None was unusually obese or thin. The subjects' ages are indicated in the relevant publications. In most cases, their habitual levels of physical activity ranged from very inactive to regular participation in recreational sport. A few of the subjects, however, were top-class athletes but there is little evidence that their inclusion influenced the conclusions drawn in the publications.

Most of the normal subjects were recruited from the author's professional and social circles. It must be recognised, therefore, that physically 'aware' people (e.g. physiotherapists, exercise physiologists and sportsmen) will inevitably be over-represented. The self-selection of subjects for a training study (Paper VII), by responding to an advertisement in the hospital newsletter, must be subject to the same general criticism.

STATISTICS

The statistical methods used in this work are indicated in the relevant sections. The choice was guided by Sokal & Rohlf (1969) except in Paper V where one of the co-authors (viz. M.J. Parker) was statistician to the Oxford Rehabilitation Research Unit.

ETHICS

The studies were conducted with the approval of the Research and Ethics Committee of the Royal Postgraduate Medical School and Hammersmith Hospital, the Army Personnel Research Committee, the

Committee of Ethics of Clinical Investigations at University College Hospital and the Nuffield Sector Ethics Committee, Oxford.

The patients and normal subjects gave their informed consent and participated on the understanding that the procedures were for the purpose of research. They were asked to sign an "informed consent form" before undergoing muscle biopsy (Appendix 3).

CHAPTER 3

NEEDLE BIOPSY OF MUSCLE

THE NEEDLE

TECHNIQUE

SPECIMEN HANDLING

Chemistry

Histochemistry

Micromorphometry

CONCLUSIONS

CHAPTER 3

NEEDLE BIOPSY OF MUSCLE

THE NEEDLE

As long ago as 1864, the French neurologist Duchenne designed an instrument for obtaining biopsy specimens of human muscle (Fig. 3.1) (Charrière & Duchesne (sic), 1864). The technique, however, was largely ignored until its re-introduction by Bergström in 1962. As it happens, Bergström was unaware of Duchenne's needle when he adapted a synovial biopsy needle in order to investigate muscle electrolytes in patients with renal disease. Bergström's needle and its descendants (e.g. Fig. 3.2) are, nevertheless, remarkably similar to Duchenne's "emporte-pièce histologique". Other designs of needle include the 'Jamshidi' needle and the 'Trucut' needle, but in most situations their disadvantages outweigh any advantages.

Initially, my colleagues and I used the design of muscle biopsy needle described by Bergström (1962). It became apparent, however, that others, performing the procedure less frequently than ourselves, were encountering difficulty in the manipulation of this instrument. I therefore modified the needle by fitting finger and thumb rings (Fig. 3.2) (Young, Wiles & Edwards, 1978a). (It later transpired (Correspondence, Lancet, 1, 153, 1979) that a very similar adaptation had already been devised and described by Nichols, Hazlewood & Barnes, 1968.) The modified needle (the "UCH muscle biopsy needle") was used almost exclusively for all needle biopsies performed by myself or my colleagues after the end of 1978. With a needle of 4.5mm outside diameter, a single pass usually yielded some 20-50mg of muscle (wet weight). The mode was about 40mg and the largest was 100mg.

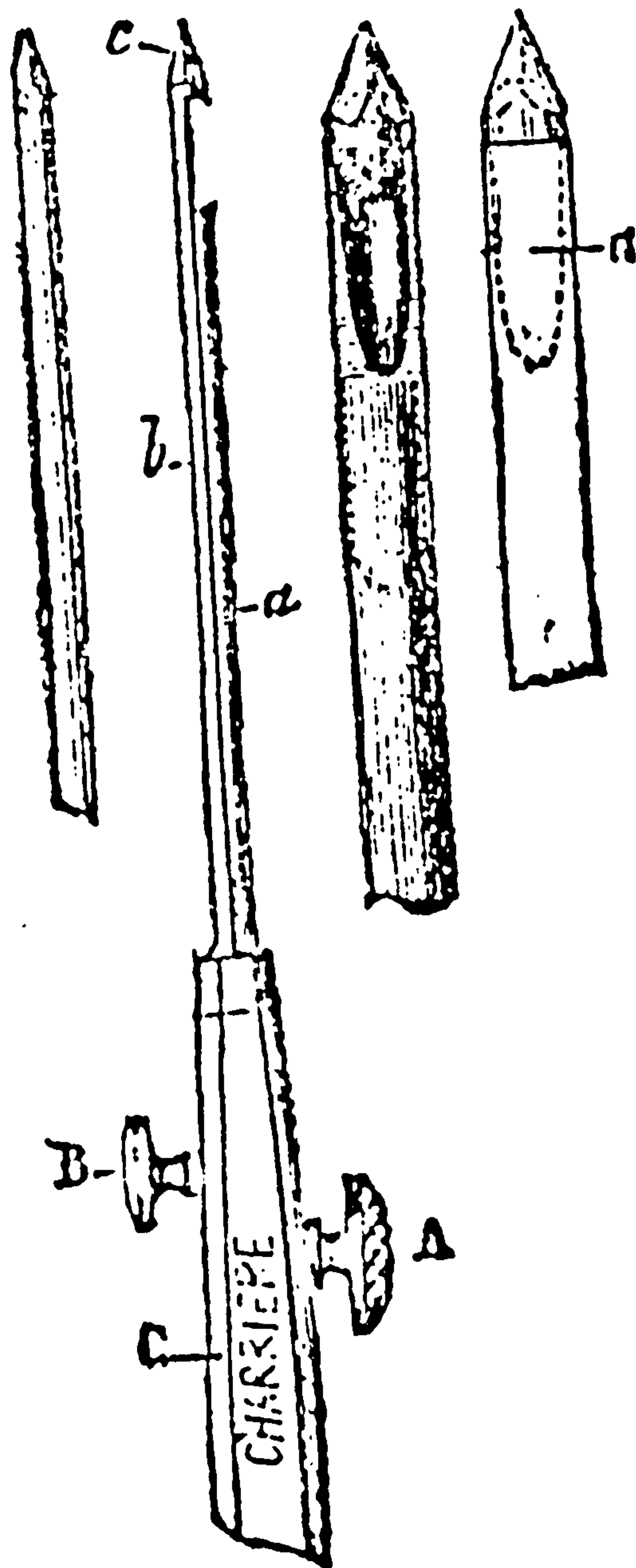


Fig. 3.1 Duchenne's "emporte-pièce histologique" for taking biopsy specimens of human muscle (Charrière & Duchesne (sic), 1864).

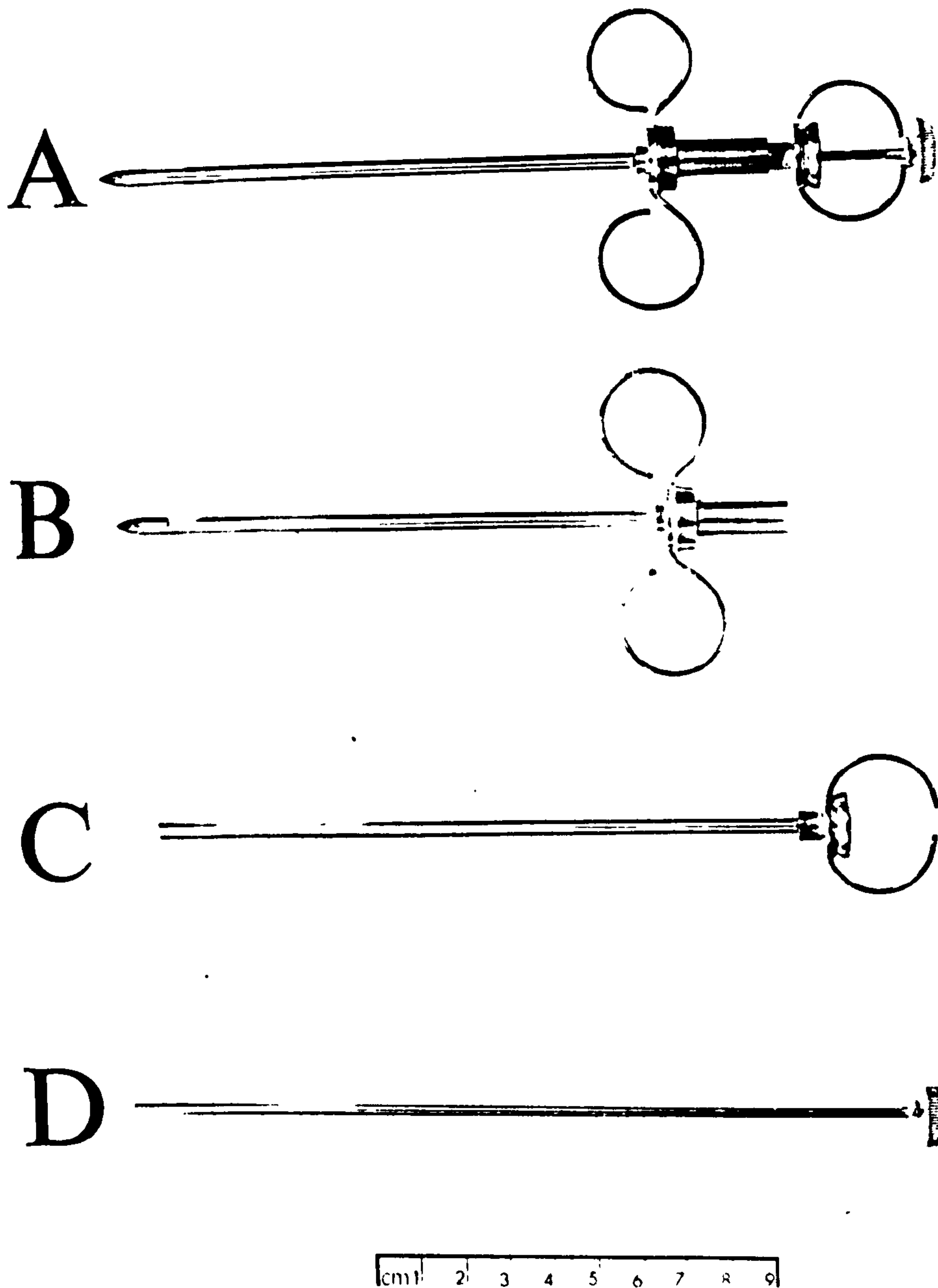


Fig. 3.2 U.C.H. muscle biopsy needle (Young et al., 1978a)

- (A) complete needle, as supplied
- (B) outer cylinder of needle (with side 'window')
- (C) inner cylinder (with cutting edge)
- (D) rod used to dislodge specimen from inner cylinder after completion of biopsy

TECHNIQUE

My colleagues and I described the technique in a recent review (Edwards et al., 1980), and a more detailed account of the technique was also prepared at the request of a colleague working in Fiji (Young, 1979b).

Biopsies were taken through an anteriorly placed incision and with a direction and depth of insertion of the needle such that specimens were taken from deep in the lateral mass of the muscle, in the same coronal plane as the femur. Judging from our own scans (Paper V) and from an atlas of cross-sectional anatomy (Eycleshymer & Schoemaker, 1911), it seems likely that in most cases the fibres sampled were taken from vastus intermedius, with occasional samples coming from vastus lateralis.

For most of this work, biopsies were taken at the level judged by eye to be approximately the junction of the distal and middle thirds of the thigh. For studies which combined scans with biopsies (Papers VI and VII), specimens were taken at the same level as the scans, viz. "mid-thigh". This was defined as half-way between the greater trochanter and the lateral joint line of the knee. In Studies VI and VII, therefore, specimens were taken at a rather more proximal level than in the earlier Studies. The difference between the sites was not great, however, since "mid-thigh" as defined is more distal than the mid-point of the thigh as judged by eye.

SPECIMEN HANDLING

Chemistry

The muscle contents of adenosine triphosphate (ATP), phosphoryl-creatine (PC), total creatine (TC), lactate (LA) and glycogen were

measured in several studies and these data are included in Papers II and III. Some unpublished results are also included in the thesis, although many of these have been reported in abstract form (Young, Jones, Maunder & Edwards, 1975 and 1977a).

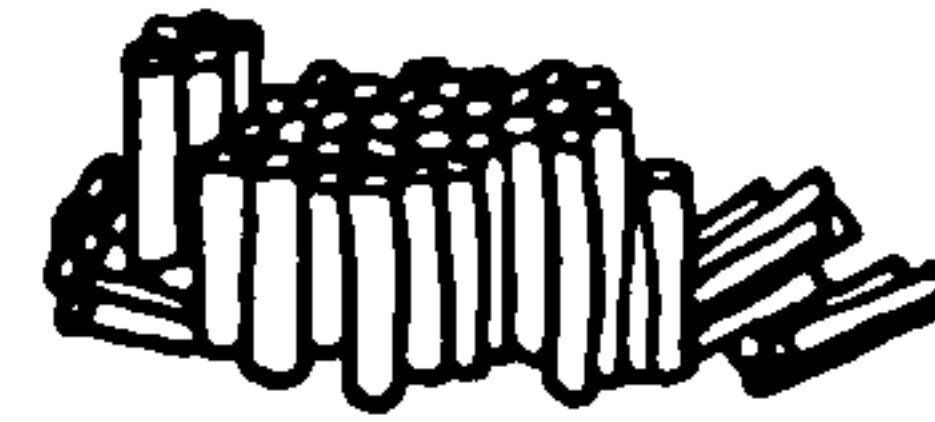
Within 2-3s of being taken, needle biopsy samples were plunged into isopentane cooled by liquid nitrogen. They were then freeze-dried while still inside the biopsy needle. After dissection to remove connective tissue, the samples were analysed by enzymic microanalytical techniques (Edwards, Jones, Maunder & Batra, 1975a). The variability and normal values for the analyses are described by Edwards et al. (1975a). The variability introduced by the sampling procedure is described by Harris, Hultman & Nordesjö (1974).

Histochemistry

Standard histological and histochemical techniques were used for Studies II-IV. The procedure for demonstrating myosin ATPase activity was improved by my colleagues during the course of this series of investigations and their modified procedure was used for Studies VI and VII (Round, Matthews & Jones, 1980). This improved the consistency with which sections were stained satisfactorily but did not alter the staining characteristics of the muscle fibres.

The other important point about the preparation of specimens is that they were all orientated at x10 to x30 magnification under a dissecting microscope before freezing (Fig. 3.3) in order that the maximum number of fibres should be cut in true transverse section (Edwards, Maunder, Lewis & Pearse, 1973).

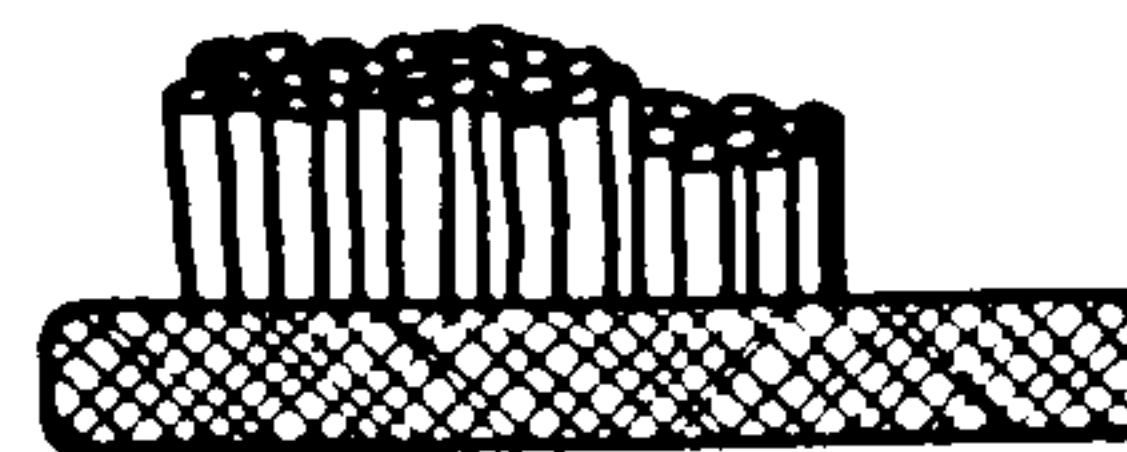
1. Sample removed from biopsy needle and left to 'rest' for 10 minutes.



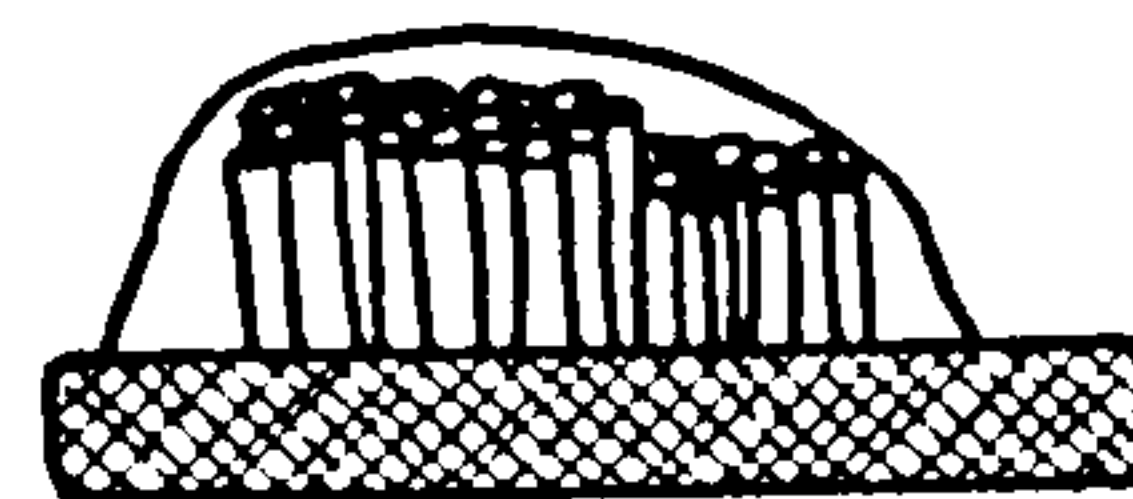
2. Small piece of sample processed for electron microscopy.



3. Large piece examined under dissecting microscope and fibres vertically orientated.



4. Covered with mounting medium to prevent drying and to support tissue.



5. Frozen in isopentane for histochemical studies.

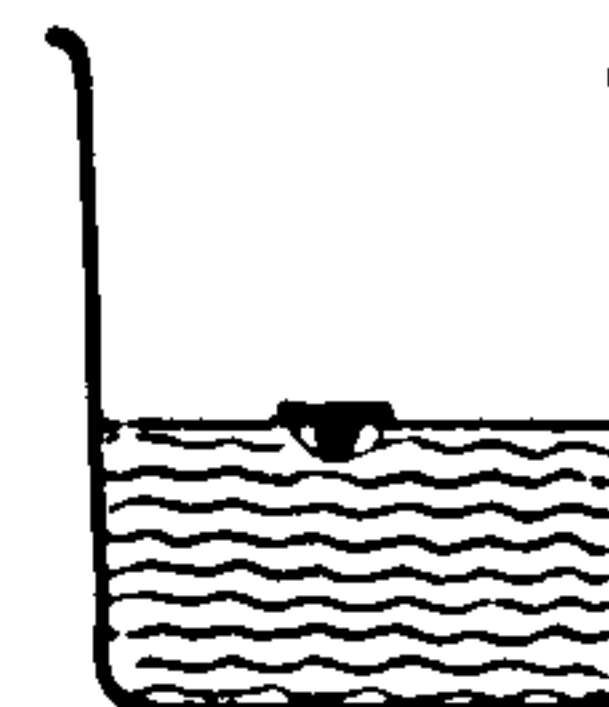


Fig. 3.3 Preparation of needle biopsy specimen of muscle for sectioning, mounted on a cork disc.

Micromorphometry

The relative frequency of type I and type II muscle fibres was established by counting at least 200 fibres in each biopsy. If two specimens were taken from the same site on the same occasion, the mean of the two results was used (giving each specimen equal weight).

Muscle fibre cross-sectional area was measured for 100 fibres of each type in each biopsy. Again, if two specimens were available, the final results were taken as the equally-weighted means of the results calculated for each specimen separately. The mean fibre area of the type I fibres (MFA I) and the mean fibre area of the type II fibres (MFA II) were combined with the relative frequency of the two fibre types to calculate the overall mean fibre area (MFA) for each biopsy and the percentage contribution of type II fibres to the cross-sectional area of the biopsy (%CSA II).

The method used for measuring muscle fibre cross-sectional area was changed in the course of this series of studies. At first, the parameter actually measured was the "lesser diameter" of each fibre (Dubowitz & Brooke, 1973) and the cross-sectional area was then calculated on the basis of an assumed circular cross-section. The results in Paper IV were obtained by measuring the lesser fibre diameter with an eye-piece micrometer. For Papers II and III the method of choice was a ruler applied to a magnified drawing of the lesser diameters, prepared with the aid of a microscope drawing tube. Four sections were measured twice by one method and twice by the other method. There was no evidence of a difference in the variability of the results obtained by the two methods ($CV < 5\%$) nor of a difference in the values obtained for the ratio MFA II/MFA I ($P = 0.5$; two-way analysis of variance, using log-transformed data). There was, however, a highly significant tendency for the second method to give values for MFA which were larger by about 20% ($P < 0.005$; two-way analysis of variance). This, coupled with the very wide range of

normal values for fibre size, explains why, in Papers II, III, IV, VI and VII, we have not placed much emphasis on direct comparisons of the MFA values for different groups of subjects. We have preferred to concentrate on bilateral or longitudinal comparisons within the same individuals.

Shortly before work started on Studies VI and VII, we became aware of work which strongly suggested that the lesser-diameter method for measuring myofibre radial size was unsatisfactory (Clancy & Herlihy, 1978). The lesser-diameter method consistently underestimates the true MFA since the fibres are not cylindrical. The principal argument for using the method had been that it would avoid any errors due to obliquity of sectioning (Dubowitz & Brooke, 1973, p. 99). Clancy and Herlihy showed, however, that obliquity of section (even up to 25°) affects fibre area to an insignificant degree by comparison with other sources of error. We therefore changed to measuring the actual cross-sectional area of the transversely sectioned muscle fibres (Papers VI and VII). This was done with the aid of the MOPPET system (Round, Jones & Edwards, 1982): the fibre outlines are superimposed, via a microscope drawing tube, on to a Reichert-Jung MOP-1 planimetry drawing-tablet and the data are ordered by means of a PET desk-top computer. The coefficient of variation of repeated MFA estimates is still less than 4% and there is a close correlation between the MFA values obtained by this method and by the second of the lesser-diameter methods ($r = 0.96$; $n = 23$). Planimetric values for MFA were 46% greater than those derived from lesser diameters. (Some of the normal MFA values in Paper VI were actually "corrected" from values derived by the lesser-diameter technique.)

After taking repeated biopsies through the same skin incision, the "within subject" coefficients of variation are: MFA - 16%, II % - 17%, %CSA II - 16% (Paper III). It is clear that sampling is a major source of variation. Particularly in the later studies

(Papers VI and VII), we attempted to reduce this by taking duplicate biopsies whenever possible and analysing them separately. No-one has examined the variation of myofibre size encountered across the thickness of the lateral quadriceps in man. Fibre-type frequency, however, has been studied by Lexell, Henrikson-Larsén & Sjöström (in press) and there is a systematic decrease in type II fibre frequency at increasing depth in the human quadriceps (Fig. 8.12). This is an unavoidable limitation to any attempt to correlate the function of the whole muscle to the structure of a tiny part.

CONCLUSIONS

Needle biopsy of the quadriceps is an acceptable and technically simple procedure.

Repeated biopsies assayed for ATP, PC, TC, LA and glycogen give consistent results.

Micromorphometric analyses of repeated biopsies are subject to rather high variability. This may be due to the structural inhomogeneity of the muscle.

CHAPTER 4

ISOMETRIC STRENGTH OF THE NORMAL HUMAN QUADRICEPS

INTRODUCTION

SUBJECTS

METHODS, RESULTS AND DISCUSSION

Technique

Validity

Effect of body weight

Effect of leg length

Repeatability

CONCLUSIONS

CHAPTER 4

ISOMETRIC STRENGTH OF THE NORMAL HUMAN QUADRICEPS

INTRODUCTION

The assessment of muscle function is a necessary part of clinical medicine. Since the 'purpose' of muscle is to develop tension, the assessment of muscle function must include studies of force generation. Manual testing is of little or no value for the study of a large muscle group, such as the quadriceps femoris, unless the patient is extremely weak (Beasley, 1961). The quadriceps is usually tested by observing the patient's performance in activities such as rising from a chair or climbing stairs. These tests are influenced by many factors other than the state of the muscle itself. In a sense, this increases their value in the management of the 'whole patient', but it also means that they do little to improve our understanding of the cause of the patient's disability.

The first and simplest requirement is the measurement of voluntary isometric strength. A wide variety of dynamometers have been described over the past two to three hundred years (Pearn, 1978) and the concept that dynamometry might have clinical applications is far from new:

"Ne pourrions-nous pas acquérir des connaissances non moins importantes, si nous avions un moyen facile pour mesurer ... nos forces relatives dans les différens âges de la vie et dans les différens états de santé?"
(Regnier, 1798)

Yet objective testing of muscle strength is almost unknown in routine clinical practice. It was necessary, therefore, to start this series of investigations by examining a simple form of dynamometer that could be readily applied to the quadriceps femoris, a clinically and functionally important muscle which is also suitable for parallel studies of its structure and chemistry.

The work then progressed to establishing the suitability of repeated measurements of the voluntary, isometric strength of the

quadriceps for following changes in muscle function during the treatment of disease, following disuse, or during physical training. Normative data were collected against which to judge the strength of individual patients.

SUBJECTS

The subjects and their ages are described in Paper I. Their levels of habitual physical activity covered the complete 'normal' range. In addition, the study included some elite athletes. The female subjects included 12 skiers from whom the British team was to be selected. Their results were similar to those for the other females studied. The male subjects included the world champion squash player, a former international breast-stroke swimmer and an elite ultra-long distance runner. For their weights, the former swimmer was the strongest man studied, the squash player was among the strongest, and the runner was among the weakest.

METHODS, RESULTS AND DISCUSSION

Technique

The published measurements of quadriceps strength (Papers I - III, and VII) were all made with the adjustable, straight-backed, muscle-testing chair described and illustrated in Paper I. The pelvis was stabilised by an adjustable lap strap. The force of a quadriceps contraction was transmitted to a strain gauge by an inextensible strap looped around the ankle, immediately proximal to the malleoli.

The strength testing procedure is described in Paper I. "Strength" was measured as the best of at least 3 maximal voluntary contractions (MVCs). The procedure evolved from experience of measuring both voluntary strength and other physiological indices dependent upon a maximal voluntary effort. For example, the repeatability of some pulmonary function tests depends upon the

operator's skill in enlisting the subject's full cooperation. Freedman & Prowse (1966) recommended a similar routine for the measurement of the forced expiratory volume in 1 second ($FEV_{1.0}$). No doubt they were also influenced by a comment made by Hutchinson (1846) when reporting his measurements of the forced vital capacity of 2130 subjects:

"Each of these individuals breathed three consecutive times into the spirometer because either from timidity or inexperience, the first observation is frequently not a correct experiment, but by three observations the point sought for is accurately determined."

Validity

An important question to be considered is whether the quadriceps is capable of producing a greater isometric tension than that seen during a maximal voluntary contraction. As discussed in Paper VII, opinion is divided. Some, basing their argument on an increase in the integrated surface electromyogram recorded during a maximal voluntary contraction, believe that the voluntary recruitment of motor units may be increased by strength-training (e.g. Komi, Viitasalo, Kauramaa & Vihko, 1978; Moritani & de Vries, 1979). Others point to the evidence - provided by Merton (1954) for adductor pollicis, and by Edwards and his colleagues for the quadriceps (Paper I; Edwards, 1975; Bigland-Ritchie, Jones, Hosking & Edwards, 1978) - that, in an isometric contraction, the well-motivated subject can activate a muscle as fully with a maximal voluntary effort as with supramaximal electrical stimulation of the motor nerve.

Edwards (1975) included a number of patients in his demonstration that the metabolic heat production by the contracting muscle was the same during maximal voluntary contractions as it was during maximal electrical stimulation of the nerves to parts of the quadriceps. Nevertheless, it is not always as easy to motivate a patient to make a maximal contraction as is the case with normal subjects. This is particularly so if the contraction causes, or is expected to cause, pain. The identification of pain-limited efforts was particularly important when studying patients with osteomalacia

although, as explained in Paper II, none of the reported measurements of strength was considered to have been limited by pain.

One of the patients with osteomalacia suffered pain in the hip during dynamometry of the opposite quadriceps to that reported in Paper II. As treatment with vitamin D gradually reduced the level of pain, the shape of her force record changed, demonstrating how the pain-limited effort can sometimes be identified (Fig. 4.1); there is a slow and tentative rise of force which cannot then be maintained. Even the release of tension may be done cautiously. The normal subject applies force rapidly, holds it steadily and releases it rapidly again (Beasley, 1961).

Submaximal efforts, whether in an attempt to deceive or because of pain, sometimes result in greater differences between successive MVC trials than are seen with genuinely maximal efforts, unhampered by pain. For 50 consecutive patients with muscle-related symptoms the median percentage difference between the best and second best MVC trials made by the stronger leg during a single test session was only 2.3%, comparing well with a median value of 2.1% obtained for 50 consecutive normal subjects (including 12 of the 15 top-class athletes who participated in Study I - vd. Chapter 2) (Young, 1979a). Although the range of differences was much greater for the patients (0 - 19%) than for the normal subjects (0 - 8%), most of the patients showed the same high degree of consistency as the normal subjects, and at least 2 of the other 8 patients were already known to have a significant psychological component to their illness. This is further suggestive evidence that, in the absence of pain, the skilled investigator can persuade most patients to make a truly maximal quadriceps contraction.

Effect of body weight

In normal subjects, muscle strength (especially for a weightbearing muscle such as the quadriceps) is usually closely related to body weight (e.g. Beasley, 1961; Tornvall, 1963; Cuddigan, 1973; Hosking, Bhat, Dubowitz & Edwards, 1976; Hosking, Young, Dubowitz & Edwards, 1978). This was also the case in Paper I, where

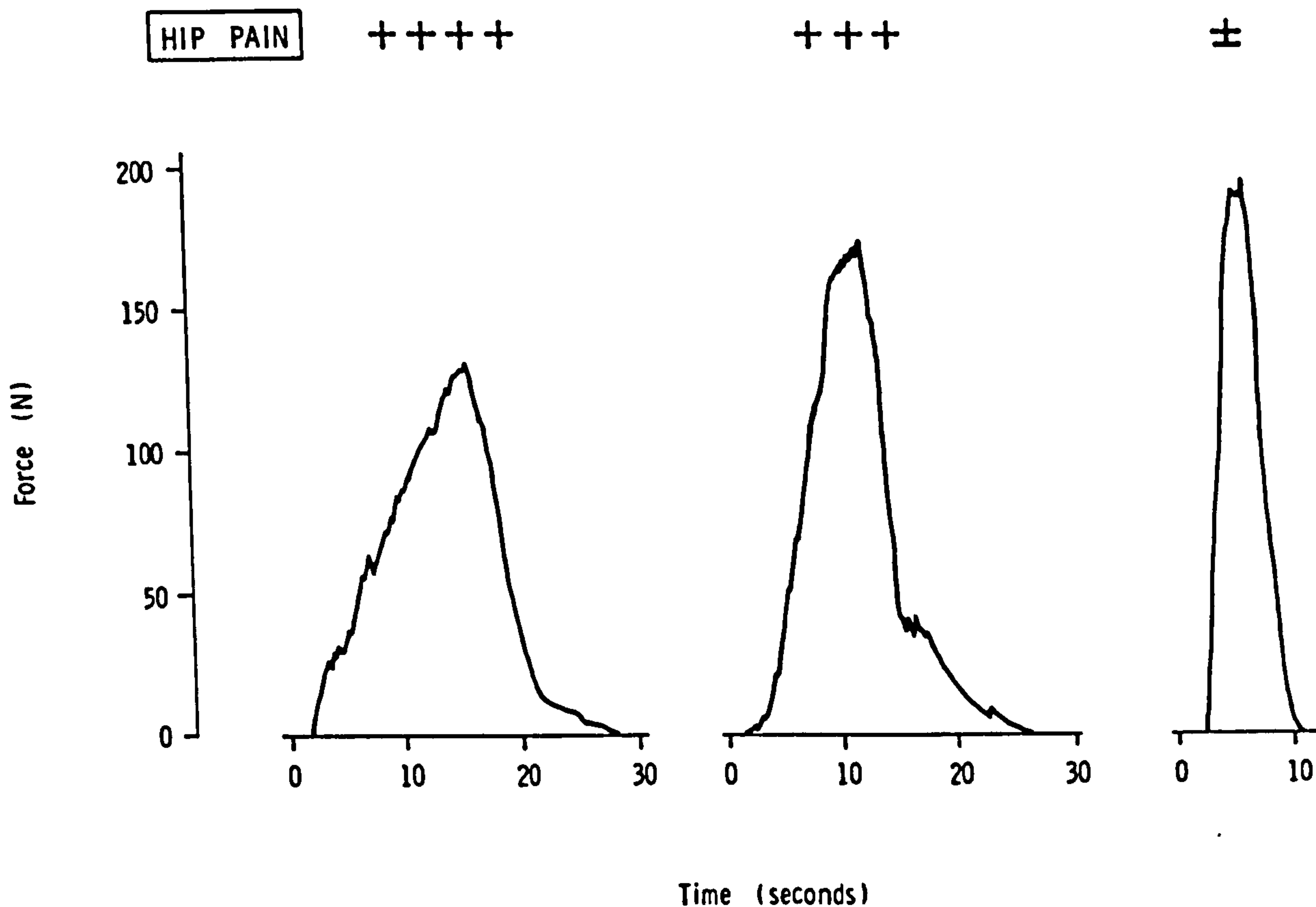


Fig. 4.1 The effect of hip pain on the shape and height of the isometric force record obtained during supposedly maximal voluntary quadriceps contractions performed by a patient with osteomalacia before, and at two stages during, her treatment with vitamin D.

The third effort is considered not to have been limited by pain but to be a genuine measure of the patient's quadriceps strength.

the wide range of body weights resulted in a high coefficient of correlation ($r = 0.92$; $n = 145$). Other indices with which quadriceps strength is correlated, such as lean body mass or, perhaps, total body potassium, are not only less convenient to measure than body weight but they offer no practical benefit as indicators of the quadriceps strength which would be expected in the absence of disease or atrophy. Quadriceps strength may also be related to height, especially when dealing with children (Hosking et al., 1976). Nevertheless, we prefer weight as a reference standard since it seems more relevant to function when judging the performance of a weight-bearing muscle.

While a reference standard, such as body weight, is necessary for comparisons between patients or between patients and normal subjects (as in Papers II and III), it is much less important when repeated strength measurements are made on the same individual(s) over a period of treatment or training (Papers II, III and VII; Edwards, Wiles, Round, Jackson & Young, 1979).

As is evident from the discussion in Paper II, the former class of comparison must be treated cautiously because of such factors as the scatter of 'normal' strength values at any one weight and the difficulties inherent in selecting normative data which are appropriate to the patients being studied. Nevertheless, it is often useful, when testing a patient's quadriceps strength, to have some indication of his expected strength. A helpful, approximate rule is that an adult's predicted 'normal' MVC (in kg force) is about 75% of his body weight, and the 'lower limit of normal' (i.e. the third centile) is about half the body weight (Paper I). At low body weights this simple guide no longer applies and performance has to be compared with the regression line (for strength on weight) obtained from comparable normal subjects.

Alternatively, it is sometimes helpful to use a graph such as that in Figure 4.2 (derived from the data in Paper I) to compare the relative severity of weakness shown by patients of different body weights (Young, 1979a).

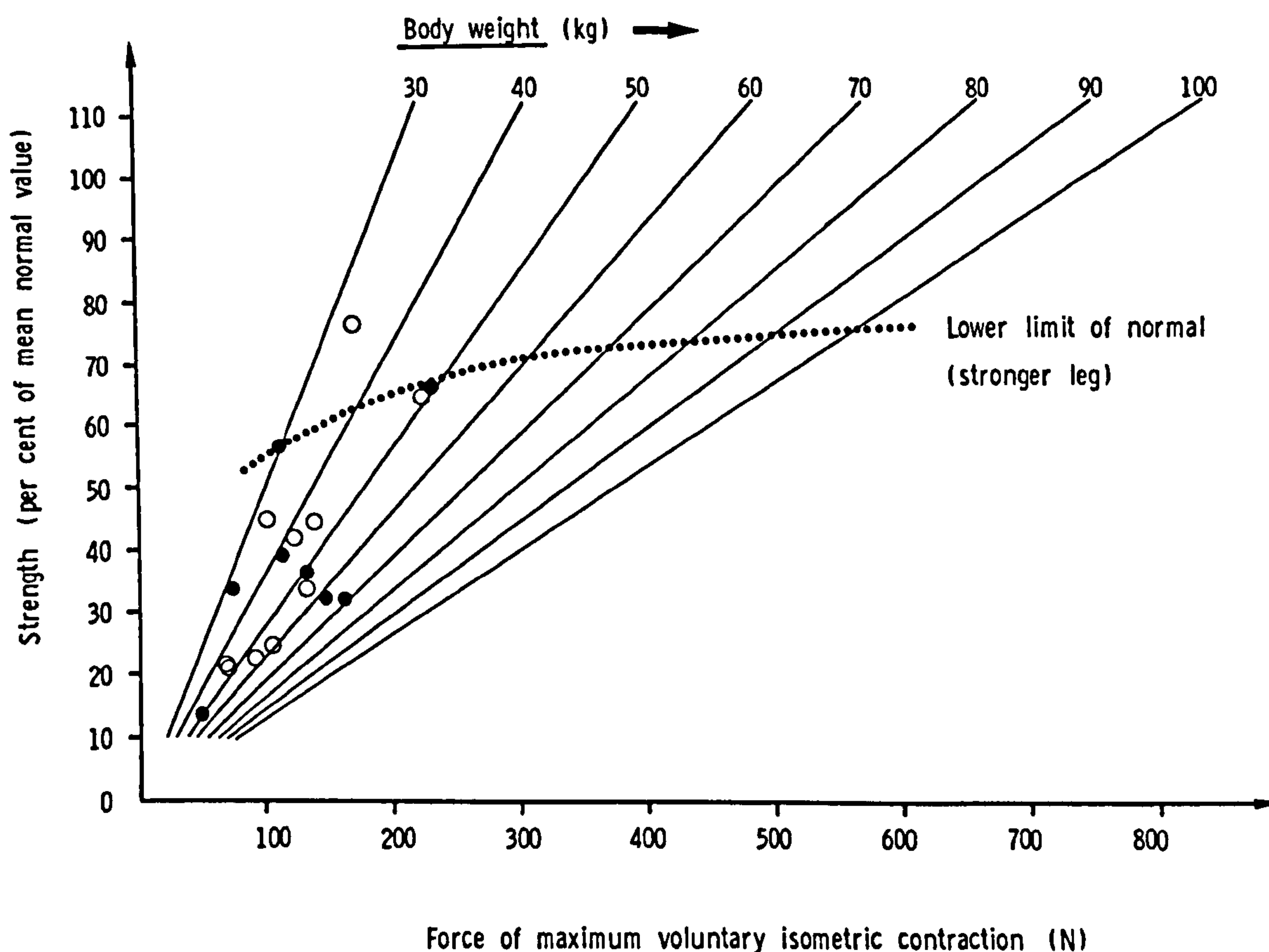


Fig. 4.2 This figure allows a rapid comparison of the relative strengths (of the stronger quadriceps) of patients of different body weights. It is based on the regression line described in Paper I, with the lower limit of normal strength being calculated as 2 SD below the mean for data groups at intervals of 10kg body weight.

The data plotted to illustrate the use of the figure are those of Figure 6.4 (● = nutritional osteomalacia, ○ = osteomalacia from other causes).

Effect of leg length

Despite the practical usefulness of body weight as a reference standard for quadriceps strength, studies designed to eliminate the need for such a standard are to be preferred, i.e. longitudinal comparisons or comparisons between the two limbs of the same individual. Such studies also eliminate any problems due to the fact that the muscle testing chair actually records the force exerted at the end of a lever, the lower leg. At first sight, it might seem preferable to record strength as the torque generated about the axis of the knee joint. In practice, however, this does not appear to be so; principally because the scatter of the strengths recorded for subjects of the same body weight is large when compared with their range of lower-leg length. Moreover, measuring the length of the lever arm introduces another potential source of error. Taking the length of the lever arm into account made little difference to the correlation between quadriceps strength and body weight in 25 normal subjects (Chapter 9).

In addition, the quadriceps is a pennate muscle. The number of sarcomeres pulling in parallel and the angle of pennation are therefore functions of muscle length (in addition, of course, to being functions of muscle cross-sectional area). The length of the quadriceps, in turn, is closely related to the length of the lower leg. The effect of being tall on the length of the lever is therefore at least partly offset by the quadriceps' fibres being more numerous and/or more longitudinally orientated. Nevertheless, the use of longitudinal or bilateral comparisons avoids the problem altogether.

Repeatability

Longitudinal studies of muscle strength require that the measurement should be reproducible. The voluntary isometric quadriceps strength of 7 normal subjects (5 female, 2 male) was measured (as the best of at least 3 MVCs) on 4 separate occasions, at least 5 days apart (to minimise any training effect), over a period

of 50 days. The 'within subject' variance gave coefficients of variation of 4.4% (right) and 5.2% (left) (Young, 1979a).

Somewhat greater 'within subject' variability was shown by the 20 subjects recruited for the training study (Chapter 9) when their strength was measured on 3 consecutive days before the start of training (CV = 6.6% and 8.2%). It is not known why these subjects' strength measurements were perhaps less reproducible ($P \div 0.05$, F_{\max} test) than those obtained before, particularly as there was no evidence of an order effect (implying training or learning) over the 3 days ($P > 0.25$, one-tailed analysis of variance).

Tornvall (1963), recording maximum, voluntary, isometric strength in much the same way as ourselves, measured 44 subjects on 2 occasions separated by 1½ hours: he found coefficients of variation of 4.4% (right) and 3.9% (left). A coefficient of variation of 4% was also reported by Thorstensson, Larsson, Tesch & Karlsson (1977).

CONCLUSIONS

In well-motivated subjects the force of the best of at least 3 maximal, voluntary contractions of the quadriceps femoris (with the knee flexed to 90°) is a repeatable and valid measure of the muscle's isometric strength. This measure has considerable potential for clinical application (vd. ensuing chapters).

In normal children and adults, the isometric strength of the quadriceps is proportional to the body weight. This is useful for the objective evaluation of a patient's complaint of weakness, allowing the recognition of weakness of quadriceps muscles which would otherwise be classed as grade 5 (Medical Research Council, 1943).

CHAPTER 5

ISOMETRIC RELAXATION RATE OF THE NORMAL HUMAN QUADRICEPS

INTRODUCTION

METHOD

SUBJECTS, RESULTS AND DISCUSSION

Normal values

Effect of stimulus strength

Repeatability

Comparison with other muscles

Effect of fibre-type composition

- (i) Experiment 1 - *in vitro* (Dr. R.F.W. Moulds)
- (ii) Experiment 2 - normal subjects
- (iii) Experiment 3 - patients
- (iv) Experiment 4 - normal subjects (Dr. C.M. Wiles)
- (v) Discussion of experiments 1-4

RELAXATION RATE AS A PREDICTOR OF FIBRE-TYPE COMPOSITION

CONCLUSIONS

CHAPTER 5

ISOMETRIC RELAXATION RATE OF THE NORMAL HUMAN QUADRICEPS

INTRODUCTION

In addition to evaluating the maximum force which a muscle can develop, it is desirable to be able to say whether it produces force normally. This led to the development of indices of muscle 'contractility' which were (i) independent of volition, (ii) independent of muscle mass (which we were unable to measure at that time), and (iii) applicable to the quadriceps. Such tests had previously been limited in their use to the examination of peripheral muscles with accessible motor nerves (references in Paper I). Paper I reports indices of muscle contractility which may be recorded for a substantial part of the quadriceps during transcutaneous electrically-stimulated contractions. It also reports normal values for these indices against which patients' results may be judged. The values are comparable with those for the adductor pollicis studied by more conventional electrophysiological means (Paper I) and with those for strips of excised human muscle studied in vitro (Moulds, Young, Jones & Edwards, 1977).

The speeds of contraction and relaxation are closely linked with the rates of development and decay of the active state. They influence the power output which can be developed by a given strength of muscle, the tension and degree of fusion of a tetanic contraction (e.g. Figs. 5.1 and 7.3), and therefore the ability to sustain a contraction (e.g. Chapter 7). When studying tetanic contractions in vivo it is technically easier to study the rate of relaxation rather than of contraction.

Measurements of speed may be made both for muscle twitches and for tetani. In vivo studies in man have tended to concentrate on twitches rather than tetani. The principal reason for this has probably been the relative discomfort of high frequencies of electrical stimulation. It seems likely that another important

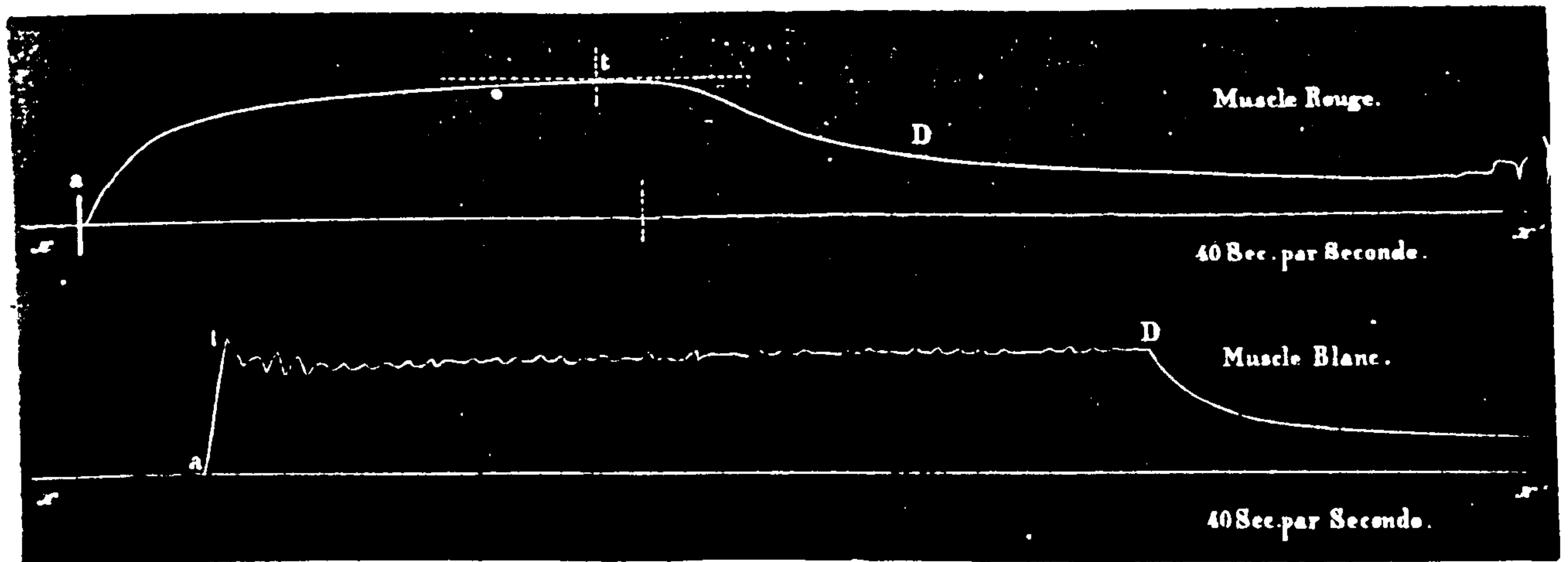


Fig. 40. — Tétanos du muscle rouge et du muscle pâle produits par un même nombre de secousses. — $x x'$, axe des temps. — a , début, — t , sommet du tétanos, — D , décontraction.

Fig. 5.1 Early demonstration (Ranvier, 1880) of the tetanic properties of "red" muscle (comprising principally type I fibres) and "white" muscle (comprising principally type II fibres).

factor has been the feeling that the single twitch is a more fundamental expression of muscle contractility than the tetanus. However, detailed study of the characteristics of the twitch is complicated by its susceptibility to a wide variety of influences which are either difficult or impossible to control when studying the intact subject. Moreover, the twitch is probably a rather artificial phenomenon; during voluntary activity it is likely that individual motor units rarely fire at less than 10Hz (Bigland & Lippold, 1954). Typical firing frequencies during voluntary muscular activity are probably within the range 20-40Hz (Bigland & Lippold, 1954; Freyschuss & Knuttsson, 1971; Marsden, Meadows & Merton, 1971; Grimby & Hannerz, 1977). The indices used in the present studies were therefore measured for tetanic contractions, induced by stimulation at 30Hz.

The other major difference between the present series of investigations and previous related work is in the use of the quadriceps femoris as the experimental muscle. As discussed in Paper I, previous work has been limited to peripheral muscles and has usually required the presence of an accessible motor nerve trunk. The only studies which had quantified the mechanical responses of a proximal muscle in response to electrical stimulation had been those of Buchthal & Schmalbruch (1970) in which they studied the contraction speeds of twitches of individual motor units in the long head of the biceps brachii and the lateral head of triceps brachii in response to motor point stimulation.

This chapter looks in particular at the measurement, in vivo, of the rate of relaxation from tetanic contractions of the human quadriceps. It also considers whether measurement of relaxation rate might provide a non-invasive means of establishing the muscle's fibre-type composition.

METHOD

Quadriceps relaxation speed was measured with the same dynamometry system as voluntary strength. The procedure for

measuring the rate of relaxation from stimulated contractions is fully described in Paper I, along with details of the actual stimulation technique and its acceptability to experimental subjects.

The speed of muscle relaxation was expressed in two different ways. In the earlier studies (Paper I) the indices used were the times elapsing from the last stimulus until the force had fallen to 95% and to 50% of its plateau value, viz. SF_{95} and SF_{50} respectively. Later, the force signal was electronically differentiated with respect to time and the chosen index of the rate of muscle relaxation became the maximum percentage of plateau force lost per 10ms (=MRR). In fact, MRR could also be calculated from the earlier indices ($MRR = 450/(SF_{50}-SF_{95})$) (Wiles, Young, Jones & Edwards, 1979). Each individual's result was the mean of three or four tetani.

SUBJECTS, RESULTS AND DISCUSSION

Normal values

Paper I reported normative data for SF_{95} and SF_{50} obtained from 82 normal subjects. The results obtained for the elite athletes (vd. Chapter 4 "Subjects") did not differ from those of the other normal subjects and have not been considered separately. Additional analysis showed no evidence of a sex difference (Table 5.1) nor of an age effect over the range 15 - 54 years (Table 5.2). In 16 children under the age of 12, however, mean SF_{95} was shorter (57.4; SD = 7.9) and SF_{50} longer (114.3; SD = 9.8) (Hosking et al., 1978).

It is not clear why children should have a shorter SF_{95} and a longer SF_{50} (and therefore a slower MRR) than adults. The former may perhaps reflect less influence of the series elastic component in the shorter muscle and the latter a lower intramuscular temperature. Nevertheless, the differences are small when compared with the changes in relaxation speed observed in thyroid disease, especially the slowing of relaxation observed in hypothyroidism (Paper III).

TABLE 5.1 Effect of sex on the time (ms) taken for the force of an electrically stimulated quadriceps contraction to fall to 95% and 50% of its plateau value (normal subjects)

		Mean	SD	n
SF ₉₅	male	60.2	4.1	46
	female	60.6	4.3	36
SF ₅₀	male	102.9	7.9	45
	female	106.9	10.7	36

TABLE 5.2 The effect of age on the time (ms) taken for the force of an electrically stimulated quadriceps contraction to fall to 95% and 50% of its plateau value (normal subjects)

	Age range	Mean	SD	n
SF ₉₅	15-24	59.9	3.6	27
	25-34	60.7	4.3	32
	35-44	61.7	5.2	10
	45-54	61.8	3.2	5
SF ₅₀	15-24	104.1	9.7	27
	25-34	103.7	10.2	32
	35-44	107.3	9.9	9
	45-54	107.6	7.7	5

Effect of stimulus strength

Two strengths of quadriceps stimulation were used to measure SF₉₅ and SF₅₀ in 9 normal subjects. Each subject was tested twice, using the same leg, but with the higher and lower stimulus strengths being applied in the opposite order. On both occasions, the voltages used were adjusted so that stimulation at 30Hz produced approximately 10% and 30% of the force of a maximal voluntary contraction.

Stimulus strength had no effect on SF₉₅ but SF₅₀ was slightly longer at the lower voltage ($0.05 > P > 0.025$) (Table 5.3). This effect, although still small, was even more clear-cut when MRR was calculated ($0.005 > P > 0.001$) (Table 5.3).

A similar experiment was conducted with measurements of MRR in 16 normal subjects (Wiles et al., 1979). Contractions equivalent to 10% and 30% of MVC were produced by stimulation at 80Hz. Once again, the mean MRR at the lower force was slower (by 0.5% of plateau force loss/10ms), but the difference was not statistically significant ($P > 0.1$). In this experiment MRR was also measured from voluntary contractions; relaxation was slower from 10% MVC than from 30% MVC than from 100% MVC, and only relaxation from 100% MVC equalled the rate of relaxation from stimulated contractions.

It would seem that stimulus strength may have a small effect on measurements of relaxation rate from stimulated contractions, possibly reflecting a degree of preferential type I fibre activation at low stimulus strengths. This might be more apparent at 30Hz (Table 5.3) than at 80Hz (Wiles et al., 1979) since, at the lower stimulus frequency, the activated type I fibres would produce a more completely fused tetanus than would the type II fibres. Any such effect, however, is small by comparison with the effect on MRR of the hierarchical recruitment of motor units in voluntary contractions (Wiles et al., 1979). For most practical purposes, stimulus strength has little or no effect on relaxation speed, provided that at least 10% of the muscle is activated.

TABLE 5.3 Effect of stimulus strength on the quadriceps' relaxation indices of 9 normal subjects, each studied twice

		Mean	SD	n
SF ₉₅ (ms)	10%MVC	63.7	4.4	18
	30%MVC	64.1	3.6	18
SF ₅₀ (ms)	10%MVC	110.4	9.8	18
	30%MVC	107.4	6.3	18
MRR (% of plateau force loss/10ms)	10%MVC	9.83	1.18	18
	30%MVC	10.49	0.96	18

Repeatability

The repeatability of in vivo measurements of quadriceps relaxation rate was assessed in 6 subjects retested on different days (17 tests in all). The 'within subject' coefficients of variation were $\leq 4\%$ for SF₉₅, SF₅₀ (Paper I) and for calculated values of MRR (Wiles et al., 1979).

Comparison with other muscles

The relaxation speeds of the quadriceps and adductor pollicis were compared in ten subjects (Paper I). They were similar, although the hand muscle had a rather shorter SF₉₅. The MRR values were extremely similar.

The speed of quadriceps relaxation from stimulated contractions in vivo was compared with the results obtained by a colleague (Dr. R.F.W. Moulds) for small strips excised from a variety of human muscles, and tested in vitro. Once again there was good overall similarity, although SF₉₅ was significantly shorter in vitro (Moulds et al., 1977).

In both instances the shorter SF₉₅ may reflect the smaller series elastic component of the shorter muscle (adductor pollicis), or of the excised piece of muscle, than in the quadriceps.

Effect of fibre-type composition

(i) Experiment 1 - in vitro (Dr. R.F.W. Moulds)

Type II muscle fibres relax faster than type I fibres, but their relative speeds are not known in man. The relaxation speed ($1/\text{SF}_{50}$) of isolated strips of human muscle was measured in vitro and compared with the percentage of type II fibres in the remainder of the 10 (surgical) biopsies from which they had been taken (Moulds et al., 1977). There was a close correlation ($r = 0.92$) and extrapolation of

the regression line estimated the ratio of the speeds of type II and type I fibres to be 4.25:1.

(ii) Experiment 2 - normal subjects

An analogous study was performed in vivo with 23 normal subjects. Quadriceps relaxation rate was compared with the contribution of type II fibres to the cross-sectional area of (needle) biopsies taken from the lateral mass of the muscle. Eight of the subjects had bilateral studies. In eight of the remaining subjects, however, biopsies and stimulation experiments were conducted on opposite legs. (This was considered acceptable since normal subjects already studied had shown substantial symmetry in respect of fibre-type composition or relaxation rate).

There was no correlation between the speed of the first 5% loss of force (i.e. $1/SF_{95}$) and the percentage contribution of type II fibres to the overall cross-sectional area of the fibres in the corresponding biopsy (%CSA II). There were, however, significant correlations between $1/SF_{50}$ and %CSA II ($r = 0.40$; $P < 0.05$) and between $450/(SF_{50} - SF_{95})$ - i.e. MRR - and %CSA II ($r = 0.49$; $P < 0.01$). Extrapolation of the model II regression lines for these relationships suggested relative speeds (II/I) of 1.22 (95% confidence limits = 1.15 - 1.27) and 1.47 (1.02 - 2.09) respectively.

For completeness, the 8 subjects in whom %CSA II and speed had been measured on opposite legs were replaced by a further 10 subjects in whom only one quadriceps was studied. There was then no correlation between relaxation rate and %CSA II (e.g. for MRR, $r = 0.13$; $P > 0.4$) (Wiles et al., 1979).

(iii) Experiment 3 - patients

The biopsy and relaxation rate data were examined retrospectively for 62 patients from the muscle clinic. Some had 'primary' muscle disease, some had unexplained muscular weakness,

fatigue or pain, and some had weakness secondary to a non-muscular primary disease. None had biopsy evidence of dystrophy or denervation. Patients with osteomalacia or thyroid disease were also excluded; they will be considered separately (Chapters 6 and 7). The patients were otherwise unselected. Most had had biopsies and stimulation studies on opposite legs.

There was a significant positive correlation between $1/SF_{50}$ and %CSA II ($r = 0.55$; $P < 0.001$) and between $450/(SF_{50} - SF_{95})$ - i.e. MRR - and %CSA II ($r = 0.49$; $P < 0.001$). Once again, the (model II) regression lines suggested that type II fibres are $1\frac{1}{4}$ - 2 times as fast as type I fibres (1.44 and 1.68 times respectively) (Fig. 5.2).

(iv) Experiment 4 - normal subjects (Dr. C.M. Wiles)

A rather different approach to the question was used by my colleague Dr. C.M. Wiles. He compared quadriceps MRR values, obtained from high and low force voluntary contractions, with the needle biopsy findings in the same muscle in ten normal subjects (Wiles et al., 1979). In each case, relaxation was faster from the high force contractions. Assuming that only type I fibres were recruited for the low force contractions (10% MVC) and that all fibres were active during maximal contractions, Dr. Wiles calculated the mean ratio of type II relaxation rate to type I relaxation rate to be 2.1 (SD = 0.46).

(v) Discussion of experiments 1 - 4

The general impression which emerges from these studies is that, during relaxation, type II fibres are probably $1\frac{1}{4}$ - $2\frac{1}{2}$ times as fast as type I fibres. The most discrepant finding is the ratio of 4.25 obtained in vitro (Moulds et al., 1977); this is more in keeping with the value of 6.0 obtained by Bolstad & Erslund (1978) for the ratio of the energy turnover rates of fast and slow fibres, calculated from measurements of the maximum rate of heat production during isometric contractions of different muscles. Essén, Jansson,

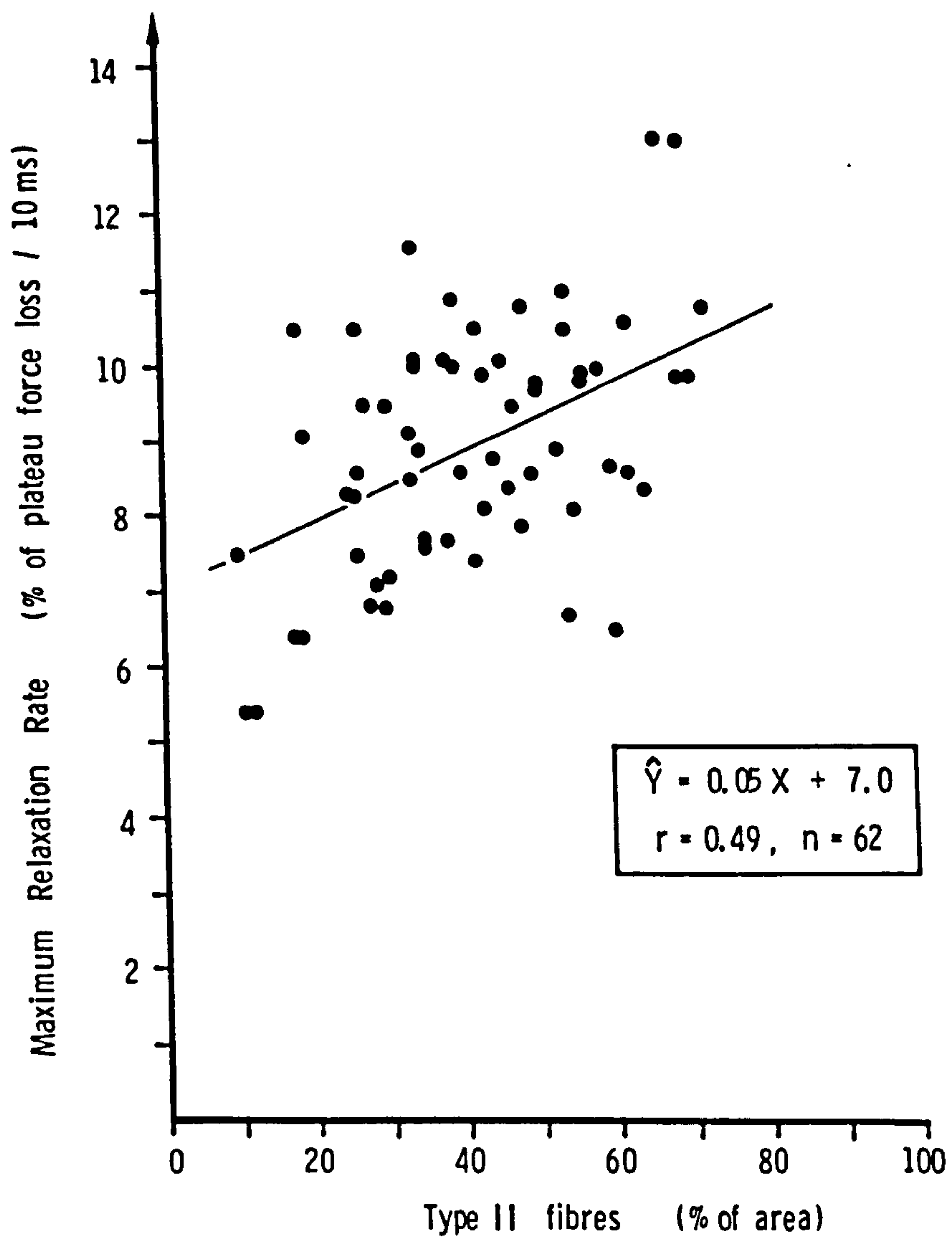


Fig. 5.2 Relationship between quadriceps' relaxation rate and its fibre-type composition in unselected patients with muscular complaints, but excluding those with denervation, dystrophy, osteomalacia or thyroid disease.

Henriksson, Taylor & Saltin (1975) measured myosin ATPase and phosphofructokinase activity in needle biopsies of human muscle. The ratio of the maximum metabolic rates of the two fibre types seemed to be between 2 and 3. This is closer to the ratio of fibre speeds obtained in most of our studies. The work of Buchthal & Schmalbruch (1970) is, in some ways, more comparable with our work, inasmuch as they made direct, in situ measurements of muscle speed (but measuring twitch contraction times rather than tetanic relaxation times). Calculating from their data, I estimate the ratio of the 'speeds' of type II and I fibres to be approximately 1.5.

The regression line of Moulds et al. (1977) is heavily weighted by two preparations with relaxation rates much slower than those recorded for the intact quadriceps. As a result, the discrepancy between our in vitro and in vivo measurements is greatest at slow relaxation rates; preparations with a high proportion of type I fibres relaxed more slowly in vitro than would have been expected from the in vivo data. This is the opposite to what might have been expected from the fact that the isolated preparations were studied at 37°C, whereas the temperature of the quadriceps in vivo is nearer 35°C (Edwards, unpublished; Sargeant, unpublished; Harris, Hultman, Kaijser & Nordesjö, 1975). A possible explanation relates to the degree of anoxia suffered by the muscle strips studied in vitro. Although Moulds et al. adduced evidence that serious anoxia was probably unusual, it seems likely that the supply of oxygen to an excised strip of muscle may sometimes be suboptimal. Under hypoxic conditions, the specimens at greatest risk are probably those with the greatest proportion of type I fibres, since these are the fibres less suited to anaerobic metabolism (e.g. Domonkos & Latzkovits, 1961). Depletion of ATP in these specimens could have resulted in unduly slow relaxation rates (Edwards, Hill & Jones, 1975b).

Before concluding that the weight of the evidence points to type II fibres relaxing $1\frac{1}{2}$ - $2\frac{1}{2}$ times faster than type I fibres, it is necessary to acknowledge a potential flaw in the logic of all the experiments described above (both in vitro and in vivo). All estimates of the relative speeds of relaxation of type I and type II muscle fibres obtained from studies of 'mixed' preparations are

subject to the assumption that both fibre types generate a similar force/cross-sectional area. It is possible, however, that type II fibres may be $1\frac{1}{2}$ - $3\frac{1}{2}$ times as strong per unit cross-sectional area. (The limited evidence available on this topic is discussed in Chapter 10). If type II fibres are significantly stronger, the relationship between the relaxation speed of mixed preparations and their proportional content of type II fibres will be non-linear. This, in turn, would lead to an overestimate of the relaxation rate of the type II fibres since the estimates were derived by linear extrapolation beyond the upper limit of the data.

RELAXATION RATE AS A PREDICTOR OF FIBRE-TYPE COMPOSITION

One of the reasons for paying so much attention to the measurement of relaxation rate was the hope that it might provide a non-invasive means for predicting the relative contribution of type I and type II fibres to a muscle's cross-section. This seemed particularly attractive in view of the good reproducibility of the indices of relaxation speed. It seems unlikely, however, that the relaxation rate of type II fibres is much more than twice that of type I fibres. This limits the discriminatory power of relaxation rate as a predictor of %CSA II.

In the experiments just discussed, there appears to be wide individual variation in the relationship between MRR and %CSA II. It is impossible to say to what extent this reflects a true variation in the relationship, and to what extent it reflects the poor reproducibility of %CSA II derived from repeated needle biopsies - coefficient of variation = 16% (Paper III and Chapter 3). The inter-person variability of MRR values from low force voluntary contractions suggests a degree of true variation in the relationship (Wiles et al., 1979). It is believed that these contractions represent 'pure' type I fibre activity, yet the inter-person coefficient of variation for MRR was 15.4%, suggesting that the absolute relaxation rate of type I fibres may vary from person to person. It seems unlikely, therefore, that measurements of MRR from stimulated contractions provide an accurate means of estimating

%CSA II in the individual subject or patient. Perhaps they may prove to have a place in following changes in the fibre-type composition of a single muscle.

Even if the absolute relaxation rates of the two fibre types vary from person to person, a constant ratio of their speeds could still permit the accurate, non-invasive prediction of %CSAII, from measurements of MRR from low- and high-force, voluntary contractions. To test this hypothesis, however, requires a means of estimating %CSA II in the whole muscle which would be more reliable than is currently possible by needle biopsy.

CONCLUSIONS

Transcutaneous electrical stimulation by pad electrodes is a safe and acceptable method of producing contractions of up to about 60% of the quadriceps.

The relaxation rate (and other contractile characteristics) of the quadriceps can be measured reproducibly from recordings of tension during isometric contractions produced by transcutaneous electrical stimulation.

The relaxation rate of the quadriceps is similar to that of the adductor pollicis and of isolated strips of human muscle studied in vitro.

Type II fibres in the human quadriceps probably have relaxation rates some $1\frac{1}{2}$ - $2\frac{1}{2}$ times as fast as type I fibres.

The practical value of measurements of relaxation rate as non-invasive predictors of fibre-type composition is limited by person-to-person variation in the absolute relaxation rate of type I fibres and by the lack of a reliable, in vivo, measure of the true fibre-type composition of the muscle.

CHAPTER 6

QUADRICEPS WEAKNESS IN OSTEOMALACIA

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- (ii) *Electromechanical activation*
- (iii) *Relaxation rate*

Muscle chemistry

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- (i) *Histology*
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After treatment

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CHAPTER 6

QUADRICEPS WEAKNESS IN OSTEOMALACIA

INTRODUCTION

Proximal muscle weakness is often a prominent feature of a number of endocrine disorders, notably hypo- and hyper-adrenalism, hyperthyroidism and osteomalacia. The muscle symptoms may be disabling and are often the patient's main complaint. It is therefore important to the patient that his recovery of strength should be as rapid and as complete as possible, irrespective of the other features of the underlying endocrinopathy. This would be justification enough for studying the mechanism(s) responsible for weakness in these conditions.

The fact that strength recovers at all provides another strong motive for examining these conditions: a similar distribution of weakness may also accompany cancer, chronic alcoholism and connective tissue diseases such as rheumatoid arthritis, scleroderma or systemic lupus erythematosus even in the absence of overt neuropathy or myositis. The primary disorders in these situations may be less amenable to successful treatment. Indeed, treatment with corticosteroids may even aggravate the muscular weakness. What are the chief mechanisms for muscle weakness in endocrine disease, and do any of them operate in other, less readily treated, debilitating disorders?

Osteomalacic myopathy was chosen for study since the weakness may be profound (Fig. 6.1 and e.g. Dent, 1956; Smith & Stern, 1967; Schott & Wills, 1976). This is also well illustrated by two descriptions of osteomalacia in India:

"Aggravated 'vata' in Bone:

It causes excessive and constant pain of the hip bones and great loss of strength."
(Vagbhatta, circa 500AD)

1^o *Laxitas* quædam & *mollities*, si non *flacciditas*, omnium partium primò affectarum in hoc affectu observari solet. Cutis tactu mollior, & ut plurimuùm lævior deprehenditur, caro musculosa minùs rigida & firma; articuli facilè flexiles sunt, sæpèque corpori sustinendo impares;

2^o *Debilitas* quædam & *languor* live *enervatio* omnibus partibus motui inservientibus accidit. Debilitas hæc multum dependet à laxitate, mollitie, & flacciditate partium jam jam dictis;

"First, A certain laxity and softness, if not a flaccidity of all the first affected parts is usually observed in this affect. The Skin also is soft and smooth to the touch, the musculous flesh is less rigid and firm; the joints are easily flexible, and many times unable to sustain the body.
Secondly, A certain debility, weakness, and enervation befallerh all the parts subservient to motion. This weakness dependeth much upon the laxity, softness, and litherness of the parts aforesaid:"

Fig. 6.1 Early description of muscle weakness in rickets (Glisson, Bate & Regemorter, 1650; translation from Glisson, Bate & Regemorter, 1668).

"In order to straighten her legs the patient wriggles her feet down the bed by laboriously flexing and extending her toes, at the same time grasping her thighs with her hands. To get up from a sitting to a standing position, the patient climbs up her own legs, in a way similar to that of a child suffering from pseudo-hypertrophic muscular atrophy."
(Scott, 1916)

Moreover, it seemed possible that altered calcium transport might have important effects on excitation-contraction coupling and on the speed of muscle relaxation; it was hoped that our 'indices of contractility' (Paper I) might be able to identify any such effects. Our other investigative tool, needle biopsy, seemed suitable for investigating the importance or otherwise of muscle atrophy. Writing chiefly about rickets, several early writers had commented on the presence of gross or microscopic atrophy (reviewed by Hagenbach-Burckhardt, 1904; Bing, 1908). Glisson et al. (1650) gave a particularly graphic description which is well worth quoting at length (Fig. 6.2). More recently, however, it has been said that weakness is often disproportionate to the muscle wasting in osteomalacia (Schott & Wills, 1976). Is it just that modern writers are seeing less severe disease and are therefore unable to recognise gross atrophy, or is atrophy less important as a cause of weakness in vitamin-D deficient adults than in children?

PATIENTS

In all, 18 patients with osteomalacia were studied (Fig. 6.3). Of the 8 Indians, 7 were women with nutritional osteomalacia. The remaining patients, again predominantly female, had a variety of underlying diagnoses although 9 of them could perhaps be loosely grouped together as having a 'malabsorptive' cause for their osteomalacia. Most of these patients had clinically apparent proximal muscle weakness, but none had any clinical evidence of neuropathy.

Twelve of these patients were studied during treatment of their disease with α -hydroxycholecalciferol, vitamin D₂ or vitamin D₃ (\pm a gluten-free diet). These 12 (6 with nutritional osteomalacia, 4 with

Partes carnosae, imprimis musculosae infra caput, quas inter primò affectas suprà numeravimus, in progressu morbi magis magisque indies atteruntur, extenuantur, macièque conficiuntur. Hoc signum non statim à morbi initio elucescit, quòd notabilem morbi motum priusquam evidenter appareat, prærequirat; certissimè tamen, tractu temporis, in conspectum venit, morbumque ad extremum terminum, seu ad vitam, sive ad mortem comitatur; optimèque motum ac gradum morbi ex incremento suo demonstrat.

"The fleshy parts, especially those which are full of Muscles beneath [i.e. caudad to] the Head which we have listed among the first affected, In the progress of the Disease are daily more and more worn away, made thin and lean. This Sign doth not presently shew it self from the beginning of the Disease, because it pre-requireth some notable motion of the Disease before it evidently appeareth; yet in time it most certainly is exposed to the senses, and accompanieth the disease to the last step be it either to life or death; excellently demonstrating the motion and degree of the Disease by its encrease."

Fig. 6.2 Early description of muscular atrophy in rickets (Glisson et al., 1650 & 1668).

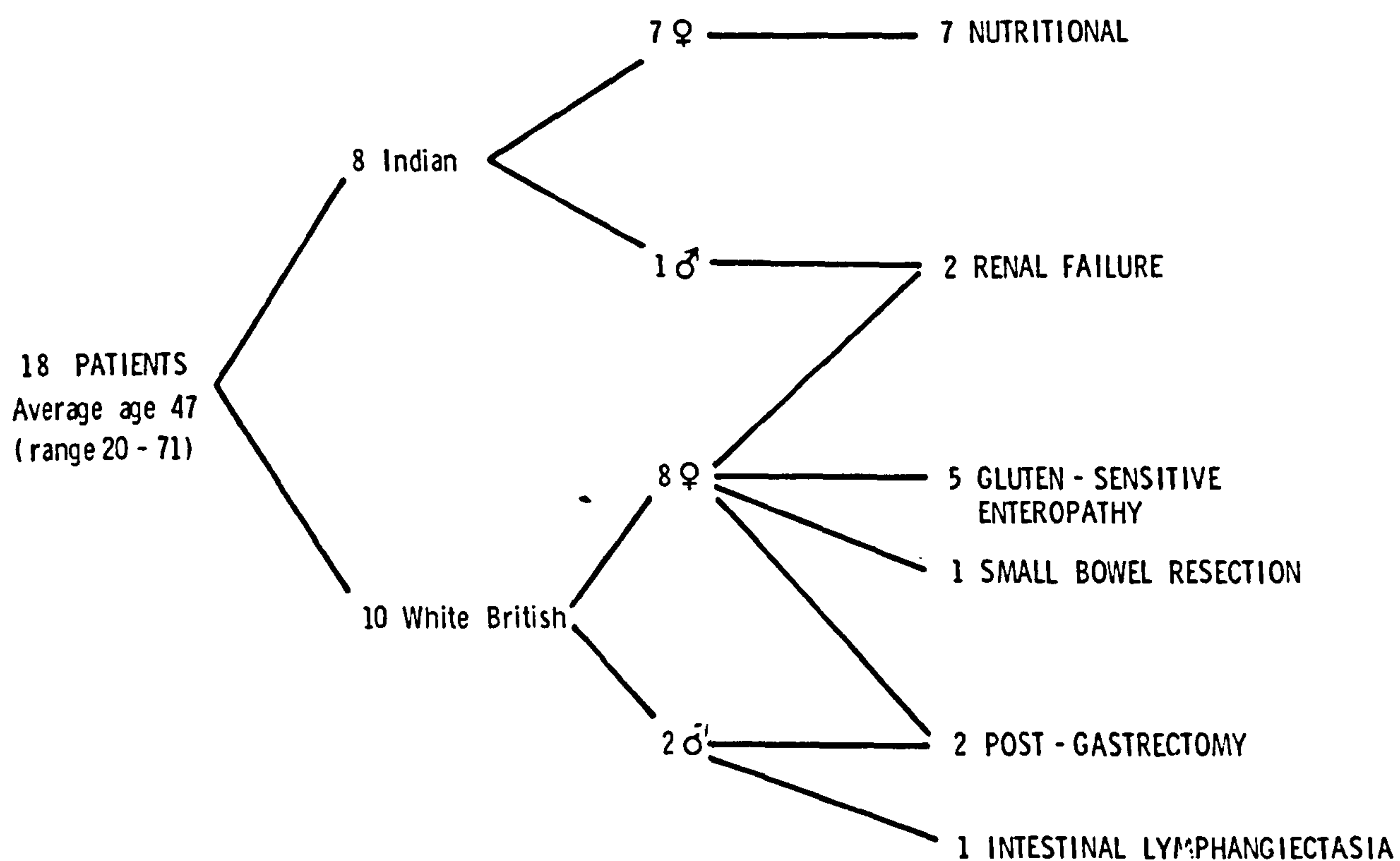


Fig. 6.3 Summary of patients with osteomalacia who were studied, and of the causes of their osteomalacia.

gluten-sensitive enteropathy and 2 with post-gastrectomy osteomalacia) are described in detail in Paper II.

It is not possible to say whether the severity of the weakness displayed by these patients is typical for osteomalacia since the majority were referred for study because of our known interest in the 'myopathy' of osteomalacia. Four of the Indian women (numbers 3-6 in Paper II) were unselected, however. They were among the 10 weakest of the 18 patients. This suggests that, as a group, the patients studied were not atypical of patients presenting to hospitals with osteomalacia.

METHODS

Nitrogen balance

Two of the patients with osteomalacia were studied by measurement of their net nitrogen balance over consecutive 4-day periods. Cuprous thiocyanate was used as a continuous internal marker (Dick, 1969) and carmine markers were taken with breakfast at the start of each 4-day period. The diet was constant throughout and the dietary intake of nitrogen was measured by analysis of an additional quarter retained from each meal.

RESULTS

Dynamometry

(i) Strength

The measurements of quadriceps strength made before treatment was started are shown in Figure 6.4. Except for those cases where it was only possible to measure the strength of one quadriceps (e.g. because of pain or because of risk of fracture through a Looser zone), the results plotted were obtained for each patient's stronger

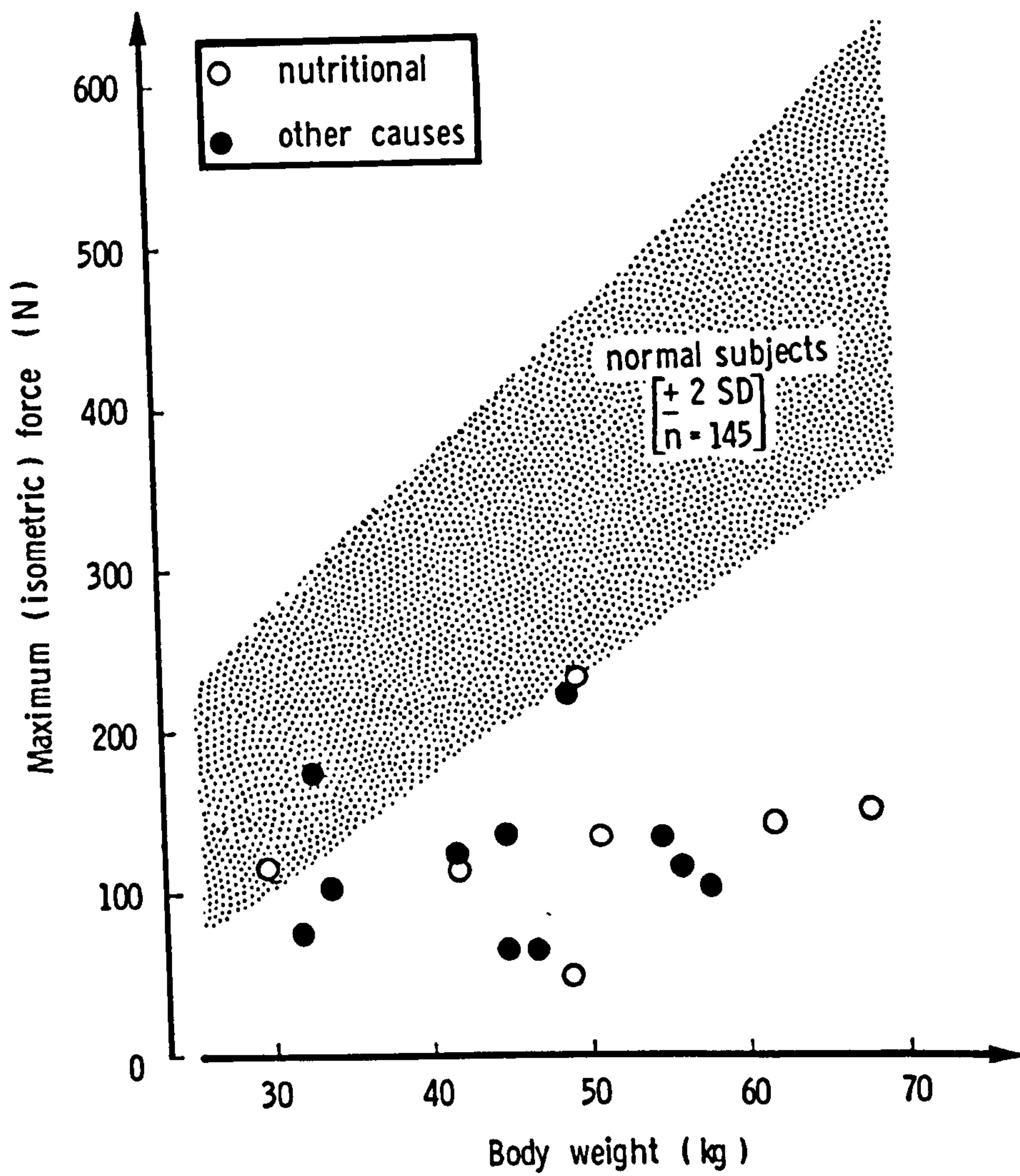


Fig. 6.4 Isometric quadriceps strength in patients with osteomalacia and in normal subjects (vd. also Fig. 4.2).

leg. They are compared with the corresponding values obtained for the stronger leg of normal subjects of the same body weight (Paper I).

None of the measurements of strength included in Figure 6.4 was considered to have been limited by pain. The patients' behaviour was carefully observed during the tests and they were also asked specifically about pain. Moreover, treatment relieved pain more rapidly than it increased strength and in the few patients where a temporary increase in pain followed the start of treatment there was no corresponding decrease in MVC.

The patients' weakness bore no relation to the degree of hypocalcaemia or hypophosphataemia. Nor was there any consistent difference in relative strength between those with nutritional osteomalacia and those with some other cause for their osteomalacia.

(ii) Electromechanical activation

Conventional electromyography was performed on 6 patients. There was no evidence of a failure of neuromuscular transmission or sarcolemmal depolarisation. There was an unduly rapid loss of force during 18s of continuous stimulation at 30Hz in 2 out of 15 patients - viz. 40% and 75% of the initial force, compared with a median value of 9.1% for 40 normal subjects (range 0-27%). Both these patients had an electromyogram but in neither case was there evidence of myasthenia. (However neither patient had a 'Tensilon' test.)

The other component of 'electromechanical activation' is excitation-contraction coupling. Impairment results in a proportionately greater reduction in force at low frequencies of stimulation than at high frequencies, a phenomenon which can be demonstrated by transcutaneous stimulation of the quadriceps (Young & Edwards, 1977; Edwards, Hill, Jones & Merton, 1977). None of the 18 patients with osteomalacia showed any such evidence of excitation-contraction uncoupling.

(iii) Relaxation rate

Quadriceps relaxation in osteomalacia was significantly slower than normal ($P < 0.001$), whether considered as SF₉₅, SF₅₀ or (calculated) MRR (Table 6.1).

Muscle chemistry

Needle biopsies from the quadriceps of patients with osteomalacia revealed the mean resting contents of ATP and PC to be 17.2 μmol per g dry weight (SD = 4.44; n = 15) and 55.9 μmol per g dry weight (SD = 17.46; n = 14) respectively (Fig. 6.5). The patients' values overlapped with much of the normal range (Edwards et al., 1975a), and there was no correlation between the severity of each patient's weakness and the degree of depletion of their muscle ATP and/or PC contents.

Muscle biopsy morphology

(i) Histology

None of the biopsies had any abnormality on conventional histology apart from a reduction in fibre size and an increased variability in fibre size. The latter was the result of some fibres retaining a near-normal cross-sectional area while others were atrophic. There was no evidence of replacement of muscle by fat or fibrous tissue (as is seen in muscular dystrophy) nor of denervation. There was no evidence of any round-cell infiltration nor of an excess of macrophages (such as might suggest the removal of necrotic myofibres). There was no histochemical evidence of unusual acid phosphatase activity. Histochemical 'typing' of myofibres demonstrated abnormalities in the relative size and frequency of the fibre types which will be described later. There was, however, no indication of fibre-type grouping.

TABLE 6.1 Indices of the rate of quadriceps relaxation in patients with osteomalacia

	SF ₉₅ (ms)			SF ₅₀ (ms)			MRR* (% plateau force loss/10ms)		
	mean	SEM	n	mean	SEM	n	mean	SEM	n
Normal (Paper I)	60.4	0.46	82	104.7	1.04	81	10.31	0.17	81
Osteomalacia	72.6	2.31	18	128.6	3.83	13**	7.90	0.37	13**

* MRR calculated as $450 / (SF_{50} - SF_{95})$

**Technically satisfactory recordings of SF₅₀ were obtained in only 13 of the 18 patients

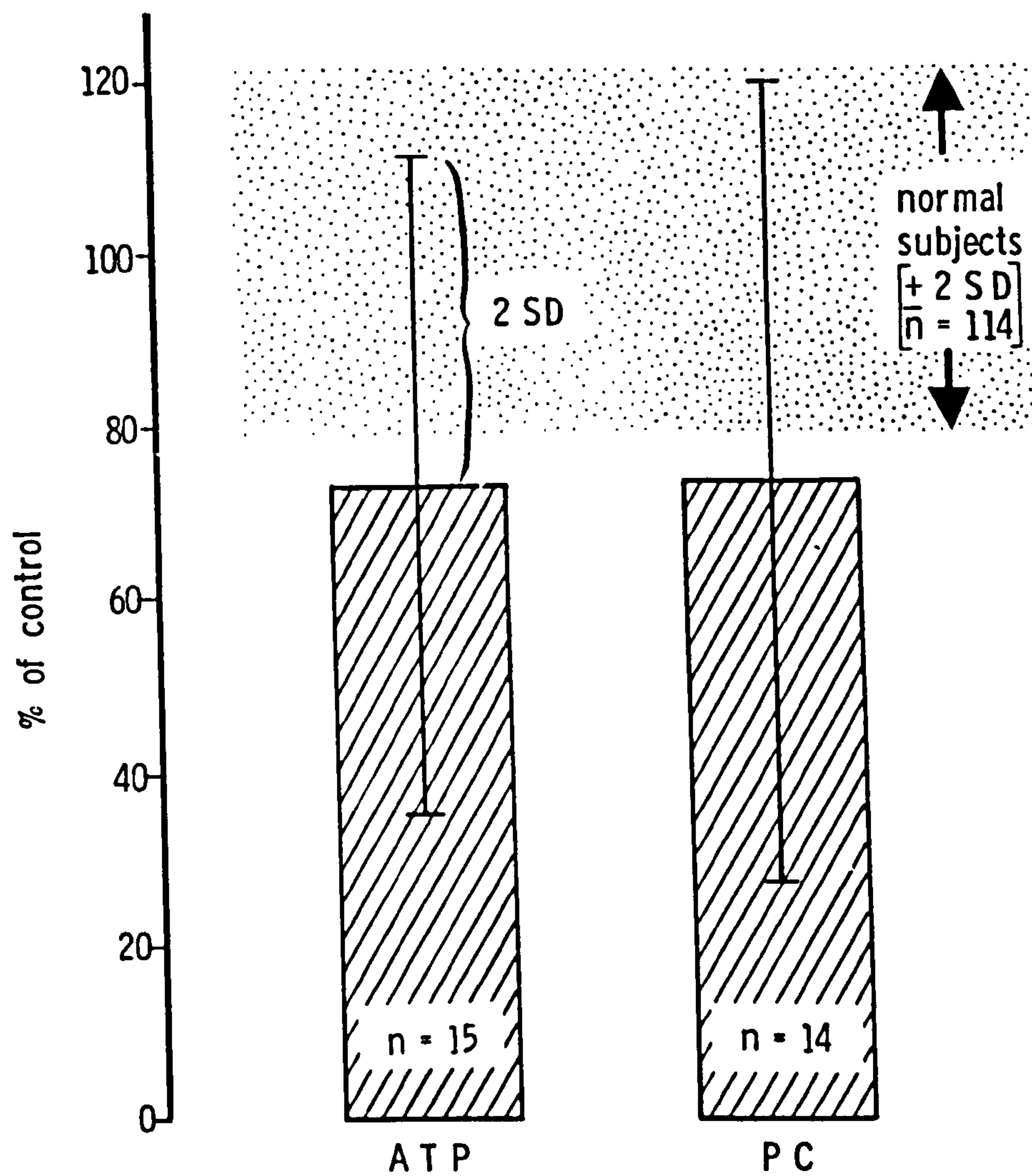


Fig. 6.5 ATP and PC contents of resting muscle in osteomalacia. The results have been calculated per gram dry weight and then expressed as percentages of the corresponding mean values of 114 normal subjects (Edwards et al., 1975a).

(ii) Muscle fibre size

One patient declined to have a muscle biopsy. The patients' mean fibre areas were smaller than those of sex-matched normal subjects ($P < 0.01$ for the men; NS for the women) (Table 6.2).

(iii) Muscle fibre types

The female patients had a significantly lower frequency of type II fibres than the normal women ($0.02 > P > 0.01$) but the male patients did not differ significantly from the normal men (Table 6.3).

Overall, the ratio MFA II/MFA I in the patients' biopsies did not differ significantly from the normal subjects' values, although the degree of preferential type II atrophy in the biopsies from some individual patients was quite dramatic (Fig. 6.6).

The patients' biopsies did not yield a positive correlation between the ratio MFA II/MFA I and the frequency of type II fibres.

(iv) Relationship of relaxation rate to fibre-type composition

Quadriceps relaxation rate, as $MRR = 450 / (SF_{50} - SF_{95})$, was compared with its fibre-type composition in the 11 patients in whom this was possible. There was no correlation ($r = -0.01$).

Effects of treatment

(i) Strength

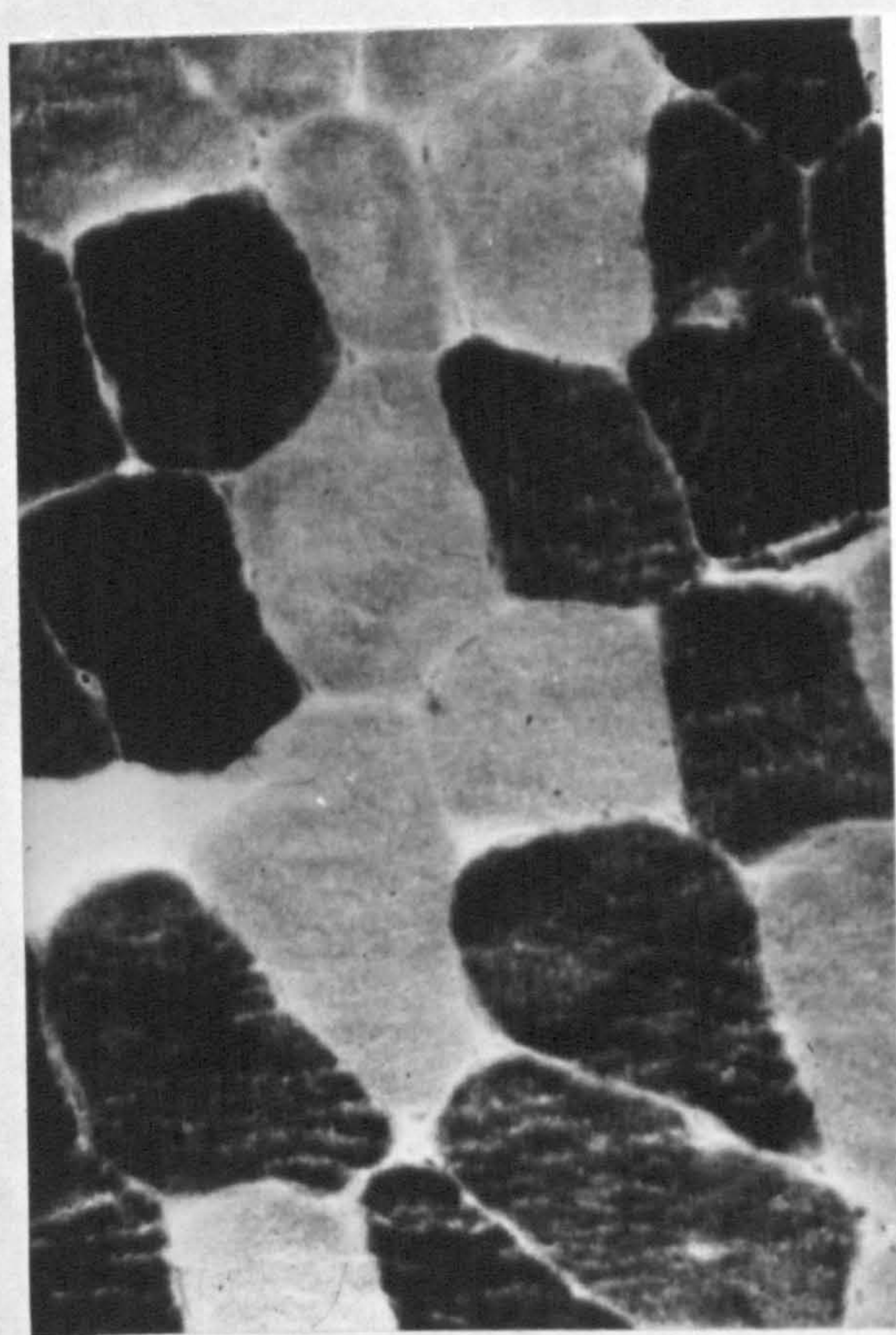
Figure 6.7 charts the progress of a 39 year old woman with nutritional osteomalacia. She was followed for nearly a year, during which time she received generous doses of vitamin D₂. Her strength is plotted as a percentage of the mean value for normal subjects of the same body weight. Even when her alkaline phosphatase had been normal for 2 months, her strength was still well below the third

TABLE 6.2 Mean fibre areas in osteomalacic patients compared with sex-matched normal controls

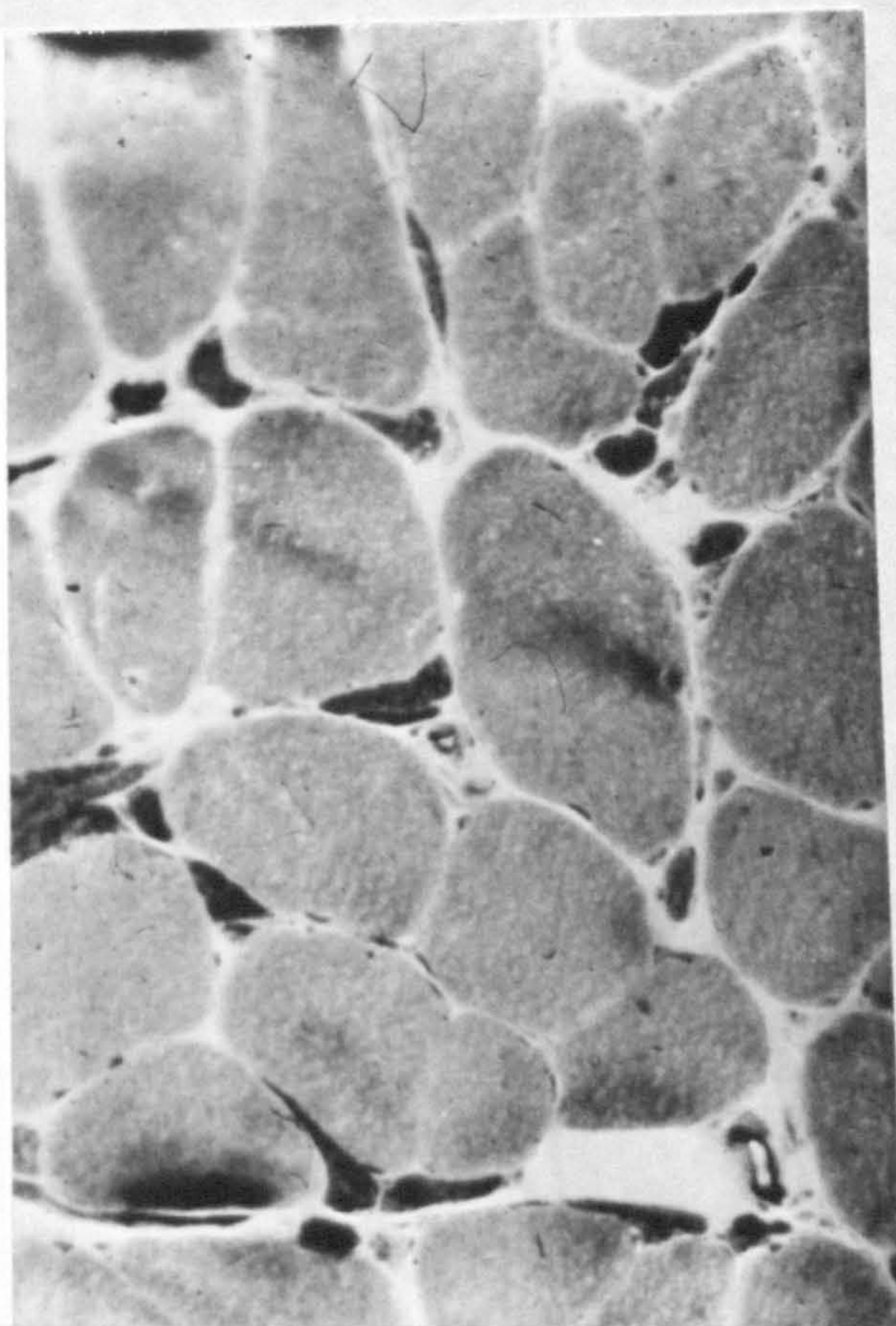
	n	Mean age (years)	Mean fibre area (μm^2)	
			\bar{x}	SD
<hr/>				
Male				
normal	24	32.5	3924	1072
osteomalacia	3	42.3	1968	471
 Female				
normal	9	31.3	2277	476
osteomalacia	14	49.4	1854	744
<hr/>				

TABLE 6.3 Fibre-type characteristics in osteomalacic patients compared with sex-matched normal controls

	n	Mean age (years)	Type II frequency (%)		MFA II/MFA I	
			\bar{x}	SD	median	range
Male						
normal	24	32.5	42.1	14.2	1.16	0.75-1.66
osteomalacia	3	42.3	47.3	18.5	0.81	0.47-1.24
Female						
normal	9	31.3	53.8	12.0	0.85	0.52-1.15
osteomalacia	14	49.4	41.5	10.5	0.63	0.28-0.97

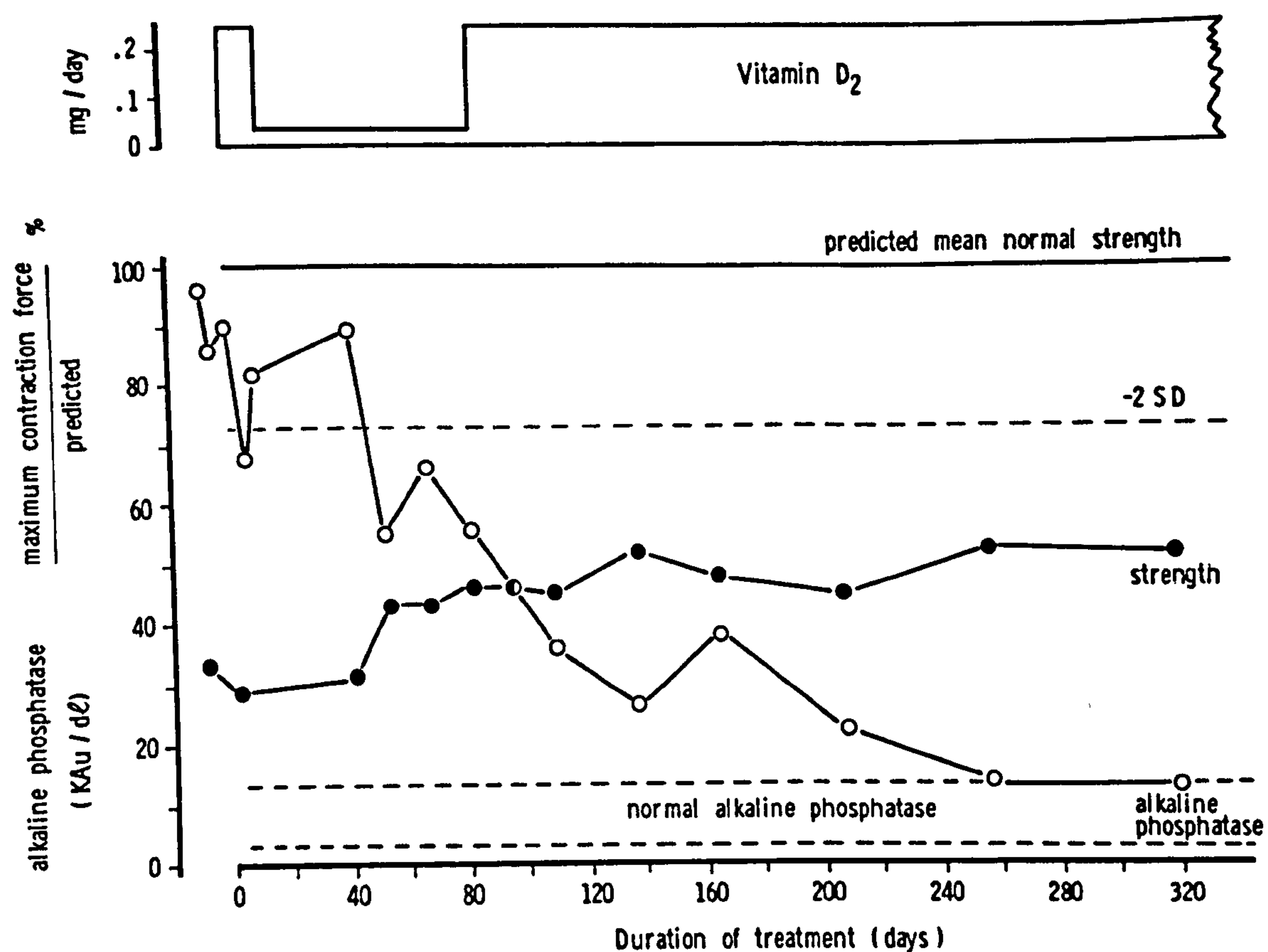


a



b

Fig. 6.6 Transverse sections of needle biopsy specimens of human quadriceps stained to show the activity of myosin ATPase (pH 9.4) in the normal subject (a) and in the patient with proximal muscle weakness secondary to osteomalacia (b). The patient's muscle shows a severe degree of preferential type II fibre atrophy.



Patient : R.S. (Q39)

Fig. 6.7 Sequential measurements of isometric quadriceps strength and plasma alkaline phosphatase in a patient with nutritional osteomalacia treated with vitamin D₂ (Paper II, patient no. 4.).

centile. The subject of Figure 6.8, a 60 year old woman with nutritional osteomalacia, was treated for 4 months with 1α -hydroxycholecalciferol, and then with vitamin D₃. The dose of 1α -hydroxycholecalciferol was as large as was possible in her case - the two reductions were both occasioned by hypercalcaemia - yet she too was still weak at a time when her alkaline phosphatase had returned to normal.

The rates of recovery of muscle strength illustrated in Figures 6.7 and 6.8 were not unusual. The effects of treatment on quadriceps strength were observed in 10 patients followed for at least 3 months, and in 2 patients followed for shorter periods. The observations made on these 12 patients form the basis of Paper II. It was striking that in only 2 cases did quadriceps strength return to within 2SD of the mean normal value within the period of study (Fig. 6.9). (The problems of assigning appropriate normal values are discussed in Paper II.)

(ii) Relaxation rate

Quadriceps relaxation rate appeared to increase during treatment of the osteomalacia, but this tendency was not confirmed statistically (Table 6.4). Pre- and post-treatment values of both MRR and %CSA II were obtained for 6 patients, followed for a mean of 117 days (range 27-300). The changes in %CSA II in these patients were too small to permit useful comment on whether changes in relaxation rate might be predictive of changes in fibre-type composition, within individual subjects.

(iii) Strength, fibre size and fibre types

The changes in quadriceps strength during treatment were compared with the changes in muscle fibre size in 8 patients (Fig. 6.10). Changes in fibre size tended to be matched by changes in strength. The percentage changes in MVC were correlated with the percentage changes in MFA ($r = 0.60$; $n = 11$; $P < 0.05$). The slope of

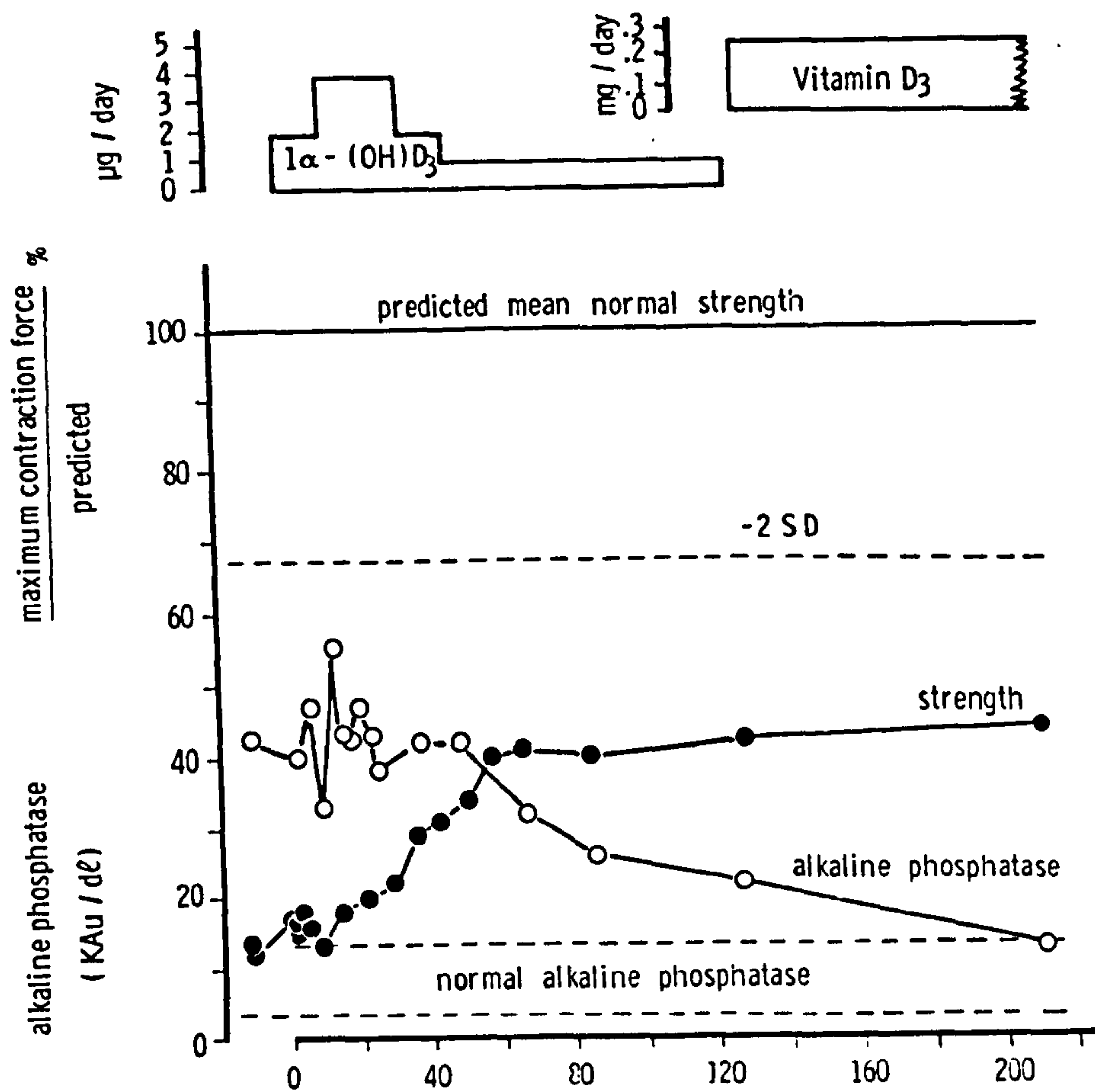


Fig. 6.8 Sequential measurements of isometric quadriceps strength and plasma alkaline phosphatase in a patient with nutritional osteomalacia treated with 1α - hydroxycholecalciferol and then vitamin D₃ (Paper II, patient no. 6).

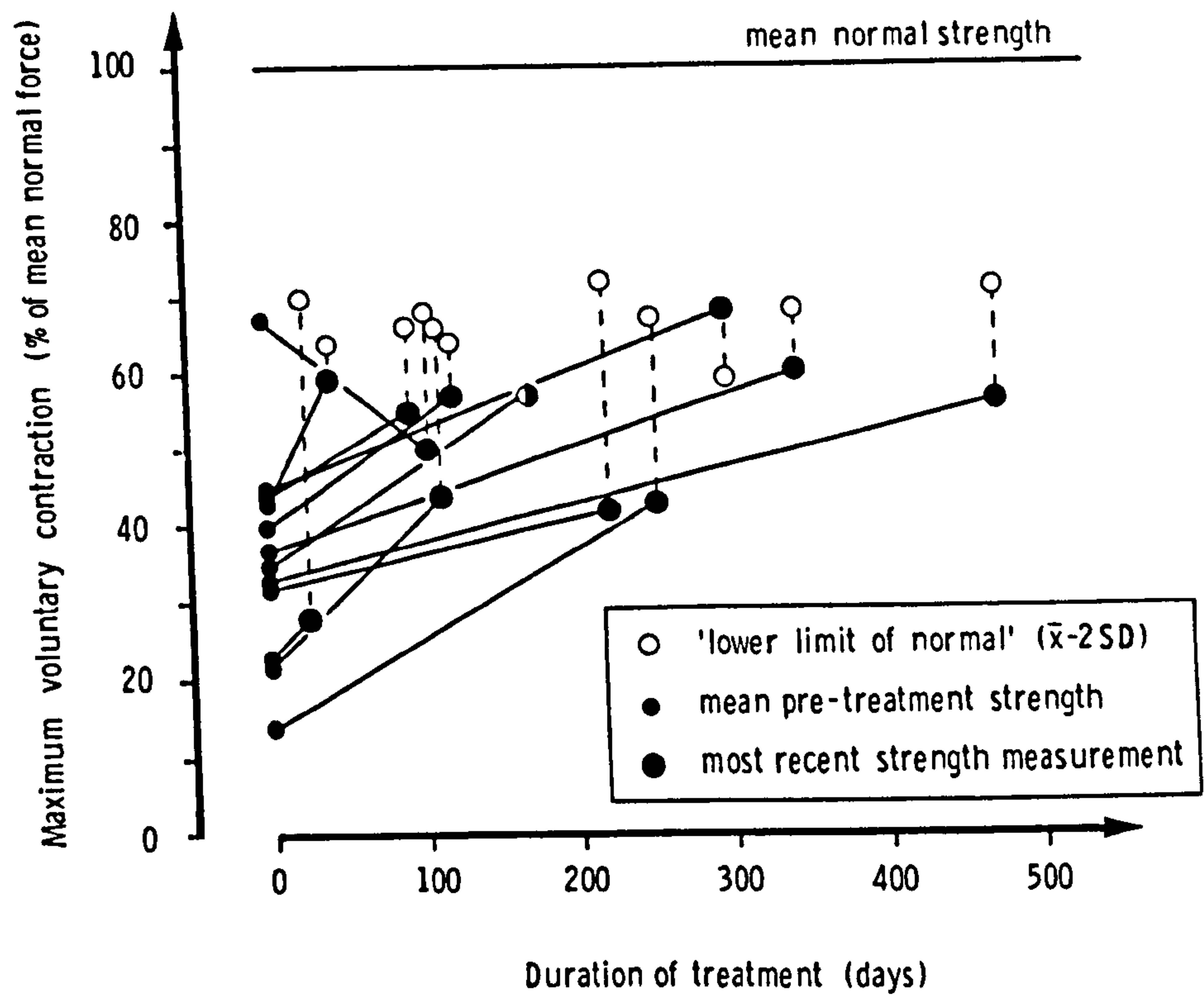


Fig. 6.9 Changes in quadriceps strength during treatment of 12 patients with osteomalacia. Each patient's strength measurements have been expressed as a percentage of the mean value for normal subjects of the same body weight.

TABLE 6.4 Comparison of the mean pre-treatment values of the relaxation indices obtained from patients with osteomalacia with the most recent values obtained from the same patients while receiving treatment

	Mean values		Mean duration of treatment (days)	n	P (in paired t-tests)
	Pre- treatment	On treatment			
SF ₉₅ (ms)	74.6	70.3	179 (range 25-388)	12	0.2>P>0.1
MRR (% plateau force loss/10ms)	7.79	8.32	212 (range 42-388)	9	0.3>P>0.2

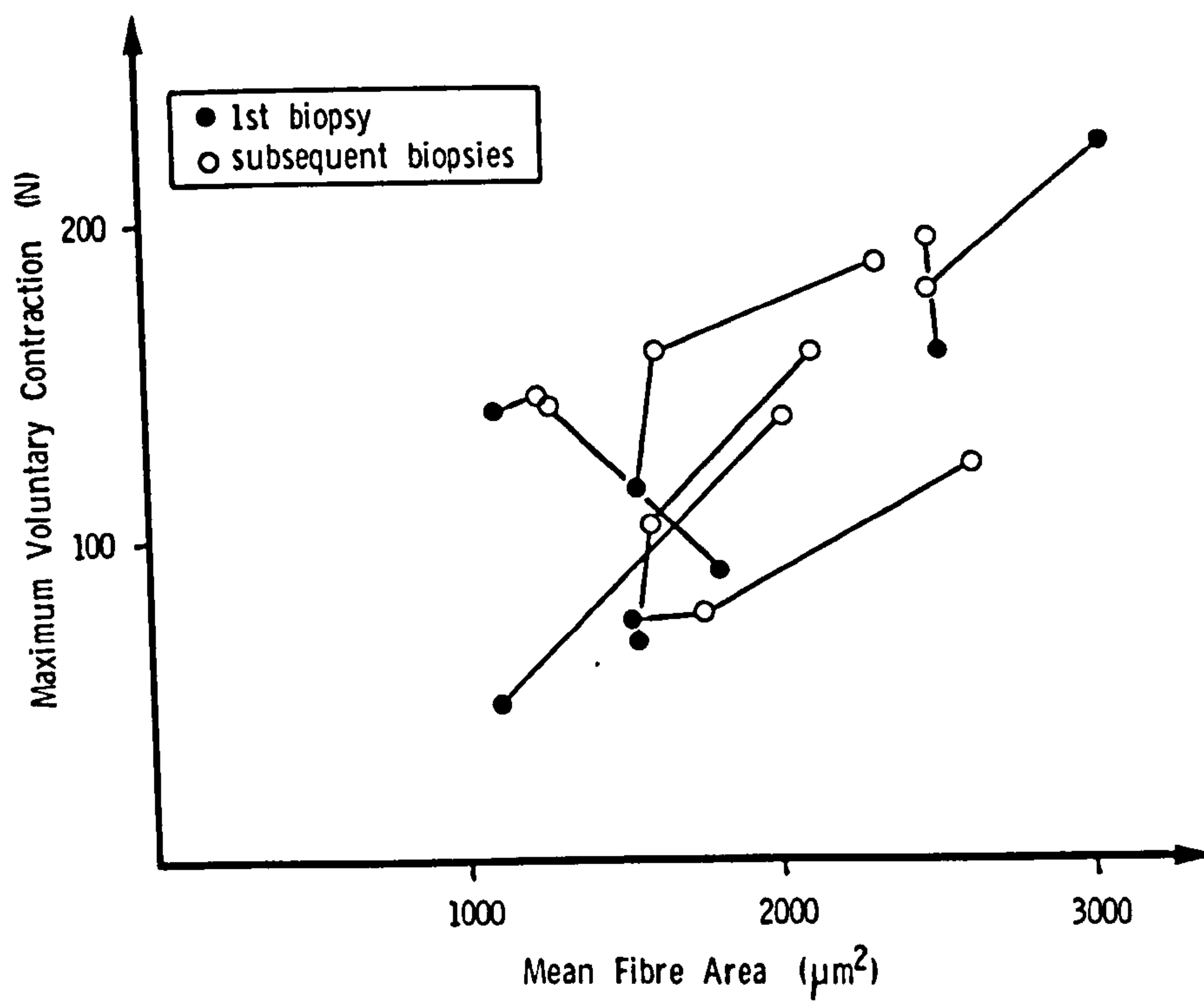


Fig. 6.10 Comparison of quadriceps strength and fibre size in 8 patients receiving treatment for osteomalacia due to nutritional deficiency (4), gluten-sensitive enteropathy (3) or gastrectomy (1).

this relation, calculated as a model II regression, is 0.87, but it has extremely wide 95% confidence limits (-1.0 to +2.0), consistent with the inherent variability of measurements of MFA from repeated biopsies (Chapter 3).

Increases in MFA were principally due to growth of the previously atrophic type II fibres (Fig. 6.11). There was a small, but statistically significant, reduction in the relative frequency of type II fibres (44% to 39%) between the first and last biopsies from the same 8 patients (Paper II for details).

(iv) Nitrogen balance

Two of the Indian women with nutritional osteomalacia (Paper II, numbers 5 and 6) underwent nitrogen balance studies. Both these ladies were obese and their total body muscle mass was assumed to be approximately 35% of body weight. If it is assumed that muscle contains 31.4g of nitrogen per kg (Dickerson & Widdowson, 1960) and that the positive nitrogen balance is attributable to the growth of muscle, it is possible to calculate a net rate of synthesis of muscle for each patient (Table 6.5). The values obtained are compared with the corresponding rate of change of quadriceps strength (calculated from the regression of MVC on duration of treatment).

DISCUSSION

Profound muscle weakness is a well recognised, but unexplained, feature of osteomalacia. Apart from loss of strength, the most prominent abnormality of quadriceps muscle function observed in this study was slowing of relaxation. The first part of this discussion is devoted to the slow relaxation. The remainder examines the weakness itself, the mechanisms by which it might be explained, and its response to treatment.

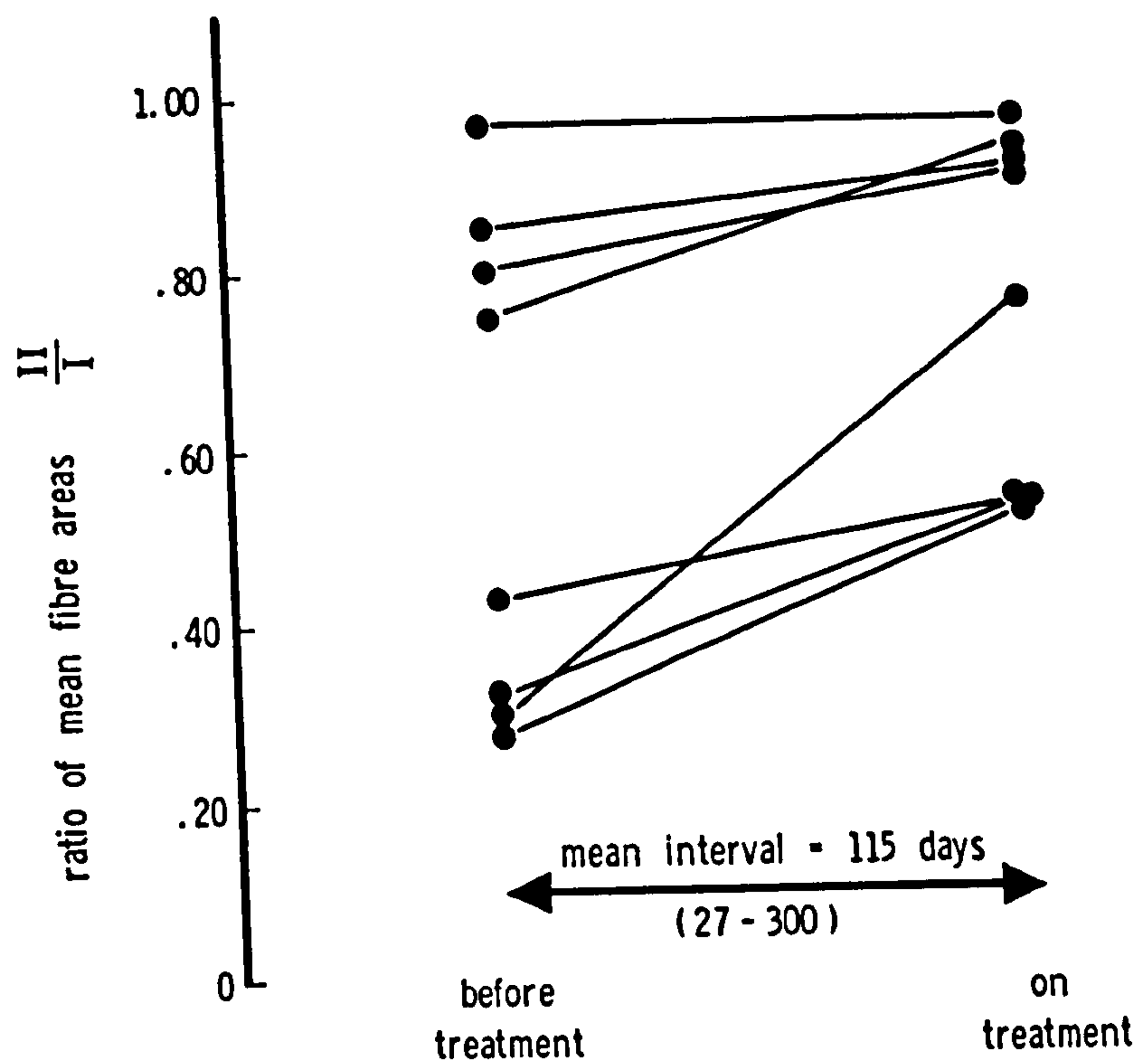


Fig. 6.11 Changes in the relative sizes of the type I and type II fibres in biopsies from the quadriceps of 8 female patients receiving treatment for osteomalacia.

TABLE 6.5 Calculated rates of change of total body muscle and quadriceps strength in 2 patients with nutritional osteomalacia being treated with 1 α -hydroxycholecalciferol
 (D.G. is also the subject of Figure 6.8)

(S.P. 46 years)

Duration of treatment (days)	19 & 20
<hr/>	
Rate of change (% of pre-treatment value per day) of:	
quadriceps strength	1.9
total body muscle	0.8

(D.G. 60 years)

Duration of treatment (days)	28 & 29
<hr/>	
Rate of change (% of pre-treatment value per day) of:	
quadriceps strength	1.5
total body muscle	0.5

Relaxation rate

Studied in vivo, relaxation of the human quadriceps from a brief stimulated tetanus is slow in osteomalacia. This is consistent with the demonstration by Rodman & Baker (1978) that the soleus muscle of vitamin D-depleted rats relaxes slowly when studied in vitro. Nevertheless, slow relaxation of intact human muscle is not confined to osteomalacia. The very slow relaxation of the quadriceps in hypothyroidism has been illustrated elsewhere (Hosking et al., 1978; Chapter 7) and a lesser degree of slowing has also been reported in Duchenne muscular dystrophy for both the adductor pollicis (Desmedt, Emeryk, Renoirte & Hainaut, 1968; Hosking et al., 1978) and the quadriceps (Hosking et al., 1978). Patients with quadriceps symptoms attributable to alcoholic myopathy or associated with conditions such as systemic lupus erythematosus, scleroderma or polymyositis/dermatomyositis also had prolonged relaxation times similar to those recorded for the patients with osteomalacia (Young & Scott, unpublished observations).

The resting concentration of ATP in the quadriceps of the patients with osteomalacia was low and the relaxation speed of the isolated rat soleus muscle is proportional to its content of ATP (Edwards et al., 1975b). Nevertheless, the present evidence offered no support whatsoever for this as a possible mechanism for the prolonged relaxation indices recorded for the patients with osteomalacia.

Following the argument developed in Chapter 5, it seemed possible that the slow quadriceps relaxation seen in osteomalacia might be due to the reduced contribution of type II muscle fibres to the overall muscle cross-sectional area. It was not possible to demonstrate a correlation between quadriceps relaxation speed in osteomalacia and the muscle's content of type II fibres. This does not exclude the possibility of such an association. For example, testing for a simple correlation assumes that force production per unit cross-sectional area is the same for the two fibre types; this may not be the case (Chapter 9). It assumes that force production by an atrophic type II fibre is reduced in proportion to its loss of

cross-sectional area; this assumption may not be valid in disease. Finally, there is the question of biopsy sampling-error; the coefficient of variation for estimates of %CSA II is high (CV = 16%) (Paper III).

Muscle relaxation requires the lowering of the sarcoplasmic calcium ion concentration. This is achieved by the uptake of calcium ions into the sarcoplasmic reticulum (SR) from the sarcoplasm (Peachey, 1968). During post-natal development, the relaxation speed of rat muscle (in vitro) and the avidity of its SR for calcium change in parallel (Drachman & Johnston, 1973). Curry, Basten, Francis & Smith (1974) have demonstrated that the uptake of calcium into the SR of rachitic rabbit muscle is impaired. The in vivo slowing of quadriceps relaxation in osteomalacia reported here might therefore be explained on the basis of a diminution in the avidity of the SR for calcium in the presence of vitamin D deficiency. This, in its turn, could still be linked with the presence of type II atrophy since fragmented SR from a muscle with numerous type II fibres (guinea pig vastus lateralis) accumulates calcium faster than that derived from a muscle with only type I fibres (guinea pig soleus) (Fiehn & Peter, 1971).

Such an explanation seems attractive, but if slow quadriceps relaxation is a specific effect of vitamin D deficiency, albeit compounded by the effect of type II atrophy, it should be substantially corrected by the administration of vitamin D. While there was a tendency for this to happen, it was not confirmed statistically and it was, in any case, a very slow process, taking several weeks or months to occur. This contrasts with the finding of Matthews, Heimberg, Ritz et al. (1977) that oral 1,25-dihydroxycholecalciferol for only 5 days before sacrifice corrected the impaired calcium transport of SR vesicles derived from the muscles of uraemic rabbits.

Quadriceps relaxation speed is slow in osteomalacia but it is also slow in other, unrelated conditions. It therefore seems unlikely that the slowing is a specific effect of vitamin D on muscle or on its SR membranes. Although the present evidence provides no

specific support for the hypothesis, it seems possible that the slowing may yet be related to the preferential loss of type II fibre area.

Cause of weakness

Patients with osteomalacia are often profoundly weak. Nevertheless, the cause of osteomalacic 'myopathy' remains unknown. In the present analysis, the theoretically possible causes of muscular weakness are considered in 3 broad categories - defective electro-mechanical activation, impaired short-term energy supply processes, or inadequate contractile machinery.

A normal electromyogram was obtained for each of the 6 patients on whom it was done. These tests were performed by experienced neurologists as part of their routine electrodiagnostic service. Dastur, Wadia & Bharucha (1972) found normal electromyograms in 6 patients with nutritional osteomalacia, but they also found an excess of short-duration, small-amplitude potentials in a further 2 patients. Most of the other published accounts describe a high incidence of "myopathic" records - i.e. with short-duration, polyphasic potentials, often of low amplitude (Ekbom, Hed, Kirstein & Åström, 1964; Prineas, Mason & Henson, 1965; Smith & Stern, 1969; Banerji & Hurwitz, 1971; Floyd, Ayyar, Barwick, Hudgson & Weightman, 1974). Skaria, Katiyar, Srivastava & Dube (1975) not only found myopathic records in 30 patients with nutritional osteomalacia, they also demonstrated that the 14 patients who were studied after treatment then had longer and larger motor unit potentials. Their study was unusual, however, in that their patients also had slowing of nerve conduction. It is possible that their patients were deficient of several vitamins; nerve conduction velocities did not change during treatment with vitamin D. However, neither the literature nor the results of the present study gives any indication that denervation or a myasthenia-like failure of neuromuscular transmission might be the cause of the weakness of osteomalacia.

The only suggestion that there might be an element of

neuromuscular junction failure was the rapid loss of force during prolonged tetanic stimulation which was observed in 2 out of 15 patients in this study. Although this phenomenon may be associated with myasthenia gravis (Solís-Cámara, Gordon-Barrios, Yankelevich & Negrete, 1965), it has also been described in other conditions, where it does not respond to edrophonium (Young & Edwards, 1977) and its explanation is not known. It was only possible to repeat the measurements of force decrement in 1 of the 2 patients in whom this index of muscle function was abnormal. Her subsequent results were less abnormal than before treatment, but were still near to, or above, the upper limit of normal after 250 days of treatment. Whatever the explanation for this phenomenon, it was not observed in the other 12 patients who were tested in this way and therefore seems unlikely to be a major cause of weakness in the majority of patients with osteomalacia.

Evidence for excitation-contraction coupling failure was sought by comparing the forces of contraction resulting from stimulation at low frequencies of stimulation (1-20Hz) with those obtained at higher frequencies (30-100Hz). There was no indication of the selective, low-frequency, force loss associated with 'uncoupling' of excitation and contraction.

The immediate source of energy for muscle contraction is ATP. The supply of ATP is maintained (in the short-term) by the break-down of PC. In vitro experiments with animal muscle have shown that the strength of a poisoned muscle may be proportional to the logarithm of its ATP content (Murphy, 1966), and the strength of a fatigued muscle may be proportional to its content of PC (Spande & Schottelius, 1970). However, the extent of the ATP and PC depletion used in these experiments was more severe than the mean reduction (25%) seen in the group of patients with osteomalacia. Further arguments against attributing the osteomalacic patients' weakness to their slightly reduced levels of muscle phosphagen (i.e. ATP + PC) are the extensive overlap of results obtained for individual patients and for normal subjects, and the absence of any correlation between the severity of each patient's weakness and the depletion of muscle phosphagen.

Indeed, some very weak patients had normal levels of ATP and PC in their muscle.

Plasma values for creatine kinase (CK) activity were normal, in keeping with the absence of any biopsy evidence of destruction of muscle cells or of unusual acid phosphatase activity. Even in patients who were so weak that they were bedridden, the only obvious abnormality in the biopsy appearance of the muscle was a reduction in the size of the muscle fibres. This is in agreement with the findings of Dastur, Gagrut, Wadia, Desai & Bharucha (1975) for 19 patients (gluteus maximus) and with those of Skaria et al. (1975) for 17 patients (10 quadriceps, 7 gluteus maximus). A few other authors have also reported muscle fibre atrophy in individual patients with osteomalacia (Ekbom et al., 1964; Prineas et al., 1965; Singhal, 1966; Smith & Stern, 1967). Dastur et al., "confirmed" the presence of atrophy in their patients' biopsies (gluteus maximus) by comparison with the size of fibres in biopsies from normal subjects (quadriceps femoris). The appropriateness of their 'controls' is dubious.

Effects of treatment

In the present study, each patient was used as her own 'control' for evaluation of the severity of the muscle fibre atrophy. Comparison of MFA with the speed of recovery of quadriceps MVC made it possible to assess the contribution of a reduced mass of contractile 'machinery' (as indicated by MFA) to each patient's weakness. The gains in strength were in proportion to the growth of the muscle fibres and were correspondingly slow. We agree with Dastur et al. (1975) that "... the basic myopathology of osteomalacia is best understood as an atrophy".

The metabolic balance results are also in keeping with the conclusion that, when osteomalacia is treated, the recovery of muscle strength is largely due to the recovery of muscle bulk. The calculated rates of recovery of total muscle mass are slightly smaller than the rates of recovery of quadriceps' strength, but the

positive nitrogen balance is unlikely to be equally distributed to all the muscles in the body. The weakness of osteomalacia is selective in its distribution, and even affected muscles seem to recover their strength at different rates (Fig. 6.12).

Henderson, Russell, Ledingham et al. (1974) recorded a slow increase in force production/integrated EMG activity in one subject with renal osteodystrophy receiving treatment with 1,25-dihydroxycholecalciferol. This would be in keeping with myofibre growth. They also reported that the change occurred after 58 days of treatment but not after 12, in keeping with the rather slow recovery of strength observed in the present study.

A significant defect in either electromechanical activation or muscle energy metabolism might be expected to respond to treatment relatively quickly. The slow return of strength argues against such a defect being the immediate cause of weakness. Nevertheless, the anabolic influence of vitamin D on D-deficient muscle probably starts promptly on the initiation of treatment. Birge & Haddad (1975) demonstrated an increased rate of uptake of tritiated leucine into the diaphragmatic muscle of D-deficient rats within 7 hours of the oral administration of cholecalciferol. Augmentation of the diaphragmatic ATP content accompanied the increased amino acid uptake, and the authors postulated that "... ATP concentrations within the cell of the vitamin-deficient animal are reduced to levels which are rate limiting in the synthesis of protein". This could apply equally to the patients in this study - their weakness might be indirectly related to their reduced muscle ATP (and PC) content, as a result of impaired muscle synthesis.

Similarly, as suggested by Swash, Schwartz & Sargeant (1979), the myofibre atrophy might be secondary to a defect in, e.g., excitation-contraction coupling. The recovery of strength would still depend on myofibre growth although the primary cause of weakness was excitation-contraction coupling failure. While abnormalities of sarcotubular function are present in osteomalacic muscle there is, as yet, no evidence that they interfere with maximal strength, excitation-contraction coupling or neuromuscular transmission.

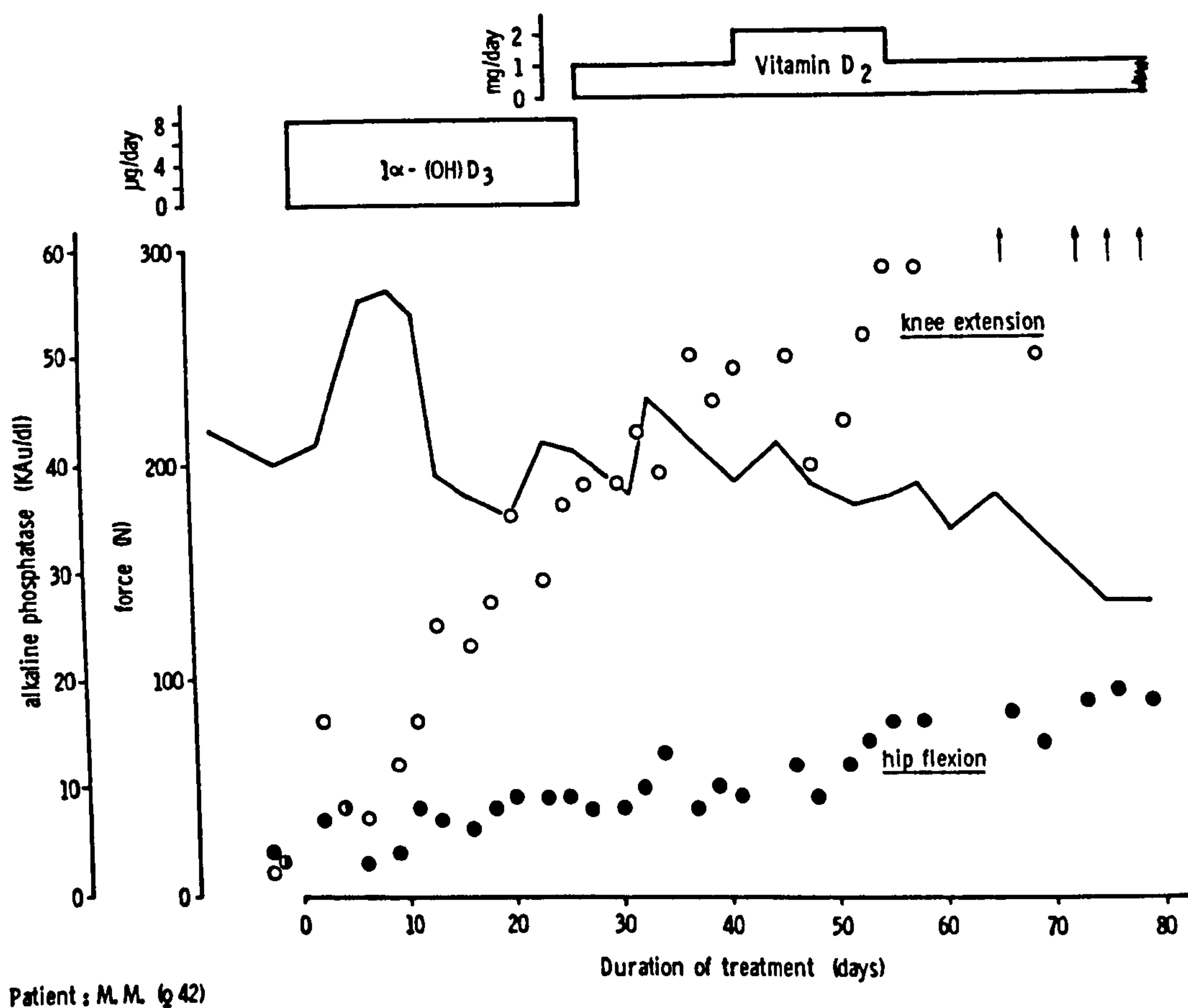


Fig. 6.12 Measurements of the strength of right hip flexion and right knee extension made with the Hammersmith Myometer (Edwards & McDonnell, 1974) during treatment of severe, anticonvulsant-induced, osteomalacia in a 42 year old woman (not included in the other studies described here). The changes in the patient's plasma alkaline phosphatase are shown by the solid lines (normal range = 3-13 KAU/dl).

(The Hammersmith Myometer is a hand-held instrument with a full-scale reading of 300N; knee extension forces in excess of this are indicated by arrows. The absolute forces achieved by different muscle groups cannot be directly compared as the mechanical conditions for measurement are not the same.)

It seems clear that muscle atrophy is a major, immediate cause of weakness in established osteomalacia (although the cause of the loss of muscle bulk remains obscure). No other contributory factors have been identified but Pleasure, Wyszynski, Sumner et al. (1979) have demonstrated that rachitic chick muscle produces a subnormal tension/muscle weight. This suggests that some factor other than atrophy also contributes to the chick myopathy. As discussed in Chapter 9, it should now be possible to test such a possibility in man: quadriceps cross-sectional area can now be measured (Paper V) and it should not be difficult to establish a reliable normal range for the strength/unit cross-sectional area of the quadriceps (Chapter 9).

After treatment

Although the slow recovery of strength reflects the time needed to 'rebuild' the contractile machinery, it seems surprising that the recovery of strength was not more complete during the period of observation. As discussed in Paper II, this may simply reflect the difficulties inherent in finding appropriate normal 'controls'. Alternatively, do the patients' quadriceps muscles have a subnormal strength/cross-sectional area even after a long period of treatment? Or have they actually lost muscle fibres, which cannot be replaced? These questions should be amenable to study with the aid of ultrasonographic measurements of quadriceps cross-sectional area.

CONCLUSIONS

The severe, proximal muscle weakness of patients with osteomalacia has been confirmed.

Quadriceps weakness in osteomalacia is not associated with evidence of muscle necrosis or failure of electromechanical activation. It is associated with slowing of muscle relaxation but neither the cause nor the relevance of the slowing is known.

Quadriceps weakness in osteomalacia is associated with reduced mean levels of muscle ATP and PC. The severity of weakness, however, bears no relation to the muscle's concentration of high-energy phosphate.

Atrophy of its muscle fibres, particularly type II fibres, is associated with quadriceps muscle weakness in osteomalacia. The recovery of strength during treatment with vitamin D is associated with growth of muscle fibres, especially of the type II fibres.

The rate of recovery of strength appears similar during treatment with vitamin D₂ or D₃ and with 1 α -hydroxycholecalciferol. The amelioration of weakness is a slow process, with a time course measured in weeks, months or possibly even years. Even then, it may be incomplete, patients not achieving the level of quadriceps strength expected for their body weight.

Future studies incorporating measurements of the cross-sectional area of the whole quadriceps will determine whether atrophy is the sole cause of weakness in osteomalacia or whether the quadriceps is weak relative to its size. These studies should also contribute to an understanding of the apparently incomplete recovery of strength in the patients studied.

CHAPTER 7

QUADRICEPS WEAKNESS IN THYROID DISEASE

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- (ii) Electromechanical activation
- (iii) Relaxation rate

Muscle chemistry

Muscle biopsy morphology

- (i) Pathology
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Cause of weakness

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CHAPTER 7

QUADRICEPS WEAKNESS IN THYROID DISEASE

INTRODUCTION

Both ends of the spectrum of thyroid gland activity are known to have distinctive effects on muscle function, producing weakness and changing muscle speed. These abnormalities are reversed by appropriate treatment. The dynamometric techniques of Paper I were therefore combined with needle biopsy studies of myofibre size, fibre-type proportions and muscle energy metabolism in order to examine the physiological basis for the muscle symptoms encountered in association with thyroid disease. It seemed likely that the slow muscular relaxation of hypothyroidism might be due to a change in the contractile mechanism itself (Lambert, Underdahl, Beckett & Mederos, 1951). This made the area particularly attractive for study since some slowing of relaxation is also found in osteomalacia (Young, Brenton & Edwards, 1978b; Chapter 6) and in Duchenne muscular dystrophy (Hosking et al., 1978).

PATIENTS

Eight patients with hypothyroidism and 5 with hyperthyroidism were studied. They are described in more detail in Paper III. Patients were studied whether or not they had symptoms or signs of muscle weakness but we were again dependent on referral of patients by physicians aware of our interest in endocrine 'myopathies'.

METHODS

Muscle energy turnover

The turnover of ATP during a prolonged, isometric muscle contraction was calculated from the changes in concentration of ATP,

PC and lactate in needle biopsy specimens taken from the quadriceps before and after the contraction (Edwards, Hill & Jones, 1975c). The contraction was made under conditions of local ischaemia, its duration measured and its average force expressed in terms of the patient's maximum voluntary isometric strength. Patients were asked to maintain the contraction for as long as possible, matching its force to an oscilloscope display of a target force equivalent to 50% of their MVC.

The concentrations of ATP, PC and lactate were all expressed relative to the total creatine (i.e. PC plus free creatine) concentration of the same biopsy sample (Edwards et al., 1975a) and total ATP turnover was calculated assuming the generation of 1.5 mole of ATP for each mole of lactate formed (Burton & Krebs, 1953):
$$\text{ATP turnover} = (1.5 \times \text{increase in lactate}) + (\text{decrease in PC}) + (\text{decrease in ATP}).$$

RESULTS

Dynamometry

(i) Strength

Four of the 5 hyperthyroid patients and 2 of the 8 hypothyroid patients had quadriceps weakness when compared with normal subjects of the same body weight (Fig. 7.1).

Quadriceps strength increased to within the normal range in the only thyrotoxic patient studied sequentially during treatment. Four of the hypothyroid patients (including 1 with subnormal strength) had their strength remeasured after replacement treatment for 2½, 3, 4 and 10 months. There was no significant change.

(ii) Electromechanical activation

None of these patients was studied electromyographically. The

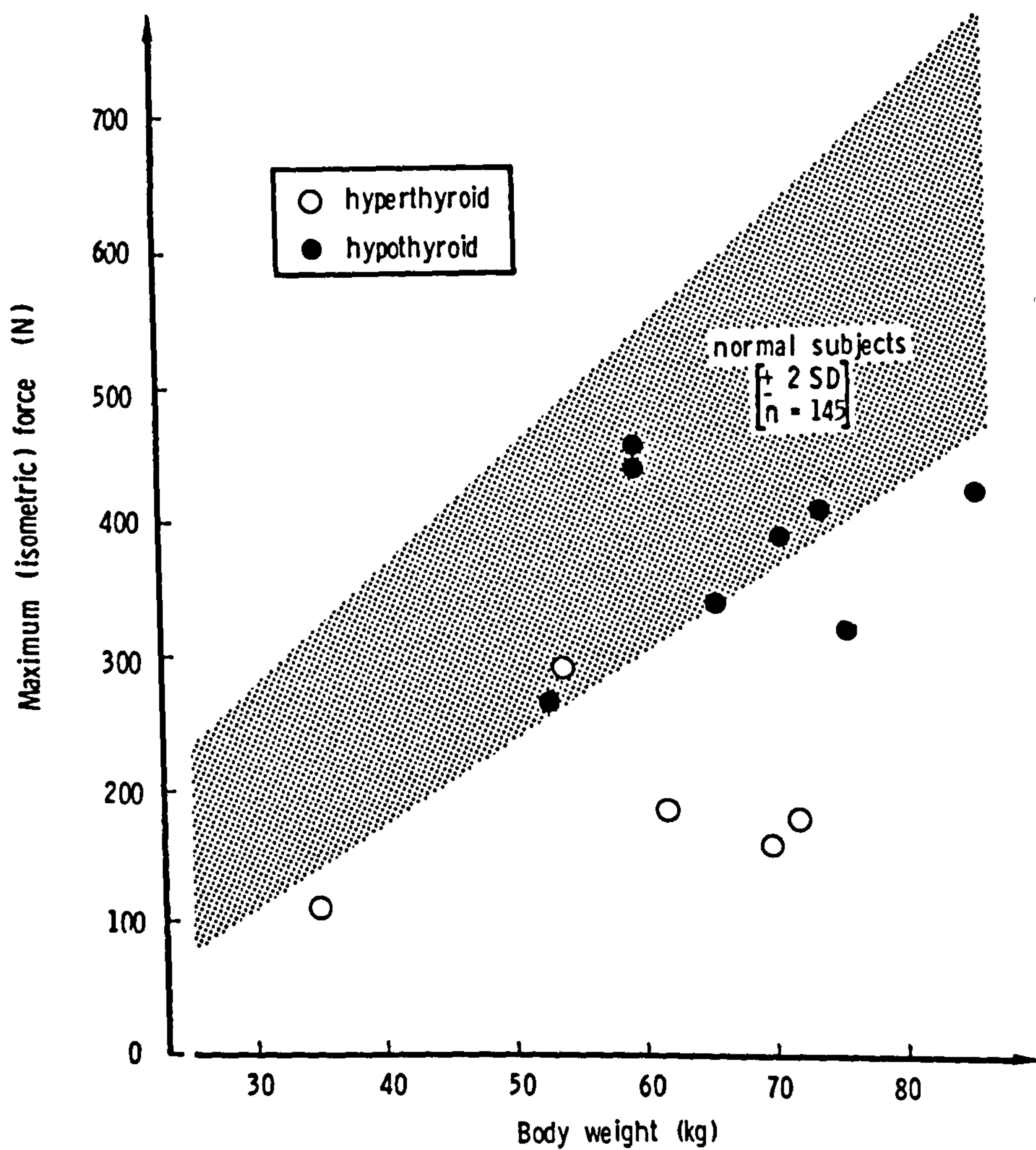


Fig. 7.1 Isometric quadriceps strength in patients with thyroid gland dysfunction and in normal subjects.

ability of the quadriceps to maintain force during continuous stimulation at 30Hz for 18s was tested in 1 hyper- and 3 hypothyroid patients: all proved normal.

(iii) Relaxation rate

There was significant slowing of relaxation in hypothyroidism and significant acceleration in hyperthyroidism (details in Paper III). The slowing was more pronounced than the acceleration, 2 thyrotoxic patients having an MRR within the normal range.

Treatment gradually normalised the relaxation rates of all 5 hypothyroid patients followed-up. The changes in MRR associated with treatment were followed in detail in 2 hypo- and 1 hyperthyroid patient (one of each described in Paper III). As expected (vd. Chapter 5, 'Introduction'), there was a close, inverse relationship between the changes in relaxation rate and changes in the degree of tetanic fusion during stimulation at 10Hz.

Muscle chemistry

Plasma creatine kinase (CK) levels were high in the hypo-, and low or low-normal in the hyper-thyroid patients (details in Paper III).

The mean resting levels of ATP and PC were lower than normal in both groups of patients (Paper III). As with osteomalacia, however, there was a large overlap of the patients' results with the normal range. Quadriceps strength (as a percentage of the corresponding mean normal value) was positively correlated with muscle ATP and PC concentrations for both the hyperthyroid patients ($r = 0.39$ and 0.70) and the hypothyroid patients ($r = 0.82$ and 0.79); neither of the correlation coefficients is statistically significant for the hyperthyroid patients but both are significant ($0.02 < P < 0.05$) for the hypothyroid group.

Hypothyroid patients were able to maintain 50% MVC for longer than the normal subjects but with less production of lactate and less consumption of PC. Their total ATP turnover was therefore slightly lower than normal ($P < 0.05$) but the dramatic difference was in their much lower rate of ATP turnover ($6.7 \mu\text{mol of ATP} \cdot \text{s}^{-1} \cdot \text{mmol}^{-1}$ of TC, $\text{SEM} = 1.5$, compared with 26.0 , $\text{SEM} = 3.4$). The proportion of the ATP turned over which was derived from anaerobic glycolysis was slightly, but not significantly, lower than normal (mean = 47.1% , $\text{SEM} = 7.2$, compared with 58.7% , $\text{SEM} = 2.9$); this is in keeping with the slightly lower total ATP turnover, as the proportion from glycolysis increases the greater the amount of ATP turned over.

The anxious nature of thyrotoxic patients explains why ATP utilisation data are available for only 1 person in this group. The ATP turnover rate in her muscle was twice normal.

Muscle biopsy morphology

(i) Pathology

In addition to abnormalities of fibre-type frequency and size, 5 hypo- and 2 hyper-thyroid patients had microscopically abnormal biopsies. A slight excess of internal nuclei was apparent in the biopsies from 3 of the hypothyroid patients, one of whom also had occasional split fibres. Two of these patients had a second biopsy after a period of treatment (10 and 40 weeks) and both repeat biopsies showed considerable improvement. Two hypothyroid patients and 2 hyperthyroid patients had histochemical evidence of excessive acid phosphatase activity in their muscle. Of these patients, only one, with hypothyroidism, was re-biopsied: his muscle still showed an abnormal acid phosphatase reaction after 10 months of treatment. Finally, 1 hypothyroid patient had a lot of 'moth-eaten' fibres in his biopsy and another had a number of fibres showing cores which stained for oxidative enzyme activity. The latter had improved greatly when re-biopsied 10 weeks later.

There was no other evidence of muscle necrosis and no evidence of either inflammation or denervation.

(ii) Muscle fibre size

The hypothyroid patients' mean fibre areas did not differ significantly from those of the sex-matched normal subjects (Table 7.1). The hyperthyroid patients' biopsies, however, showed a significant degree of myofibre atrophy ($0.02 > P > 0.01$ for the man, $0.05 > P > 0.02$ for women) (Table 7.1).

(iii) Muscle fibre types

The mean frequency of type II fibres in the biopsies from the hypothyroid patients was lower than in those from the normal subjects (NS for men; $0.05 > P > 0.02$ for women) (Table 7.2, Fig. 7.2). For the hyperthyroid patients, the man had a significantly higher type II frequency than normal subjects ($P < 0.01$), but the women had a non-significantly lower mean value (Table 7.2).

The 5 hypothyroid men all had a smaller ratio MFA II/MFA I than any of the normal men ($P < 0.001$) (Table 7.2, Fig. 7.2). This tendency, although apparent, was not significant in the women. There was no difference between the hyperthyroid patients and the normal subjects in respect of the ratio MFA II/MFA I (Table 7.2).

Taking all the patients together, there is a significant tendency for those with the most selective type II atrophy to have the fewest type II fibres; the ratio MFA II/MFA I is positively correlated with the frequency of type II fibres (Spearman's coefficient of rank correlation = 0.58, $P < 0.01$).

Five hypothyroid patients also had muscle biopsies taken after 72 - 303 days of treatment. In 4 the low pre-treatment value of %CSA II had increased towards normal and in 1 it was unchanged. The

TABLE 7.1 Mean fibre areas in hypo- and hyperthyroid patients
compared with sex-matched normal controls

	n	Mean age (years)	Mean fibre area (μm^2)	
			\bar{x}	SD
Male				
normal	24	32.5	3924	1072
hypothyroid	5	40.5	3402	1128
hyperthyroid	1	32.0	1220	-
Female				
normal	8	31.0	2278	509
hypothyroid	3	48.0	2720	225
hyperthyroid	4	45.5	1648	248

TABLE 7.2 Fibre-type characteristics in hypo- and hyperthyroid
patients compared with sex-matched normal controls

	n	Mean age (years)	Type II fibre frequency (%)		MFA II/MFA I	
			\bar{x}	SD	median	range
Male						
normal	24	32.5	42.1	14.2	1.16	0.75-1.66
hypothyroid	5	40.5	31.0	17.0	0.63	0.21-0.68
hyperthyroid	1	32.0	81.8	-	0.90	-
Female						
normal	8	31.0	56.7	12.3	0.84	0.52-1.04
hypothyroid	3	48.0	25.7	12.1	0.53	~0.05-0.74
hyperthyroid	4	45.5	46.7	13.0	0.95	0.73-1.01

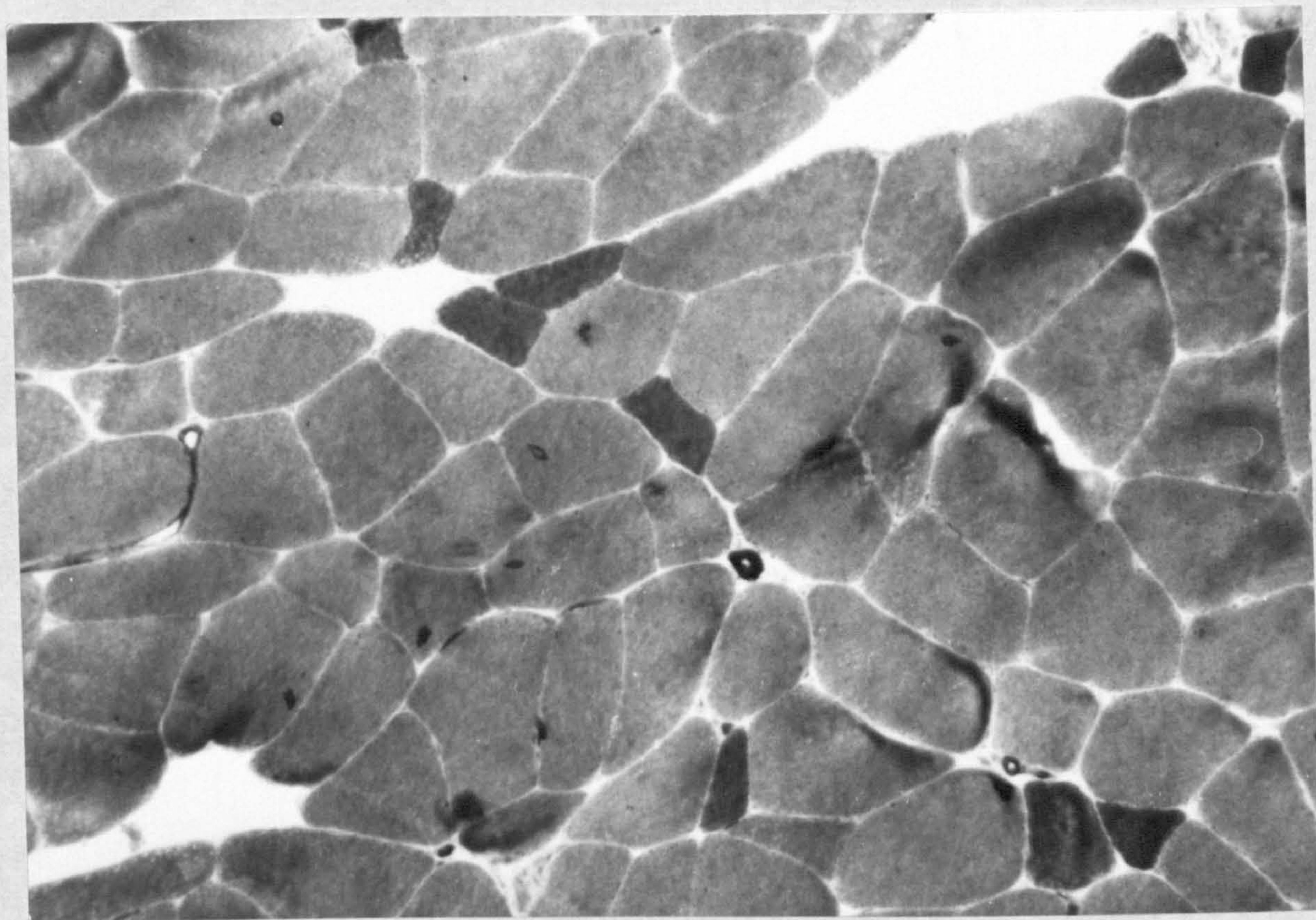


Fig. 7.2 Transverse section of a needle biopsy specimen from the lateral quadriceps of a 50-year-old man (V.A. in Paper III) with hypothyroidism. (Myosin ATPase, pH 9.4.)

improvements were probably principally the result of an increased frequency of type II fibres.

DISCUSSION

Cause of weakness

The patients with thyroid disease were studied principally with a view to examining energy turnover in muscle as a possible cause of weakness. There are therefore no follow-up data to allow a comparison of changes in fibre size with changes in muscle strength during treatment. Nevertheless, it seems likely that the small size of the fibres found in the quadriceps of the thyrotoxic patients contributed significantly to their weakness. (Both strength and fibre size were relatively normal in the hypothyroid patients studied.) Total body potassium content is low in thyrotoxicosis and returns to normal as the gland is suppressed (Bayley, Harrison, McNeill & Mernagh, 1980; Edmonds & Smith, 1981). The rate at which this occurred in relation to the rate of increase in body weight led Edmonds & Smith to conclude that their findings were consistent with atrophy being the major cause of the weakness of thyrotoxicosis, although they also indicated that intracellular potassium depletion was probably also present in the weakest patients. It is a great pity that they did not measure muscle strength but used only a crude, 3-point scale for assessing the performance of various muscular tasks.

Elevation of the circulating level of creatine kinase (CK) is a well-recognised phenomenon in hypothyroidism (Graig & Ross, 1963). Quite high levels may be seen and most of the circulating enzyme is of skeletal-muscle origin (Doran & Wilkinson, 1975). At first sight this seems indicative of muscle fibre damage. The microscopic evidence for damage in the present study, however, was much the same in both hypo- and hyper-thyroid patients and the latter had CK levels at or below the lower limit of normal. (Low CK levels in thyrotoxicosis have also been noted by Graig & Smith, 1965.) Like CK, the other enzymes showing elevated circulating levels are of cytosolic origin (Doran, 1978; O'Malley, Davies & Rosenthal, 1981).

The absence of elevated levels of mitochondrial enzymes suggests that neither muscle destruction nor reduced plasma clearance is the explanation. Moreover, serum amylase levels are often mildly elevated in hypothyroidism although muscle is not a rich source of this enzyme (Doran & Wilkinson, 1975).

Because the level of ATP is known to influence the rate of leakage of enzymes from cells (Wilkinson & Robinson, 1974), it has been suggested that this is an explanation for the raised CK levels in hypothyroidism (Doran & Wilkinson, 1975; O'Malley et al., 1981). The muscle concentration of ATP was just as low in our osteomalacic patients (Chapter 6) as in our hypothyroid patients. The CK values in osteomalacia were normal, however, thus eliminating ATP depletion as the cause. The other explanation which has been advanced is that the high CK levels are related to the hypothyroid patients' low body temperature (Doran & Wilkinson, 1975; O'Malley et al., 1981). The evidence reported by O'Malley was consistent with this, as are the rather low CK values recorded for hyperthyroid patients. The actual mechanism remains obscure.

It seems unlikely that the alterations in the relative numbers and sizes of type I and type II fibres contributed much to loss of strength. If the fibre types do differ in their specific strength, it is probably the type II fibres which are stronger (vd. Chapter 10). The hypothyroid patients showed much the greater relative loss of type II fibre area but mostly had normal strength.

There was a tendency for the weaker patients to have lower levels of muscle phosphagen. Nevertheless, as has been discussed already for the patients with osteomalacia, it seems unlikely that the ATP or PC levels were low enough to impair the patients' maximal strength. Moreover, the mean phosphagen levels of the hypothyroid patients were similar to those of the toxic group although the 2 groups differed markedly in their strength, and the high rate of ATP turnover in the one thyrotoxic patient so studied suggests that her MVC was probably not limited by the availability of ATP.

The rate of high-energy phosphate regeneration does not appear to have been limiting for the hypothyroid patients since their post-contraction biopsies had a PC concentration which was less depressed than normal. The rather low muscle lactate at fatigue represents a reduced ATP requirement rather than a block in its anaerobic regeneration from glycogenolysis, as has been suggested (McDaniel, Pittman, Oh & di Mauro, 1977). The slow relaxation rate is associated with a reduced rate of ATP utilization for the maintenance of a constant force.

As argued in Paper III, the rapid relaxation rate of thyrotoxic muscle, and the correspondingly high stimulus frequency required for tetanic fusion, may well contribute to subjective "weakness" experienced during submaximal, everyday muscle contractions. Such contractions are probably performed with a firing rate of 10-40Hz (Bigland & Lippold, 1954; Freyschuss & Knuttson, 1971; Marsden et al., 1971; Grimby & Hannerz, 1977) and therefore lie on the steep part of the frequency/force curve - i.e. the part where force production is most sensitive to stimulus frequency (Fig. 7.3). It seems unlikely, however, that this mechanism would be a factor in the weakness observed during a maximal voluntary contraction (probably performed with a firing frequency of more than 50Hz, i.e. on the plateau of the frequency/force curve). The clinical dissociation between the degree of muscle atrophy and muscle strength which Ramsay (1966) considered to exist might be explained if his estimates of a patient's strength were formed during the performance of submaximal or prolonged contractions or on the basis of the patient's subjective assessment of his weakness. It is significant, therefore, that Ramsay made use of a test which involved timing the patient's ability to maintain a submaximal isometric contraction (Ramsay, 1966; Lahey, 1926).

Relationship of relaxation rate to fibre-type composition

Considering all 13 thyroid patients together, there seems to be an extremely close link between the relaxation rate and fibre-type composition of the quadriceps ($r = 0.93$). The (model II) regression

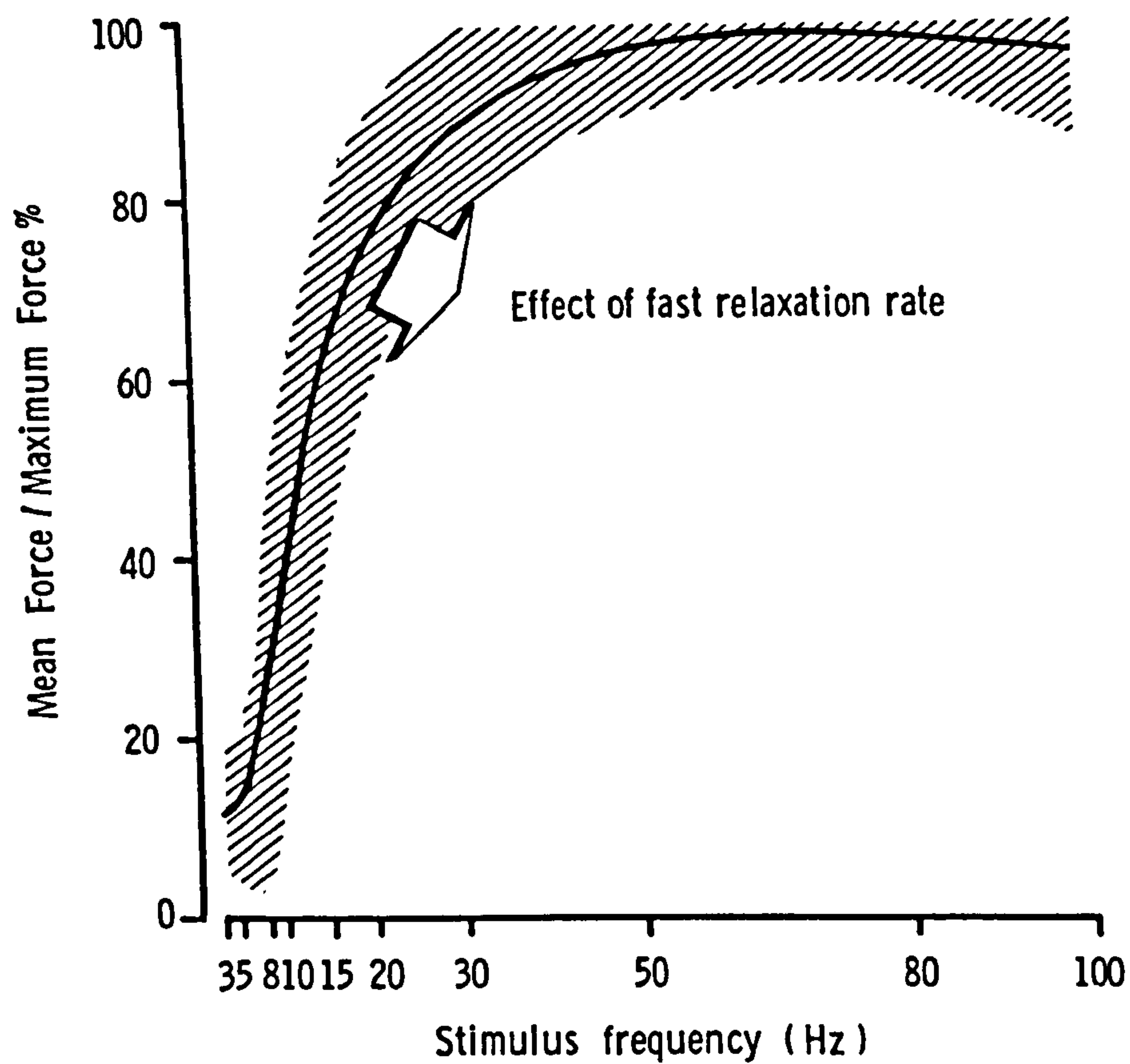


Fig. 7.3 A rapid relaxation rate increases the stimulus frequency required for tetanic fusion and shifts the frequency/force curve to the right. (Normal frequency/force curve for the human quadriceps taken from Paper I.)

line, however, is very different from that calculated for a large group of patients with other conditions (Fig. 5.2), having a smaller Y-intercept and a steeper slope (Fig. 7.4). As discussed in Chapter 5, it seems likely that the relationship observed in these other patients is similar to that which obtains in normal muscle. The steep relationship in Figure 7.4 suggests that thyroid imbalance not only alters the fibre-type composition of the quadriceps, but also alters the mean relaxation rates of type I and type II motor units. The low frequency of type II fibres in hypothyroid muscle is well established in man (McKeran, Slavin, Andrews, Ward & Mair, 1975). The rest of this hypothesis, however, relies on animal experiments for support.

Hyperthyroid rat muscle has an increased frequency of type II fibres and increased speeds of tetanic contraction, twitch contraction and twitch relaxation (Johnson, Mastaglia & Montgomery, 1980). (Tetanic relaxation rate was not reported.) Thirty per cent of the individual motor units sampled in soleus had twitch contraction times which were shorter than any seen in the normal soleus (Montgomery & Webb, 1982). Since the frequency of type II fibres probably only increased from about 20% to 40% (Johnson et al., 1980), this implies that there was also an increase in the intrinsic speed of the type II units. Similarly, the slowing of motor units observed in the rat soleus after thyroidectomy is greater than can be explained by the reduction in the type II fibre frequency (Ashton, Montgomery & Webb, 1981). From this and from the data in Figure 7.4, it would seem that the alterations of muscle speed observed in patients with thyroid disease are due to more than just the interconversion of fast and slow motor units.

CONCLUSIONS

A reduced maximal quadriceps strength is not uncommon in patients with thyrotoxicosis and is associated with a reduction in the cross-sectional area of muscle fibres. The atrophy affects both fibre types equally.

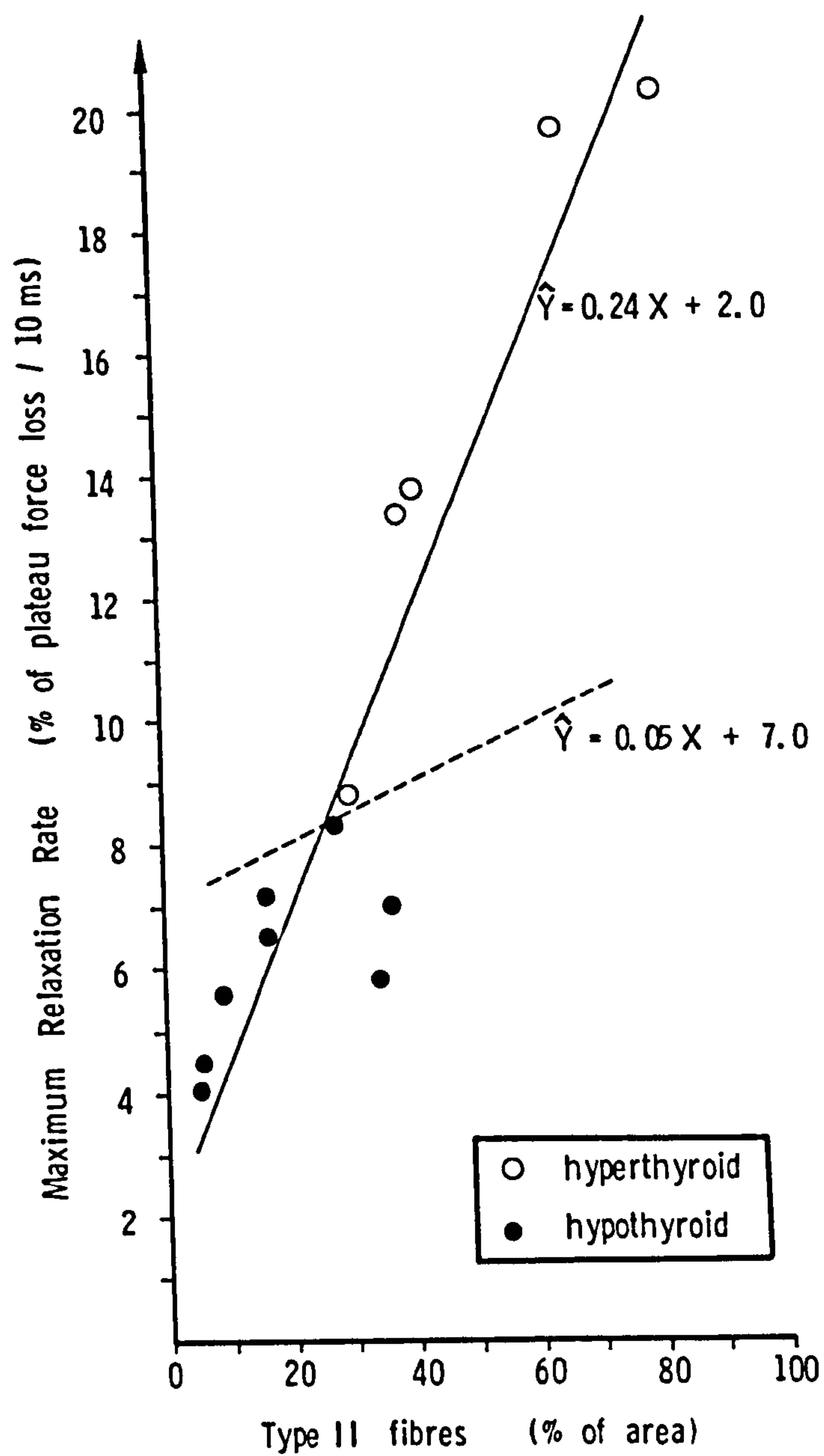


Fig. 7.4 Relationship between quadriceps relaxation rate and fibre-type composition in 8 hypothyroid and 5 hyperthyroid patients. The broken line indicates the regression calculated for 62 other, assorted patients (Fig. 5.2).

In hyperthyroidism, subjective weakness in submaximal contractions is probably also due to the higher stimulus frequency required to achieve tetanic fusion.

The high circulating levels of creatine kinase found in hypothyroidism are not indicative of active muscle destruction.

Slightly reduced mean concentrations of ATP and PC are found in quadriceps biopsies from patients with hypothyroidism and with hyperthyroidism. The depression of the concentrations of phosphagen tended to correlate with the severity of weakness, but only within each group of patients.

Hypothyroid patients were able to sustain a submaximal contraction for longer than normal, for a rather lower than normal total ATP turnover. Their rate of ATP turnover was therefore much lower than normal, presumably because their slow muscle-relaxation made their muscle more efficient (in terms of force x time/ATP-used). The rate of lactate production by hypothyroid muscles was correspondingly slow; there was no evidence that glycolysis was slower than was necessary to meet the needs of ATP regeneration.

The frequency and the mean cross-sectional area of type II fibres relative to type I fibres is reduced in hypothyroidism.

The type II frequency was higher than normal in the 1 male patient with hyperthyroidism and in 1 of the 4 thyrotoxic women.

The slowing of quadriceps relaxation in hypothyroidism and the acceleration observed in hyperthyroidism cannot be fully explained by the changes in fibre-type composition. It is likely that the mean relaxation rates of type I and type II motor units are also changed.

CHAPTER 8

QUADRICEPS ATROPHY AND WEAKNESS AFTER KNEE INJURY AND/OR IMMOBILIZATION

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CHAPTER 8

QUADRICEPS ATROPHY AND WEAKNESS AFTER KNEE INJURY AND/OR IMMOBILIZATION

INTRODUCTION

Weakness associated with loss of muscle mass is a feature of joint injury and/or immobilization. It contributes significantly to disability and probably also renders the joint vulnerable to further damage. Following knee injury and/or immobilization, quadriceps weakness and wasting may sometimes persist most stubbornly.

Much of the time spent by physiotherapists attempting to correct joint-induced ('arthrogenous') muscle wasting may be futile. Little is known about the underlying pathophysiology and the putative therapeutic techniques have not been properly evaluated. The treatments given by physiotherapists to patients with arthrogenous quadriceps weakness are largely empirically-based and their effectiveness is assessed either subjectively or with a tape measure - a technique which is of limited accuracy (Nicholas, Taylor, Buckingham & Ottonello, 1976; Kirwan et al., 1979) and, as will be discussed in this chapter, of dubious relevance. This chapter describes a series of studies into the nature of arthrogenous quadriceps atrophy and the development of a method of measuring the size of the muscle. Chapter 9 then discusses the first of a new series of experiments to apply this knowledge and technique to studies of muscle growth.

The muscular effects of generalised conditions (e.g. starvation, muscular dystrophy or myositis) can be followed most informatively with 'whole-body' techniques such as metabolic balance studies (Edwards et al., 1979) or the measurement of 3- methylhistidine excretion (Edwards et al., 1981). Such an approach is less helpful when the atrophy is limited to a single limb or to a single muscle or muscle group. Evaluation of events in these circumstances demands an approach at the level of the individual muscle.

Davies & Sargeant (1975a,b,c) had already attempted to identify structural and functional changes at the level of the single limb, using measurements of total fat-free limb volume and of maximal oxygen uptake ($\dot{V}O_2$ max) during one-legged pedalling. Doubtful if this approach was sufficiently specific, I began my work in this area by comparing 'individual-muscle' results (obtained by needle biopsy and dynamometry) with 'single-limb' results (obtained by anthropometry and ergometry). This confirmed the need for a means of actually measuring the severity of atrophy localised to the quadriceps. A means of measuring the whole muscle would also be preferable to extrapolation from measurements of myofibre size. After my move from London to Oxford, therefore, I concentrated on developing and appraising ultrasound scanning for this purpose. As a result, this chapter also includes an account of how the simultaneous use of ultrasonography extends the conclusions which can be drawn from needle-biopsy information.

Throughout this chapter I have tacitly assumed that the tension developed per unit cross-sectional area is the same for both type I and type II muscle fibres. Some recent evidence, however, suggests that this may not be the case. The possibility that type II fibres may be twice, or even three times, as strong as type I fibres is discussed in Chapter 10, when the effect on the data in this chapter is also considered.

PATIENTS

A summary of the patients described in this chapter is given in Figure 8.1.

The 7 patients described in Paper IV were all attending the Joint Services Medical Rehabilitation Unit, Chessington. They were otherwise healthy, young servicemen who had fractured the tibia and fibula of one leg and had been treated by immobilization of the injured limb in a long-leg-plaster. One patient still had non-union of the tibia at the time of study and was still not permitted to bear weight through the injured limb; his knee had then been immobilized

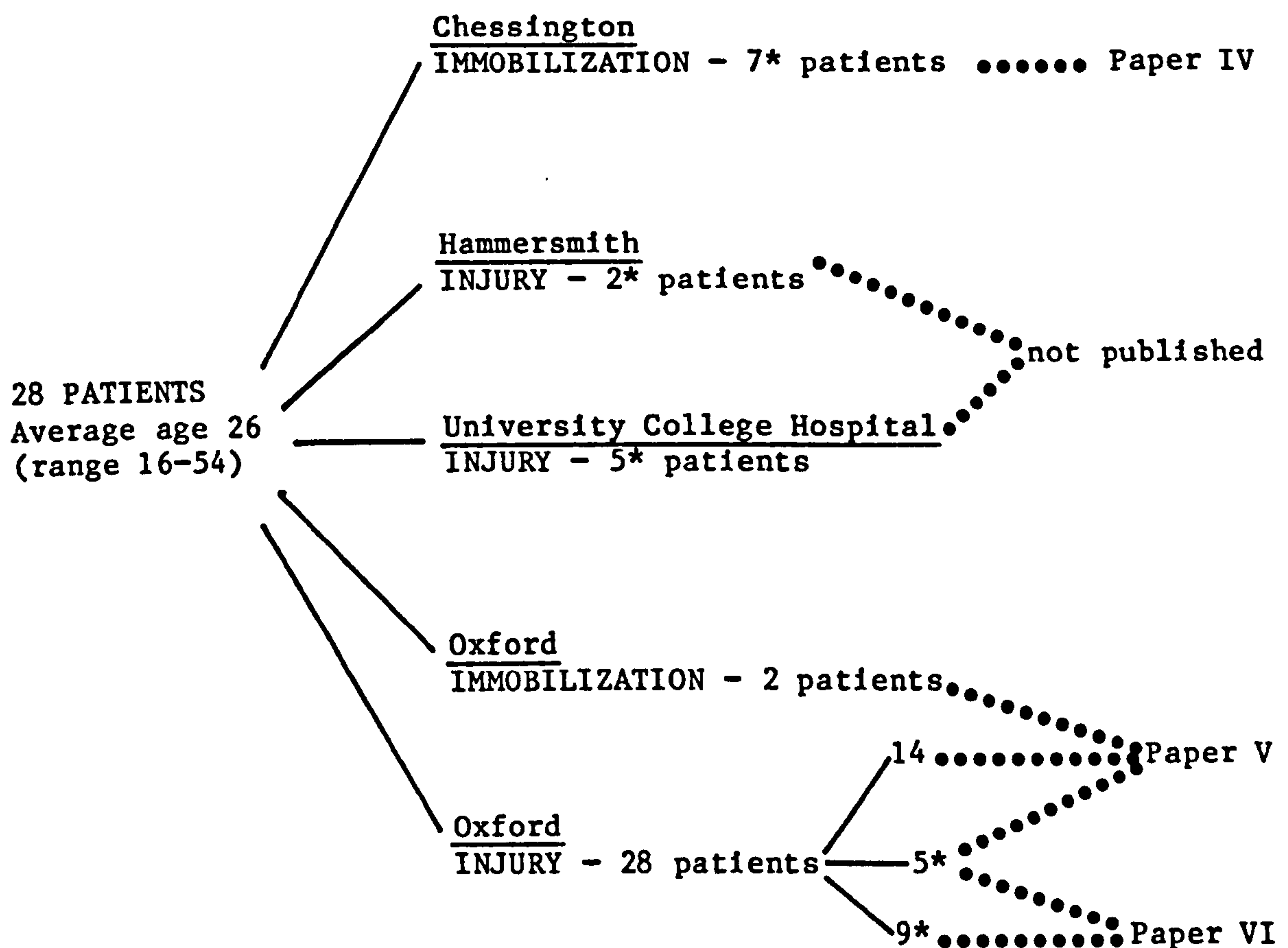


Fig. 8.1 Summary of patients with thigh muscle wasting following knee immobilization or knee injury (+ immobilization), the places at which they were studied and the Papers in which their data have been published.

* Patients who underwent needle biopsy of both quadriceps.

and/or non-weight-bearing for the longest period of this group of patients (213 days).

In the present discussion, the data in Paper IV will be considered along with unpublished data from a further 7 patients (5 male, 2 female). Their quadriceps wasting was secondary to a variety of knee injuries and they were attending either Hammersmith Hospital or University College Hospital (Fig. 8.1). Three of the 4 patients who had undergone meniscectomy were studied as soon as they could achieve 90° of flexion following removal of the Robert Jones bandage (2) or plaster cast (1) which had been applied at operation. (One of these patients was a female professional footballer.) The fourth meniscectomy patient was studied 2 months after his operation. One patient was seen 4 days after the resumption of full weight-bearing, having torn a medial collateral ligament 7 weeks previously. One was studied 6 months after developing a septic arthritis (secondary to arthroscopy for a suspected meniscus tear). The remaining patient (female) was seen 14 weeks after undergoing patellectomy as the treatment for a 6 year history of chondromalacia patellae. The average age of the second 7 patients was 29 years (range 16 - 54 years).

For Papers V and VI, patients were recruited from the physiotherapy, orthopaedic and soft-tissue injury out-patient clinics which they were attending (at the Nuffield Orthopaedic Centre and the John Radcliffe Hospital, Oxford) after suffering unilateral knee injury and/or immobilization (Fig. 8.1). They had a wide range of underlying orthopaedic diagnoses (listed in the 2 papers) but only 2 patients, with tibial fractures (Paper V), might be said to have had knee immobilization without knee injury. Five of the patients described in Paper VI were also included in Paper V. The patients were all essentially healthy, young or middle-aged adults and had been asked to participate in the studies if a difference in mid-thigh circumference of at least 2cm was recorded by the physiotherapist in the clinic. Failure of the investigators to confirm this difference, as was frequently the case, was not a reason for excluding patients from the study. Criteria for exclusion were (1) clinical evidence suggestive of denervation, muscle disease or inflammatory joint

disease, (2) a history of injury to the other lower limb indicative of reduced hip, knee or ankle mobility for more than 1 week in the preceding 2 years, (3) the presence of a femoral or pelvic fracture.

METHODS

Dynamometry

The 5 patients who were attending the Hammersmith and University College Hospitals had their voluntary isometric strength measured with the muscle testing chair described in Paper I. Similar, but not directly comparable, equipment (Doré, Hackett, Imms & Prestidge, 1977) was used, by Dr. Imms, to measure the voluntary, isometric, quadriceps strength of 6 of the Chessington patients. The apparatus differs in that it avoids stress on the fracture site and measures isometric torque about the knee rather than the force exerted at the ankle.

Ergometry

Maximum oxygen uptake ($\dot{V}O_2$ max) in one-legged cycling was measured by Dr. A.J. Sargeant at Chessington (Paper IV). The technique has been described in detail elsewhere (Davies & Sargeant, 1975a).

Anthropometry

(i) Thigh circumference

In routine clinical practice, tape measure estimates of limb circumference are commonly used to record muscle growth or atrophy. The measurements of thigh circumference (C) reported in Papers V and VI were made at "mid-thigh" (defined here as half-way between the greater trochanter and the lateral joint line of the knee).

Repeated measurements were made on 2 male and 6 female subjects (a total of 29 sets of measurements). The 'within-limbs' coefficient of variation for C^2 and the 'within subjects' coefficient of variation for $C_{\text{left}}^2/C_{\text{right}}^2$ were both $\leq 2\%$.

(ii) Fat-free limb volumes

The fat-free volume of each lower limb was estimated anthropometrically, treating the limb as a series of truncated cones and using measurements of skinfold thickness to allow for subcutaneous fat (Jones & Pearson, 1969; Davies & Sargeant, 1975c). Together, the two most proximal 'cones' extend from the gluteal fold to the narrowest point proximal to the knee joint. The sum of these two 'cones' was taken to be the fat-free thigh volume (FFTV).

The repeatability of this technique in our hands was checked: the coefficients of variation were again $\leq 2\%$.

Ultrasonography

Ultrasound scanning was first used to measure muscle cross-sectional area (CSA) in 1968 (Ikai & Fukunaga, 1968), but was technically difficult. Even in 1979, Dons, Bollerup, Bonde-Petersen & Hancke reported difficulty in using ultrasonography to measure quadriceps CSA. Nevertheless, we have found that our scanning technique, with a conventional diagnostic ultrasound B-scanner with grey scale attachment (Nuclear Enterprises, 'Diasonograph NE 4200') provides an image of a transverse section through the quadriceps from which its CSA can be accurately measured. Examples of scans are included with the description of the technique in Paper V. A more general account of ultrasonography of muscle is given in Young & Hughes (1982).

Except where specifically stated, scanning was done at the mid-thigh level (i.e. halfway between the greater trochanter and the

lateral joint line of the knee. Scans were only performed with the muscle relaxed.

The scan image was photographed from the oscilloscope to give an ultrasonogram on Nuclear Medicine NMB film (Kodak) which was about half life-size. The outline of the quadriceps group was then traced from the ultrasonogram. An electronic caliper of known separation was incorporated into a scan on each occasion that the instrument was used.

At first, the area enclosed by the traced outline was measured by cutting out and weighing the tracings. (Calibration was by cutting out and weighing circles of tracing paper of diameter equal to the caliper separation.) Later, the areas were measured from the tracings by means of an MOP electronic planimeter. This change did not influence the variability of repeated measurements of quadriceps CSA but greatly reduced the time taken to measure the tracings. The 'within subject' coefficient of variation for quadriceps CSA measured on single scans on different days was 6.1%. Our procedure, therefore, was to record quadriceps CSA as the mean of 4 scans on each occasion, thus reducing the coefficient of variation to 4.0% (Paper V).

RESULTS

Biopsy morphology

The histological appearance of sections cut from the patients' biopsies was normal apart from a reduction in mean myofibre size and, often, an increased variation in fibre size. Histochemical studies often showed a loss of contrast between fibre types in respect of their oxidative enzyme reactions. Histochemical evidence of even a slight degree of abnormal acid phosphatase activity was rare. Fibre-typing according to myosin ATPase reactivity revealed changes in the relative sizes of type I and type II fibres which will be described later.

Muscle chemistry

Bilateral biopsies from 6 of the Chessington patients were analysed for their content of ATP and PC. There was no consistent difference between the injured and uninjured leg for either metabolite, as was also the case for patients studied some 14 months after knee ligament surgery (Grimby, Gustafsson, Peterson & Renström, 1980).

Myofibre size, lower limb volumes and ergometry

Satisfactory ergometric studies were achieved for 6 of the 7 post-fracture patients. Except for 1 subject, the values of $\dot{V}O_2$ max fell within the limits previously reported for the relationship between leg volume and one-legged $\dot{V}O_2$ max (Davies & Sargeant, 1975a).

The levels of $\dot{V}O_2$ max in one-legged cycling which were achieved averaged 17% less with the injured limbs. The difference in the fat-free volume of their limbs averaged 12% (Paper IV). In contrast, the mean reduction in myofibre cross-sectional area was 43%.

In order to look more closely at this apparently anomalous result, the fat-free thigh volume was calculated (instead of fat-free limb volume) and, in addition, the relationship between FFTV and MFA was examined in a further 7 patients (at Hammersmith and University College Hospitals). Considering all 14 patients with unilateral wasting, the MFA in the biopsy from the wasted side was, on average, 38% less than that for the opposite side (Fig. 8.2). The mean difference in FFTV was only 11%. If representative of events in the quadriceps as a whole, the fibre size measurements showed the atrophy to be much greater than would be suspected from estimates of fat-free thigh volume (Fig. 8.3).

Myofibre size and knee-extension strength

Bilateral measurements of isometric knee-extension strength

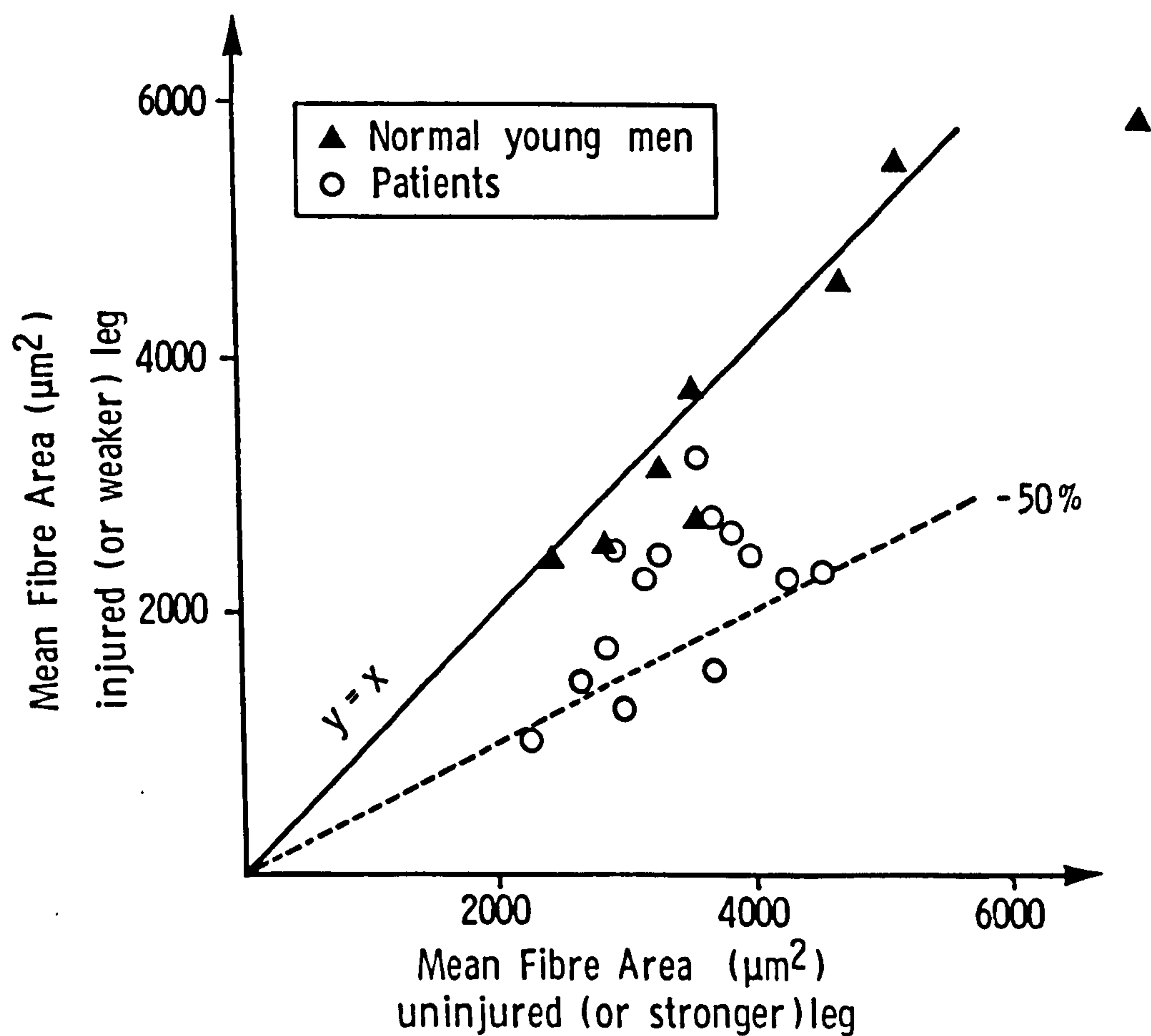


Fig. 8.2 Comparison of the mean cross-sectional area of the muscle fibres in needle biopsies taken from both quadriceps of 14 patients with unilateral muscle wasting secondary to knee immobilization or injury. (The corresponding weaker limb/stronger limb comparison has been made for 8 normal young men.)

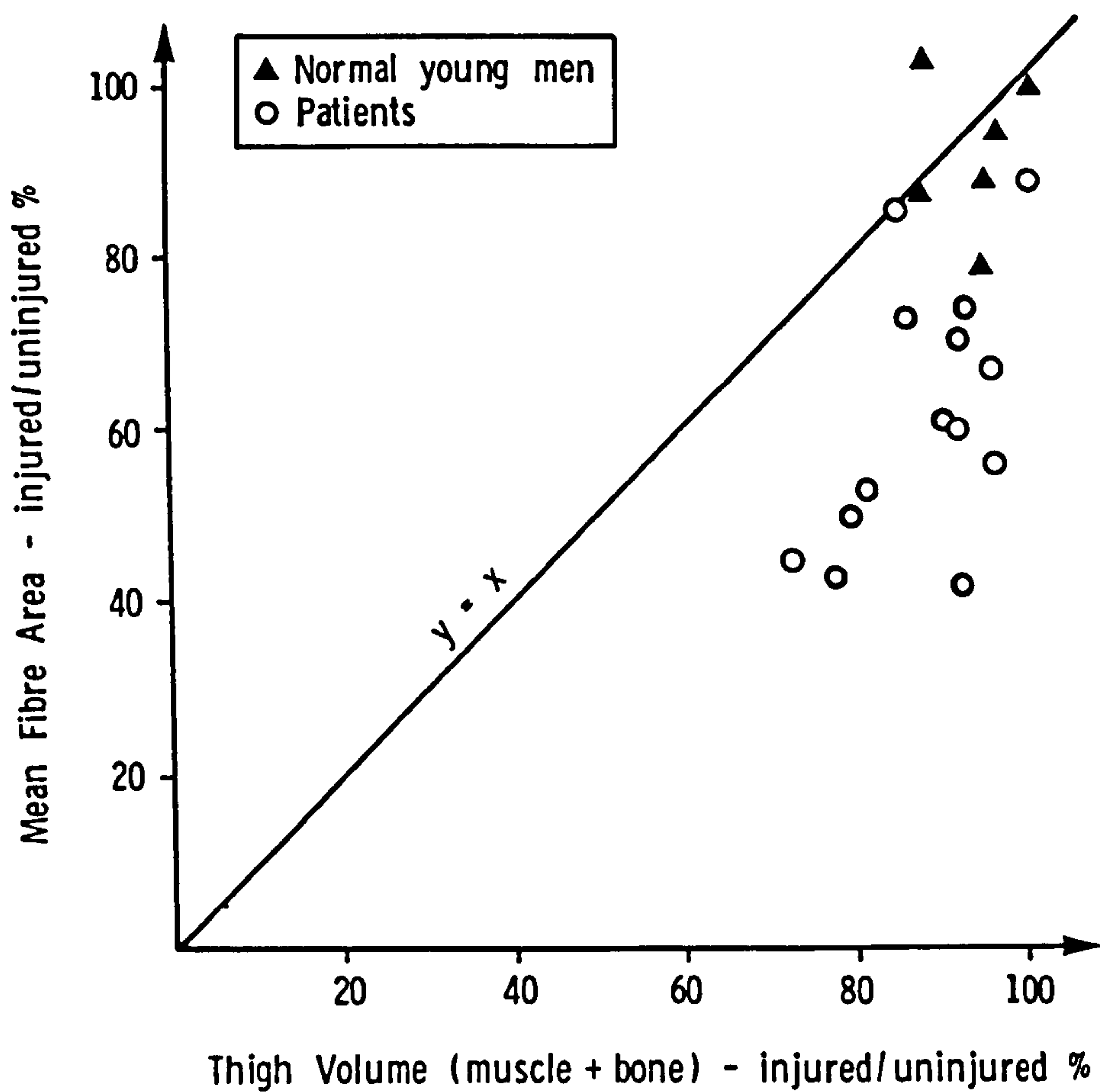


Fig. 8.3 Anthropometric estimates of fat-free thigh volume appear to underestimate the severity of quadriceps muscle wasting which follows knee immobilization or injury. (Weaker quadriceps/stronger quadriceps ratios have been plotted for the normal subjects.)

which were judged not to have been limited by pain were obtained for 6 of the patients with tibial fractures (Chessington) and for 5 of the patients with knee injuries (Hammersmith and UCH). The former measurements were made by Dr. F.J. Imms (vd. 'Methods') and the latter by myself. Although different methods were used for the 2 groups of patients, the results can all be considered together if the strength of the injured limb is expressed as a percentage of the strength of the contralateral, uninjured limb (Fig. 8.4).

None of these strength measurements is included in my published work, largely because the subjective selection of "satisfactory" measurements of strength (i.e. those not limited by pain or fear of pain) was done retrospectively. Nevertheless, they are considered here since they help to put the other studies in context. They are consistent with the argument, developed in Papers II and III, that the severity of muscle weakness in these conditions is related to the reduction in MFA. There was a mean difference in quadriceps strength of 38% and in MFA of 37% for the same 11 patients.

The important practical conclusions drawn from these data were that one-legged cycle ergometry might not be sufficiently sensitive to changes in quadriceps function and that the same might also apply to thigh anthropometry as a measure of changes in quadriceps bulk. Hence the next study.

Quadriceps cross-sectional area and thigh volumes

The patients with unilateral thigh muscle wasting who are described in Paper V had differences in mid-thigh circumference ranging from 0.4cm to 6.1cm (median = 1.8cm). In many cases, therefore, the difference was not significant at the 95% level (Nicholas et al., 1976; Kirwan et al., 1979). Yet the difference in quadriceps CSA in these same 21 patients ranged from 8% to 47% (median = 24%). These data indicate that, after knee injury or immobilization, simple thigh circumference measurements seriously underestimate the amount of quadriceps wasting which has taken place (Paper V, Fig. 3).

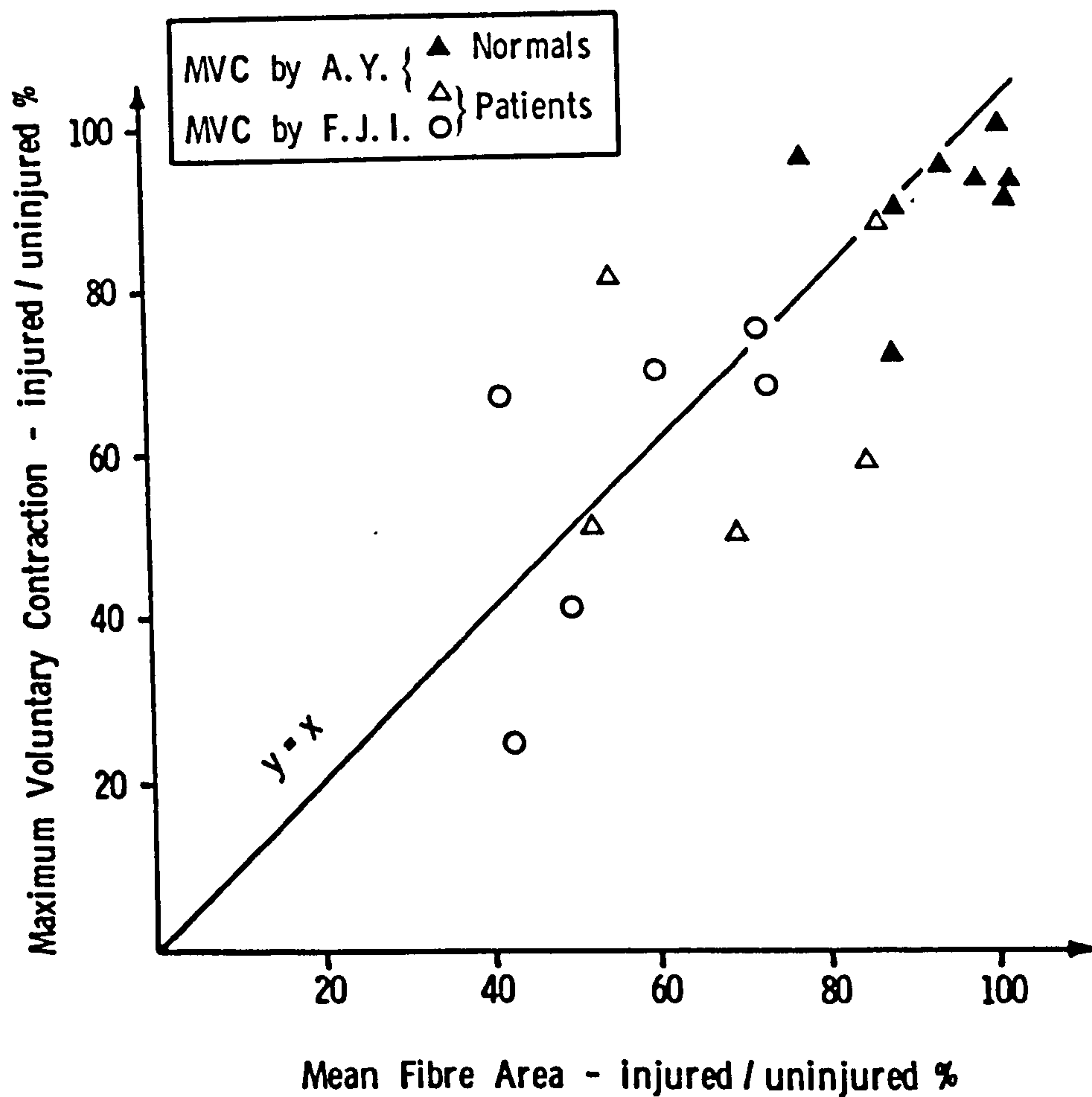


Fig. 8.4 Comparison of the injured/uninjured limb ratios for quadriceps fibre size and strength. (Weaker quadriceps/stronger quadriceps ratios have been plotted for normal young men.)

When allowance is made for differences in the thickness of subcutaneous fat in the two limbs, by measuring FFTV, anthropometry still underestimates the true severity of quadriceps atrophy (Paper V, Fig. 4).

Quadriceps cross-sectional area and myofibre size

The between-limb differences in quadriceps CSA and MFA were compared in 14 patients with unilateral wasting following knee injury and with a wide range of values for their 'normal' quadriceps CSA ($43 - 104\text{cm}^2$) (Paper VI, Fig. 1). The reductions in quadriceps CSA varied from 11% to 46% and were matched by similar reductions in MFA (Fig. 8.5).

These data were presented in a slightly different way in Paper VI in order to indicate that, despite the severity of the wasting in some cases, there was no need to invoke hypoplasia as a contributory factor. If the cross-sectional area of a whole muscle is divided by its mean fibre area, the quotient is a function of the number of fibres it contains. There was a close correlation between the values of CSA/MFA obtained for the two limbs of each patient ($r = 0.75$, $P < 0.005$) and the regression of $\text{CSA/MFA}_{\text{injured}}$ on $\text{CSA/MFA}_{\text{uninjured}}$ was virtually indistinguishable from the line of identity (Paper VI, Fig. 2).

Selective atrophy of type I or type II muscle fibres

(i) Patients recovering from a tibial fracture

The biopsies from the 7 patients studied at Chessington (Paper IV) indicated that the atrophic process involved both of the principal myofibre types, as distinguished by their myosin ATPase activity. In 6 patients the difference between the injured and uninjured limb was greater for the type I fibres than for the type II fibres (Fig. 8.6). The exception was the only patient who had not yet started weight-bearing. His knee had been immobilized for 7

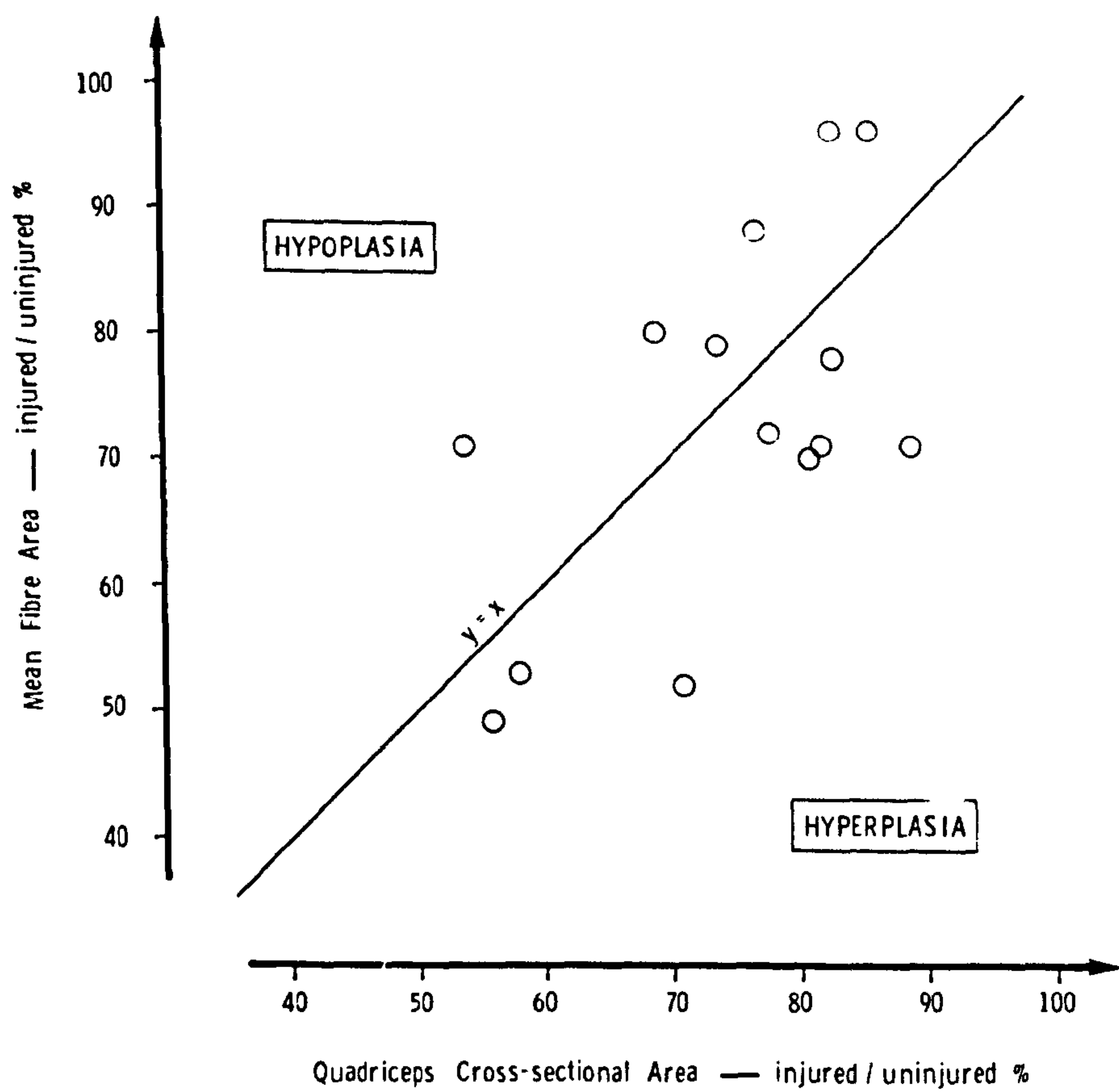


Fig. 8.5 Comparison of the injured/uninjured limb ratios for quadriceps cross-sectional area and fibre size in 14 patients who had suffered unilateral knee injury.

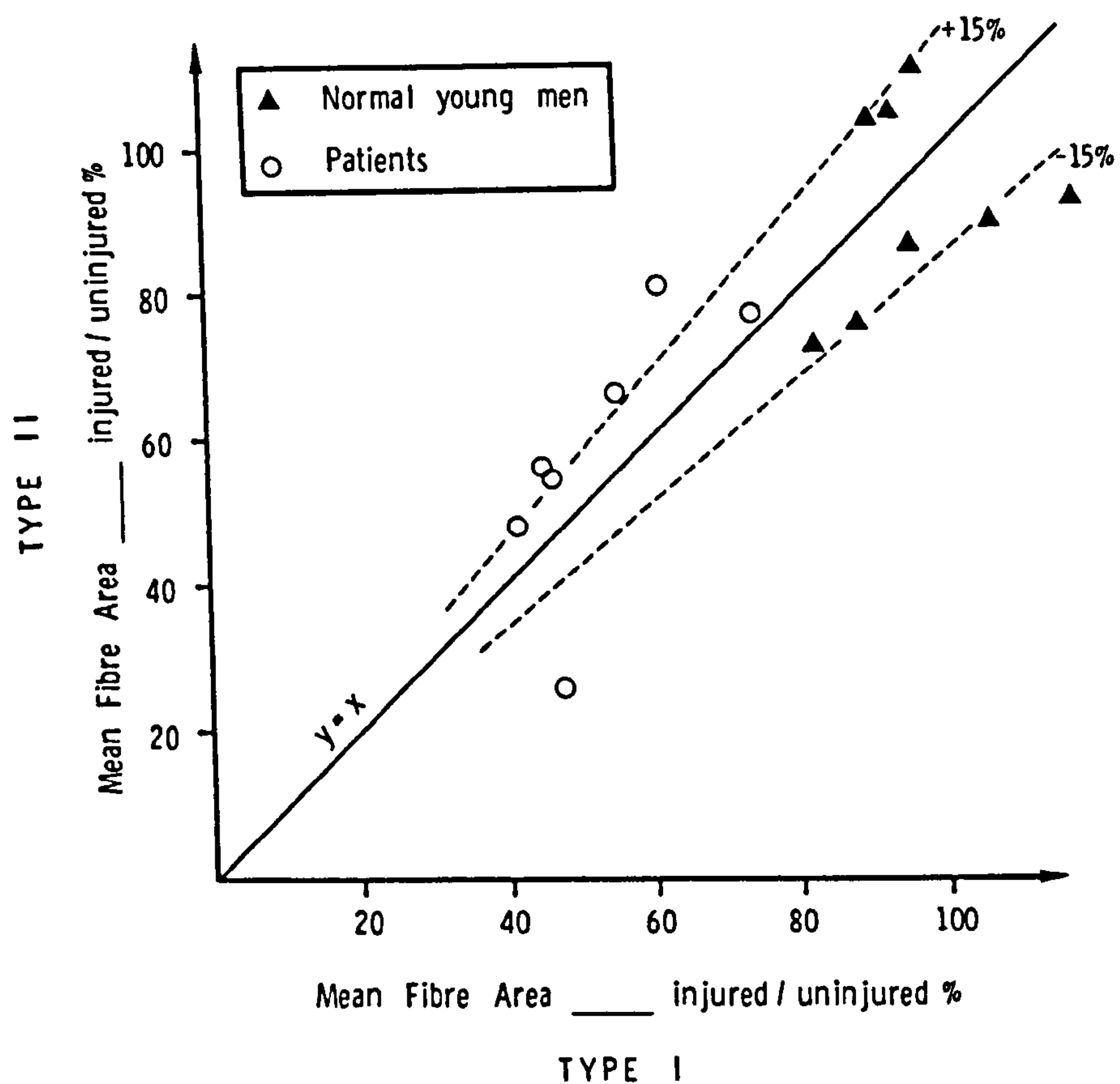


Fig. 8.6 Comparison of the injured/uninjured limb ratios for the size of type I and type II fibres from the quadriceps of 7 young men recovering from unilateral tibial fractures. (Weaker quadriceps/stronger quadriceps ratios have been plotted for normal young men.)

months and he still had non-union of his tibia. The 6 others had been weight-bearing for at least a week and were making uncomplicated recoveries.

Taking the 7 patients as a group, the apparent tendency to selective type I atrophy is not statistically significant (Wilcoxon signed-ranks test). Nevertheless, it is in marked contrast to the selective atrophy of type II fibres commonly encountered in patients with muscle weakness secondary to systemic conditions such as osteomalacia (Paper II), thyroid disease (Paper III; McKeran et al., 1975), primary hyperparathyroidism (Patten, Bilezikian, Mallette et al., 1974), corticosteroid excess and a variety of other non-muscular disorders (Dubowitz & Brooke, 1973, pages 78-79). This contrast was examined by comparing the bilateral biopsies from the 7 young men who had had fractures with unilateral biopsies from 17 normal young men and from 19 young men with muscle weakness secondary to a variety of systemic disorders (Fig. 8.7). The comparison is based on an alternative method of evaluating atrophy, calculating 'atrophy factors' to identify biopsies with an abnormal number of small fibres (Brooke & Engel, 1969). (The 'atrophy factor' is simply a weighted score of the number of fibres in a biopsy which are unusually small, weighted according to just how small they are.) Atrophy factors calculated separately for each fibre type confirmed that in 6 of the 7 orthopaedic patients the type I fibres were the more severely affected, and in only 2 was there a significantly elevated atrophy factor for the type II fibres. This is quite unlike the findings in their own uninjured limbs, in the normal controls (with 1 exception) and in the 'patient-controls' (also with 1 exception).

(ii) Patients who had suffered a knee injury

The biopsies from the 7 'knee injury' patients studied at Hammersmith and University College Hospitals (unpublished data) are considered together with those from the 14 'knee injury' patients biopsied in Oxford (Paper VI). Examples of predominantly type I fibre atrophy and of predominantly type II fibre atrophy were found in both groups of patients, with the latter being more common (Fig. 8.8).

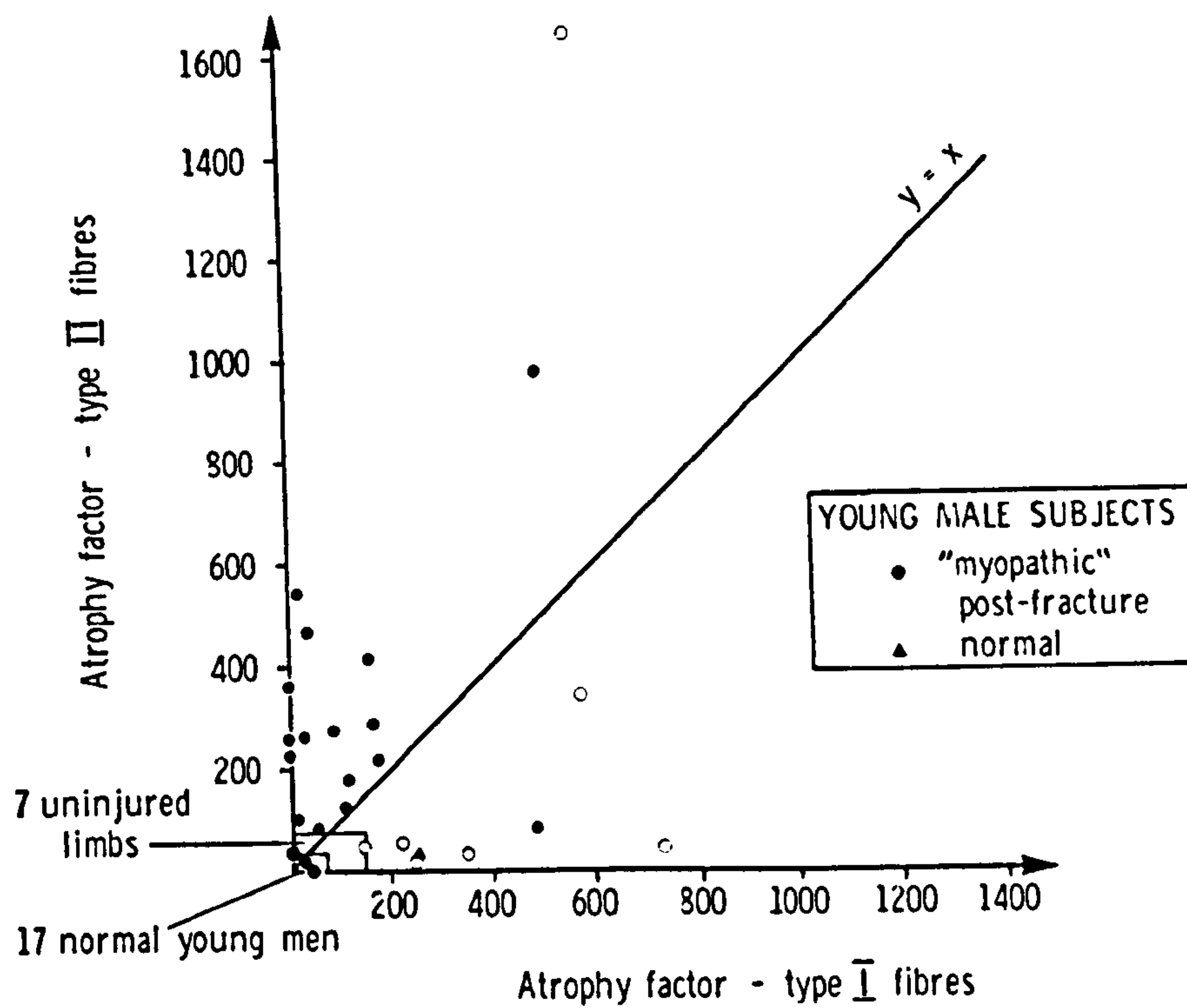


Fig. 8.7 Atrophy factors for type I and type II fibres in bilateral quadriceps biopsies from 7 young men recovering from tibial fractures and in unilateral biopsies from 17 normal young men and 19 young men with muscle weakness secondary to a non-muscular systemic disease.

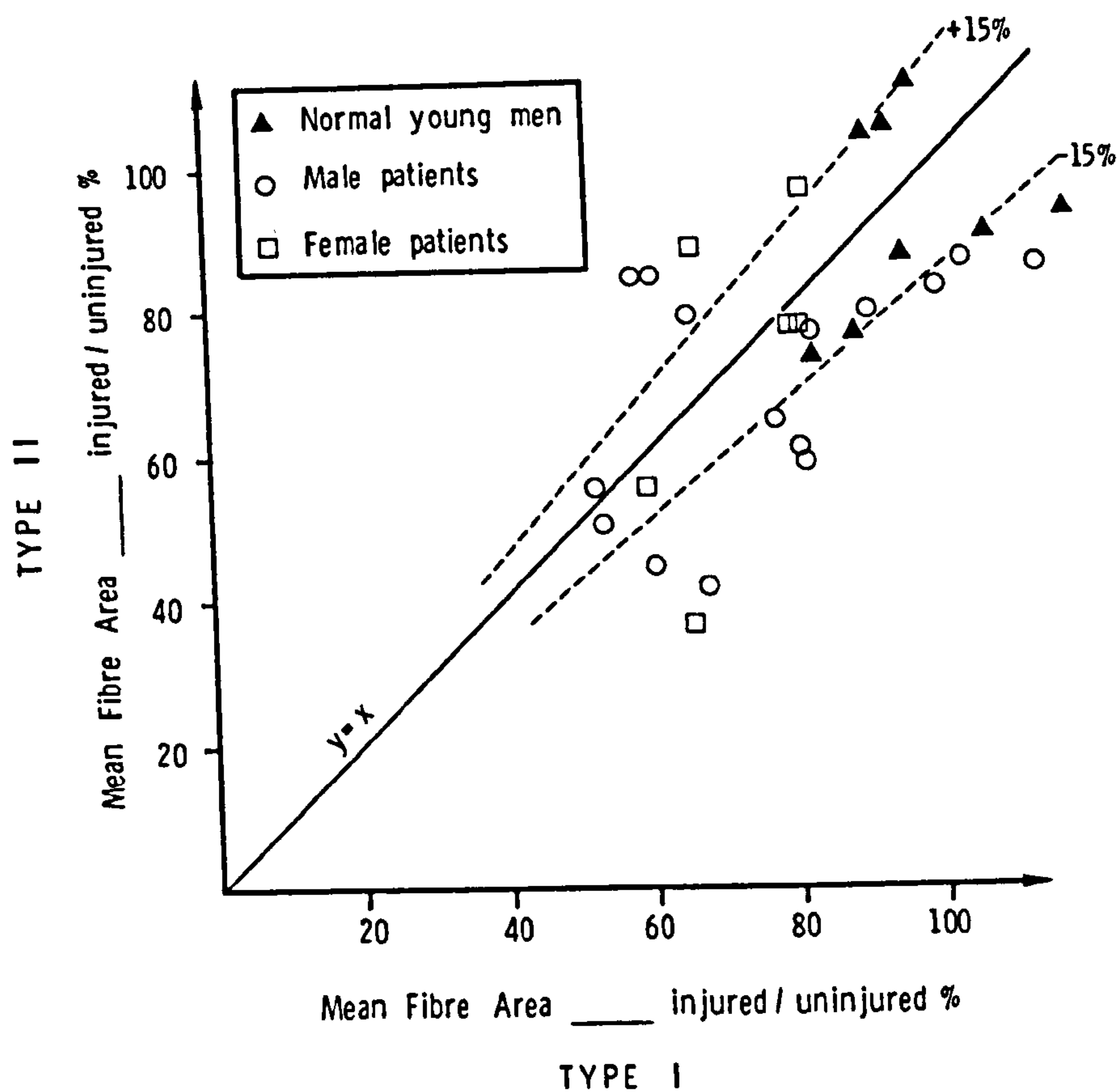


Fig. 8.8 Comparison of injured/uninjured limb ratios for the size of type I and type II fibres from the quadriceps of 6 women and 15 men who had suffered a unilateral knee injury. (Weaker quadriceps/stronger quadriceps ratios have been plotted for normal young men.)

In some instances the selective nature of the atrophy was quite prominent (Figs. 8.9 and 8.10). It was not possible to recognise any consistent relationship between the more severely affected fibre type and clinical characteristics such as the nature of the injury, the time since the injury, the time since resumption of weight-bearing, the presence of an effusion, a limited range of knee flexion or the estimated nature and frequency of rehabilitation exercise.

Frequency of type II fibres in the quadriceps following knee injury and/or immobilization

The wasted quadriceps of 5 of the 7 post-fracture patients studied at Chessington (Paper IV) showed a higher frequency of type II fibres than the contralateral muscle. In the other 2 patients the frequency of type II fibres was lower in the wasted muscle. The mean difference was +3% and was not statistically significant.

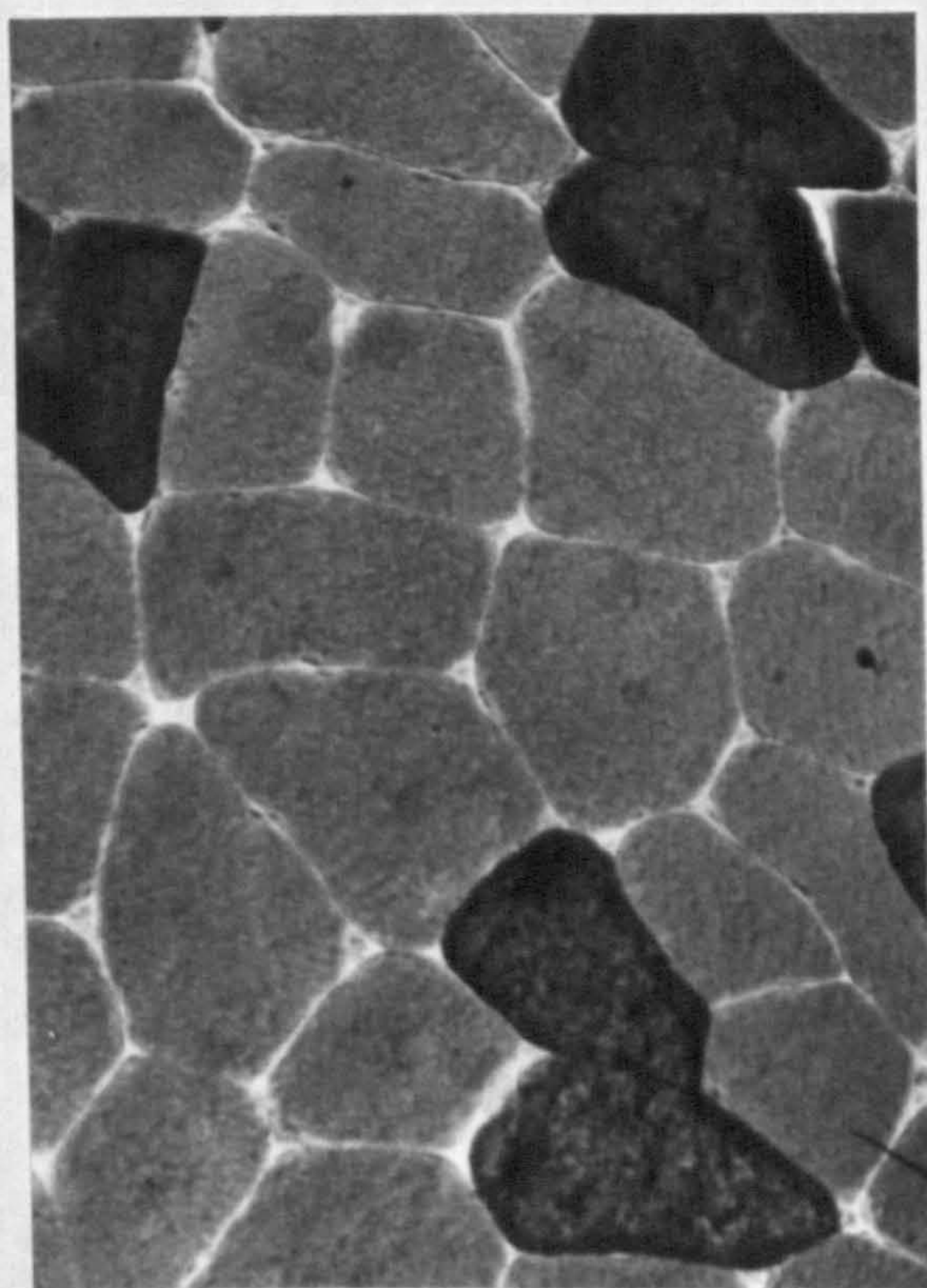
The mean difference between the limbs of the 7 knee-injury patients studied at Hammersmith and University College Hospitals was +1% and was not statistically significant.

The 14 knee-injury patients studied in Oxford (Paper VI) had a mean difference of +7% (median = +8%; range = -10 to +23) (Fig. 8.11). This difference was statistically significant ($P = 0.03$, two-tailed Wilcoxon signed-ranks test). The tendency to an increased type II frequency in the wasted muscle appeared unrelated to whether there was preferential atrophy of the type I or the type II fibres (e.g. Figs. 8.9 and 8.10).

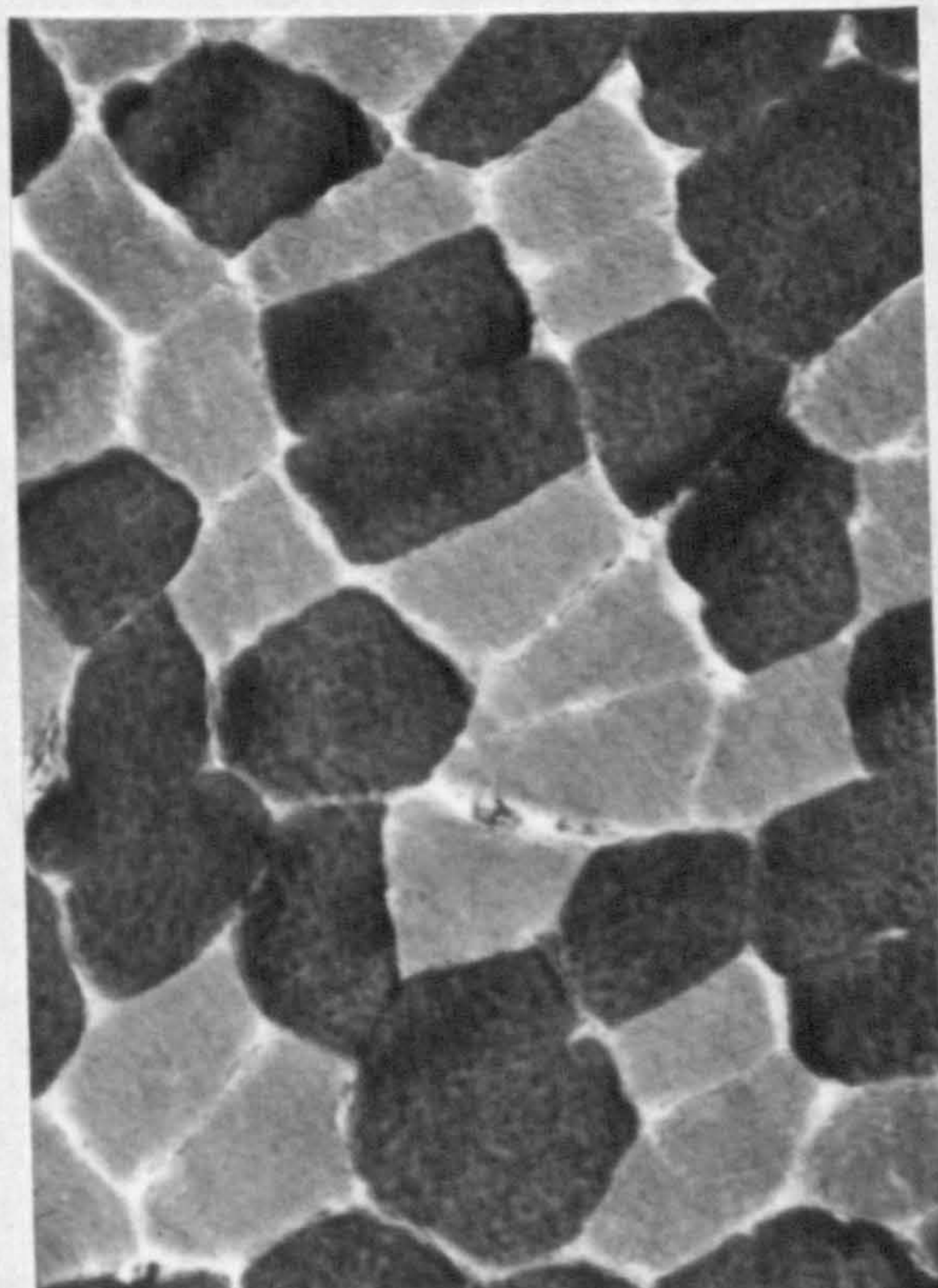
DISCUSSION

Muscle size and function

Traditionally, the thigh muscle wasting that follows knee injury is recorded in terms of changes in thigh circumference. Yet the tape measure encloses not only the quadriceps but also a large bulk of other muscles and a layer of subcutaneous fat. Some allowance can be



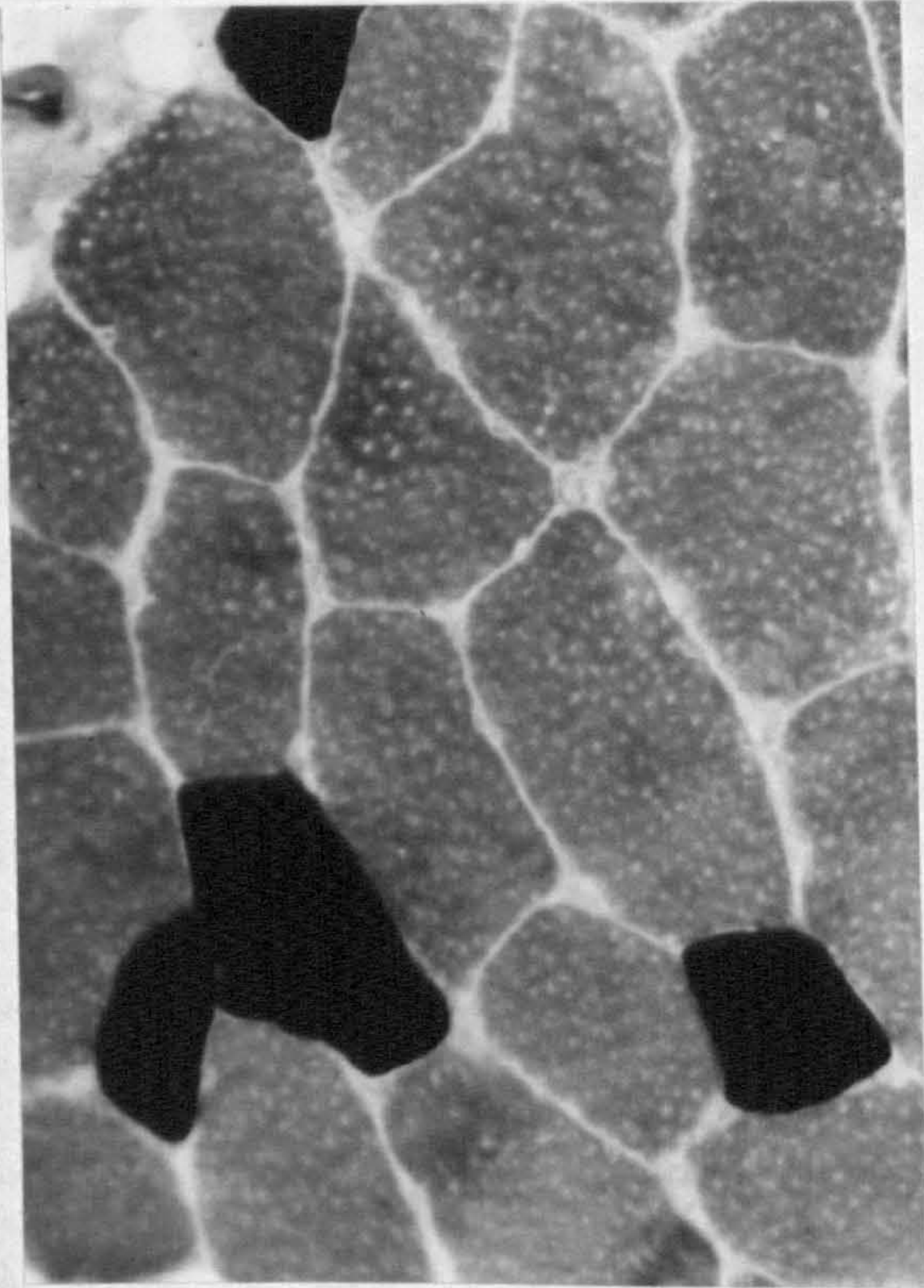
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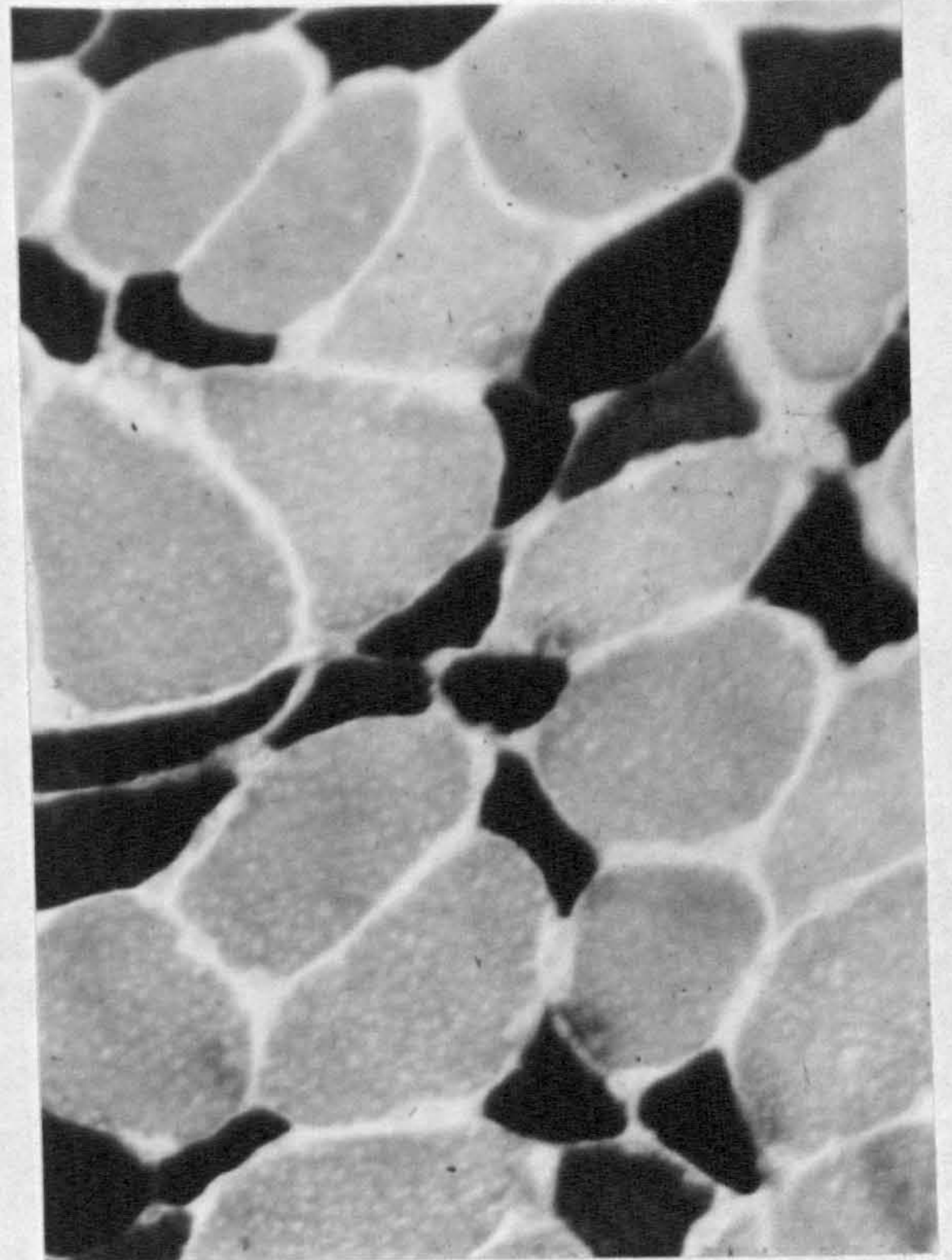
b

Fig. 8.9 PREFERENTIAL TYPE I FIBRE ATROPHY

Transverse sections of needle biopsy specimens taken from the quadriceps of the uninjured (a) and injured (b) limbs of a 24-year-old man, 4 days after the resumption of full weight-bearing, having torn a medial collateral ligament 7 weeks previously. (Myosin ATPase, pH 9.4; equal magnification.)



a



b

Fig. 8.10 PREFERENTIAL TYPE II FIBRE ATROPHY

Transverse sections of needle biopsy specimens taken from the quadriceps of the uninjured (a) and injured (b) limbs of a 33-year-old woman before the resumption of full weight-bearing, 7 weeks after a medial meniscectomy and 'pivot shift repair'. (Myosin ATPase, pH 9.4; equal magnification.)

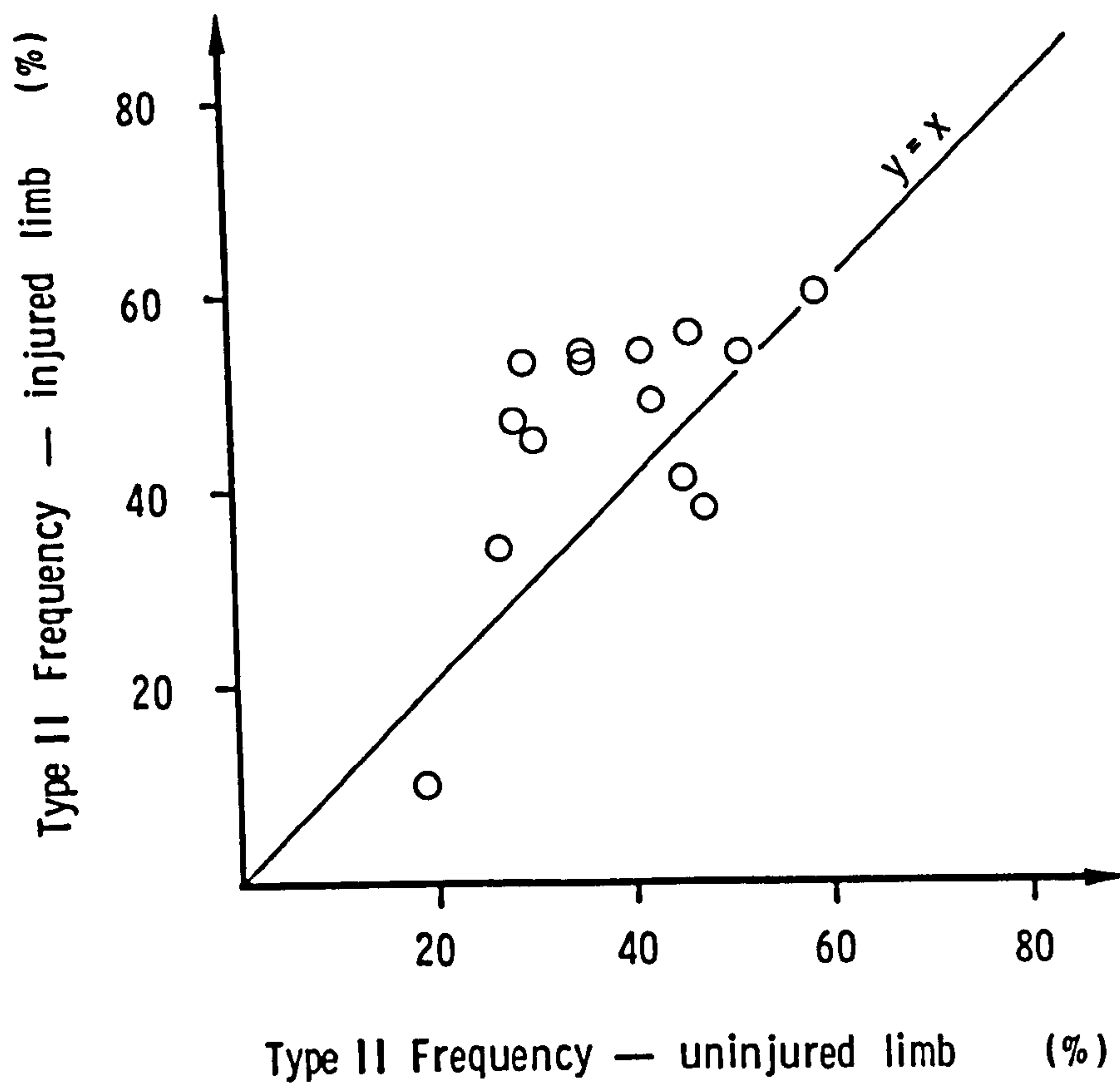


Fig. 8.11 The frequency of type II muscle fibres in bilateral needle biopsy specimens from the lateral quadriceps of 14 patients with unilateral knee injury.

made for the latter by means of soft-tissue X-rays or skinfold thickness measurements. Nevertheless, these techniques are still unable to differentiate between changes localised to the quadriceps and those also affecting the hamstrings and the adductors.

An image of a transverse section through the thigh, sufficiently detailed to allow accurate measurement of quadriceps CSA, can now be obtained by computerised axial tomography or by grey-scale ultrasound B-scanning. Computerised tomography has now been used in a study of 2 patients with thigh muscle wasting following knee trauma (Ingemann-Hansen & Halkjaer-Kristensen, 1980) and in a study of calf muscle wasting following rupture of the achilles tendon (Häggmark & Eriksson, 1979a). As discussed in Paper V, ultrasonography had already been used to estimate approximate muscle size in 2 strength-training studies (Ikai & Fukunaga, 1970; Dons et al., 1979) when we first reported its application to the accurate measurement of muscle wasting (Young, Hughes, Russell & Parker, 1979). Ultrasonography has two major advantages over computerised tomography: it uses equipment which is widely available in hospitals throughout the country and it does not involve any exposure to ionising radiation.

Having developed a technique for measuring quadriceps CSA by ultrasonography (and having confirmed the need for such a measurement - Paper V), it was possible to return to our earlier, rather tentative conclusions concerning the disparity encountered in patients with unilateral, arthrogenous thigh-muscle wasting (Paper IV), between the marked reduction in MFA and the much more modest reductions in fat-free limb volume and in $\dot{V}O_2$ max during one-legged pedalling.

In their previous studies of quadriceps wasting, Davies & Sargeant (1975a,b) had shown that 15 weeks' immobilization in a long-leg cast after lower leg fracture reduced the fat-free volume of the affected limb by 12% and that this was accompanied by a similar fall in the $\dot{V}O_2$ max achieved with one-leg pedalling. Since the work of pedalling was believed to be performed mainly by the quadriceps, the implication seemed to be that the degree of quadriceps atrophy was similar to that occurring in the other leg

muscles and was also similar to the reduction in quadriceps work capacity. The changes in $\dot{V}O_2$ max and thigh volume in Paper IV were comparable to those previously obtained by Davies & Sargeant, but the measurements of quadriceps MFA suggested a much greater degree of atrophy. In fact, a difference in fibre size of about 40% for a difference in FFTV of 11-12% suggested that the loss of muscle bulk was virtually confined to the quadriceps. At that time, however, there was no evidence from human studies to support this extrapolation from myofibre size to whole muscle size. Such evidence is now available.

Justification for the use of MFA as an indicator of the size of a muscle has now come from 4 studies. In the first of these, Häggmark, Jansson & Svane (1978) combined needle biopsy and computerised tomography and showed a close linear relationship between MFA and the cross-sectional area of vastus lateralis plus vastus intermedius in a group of 9 men chosen for their different degrees of muscular development. Häggmark & Eriksson (1979a) then described 7 patients whose ankles had been immobilized in plaster for 6 weeks following surgical repair of a ruptured achilles tendon. The injured/uninjured limb ratios for soleus MFA and calf-muscle CSA (i.e. all muscles posterior to the tibia and fibula) were 23% and 25-30% respectively. (The latter figure is reported as 25% but recalculation of the published data gives a figure closer to 30%.) A brief abstract (Halkjaer-Kristensen, Ingemann-Hansen & Saltin, 1980) describes very similar changes in quadriceps CSA and MFA in 2 men undergoing minor knee surgery and subsequent rehabilitation-training. The fourth study is Paper VI of the present series and its findings are the most relevant: the mean differences in quadriceps CSA and MFA were 26% and 27%, respectively, in 14 patients who had suffered a knee injury (Fig. 8.5). Moreover, the injured/uninjured limb ratios were significantly correlated ($r = 0.6$, $P < 0.025$) with a regression coefficient not significantly different from unity ($= 0.92$, Bartlett's 3-group method for model II regression).

It is clear that fat-free limb volumes have been discredited as a means of evaluating arthrogenous quadriceps atrophy. Does this mean that $\dot{V}O_2$ max during single-leg pedalling, which usually mirrored

the changes in fat-free volumes, is not a good index of the functional state of the quadriceps? Or does it mean that knee injury/immobilization reduces quadriceps function by less than it reduces quadriceps size? First, let us consider the effect of quadriceps atrophy on other aspects of the muscle's function.

Sixteen patients studied at meniscectomy and again 4 weeks later showed a mean reduction in MFA (vastus medialis) of 16% and in knee extension strength (measured in extension) of 32% (Karumo, Rehunen, Näveri & Alho, 1977). There was no attempt, however, to exclude strength measurements which had been limited by pain. The effect of unilateral knee injury and/or immobilization on the injured/uninjured limb ratios for quadriceps MFA and the strength of pain-free, isometric knee extension (at 90°) was examined retrospectively in 11 patients (Fig. 8.4). Fibre size (and therefore the size of the whole muscle) and quadriceps strength appeared to have been affected equally. In another study, 7 normal young men underwent a period of elbow immobilization (MacDougall, Elder, Sale, Moroz & Sutton, 1980). The average decrease in triceps MFA was 30% and in the force of a maximal, voluntary, concentric contraction, 41%. There may be a physiological explanation for strength being reduced more than fibre size: several workers have shown a greater loss of myofibrillar than of sarcoplasmic proteins in atrophied animal muscle (e.g. Helander, 1957; Herbison, Jaweed & Ditunno, 1978).

It will be apparent in Chapter 9 that the relationship between quadriceps hypertrophy and increments in strength is not a simple one. Nevertheless, in the case of atrophy, and in the absence of pain or an effusion, such evidence as is available suggests that isometric strength is an aspect of the quadriceps' function which is reduced to at least the same degree as the size of its fibres and, therefore, the size of the whole muscle. If this is true, one would expect similar patients to show a greater reduction in knee-extension strength than in knee-flexion strength, because of the apparently selective nature of the quadriceps atrophy (Paper IV; Paper V; Ingemann-Hansen & Halkjaer-Kristensen, 1980). Yet young servicemen recovering from tibial fractures have been reported to have equal injured/uninjured limb ratios for the strength of isometric knee-

flexion and knee-extension (Imms, Hackett, Prestidge & Fox, 1977). Even at the time of discharge from the rehabilitation unit the ratios were still equal (75% and 73%) although there was presumably no longer any significant pain or anxiety to interfere with the measurements. The same group has also reported that knee-extension and knee-flexion strengths were about equally impaired for as long as 6 months after meniscectomy (Jenkins, Imms, Prestidge & Smalls, 1976). These findings are unexplained. They emphasise, however, the need for a prospective study which includes measurements of the strength and of the size of both the extensors and flexors of the knee.

Such a study should also include other methods of evaluating the functional state of the muscles. The isokinetic recording of maximal voluntary strength is often advocated, but has never been subjected to the kind of analysis described in the preceding paragraphs.

Investigating the rather small difference in single-leg $\dot{V}O_2$ max, Sargeant & Davies (1977) applied an alternative form of dynamic dynamometry. During both one- and two-legged cycling, the forces applied to the cranks were monitored separately for the atrophic limb and the normal limb. A further 6 analogous post-fracture patients were studied: they showed normal-atrophic limb differences of 11% for fat-free limb volume, 12% for $\dot{V}O_2$ max in single-leg pedalling but 38% for the proportion of the work rate applied to the cranks during two-legged cycling. The peak force exerted at each submaximal rate of two-legged work was also reduced by about 40%, similar to the reduction in isometric knee-extension strength which might be expected for such patients.

Is the 11% change in $\dot{V}O_2$ max the truer reflection of the change in the quadriceps' functional capacity and the 40% difference in work-sharing merely the result of an attempt to protect the injured limb? Or, does the apportionment of work give a truer picture of the functional capacity of the muscle while the small difference in $\dot{V}O_2$ max is the result of activity in other muscles? Sargeant & Davies (1977) demonstrated that the 2 limbs had the same 'patterns' of force production over a range of submaximal one- and two-legged

work rates. Undue activity in other muscles might seem unlikely, therefore, except that the 'patterns' of force production were not reported for work rates greater than what I calculate to have been about 75-80% of maximum.

This still leaves unanswered some fundamental physiological questions, such as why the atrophic limb has a higher $\dot{V}O_2$ than the normal limb at submaximal, single-leg work-rates and whether this reduced efficiency also applies at its maximal work rate. Nevertheless, the important point in the present context is that the unconscious sharing of submaximal work between the 2 limbs was in a ratio similar to that which would be expected for quadriceps size. This suggests that the amount of atrophy may be highly relevant for the performance of normal, submaximal, bilateral, everyday activities. The division of labour between limbs during submaximal exercise should therefore be included in future studies of atrophy, when it can be directly compared with ultrasonic or tomographic measurements of quadriceps CSA.

Muscle fibre number

There is some animal evidence that immobilization may sometimes alter the number of myofibres in a muscle. One study has shown a reduced number of fibres in the rat soleus (Booth & Kelso, 1973) and another has produced evidence suggestive of an increased number of fibres in the same muscle (Herbison et al., 1978).

Our data for the relationship between the MFA and CSA of the quadriceps was presented in Paper VI as evidence for an unchanged total number of fibres in the muscle despite the presence of pronounced wasting following knee injury. This conclusion depends on 4 important assumptions:

1. that the relationship between quadriceps CSA and MFA in the uninjured limb at the time of the study is similar to that in the other limb before it was injured,

2. that the biopsied fibres are representative of those in all 4 heads of the muscle,
3. that measurements at mid-thigh are representative of the whole length of the muscle, and
4. that both quadriceps have the same myofibre length.

The justification for these assumptions is fully discussed in Paper VI and need not be rehearsed here.

The importance of the conclusion is that schemes of therapeutic exercise based on the changes which they are known to produce in MFA might not be as appropriate if a significant part of the wasting was due to a loss of muscle fibres. There is animal evidence which suggests that there may perhaps be forms of exercise which can influence muscle fibre number (discussed in Paper VI). As far as the present patients are concerned, however, it seems that there is no need to look for a form of exercise that will increase muscle fibre number in man.

Selective atrophy of type I or type II muscle fibres

Motor units comprising type I muscle fibres are used for low-force voluntary activity, whereas high-intensity activity also involves the recruitment of the motor units which comprise type II muscle fibres (e.g. Piehl, 1974; Gollnick et al., 1974a,b). The effects of physical training appear to be specific, not only to the particular muscle involved, but also to the individual muscle fibres used to perform the training exercise. In normal subjects, endurance-training may increase the cross-sectional area of type I fibres, while sprint- or strength-training may increase the size of both fibre types but particularly the type II fibres. These adaptations have even been demonstrated in the opposite legs of the same subjects (Saltin, Nazar, Costill et al., 1976). This confirms that the differences observed in the sizes of type I and type II fibres in the muscles of established athletes may be the result of their patterns of training exercise (Edström & Ekblom, 1972;

Gollnick, Armstrong, Saubert, Piehl & Saltin, 1972; Costill, Fink & Pollock, 1976).

Selective atrophy of one or other fibre type may also be observed. Atrophy of type II fibres has been described in patients with a wide variety of conditions, including several systemic diseases not primarily involving muscle (vd. 'Results' and also Papers II and III). Since type II fibres are 'high-threshold' fibres, it has been suggested that their selective atrophy merely reflects a sick patient's general inactivity (Dubowitz & Brooke, 1973, page 79). It is no surprise, therefore, that many of the patients described in this chapter showed preferential atrophy of their type II fibres. This has also been the experience of others (e.g. Hultén, Renström & Grimby, 1981).

Preferential atrophy of type I fibres is not a common feature in muscle histopathology. Nevertheless, it was clearly present in the biopsies from several of the patients described in this chapter, especially those recovering from an uncomplicated tibial fracture (Paper IV). Edström (1970) reported finding preferential type I atrophy in the medial vastus muscle of patients with chronic dysfunction of the cruciate ligaments. It has also been observed in the medial or lateral vastus of 3 out of 9 patients with knee dysfunction, none of whom showed type II atrophy (Staudte, 1978), in the lateral vastus after reconstruction of the anterior cruciate (Häggmark, 1978; Häggmark & Eriksson, 1979b), and in soleus after repair of ruptured achilles tendons (Häggmark & Eriksson, 1979a).

It is not apparent which clinical features determine whether there is selective atrophy of one or other fibre type. It seems possible that the absence of muscle stretch might be important for the development of type I atrophy; muscle stretch produces reflex activation of type I and possibly type IIA muscle fibres (Burke & Edgerton, 1975). This would accord with the observation that type I atrophy in the human soleus after repair of a ruptured achilles tendon was worse in a patient whose ankle was immobilized in a relatively plantar-flexed position (Häggmark & Eriksson, 1979a). It would also tally with the observation that following cruciate

ligament surgery, the use of a hinged brace rather than a rigid plaster cylinder prevented the occurrence of type I atrophy in the quadriceps (Häggmark & Eriksson, 1979b). Similarly, it seems that in rheumatoid arthritis, active joint inflammation may be associated with type II atrophy whereas joint damage or deformity may be associated more often with type I atrophy (Brooke & Kaplan, 1972). Perhaps type II fibres are more subject to reflex inhibition of their activity by the presence of an effusion or joint pain.

The notion that type I atrophy is related to the absence of muscle stretch, while general inactivity, pain or a joint effusion may produce type II atrophy would be consistent with the biopsy findings for many of our patients. It would not be universally consistent, however. Moreover, it would not explain the occurrence of type I atrophy in patients with chronic cruciate ligament dysfunction (Edström, 1970). Perhaps time is also an important factor; the duration of cruciate ligament dysfunction in Edström's patients was measured in years. This would also be consistent with the situation in rheumatoid arthritis (vd. previous paragraph) and with the findings of Staudte & Brussatis (1977) in their study of quadriceps fibre-type atrophy in 32 patients with a wide variety of knee problems. They concluded that type II atrophy was associated with "a moderate impairment but still ambulatory condition", type I atrophy with "frequent sudden short-lasting pain in the knee joint" and combined I and II atrophy with "severe impairment and [near immobilization]".

Finally, if there is a relationship between the pattern of rehabilitation exercise and its effects on the size of each fibre type (vd. infra), then any search for the cause(s) of preferential atrophy of one or other fibre type must take into account the nature of the rehabilitation exercise being performed by the patients. As far as the present data are concerned, it may be of considerable significance that the 7 post-fracture patients in Paper IV were all being treated in the same residential, military, rehabilitation unit.

The selective effects of different patterns and intensities of exercise on the growth of type I and type II fibres suggest that the

exercise treatment of quadriceps wasting might be improved by tailoring the exercise to the relative degree of wasting of each type of fibre. It is attractive to suppose that there is little point in encouraging a patient to make maximal muscle contractions if his wasting is largely due to atrophy of the type I fibres. Conversely, it seems reasonable that type II atrophy might be an indication for the prescription of strictly maximal muscle contractions plus, of course, the treatment of any underlying systemic disease. It is some time since we raised this question (e.g. Young, Maunder & Edwards, 1977b). We were not alone in doing so (Staudte & Brussatis, 1977; Staudte, 1978). Others have also argued that the greater importance of type II fibres for tension development in fast contractions (Grimby & Hannerz, 1977; Thorstensson, Grimby & Karlsson, 1976a) may mean that high-speed, isokinetic training should be prescribed to treat type II atrophy (Hultén et al., 1981).

The concept that muscle wasting may be corrected faster by exercise designed to stimulate growth of the affected fibre type still lacks experimental proof. Nevertheless, the first step has been made; there is significant recruitment of type II fibres when the atrophic quadriceps performs isometric or isokinetic exercise at 50% or more of its maximal isometric strength (Hultén et al., 1981). This is essential information for anyone planning a trial of therapeutic exercise prescribed according to which type of fibre is more atrophic. The other prerequisite is some knowledge of the clinical factors which determine the microscopic pattern of atrophy. For example, high-resistance exercise would be no more effective than any other so long as a factor responsible for type II atrophy continued to operate. Alternatively, if muscle stretch is important for the growth of type I fibres, any comparison of quadriceps exercise regimes must also take account of the frequency and extent of both active and passive knee flexion.

Ultimately, an understanding of the mechanisms responsible for preferential fibre-type atrophy will allow the individual patient to be given the most appropriate treatment without first undergoing a biopsy.

Frequency of type II fibres in atrophic muscle

Our data suggest a tendency for the atrophic quadriceps to have a higher proportion of type II fibres than the contralateral muscle. Even if true, the difference is small and is probably of little clinical importance. The implied change in type II frequency following knee injury would nevertheless be of considerable physiological interest.

The physiological determinants of a muscle's fibre-type composition are poorly understood (vd. recent, extensive review of the subject by Salmons & Henriksson, 1981). It is known that experimental manoeuvres which alter the pattern of motor-nerve activity (e.g. chronic electrical stimulation or cross-innervation) can change the fibre-type composition of animal muscle. Nevertheless, of the numerous training studies which have been conducted in animals, only 3 have shown changed fibre-type proportions in the trained muscle. None of the longitudinal training studies performed in man has shown evidence of interconversion between type I and type II muscle fibres. The high proportion of type I fibres in the muscles of marathon runners and other endurance athletes (Gollnick et al., 1972; Costill et al., 1976) is said to be 'inherited' rather than 'acquired' (Komi, Viitasalo, Havu et al., 1977). The implication is that the results of cross-sectional studies of established athletes simply reflect their self-selection for the kind of sporting event to which their muscle fibres are best suited; type I fibres are particularly suited for aerobic metabolism and are relatively resistant to fatigue.

Salmons & Henriksson (1981) argue convincingly that the apparent immutability of the relative frequency of type I and type II fibres with exercise training merely reflects the discontinuous nature of the stimulus. They point out that those properties of muscle which are most stable under exercise conditions are also those which can be changed only by long periods of electrical stimulation. They contend that the difference between endurance exercise training and chronic low-frequency electrical stimulation is quantitative rather than qualitative. This is borne out by the increased type I frequency

found in the soleus of rats kept continuously under conditions of increased gravity (Martin & Romond, 1975; Salmons & Oyama, work in progress cited by Salmons & Henriksson, 1981), in both soleus and plantaris after excision of gastrocnemius (Ianuzzo, Gollnick & Armstrong, 1976; Ianuzzo & Chen, 1979), and in the diaphragm of rats after chronic tracheal banding (Keens, Chen, Patel et al., 1978).

Knee injury or the application of a long-leg plaster results in a change of muscle activity which is much more continuous than that produced by exercise training. Indeed, cast immobilization of the hind-limbs of rats has been shown to increase the frequency of type II fibres in the soleus muscle (Booth & Kelso, 1973; Herbison et al., 1978) and hind-limb fixation has the same effect on the guinea-pig soleus (Maier, Crockett, Simpson, Saubert & Edgerton, 1976).

Two studies in man had already suggested that disuse might increase the proportion of type II fibres. In patients with spinal-cord transection, the proportion of type II fibres in the paralysed muscles was very much higher than in those which still had supraspinal control (Grimby, Broberg, Krotkiewska & Krotkiewski, 1976). In addition, Jansson had described an elite cross-country skier whose very high percentage of type I fibres (typical of an endurance athlete) was reduced following knee injury (Jansson, Sjödin & Tesch, 1978). After a return to training, his quadriceps regained its previous, high percentage of type I fibres and he went on to win an Olympic gold medal (E. Eriksson, personal communication).

Our data were the first reported showing a statistically significant increase in quadriceps type II frequency with knee injury and/or immobilization in man (Young, Hughes, Round, Edwards & Maunder-Sewry, 1980; Paper VI). Our findings have now been confirmed in a much larger, Scandinavian series (Ingemann-Hansen & Halkjaer-Kristensen, 1981). The small size of the change and the high variability of fibre-type percentages measured in repeated biopsies (Paper III) may explain why earlier studies had failed to show it (e.g. Karumo et al., 1977). A similar magnitude of mean change was seen in the immobilized soleus muscles of the patients studied by

Häggmark & Eriksson (1979a) (from 16% type II to 23%) but, with only 7 patients, it did not reach statistical significance.

It is necessary also to consider the possibility that an injured limb's biopsy may be taken from a consistently different part of the quadriceps, a part with a naturally higher percentage of type II fibres. As discussed in Paper VI, there is some evidence that deeper parts of vastus lateralis comprise a slightly higher proportion of type I fibres (Johnson, Polgar, Weightman & Appleton, 1973), a phenomenon which is quite pronounced in some animal muscles. This has now been confirmed (Fig. 8.12) by a much more detailed study (Lexell, Henrikson-Larsén & Sjöström, in press). It is hard to refute this possibility, although Lexell's data could equally explain the high variability of repeated biopsies and hence the delay in recognising that type II frequency is increased in the atrophic quadriceps.

The apparent increase in type II frequency is in keeping with the theoretically-expected direction of change (Salmons & Henriksson, 1981). If the apparent change is real, it could have come about by the loss of type I fibres, the formation of new type II fibres, a combination of these two processes, or the conversion of type I fibres into type II fibres. The first two possibilities would result in a decrease or increase, respectively, in the total number of fibres in the muscle. As has already been discussed, the patients in Paper VI had similar numbers of fibres in their two quadriceps. Considering specifically the 10 patients in Paper VI whose wasted quadriceps showed an increased type II fibre frequency, it is possible to calculate the magnitude of change in fibre number which would be required in order to produce the observed changes in fibre-type frequency (Fig. 8.13). It is clear that the increased type II frequency cannot be explained on the basis of the selective formation or loss of muscle fibres.

It is possible that the loss of type I fibres has been matched by the formation of an equal number of new type II fibres. The most likely explanation, however, would still seem to be that there has been a conversion of type I fibres into type II fibres.

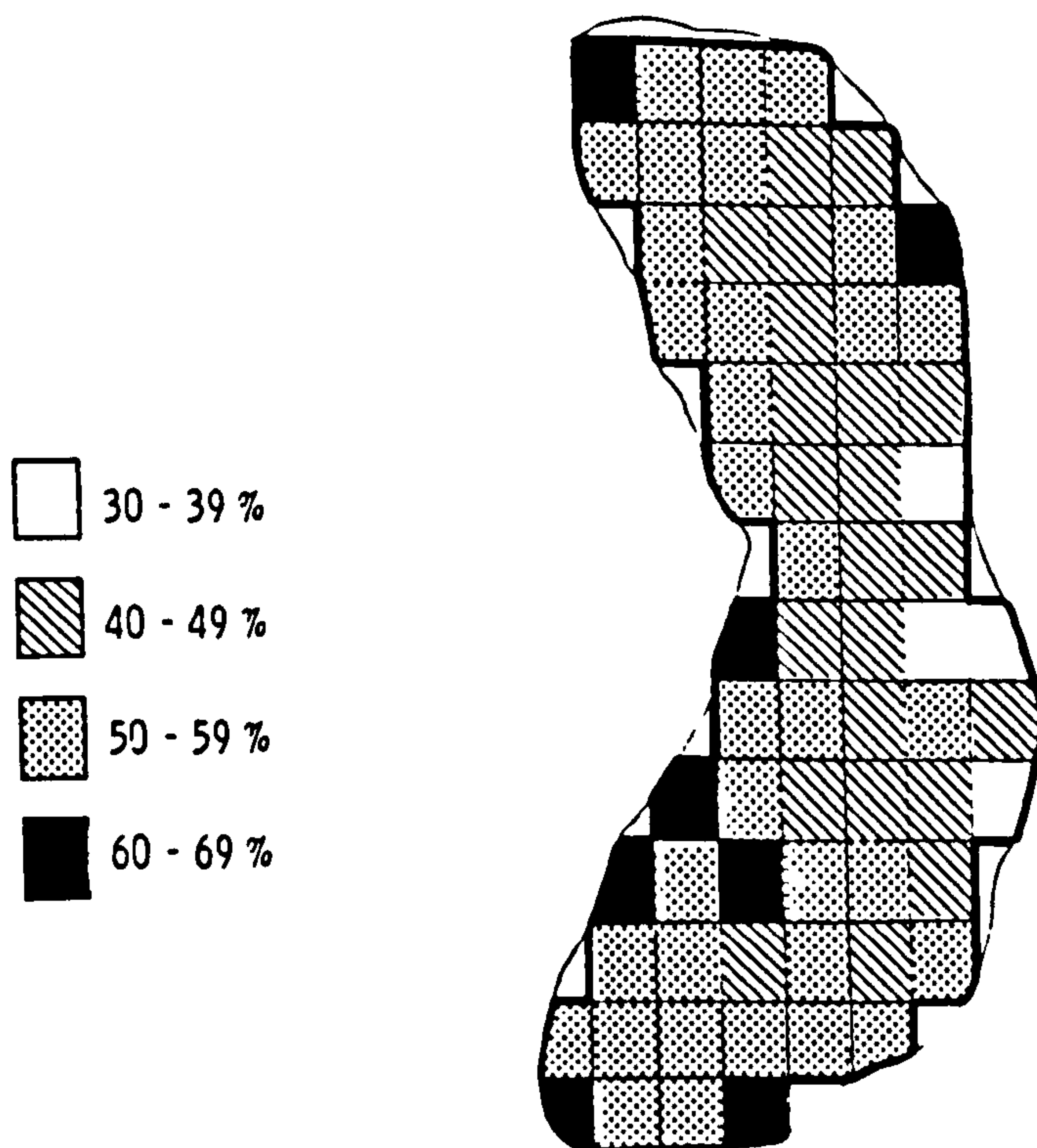


Fig. 8.12 The frequency of type I fibres seen in a transverse section of a single human vastus lateralis. (Anterior aspect to top of page and lateral aspect to the reader's right.)

(Figure drawn from data kindly supplied by J. Lexell, personal communication.)

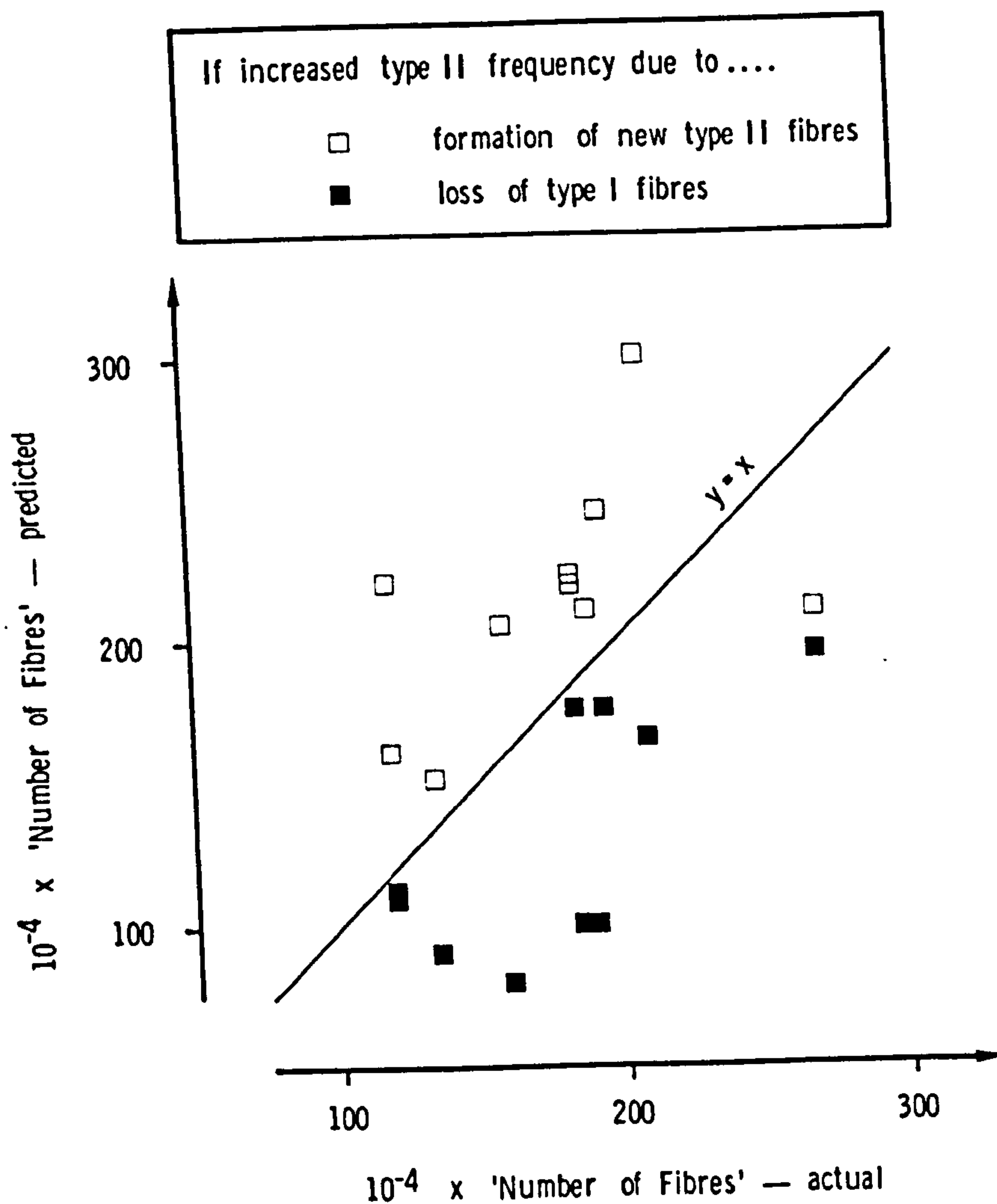


Fig. 8.13 Predicted total 'number of fibres' required to explain the increased percentage of type II fibres observed in the wasted quadriceps of 10 patients, compared with the observed 'number of fibres'. ('Number of fibres' is defined here as the ratio of whole muscle cross-sectional area to its mean fibre area.)

Circumstantial evidence in favour of such an explanation might be provided if the wasted muscles were shown to contain an increased percentage of type IIC fibres, normally rare and suggested to be an intermediate stage between type I and type II fibres (Jansson, Sjödin & Tesch, 1978). Somewhat stronger evidence would be provided by the immunohistochemical demonstration in the atrophied muscle of fibres containing both the type I and type II forms of myosin, tropomyosin and the troponins (Dhoot & Perry, 1979; Salmons & Henriksson, 1981).

CONCLUSIONS

Quadriceps cross-sectional area may be measured by computerised axial tomography or by ultrasound B-scanning. The latter is particularly suitable for repeated measurements since it does not involve exposure to radiation.

The severity of the quadriceps wasting which follows knee injury is seriously underestimated by measurements of thigh circumference, even when they are supplemented by caliper measurements of subcutaneous fat. It would be a waste of time to base any research study of quadriceps wasting on measurements of thigh circumference.

In the absence of pain or an effusion, knee injury probably results in a loss of isometric strength of the quadriceps similar in severity to the loss of its cross-sectional area.

The reduction in maximal oxygen uptake during one-legged exercise is less than the reduction in quadriceps size but is similar to the reduction in the fat-free mass of the limb. This can probably be explained by activity in muscles other than the quadriceps during maximal, one-legged pedalling.

The involuntary sharing of work between the two limbs in sub-maximal, two-legged pedalling probably reflects the reduction in quadriceps size and in its isometric strength. This should be investigated further, as a potentially valuable index of muscle function in rehabilitation practice.

The severity of quadriceps wasting following knee injury can be explained entirely by the reduction in the size of the individual muscle fibres. There is no evidence of a change in the total number of fibres in the muscle.

Measurements of fibre size in repeated muscle biopsies have a higher coefficient of variation (16%) than measurements of whole muscle cross-sectional area by ultrasonography (4%). The place of muscle biopsy is to provide information about the nature, rather than the severity, of the atrophy, indicating, for example, the relative involvement of type I and type II fibres.

There is a tendency for arthrogenous muscle wasting to be associated with an increase in the frequency of type II muscle fibres, possibly as a result of a type I/type II interconversion. This seems unlikely to be of major clinical importance except for the injured endurance athlete, whose athletic performance depends on his muscles' high proportion of type I fibres.

Preferential atrophy of one or other fibre type may occur in the quadriceps following knee injury and/or immobilization. It is not known what particular clinical features determine whether an individual patient's muscle will show type I atrophy, type II atrophy or combined atrophy of both fibre types. In the present series, type I fibre atrophy was particularly common in young men making an uncomplicated recovery from a tibial fracture, but the relevance of this observation is uncertain. The relative severity of atrophy of each type of fibre in a patient's muscle may indicate the type and pattern of therapeutic exercise best suited to his rapid recovery. This concept awaits experimental proof once the causes of selective fibre-type atrophy have been established.

CHAPTER 9

QUADRICEPS SIZE AND STRENGTH IN NORMAL SUBJECTS AND THE EFFECT OF HIGH-RESISTANCE TRAINING

INTRODUCTION

SUBJECTS

Quadriceps cross-sectional area and isometric strength
Effects of strength-training

METHODS

Measurement techniques

- (i) *Dynamometry*
- (ii) *Ultrasonography*

Quadriceps cross-sectional area and isometric strength

Plan of training experiment

- (i) *Timing and frequency of strength measurements*
- (ii) *Timing of scans and biopsies*
- (iii) *Initial training programme*
- (iv) *Modified training programme*

RESULTS

Quadriceps cross-sectional area and isometric strength

Effects of strength training

- (i) *Pre-training strength measurements*
- (ii) *Quadriceps cross-sectional area and strength*
- (iii) *Quadriceps cross-sectional area and thigh circumference*
- (iv) *Myofibre size and number*

DISCUSSION

Quadriceps size and strength

Effects of strength-training

- (i) *Measurement of hypertrophy*
- (ii) *Muscle size and strength*
- (iii) *'Cross-education'*

CONCLUSIONS

CHAPTER 9

QUADRICEPS SIZE AND STRENGTH IN NORMAL SUBJECTS

AND

THE EFFECT OF HIGH-RESISTANCE TRAINING

INTRODUCTION

The work described in this chapter uses ultrasonography for a preliminary study of the relationship between the size and strength of the normal human quadriceps (Young, Stokes, Walker & Newham, 1981) and of the influence of the muscle's fibre-type composition on this relationship (unpublished data). It then goes on to examine the effect of high-resistance training ("strength-training") on quadriceps size and strength. Virtually the same subjects were used throughout (vd. inf.).

The linear relationship between the voluntary isometric strength of the quadriceps and the body weight of normal subjects (Paper I) has proved extremely useful for the objective evaluation of weakness of patients (e.g. Papers II and III). Patients with weakness secondary to such conditions as thyrotoxicosis or osteomalacia often show microscopic evidence of muscle fibre atrophy. Extrapolation from measurements of fibre size, however, is not a satisfactory way to judge the extent to which their weakness can be explained by the loss of muscle tissue. A similar problem arises when trying to gauge the relative contributions of reflex inhibition and atrophy to the quadriceps weakness of a patient with knee joint pathology. The first part of this work was therefore a limited, cross-sectional study of the relationship between quadriceps strength and ultrasonographic measurements of its cross-sectional area, asking whether a larger study would be justified in order to establish a normal range for strength standardized for muscle CSA, to complement that for strength standardized for body weight.

Most of the subjects who provided the data for the cross-sectional study then went on to train one quadriceps with a programme

of high-resistance training. Almost all previous studies purporting to examine the effect of training on the size and strength of a muscle have been limited by their dependence on limb circumference measurements as an index of muscle growth. The use of ultrasound scanning in this longitudinal study meant that the subjects' strength gains could be compared with accurate measurements of the growth of their quadriceps muscles.

Finally, further needle biopsy specimens were taken at the end of the period of training in an attempt to describe the effects of the strengthening exercise on the size and number of the quadriceps' muscle fibres.

SUBJECTS

Quadriceps cross-sectional area and isometric strength

The cross-sectional comparison of quadriceps size and strength was conducted with 25 healthy volunteers. The median age of the 11 men was 27 years (range 21 to 48) and of the 14 women, 25 years (range 19 to 48). None had suffered an injury to either lower limb necessitating immobilization of a joint for more than a week within the preceding 2 years. All except one were students or staff at University College Hospital Medical School or the Oxford Hospitals. The one exception was the coach of an Oxford swimming club.

Along with 2 of my colleagues, I acted as both investigator and experimental subject. The other 22 subjects all volunteered in response to advertisements (in hospital newsletters or on hospital notice-boards) asking for people to participate in a training study. This element of self-selection must be considered when attempting to generalise from the present results (vd. Chapter 2).

Effects of strength-training

Twenty-two of the volunteers performed unilateral high-

resistance training of the quadriceps. The first 5 subjects performed particularly vigorous training (vd. 'Methods') with the result that the post-training strength measurements of at least 1 of them were limited by knee-joint discomfort. The post-training dynamometry results from all 5 have therefore been discarded and only the data from the ensuing 17 subjects have been used to compare the gains in quadriceps strength and in its CSA.

A comparison of pre- and post-training biopsies proved possible for 12 subjects (including the 5 whose post-training strength measurements were discarded). For 2 of these 12, however, it was only possible to compare the fibre-type frequency; fibre size measurements were not possible, because of ice artefact.

METHODS

Measurement techniques

The techniques used have already been described in Chapter 3 (muscle biopsy and morphometry), Chapter 4 (dynamometry), and Chapter 8 (thigh circumference and ultrasonography). Only departures from these descriptions are noted here.

(i) Dynamometry

The version of the muscle-testing chair which was used for this phase of the work had a rather narrower ankle strap than the original. A broad canvas sling was therefore wound in a figure-eight around the ankle and attached to the strap, in order to avoid discomfort. This did not alter the forces recorded for normal subjects' strength (Stokes & Young, unpublished data) but it did ensure the continued acceptability of the procedure.

"Strength" was not only expressed as the force (MVC) exerted on the ankle strap (as in Paper I), it was also expressed as the product of the force on the strap and the length of the lower leg (from the

floor to the lateral joint line of the knee, while standing). The latter index of strength is referred to as "torque" (MVT) although the point of application of the force was slightly proximal to the sole and no attempt was made to define the fulcrum of the lever. The value of MVT will be consistently larger than the true torque but is much more easily measured. Moreover, comparisons of the torque developed by individuals of different leg lengths are not affected by this expediency. For example, expressing the tallest subject's strength as a percentage of the shortest subject's strength, the figures are 124.8% for MVC, 162.6% for MVC x knee-to-floor distance (i.e. MVT) and 161.9% for MVC x knee-to-strap distance.

(ii) Ultrasonography

For the post-training measurements, mid-thigh was relocated with the aid of a transparent sheet on which its position had been recorded with respect to naevi, scars or other skin blemishes (Dons et al., 1979). Using this technique, the coefficient of variation of CSA measurements is probably less than 2%, rather than the 4% reported in Paper V (Stokes & Young, unpublished data).

Quadriceps cross-sectional area and isometric strength

The first set of strength measurements made on each subject (vd. infra) was used for comparison of the untrained quadriceps' strength with its cross-sectional area and its morphology.

Plan of training experiment

(i) Timing and frequency of strength measurements

The strength of each subject in the training study was measured on 3 occasions before the start of the training period and again on 2 occasions after the end of training. In most cases, the pre-training measurements were made on consecutive days, as were the post-training

measurements. The change in strength over the training period was calculated for each quadriceps as the difference between the greatest pre-training MVC and the greatest post-training MVC.

At least 24 hours (usually 48) elapsed between the last training session and the first set of post-training strength measurements.

(ii) Timing of scans and biopsies

Scans were performed on one occasion before training and on one occasion after training. The pre-training scans were done on the same day as the third set of pre-training strength measurements. The post-training scans were done between the two sets of post-training strength measurements.

Biopsies were taken only after all the other pre- and post-training measurements had been obtained. Duplicate biopsies were taken on 11 occasions and single biopsies on the other 3 occasions.

(iii) Initial training programme

The first 5 subjects performed a more vigorous training programme than the other 17. As explained in the 'Subjects' section of this chapter, the post-training dynamometry data for these 5 subjects have been discarded, but their microscopic data have been retained, and have been compared with the measurements of quadriceps CSA.

The first 5 subjects all trained the weaker quadriceps. They trained 3 times weekly for 5 weeks. Four training exercises were used. The subjects performed 4 sets of 6 repetitions of each exercise. The loads were the heaviest with which the subjects could perform 6 repetitions and were progressively increased throughout the training period.

Each training session began with a 'warm-up' of running and hopping activities. The exercises themselves were:

1. single-leg knee-extension, using apparatus similar to that illustrated in Paper VII,
2. single-leg vertical press, using a leg-press machine,
3. single-leg step-ups, using a 14 inch bench and carrying a weighted barbel,
4. single-leg vertical jump.

(iv) Modified training programme

In order to minimise the likelihood of knee discomfort, the 17 subjects described in Paper VII used a rather milder training programme, using only the first of the exercises described above (Paper VII, Fig. 1). Training was again performed thrice weekly for about 5 weeks. Each exercise session comprised 3 sets of 6-8 repetitions. After the first 3 training sessions, the training weight was increased whenever a subject successfully completed 3 sets of 8 repetitions.

During the performance of the training exercise, the untrained thigh was supported by the training bench, with the knee flexed and the lower leg freely suspended. With this arrangement, subjects tended to flex the knee of the untrained limb while exercising the other side. It had been found that the provision of support under the foot of the untrained limb meant that subjects tended to push the foot against it, so performing an isometric quadriceps contraction.

RESULTS

Quadriceps cross-sectional area and isometric strength

The strength values for the stronger quadriceps of 19 of the 25 subjects lay within the previously described normal range (Paper I). Four women were just below the normal range for their body weight.

Nevertheless, there was, as expected, a positive correlation between isometric quadriceps strength, as MVC, and body weight ($r = 0.45$, $P < 0.05$) and the regression line ($MVC = \text{weight} \times 7.75 - 47$) was very similar to that reported in Paper I ($MVC = \text{weight} \times 7.91 - 38$). As discussed in Chapter 4, the correlation was not greatly improved by taking leg length into account and plotting MVT against body weight (Fig. 9.1, a and b). There was a tendency for the male subjects to be stronger for their weight than the females.

The sex difference disappears and the correlation is much stronger when the strength of the stronger quadriceps (as MVC or MVT) is plotted against the mid-thigh measurement of its CSA (Fig. 9.2, a and b). A similar close relationship held for the subjects' weaker quadriceps. The difference in strength/CSA between an individual's two sides was significantly less than the among-subjects variation ($P < 0.001$). The median difference in strength/CSA was 6% (ranging from 0 to 25%).

Effects of strength-training

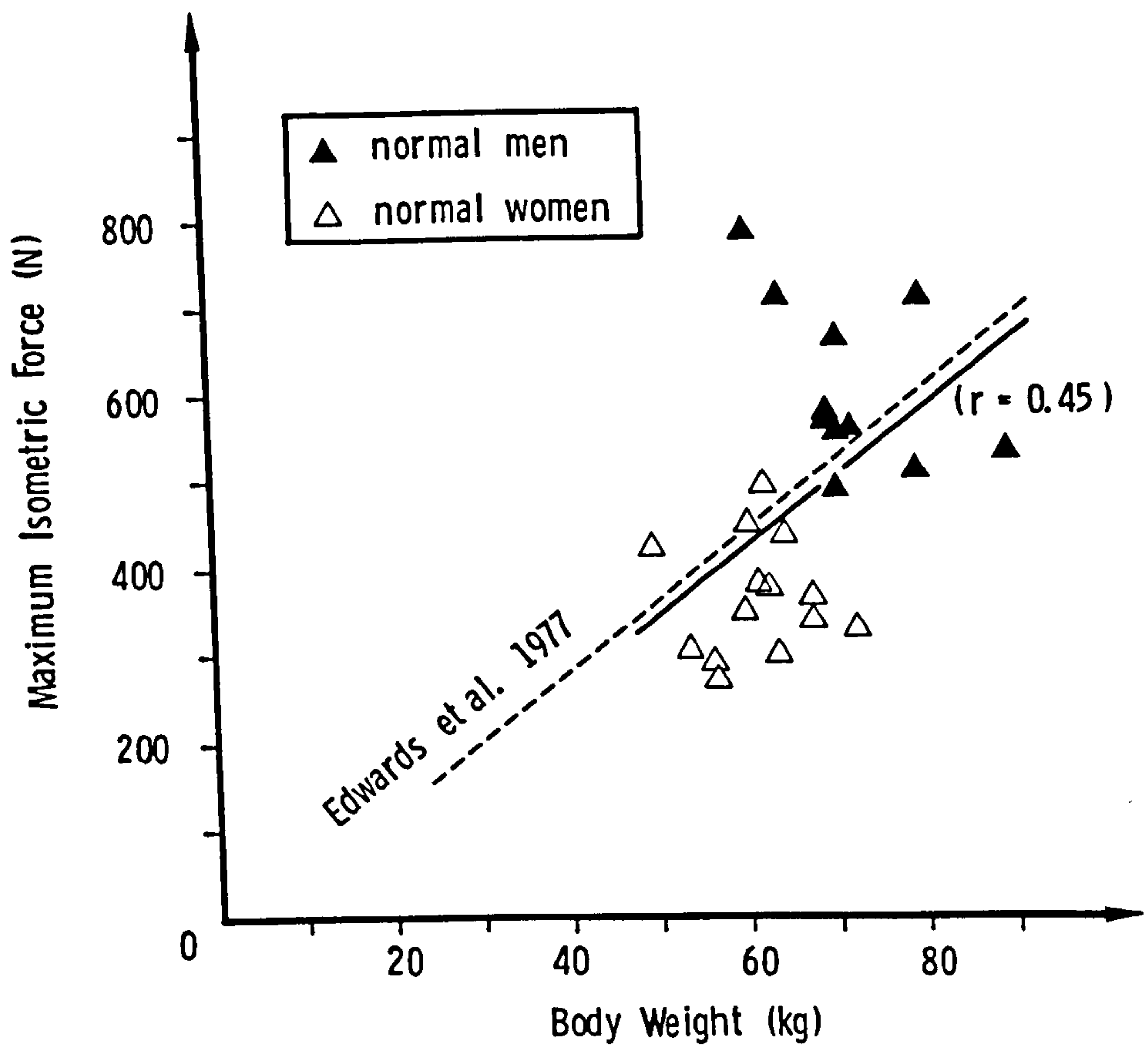
(i) Pre-training strength measurements

Twenty subjects had their strength measured on 3 consecutive days before the start of training. The results for all 20 were mentioned in Chapter 3, with respect to the variability of this measurement. Three of the 20 were only prepared to perform bilateral training; it is the other 17 who are considered here and in Paper VII. There was no significant difference in strength over the 3 days for either the leg which was to be trained or for the leg which was to remain untrained (Table 9.1).

(ii) Quadriceps cross-sectional area and strength

There was no significant change in the size or strength of the untrained quadriceps. On the trained side, however, there were

a



b

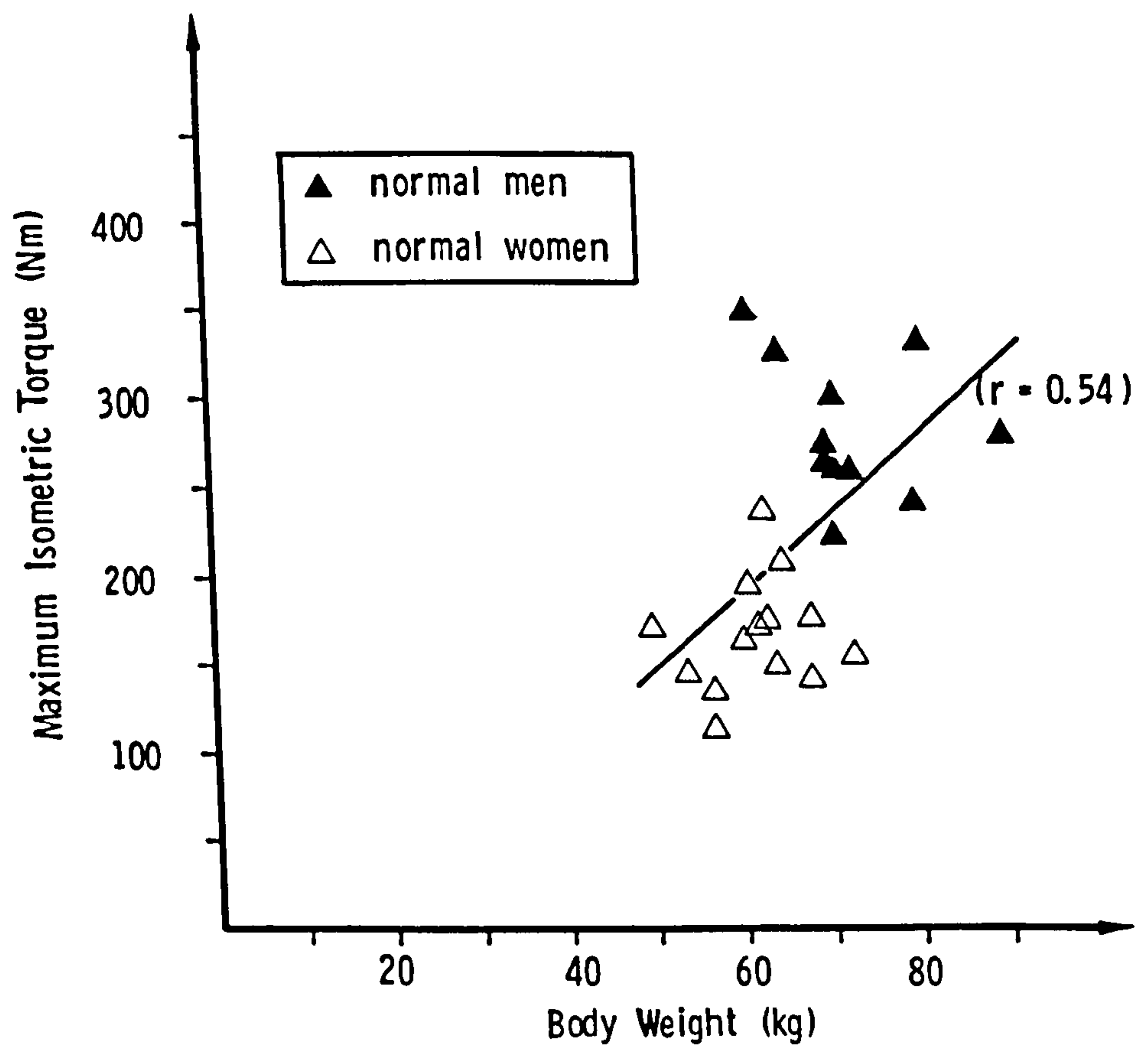
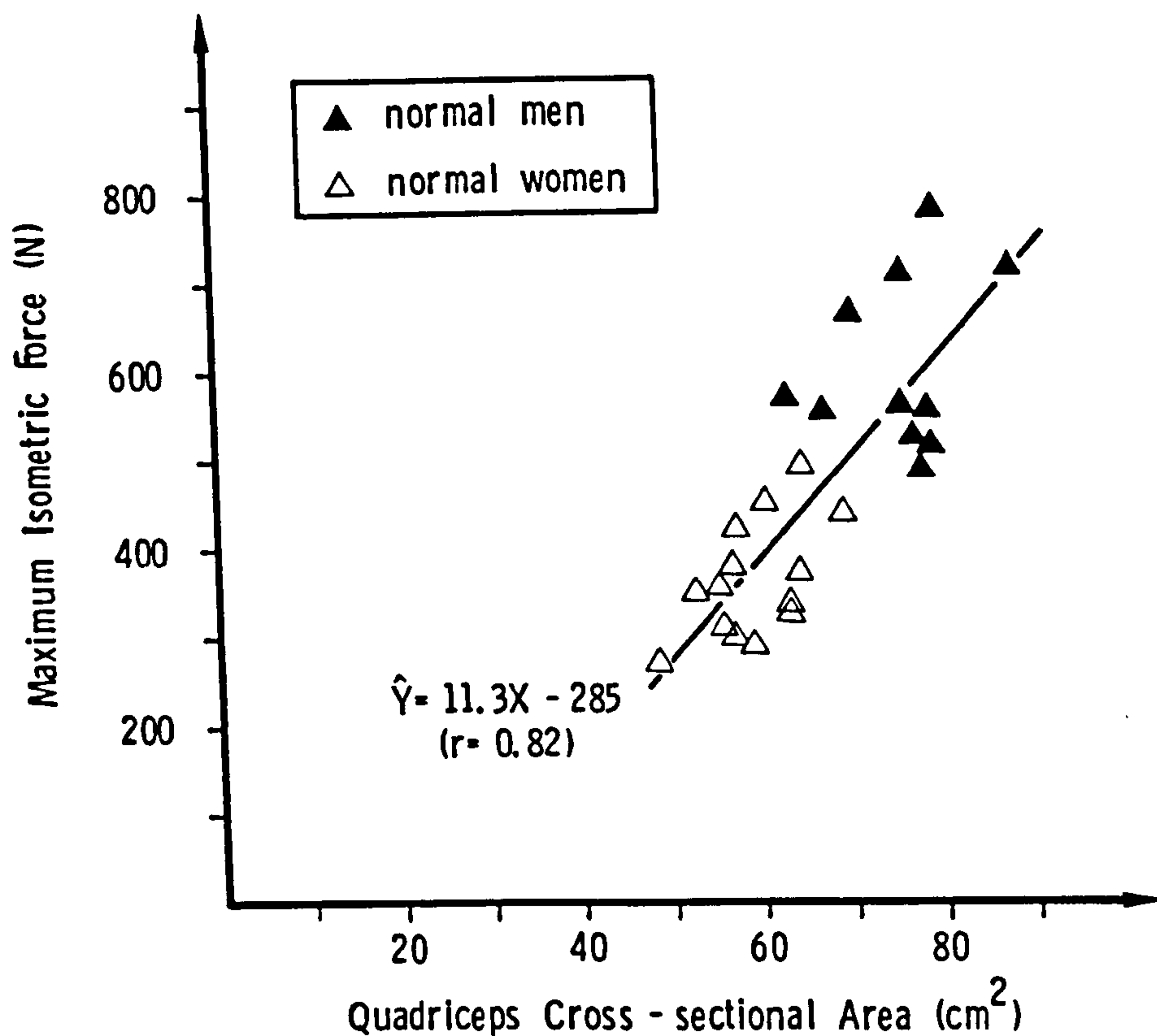


Fig. 9.1 The relationship between quadriceps strength and body weight for 25 normal subjects, aged 19-48 (median = 25).

In (a), "strength" = force = 7.73×-46.5 , ($r^2 = 0.20$).

In (b), "strength" = torque = 4.40×-75.0 , ($r^2 = 0.29$).

a



b

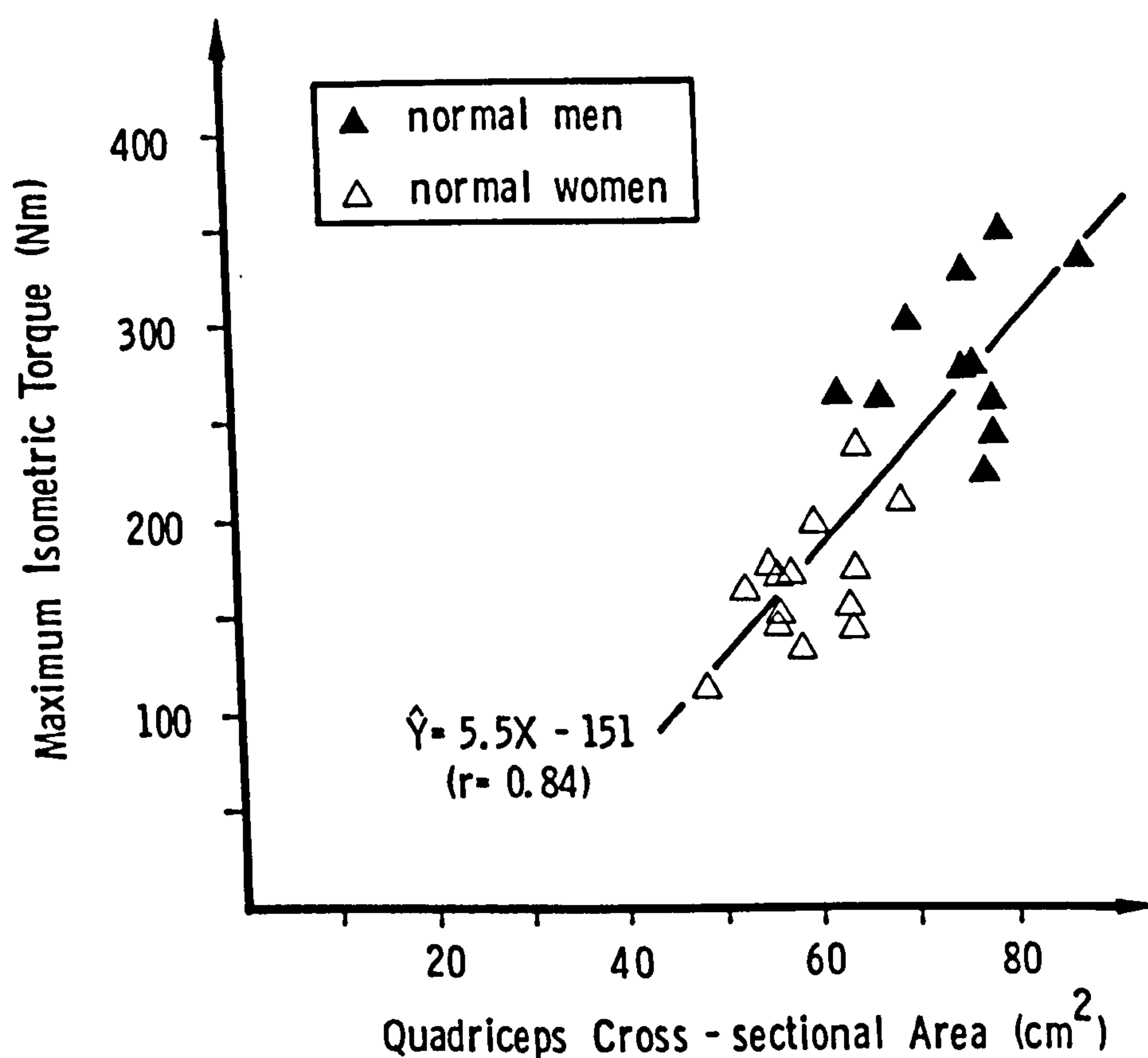


Fig. 9.2 The relationship between quadriceps strength and cross-sectional area for 25 normal subjects, aged 19-48 (median = 25).

In (a), "strength" = force ($r^2 = 0.67$).

In (b), "strength" = torque ($r^2 = 0.71$).

TABLE 9.1 Strength measurements recorded on three occasions
before the start of training

The mean values (and SDs) given in the table were calculated after each subject's strength measurements had been expessed as percentages of his/her best pre-training measurement for the same limb.

	test 1	test 2	test 3
Limb to be trained	92 (8.1)	96 (4.6)	96 (6.2)
Limb to remain untrained	93 (8.2)	96 (5.2)	95 (4.9)

highly significant increases ($P < 0.001$) in quadriceps CSA (mean = 6.3%) and MVC (mean = 15.0%) (Paper VII, Table 3).

The changes in CSA were smaller than the changes in strength ($P < 0.001$) and it was not possible to predict the amount of strength gained from the increase in muscle mass (Paper VII, Fig. 3).

(iii) Quadriceps cross-sectional area and thigh circumference

In the untrained limb, thigh circumference-squared did not change significantly but on the trained side it increased by an average of 3.7% ($P < 0.001$) (Paper VII, Table 3). The training-induced changes in quadriceps CSA were underestimated by the circumference measurements ($P < 0.001$) and could not be predicted from them (Paper VII, Fig. 2).

(iv) Myofibre size and number

There were no morphological abnormalities evident in the biopsies from the trained quadriceps. In particular, there was no evidence of fibre 'splitting'.

There were no significant changes in fibre size, the relative size or frequency of type I and type II fibres, nor in the ratio of quadriceps CSA to fibre size (Table 9.2). The mean change in MFA was +5.2% but the variability was considerable (range = -31% to +37%). Seven of the subjects in Table 9.2 participated in the main training study. They showed a mean increase in MFA of 6% and mean increases in strength (15.7%) and CSA (8.1%) which were similar to the other 10 subjects in that study.

The increase in CSA produced by the training was small compared to the inherent variability of fibre size measurements made on repeated biopsies. On the strength of the data from the 10 subjects in Table 9.2, it seems that 40 subjects would have to be studied in order to reduce to a reasonable level ($\beta < 0.1$) the likelihood of a

TABLE 9.2 The effect of approximately 6 weeks of strength-training on the fibre sizes and fibre-type composition of needle biopsies from the lateral quadriceps of 12 subjects

(Biopsies from 2 subjects could be analysed only for fibre-type frequency because of ice artefact.)

	mean fibre area (μm^2)				type II fibres		$\frac{\text{CSA}}{\text{MFA}} \times 10^{-4}$
	all fibres	type I	type II	$\frac{\text{type II}}{\text{type I}}$	frequency (%)	%CSA II (%)	
Mean (pre-training)	4645	4444	5003	1.085*	40.1	43.0	157
Mean increase	241 (5.2%)	262 (5.9%)	245 (4.9%)	-0.05* -	-1.5 (-3.6%)	-1.6 (-3.7%)	-12.2 (-7.8%)
n	10	10	10	10	12	10	10
P	NS	NS	NS	NS	NS	NS	NS

* median

'type II' error concealing an increase in fibre number large enough to account for a statistically significant ($\alpha < 0.05$) mean increase in CSA of 10%.

DISCUSSION

Quadriceps size and strength

Voluntary isometric quadriceps strength, whether expressed as the force applied at the ankle or as torque, is closely related to the cross-sectional area of the muscle. The fact that considering strength as torque makes little difference to the closeness of this relationship has already been considered (Chapter 4).

It seems that the intrinsic strengths of male and female muscle may be similar, as concluded by Ikai & Fukunaga (1968) for the elbow flexors. Differences in strength seem to be explained by the female subjects' quadriceps muscles being smaller in cross-section, for their body weight, than the men's muscles. Proper confirmation of this conclusion, however, requires that more small men and large women should be studied. Direct comparison of our values for the specific strength of the quadriceps with those of Ikai and Fukunaga for the elbow flexors is not possible. The same applies to values based on cadaver studies (e.g. Morris, 1948). While it might be possible to calculate the effects of the different lever systems, there is no satisfactory method of allowing for the differences in the muscles' internal architecture. There is no satisfactory method of determining the angle of pennation in the intact individual.

As suggested in the introduction to this chapter, a close relationship between the quadriceps' strength and its cross-sectional area could be useful for the evaluation of a patient's weakness, complementing the information gained by comparing the patient's strength with his body weight. For example, consider the patients whose osteomalacia had been treated with vitamin D for over a year but whose strength was still below 'normal' for their body weight (Paper II). It should be possible to judge whether the quadriceps

strength of such patients is commensurate with the size of the muscle or whether the muscle itself is intrinsically weaker than normal. First, however, the normative data would have to be extended to include smaller CSA values than the present series. Extrapolation of the present strength/CSA relationship to smaller values of CSA would not be justifiable.

The close similarity in quadriceps strength/CSA between the two sides of a normal individual could well be applied to the evaluation of reflex inhibition of quadriceps activity in the patient with a unilateral knee-joint effusion; the median difference in the present series of 25 subjects was only 6%. Once again, one would have to be circumspect about extrapolating from the present, 'symmetrical' subjects to patients with a large difference in the size of their two quadriceps. The appropriate 'normal' data for comparison might be obtained by examining a group of patients with unilateral wasting but who are judged to be 'inhibition-free'.

The regression lines in Figure 9.2 (a and b) have a positive X-intercept. This might imply that a part of what is measured as quadriceps CSA does not influence the production of force by the muscle. Quadriceps CSA when MVC is zero, predicted from the regression of CSA on MVC, is 40.1cm^2 (= 60% of CSA). This figure seems improbably large, emphasising the importance of not extrapolating the regression line beyond the lower limits of the available data. It also emphasises the need for caution when interpreting a regression line drawn through 2 groups of data (in this case, from men and from women).

Effects of strength-training

(i) Measurement of hypertrophy

The training-induced increases in the cross-sectional area of the quadriceps were greater than the changes in thigh circumference-squared. Of considerable practical importance is the absence of any correlation between the two changes. When a selective training

routine is used, as in this study, the extent of quadriceps growth cannot be reliably inferred from the changes in thigh circumference. This is analogous to the findings of Paper V with respect to selective quadriceps wasting.

(ii) Muscle size and strength

Although one study failed to show any change in isometric knee-extension strength after training thrice weekly for 7 weeks (Dons et al., 1979), most other authors have reported isometric strength gains similar to those in the present study, after roughly comparable training periods (e.g. Thorstensson, Sjödin & Karlsson, 1975; Thorstensson, Hultén, von Döbeln & Karlsson, 1976b; Komi et al., 1978; Aniansson & Gustafsson, 1981).

A striking finding of this study was the confirmation that the changes in quadriceps CSA were insufficient to account for its enhanced isometric strength. Numerous other authors have claimed that appropriate training can increase strength by more than it increases muscle bulk (e.g. Rose, Radzyminski & Beatty, 1957; Ward & Fisk, 1964; Stoboy, Friedebold & Strand, 1968; Penman, 1970; Moritani & de Vries, 1979). Only 2 previous studies, however, have actually measured muscle strength and cross-sectional area before and after a period of training. One of these (Dons et al., 1979) may be discounted since there was no change in either CSA or isometric strength and, as discussed in Paper VII, the claimed increase in dynamic knee-extension strength was very questionable. In the other study, Ikai & Fukunaga (1970) demonstrated a 23% increase in the cross-sectional area of the anterior compartment of the upper arm in 5 men whose elbow-flexion strength had increased by 92%.

As discussed in slightly greater detail in Paper VII, there are several possible mechanisms whereby strength might be increased more than cross-sectional area. Some authors have reported that strength-training increases the integrated EMG signal recorded during a maximal isometric contraction (e.g. Komi et al., 1978; Moritani & de Vries, 1979). They have inferred that this is indicative of an

increased maximal level of voluntary recruitment of motor units: it is not known, however, what the effect would be of muscle hypertrophy and attenuation of subcutaneous fat on the EMG signal. Against their conclusion, there is evidence which strongly suggests that, in an isometric contraction, the well-motivated subject's maximal voluntary effort will activate adductor pollicis or quadriceps femoris as fully as supramaximal electrical stimulation of its motor nerve (vd. Chapter 4).

The subjects' motivation was good but the acquisition of skill cannot be ignored as a possible explanation. It seems likely that any such 'learning' effect would have occurred early in the study; indeed Moritani & de Vries (1979) claim that this is the case. Yet the subjects in Paper VII showed no detectable increase in strength over the 3 pre-training tests. Moreover, their pre-training strength was taken to be the best recorded over all 3 test occasions. Warshal's demonstration of an increase in isometric "strength" over 3 consecutive test days (Warshal, 1979) is easily explained by her definition of "strength" as the mean of the 3 MVCs in each test. The present subjects made some very tentative MVC efforts the first time they were tested, but their best MVC did not change over the 3 days.

If the specific strength of type II fibres is greater than that of type I fibres, a preferential hypertrophy of type II fibres would result in an increment in strength greater than that in muscle CSA. The possibility of such a difference in the intrinsic strengths of type I and type II fibres is considered in Chapter 10. The biopsy data from the present study, however, showed no evidence of selective hypertrophy of either fibre type, although fibre hypertrophy equivalent to the observed mean increase in CSA (6%) could easily have been obscured by the variability inevitable when measuring MFA in repeated biopsies (Chapter 3). Cross-sectional studies of established athletes (Edström & Ekblom, 1972; Gollnick et al., 1972) certainly suggest that strength-training should produce a predominantly type II fibre hypertrophy and there is some evidence from longitudinal studies that this is so (Costill, Coyle, Fink, Lesmes & Witzmann, 1979; Aniansson & Gustafsson, 1981).

A selective increase in myofibrillar density is another possible explanation. Once again, there is evidence in the literature both to support and to challenge such a suggestion. Animal studies provide both microscopic and biochemical evidence of an increased concentration of myofibrillar material (Denny-Brown, 1960; Gordon, Kowalski & Fritts, 1967; Rowe, 1969). In contrast, 6 men who performed strength-training for 6 months, producing a 91% increase in elbow-extension strength with only a 31% increase in MFA in triceps brachii, had the same myofibrillar volume density in pre- and post-training electron micrographs (MacDougall, Sale, Moroz et al., 1979).

A further possibility is that the disparity between the mean gains in quadriceps strength and in its CSA might be explained if the initial effect of training was "not to produce hypertrophy in the muscle as a whole but to consolidate the tissue". This was the explanation proposed by Goldspink (1964) when he found that weight-lifting increased the weight of mouse muscle more than it increased the girth of the muscle fibres. Any such "consolidation" would have to be at the expense of interfascicular or intermuscular connective tissue. In studies where large changes are produced, any consolidation would have only a small effect on the final picture; in rat muscles hypertrophied by a combination of synergist-ablation and running, the changes in myofibre dry weight, whole muscle dry weight and whole muscle wet weight were very similar (Gollnick, Timson, Moore & Riedy, 1981). So long as the training-induced changes are relatively small, however, as in the present study, Goldspink's suggestion remains a possibility. It would be in keeping with the training-induced decrease in the Y-intercept of the regression of CSA on MVC observed in the present study; the intercept decreased from 31cm^2 to 19cm^2 , i.e. from 46% of the pre-training mean CSA to 27% of the post-training mean CSA.

(iii) 'Cross-education'

The untrained limbs of our 17 subjects did not show a significant change in their isometric strength, quadriceps CSA or mid-thigh circumference. There was no evidence of a 'cross-

education' effect, a phenomenon whereby unilateral strength-training is said to enhance the strength of the contralateral limb (e.g. Hellebrandt, Parrish & Houtz, 1947; Darcus & Salter, 1955; Rose et al., 1957; Komi et al., 1978). Unilateral practice of a skilled movement facilitates the contralateral performance of the movement (Fechner, 1858; Scripture, Smith & Brown, 1894; Davis, 1898). Isometric quadriceps contraction is not a complex act, however. Moreover, we have already argued that maximal, voluntary, isometric contractions of the quadriceps are not improved by the acquisition of skill. A large and carefully conducted study also failed to demonstrate any 'cross-education' effect on isometric strength (Kruse & Mathews, 1958). It appears that the occurrence of contralateral strengthening in response to unilateral training is inversely related to the efforts of the investigators to preclude contraction of muscles other than those expressly being trained (vd. 'Methods'). Indeed, one of the studies which reported a 'cross-education' effect (Hellebrandt et al., 1947) described the subjects "gripping the table with the leg of the contralateral side".

CONCLUSIONS

The tape measure underestimates the increase in muscle mass which occurs with strengthening exercise. Studies of muscle hypertrophy, like those of atrophy (Chapter 8), must include direct measurement of the individual muscle or muscle group being considered. This can be done with ultrasound B-scanning.

Normal subjects show a close correlation between the isometric strength and the cross-sectional area of the quadriceps femoris. This close relationship may prove clinically useful if it can be shown to hold good for older age groups and for smaller quadriceps than those so far studied.

The strength/cross-sectional area of the quadriceps is especially constant between the 2 limbs of the same subject. This observation will also prove valuable clinically if it is true for

individuals who are less symmetrical in quadriceps size than those in the present study.

High-resistance, low-repetition, dynamic training can increase the isometric strength of the normal quadriceps by more than it increases its total cross-sectional area. To investigate this phenomenon further, the changes produced by training will need to be greater than in the present study. The training will therefore have to be continued for longer. For example, the changes in the present study were too small (in comparison with the sampling and measurement errors involved in fibre size evaluation) for any conclusions to be drawn regarding changes in fibre number or in the relative size of type I and type II muscle fibres.

CHAPTER 10

THE RELATIVE SPECIFIC STRENGTH OF TYPE I AND TYPE II FIBRES IN THE HUMAN QUADRICEPS

INTRODUCTION

SUBJECTS

Normal muscle

Unilateral wasting

Athletes

ANALYSES

Normal muscle

Unilateral wasting

Athletes

DISCUSSION

CONCLUSIONS

CHAPTER 10

THE RELATIVE SPECIFIC STRENGTH OF TYPE I AND TYPE II FIBRES IN THE HUMAN QUADRICEPS

INTRODUCTION

Type I and type II muscle fibres may differ in their intrinsic strength per cross-sectional area, i.e. their "specific strength". Scant attention has been paid to this possibility although it is potentially relevant throughout the preceding chapters, for example when calculating the relative relaxation rate of type I and type II fibres (Chapter 5), when comparing the rate of growth of muscle fibres with the rate of recovery of strength during the treatment of osteomalacia (Chapter 6), or when comparing the effect of strength-training on muscle size with its effect on strength (Chapter 9). The greater speed of type II fibres means that they contribute a greater proportion of the total tension in fast isokinetic contractions than they do in isometric contractions (Thorstensson et al., 1976a; Thorstensson et al., 1977) but it is not known how the fibre types compare in isometric contractions.

In the rat, the maximum tetanic tension/cross-sectional area of the (fast) extensor digitorum longus is about 1.4 times that of the (slow) soleus (Bárány & Close, 1971). Cross-innervation reduces the strength/CSA of extensor digitorum longus and increases that of soleus (Bárány & Close, 1971; Hoh, 1974). In the cat, the (fast) flexor digitorum longus is normally stronger/CSA than soleus but cross-innervation reverses the relationship (Bagust, Lewis & Westerman, 1981). Within the cat gastrocnemius, type II fibres may be 2.5 to 5 times as strong as type I (Burke & Tsairis, 1973).

No equivalent data have been reported for man. Two studies have reported significant positive correlations between the isometric strength of the quadriceps and the percentage of type II fibres (Komi, Rusko, Vos & Vihko, 1977b; Tesch & Karlsson, 1978). In neither were the strength measurements standardized for the size of

the whole muscle, making worthwhile conclusions impossible. The same criticism also applies to two other studies, in which it was the type I fibre frequency which correlated with quadriceps strength (Clarkson, Kroll & McBride, 1980; Aniansson, Grimby, Hedberg & Krotkiewski, 1981).

This chapter examines the influence of fibre-type composition on the strength/CSA of the quadriceps. It uses retrospective analyses of two sets of data collected for other purposes and an analysis of the data of Maughan, Watson & Weir (in press).

SUBJECTS

Normal muscle

Complete data on fibre-type composition, strength and CSA were available for 15 normal quadriceps of 13 subjects. Twelve of these subjects were entirely normal, healthy adults (9 male, 3 female, median age 27, range 21-48). Their data were obtained in the course of the studies described in Chapter 9. To these have been added data for the uninjured limb of a patient with unilateral knee pain, the only patient so far studied with simultaneous scans, biopsies and strength measurements.

Unilateral wasting

The data used in the second analysis were obtained from 11 otherwise healthy adults with unilateral thigh muscle wasting secondary to knee injury and/or immobilization (Fig. 8.4). Six men had suffered unilateral tibial fracture followed by lower-limb immobilization in a plaster cast. A further 4 men and the only woman had suffered unilateral knee injury (3 meniscectomies, 1 partially torn medial collateral ligament, and 1 septic arthritis).

Athletes

The third analysis uses the findings of Maughan et al. (in press) concerning the strength/CSA of the quadriceps of 6 elite sprinters and 6 elite marathon runners.

ANALYSES

Normal muscle

There is a weak, positive correlation between the strength/CSA of the quadriceps and %CSA II (Fig. 10.1). This correlation achieves statistical significance when the data are treated as coming from 15 independent limbs ($r = 0.55$, $P < 0.05$). The slope and intercept of the regression line (calculated by Bartlett's 3-group method for model II regression) are such that type II fibres appear to be 3.2 times as strong as type I fibres, per unit cross-sectional area (Fig. 10.1). The 95% confidence limits for the slope, however, are so wide that the factor relating type I fibre strength to type II fibre strength could lie anywhere between zero and infinity.

Unilateral wasting

The strength of each wasted quadriceps (expressed as a percentage of the strength of the contralateral muscle) was approximately equal to its MFA (again expressed as a percentage of the MFA of the contralateral muscle). There were, however, some quite marked differences in the fibre type composition of the 2 muscles in some individuals.

The strength of each patient's wasted muscle is a function of its total CSA, its fibre-type composition and the specific strength of each fibre type. The ratio of the 2 muscles' MFA can be assumed to be the same as the ratio of their CSA (Paper VI). If the 2 fibre types have the same specific strength, the injured/uninjured limb ratio for strength will be the same as the injured/uninjured limb

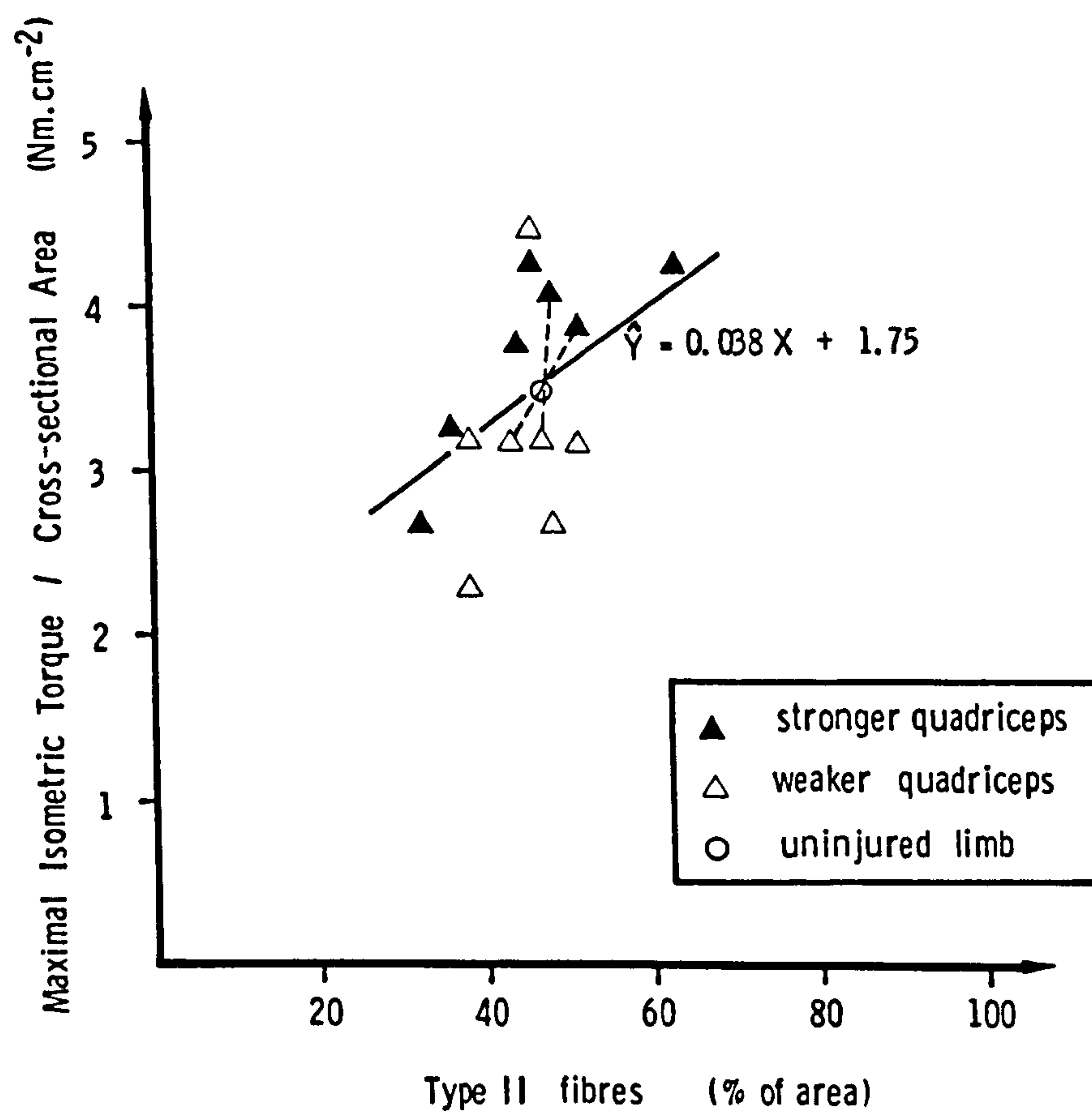


Fig. 10.1 The effect of the quadriceps' fibre-type composition, as judged from needle biopsies, on its strength per cross-sectional area. Data from 14 limbs of 12 normal subjects and the uninjured limb of a patient with unilateral knee pain.

ratio for MFA, irrespective of fibre-type composition. If type II fibres have greater specific strength than type I fibres, the injured/uninjured limb ratio for MFA will be lower than that for strength in a patient whose wasted muscle has a lower %CSA II. The converse will be true if the wasted muscle has a higher %CSA II (perhaps due to selective type I fibre atrophy). We have used our measurements of MFA and %CSA II to predict the expected injured/uninjured limb ratio for strength, for each patient, for a number of hypothesized values of the ratio of the specific strength of type II fibres to the specific strength of type I fibres. We have then examined the data to see which hypothesized ratio of specific strengths gave the closest agreement between the predicted and measured injured/uninjured limb ratios for strength. The closest agreement (by least squares) was obtained if it was hypothesized that type II fibres were approximately twice as strong for their cross-sectional area as type I fibres (Fig. 10.2).

Athletes

Maughan and his colleagues have found the quadriceps of 6 elite sprinters to be slightly, but significantly, stronger for their CSA than those of 6 elite marathon runners (Maughan et al., in press).

Had biopsies been taken from their subjects, it is highly likely that their sprinters would have had values for %CSA II between 50% and 80% while their marathon runners would have had values between 5% and 35% (Gollnick et al., 1972; Thorstensson et al., 1977). It is possible that the observed difference between the quadriceps strength/CSA of Maughan's sprinters and of his marathon runners might be due to their presumed differences in fibre-type composition. If this is assumed to be the case, it is possible to calculate the values which must be invoked for the ratio of the specific strengths of type II and type I fibres (Fig. 10.3). This analysis suggests that type II fibres are 1.2 to 2.1 times as strong per cross-sectional area as type I fibres, a range which is in close agreement with the other analyses and with the animal data.

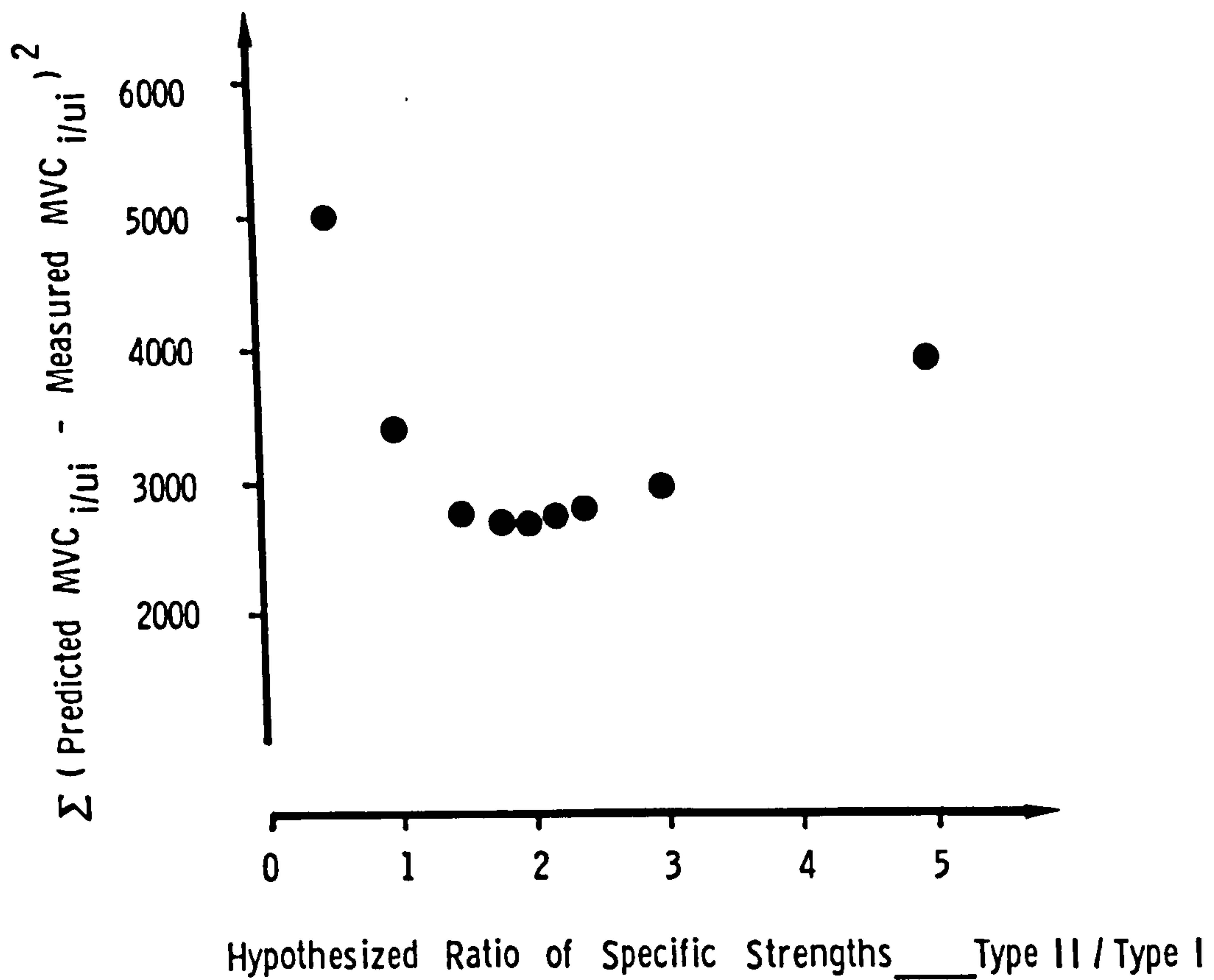


Fig. 10.2 Least squares analysis of the difference between the injured/uninjured limb ratios for MVC and the values for this ratio predicted on the basis of biopsy information and different hypothetical values for the relative specific strengths of type I and type II fibres. Data from 11 patients with unilateral arthrogenous quadriceps wasting (vd. Fig. 8.4).

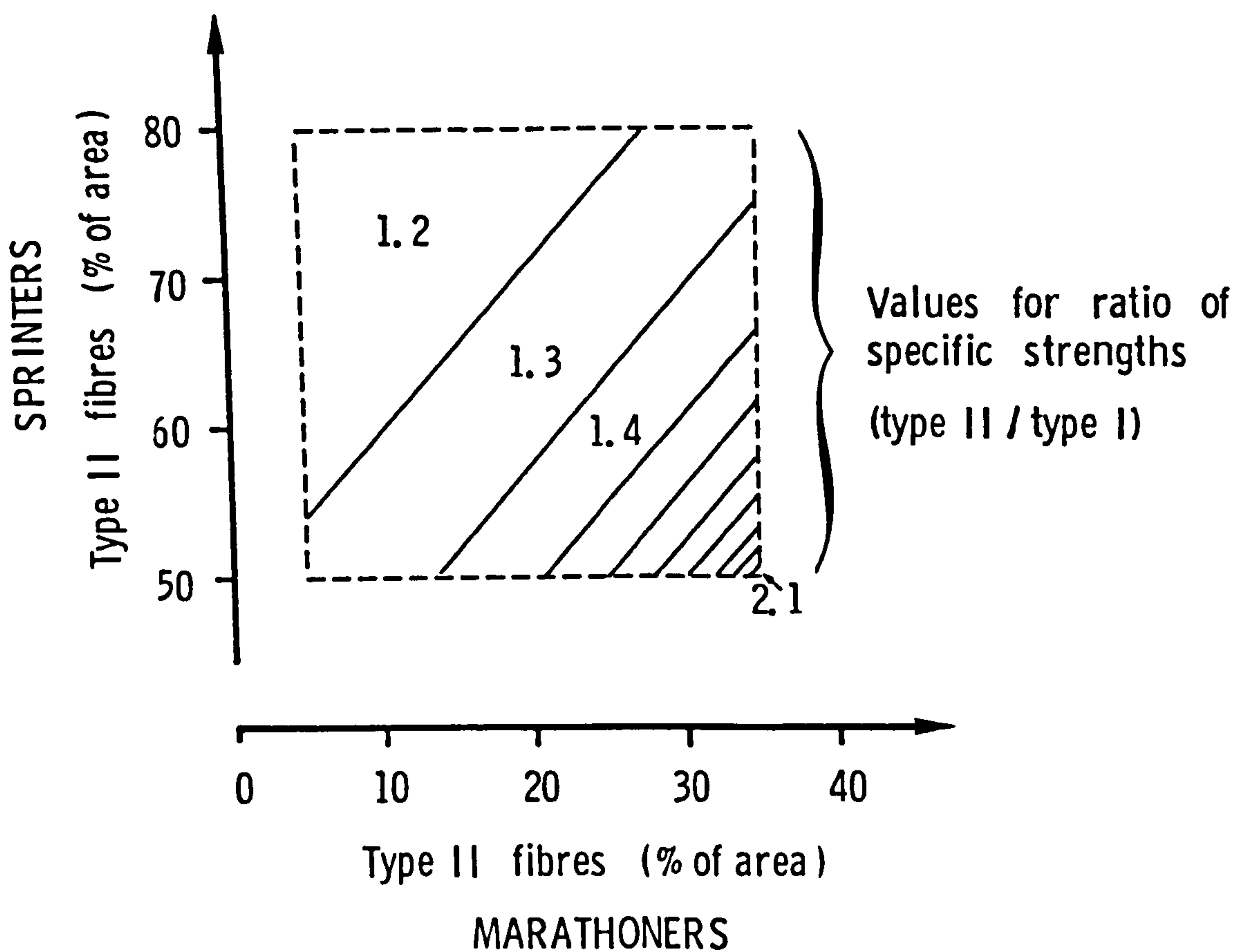


Fig. 10.3 Values for the relative specific strength of type II and type I fibres which might explain the greater strength/CSA of the quadriceps of sprinters compared with those of marathon runners. (Calculations based on data of Maughan et al., in press.)

DISCUSSION

An adequate understanding of the functional significance of preferential atrophy or hypertrophy of one or other fibre type requires that their relative specific strength should be established within reasonably narrow limits. Animal data suggests that type II fibres may be $1\frac{1}{2}$ to 5 times as strong per cross-sectional area as type I fibres. The present analyses all suggest that a similar relationship holds in the human quadriceps.

Although the concordance of these figures is very encouraging, it is impossible to ignore the fact that each analysis, taken separately, has very wide confidence limits. Considering the first of the three analyses (Fig. 10.1), narrower confidence limits might, theoretically, be achieved by (1) increasing the number of subjects studied, (2) studying subjects with a wider range of values of %CSA II or (3) reducing the measurement error for %CSA II, CSA and strength.

The derivation of confidence limits narrow enough to be useful would require an extremely large increase in the number of subjects studied. If the true ratio of type II fibre strength to type I fibre strength is assumed to be 3.2 (as in Fig. 10.1), the use of 60 subjects would still leave the 95% confidence limits for the ratio at 1.6 and 7.3. With 120 subjects they would be 2.0 and 5.5. Satisfactory confidence limits, therefore, could not be achieved with less than 100 subjects.

The second analysis (using data from patients with unilateral quadriceps wasting) was an attempt to widen the range of values of %CSA II, and so narrow the confidence limits for the calculated relative specific strength of the 2 fibre types. Since this analysis uses strength measurements made in patients with recent joint injury and/or immobilisation it is subject to error as a result of reflex inhibition of muscle contraction or the patients' unwillingness to make a truly maximal contraction for fear of experiencing pain.

The third analysis was also an attempt to widen the range of values of %CSA II, by pre-selecting subjects whose muscles were

expected to be near the upper and lower limits of %CSA II, i.e. top-class sprinters and endurance athletes respectively. It is essential to remember, however, that differences between groups of highly trained athletes may reflect not only their muscles' different fibre-type compositions but also the effects of their highly specific training programmes. Conclusions drawn from groups of athletes cannot be assumed to hold for normal, untrained muscle. It is conceivable, moreover, that successful sprinters and marathon runners may differ in their quadriceps' strength/CSA as a result of differences in the biomechanics of their lower-limb 'levers' or as a result of a different relationship between the quadriceps' mass and its mid-thigh CSA. A further problem is that even the widest possible spread of %CSA II values would not bring the confidence limits sufficiently close unless the number of subjects was also increased. This would be difficult to achieve since, by definition, there are only a few elite athletes.

The greatest source of measurement error is probably the determination of %CSA II (vd. Chapter 3). The high variability of repeated measures of %CSA II is an unavoidable limitation to any attempt to correlate the function of the whole quadriceps to the structure of a tiny part. This error could be reduced by taking multiple biopsies from each quadriceps under study. A more practicable approach would be to change from the quadriceps to a muscle with a more uniform fibre-type composition, so that single or paired biopsies would be more representative of the whole muscle. The alternative muscle would also have to be suitable for satisfactory measurements of both strength and CSA. With this in mind, perhaps the fibre-type distribution in triceps brachii should be examined; Henrikson-Larsén, Lexell & Sjöström (1983) have described a technique whereby this might be done.

It seems unlikely that future studies of the quadriceps will be able to improve significantly upon the conclusions reached in the present, retrospective analyses. It seems reasonable to conclude, therefore, that the type II fibres of the human quadriceps are probably stronger for their cross-sectional area than the type I fibres and that they may be about twice as strong.

CONCLUSIONS

The strength/CSA of the normal quadriceps is weakly correlated with the proportion of its cross-sectional area comprising type II muscle fibres.

In isometric contractions, type II fibres in the human quadriceps may have about twice the specific strength of type I fibres.

Confirmation of this relationship would require that at least 100 normal subjects should be studied. A preferable alternative might be to study a different muscle, seeking one whose %CSA II would be more accurately predicted from a biopsy.

CHAPTER 11

CLINICAL IMPLICATIONS

ISOMETRIC DYNAMOMETRY

Voluntary strength

Electrically stimulated contractions

ULTRASONOGRAPHY

Measurement of atrophy and hypertrophy of muscle

Relationship between muscle size and strength

Assessment of inflammatory muscle disease

Haematoma localisation

MANAGEMENT OF ENDOCRINE 'MYOPATHIES'

MANAGEMENT OF ARTHROGENOUS MUSCLE WASTING

CONCLUSIONS

CHAPTER 11

CLINICAL IMPLICATIONS

ISOMETRIC DYNAMOMETRY

Voluntary strength

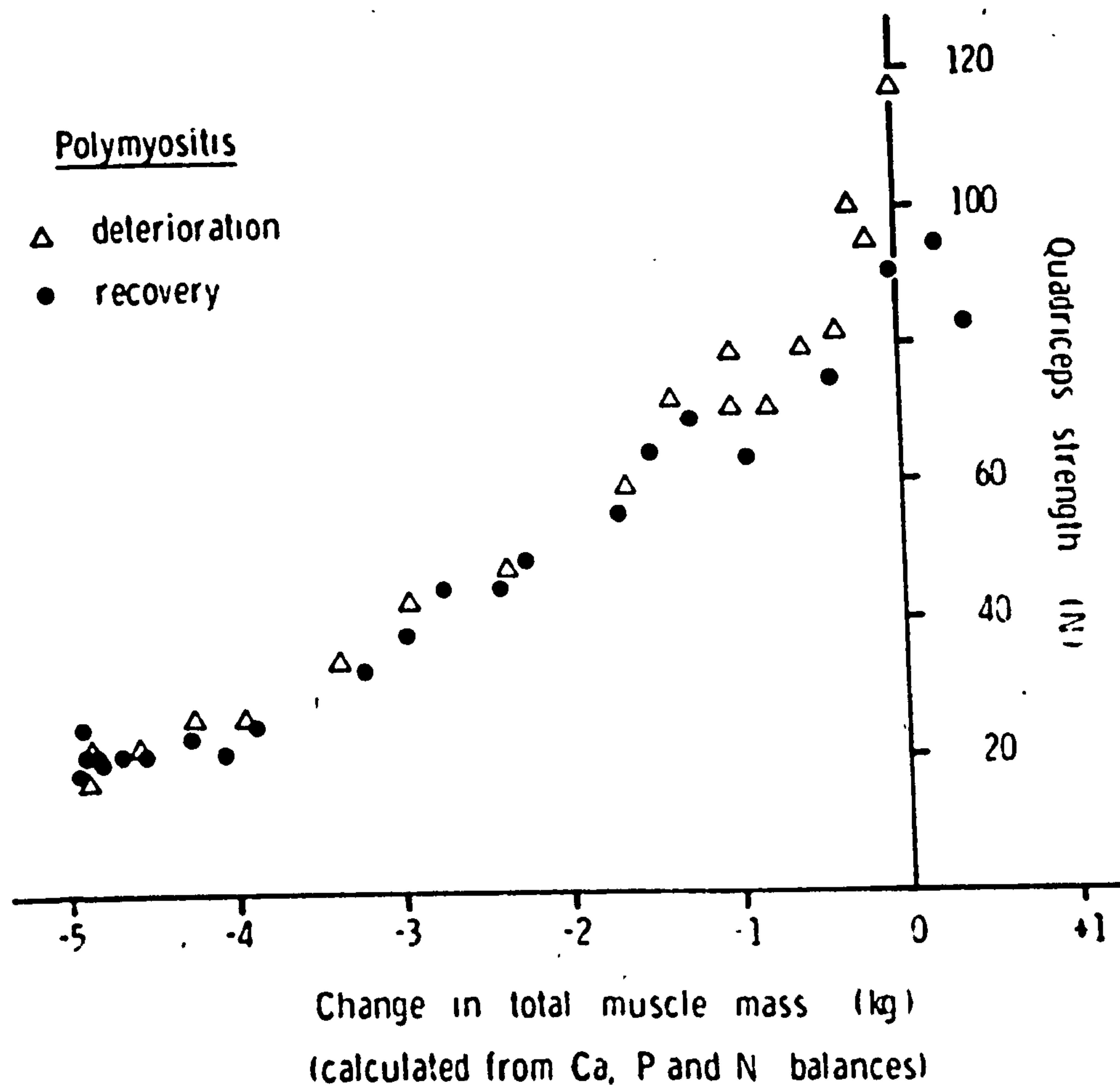
In the hands of an investigator who can elicit a good level of cooperation from the patient, voluntary isometric dynamometry allows an objective comparison of the patient's strength with the forces achieved by normal subjects of the same body weight. Not only is manual testing crude and largely subjective, it is also manifestly incapable of detecting any but the most severe degrees of weakness in many of the body's muscle groups (Beasley, 1961). Experience with the hand-held Hammersmith Myometer has confirmed that the highest force which most clinicians could exert during manual muscle testing is 300N and in many cases it is a good deal less. In the muscle-testing chair, the normal person can generate a knee-extension force, measured at the ankle, which is about 0.75 of body weight, i.e. a force of about 450-600N. Similarly, unilateral plantarflexion can generate sufficient force to raise the entire body weight.

People have attempted to find a solution in the use of timed, anti-gravity tests, such as the length of time for which the supine subject can hold his lower limb elevated to 45° with the knee fully extended (Fessel, Taylor & Johnson, 1970). Normal values have been described for this test, both for adults (Fessel et al., 1970) and for children (Hosking et al., 1976) but are of limited value because of their wide scatter. Few normal subjects would give an abnormal result but there can be many 'false negatives' (Young & Edwards, 1981). The other criticism of such tests is that they do not measure just strength. This is particularly obvious in thyrotoxicosis where the muscle's high rate of ATP turnover precludes a prolonged contraction (Chapter 7). In hypothyroidism, and presumably also in other conditions where muscle relaxation is slow, the slow ATP turnover rate might conceal weakness.

Isometric dynamometry can also identify even mild degrees of unilateral weakness if there is a greater than normal asymmetry of strength. Again, this was recognised and illustrated by Beasley (1961).

The most valuable, practical, clinical application of isometric dynamometry is not so much in the cross-sectional identification of weakness but in the longitudinal monitoring of changes in strength, whether due to treatment or to the natural course of disease. It is for this application that it is particularly important to have some knowledge of the inherent variability of the measurement. The practical value of repeated measurements of isometric strength is well shown by the case of a 37-year-old woman with severe polymyositis (Edwards et al., 1979). Metabolic balance studies confirmed that her quadriceps strength measurements were an accurate reflection of the net losses or gains in her total muscle mass during her deterioration and subsequent recovery (Fig. 11.1). Plasma creatine kinase measurements were much less useful as a guide to her progress. The same comments apply to childhood dermatomyositis (Resnick, Mundale, Mammel & Kottke, 1981). To treat myositis without measuring strength is like treating hypertension without measuring blood-pressure.

A striking feature of the study of patients receiving treatment for osteomalacia (Paper II) was the disparity between the rate of recovery of the measurements of their isometric quadriceps strength and the rate of recovery of their subjective well-being and their general physical ability. This is because even a small increase in strength may result in an enormous increase in a patient's functional ability. Strength changes occur on a continuous scale whereas functional changes are quantal. Thus, a very small strength gain may change the patient from being just unable to perform some functionally important activity to being just able to do it. This also applies in reverse; a steady loss of strength may not be apparent until the patient is suddenly unable to perform a crucial function. Moreover, the patient who is losing strength may, unconsciously, develop compensatory trick movements which maintain function and so conceal his deterioration. This underlines the



Patient : A.B. (q 37)

Fig. 11.1 Repeated measurements of isometric quadriceps strength (mean of left and right) accurately reflected the changes in the total muscle mass of a patient with polymyositis over a 3 month period during which her condition deteriorated to a very low level (despite a normal plasma creatine kinase) and then began to recover.

importance of objective measurements of muscle strength for monitoring a patient's condition.

The 'normal' rate of progression of diseases such as the muscular dystrophies may be used as the standard against which to judge a patient's strength. A profoundly weak, 17-year-old boy with severe joint contractures was recently referred to my muscular dystrophy clinic bearing the diagnosis of "Duchenne muscular dystrophy". His outlook on life and the attitude of his parents were directly related to the expectation of his imminent death. When measured in the muscle-testing chair, however, his isometric knee-extension strength was 71N - i.e. very severely reduced but still significantly higher than would be found in a 17-year-old with Duchenne muscular dystrophy (Hosking et al., 1976). This led to a detailed review of his history and past investigations, revision of his diagnosis (and therefore his prognosis), and a drastic change in morale and attitude. He has now applied for, and been offered, a place at university.

Dynamometry has an obvious place in comparative trials of physiotherapeutic programmes intended to correct muscle atrophy. Its careful use may be most informative (e.g. Jenkins et al., 1976). Yet dynamometry is frequently omitted from scientific investigations which are directly concerned with changes occurring in muscle. A case in point is the study by Edmonds & Smith (1981), discussed in Chapter 7, where sophisticated measurements of thyroid status and total body potassium were related to changes in "strength" measured on a subjective scale from 0 to 2. Another recent study from a 'centre of excellence' compared muscle biopsy findings with electromyographic changes, CK levels and "muscle strength" in 30 patients with myositis (Schwarz, Slavin, Ward & Ansell, 1980). Yet "strength" was only estimated on the MRC scale, with the patients' proximal muscles typically scoring 4 or 5 - i.e. in the range where the scale is least sensitive and most subjective. (Knee extension strength can be less than 30% of normal and yet still be correctly categorised as MRC grade 4).

Voluntary dynamometry is also possible during joint movement. Force measurements during different speeds of isokinetic muscle contraction are influenced by muscle fibre-type composition. Nevertheless, just as with isometric relaxation rate, the correlations are not adequate to allow accurate predictions of fibre-type composition from dynamometric recordings. Isokinetic recordings are also much more complex than isometric recordings. The analysis of the force records obtained from the isokinetic bicycle ergometer (Sargeant, Hoinville & Young, 1981) is a slow process and 'Cybex' recordings have to be corrected to allow for the changing effect of limb-segment mass during joint movement (Knutsson & Mårtensson, 1980). While isokinetic dynamometry warrants further research, it has, as yet, no clinical advantages to justify its greater complexity and expense. This is especially true as long as isometric dynamometry is underused in clinical practice.

Dynamometry during maximal, voluntary, isometric contractions should be part of the objective assessment of any patient with muscle wasting or weakness. Repeated measurements of voluntary strength may be used to follow changes in muscle function and, in some circumstances, in muscle mass. They would allow an objective evaluation of physiotherapy treatments, which are so often prescribed without any real understanding of what they can (or cannot) achieve. They are an essential part of the evaluation of any putative drug treatment for a muscle disease, whether in routine, clinical practice or in the context of a double-blind, controlled trial (Young & Edwards, 1981).

Electrically-stimulated contractions

It sometimes happens that it would be helpful to compare a patient's voluntary strength with the force of a supramaximal, electrically-stimulated contraction of the same muscles, for example when the patient's motivation is suspect. Unfortunately, there are only a few, peripheral, muscles (e.g. adductor pollicis) whose maximal strength can reasonably be tested by supramaximal, electrical stimulation. While it is technically possible to activate the whole

quadriceps femoris by stimulation of the femoral nerve, the procedure is too painful and too hazardous for routine use. Nevertheless, a voluntary contraction weaker than that produced by stimulating (with pad electrodes) the terminal ramifications of the quadriceps' motor nerve is a clear indication of a failure somewhere 'proximal' to the point of stimulation.

Useful as such information may be, the usual purpose of dynamometry during electrically-stimulated contractions is to allow examination of other aspects of a muscle's contractility by giving the investigator precise control over the timing and frequency of motor end-plate activation (Paper I). Some clinical applications of this facility have been described elsewhere (Young & Edwards, 1977) but it remains essentially a research technique (Edwards, 1978). The 'index of contractility' which seems most likely to prove useful in routine clinical practice is the frequency/force curve (Paper I). It may be used to monitor the dosage of the anti-spasticity drug dantrolene-sodium (Young & Edwards, 1977). It may be used to identify a particular 'type' of fatigue in limb muscles or, perhaps more importantly, in respiratory muscles (Moxham, Wiles, Newham & Edwards, 1981).

ULTRASONOGRAPHY

Measurement of atrophy and hypertrophy of muscle

As demonstrated in chapters 8 and 9, measurements of limb circumference are inadequate for studies of localised muscle atrophy or hypertrophy. These require the use of a technique which allows measurement of changes in size of individual muscles or muscle groups. This may be achieved by ultrasonography or by computerized x-ray tomography. The former is preferable since it is much cheaper, much more widely available and does not involve exposure to x-rays. In practice, however, ultrasound B-scanning is too time-consuming to be used routinely as a measure of muscle size.

In real-time ultrasonography, a linear array of crystals is held against the area to be scanned and the operator is provided with an immediate image of the structures lying in the plane of the ultrasound beams. Because of the sharply curved surface of the thigh, this very rapid and simple technique cannot provide a satisfactory image of a transverse section through all 4 heads of the quadriceps simultaneously. Unfortunately, therefore, real-time scanning is not suitable for making measurements of the cross-sectional area of the whole muscle. It is possible that a linear parameter of the quadriceps' cross-section, measurable with the real-time scanner, might be used to predict the cross-sectional area of the whole muscle (Young & Hughes, 1982). This would put the evaluation of muscle atrophy by ultrasonography within the scope of routine clinical practice.

Bilateral, mid-thigh, ultrasound B-scans previously obtained from 20 patients with unilateral thigh muscle wasting have been reviewed. This confirmed that there are linear parameters of the quadriceps' cross-section from which the relationship between the left and right quadriceps' cross-sectional areas may be predicted much more accurately than is possible from thigh circumference measurements, e.g. where ...

d = greatest 'diameter' of the quadriceps group at mid-thigh

CSA = cross-sectional area of the quadriceps group at mid-thigh

$$\text{CSA}_{\text{injured}}/\text{CSA}_{\text{uninjured}} = d_{\text{injured}}^2/d_{\text{uninjured}}^2 \times 0.89 + 2.83$$

($r = 0.89$)

It is necessary now to test which of such linear parameters it is technically possible to measure reliably with a real-time ultrasound scanner. The chosen parameters, (measured with a real-time scanner) can then be tested in a prospective study for their ability to predict the between-limbs difference in quadriceps cross-sectional area (measured from B-scans).

Relationship between muscle size and strength

Elucidation of the relationship between muscle strength and

cross-sectional area will continue to require B-scanning. As explained in Chapter 9 ('Introduction' and 'Discussion') it would be useful to have a normal range for quadriceps strength standardised for its cross-sectional area, to complement that for strength standardised for body weight (Chapter 4). It would then be possible to judge whether a patient's reduced quadriceps strength was commensurate with the size of the muscle or whether the muscle was either intrinsically weaker than normal or, perhaps, subject to reflex inhibition. As discussed in Chapter 9, further normative data, covering a wider range of muscle size, are required before this can be done. The fact that type II fibres may well be stronger than type I fibres (Chapter 10) means that the interpretation of any departure from normal would have to take into account any major change in the muscle's fibre-type composition.

Assessment of inflammatory muscle disease

Polymyositis can be a difficult condition to manage. The assessment of disease activity can be particularly difficult (Edwards et al., 1981). For example, the plasma creatine kinase may remain normal in the presence of active disease. Strength measurements are valuable indicators of progress or deterioration (vd. supra) but it may still be difficult to distinguish deterioration due to relapse from weakness as a side effect of steroid treatment. A possible approach to this problem is illustrated here by some preliminary results.

The relationship between MVC and CSA was studied in the quadriceps muscles of a 48 year-old man receiving treatment for polymyositis (Fig. 11.2). In his case, "CSA" includes not only healthy muscle but also degenerating and regenerating muscle fibres, oedema and inflammatory cell infiltrates. His improvement is reflected in a steadily increasing ratio of the strength of the quadriceps to its cross-sectional area. It seems possible that loss of strength due to a recrudescence of his disease might cause the ratio to fall again, whereas loss of strength due to the onset of a steroid-induced atrophy might be accompanied by a corresponding

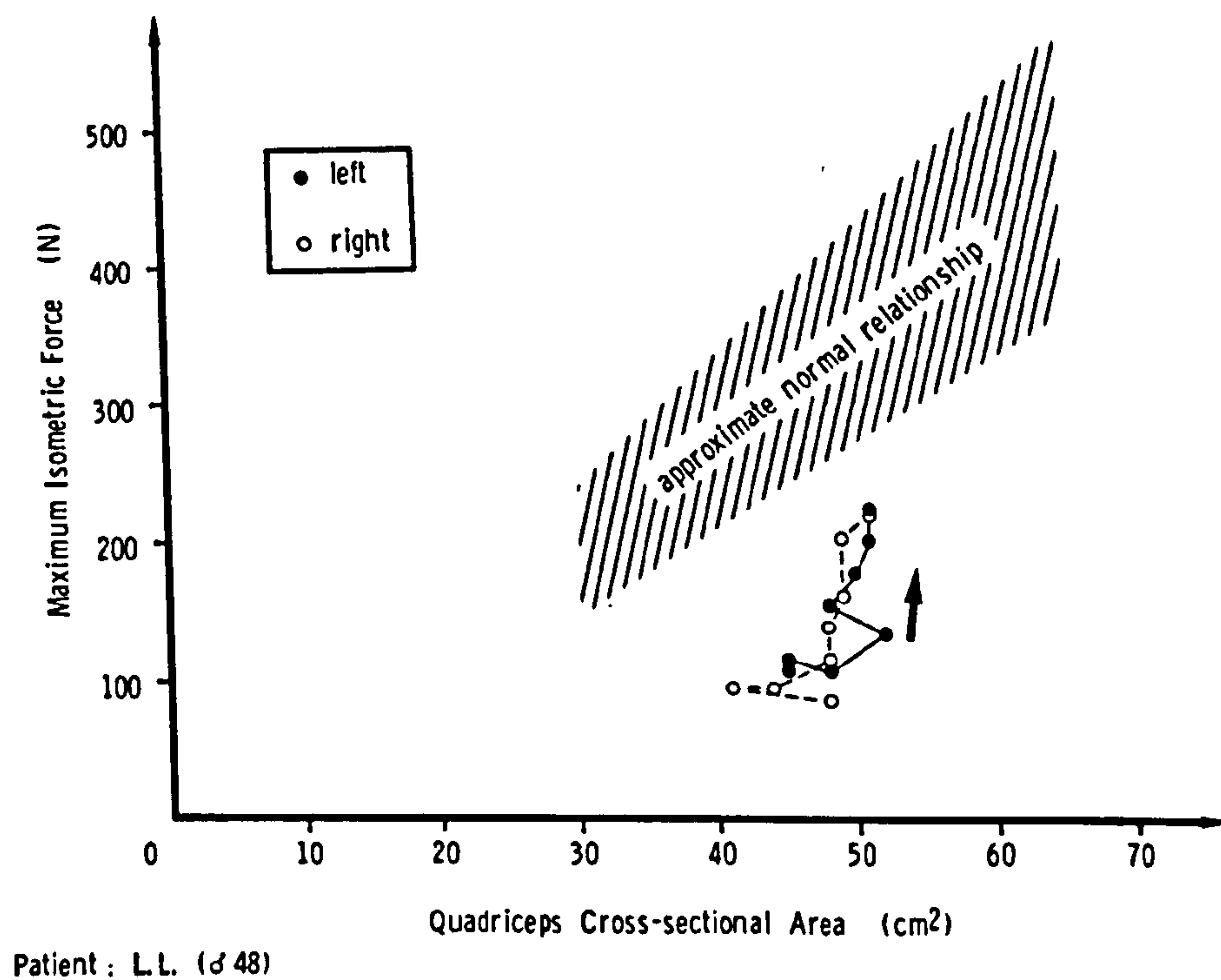


Fig. 11.2 Relationship between the strength and the cross-sectional area of the quadriceps muscles of a 48-year-old man receiving treatment for polymyositis. (The approximate normal relationship is taken from Figure 9.2).

decline in CSA, parallel to the normal relationship between MVC and CSA. Fortunately, neither situation has yet arisen; the possible value of this approach, therefore, remains conjectural.

Haematoma localisation

Clinically, an intra- or inter-muscular haematoma appears as a painful swelling with limits which are hard to define and at a depth which is usually unknown. An ultrasound scan, however, can demonstrate the depth and extent of a haematoma (Young & Hughes, 1982). This would allow the therapist to make a rational choice of frequency for therapeutic ultrasound: the deeper the lesion, the lower the frequency which would be required to ensure adequate penetration.

The localisation of an intramuscular haematoma does not require that all parts of the muscle should be examined simultaneously, nor do they have to be examined in transverse section. It would therefore be possible to take advantage of the speed and simplicity of real-time scanning for this particular application of ultrasonography.

MANAGEMENT OF ENDOCRINE 'MYOPATHIES'

The studies of osteomalacia and of thyroid disease have improved our understanding of the mechanism of proximal muscle weakness in these conditions. Loss of muscle mass is a major factor, although an increased muscle 'speed' and an increased ATP-turnover rate may contribute to fatiguability and to the sensation of weakness in hyperthyroidism. It would be interesting to use ultrasonography to determine more precisely the full extent to which osteomalacic and thyrotoxic weakness may be explained by muscle atrophy. Of more immediate, practical, clinical importance is the completeness, or otherwise, of the recovery of strength which results from vitamin D replacement. This recovery is slow and, it seems, may not be complete. It seems possible that some other stimulus to muscle

recovery is necessary. By the time osteomalacia is diagnosed, patients have often been subject to enforced inactivity for many months. Perhaps their complete recovery requires the performance of rehabilitative exercise in addition to the prescription of vitamin D. This is possible, especially since it seems that strength-training can increase strength to a greater extent than it increases muscle size (see Paper VII).

MANAGEMENT OF ARTHROGENOUS MUSCLE WASTING

One of the clearest messages to emerge from this series of studies is that limb circumference measurements are inadequate for the objective evaluation of arthrogenous muscle wasting. Quadriceps atrophy may be highly selective and may be much more severe than the difference in thigh circumference would suggest. Trials of different techniques and regimes of orthopaedic after-care are meaningless if they depend on measurements of limb circumference. Muscle cross-sectional area should be measured accurately and, where possible, voluntary isometric strength should also be measured.

Joint injury and/or immobilization may be associated with selective atrophy of type I or type II muscle fibres. As discussed in Chapter 8, the more atrophied fibre type may indicate the more important pattern of therapeutic exercise for an individual patient. This can only be tested when the causes of selective type I and type II fibre atrophy are better understood. In the meantime, it would seem wise to supplement the usual high-intensity muscle contractions with activities which might be more effective at stimulating type I fibre growth (e.g. high-repetition, low-force contractions and passive muscle stretch). This implies that following acute injury or surgery, controlled joint movement and muscle stretch should be encouraged as early as the condition of the joint permits, possibly through the greater use of hinged bracing.

As discussed earlier in this chapter, it may be possible to use the normal relationship between muscle size and muscle strength to determine whether an individual example of arthrogenous muscle

weakness owes more to atrophy or to reflex inhibition (due perhaps to the presence of a joint effusion). The possibility that type II fibres may have a greater specific strength than type I fibres means that one would have to be cautious when interpreting the relationship between a muscle's size and its strength in the presence of selective atrophy of one or other fibre-type. Nevertheless, this may provide information of practical importance for the management of intransigent arthrogenous weakness (Young, 1982).

CONCLUSIONS

This series of studies has indicated numerous areas for important future research and it has contributed to our understanding of muscle weakness. It has also yielded two fundamental messages of immediate, practical, clinical significance.

Measurements of muscle strength are essential for the rational management of neuromuscular disorders and for any research related to changes in muscle strength.

Limb circumference measurements are inadequate for following changes in muscle size. Any clinical trials of measures to minimise or reverse atrophy must use an imaging technique (such as ultrasonography or computerized X-ray tomography) which allows accurate measurement of the cross-sectional area of individual muscles or muscle groups.

CHAPTER 12

CONCLUSIONS

QUADRICEPS DYNAMOMETRY

WEAKNESS IN OSTEOMALACIA

MUSCLE FUNCTION IN THYROID DISEASE

ARTHROGENOUS WASTING

QUADRICEPS SIZE

QUADRICEPS SIZE AND STRENGTH

SPECIFIC STRENGTH OF TYPE I AND TYPE II FIBRES

CHAPTER 12

CONCLUSIONS

QUADRICEPS DYNAMOMETRY

The voluntary isometric strength of the quadriceps femoris is a repeatable and valid measure, essential for the rational management of neuromuscular disorders and for any research related to changes in muscle strength.

The relaxation rate (and other indices of contractility) of the quadriceps can be measured reproducibly by isometric dynamometry during transcutaneous electrical stimulation. Type II fibres in the human quadriceps relax faster than type I fibres but there is, as yet, no reliable way of using measurements of relaxation rate as non-invasive predictors of fibre-type composition.

WEAKNESS IN OSTEOMALACIA

Muscle atrophy is a major, immediate cause of weakness in osteomalacia. The recovery of strength during treatment with vitamin D is associated with growth of muscle fibres, is a slow process, and may be incomplete. The isometric relaxation rate of the quadriceps is slow in osteomalacia, perhaps reflecting selective atrophy of type II muscle fibres.

MUSCLE FUNCTION IN THYROID DISEASE

Quadriceps weakness in thyrotoxicosis is associated with myofibre atrophy. Subjective weakness in submaximal contractions is probably also due to the higher stimulus frequency required to achieve tetanic fusion, as a result of the muscle's increased rate of relaxation. In hypothyroidism, the rate of ATP turnover and the production of lactate during isometric contractions held to fatigue is slow, as a

result of the muscle's reduced rate of relaxation. There is no evidence of impaired ATP generation. There are abnormalities of the quadriceps' fibre-type composition in both hyper- and hypo-thyroidism but they do not fully explain the changes in the muscle's relaxation rate.

ARTHROGENOUS WASTING

The severity of quadriceps wasting following knee injury can be explained entirely by atrophy; there is no evidence of a change in the total number of fibres in the muscle. It is not known what particular clinical features determine whether an individual patient's quadriceps will show type I atrophy, type II atrophy or combined atrophy of both fibre types following knee injury and/or immobilisation; all 3 patterns of atrophy were observed. In the absence of pain or an effusion, the loss of quadriceps strength is similar to the degree of atrophy.

QUADRICEPS SIZE

Quadriceps CSA may be measured by ultrasound B-scanning. The severity of the quadriceps wasting which follows knee injury is seriously underestimated by measurements of thigh circumference, even when they are supplemented by caliper measurements of subcutaneous fat. The tape measure also underestimates the increase in muscle mass which occurs with strengthening exercise. Studies of muscle hypertrophy or atrophy must include a direct measurement of the size of the individual muscle or muscle group being considered.

QUADRICEPS SIZE AND STRENGTH

Preliminary studies of normal subjects show a close correlation between the quadriceps' strength and its CSA but high-resistance training may increase the isometric strength of the normal quadriceps by more than it increases its total CSA.

SPECIFIC STRENGTH OF TYPE I AND TYPE II FIBRES

In isometric contractions of the human quadriceps, type II fibres may be about twice as strong, for their cross-sectional area, as type I fibres.

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APPENDIX 1

CORE PUBLICATIONS

- I EDWARDS, R.H.T., YOUNG, A., HOSKING, G.P. & JONES, D.A. (1977)
Human skeletal muscle function: description of tests and
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- II YOUNG, A., EDWARDS, R.H.T., JONES, D.A. & BRENTON, D.P. (1981)
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The effect of knee injury on the number of muscle fibres in
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- VII YOUNG, A., STOKES, M., ROUND, J.M. & EDWARDS, R.H.T. (in
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APPENDIX 2

OTHER, RELEVANT, PERSONAL PUBLICATIONS

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YOUNG, A., JONES, D.A., MAUNDER, C.A. & EDWARDS, R.H.T. (1975) Needle biopsy studies of weakness and fatigue in patients with myopathy. Clinical Science and Molecular Medicine, 49, 4-5P. (Abstract)

YOUNG, A., BRENTON, D.P. & EDWARDS, R.H.T. (1978) Analysis of muscle weakness in osteomalacia. Clinical Science and Molecular Medicine, 54, 31P. (Abstract)

YOUNG, A., STOKES, M., WALKER, I.C.R. & NEWHAM, D. (1981) The relationship between quadriceps size and strength in normal young adults. Annals of the Rheumatic Diseases, 40, 619-620. (Abstract)

YOUNG, A., WILES, C.M. & EDWARDS, R.H.T. (1978) University College Hospital muscle-biopsy needle. Lancet, ii, 1285.

APPENDIX 3

CONSENT FORM

Title of Study: The Treatment and Prevention of Quadriceps Muscle Wasting.

Purpose of Study: A research study to improve our understanding of the shrinkage that occurs in the thigh muscles when the knee is injured or immobilised and to study ways of building the muscle up again.

Nature of Procedure: The taking of very small specimens ("biopsies") from the muscle on the front of one/both thighs. The specimens are taken with a large needle through a 5mm (1/5 inch) nick made in the skin after the insertion of local anaesthetic.

You will not feel the nick being made in the skin but you will feel the specimen being taken. The sensation varies from a very mild "deep pressure" to something like the dull discomfort caused by walking into the corner of a table. This discomfort only lasts for a few seconds. There may be a little stiffness the next day but this will not interfere with your normal activities.

CONSENT OF SUBJECT

Patient/Normal subject

I, of
.....
give my consent for Dr. A. Young to take needle biopsy specimens from one/both of my quadriceps muscles. Dr. Young has explained to me the nature, purpose and possible consequences of the procedure. I understand that the study is being carried out for reserch purposes and is not required for the management of my condition.

Signed: Date:

Witness:

I confirm that I have explained to the nature, purpose and possible consequences of taking needle biopsy specimens from his/her quadriceps muscles.

Signed:

Witness:

Human skeletal muscle function: description of tests and normal values

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Summary

1. The force produced by isometric contractions of the quadriceps muscle have been studied during maximal voluntary contractions and when a substantial part of the muscle was electrically stimulated via surface electrodes.

2. In normal children and adults, the force of a maximal voluntary contraction of the quadriceps was proportional to body weight.

3. The function of the quadriceps has been described in terms of the force/frequency curve, speed of relaxation and the rate of loss of force during 18 s stimulation at 30 Hz and 100 Hz.

4. The functional characteristics of adductor pollicis when stimulated via the ulnar nerve were essentially similar to those of the quadriceps.

5. Studies of the function of these two muscles are complementary since quadriceps femoris is amenable to needle biopsy investigations of its structure and chemistry whereas adductor pollicis is more suitable for electrophysiological studies.

Key words: human skeletal muscle, muscle function, strength.

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Abbreviations: MVC, maximum voluntary contraction; PTP, post-tetanic potentiation; SF₅₀, SF₉₅, relaxation indices quantifying time for relaxation from brief tetanic contractions.

Introduction

Assessment of skeletal muscle function should have the objective of characterizing its contractile properties but in man there are severe constraints on the feasibility of measuring contractile properties of muscle. Perhaps it is for this reason that there are no accepted methods for quantifying muscle function in clinical use today. Attempts to assess the contractile function of muscle have been made by electrophysiologists studying peripheral muscles from which electrical recordings can reliably be made—the adductor pollicis (e.g. Merton, 1954; Desmedt, Emeryk, Renoirte & Hainaut, 1968; Takamori, Gutmann & Shane, 1971), abductor digiti minimi of the hand (Burke, Skuse & Lethlean, 1974), first dorsal interosseous of the hand, lateral gastrocnemius and extensor digitorum brevis (McComas & Thomas, 1968) and extensor hallucis brevis (Sica & McComas, 1971). But many disorders of muscle have a predominantly proximal distribution and it is for this reason that the quadriceps, often preferentially affected, has been chosen for study. Moreover, impairment of quadriceps function can have serious practical implications for the patient as it is important for everyday activities. The muscle is sufficiently large for needle biopsies to be performed

for histochemical and electron-microscopical studies of structure (Edwards, Maunder, Lewis & Pearse, 1973), and a proximal tourniquet allows the quadriceps to be used as a 'closed system' for studies of muscle chemistry and for myothermal investigations (Edwards, 1975; Edwards, Hill & Jones, 1975).

The maximum contractile force of the quadriceps muscle is assessed as the force of a maximum voluntary contraction since supra-maximal (tetanic) femoral nerve stimulation is painful and potentially dangerous. Muscle tears, patellar dislocation and fractures in persons with brittle bones are possible hazards. A substantial portion of the quadriceps femoris can be caused to contract maximally without risk by percutaneous stimulation of the superficial branches of the femoral nerve (Edwards, Hill & McDonnell, 1973), allowing the function of this large proximal muscle to be described in terms of the force/frequency curve, speed of relaxation and rate of force fatigue during prolonged stimulation.

This paper reports measurements of voluntary and electrically stimulated contractions of the quadriceps femoris in normal subjects that may be used as criteria for the assessment of patients. The results are compared with those from the adductor pollicis studied by more conventional electrophysiological methods.

Methods

Subjects

The subjects were all normal, healthy volunteers. None was unusually obese or thin. For the reported measurements of voluntary strength, the subjects' ages ranged from 5 to 63 years, and for the electrical stimulation tests, from 12 to 63 years, although few were over 50 years of age.

The investigations were performed with the informed consent of the subjects or their parents and the approval of the Research Ethics Committee of the Royal Postgraduate Medical School and Hammersmith Hospital.

Procedure

Both voluntary and stimulated isometric contractions of quadriceps femoris were studied with the subject seated in an adjustable,

straight-backed chair with the lower leg dependent and the knee flexed to 90° (Tornvall, 1963) (Fig. 1). The pelvis was secured by an adjustable belt. Force was measured with a strap looped round the leg just proximal to the malleoli. The amplified output from the strain gauge was recorded with a rapid response oscillograph.

For electrical stimulation of the quadriceps two large, flexible, saline-soaked pad electrodes (approximately 13 cm square) were closely applied proximally and distally to the antero-lateral thigh. Stimulation was with unidirectional, square-wave pulses of 50 μ s duration and

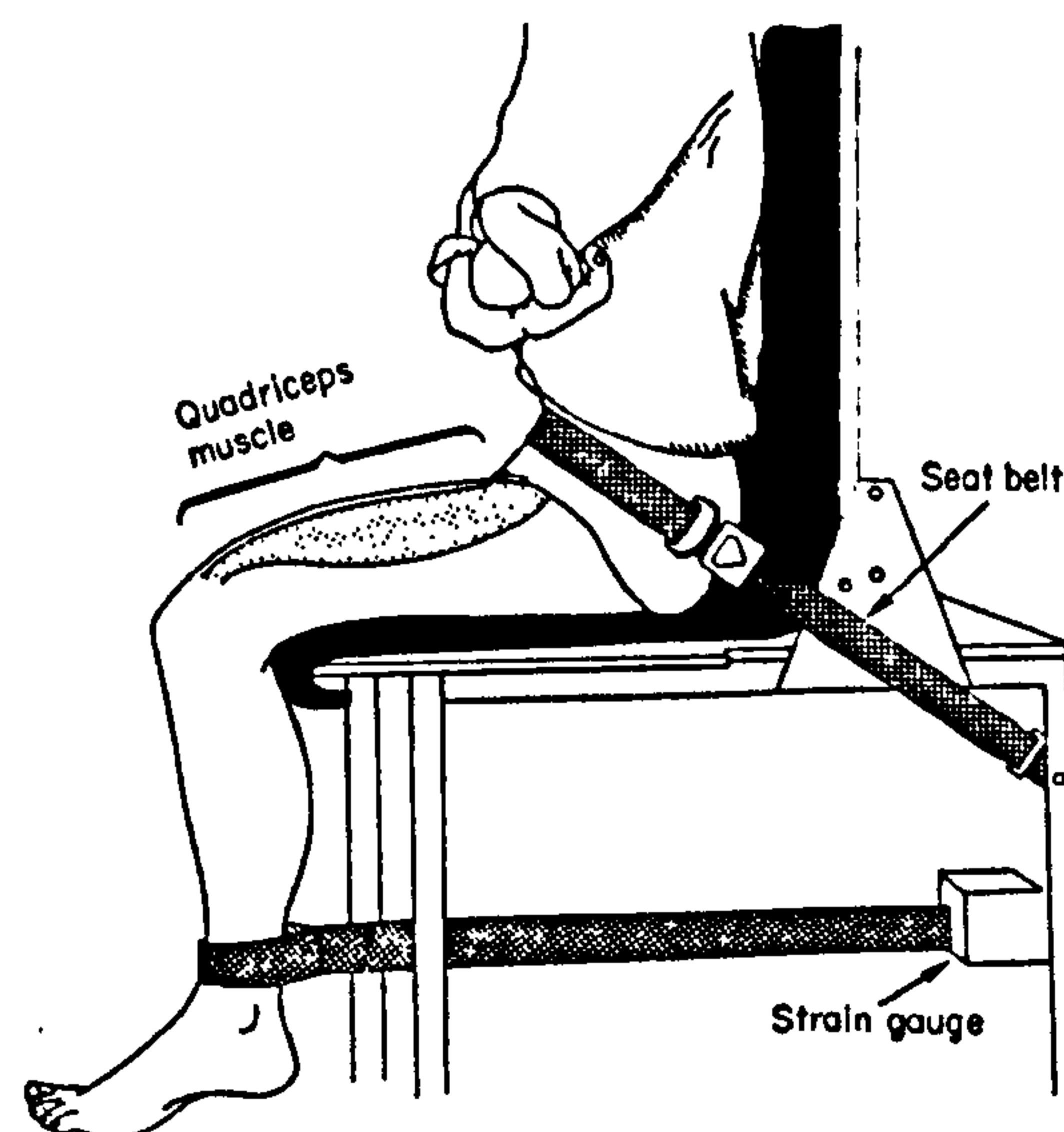


FIG. 1. Adjustable chair used for testing quadriceps muscle function.

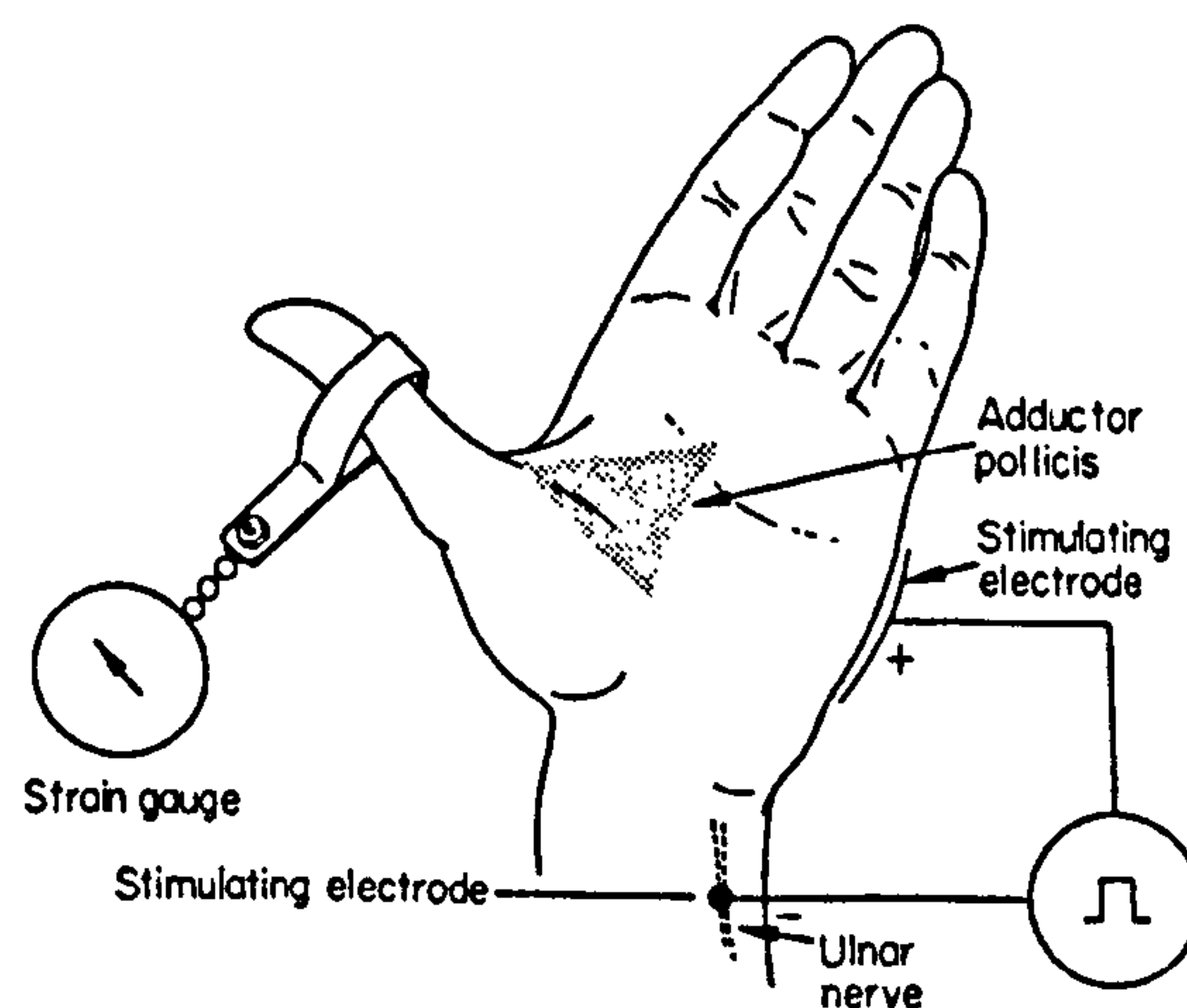


FIG. 2. Arrangement used for testing adductor pollicis (the restraining structure around the fingers has been omitted for clarity).

up to 70 V (maintained between the electrodes). This method of electrical stimulation via large-area electrode pads is similar to the conventional faradic stimulation technique used by physiotherapists and proved to be both safe and acceptable in terms of the pain elicited.

Adductor pollicis was studied in the left hand with a dynamometer based on that of Merton (1954). A strain gauge was linked by a chain to a loop around the interphalangeal joint of the thumb (Fig. 2). The hand was secured in supination with the fingers slightly flexed, the thumb abducted and its metacarpophalangeal and interphalangeal joints fully extended. The ulnar nerve was stimulated at the wrist with unidirectional, square-wave pulses of 50 μ s duration and up to 80 V. The same standard order of testing was used in the adductor pollicis as for the quadriceps:

- (1) Maximal voluntary contractions $\times 3$.
- (2) Short tetani at 30 Hz, increasing the voltage gradually until a suitable stimulus voltage was reached.
- (3) Three periods of stimulation at 30 Hz for 1 s every 15–30 s for measurement of relaxation rates.
- (4) Stimulation for 10 s at 1 stimulus/s and for 2–4 s each at 20 Hz and at 50 Hz (15–30 s rest between each period of stimulation).
- (5) Stimulation for 2–5 s at 3, 5, 8, 10, 15, 20, 30, 50, 80 and 100 Hz, with 15–30 s between each period of activity.
- (6) Stimulation for 30 s at 30 Hz and again 1–2 min later at 100 Hz.

Precautions

The mechanical properties of muscle which have been examined in this study are influenced by a number of factors. Relaxation is slowed when the muscle is cooled and accelerated when it is warmed (Lambert, Underdahl, Beckett & Mederos, 1951). A low muscle temperature is not an important practical problem when studying a large, proximal muscle such as quadriceps, insulated by a layer of subcutaneous fat, but when dealing with a small, distal muscle such as the adductor pollicis it is essential to take steps to standardize the temperature of the muscle at the time of study. The temperature of the quadriceps at rest is about 35°C (unpublished observations

from this laboratory and Harris, Hultman, Kaijser & Nordesjö, 1975) but even with an ambient temperature of 20°C the temperature of the adductor pollicis in a normal subject may be as low as 29°C. Testing of this muscle was therefore always preceded by 10 min immersion of the hand and forearm in a water bath at 44°C to bring the muscle temperature to about 35°C. Relaxation is also slowed when the muscle is fatigued (Edwards *et al.*, 1975). This slowing recovers rapidly (half-recovery time about 10 s) and need not influence results provided that the contractions are suitably spaced.

After even a brief high-frequency tetanus there is post-tetanic potentiation of the force of a twitch (PTP) and of low-frequency tetani. Although the initial reversal of PTP is fast (less than 30 s) a complete return to unpotentiated twitch tension takes at least 5 min. The effect of PTP was apparent but standardized by the fixed order of testing described.

Results and discussion

Voluntary strength of the quadriceps

Maximum voluntary contractions were maintained until the examiner was satisfied (usually in 2–4 s) that the force produced was no longer increasing. The value of each maximal voluntary contraction (MVC) was measured as the greatest force held for 1 s. Three MVC trials were made with each quadriceps. The first trial was often rather tentative as the subject learned what was required. The second and third attempts were usually very close but a fourth effort was made if the second and third values were widely different, or if the examiner was not confident that a maximal effort had been made. The difference between the two highest values obtained on the stronger leg was small (coefficient of variation = 2.8%, $n = 62$), suggesting that a consistent 'maximum' effort was being made on each occasion. (A lesser degree of consistency might be expected if the effort was less than maximal.) This practice is similar to the routine recommended by Freedman & Prowse (1966) for measuring another physiological index which is dependent upon a patient's co-operation—forced expiratory volume in 1 s (FEV_{1.0}).

In this paper only the strength of the stronger

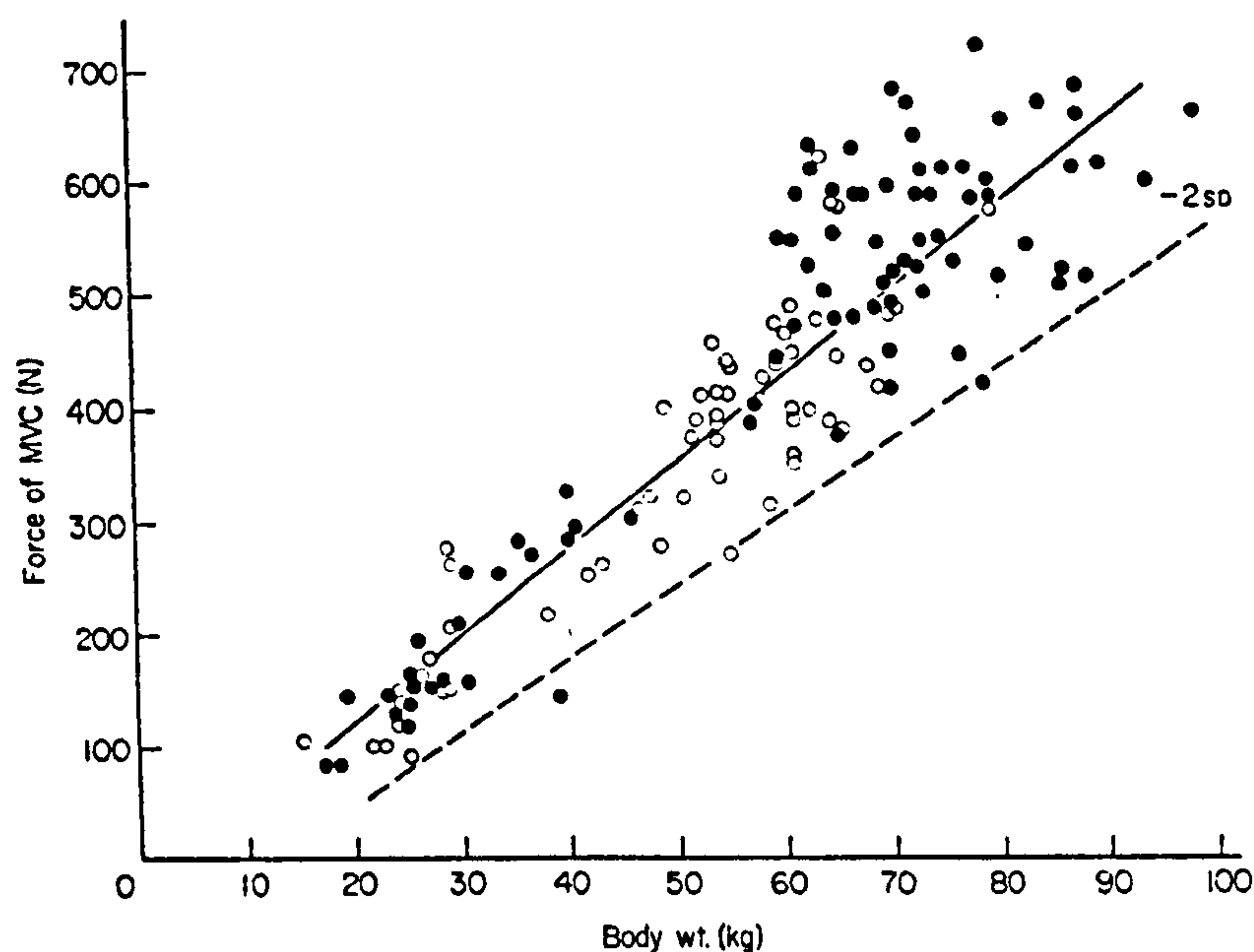


FIG. 3. Relationship between body weight and the force of a maximal voluntary isometric contraction of the quadriceps. The continuous line is the regression line based on all points [$y = 7.91x - 37.7$ ($r = 0.92$; $n = 145$)]. The broken line is the lower limit of normal calculated for data groups at 10 kg intervals distributed throughout the body-weight range. ●, Males aged 6–63 years ($n = 84$); ○, females aged 5–46 years ($n = 61$).

leg is reported. For 59 normal adult subjects the MVC of the weaker leg was 8.5% (SD 8.2) less than the stronger. The force of the MVC was related to body weight (Fig. 3). This was chosen as a standard for strength since quadriceps is a weight-bearing muscle. Results in the females fell close to those of the males and for this reason have been combined with them.

An approximate rule, useful in testing patients, is that an adult's predicted 'normal' MVC (in kg force) is about 75% of his body weight, and the 'lower limit of normal' (i.e. the third centile) is approximately half the body weight. At low body weights this simple rule is no longer satisfactory and a child's performance has to be compared with the regression line.

Electrical stimulation studies

Electrical stimulation produced contractions of up to 60% of the quadriceps MVC. Tests were usually performed with a voltage such that 15–40% of the muscle was activated, as judged by the force obtained. Discomfort experienced at the voltages used for testing was mild and largely due to the unfamiliar character of the sensations produced not only by the

stimulus itself but also by the involuntary contraction of a large muscle. Repeated testing was well tolerated. No injuries resulted from such stimulation studies though it was occasionally reported that the muscle felt a little stiff the following day.

Stimulus frequency

The response of the quadriceps to different frequencies of stimulation is illustrated in Fig. 4. The force produced at the different frequencies is expressed as a percentage of maximum tetanic force and plotted on linear scales (Fig. 5). A logarithmic scale may also be used for the frequency (Moulds, Young, Jones & Edwards, 1977) and this may sometimes be useful when examining the effects of drugs (R. F. W. Moulds, unpublished work).

As a numerical summary of this force/frequency curve the value has been obtained for the ratio of the mean force produced by an incompletely fused tetanus at 20 Hz to that developed by a fused tetanus at 50 Hz (Table 1A). The forces were recorded in the standard order given in the Procedure section above to minimize the effects of fatigue and post-tetanic

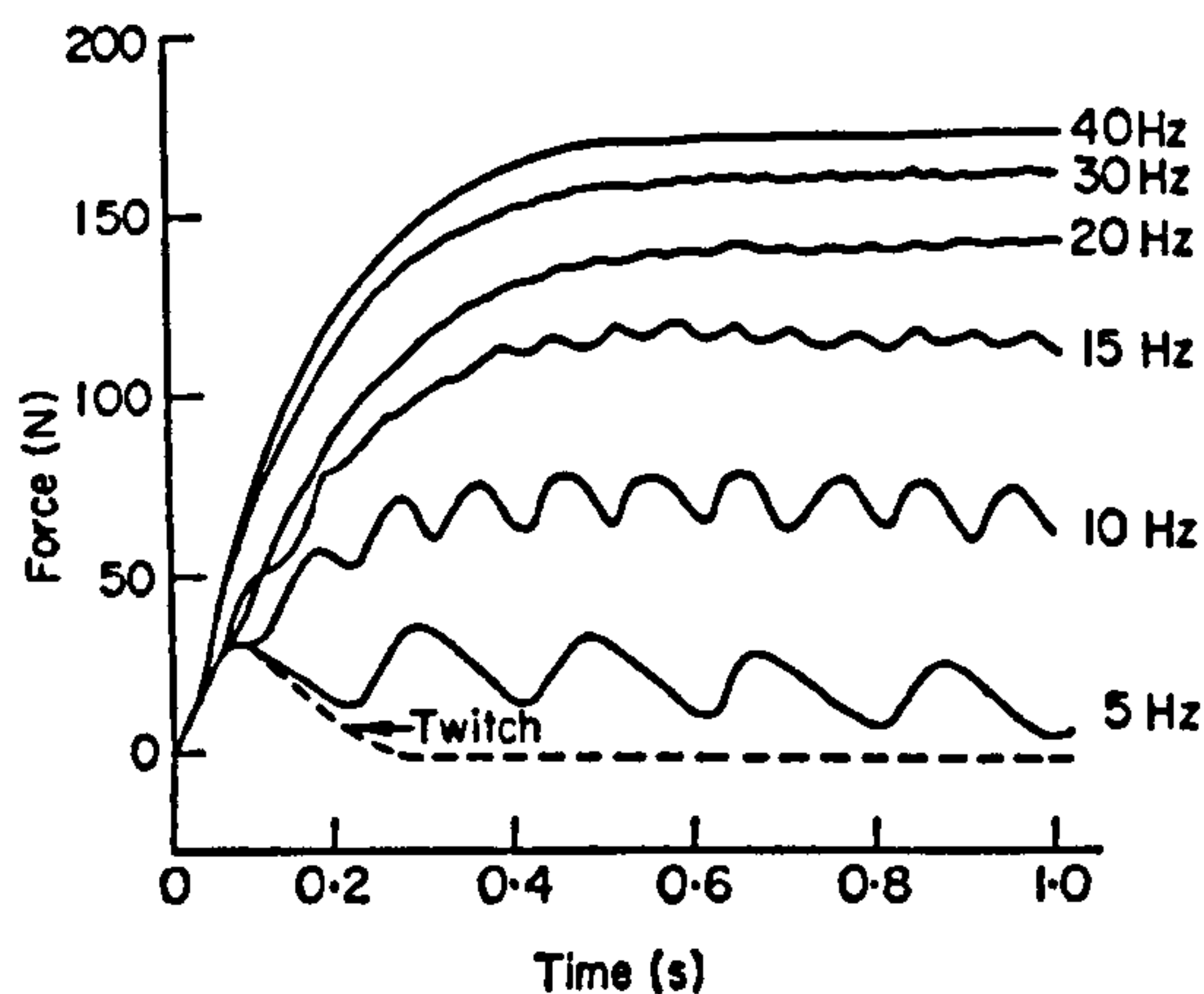


FIG. 4. Effect of stimulus frequency on the tension developed by normal human quadriceps.

potentiation. There was no consistent difference in the $\text{tetanus}_{20}/\text{tetanus}_{50}$ ratio that could be ascribed to either age or sex and for this reason the mean value for the combined data has been calculated.

Time-course of relaxation

The concept of examining the speed of relaxation of a muscle from a contraction has already been applied to studies of tendon reflexes and brief electrically stimulated contractions (e.g. Lambert *et al.*, 1951; Desmedt *et al.*, 1968; Takamori *et al.*, 1971). Since most voluntary muscle activity involves sustained contractions we have concentrated on examining the rate of relaxation from tetani produced by stimulation at 30 Hz. Higher frequencies sometimes failed to produce a satisfactory force 'plateau', either as a result of an early loss of force or as a result of superimposed movement in response to discomfort.

The indices of muscle relaxation which have been used in this study are the times, measured

from the last stimulus, taken for the force of a tetanus to fall to 95% and 50% of its plateau value, i.e. SF_{95} and SF_{50} (Fig. 6).

The values of SF_{95} reported for individual subjects were the mean values of three or four tetani at 30 Hz. Eighty-two normal subjects were tested in this way and the mean results are listed in Table 2B. Six subjects were re-tested on different days (17 tests in all). The coefficient of variation between tests was $<4\%$. This agrees well with the previously reported coefficients of variation for indices of the time-course of relaxation (Edwards *et al.*, 1973).

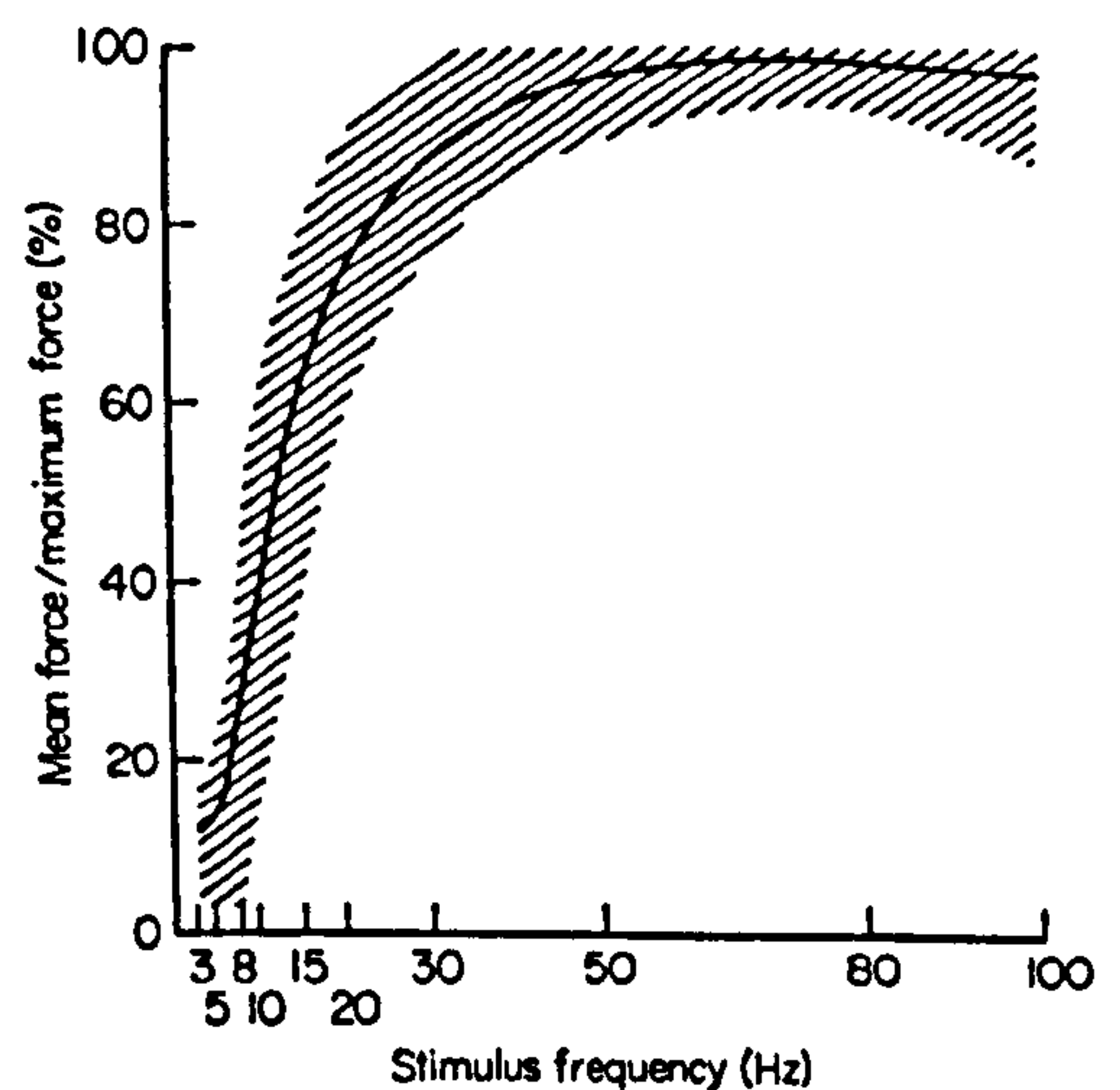


FIG. 5. Force/frequency curve of normal human quadriceps constructed from stimulated isometric contractions and showing the normal range (40 subjects, mean \pm SD). For the unfused tetani (Fig. 4) the mean force was taken as the mean value of the oscillation.

Since only a portion of the quadriceps was stimulated, the possibility was considered that different parts of the muscle might vary in their compositions and functional characteristics and the part stimulated might not be representative of the muscle as a whole. The results

TABLE 1. Normal values for indices of quadriceps function

	Mean	SD	n
(A) Force (%)			
Tetanus ₂₀ /tetanus ₅₀	76.9	6.5	78
(B) Relaxation time (ms)			
SF ₉₅	60.4	4.2	82
SF ₅₀	104.7	9.4	81

TABLE 2. Individual values of the relaxation indices of quadriceps femoris with different intensities of electrical stimulation at 30 Hz

Stimulus duration = 1 s. Subject: RHTE.

% of MVC force:	Femoral nerve stimulation		Surface pad electrodes		
	Supramaximal voltage	Reduced voltage			
	100	35	55	30	10
SF ₉₅ (ms)	67	67	65	66	62
	75	66	63	68	56
	69	62	66	66	58
SF ₅₀ (ms)	113	114	113	113	118
	115	114	112	115	116
	115	119	112	112	118

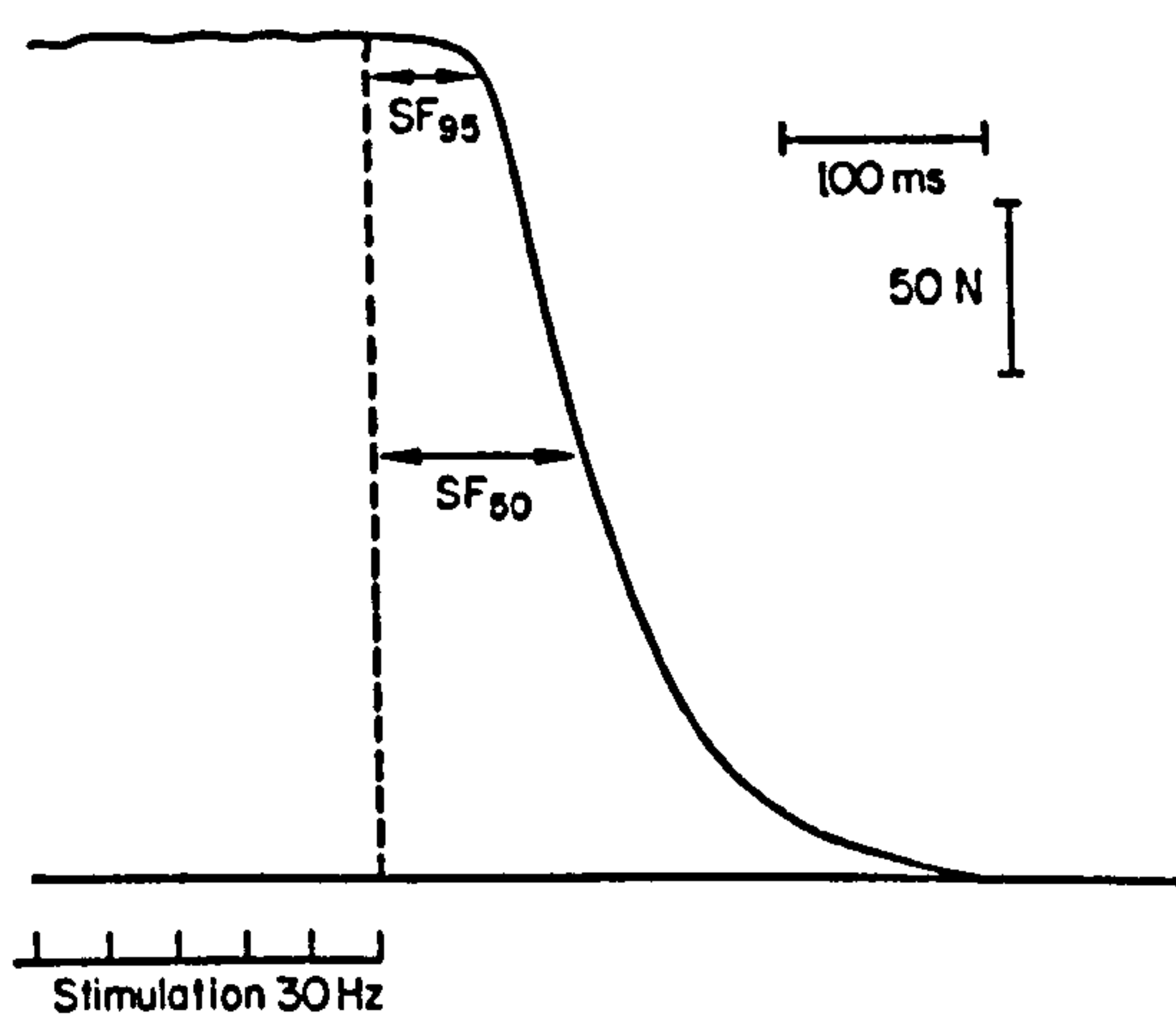


FIG. 6. Record of the relaxation of quadriceps muscle from a stimulated isometric contraction showing the indices used to describe the time-course of relaxation.

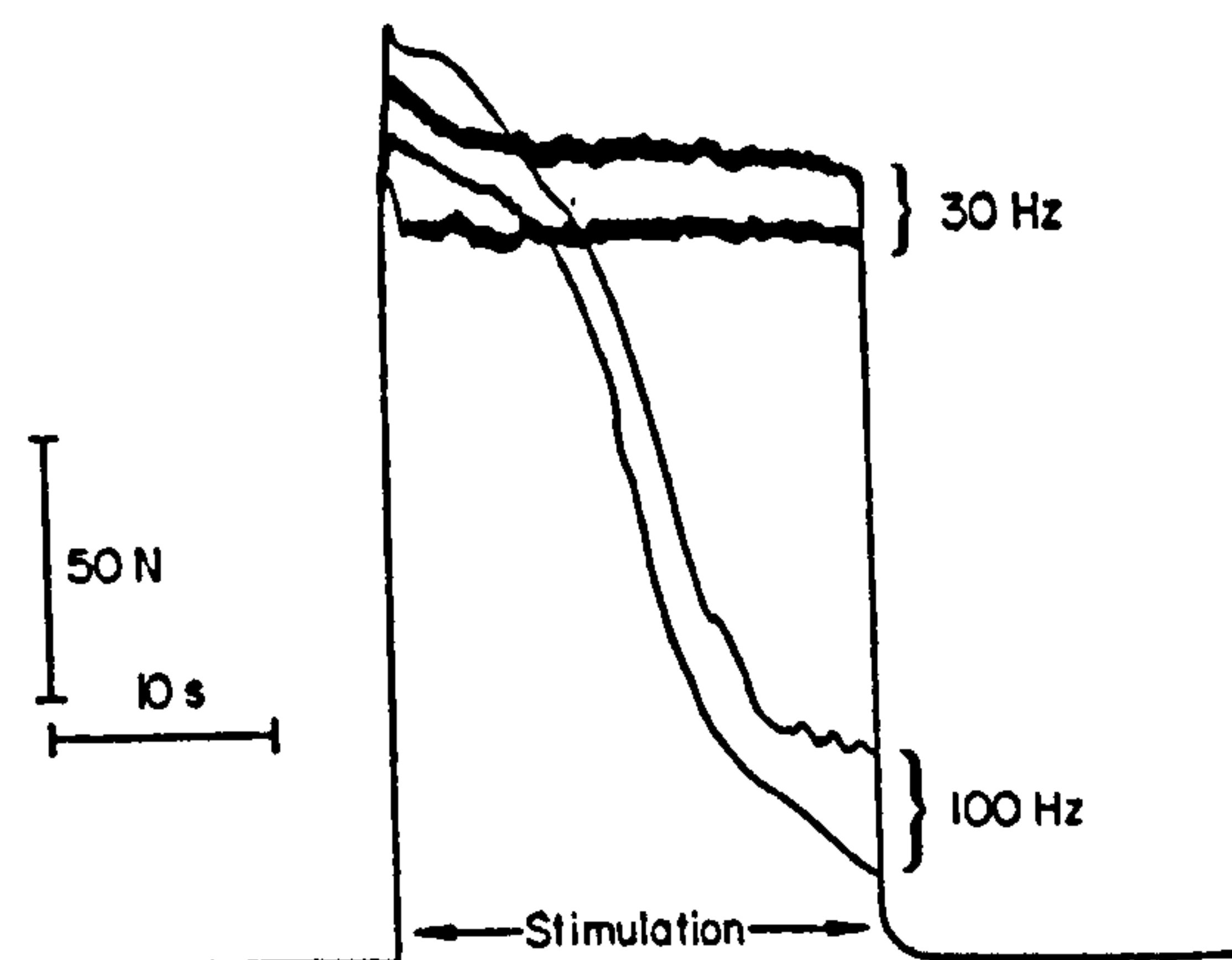


FIG. 7. Comparison of the maintenance of force during prolonged stimulation of quadriceps femoris at 30 Hz with the much more rapid loss of force during stimulation at 100 Hz (two normal subjects).

obtained by percutaneous stimulation of various proportions were therefore compared with those obtained when the whole muscle was activated by stimulation of the femoral nerve. No consistent differences were found (Table 2). Femoral nerve stimulation at high frequency is unpleasant and for this reason was performed on a single subject only, one of the investigators.

Maintenance of tension

Prolonged stimulation of the quadriceps at 30 Hz and at 100 Hz produced characteristic patterns of loss of force (Fig. 7). Similar results were obtained for the whole muscle stimulated via the femoral nerve trunk as for part of the muscle stimulated by pad electrodes. The patterns were also similar to those produced by

adductor pollicis. During the first 18 s of continuous stimulation at 30 Hz the force produced by the quadriceps decreased by 9.7% (mean) of the maximal force (sd 7.1%; $n = 40$). At 100 Hz the corresponding figure was 59.6% (sd 16.2%; $n = 27$).

Adductor pollicis and quadriceps muscles compared

The maximum voluntary contraction force of the adductor pollicis has not been reported because of the difficulty of isolating the MVC force of that muscle from possible (unintentional) augmentation by the long flexor muscles of the thumb. Electrical stimulation via the ulnar nerve allows the adductor pollicis to contract independently of the long flexors but it

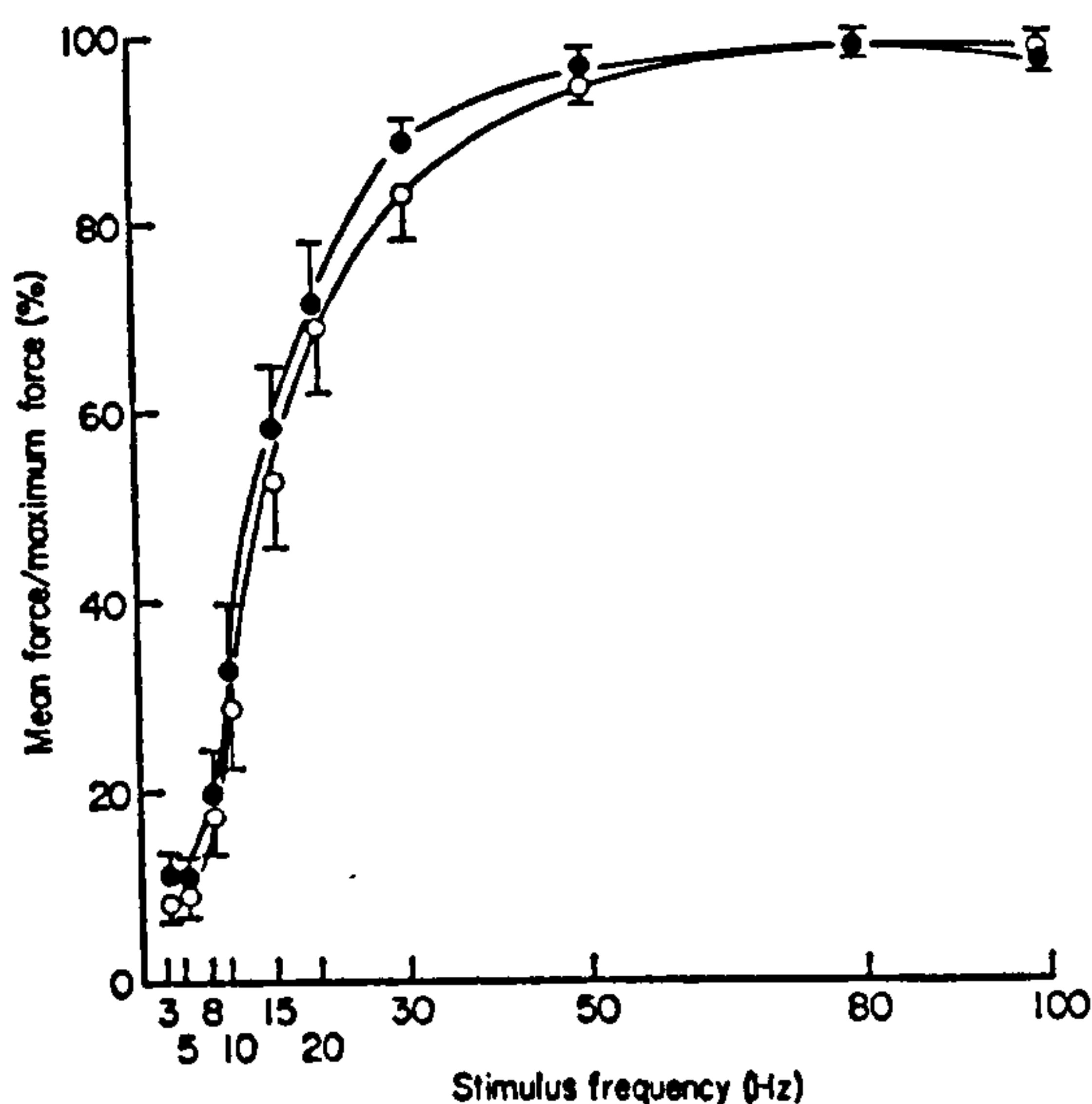


FIG. 8. Comparison of the mean force/frequency curves obtained for the quadriceps femoris (●) and adductor pollicis (○) muscles of ten normal subjects. Vertical bars show ± 2 SEM.

is not useful to quote a normal range for the peak tetanic tension since there is no obvious standard (cf. body weight in the case of quadriceps) which can be used to interpret adductor pollicis strength in different individuals.

The force produced by the adductor pollicis is also strongly influenced by factors which are not readily quantified (e.g. occupation), and a valid comparison of its intrinsic strength with that of the quadriceps is rendered impossible by the difficulty of assessing the relative sizes of the two muscles. Both muscles were studied in ten normal subjects (eight males, two females). With paired *t*-tests, only the values for SF_{95} differed significantly ($P < 0.001$). If the initial

phase of relaxation is excluded (by calculating $SF_{50} - SF_{95}$) the relaxation times for the two muscles are very similar (Table 3). The delayed initial relaxation in the quadriceps may be a reflection of the unavoidably looser connection with the recording system (Fig. 1) for this muscle in contrast to the more direct link between adductor pollicis contraction force and tension transducer (Fig. 2).

The force/frequency curve for adductor pollicis was the same as that for quadriceps femoris (Fig. 8). This measurement was made when muscle force was steady hence differences in compliance of the recording systems were unimportant.

Each index of muscle function obtained for the adductor pollicis and for quadriceps muscles of the same ten subjects were also examined by linear regression analysis to see whether results obtained for one muscle correlated with those for the other muscle (for example, if a subject has a fast-relaxing adductor pollicis, will he also have a fast quadriceps femoris?) but no such correlation was found.

The indices of muscle function obtained by electrical stimulation were thus similar in the two muscles. This finding and the result of stimulating various amounts of the quadriceps muscle (Table 2) suggest that these indices are essentially independent of muscle mass and unaffected by the presence of inactive muscle in parallel.

Conclusion

Earlier investigations of the mechanical properties of human muscle electrically stimulated

TABLE 3. Comparison of the responses of quadriceps femoris and adductor pollicis to electrical stimulation

Mean values \pm SEM are shown; $n = 10$ (both measurements were made in all individuals). $SF_{50} - SF_{95}$ represents most closely the true rate of relaxation (see Fig. 6). Note that it was the same for both muscles though different delays were recorded in the initiation of relaxation as principally reflected by SF_{95} .

	Quadriceps femoris	Adductor pollicis
Force (%)		
Tetanus ₂₀ /tetanus ₅₀	75.6 \pm 2.0	73.1 \pm 2.8
Relaxation time (ms)		
SF_{95}	60.0 \pm 1.3	51.2 \pm 1.6
SF_{50}	103.3 \pm 3.9	95.8 \pm 3.4
$SF_{50} - SF_{95}$	43.3 \pm 2.7	44.6 \pm 1.9

in vivo were concerned with the function of distal muscles. This report has described the response of both a proximal muscle group (quadriceps femoris) and a distal muscle (adductor pollicis) to electrical stimulation extended to include the effects of repetitive stimulation over a range of frequencies and to the time-course of relaxation. The tests described proved to be practicable and acceptable, and their place in the assessment of patients is being evaluated in the Muscle Clinics at Hammersmith Hospital.

Acknowledgments

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MECHANICAL FACTORS AND THE SKELETON

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Quadriceps muscle strength and fibre size during the treatment of osteomalacia

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INTRODUCTION

Vitamin D deficiency has profound effects on the mechanical properties of bone. It also impairs the function of skeletal muscle, altering the forces acting on the bones.

The weakness has a predominantly proximal distribution and may even be the presenting symptom (Skaria et al., 1975). In ancient Indian literature, the author Vagbhatta (circa 500 A.D.) remarked on the combination of 'excessive and constant pain of the hip bones and great loss of strength' (translation by Professor B.C. Katiyar). In 1916, Agnes Scott published a vivid description of Indian women with osteomalacia, including the observation that 'to get up from a sitting to a standing position, the patient climbs up her own legs in a way similar to that of a child suffering from pseudo-hypertrophic muscular atrophy'. More recently, several medical authors have made a particular point of the muscular consequences of the disease (eg Dent, 1956; Smith & Stern, 1967; Schott & Wills, 1976). Nevertheless, the cause of osteomalacic 'myopathy' remains unknown.

This report describes measurements of quadriceps muscle strength and fibre size in a group of 12 patients with osteomalacia receiving treatment with vitamin D or one of its metabolites. An abstract based, in part, on the data reported here has previously been published (Young, Brenton & Edwards, 1978).

PATIENTS

Twelve patients (11 female) were studied (Table 1). Six were Indian women with nutritional osteomalacia, four were women with gluten-sensitive enteropathy and two had post-gastrectomy osteomalacia. Most had clinically apparent proximal muscle weakness.

METHODS

Strength

Quadriceps strength was measured with the patient seated in an adjustable, straight-backed chair with the pelvis secured by an adjustable lap-strap, the lower leg dependent and the knee flexed to 90°

(Edwards et al., 1977b). The force exerted during a maximal, voluntary, isometric contraction of the quadriceps was transmitted to a strain gauge by an inextensible strap looped around the ankle. The value of each maximal voluntary contraction (MVC) was measured as the greatest force held for one second. The strength of the quadriceps was taken to be the best of three MVC trials. The validity and repeatability of this procedure has been discussed elsewhere (Young, 1980).

Table 1. Details of patients, including plasma biochemistry and isometric quadriceps strength (MVC). (Normal MVC values are those of Edwards et al., 1977b). Calcium values are corrected to specific gravity 1.027 or to albumin 46 g/l (Berry et al., 1973).

patient no.	cause of osteomalacia	race	sex	age yr	Ca mM/l	P mM/l	alkaline phosphatase iu/l KAu/100ml	MVC % of mean normal
1	nutritional	Ind	F	20	2.06	0.64	61	40
2	nutritional	Ind	F	23	1.64	1.45	83	67
3	nutritional	Ind	F	30	2.70	0.71	75	37
4	nutritional	Ind	F	39	1.79	0.88	91	33
5	nutritional	Ind	F	46	2.18	0.80	11	32
6	nutritional	Ind	F	60	2.34	0.65	42	14
7	gluten-sensitive enteropathy (GSE)	Cauc	F	47	2.18	0.64	400	35
8	GSE	Cauc	F	52	2.37	0.86	348	43
9	GSE	Cauc	F	60	2.39	1.44	188	22
10	GSE	Cauc	F	71	2.38	0.69	271	44
11	gastrectomy	Cauc	F	65	2.43	1.09	136	45
12	gastrectomy	Cauc	M	70	1.81	0.99	249*	28

*includes excessive liver phosphatase

Except for those cases where it was only possible to measure the strength of one quadriceps (eg, because of pain or because of risk of fracture through a Looser zone), the results reported were obtained for each patient's stronger leg. They were compared with the corresponding values obtained for the stronger leg of normal subjects of the same body weight (Edwards et al., 1977b).

Muscle Biopsy

Needle-biopsy samples were taken from the lateral quadriceps at the junction of the middle and distal thirds of the thigh (Edwards, Young & Wiles, 1980).

Microscopy

After preliminary orientation of the biopsy (Edwards et al., 1973), transverse sections were examined with standard histochemical techniques (Dubowitz & Brooke, 1973). Type I and type II fibres were identified by their reaction for myosin ATPase after pre-incubation at pH 9.4 and pH 4.3 (Hayashi & Freimau, 1966; Brooke & Kaiser, 1969).

The 'lesser fibre diameter' (Dubowitz & Brooke, 1973) was measured for 100 fibres of each type and a mean cross-sectional area was calculated for both fibre types in each biopsy, assuming a circular cross-section. Combining this with the frequency of each fibre type, it was possible to calculate an overall mean fibre area (MFA) for each biopsy.

Treatment

Four patients with nutritional osteomalacia (nos. 1, 2, 5 and 6) were treated with oral 1α -hydroxycholecalciferol (1-5 μ g/day). The other patients were treated with vitamin D₂ or D₃ in doses ranging from 500 to 10 000 units/day, according to their physician's opinion of their radiological and biochemical progress. Patients 7-10 were also put on a gluten-free diet.

Ethics

The patients gave their informed consent before participating in the studies described. The procedures were carried out with the approval of the Research Ethics Committee of the Royal Postgraduate Medical School and Hammersmith Hospital, and the Committee of Ethics of Clinical Investigations at University College Hospital.

RESULTS

Before Treatment

All 12 patients were weaker than normal subjects of the same body weight. The degree of weakness was unrelated to the cause of osteomalacia, the degree of hypocalcaemia or hypophosphataemia, and the serum parathormone level.

Needle-biopsy specimens from the patients' quadriceps muscles contained significantly less adenosine triphosphate (ATP) and phosphoryl creatine (PC) than found in normal muscle by Edwards et al. (1975), but there was no correlation between the severity of weakness and the degree of depletion of muscle ATP and PC.

Other investigations (not reported in detail here) suggested essentially normal electromechanical activation: (1) a normal electromyogram was obtained for each of the six patients tested, (2) only one patient's quadriceps showed an unduly rapid loss of force during prolonged tetanic stimulation, (3) in all 12 patients, quadriceps stimulation at low frequencies (3-20 Hz) and high frequencies (30-100 Hz) gave no indication of the selective, low-frequency, force loss associated with failure of excitation-contraction coupling (Edwards et al., 1977a).

None of the measurements of strength was considered to have been limited by pain. The patients' behaviour was carefully observed during the tests and they were also asked specifically about pain. Moreover,

treatment relieved pain more rapidly than it increased strength and, in the few patients in whom a temporary increase in pain followed the start of treatment, there was no corresponding decrease in MVC.

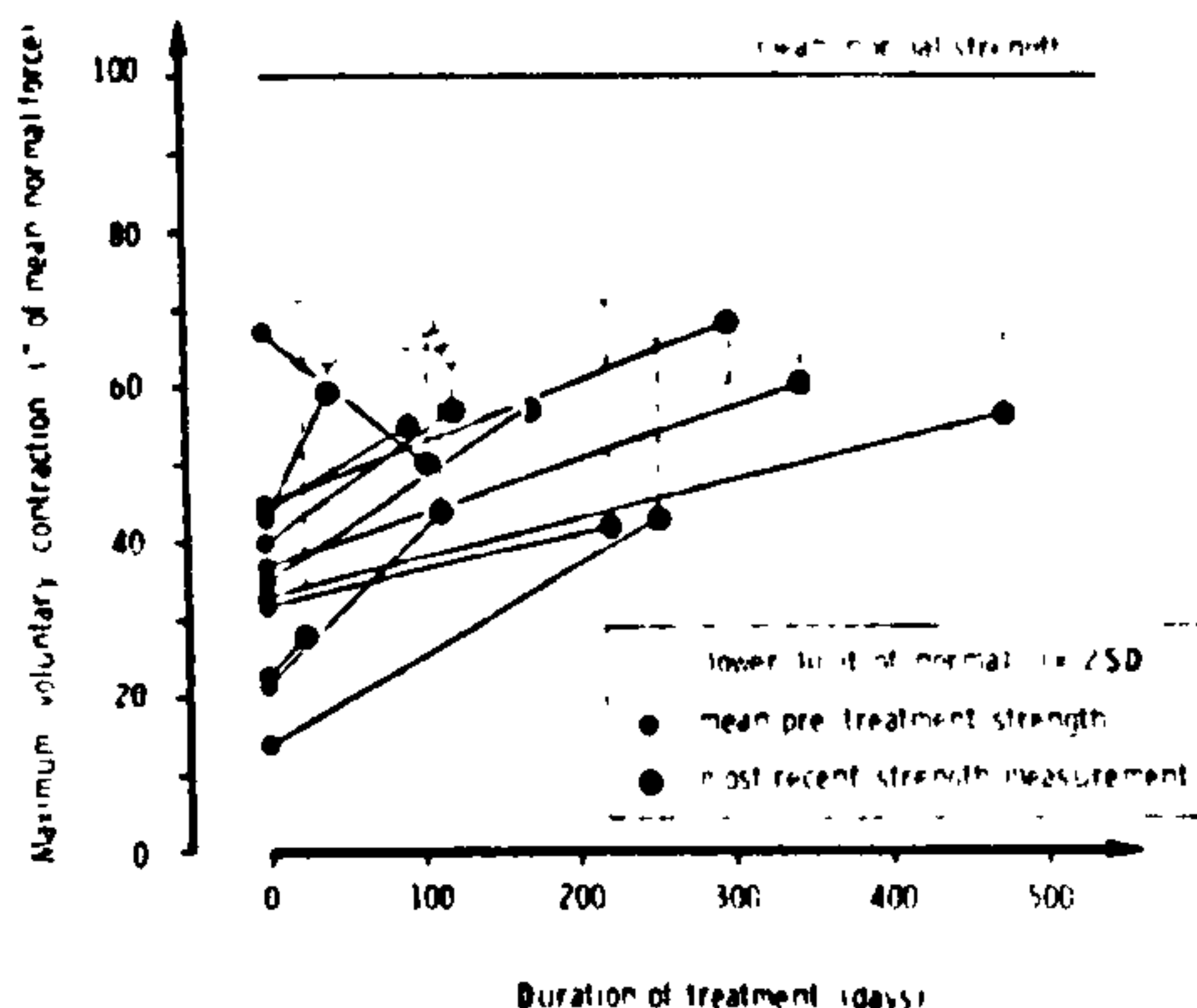


Fig. 1. Changes in quadriceps strength during treatment of 12 patients with osteomalacia. Each patient's strength measurements have been expressed as a percentage of the mean value for normal subjects of the same body weight

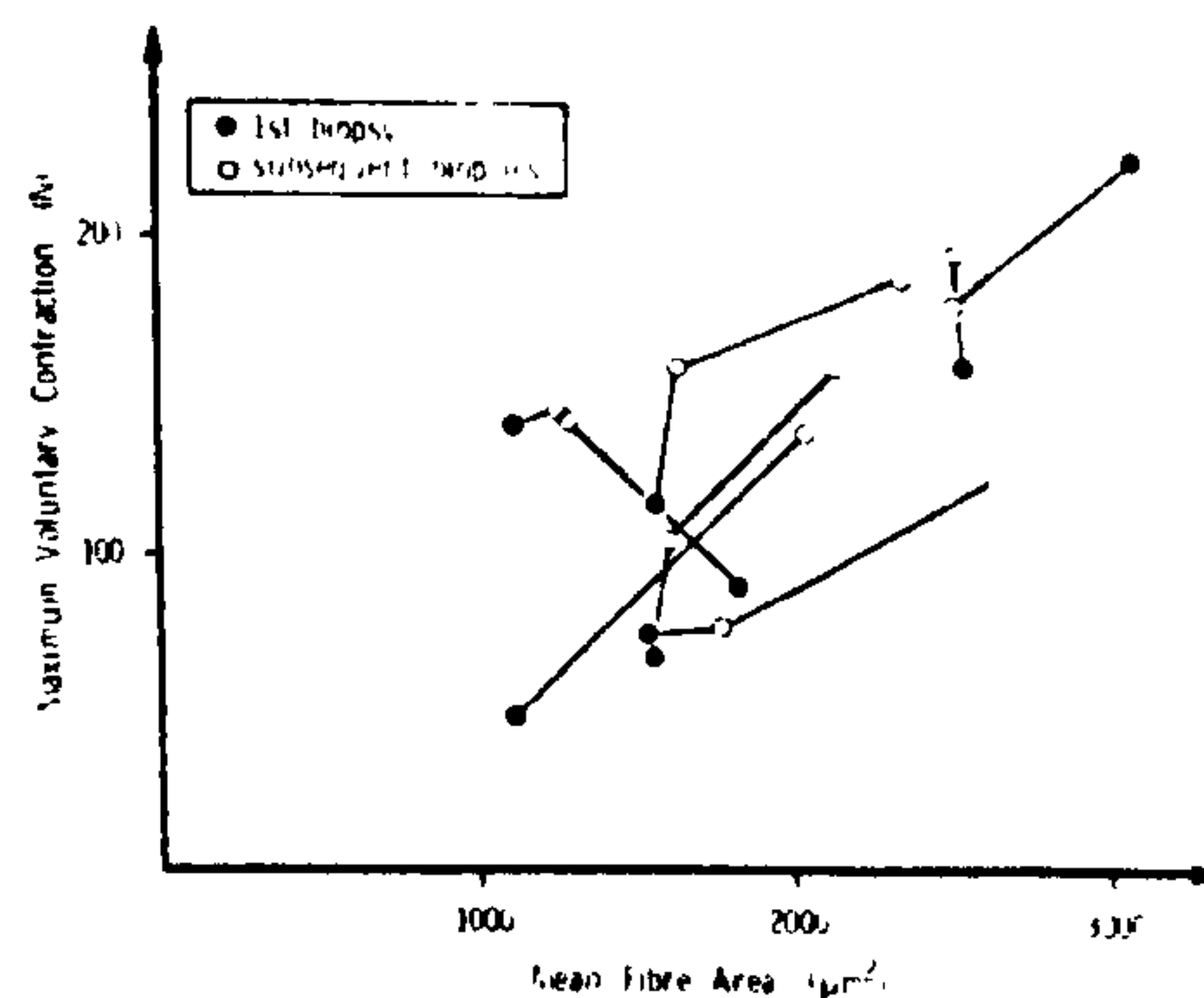


Fig. 2. Comparison of quadriceps strength and fibre size in eight patients receiving treatment for osteomalacia due to gluten-sensitive enteropathy (four patients) or nutritional deficiency (four patients)

During Treatment

The effects of treatment on quadriceps strength were observed in the patients followed for at least three months and in patients 8 and 12, followed for shorter periods (Fig. 1). During treatment, all but one of the patients gained strength. Patient no. 2 appeared to become weaker, but, since she was still hypocalcaemic after 105 days of treatment with 1 α -hydroxycholecalciferol, her compliance with treatment must be suspect.

The recovery of isometric quadriceps strength appeared to have a time course measured in weeks, months or even years and in only two cases did quadriceps strength return to within 2 s.d. of the mean normal value within the period of study (Fig. 1). The normal subjects were all Caucasian; but, in a group of 15 symptom-free young adult Indian women, all ten who had normal plasma values for calcium, phosphorus and alkaline phosphatase also had a quadriceps strength within the 'normal' range (\bar{x} = 87 per cent of mean 'normal' value) - Newham, Isenberg, Young, Wiles & Edwards (unpublished observations).

Changes in quadriceps strength during treatment were compared with the changes in muscle fibre size in eight patients (Fig. 2). Five patients underwent one follow-up study and three had two follow-up studies. The time intervals between first and last biopsy ranged from 27 to 300 days (mean = 115 days). Gains in strength were usually accompanied by increases in the mean cross-sectional area of the biopsied muscle fibres. The decrease in the strength of patient no. 2 was matched by a reduction in muscle fibre size.

The first and last biopsies from the same eight patients were compared with respect to the values of the ratio MFA II/MFA I (Table 2). The

degree of preferential type II fibre atrophy in some of the pre-treatment biopsies was dramatic. In only one case did the ratio MFA II/MFA I fail to increase with treatment - it remained constant in the patient whose initial value (0.97) was highest. The difference between the two sets of ratios was statistically significant ($P \approx 0.01$) (two-tailed, Wilcoxon two-sample test).

Table 2. Changes in the relative sizes and numbers of type I and type II fibres during treatment of osteomalacia

patient no.	MFA II/MFA I		frequency of type II fibres (%)		days of treatment
	first biopsy	last biopsy	first biopsy	last biopsy	
1	0.75	0.94	36	38	121
2	0.85	0.92	46	43	105
5	0.97	0.97	56	52	27
6	0.43	0.54	43	33	58
7	0.30	0.77	54	49	170
9	0.32	0.54	46	40	111
10	0.80	0.91	22	20	28
11	0.28	0.53	51	37	300

There was a reduction in the relative frequency of type II fibres from 44 to 39 per cent between the first and last biopsies from the same eight patients ($0.05 > P > 0.02$ in a paired t-test) - see Table 2.

DISCUSSION

Plasma values for creatine phospho inase (CPK) were consistently normal, in keeping with the absence of any biopsy evidence of extensive destruction of muscle cells. Even in patients who were so weak that they were bed-ridden, the reduction in size of the muscle fibres was the only obvious structural abnormality. This is in agreement with the findings of Dastur et al. (1975) for 19 patients (gluteus maximus) with osteomalacia and with those of Skaria et al. (1975) for 17 patients (ten quadriceps, seven gluteus maximus). A few other authors have also reported muscle fibre atrophy in individual patients with osteomalacia (Ekblom et al., 1964; Prineas, Mason & Henson, 1965; Singhal, 1966; Smith & Stern, 1967) but opinions differ as to the significance of muscle wasting as a cause for osteomalacic weakness - for example ... 'Uniform generalised wasting proportional to muscle weakness was observed in every affected patient' (Skaria et al., 1975) and '...muscle weakness which, as in many instances of osteomalacic myopathy, was often disproportionate to the muscle wasting' (Schott & Wills, 1976).

Dastur et al. (1975) 'confirmed' the presence of atrophy in their patients' biopsies by comparison with the size of fibres in biopsies

from normal subjects. However, the appropriateness of their controls is dubious. In the present study, each patient was used as her own control for evaluation of the severity of the muscle fibre atrophy: measurements of MFA made on biopsies taken during treatment with vitamin D were compared with those made on the pre-treatment biopsies from the same eight patients. Comparison of changes in MFA with the increase of quadriceps MVC during treatment made it possible to assess the probable contribution of a reduced mass of muscle (as indicated by MFA) to the patients' initial weakness.

It is possible that the measured rates of recovery of strength observed in this study may seem slow in comparison with subjective clinical experience. This is because even a small increase in strength may result in an enormous increase in the patient's functional ability. For example, it can mean the difference between success and failure in walking or in rising from the toilet unaided. Strength changes occur on a continuous scale whereas functional changes are quantal.

The uniformly slow return of strength argues against the existence of a significant defect in either electromechanical activation or muscle energy metabolism since such a defect might be expected to respond to treatment relatively quickly. Instead, the gains in strength were in proportion to the growth of muscle fibres and were correspondingly slow.

The effect of vitamin D on muscle growth in osteomalacia is slow to become apparent as a measurable increase in strength or fibre size. However the anabolic influence of vitamin D on D-deficient muscle probably starts promptly on the initiation of treatment. Birge & Haddad (1975) demonstrated an increased rate of uptake of tritiated leucine into the diaphragmatic muscle of D-deficient rats within seven hours of the oral administration of cholecalciferol. Augmentation of the diaphragmatic ATP content accompanied the increased amino acid uptake, and the authors postulated that '...ATP concentrations within the cell of the vitamin-deficient animal are reduced to levels which are rate-limiting in the synthesis of protein'. This could apply equally to the patients in this study - their weakness might be indirectly related to their reduced muscle ATP (and PC) content as a result of impaired muscle synthesis. However, Birge & Haddad (1975) also demonstrated that the ATP content of D-deficient rat muscle and its rate of uptake of leucine could be increased by the in-vitro application of 25-hydroxycholecalciferol, but not 1,25-dihydroxycholecalciferol. Yet in this study, three of the four patients treated with 1α -hydroxycholecalciferol showed a strength recovery similar to those treated with cholecalciferol.

It is not clear which metabolite of vitamin D is responsible for the effect on muscle strength. In the osteomalacia of chronic renal failure, 25-hydroxycholecalciferol improves muscle strength (Eastwood et al., 1977) suggesting that 1α -hydroxylation is unnecessary for this effect. Yet, muscle weakness is a cardinal feature of vitamin D-dependent rickets ('pseudomangel rachitis') - a condition due to specific failure of renal 1α -hydroxylation and readily treated with 1α -hydroxycholecalciferol.

It is also hard to explain why, even after prolonged treatment with vitamin D and despite a considerable recovery in muscle fibre cross-sectional area, the patients' quadriceps remained weaker than those of

normal subjects.

The pre-treatment muscle fibre hypotrophy was more pronounced in the type II fibres, as is the case in a variety of other disorders (Dubowitz & Brooke, 1973; Edström & Nordemar, 1974; Patten et al., 1974; Young et al., 1975). This would be an additional factor contributing to the initial weakness if type II fibres produce more force per unit cross-sectional area than type I fibres. The post-treatment biopsies, however, gave values for MFA and MFA II/MFA I very similar to those obtained from normal female subjects, ruling out persistence of preferential type II hypotrophy as an explanation for the persisting quadriceps weakness.

On the other hand, the frequency of type II fibres in the patients' biopsies was less after treatment (39 per cent) than before treatment (44 per cent). This change was only a small one and it seems unlikely that it would be adequate to explain the apparently large shortfall in recovery of strength. The reduction in type II fibre frequency becomes more pronounced, however, when the patients' biopsies are compared with those of nine normal Caucasian females (mean age 31, range 16-50 years) in which the mean frequency of type II fibres was 54 per cent (s.d. = 12.1). It is hard to understand why treatment seems to have exaggerated this difference, but this might explain the patients' persisting weakness, especially if it represents an actual loss of muscle fibres rather than an interconversion of fibre types.

It might be possible to establish whether there has been an actual loss of muscle fibres by comparing the ratio of MFA to whole muscle cross-sectional area with the same ratio in normal subjects. Quadriceps cross-sectional area can now be measured from computed axial tomograms (Häggmark, Jansson & Svane, 1978) or from ultrasonograms (Young et al., 1979). However it will still be difficult to find a sufficiently comparable control group. For example, matching for race is complicated by the fact that subclinical osteomalacia is probably common in 'normal' Indian women living in this country (Holmes et al., 1973) and vitamin D-deficiency in Caucasian Britons is usually secondary to some other disease which might have its own effect on muscle.

Not only are the mechanical properties of bone altered in vitamin D-deficiency, but the bones are subjected to altered muscle forces whose return to normal during treatment is slow and may be incomplete.

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Muscle relaxation rate, fibre-type composition and energy turnover in hyper- and hypo-thyroid patients

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Summary

1. Quadriceps strength, relaxation rate, fibre-type composition and energy-turnover rate during a submaximal contraction have been measured in hypo- and hyper-thyroid patients and compared with findings in normal subjects.
2. Six out of eight hypothyroid patients had normal strength whereas four out of five hyper-thyroid patients were weak.
3. Relaxation rate was decreased in all the hypothyroid patients but increased in only three out of five hyperthyroid patients.
4. In hypothyroidism there was a marked reduction in the percentage contributed by type II fibres to muscle cross-section, partly due to type II atrophy but also due to a decrease in the relative frequency of type II fibres. In hyperthyroidism both fibre types tended to atrophy.
5. The rate of ATP turnover during submaximal contraction held to fatigue was reduced in hypothyroidism. This was probably due to decreased ATP utilization rather than an impaired supply of energy-supplying substrates. In hyperthyroidism the rate of ATP turnover was increased.
6. Altered relaxation rate and ATP-turnover rate may be explained on the basis of changes in myosin ATPase activity with thyroid status.

Changes in muscle-fibre-type composition, as determined histochemically, could not *per se* account for the functional abnormalities.

Key words: energy turnover, human quadriceps, hyperthyroidism, hypothyroidism, muscle-fibre types, relaxation rate.

Introduction

Complaints by patients of muscle weakness and/or fatigue are common in both hypo- and hyperthyroidism, but the mechanisms by which altered thyroid status may cause these symptoms are poorly understood. A possible clue to these mechanisms may lie in the well-known clinical sign of slow muscular relaxation in hypothyroidism which was first thoroughly investigated by Lambert, Underdahl, Beckett & Mederos (1951). The slowed relaxation of the muscle in the Achilles tendon reflex appeared to be of muscular origin and could not be explained by delayed neural conduction or reduced intramuscular temperature. Concurrent electrical discharges were absent during relaxation, suggesting a possible change in the contractile mechanism itself. In their concluding paragraph, Lambert *et al.* (1951) suggested that slowed relaxation might be due to 'a decrease in the rate of energy liberation in the contractile process'.

A cause of slowed relaxation in hypothyroid myopathy which is compatible with Lambert's suggestion comes from the observation by McKeran, Slavin, Andrews, Ward & Mair (1975) of atrophy and reduction in frequency of type II

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fibres. Since the type II fibres have fast-twitch contractile characteristics (Close, 1972) and a high rate of energy utilization (Bolstad & Ersland, 1975) compared with type I, their decrease could result both in slowed relaxation and a reduction in energy liberation.

In recent years it has become possible to investigate the human quadriceps *in vivo* in terms of its contractile properties (Edwards, Young, Hosking & Jones, 1977) and, with the needle-biopsy technique, its structure (Edwards & Maun-der, 1977) and chemistry (Edwards, Jones, Maun-der & Batra, 1975c). Energy turnover may be assessed by measuring changes in muscle content of high-energy phosphate compounds and lactate during fatiguing contractions (Edwards, Hill & Jones 1975a; Edwards 1976, 1977). We have used these techniques to assess whether thyroid status influences the rate of energy liberation during muscular contraction and have tried to see whether the changes observed can be attributed to altered relaxation rate or fibre-type composition.

Methods

Patients and normal subjects

Eight patients with hypothyroidism (five male, three female, mean age 43 years) and five patients with hyperthyroidism (one male, four female, mean

age 43 years) were studied. All had a clear diagnosis based on compatible clinical features and unequivocal biochemical findings (Table 1) in the absence of any notable diseases. Patients were studied whether or not they had muscle symptoms or signs attributable to altered thyroid status. In hypothyroidism an elevated plasma creatine phosphokinase was found in all cases where it was measured (Table 1). None of the hyperthyroid group had a raised creatine phosphokinase but three hyperthyroid patients had values at or below the lower limit of normal.

All investigations were performed with the informed consent of the patients and the normal subjects. Procedures were approved by the Research Ethics Committee of the Royal Post-graduate Medical School and Hammersmith Hos-pital or the Committee of Ethics of Clinical Investigation at University College Hospital.

Procedures

Muscle function. The force of a maximum voluntary isometric quadriceps contraction was determined for each patient and compared with the mean value for normal subjects of the same body weight (Edwards *et al.*, 1977).

The maximum relaxation rate of the quadriceps muscle from a 30 Hz electrically stimulated isometric contraction (force = 20–40% of the force

TABLE 1. *Age, sex and biochemical indices of thyroid function and plasma creatine phosphokinase in hypo- and hyper-thyroid patients*

Normal plasma values: thyroxine (T4) 70–160 nmol/l; tri-iodothyronine (T3) 1.2–2.8 nmol/l; protein-bound iodine (PBI) 315.2–630.4 nmol/l; thyroid-stimulating hormone (TSH) 0–4 m-units/l; creatine phosphokinase 10–120 i.u./l. N.D., Not done.

Patient	Age (years)	Sex	Thyroid status		Plasma creatine phosphokinase (i.u./l)
			nmol/l	m-units/l	
Hypothyroid					
V.A.	50	M	PBI 86.7	N.D.	432
D.R.	22	M	T4 58.0	N.D.	660
V.C.	68	M	T4 13.0	TSH > 128	2030
R.K.	30	M	T4 < 10	TSH 128	670
J.K.	32	M	T4 < 10	TSH 58	425
Y.L.	57	F	T4 < 10	TSH 36	245
M.L.	34	F	T4 < 10	N.D.	N.D.
H.B.	53	F	PBI 118.2	N.D.	270
Hyperthyroid					
H.E.	32	M	T4 520.0	nmol/l T3 > 12.0	16
E.A.	31	F	T4 181.0	T3 4.5	8
O.B.	60	F	⁹⁹ Tc ^m uptake 7.6%* at 20 min	T3 6.9	4
S.F.	36	F	T4 484.0	T3 > 12.0	N.D.
A.L.	55	F	PBI 851.0	N.D.	10

* Technetium 99 thyroid uptake at 20 min; normal: 0.4–3.0%.

of a maximum voluntary contraction) was determined either from the differentiated force record or from the rate of force loss between 95% and 50% of the plateau after the last electrical stimulus (Wiles, Young, Jones & Edwards, 1979). In either case the maximum relaxation rate was taken as the mean of three determinations and expressed as the percentage of the plateau force lost/10 ms. The coefficient of variation for relaxation rate values obtained in this way had previously been found to be 3.6% (Wiles *et al.*, 1979).

Normal values for maximum relaxation rate were determined in 28 healthy subjects (23 male, five female, mean age 29.6 years), none of whom had any evidence of a muscle disorder. The majority of these subjects had participated in a previous study (Wiles *et al.*, 1979).

Fibre-type composition. Needle-biopsy specimens of muscle were obtained from the lateral part of the quadriceps (Edwards & Maunder, 1977) at approximately the junction of the middle and distal thirds of the thigh. Muscle fibres were classified as type I or type II on the basis of their histochemical reaction for myosin ATPase after preincubation at pH 9.4 (Dubowitz & Brooke, 1973). The 'lesser fibre diameter' (Dubowitz & Brooke, 1973) of 100 fibres of each type and the numerical proportions were measured. Assuming circular cross-section of diameter equal to the 'lesser fibre diameter', the contribution of each fibre type to overall muscle fibre cross-section was calculated. Normal values were ascertained in similarly prepared biopsy specimens from 30 male (mean age 32.5 years, range 18–51 years) and eight female subjects (mean age 31 years, range 15–50 years) who were all healthy but who did not regularly undergo any form of special physical training. For the purpose of relating the percentage type II fibre cross-sectional area to maximum relaxation rate (Fig. 2), a more restricted group of normal subjects was used, being the same 28 subjects as were used to provide normal relaxation rate values (see 'Muscle function', above). In this latter group fibre-type composition was determined in the same leg as relaxation rate.

The coefficient of variation for the fibre characteristics was obtained from duplicate samples in 30 quadriceps biopsies in both normal subjects and patients with muscular conditions but who did not have evidence of muscle replacement with fat or fibrous tissue. The results were: mean fibre diameter, 8%; frequency (%) type I or II, 17%; cross-sectional area (%) type I or II, 16%.

Muscle-energy stores and metabolites. The

muscle content of ATP, phosphorylcreatine, free creatine, lactate and glycogen was determined in needle-biopsy specimens of the quadriceps as previously described (Edwards *et al.*, 1975c). The coefficient of variation for the analyses was 5%. Normal values for these compounds were taken as those given by Edwards *et al.* (1975c).

In order to measure ATP turnover, specimens of muscle were obtained from closely adjacent sites before and at the end of submaximal (35–60% of maximum) voluntary isometric quadriceps contractions held to fatigue. A pneumatic cuff was inflated around the proximal thigh to ensure local ischaemia. The five hypothyroid patients so studied (four males, one female, mean age 46 years, range 22–68 years) made voluntary contractions with a mean force of 48% of their maximum and the single hyperthyroid patient 45% of her maximum. The results were compared with those in six normal male subjects (mean age 31 years, range 25–36 years) maintaining comparable voluntary contraction forces (mean 52.7% of their maximum) as reported by Edwards *et al.* (1975a).

The turnover of ATP was calculated from the change (Δ) in muscle concentration of ATP, phosphorylcreatine and lactate (Edwards *et al.*, 1975a) after standardization of these values to the total creatine (=free creatine + phosphorylcreatine) content of the biopsies (Edwards *et al.*, 1975c; Edwards, 1976b): namely

$$\text{ATP turnover} = 1.5\Delta\text{lactate} - \Delta\text{phosphorylcreatine} - \Delta\text{ATP}$$

The ATP-turnover rate was obtained by dividing the ATP turnover, expressed as μmol of ATP/mmol of total creatine, by the duration of the contraction in seconds. The units for ATP turnover rate were therefore μmol of ATP s^{-1} mmol^{-1} of total creatine.

Results

Muscle function. The strengths of maximum voluntary isometric contractions of quadriceps are shown in Fig. 1. Two of the hypothyroid patients and four of the hyperthyroid patients were weak when compared with normal subjects of the same body weight. The mean maximum relaxation rate in 28 healthy subjects was 11.3% force loss/10 ms, the normal range (mean \pm 2 SD) being 8.8–13.8% force loss/10 ms. These values are not significantly different from those calculated from results for a much larger group of 81 subjects, aged 12–63 years, previously published (Edwards *et al.*, 1977).

In all the hypothyroid patients maximum relaxation rate was significantly reduced with a group mean of 6.14% force loss/10 ms (Table 2). In the five hyperthyroid patients the maximum relaxation rate for the group was significantly ($P < 0.005$) elevated at 15.2% force loss/10 ms, but two subjects fell within the normal range.

Muscle-fibre composition. The diameters and frequencies of type I and II fibres together with the calculated percentage cross-sectional area of type II fibres are shown for individual patients in Table 2 and the means grouped according to sex are shown together with normal values in Table 3. Analysis by sex was necessary because normal males had significantly larger type I and II fibres ($P < 0.001$) and fewer type II fibres ($P < 0.05$) than females in this study. There was, however, no significant difference in the percentage cross-sectional area constituted by type II fibres in males and females.

Hypothyroid patients showed a marked reduction in the contribution of type II fibres to overall muscle fibre cross-section, which in males was attributable to reduced type II fibre size and possibly to reduced frequency of type II fibres, although this latter did not reach statistical significance. In females one patient (Y.L.) had gross type II atrophy, whereas in the group as a whole type I fibres were slightly larger than normal and there was a reduction in the frequency of type II fibres.

In the four hyperthyroid females, type I fibres were reduced in size. Type II fibres were slightly (but not significantly) reduced in size, as was their contribution to overall fibre cross-section. The single male hyperthyroid patient showed atrophy of both fibre types and a striking increase in type II fibre frequency.

The relationship between the muscle-fibre cross-section composed of type II fibres and maximum relaxation rate is shown in Fig. 2. As previously

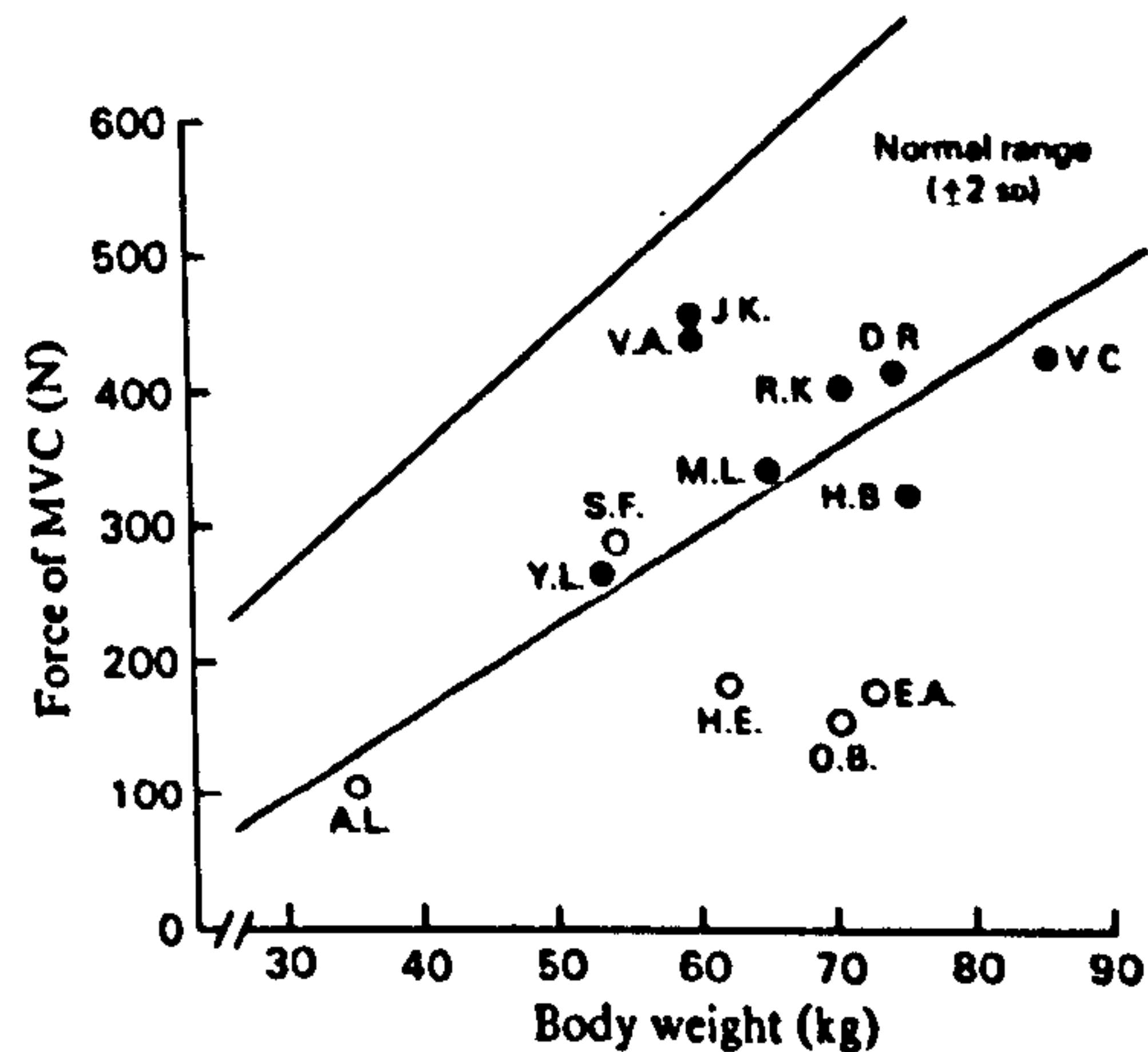


FIG. 1. Force (newtons) of maximum voluntary isometric contractions (MVC) of quadriceps in eight hypo- (●) and five hyper-thyroid (○) patients is compared with that of normal subjects matched for body weight. The straight lines enclose ± 2 SD quadriceps strength for 84 males (aged 6–63 years) and 61 females (aged 5–46 years) (Edwards *et al.*, 1977).

TABLE 2. Quadriceps relaxation rate and fibre-type composition in hypo- and hyper-thyroid patients

	Maximum relaxation rate (% force loss/10 ms)	Fibre diameter (μm)		Fibre frequency (%)		Contribution of type II fibres to overall fibre cross-sectional area (%)
		I	II	I	II	
Hypothyroid						
V.A.	4.5	69.2	32.0	76	24	5.8
D.R.	7.2	61.2	50.5	80	20	16.5
V.C.	5.8	96.1	68.1	49	51	34.5
R.K.	5.6	61.6	49.2	87	13	9.0
J.K.	7.0	67.4	53.7	53	47	36.5
Y.L.	4.1	64.4	Gross atrophy	87	13	5.0
M.L.	6.5	59.8	43.7	73	27	16.5
H.B.	8.3	63.2	54.4	63	37	27.8
Hyperthyroid						
H.E.	20.3	41.0	39.0	17	83	81.8
E.A.	13.8	44.3	43.6	58	42	41.1
O.B.	13.4	51.1	49.4	59	41	38.7
S.F.	19.7	42.7	42.9	34	66	66.0
A.L.	8.8	48.1	41.0	62	38	30.5

Normal range: 8.8–13.8 (mean \pm SD)

See Table 3 for normal values

TABLE 3. Grouped mean values for fibre-type characteristics in hypo- and hyper-thyroid patients compared with sex-matched normal controls

Fibre measurements are expressed as mean \pm SD.

	<i>n</i>	Age (years)	Fibre diameter (μ m)		Fibre frequency (%)		Contribution of type II fibres to overall cross-sectional area (%)
			I	II	I	II	
Male							
Normal	24	32.5	68.9 \pm 7.1	69.2 \pm 8.5	57.9 \pm 14.2	42.1 \pm 14.2	41.3 \pm 13.0
Hypothyroid	5	40.5	71.1 \pm 14.4	50.7 \pm 12.9*	69.0 \pm 17.0	31.0 \pm 17.0	20.5 \pm 14.3*
Hyperthyroid	1	32.0	41.0 —	39.0 —	17.0 —	81.8 —	81.8 —
Female							
Normal	8	31.0	54.9 \pm 4.9	50.1 \pm 6.5	46.3 \pm 12.3	56.7 \pm 12.3	49.2 \pm 14.0
Hypothyroid	3	48.0	62.5 \pm 2.4*	49.1 \pm 7.6†	74.3 \pm 12.1*	25.7 \pm 12.1*	16.4 \pm 11.4*
Hyperthyroid	4	45.5	46.5 \pm 3.8*	44.2 \pm 3.6	53.3 \pm 13.0	46.7 \pm 13.0	44.1 \pm 15.3

* Significant difference from normal value of same sex ($P < 0.05$, Student's *t*-test).

† Mean value from two patients only, the third having gross type II atrophy.

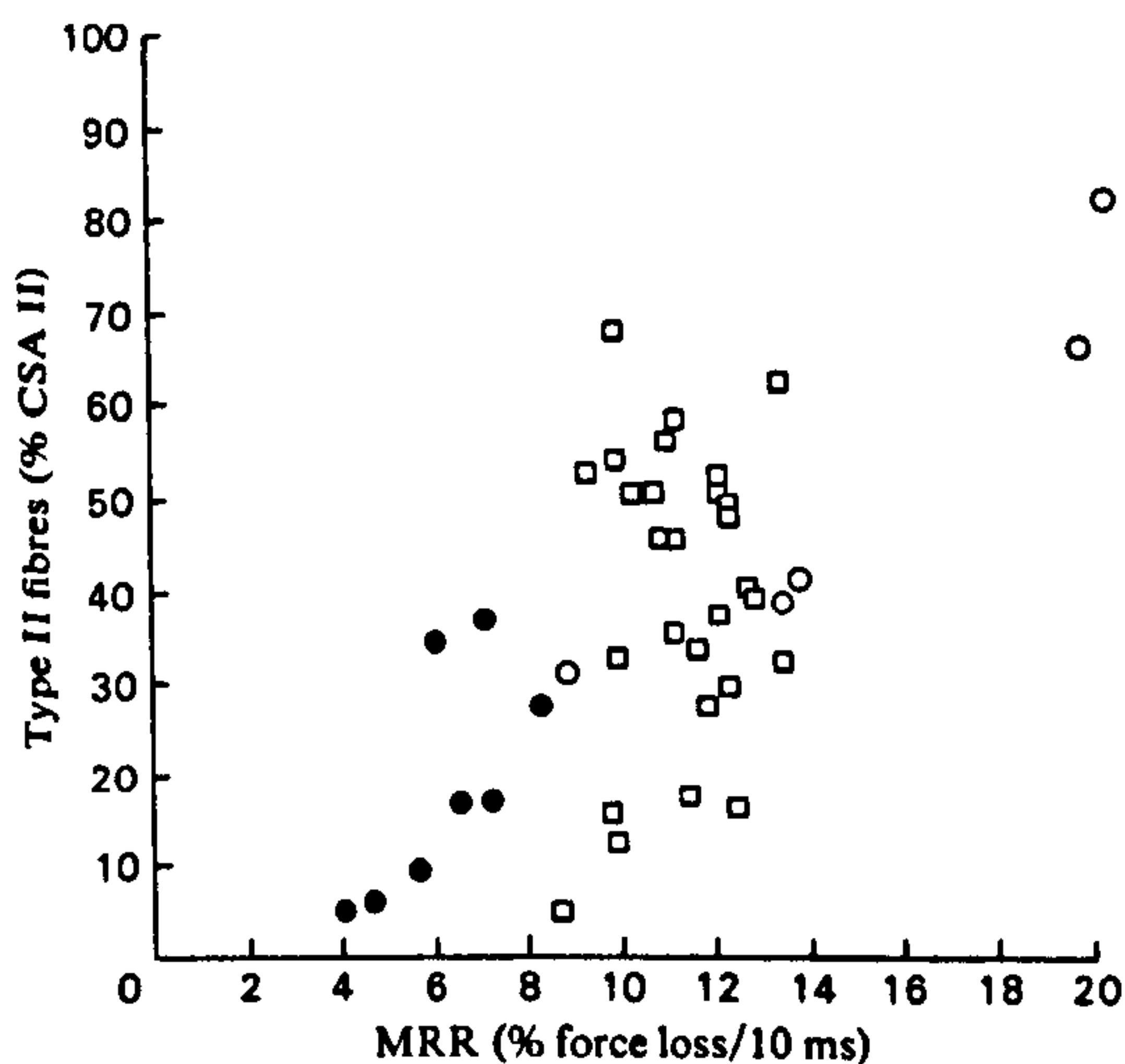


FIG. 2. Percentage contribution of type II muscle fibres to overall fibre cross-section area (CSA) is related to maximum relaxation rates (MRR) in eight hypo- (●) and five hyper-thyroid (○) patients and 28 normal subjects (□).

reported (Wiles *et al.*, 1979), there is no clear correlation in normal subjects. However, in the thyroid patients there was a tendency for the contribution of type II fibres to overall muscle-fibre cross-sectional area to increase with maximum relaxation rate.

Muscle energy stores and ATP turnover. The resting muscle concentrations of ATP, phosphorylcreatine, total creatine, lactate and glycogen in hypo- and hyper-thyroid patients are compared with those of normal patients in Table 4. ATP and phosphorylcreatine were reduced in both groups of patients and total creatine and glycogen were reduced in those with hyperthyroidism. There was

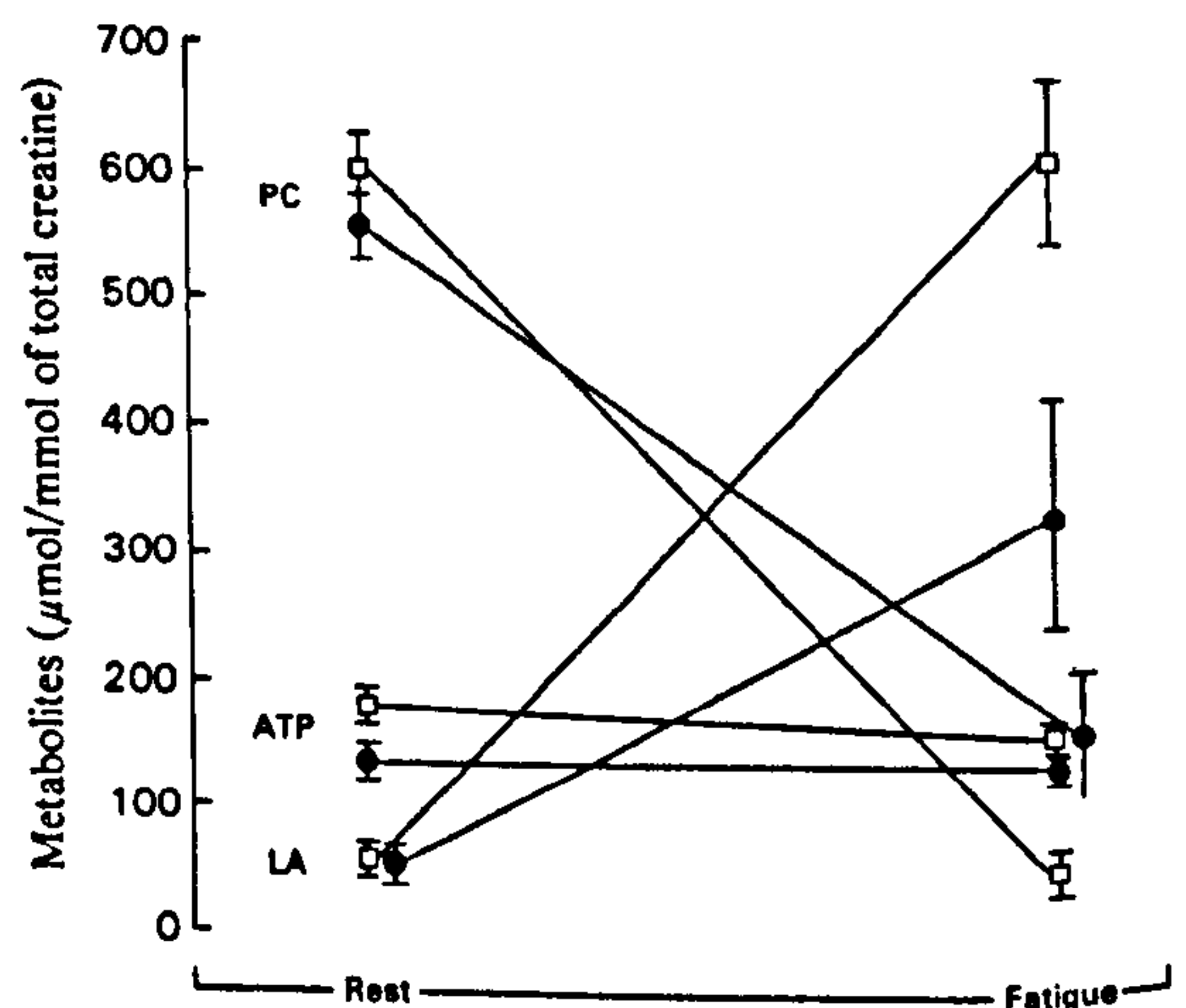


FIG. 3. Mean changes occurring in muscle content of ATP, phosphorylcreatine (PC) and lactate (LA) during submaximal voluntary isometric quadriceps contractions held to fatigue (defined as failure to sustain the target force) are shown. □, Mean changes in six normal subjects making a contraction at, on average, 52.7% of maximum force; ●, mean changes in five hypothyroid patients at an average of 48.0% of maximum force. Horizontal bars represent \pm 1 SEM. For clarity the values in a single hyperthyroid patient (S.F.) are given separately; ATP 170 \rightarrow 154, phosphorylcreatine 633 \rightarrow 126, lactate 35 \rightarrow 588 μ mol mmol of total creatine (rest to fatigue) during a contraction 45% of maximum.

no significant difference in resting lactate concentrations.

During submaximal isometric quadriceps contractions held to fatigue, phosphorylcreatine breakdown and lactate formation (and hence ATP turnover) were both reduced ($P < 0.05$) in the hypothyroid patients compared with normal subjects (Fig. 3). When the ATP turnover was related to the force \times time performed before fatigue, it was

apparent that on average the hypothyroid patients tended to sustain comparable levels of force for longer (i.e. greater force \times time) than the normal subjects, but at reduced energetic cost (Fig. 4). Thus the ATP-turnover rate was reduced in all hypothyroid patients at $6.7 \pm 1.5 \mu\text{mol}$ of ATP $\text{s}^{-1} \text{mmol}^{-1}$ of total creatine (mean \pm SEM) compared with that in normal subjects of $26.0 \pm 3.4 \mu\text{mol}$ of ATP $\text{s}^{-1} \text{mmol}^{-1}$ of total creatine (mean \pm SEM). The proportion of ATP turnover derived from anaerobic glycolysis ($1.5 \times \Delta\text{lactate}/\text{total ATP turnover}$) was $58.7 \pm 2.9\%$ (mean \pm SEM) in normal subjects and $47.1 \pm 7.2\%$ (mean \pm SEM) in hypothyroid subjects, but the difference did not reach statistical significance at the 5% level.

The single hyperthyroid patient (S.F.) so studied maintained the target force for only a short time compared with normals (i.e. a low force \times time) for a high ATP turnover (Fig. 4). The ATP-turnover rate in this patient was strikingly elevated at $55.2 \mu\text{mol}$ of ATP $\text{s}^{-1} \text{mmol}^{-1}$ of total creatine. Thyroid status therefore had a marked effect on the rate of ATP hydrolysis during ischaemic muscle contractions.

Changes during treatment. Detailed contractility studies were performed on patients J.K. (hypothyroid) and H.E. (hyperthyroid) during treatment. Maximum relaxation rate returned to normal in both cases. It was observed that, during

quadriceps stimulation at 10 Hz, the degree of tetanic fusion altered correspondingly (Fig. 5), falling in J.K. and increasing in H.E. In the hypothyroid patient (J.K.), the reduction in tetanic fusion with treatment, resulting in increased internal work, was expected to increase energy-turn-

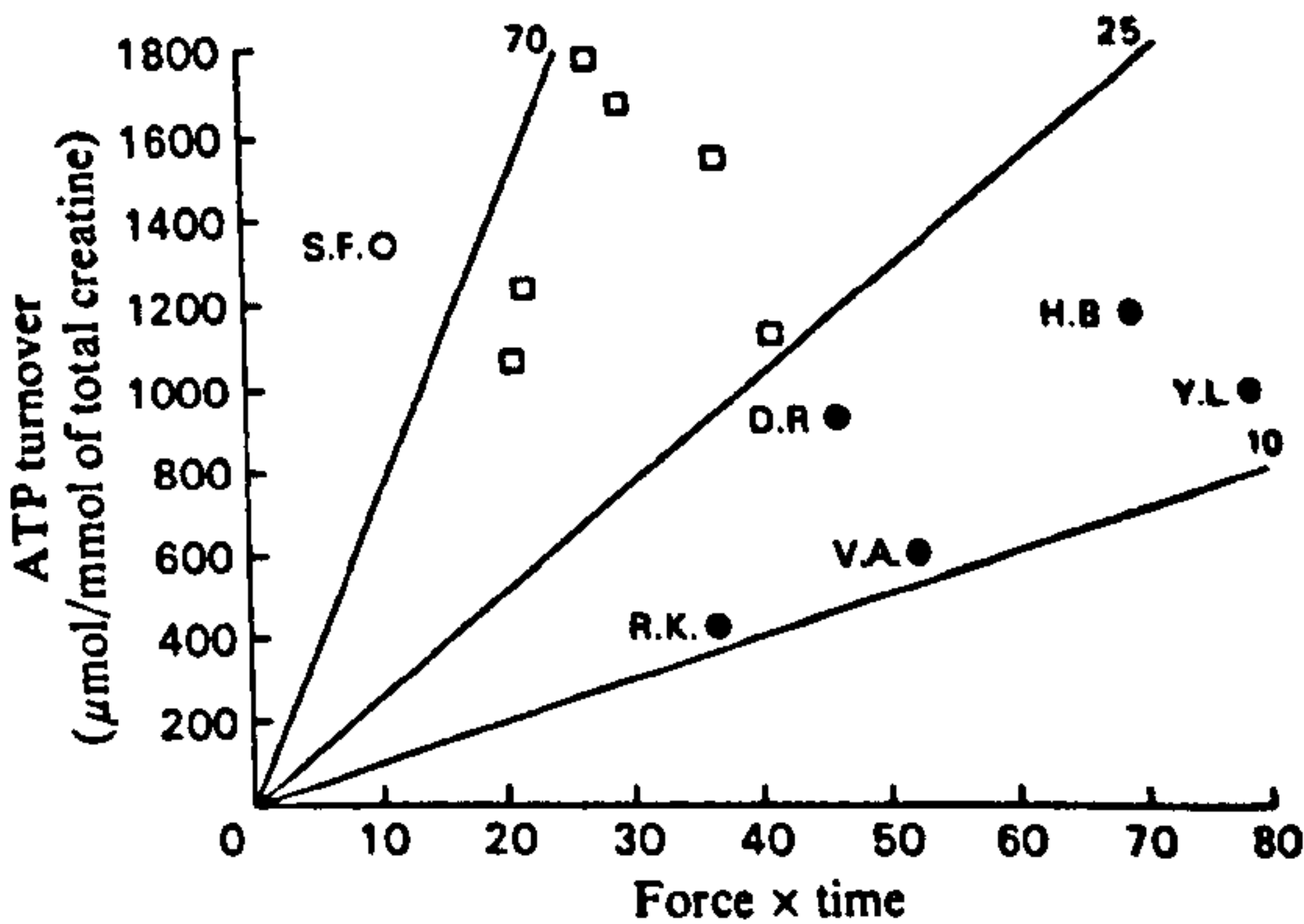


FIG. 4. Actual ATP turnover is related to the force \times time [calculated as the (actual force sustained/maximum force) \times length of contraction in seconds] sustained before fatigue for hypo- (●) and hyper-thyroid (○) patients and normal subjects (□). The straight-line gradients are isopleths whose values 70, 25 and 10 represent theoretical maximum ATP-turnover rates ($\mu\text{mol s}^{-1} \text{mmol}^{-1}$ of total creatine) and imply increasing economy of energy substrate utilization. The actual ATP-turnover rates for each group are based on ATP turnover during submaximal contractions (see the text).

TABLE 4. Resting quadriceps energy stores and metabolites ($\mu\text{mol g dry weight}$) in hypo- and hyperthyroid patients compared with normal

	ATP	Phosphorylcreatine	Total creatine	Lactate	Glycogen
Hypothyroid					
V.A.	23.0	91.3	142.3	1.7	309
D.R.	19.2	80.4	135.0	13.7	474
R.K.	18.2	70.5	122.8	2.3	413
J.K.	18.9	87.1	135.9	3.6	309
Y.L.	14.7	43.2	84.0	7.7	281
M.L.	16.8	40.2	106.8	9.3	224
H.B.	14.0	53.0	107.0	2.7	282
Mean	17.8*	66.5*	119.1	5.9	319
SD	3.0	21.1	20.9	4.5	90
Hyperthyroid					
H.E.	19.5	41.4	76.0	11.7	178
E.A.	17.5	50.1	98.0	4.4	201
O.B.	16.0	60.3	111.3	9.4	170
S.F.	23.3	86.7	137.0	4.8	207
A.L.	12.7	39.4	130.7	5.1	138
Mean	17.8*	55.6*	110.6*	7.1	179*
SD	4.0	19.3	24.8	3.3	28
Normal values					
(mean \pm SD, $n = 114$)	23.6 \pm 2.56	76.0 \pm 7.90	125.3 \pm 17.00	5.4 \pm 3.57	334 \pm 72.8 ($n = 62$)

* Significant difference: $P < 0.05$. Patient V.C. has been excluded from this analysis because of a technically unsatisfactory specimen.

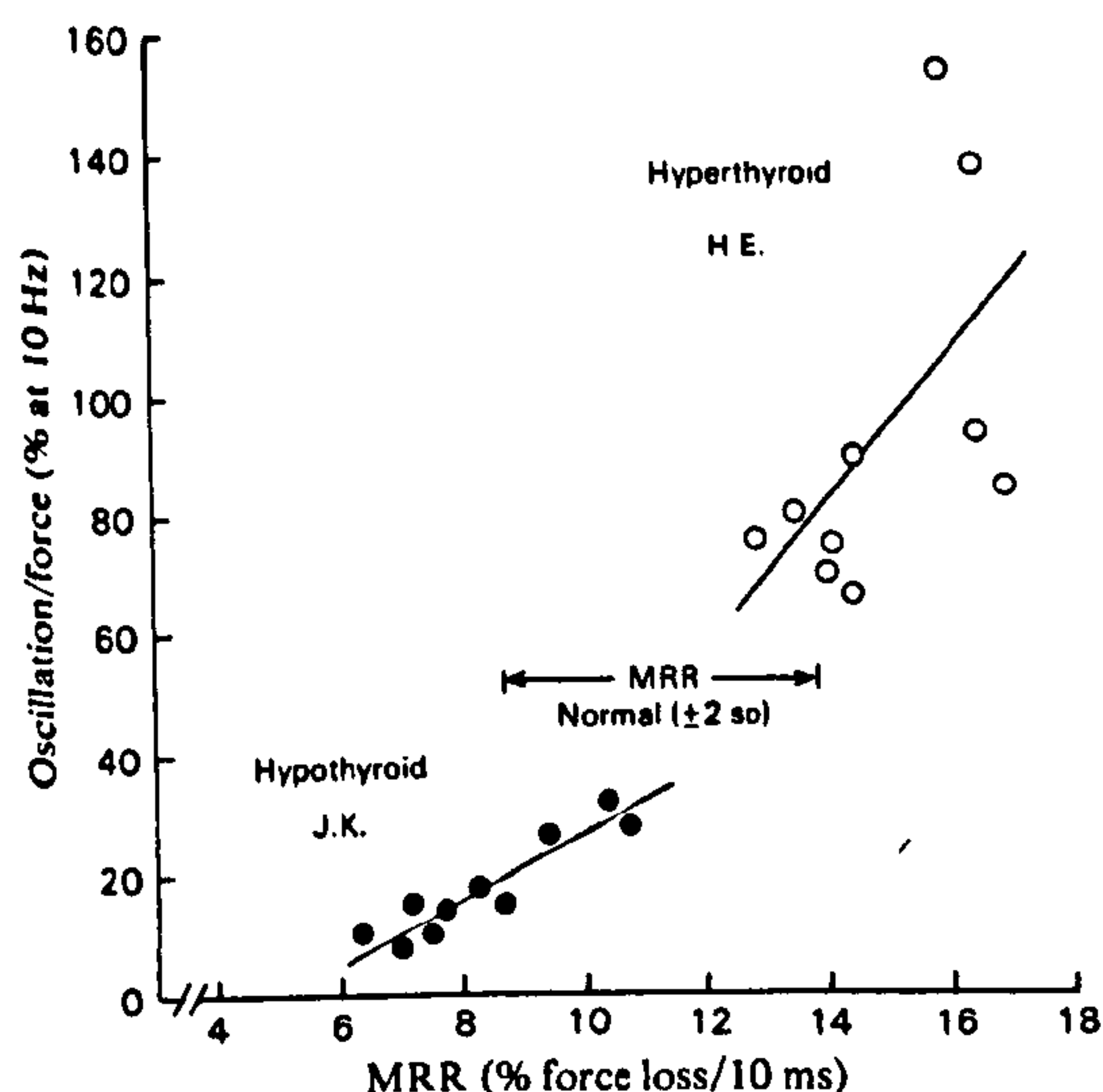


FIG. 5. Relationship between degree of tetanic fusion [mechanical oscillation/mean force (%)] during a 10 Hz tetanus and relaxation rate, is illustrated with reference to a hypo- (J.K.) and a hyperthyroid (H.E.) patient. In each case these contractility indices were obtained during the course of treatment (over a 3 months period for J.K., over a 10 months period for H.E.) as relaxation rate returned into the normal range. The regression lines have correlation coefficients of 0.9 (J.K.) and 0.6 (H.E.).

over rate. This patient had, in addition to studies before treatment, biopsies performed to measure ATP-turnover rate and fibre-type composition after the return to euthyroid status, at which time the maximum relaxation rate was well within the normal range. The ATP-turnover rate during fatiguing maximum contractions was found to have increased threefold (Table 5). Fibre-type composition, however, as judged from duplicate samples on each occasion remained essentially unchanged (Table 5). Therefore changes in contractility and energy-turnover rate had occurred in the absence of any change in fibre-type composition as determined histochemically.

Similar studies have been performed before treatment in only one hyperthyroid subject (S.F.), principally because of the high degree of anxiety which such patients experience. The investigations were more easily performed in, and better tolerated by, the hypothyroid patients.

Discussion

Our results indicated that the quadriceps muscle of hypothyroid patients, although not notably weak, was slow in relaxing and was more economical than normal in the utilization of its energy substrates. The quadriceps of hyperthyroid patients

TABLE 5. Changes in quadriceps after treatment of hypothyroidism

Patient J.K.; age 32 years.

	Before treatment	After 90 days' treatment (euthyroid)
Strength (% of predicted normal)	104	91
Maximum relaxation rate (% force loss/10 ms)	7.0	10.3
Oscillation at 10 Hz (%)	7.9	31.6
mean force		
ATP-turnover rate ($\mu\text{mol of ATP s}^{-1} \text{ mmol}^{-1}$ of total creatine)	8.4	26.6
Contribution of type II fibres to overall fibre cross-section (%)	37	42

tended to be weak, had a normal or fast relaxation rate, and had an increased rate of energy consumption. Both groups had change in fibre-type composition.

Control data

The results for patients in this study have been compared with data from control populations of normal subjects obtained retrospectively. In comparing quadriceps strength, patients were matched only for body weight although they were within the age span of the controls (Edwards *et al.*, 1977). However, since on average hypothyroid patients are likely to be more obese and less habitually active than controls (and vice versa for hyperthyroid patients), the results tend to emphasize the relatively normal strength of the hypothyroid group and the weakness of the hyperthyroid group. Relaxation rate data were not matched against those from controls of similar age or sex, but analysis of the relaxation rates of 82 normal subjects by age and sex (the normal subjects in Edwards *et al.*, 1977) suggest that there is no systematic change due to sex or age over 12 years. As regards ATP-turnover rate, assuming matched relative contraction forces (see the Methods section), there is no reason *a priori* for assuming that this should differ by virtue of age or sex. Indeed the fact that hypothyroid patients performed on average more force \times time before fatigue is the more remarkable in view of their greater age and likely lower habitual activity than the younger male controls. It is possible that a proportion of the fibre atrophy seen in patients was a result of their greater average age, although the results of McKeran *et al.* (1975) make this unlikely as the sole explanation.

Muscle contractility and fibre-type composition

Relaxation rate of the quadriceps muscle was slowed in all hypothyroid patients but was significantly increased in only three out of five hyperthyroid patients. These findings are consistent with those of Lambert *et al.* (1951), who found ankle-jerk time to be an insensitive indicator of excess thyroid activity (75% of their hyperthyroid patients falling within the normal range).

We found that thyroid status appeared to have influenced fibre-type composition in the quadriceps. In hypothyroidism there was a reduction in the contribution of type II fibres to muscle-fibre cross-section attributable mainly to a reduced type II fibre diameter in males, but to a reduced frequency of type II fibres in females, although this also tended to occur in males. Whether the type II fibres atrophy and eventually disappear cannot be stated with certainty, but the fact that the patients with the lowest type II fibre counts (Table 2) tended to have normal quadriceps strength (Fig. 1), suggests that large numbers of type II fibres have not been lost but may have been converted into type I. In the hyperthyroid patients one male, with the fastest relaxation rate, had marked type II predominance of fibres with atrophy of both types, and there was fibre atrophy, notably of type I fibres, in the females.

Changes in fibre size and fibre-type composition with altered thyroid status have previously been described in both animal (Ianuzzo, Patel, Chen, O'Brien & Williams, 1977) and human studies (Ramsay, 1966; McKeran *et al.*, 1975). This prompts the question as to whether changes in relaxation rate might be related to the altered proportions of the type I (slow-twitch) and type II (fast-twitch) fibres. There are two lines of evidence which suggest that this is not the case. If, first, the patients are considered as a single group with altered thyroid function (Fig. 2), a highly significant regression ($y = 3.80x - 4.98$, $r = 0.91$, $P < 0.001$) relates percentage fibre cross-section contributed by type II fibres to maximum relaxation rate (model II regression: Sokal & Rohlf, 1969). When $y = 0$ (that is, the muscle comprises only type I fibres) the maximum relaxation rate is predicted to be 1.31 and at $y = 100$ (that is all type II fibres) it is 27.6. Therefore, in this group of patients, type I and II fibres appear to differ by a factor of 21 in their relaxation rates. This is in contrast with the twofold range found in normal subjects (Wiles *et al.*, 1979). Secondly, in patient J.K. (Table 5) duplicate samples of muscle (to reduce sampling

error) taken before the start of treatment and after relaxation rate, and ATP-turnover rate had returned to normal, showed no changes in fibre-type composition. This suggests that relaxation rate and ATP turnover may change independently or with a different time course from those characteristics reflected by fibre typing based on histochemical demonstration of myosin ATPase.

Relaxation rate might be influenced by thyroid status as a result of changed rates of calcium ion binding by the sarcoplasmic reticulum, since this has been thought to be rate limiting in normal relaxation (Sandow, 1965). It has been estimated that ATP turnover attributable to the processes of activation (calcium release and re-accumulation by sarcoplasmic reticulum) probably accounts for no more than 30% of the total (Homsher, Mommaerts, Ricchiuti & Wallner, 1972). In our hypothyroid patients, ATP-turnover rate was three to four times less than in normal subjects. A study of cat heart papillary muscle (Skelton, Pool, Seagren & Braunwald, 1971) showed a similar degree of change in hypothyroid animals. Hence even a very large reduction in calcium-uptake rate would not account for more than a 20% decrease in ATP-turnover rate. Furthermore, although isolated sarcotubular vesicles from hypo- and hyper-thyroid rat skeletal muscle showed a reduction and increase respectively in calcium ion-uptake rate (Peter, Worsfold & Stempel, 1970) these authors found no increase in maximal transport rates in vesicles from human hyperthyroid muscle, and uptake has been found to be reduced in hyperthyroidism in cat skeletal muscle (Ash, Besch, Harigaya & Zaimis, 1972) and dog heart muscle (Conway, Heazlitt, Fowler, Gabel & Green, 1976).

Relaxation rate and energy turnover

It has been argued (Edwards, Hill & Jones, 1975b) that rates of relaxation reflect actomyosin cross-bridge turnover and hence ATP-turnover rates. In the present study, the low rates of ATP turnover in hypothyroid patients were always associated with a low relaxation rate. The single hyperthyroid patient so studied (S.F.) had a high relaxation rate and ATP-turnover rate. In respect of this patient, it would be of considerable interest to establish whether or not hyperthyroid patients with normal relaxation had high ATP-turnover rates, since previous experience of the relationship between relaxation and energy liberation suggests that a high ATP-turnover rate would be seen only when the relaxation was fast (Edwards *et al.*,

1975a, b). In the hypothyroid patients, ATP-turnover rate could be reduced either because of a reduced supply of energy substrates (ATP, phosphorylcreatine, lactate) or because of reduced utilization by myosin ATPase.

Resting concentrations of ATP and phosphorylcreatine were reduced in both hypo- and hyperthyroid patients but similar degrees of ATP and phosphorylcreatine depletion are seen in a wide range of muscular disorders (Edwards, 1976b). If they were limiting ATP-turnover rate, one would expect to find a greater than normal reduction in muscle concentrations at fatigue, whereas phosphorylcreatine was broken down to a lesser extent than normal in the hypothyroid patients (Fig. 3). It also seems unlikely that these stores would be limiting in the short contractions necessary to test muscle strength. McDaniel, Pittman, Oh & Di Mauro (1977) found, as we do (Fig. 3), reduced lactate production by muscle during ischaemic contractions in hypothyroid patients and interpreted this as indicating a block in glycogenolysis. If the glycolytic flux were reduced with a consequent reduction in the ATP supplied from this source one would expect that hypothyroid patients would either make contractions of reduced force \times time before fatigue or would have depleted, more than is usually found in such contractions, their short-term stores of ATP and phosphorylcreatine. The reverse was the case, since they performed on average more force \times time with less decrease in phosphorylcreatine at fatigue than normal (Fig. 4). The proportion of ATP turnover derived from anaerobic glycolysis was normal in hypothyroid patients and there appears therefore to be no reason to suggest a limitation in glycolytic flux. The reduced lactate production reflects the reduced ATP utilization for a given force \times time, i.e. in hypothyroidism there is a metabolic economy in ATP hydrolysis.

The reduced rate of utilization of ATP and low relaxation rate (and possibly the reverse situations in hyperthyroidism) could be explained by a change in character of the myosin ATPase. In the skeletal muscle of hypothyroid rabbits and rats (soleus) myosin ATPase activity is reduced (Jacobson, Humphrey & Grey, 1972; Patel, Chen, Gollnick & Ianuzzo, 1978). Conversely myosin ATPase activity is increased in hyperthyroid rat soleus (Patel *et al.*, 1978) as are rates of actomyosin syneresis in cat muscle (Ash *et al.*, 1972).

Whatever its cause a change in relaxation rate influences the degree of mechanical fusion during unfused tetani (Fig. 5) and consequently the fre-

quency-force relationship (Edwards *et al.*, 1977). This accounts for changes in fusion frequency with thyroid status found in animal studies (Gold, Spann & Braunwald, 1970; Fitts, Brooke, Winder & Holloszy, 1978). Slowing of relaxation rate in hypothyroidism, by reducing the internal work against series elastic elements and increasing the force produced at a given firing frequency should reduce the ATP turnover requirement/unit force, i.e. improve energy economy, the converse being expected in hyperthyroidism.

Muscle strength

Quadriceps weakness was a feature of four out of five of the hyperthyroid patients (Fig. 1). Ramsay (1966) thought that clinically there was a dissociation between the degree of muscle atrophy and muscle strength in hyperthyroid myopathy and suggested that the energy supply for contraction was impaired. The ATP-turnover rate in the one hyperthyroid patient so tested in this study was twice normal, implying that this is unlikely to be limiting. It remains to be established whether muscle weakness in hyperthyroidism can be attributed solely to muscle atrophy (i.e. a reduction in the total muscle-fibre cross-section) due, for instance, to increased protein degradation (De Martino & Goldberg, 1978), or whether the intrinsic contractile strength of the muscle is reduced per unit cross-sectional area. Studies in hyperthyroid rat muscle (Gold *et al.*, 1970; Fitts *et al.*, 1978) have shown normal force production/unit cross-sectional area. However, such findings, based on measurement of force produced during a fused tetanus, disguise the possibility that at lower (physiological) stimulation frequencies, e.g. 10–30 Hz in man, the increased relaxation rate in hyperthyroidism may result in lower force production than normal, i.e. there is a shift in the frequency-force relationship to the right. Correction of relaxation rate with control of the hyperthyroid state would therefore enhance mean force production by motor units for a given (low) firing rate in voluntary contractions and might offer an explanation for the rapid clinical improvement on treatment noted by Ramsay (1966).

Similar considerations in hypothyroidism suggest that slowed relaxation should tend to offset any reduction in intrinsic muscle-fibre strength (Gold *et al.* (1970) at low or intermediate firing rates. Furthermore, since a given submaximal force should then be sustained by a lower average firing rate than normal, such hypothyroid patients might

be predicted to maintain such a force for a longer period before fatigue. In fact hypothyroid patients did maintain force for greater than normal periods on average during the energy-turnover measurements (Fig. 4).

These physiological arguments apart, reduced maximum strength in hyperthyroidism seems likely to be determined by the degree of muscle-fibre atrophy which has occurred, and hence depends in part on the duration and severity of the disorder.

Conclusion

We have confirmed that energy liberation is reduced in association with relaxation rate in hypothyroidism and have found that the reverse may occur in hyperthyroidism. Reduced energy liberation appears to be due to a reduction in ATP utilization, possibly explained by altering myosin ATPase activity. These findings imply that hypothyroid muscle can function more economically than normal and as a result is potentially less fatiguable, the converse being the case in hyperthyroidism. The combination of increased relaxation rate and fibre atrophy in hyperthyroidism could explain why weakness tends to be a prominent complaint in these patients.

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Functional and structural changes after disuse of human muscle

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Summary

1. Seven patients who had suffered unilateral leg fracture were studied after removal of immobilizing plaster casts.

2. Leg volume measured anthropometrically was reduced by 12% in the injured leg (5.68 ± 1.05 litres) compared with the uninjured (6.43 ± 0.87 litres). Associated with this loss was a similar reduction in the net maximum oxygen uptake achieved in one-leg cycling, from 1.89 ± 0.21 l/min in the uninjured leg to 1.57 ± 0.18 l/min in the injured.

3. Measured by a percutaneous needle biopsy technique, a reduction of 42% was found in the cross-sectional area of the muscle fibres sampled from the vastus lateralis of the injured compared with the uninjured leg.

4. Staining for myosin adenosine triphosphatase activity showed that both type I and II fibres were affected, being reduced respectively from 3410 to 1840 μm^2 and from 3810 to 2390 μm^2 cross-sectional area.

5. Possible reasons and implications are discussed for the discrepancy between the magnitude of the difference observed in the gross measurement of leg function (maximum oxygen uptake) and structure (leg volume) as compared with the cellular level (cross-sectional fibre area).

Key words: atrophy, muscle, oxygen uptake.

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Introduction

Atrophy of the affected limb and loss of muscle power follows bone fracture and subsequent immobilization. Years of experience have enabled the rehabilitation professions to develop empirical programmes to reverse these changes. However, the efficacy of such programmes may be further improved if we can increase our understanding of the atrophic response to disuse in human muscle.

Recent studies showed that 15 weeks immobilization in a long-leg plaster cast after fracture reduced the fat-free volume of the affected leg by 12%, which was accompanied by a similar fall in the maximum oxygen uptake ($\dot{V}\text{O}_{2\text{max}}$) achieved with one-leg pedalling (Davies & Sargeant, 1975a,b). However, it was not known how far these changes in gross structure and function were reflected at a cellular level within the affected muscles.

Since the work of pedalling is performed mainly by the leg extensors (A. J. Sargeant & C. T. M. Davies, unpublished work) needle biopsy was used (Edwards, Maunder, Lewis & Pearse, 1973) to study fibre atrophy in the quadriceps femoris muscle and to compare this with measurements of the gross leg volume and maximal oxygen uptake of patients recovering from unilateral leg fracture.

Methods

The patients, seven otherwise healthy young servicemen, had tibia and fibula fractures of one leg, treated by immobilization of the injured

limb in a long leg plaster, for a mean period of 131 days (range 53–213 days). Our measurements were made from 0 to 42 days after effective mobilization (calculated from the time the patients were assessed as being weight-bearing on the affected leg after removal of the plaster). We also studied eight healthy male subjects who had no recent leg injuries. All subjects, both normal and patients, were volunteers and gave their free informed consent.

The investigation and the techniques involved were approved by the Research and Ethics Committee of the Royal Post-graduate Medical School, Hammersmith Hospital.

The volume of each leg (muscle plus bone) was determined anthropometrically (Davies & Sargeant, 1975c). The estimation of the maximum oxygen uptake ($\dot{V}O_{2max}$) in one-leg cycling has been described in detail (Davies & Sargeant, 1975a). The subjects pedalled a fixed-wheel stationary bicycle ergometer in a continuous progressive test spanning the subjects' working capacity from zero up to maximum in four or five work loads each lasting 5 min. $\dot{V}O_{2max}$ was measured by the Douglas bag technique over the last minute of the final 3 min of work as the subject was encouraged to pedal at maximal effort. If this could be sustained the load was increased and $\dot{V}O_2$ measurements were repeated. Owing to the difficulty of obtaining maximal measurements in one-leg exercise which meet the 'plateau' criterion developed for two-leg exercise, it was sometimes necessary to take duplicate measurements on subsequent days. $\dot{V}O_{2max}$ was expressed after subtracting the $\dot{V}O_2$ during pedalling at a constant speed against zero load (Hill, 1965; Whipp & Wasserman, 1969).

Needle biopsies were taken from the lateral part of the quadriceps muscle at the junction of the distal and middle thirds of the thigh, with a Bergström needle inserted through a 4 mm skin incision (Bergström 1962, 1975; Edwards, 1971). The biopsy was prepared as previously described (Edwards *et al.*, 1973). Transverse 10 μ m frozen sections were stained for myosin adenosine triphosphatase activity at pH 9.4 and after preincubation at pH 4.3 (Hayashi & Friemau, 1966; Brooke & Kaiser, 1969) to identify type I and type II muscle fibres. The relative frequency of the two fibre types was counted in each biopsy and the mean cross-sectional area of each type of fibre calculated

from measurement of the 'lesser fibre diameter' in 50–100 fibres (Dubowitz & Brooke, 1974), to give the overall mean cross-sectional fibre area for each biopsy.

Results

Mean results \pm SD are given.

Leg volume and oxygen uptake

After immobilization the total leg volume (which is muscle plus bone) measured anthropometrically was on average 12% less in the injured (5.68 ± 1.05 l) compared with the uninjured (6.43 ± 0.87 l) leg. The loss was similar in both upper and lower leg measurements (Table 1).

The $\dot{V}O_{2max}$ achieved in one-leg cycling was also reduced from 1.89 ± 0.21 l/min in the uninjured to 1.57 ± 0.18 l/min in the injured leg. All values of $\dot{V}O_{2max}$, except in subject no. 3, fell within the limits for the relationship leg vol./ $\dot{V}O_{2max}$ previously reported (Davies & Sargeant, 1975a), for both injured and uninjured legs (Fig. 1).

Changes in muscle fibres

The frequencies of type I and type II fibres (mean 61 and $39 \pm 10\%$ respectively) are not significantly different between the injured and uninjured legs. Both type I and type II fibres of the injured leg show a significant ($P < 0.01$) and similar reduction in the mean cross-sectional area, when compared with the uninjured leg, type I fibres being reduced from 3410 ± 530 μ m² in the uninjured to 1840 ± 410 μ m² in the injured leg, and type II fibres from 3810 ± 940 μ m² to 2390 ± 910 μ m². A combined mean fibre area calculated for each leg, taking into account the frequency and mean area of both fibre types, shows on average a reduction of 42%, from 3570 ± 630 μ m² in the uninjured to 2080 ± 550 μ m² in the injured leg (Fig. 2). The degree of atrophy, as indicated by the mean fibre area, was correlated ($r = 0.82$, $P < 0.05$) with the length of time (from 0 to 42 days) that the patients had been weight-bearing (Fig. 3).

The percentage reduction in mean fibre area of the injured leg is proportionally greater than is indicated by anthropometric assessment

TABLE 1. *Structure and function in injured and uninjured legs in seven patients and eight normal subjects*
ui = uninjured leg; i = injured leg; s = stronger leg; w = weaker leg.

Patients										Normal subjects					
No.	Age (years)	Time immobile (days)	Time wt. bearing (days)	Leg volume (l)		$\dot{V}O_{2max}$ net (l/min)	Fibre type frequency (%)		$10^{-2} \times$ Mean fibre area (μm^2)	No.	Age (years)	Fibre type frequency (%)		$10^{-2} \times$ Mean fibre area (μm^2)	
				Total	Upper		I	II				I	II	I	II
1	19	54	28 ui i	6.96 6.37	4.08 3.73	1.86 1.68	67 62	33 38	39 22	N1	25 s w	72 64	28 36	49 46	60 63
2	18	53	7 ui i	5.33 5.00	3.11 2.90	— —	55 62	45 38	27 13	N2	20 s w	58 62	42 38	37 36	43 37
3	29	213	0 ui i	5.46 4.45	3.18 2.47	1.88 1.44	66 79	34 21	30 14	N3	22 s w	45 57	55 43	22 19	27 30
4	26	183	14 ui i	6.20 5.19	3.60 3.01	1.66 1.42	54 38	46 62	37 16	N4	22 s w	53 58	47 42	33 27	37 27
5	30	180	42 ui i	7.31 6.88	3.99 3.74	2.28 1.87	59 58	41 42	29 22	N5	32 s w	64 75	36 25	33 30	35 36
6	18	85	30 ui i	7.64 7.03	4.78 4.16	1.79 1.57	71 51	29 49	37 23	N6	33 s w	78 50	22 50	27 24	36 27
7	20	150	14 ui i	5.92 4.87	3.49 2.79	1.87 1.41	64 63	36 37	40 19	N7	23 s w	70 70	30 30	42 45	58 52
										N8	27 s w	56 57	44 43	32 37	40 37

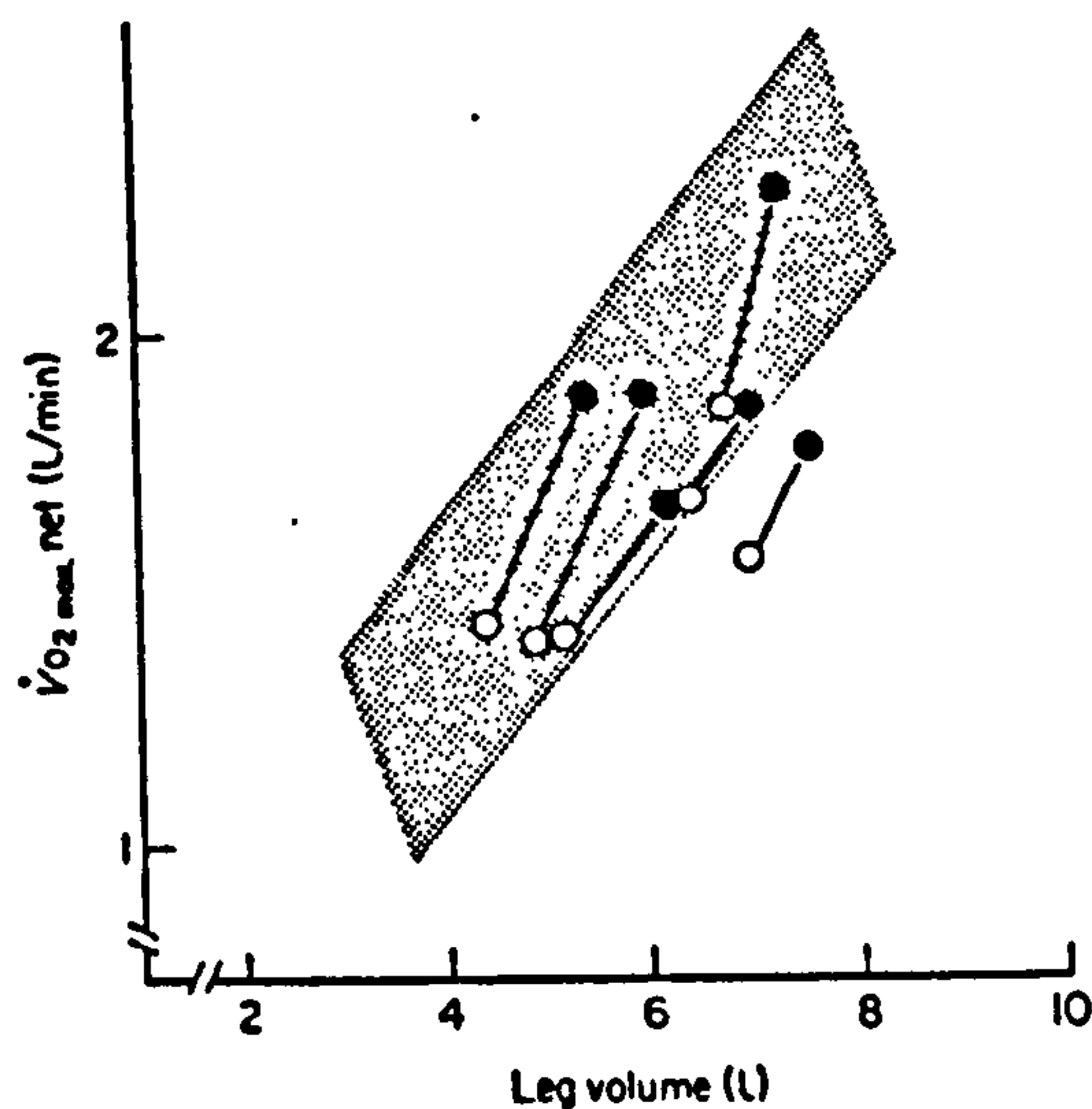


FIG. 1. Relationship of $\dot{V}O_{2\max}$ (net) of one-leg exercise to leg volume (muscle plus bone). Data points are given for patients' injured (○) and uninjured (●) legs, linked with a bar. The stippled area gives the confidence limits of the relationship in normal subjects (Davies & Sargeant, 1974).

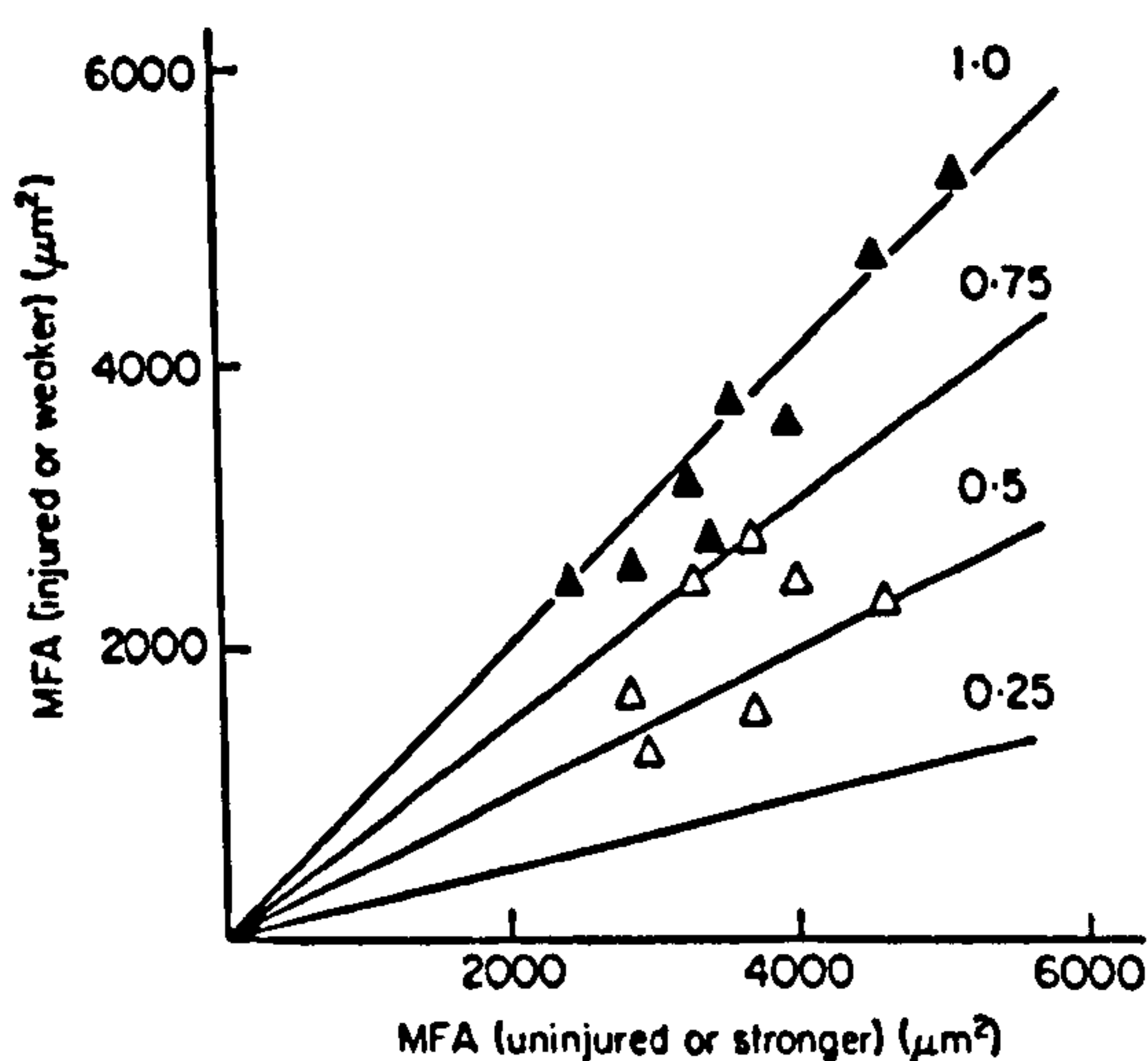


FIG. 2. Mean fibre area (MFA) of the injured (or weaker) leg in relation to that of the uninjured (or stronger) leg for the patients (Δ) and the normal subjects (▲).

of leg volume (Fig. 4). Similarly, since $\dot{V}O_{2\max}$ is related to leg volume (Fig. 1) the reduction in mean fibre area is also proportionally greater than the reduction of $\dot{V}O_{2\max}$ when the injured leg is compared with the uninjured (Table 1).

Discussion

The atrophic response of human muscle to

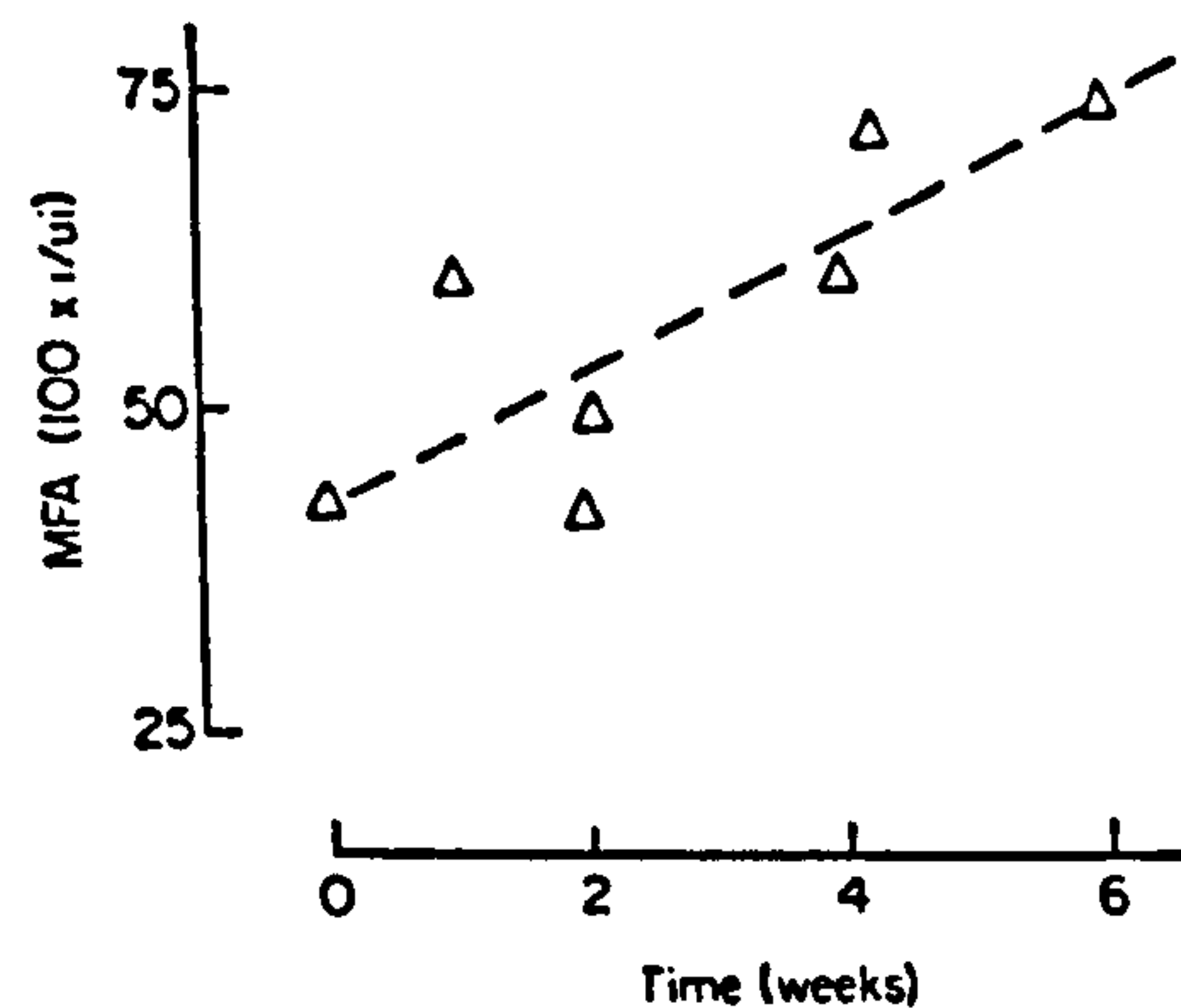


FIG. 3. Degree of muscle fibre atrophy in relation to the time spent weight-bearing after removal of plaster cast. Mean fibre areas (MFA) of the injured (i) legs are expressed as percentages of those in the uninjured (ui) legs. The regression line (---) is $y = 43.4 + 5.12x$; $r = 0.82$.

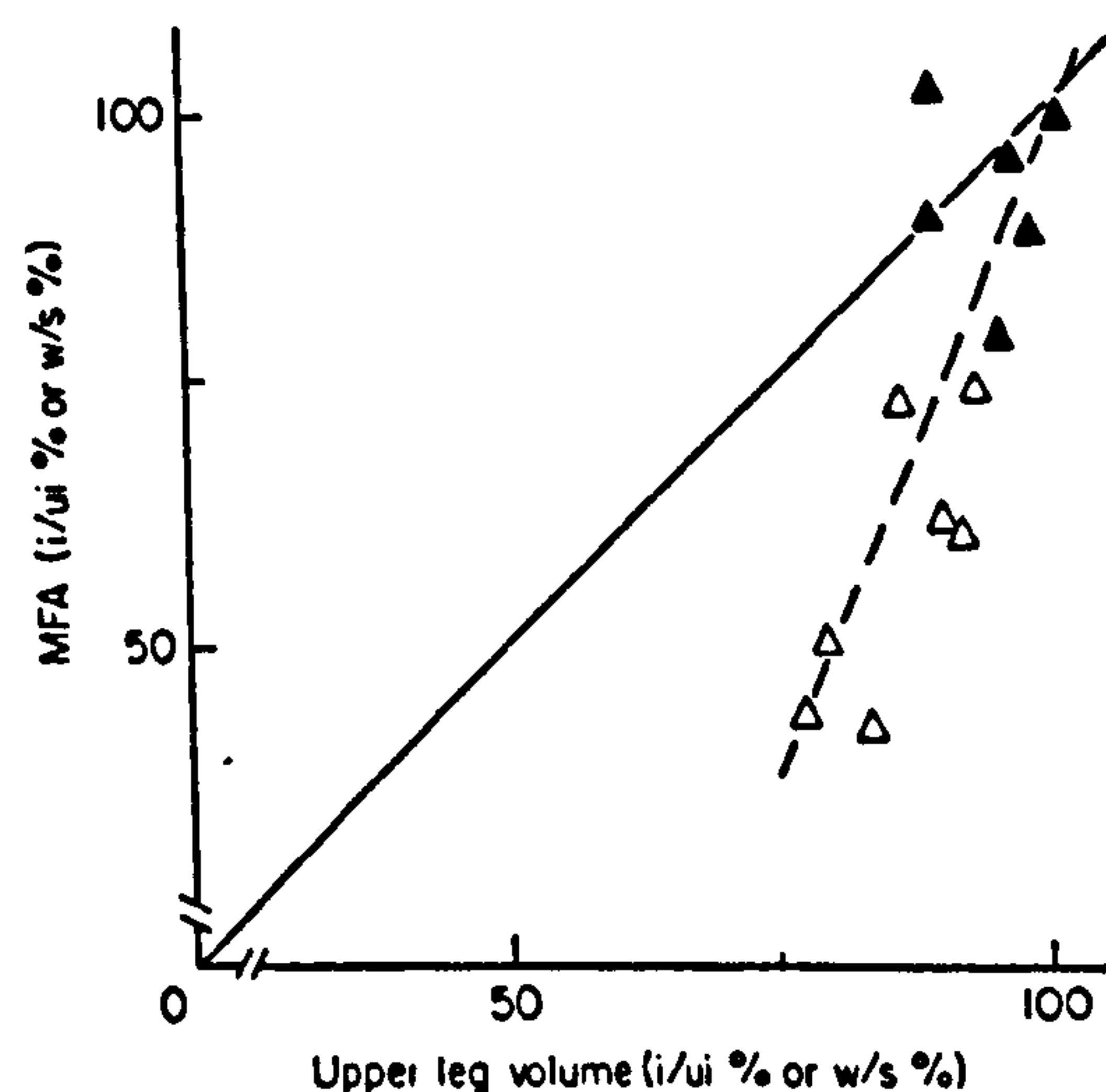


FIG. 4. Degree of muscle fibre atrophy (MFA, see Fig. 3) related to the reduction in anthropometric estimation of the thigh (muscle plus bone) volume. Δ, Patients [injured leg measurements (i) are expressed as a percentage of the uninjured (ui)]; ▲, normal subjects [weaker leg measurement (w) is expressed as a percentage of the stronger (s)]. The regression line for the combined data (---) is $y = 2.287x - 134.2$; $r = 0.78$.

disuse is a common clinical observation, but there are surprisingly few data available on the functional and structural implications of disuse atrophy in man. In recent investigations (Davies & Sargeant, 1975a, b, c) aspects of disuse atrophy were examined in patients who had had one leg immobilized in a plaster cast after unilateral leg fracture. This enabled direct comparisons between the injured and the uninjured legs after a known period of im-

mobilization and muscle disuse. By the same method we have made direct comparisons of the changes in the muscle fibres. Unlike earlier studies on disuse atrophy, where the biopsy samples were taken at the time of surgical intervention in an affected limb (Patel, Razzak & Dastur, 1969; Edstrom, 1970), by use of percutaneous needle biopsy we obtained samples from both affected and unaffected limbs.

The changes in both $\dot{V}O_{2\max}$ of one-leg exercise and leg volume (muscle plus bone) in our patients (Fig. 1) confirm the previous findings, a fall of approximately 15% in both variables being indicated when the injured is compared with the uninjured leg.

The frequency and size of muscle fibre types in the patients' uninjured leg were similar to those for the healthy male control subjects. There was no systematic difference in the frequency of fibre types between the two legs in the patients, and the variation was no greater than found in bilateral samples from the normal control subjects. The cross-sectional areas of both fibre types were significantly reduced in the injured legs of all the patients when compared with their own uninjured legs (Table 1, Fig. 2). In six of the seven cases, type I fibres showed relatively greater atrophy than type II fibres; thus the mean reduction in type I fibres was 46% compared with 37% in type II fibres. Edstrom (1970) observed a similar tendency for red muscle fibres to atrophy more than white. However, he studied patients with long-term (~2 years) knee-joint dysfunction who cannot be considered comparable with our patients.

These findings differ from the predominantly type II fibre atrophy in the muscle of patients with a variety of non-muscular disorders, e.g. osteomalacia, chronic alcoholism, corticosteroid overdose etc. (Dubowitz & Brooke, 1974). The suggestion, therefore, that type II fibre atrophy in these conditions may merely reflect their reduced habitual activity with relative disuse, due to pain or to general ill-health, may be open to question.

We obtained biopsies from 0 to 42 days after the patients started weight-bearing, and were able effectively to exercise the atrophied limb. Not surprisingly we found a significant correlation (Fig. 3) between the overall degree of fibre atrophy and the time that the patients had been weight-bearing. However, our study

includes patients with periods of preceding immobilization ranging from 53 to 213 days, and more, particularly longitudinal, information is needed on the time-course of the recovery process.

The measurements of cross-sectional area of individual fibres indicate that atrophy in the quadriceps muscle is much greater than would be suspected from measurements of either the total leg or thigh volume (Fig. 4), and might imply that when thigh volume was reduced by approximately 50% fibre area would be zero. Part of this discrepancy may be accounted for by the proportion of non-contractile tissue, but this clearly cannot account for 50% of the leg (muscle plus bone) volume since correction has already been made for the subcutaneous fat, and bone accounts for only about 11% of muscle plus bone volume in the leg (Davies & Sargeant, 1975c).

More importantly, immobilization of the knee in a long leg plaster may produce greater atrophy of the quadriceps muscle than of the other thigh muscles. Also the deep muscle site of biopsy in these patients may not be typical of the whole of the quadriceps under these conditions, although the few available studies (Johnson, Polgar, Weightman & Appleton, 1973; Harris, Hultman & Nordesjö, 1974) suggest that the normal quadriceps in man are relatively homogeneous both structurally and functionally.

A 17% reduction of the $\dot{V}O_{2\max}$ achieved in one-leg exercise with the injured leg is associated with the change in leg volume. This again contrasts with the greater reduction in mean fibre area, suggesting that the functional effect of immobilization may be obscured by metabolic activity of other muscles or other regions of the quadriceps in one-leg cycling. However, in a similar group of patients performing one-leg cycling the pattern of forces applied to the cranks with the injured leg was the same as with the uninjured leg (A. J. Sargeant & C. T. M. Davies, unpublished work). This argues against the view that there is markedly greater atrophy (as indicated by the mean fibre area) in the quadriceps as compared with other leg muscles, since it would be surprising if substitution of the hamstrings or gluteal muscles for the quadriceps did not affect the pattern of force application in cycling. Further studies are needed to study any regional

differences in structure and function within the quadriceps, as well as between these and other muscle groups after immobilization.

In conclusion, there is a large degree of fibre atrophy in the quadriceps femoris resulting from disuse after immobilization, and this affects both fibre types. Atrophy is greater than gross measurements of leg volume or function would suggest, possibly because there is relatively less atrophy either in the other thigh muscles, or in other regions of the quadriceps femoris. The nature and functional implications of this differential atrophy require further investigation.

Acknowledgments

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MEASUREMENT OF QUADRICEPS MUSCLE WASTING BY ULTRASONOGRAPHY*

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SUMMARY

Grey-scale ultrasonography can produce an image of the quadriceps muscle from which it is possible to measure its cross-sectional area (CSA). The between-days coefficient of variation for quadriceps CSA (at mid-thigh) in 14 legs of seven subjects each scanned on four days was reduced to 4.0% by averaging four scans on each day.

Bilateral scans (at the mid-thigh level) were used to measure the severity of quadriceps wasting in 21 otherwise healthy adult patients with a difference in thigh circumference following unilateral knee immobilization or injury.

Quadriceps wasting as demonstrated by the scans was consistently more severe than the disparity in whole thigh cross-sectional area at the same level or the disparity in anthropometric estimates of fat-free thigh volume. Investigations concerned with changes in quadriceps muscle bulk must therefore use a technique (such as ultrasonography) which allows measurement of the quadriceps itself.

IMMOBILIZATION or injury of the knee joint quickly results in weakness and wasting of the ipsilateral quadriceps muscle, and the restoration of quadriceps strength is important for the recovery of normal knee function and control (Duthie and Ferguson, 1973). Since the measurement of quadriceps strength in patients with a recent history of lower-limb trauma is often difficult, the need for, and the effectiveness of, physiotherapeutic exercise are usually assessed on the basis of estimates of muscle bulk and not of strength. Although their reproducibility is poor (Nicholas *et al.*, 1976; Kirwan *et al.*, 1979), thigh circumference measurements are used to estimate changes in quadriceps bulk. Moreover, quite large changes in the size of the quadriceps may be obscured by the variable thickness of the surrounding layer of subcutaneous fat (Ingemann-Hansen and

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† Dr. Nichols died on 8 September 1979.

Requests for reprints to Dr. A. Young.

Halkjaer-Kristensen, 1977) and the presence in the thigh of a large bulk of other muscles. The latter remains a potential problem even if skinfold thickness measurements are used to estimate the volume of subcutaneous fat.

Evidence from a muscle-biopsy study suggests that after tibial fracture the wasting process may often be highly selective, affecting the quadriceps much more than the other thigh muscles (Sargeant *et al.*, 1977). However, any attempt to extrapolate from observations of muscle fibre size to conclusions about whole muscle size depends on major assumptions regarding the number of fibres in the muscle and the extent to which the biopsy findings are representative of events in the muscle as a whole.

This paper describes how ultrasonography may be used to provide an image of a cross-section of the quadriceps and how the cross-sectional area of this image is affected by knee injury and/or immobilization.

METHODS

Quadriceps scanning

An image of a transverse section through the quadriceps muscle group was obtained by means of a conventional diagnostic ultrasound scanner with grey scale attachment (Nuclear Enterprises, 'Diasonograph NE4200'). Scans were made at a point (hereafter referred to as 'mid-thigh') half-way between the greater trochanter and the lateral joint line of the knee. The height of this point above the floor was recorded so that it could be identified on future occasions.

Scanning was performed with the subject supine and his leg supported with the knee extended. The leg was supported with the hip flexed to 5° (or sometimes 10°) to allow scanning from more posterior points on the thigh circumference, thus ensuring that an adequate image could be compounded. The scanning gantry was tilted through an equal angle so that a transverse scan was still obtained. The best images were usually obtained with a much attenuated sound input of frequency 2.5 MHz, and a compensation rate of 5 dB/cm, with care being taken not to distort by compression. The last condition was easier to meet when using a probe of small face area. The probe used was Nuclear Enterprises NE4328 (13 mm LIF, 2.25 MHz). Arachis oil was used as the coupling medium.

The scan image was photographed to give an ultrasonogram on Nuclear medicine NMB film (Kodak) which was about half life-size.

Quadriceps cross-sectional area

The outline of the quadriceps group was traced from the photograph of each scan and the cross-sectional area (CSA) of the quadriceps was measured by cutting-out and weighing the tracing.

Thigh circumference

Thigh circumference was measured by tape measure, at 'mid-thigh', with the subject standing.

Fat-free thigh volume

Thigh volume was estimated by measuring its circumference at three levels and

reating the thigh as two truncated cones (after Jones and Pearson, 1969). The levels were defined on the dominant or uninjured leg (normal subjects or patients respectively) as (i) gluteal fold, (ii) knee plus one third sub-ischial height and (iii) narrowest point above the knee. The circumferences of the non-dominant (or injured) limb were measured at the same heights above the ground.

The thickness of subcutaneous fat was estimated by applying the regression equations of Davies and Sargeant (1975) to skinfold thickness measurements made anteriorly and posteriorly at level (ii). These measurements were made with Harpenden skinfold calipers (British Indicators Ltd). The anterior skinfolds were measured with the subjects standing, but for the posterior measurements it was necessary for them to lie prone with the knee flexed, so that a measureable skinfold could be lifted. The fat-free thigh volume was then calculated by subtraction of the estimated fat volume from the estimated total thigh volume.

SUBJECTS

Repeatability study

Four female and three male adults participated in a study of the repeatability of ultrasonographic measurements of quadriceps cross-sectional area. Two of the male subjects had mild unilateral wasting secondary to knee injury.

Cross-sectional study of quadriceps wasting

Patients (five female and 16 male) were recruited from the physiotherapy 'knee class'

TABLE
PATIENTS WITH UNILATERAL THIGH MUSCLE WASTING

Diagnosis	Age (years)	Sex	Difference in mid-thigh circumference	
			(cm)	(%)
Meniscectomy	19	F	4.3	-7.9
Meniscectomy	19	M	1.8	-3.5
Meniscectomy	20	M	1.7	-3.1
Meniscectomy	26	M	0.5	-1.0
Meniscectomy	27	M	1.9	-3.9
Meniscectomy	28	M	1.4	-3.0
Meniscectomy	33	M	0.4	-0.8
Knee injury	19	M	3.3	-6.8
Knee injury	23	M	1.3	-2.6
Knee injury	44	M	0.5	-1.0
Knee injury + POP	22	F	1.7	-3.3
Knee injury + POP	27	M	1.8	-4.2
Knee injury + POP	27	M	2.0	-3.7
Knee injury + POP	34	F	1.6	-3.3
Fractured tibia	23	M	6.1	-12.4
Fractured tibia	29	F	2.7	-5.9
Fractured tibia	31	M	2.5	-4.5
Fractured tibia + pes anserinus transfer	35	M	2.9	-5.4
Pes anserinus transfer	39	M	1.6	-3.4
Arthrotomy	19	F	1.8	-3.6
Arthrotomy	36	M	0.5	-0.9

and from an orthopaedic out-patient clinic. These were essentially healthy young adults (median age 27 years; range 19–44) who were referred to us if their therapist had measured at least 2 cm discrepancy in mid-thigh circumference following knee injury or immobilization (Table). (This difference was not always confirmed when the patients were remeasured by the investigators.)

Patients with a history of injury to the other lower limb within the preceding two years were excluded, as were any with clinical evidence to suggest denervation, muscle disease, or inflammatory joint disease. The patients had a wide range of underlying orthopaedic diagnoses (Table), with post-menisectomy patients forming the largest single group.

RESULTS

Quadriceps scanning

It was always easy to distinguish the femur and the four parts of the quadriceps in the transverse ultrasonograms (Fig. 1, Plate IX and Fig. 2, Plate X). Difficulties in scan interpretation arose in two areas. The boundary between biceps femoris and the quadriceps was sometimes hard to define where the short head of the biceps is closely applied to the posterior aspect of the lateral mass of the quadriceps. A more difficult boundary to define was that of vastus medialis with adductor longus and sartorius.

Satisfactory scans were obtained more readily for female subjects than for male subjects. Of the males, those with the smallest skinfolds tended to be the hardest to scan—distortion by compression was harder to avoid and, in the very lean, the transducer ringing period obscured the outer border of the muscle.

Repeatability study

Each thigh of each of the seven subjects was scanned four times on each of four visits. The 'between-scans' variance gave a coefficient of variation of 5.2%. (Errors in cutting out and weighing of the tracings made only a minimal contribution to this.)

The net 'between visits' coefficient of variation was 6.1%. If the component of variance attributable to the 'between-scans' variation was reduced by averaging each subject's four scans on each occasion, the net 'between-visits' coefficient of variation was reduced to 4.0%.

Although the female subjects were easier to scan, there was no significant difference between the coefficients of variation calculated for males and females. However this could, of course, simply reflect the small number of subjects.

Cross-sectional study of quadriceps wasting

The same pattern of results was obtained for the post-menisectomy group as for the other patients and the results will therefore be reported together.

The injured/uninjured limb ratio for the mid-thigh circumference-squared was consistently greater than the corresponding ratio for quadriceps cross-sectional area (mean of four scans on each thigh) (Fig. 3). The two ratios show a weak, linear correlation ($r = 0.50$; $0.05 > P > 0.01$).

It appears that in these patients, the reduction in the cross-sectional area of the whole thigh was consistently less than the reduction in quadriceps cross-sectional area. To test whether this discrepancy might be explained simply by a greater thickness of subcutaneous fat in the injured limb, the injured/uninjured limb ratios for quadriceps

PLATE IX

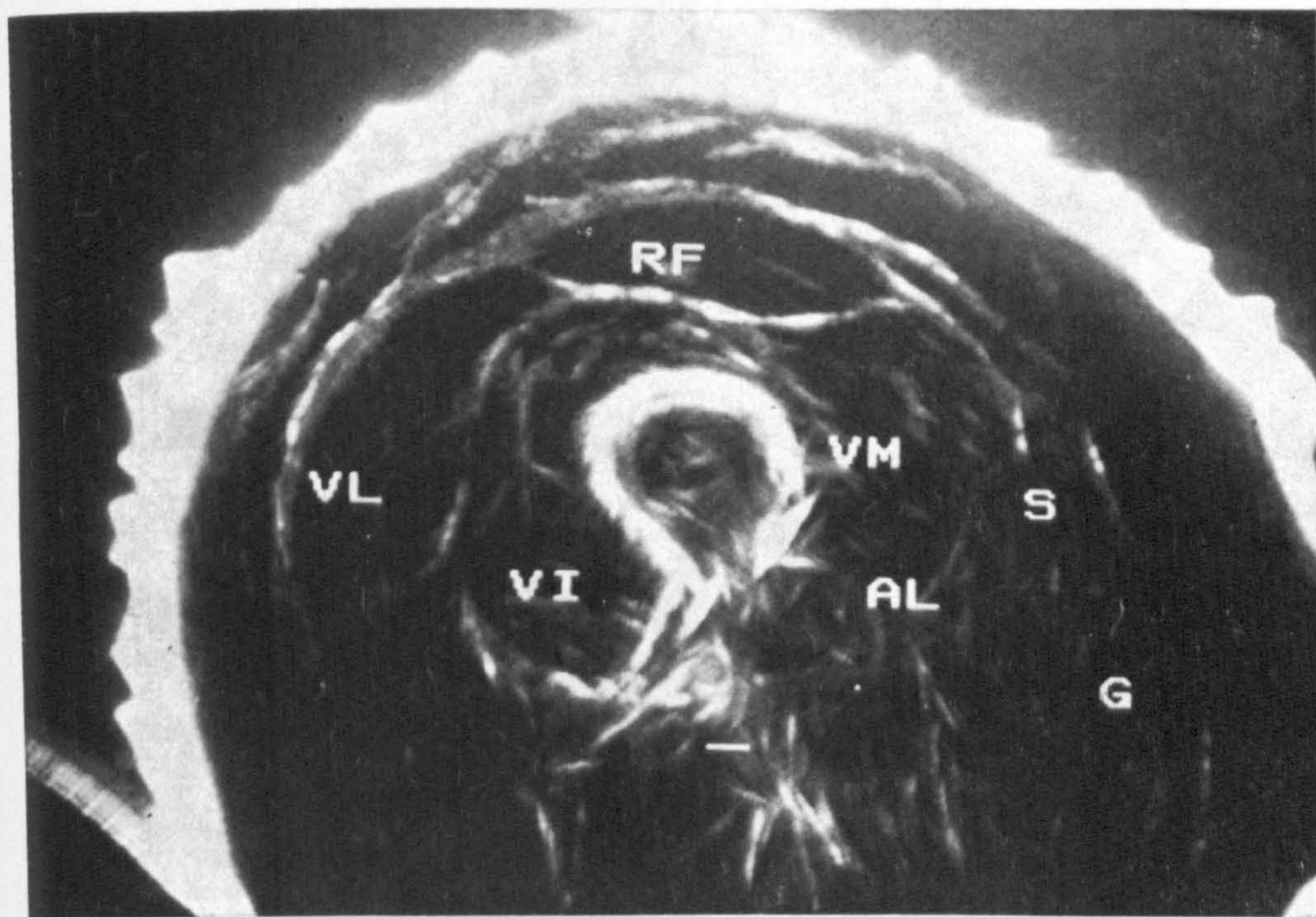


FIG. 1.—Normal transverse ultrasonogram made at the mid-thigh level in a 19-year-old girl (VI = vastus intermedius; VL = vastus lateralis; RF = rectus femoris; VM = vastus medialis; AL = adductor longus; S = sartorius; G = gracilis).

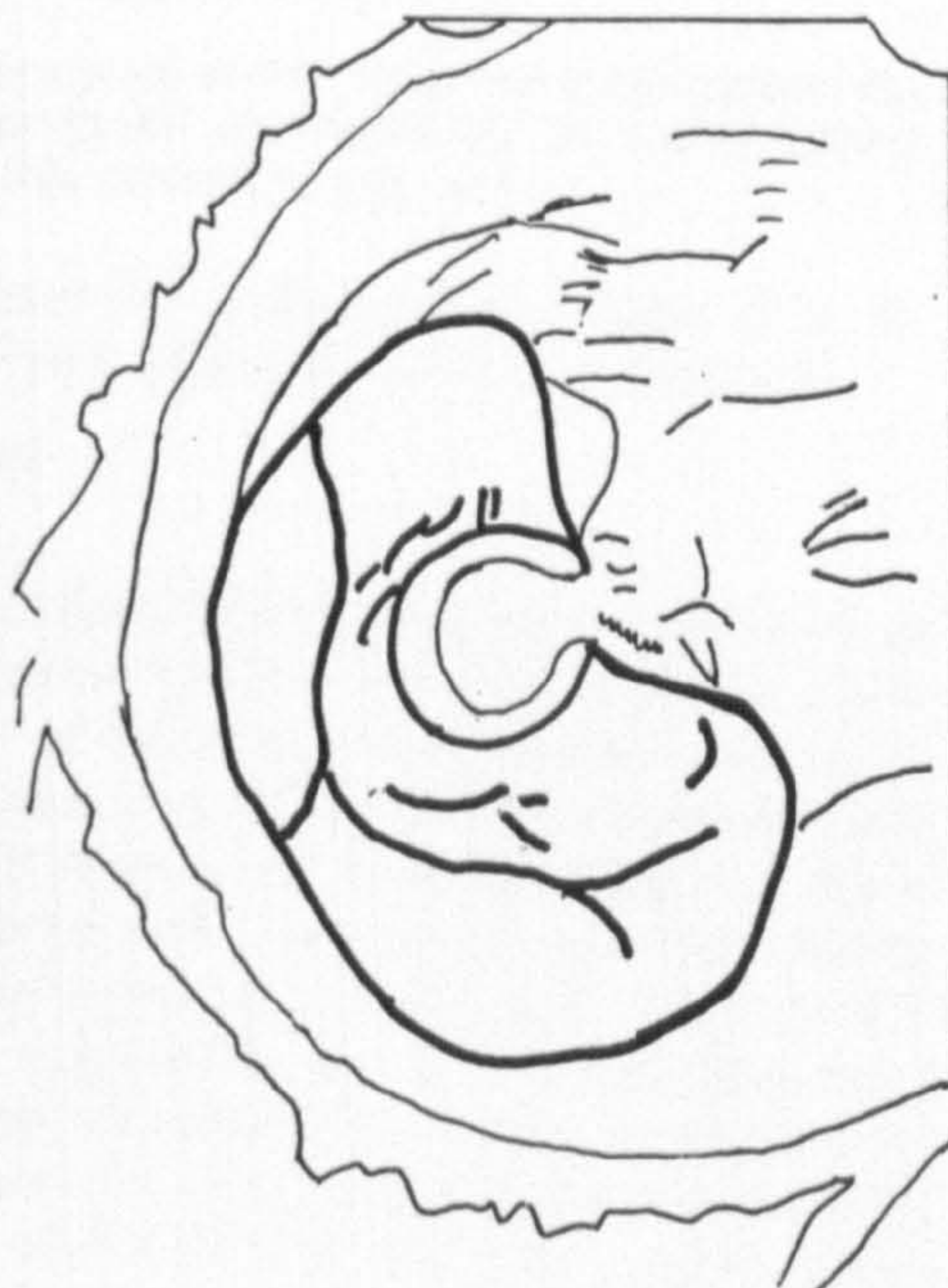
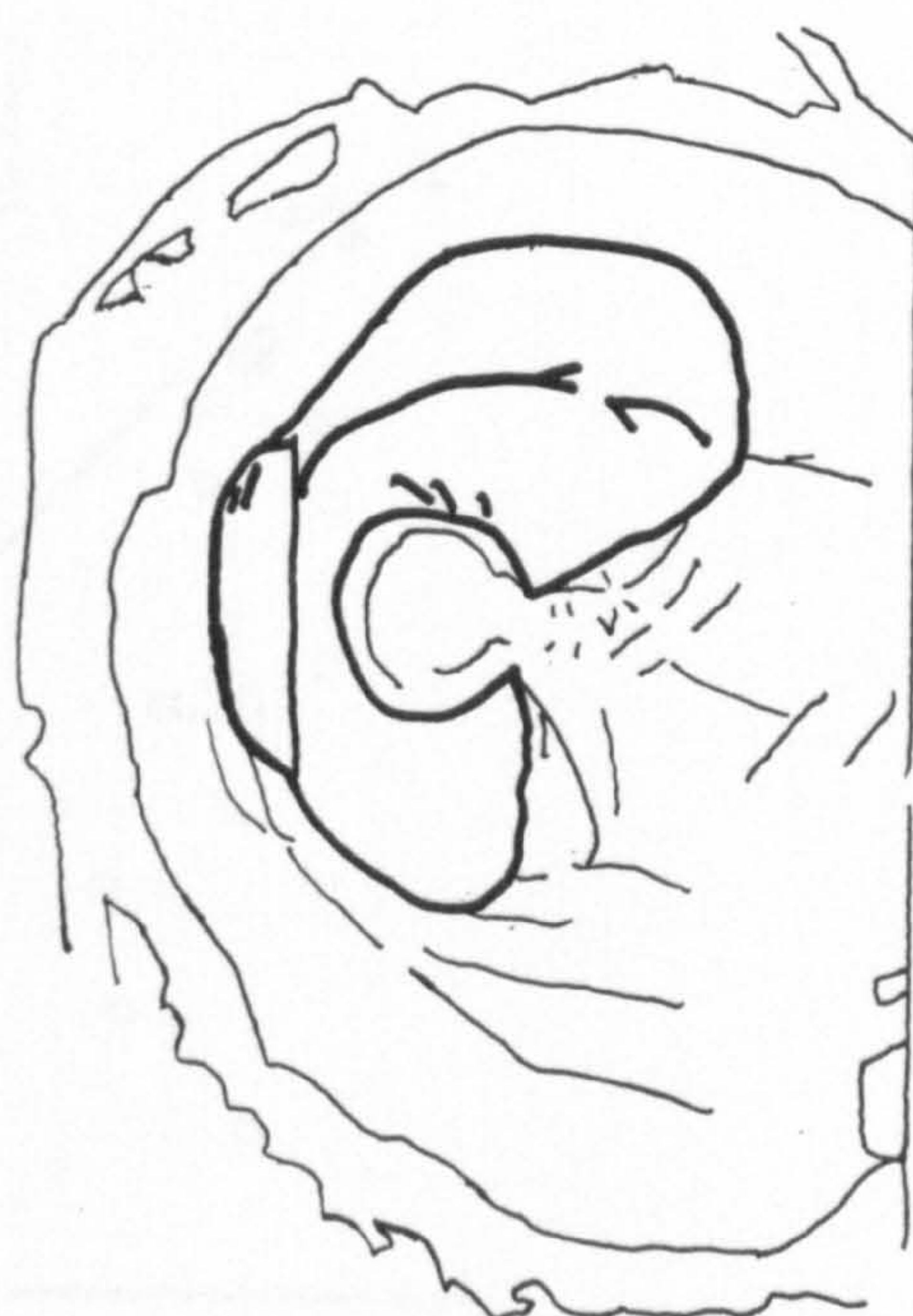
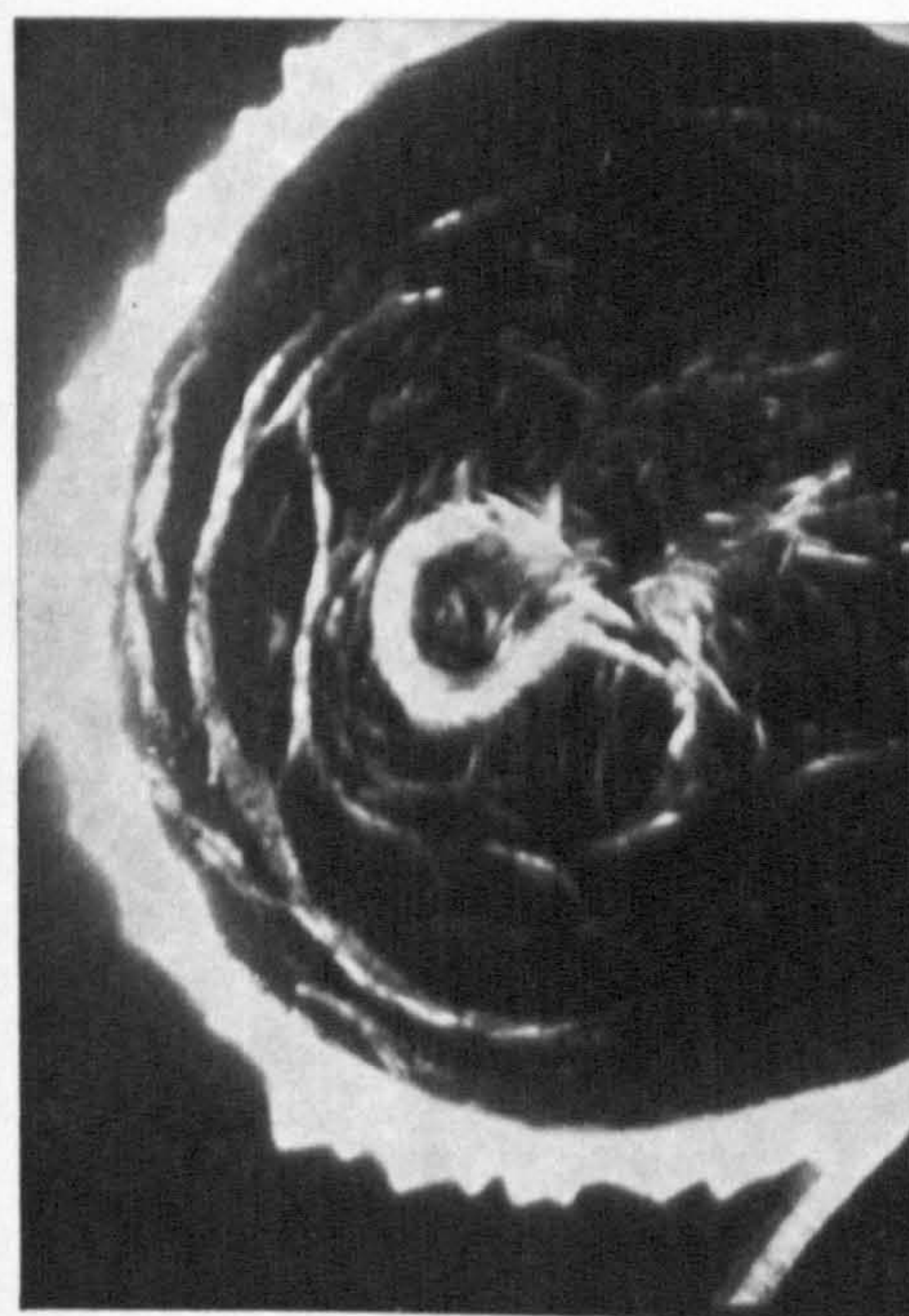
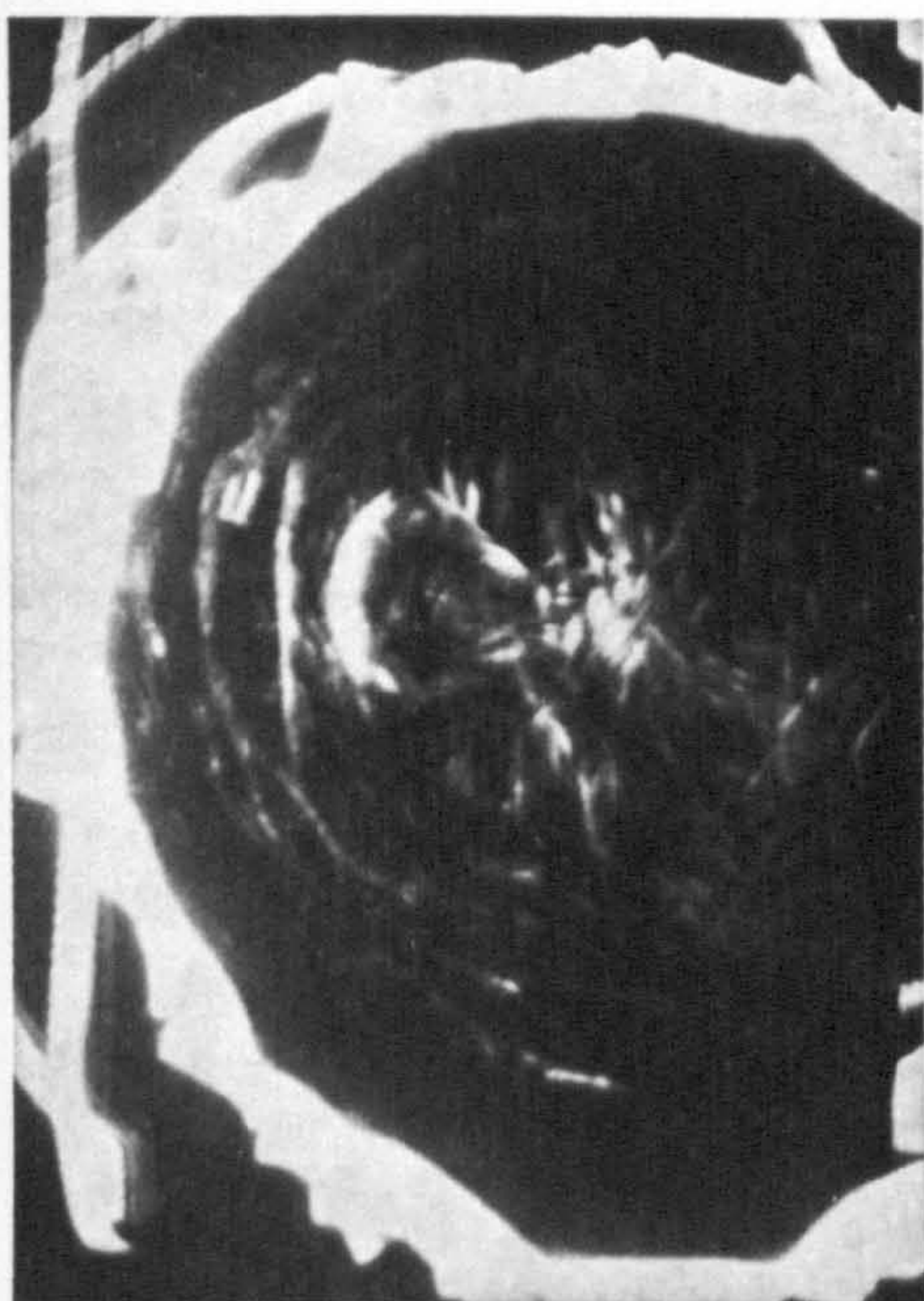


FIG. 2.—Bilateral transverse ultrasonograms made at the mid-thigh level in a patient with unilateral thigh muscle wasting following a meniscectomy. The quadriceps' outlines are indicated by the heavier lines in the diagrams. Measurement shows a difference in quadriceps cross-sectional area of -22% .

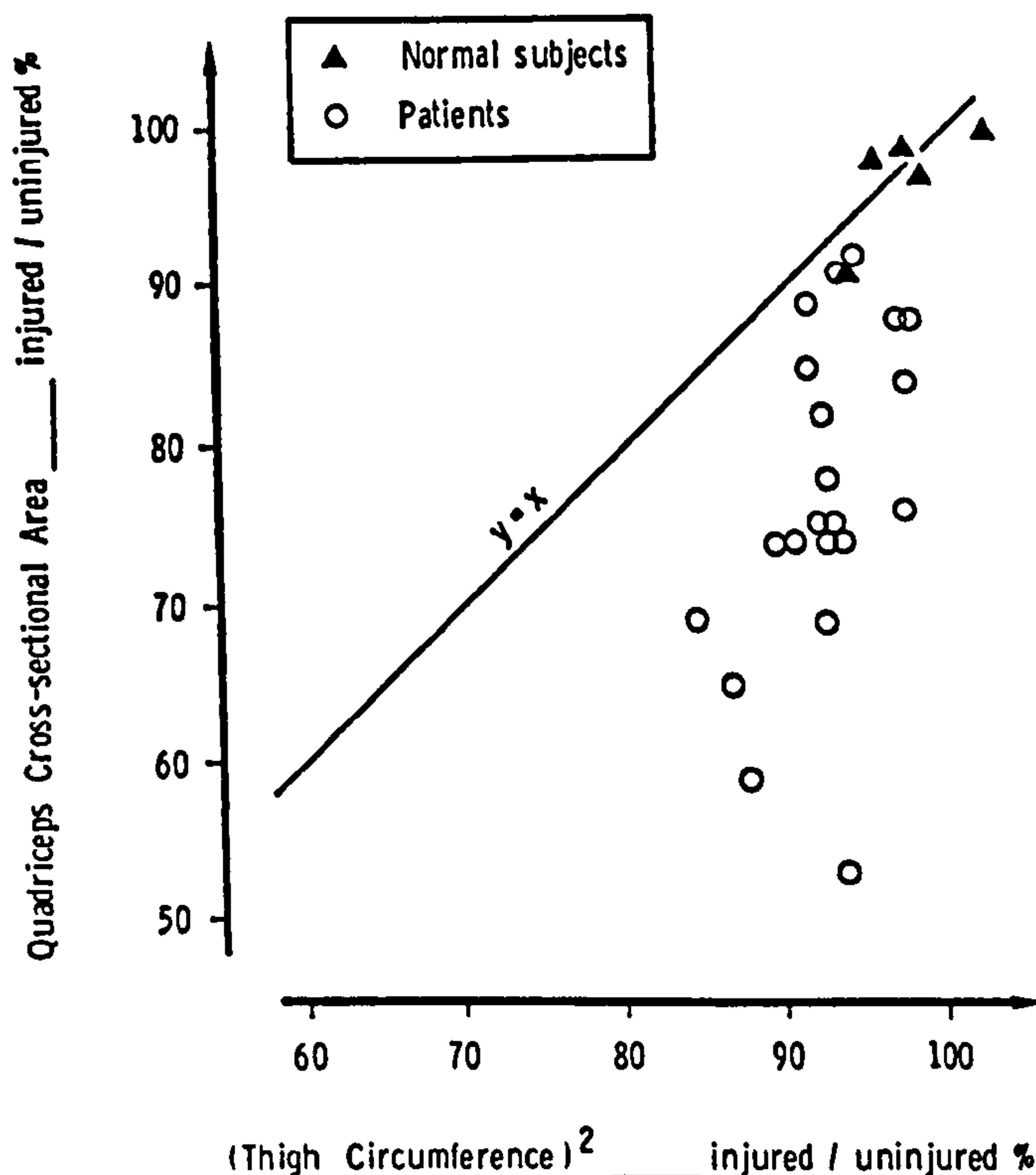


FIG. 3.—The injured/uninjured limb ratio for quadriceps cross-sectional area plotted against the equivalent ratio for thigh circumference-squared. (The graph also includes the corresponding weaker limb/stronger limb ratio for five normal young men.)

cross-sectional area were replotted against the ratios for fat-free thigh volume (Fig. 4). The discrepancy between the ratios persists ($P < 0.001$), although it is less pronounced.

DISCUSSION

Quadriceps scanning

The use of ultrasonography to delineate individual skeletal muscles in man, and so measure their cross-sectional area, was first described by Ikai and Fukunaga in 1968. They subsequently used the technique in a study of the effects of physical training on the strength of elbow flexion and the cross-sectional area of the anterior compartment of the upper arm (Ikai and Fukunaga, 1970). However, their technique did not allow adequate differentiation between muscles. Moreover, the procedure was not widely applicable as it involved scanning the limb submerged in a water-bath. As a result, there were no other published reports of this application of ultrasonography until Dons *et al.* (1979) used it to obtain an indication of quadriceps cross-sectional area. Difficulty in distinguishing muscle boundaries meant that they were limited to measuring skin to bone thickness at 33° intervals around a 100° sector of the antero-lateral thigh and did not actually measure quadriceps cross-sectional area.

Human muscle cross-sectional area has been measured *in vivo* by computerized axial tomography (Häggmark *et al.*, 1978; Häggmark and Eriksson, 1979; Bulcke *et al.*, 1979). However this technique requires sophisticated and expensive equipment of very

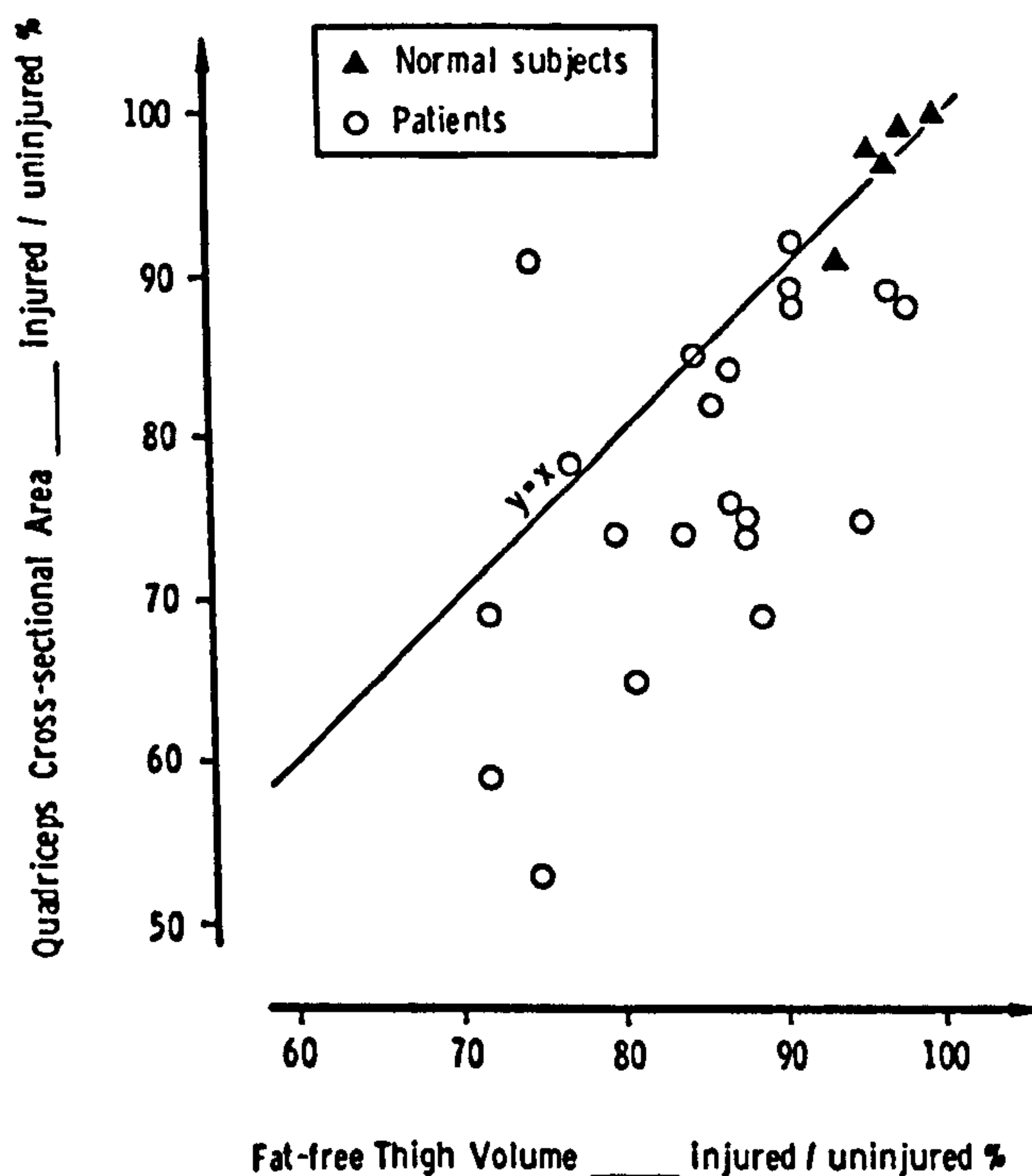


FIG. 4.—The injured/uninjured limb ratio for quadriceps cross-sectional area plotted against the equivalent ratio for fat-free thigh volume. (The graph also includes the corresponding weaker limb/stronger limb ratio for five normal young men.)

limited availability. Moreover, the radiation exposure entailed in axial tomography of an extremity, although small (Perry and Bridges, 1973), still places significant ethical limitations on the use of the technique for sequential measurements.

The principal advantage of computed tomography would be its superior image resolution (Ferrucci, 1979a). However this study demonstrates that a practised operator using a modern ultrasonic scanner can obtain a satisfactory image of a transverse section through the quadriceps muscle group. Our experience of scanning the quadriceps suggests that other muscles could probably also be studied in this way, after a period of preliminary experimentation to establish the appropriate details of scanning technique.

The high reflectivity of fat for ultrasound means that an obese abdomen is difficult to scan successfully (Ferrucci, 1979b). However, a substantial layer of subcutaneous fat facilitates thigh ultrasonography by minimizing deformation of the underlying muscle during scan compounding. This is particularly relevant when scanning a limb of small radius since adequate compounding is then impossible without significant compression of the skin surface. Whatever its radius, a very lean thigh cannot be scanned successfully by the technique described, since the ringing period of the transducer obscures the outer boundary of the muscle. Such a limb would have to be scanned through a water-bath.

It also seems likely that a thigh with a substantial layer of subcutaneous fat also has a greater amount of intermuscular fat which may make it easier to delineate the intermuscular boundaries.

Cross-sectional study of quadriceps wasting

In a biopsy study of seven patients with quadriceps wasting following lower-leg fracture

and subsequent knee immobilization (Sargeant *et al.*, 1977), measurements of muscle fibre size suggested that quadriceps atrophy might sometimes be much greater than would be expected from anthropometric estimates of fat-free thigh volume. This conclusion depended on the assumption that the fibre size measurements were representative of events in the quadriceps as a whole. The work of Häggmark *et al.* (1978) lent some weight to this assumption: they showed that for nine male subjects there was a linear relationship between MFA and the CSA of vastus intermedius plus vastus lateralis as measured by computerized axial tomography.

This study has now confirmed that, after knee injury or immobilization, estimates of muscle wasting based on thigh circumference measurements seriously underestimate the severity of the quadriceps wasting actually present. Moreover, this discrepancy cannot be explained simply by a greater thickness of subcutaneous fat in the injured limb. It seems that in these patients the wasting process is largely localized to the quadriceps itself, sparing the other thigh muscles. Strictly speaking, confirmation of this requires a longitudinal study since changes may also be occurring in the uninjured limb.

Small differences in thigh circumference are not only difficult to demonstrate reliably (Nicholas *et al.*, 1976; Kirwan *et al.*, 1979) but they may conceal large differences in quadriceps cross-sectional area. A 2.5 cm difference in mid-thigh circumference probably means a 22–33% difference in quadriceps CSA (95% confidence limits of prediction, if larger thigh circumference = 50 cm).

For routine clinical practice, thigh muscle ultrasonography is too time-consuming to have an important place as a measure of muscle wasting. Nevertheless, studies such as this give a better appreciation of the true implication of a clinically demonstrable difference in thigh circumference. On the other hand, ultrasonography is a potentially useful tool for physiotherapy research since a study of the treatment of muscle wasting demands measurement of the individual muscle group involved. Indeed, we should suggest that it would be a waste of time to base any research study of quadriceps physiotherapy on measurements of thigh circumference.

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The effect of knee injury on the number of muscle fibres in the human quadriceps femoris

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Summary

1. By means of ultrasound scanning, bilateral measurements of the cross-sectional area of the quadriceps muscle group were made in 14 young adults with unilateral thigh muscle wasting after knee injury. Needle biopsy specimens from the lateral mass of the muscle were used to estimate the myofibre cross-sectional area for both quadriceps of each subject.

2. The cross-sectional area of the quadriceps of each patient's injured limb was always smaller than that of the contralateral muscle. The wasting was largely localized to the quadriceps, with relative sparing of the other thigh muscles.

3. None of the biopsies showed any abnormality apart from a reduction in fibre size. In each case, the injured limb's reduced quadriceps cross-sectional area was associated with a reduced mean fibre area.

4. The ratio of the cross-sectional area of a muscle to its mean fibre area is a function of the number of fibres it contains. The ratio varied considerably from patient to patient but there was close agreement between the values obtained for the two limbs of each patient.

5. The quadriceps wasting produced by knee injury was due to muscle fibre atrophy. There was no evidence for a change in the number of fibres in the muscle.

Key words: atrophy, hyperplasia, hypoplasia, knee injury, muscle biopsy, ultrasonography.

Abbreviations: CSA, muscle cross-sectional area; MFA, mean fibre area.

Introduction

Muscle wasting is a common problem in the presence of joint disease, or after joint injury. The resulting muscle weakness may contribute to further joint damage. It is therefore important to be able to correct the wasting. If this is to be done more efficiently than at present, it is necessary to know more about the nature of the wasting which takes place.

An even more fundamental requirement is the ability to measure how much wasting has occurred. Techniques are now available which make this possible. Ultrasonography [1] and computerized axial tomography (CAT) [2] have both been used to measure the cross-sectional area of the quadriceps muscle after knee injury and/or immobilization; the relative merits of these two techniques have also been discussed [1].

Provided there is no infiltration by some other tissue, the size of a muscle depends on the size of the muscle fibres and on the total number of fibres in the muscle. Theoretically, muscle wasting may therefore be due to shrinkage of muscle fibres, or to a reduction in their total number, or to some combination of the two processes. Schemes of therapeutic exercise have been proposed [e.g., 3] on the basis of the changes which they are known to produce in muscle fibre cross-sectional area. These schemes are relevant only if the wasting is due to a reduction in fibre

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size: they are not relevant if a significant part of the wasting is due to loss of muscle fibres.

The present study examines whether the reduction in the size of the quadriceps which follows knee injury is associated with a commensurate change in the size of its constituent fibres. It therefore asks, indirectly, whether there is any evidence of a change in the number of fibres in the quadriceps. A preliminary account has been published elsewhere in abstract form [4].

Patients

Patients were recruited from the outpatient clinics which they were attending after suffering unilateral knee injury. They were asked if they would participate in the study if the clinic measurement of their mid-thigh circumference showed a discrepancy of at least 2 cm. Patients with a history of injury to the other lower limb within the preceding 2 years were excluded, as were any with clinical evidence to suggest denervation, muscle disease or inflammatory joint disease.

A total of 15 patients (11 males, four females) agreed to take part in the study. The presence of ice artifact in the biopsies from one male patient meant that meaningful fibre area measurements were not possible in his case. All the results reported refer therefore to the other 14 patients. They had a wide range of underlying orthopaedic diagnoses, all involving an insult to the joint and various degrees of immobilization of the joint (Table 1).

Bilateral measurements of mid-thigh quadriceps cross-sectional area were also made on 12 normal males (median age 32 years, range 21–48) and 13 normal females (median age 25 years, range 19–47). The maximum voluntary isometric quadriceps strength of the normal subjects was also measured [5] in order that patients' uninjured limbs might be compared with the stronger limbs of the normal subjects.

The study was carried out under the auspices of the Nuffield Sector Ethics Committee, Oxford and the Committee of Ethics of Clinical Investigation at University College Hospital, London.

Experimental subjects were asked to signify their 'informed consent' to the needle biopsy procedure by signing a consent form which included a statement that the study was being carried out for research purposes and was not required for the management of their condition.

Methods

Whole muscle cross-sectional area

Bilateral transverse ultrasound scans were made through the quadriceps muscle group at a point (hereafter referred to as 'mid-thigh') halfway between the greater trochanter and the lateral joint line of the knee. Scanning was performed with the patient supine and the leg to be scanned supported at an angle of 5° with the knee extended and the muscles relaxed.

The scan image was photographed to give an ultrasonogram which was about half life size.

TABLE 1. *Description of patients studied*
PAT, Pes anserinus transfer.

Patient no.	Diagnosis	Age (years)	Sex	Difference in mid-thigh circumference	
				cm	%*
1	Patellar tendinitis	44	M	1.8	3.6
2	Knee injury + plaster cylinder	23	M	1.8	4.2
3	Knee injury + plaster cylinder	25	M	2.1	4.7
4	Torn meniscus	23	M	1.6	2.9
5	Torn meniscus	27	M	2.0	3.8
6	Meniscectomy	19	F	4.3	8.6
7	Meniscectomy + PAT	33	F	3.6	8.4
8	Meniscectomy + PAT + recurrent effusions	19	M	7.0	16.8
9	Medial collateral ligament repair	23	M	2.8	6.6
10	Medial collateral ligament repair	35	M	2.5	5.2
11	Arthrotomy (for loose body)	19	F	1.8	3.7
12	Arthrotomy (after gunshot wound)	32	M	3.0	6.1
13	Fractured tibial plateau	29	F	2.7	6.3
14	Fractured tibia + Küntscher nail	25	M	4.5	10.0

* (Uninjured – injured) × 100/uninjured.

Four transverse ultrasonograms were made at the mid-thigh level of both lower limbs of each patient. The outline of the quadriceps group was traced from each ultrasonogram and the tracing was then cut out and weighed. The cross-sectional area of each quadriceps group (CSA) was taken to be the mean of the weights of the four scans. Repeated sets of four scans, made on successive days, have shown the coefficient of variation for CSA to be 4%. The scanning technique and the repeatability of the measurements have been described in more detail elsewhere [1].

Muscle fibre cross-sectional area

Needle biopsies were taken from the lateral part of each quadriceps at the mid-thigh level with a UCH muscle biopsy needle of outside diameter 4.5 mm [3, 6]. The direction and depth of insertion of the biopsy needle was such that biopsies were taken from deep in the lateral mass of the muscle, in the same coronal plane as the femur. It seems likely, therefore, that in most cases the fibre samples were taken from vastus intermedius [1, 7].

If the procedure was well tolerated by the patient, duplicate biopsies were taken: duplicate biopsies were taken from 10 of the 28 limbs and single biopsies were taken from the other 18 limbs.

After preliminary orientation under a dissecting microscope [8], specimens were rapidly frozen in isopentane cooled to its melting point by liquid nitrogen. Transverse cryostat sections were prepared from each biopsy and stained to show myosin adenosine triphosphatase activity at pH 9.4 [9] to identify type I and type II muscle fibres.

The mean area of each type of fibre was calculated after measuring 100 fibres of each type with the MOPPET planimetry system [10]. The relative frequency of the two fibre types was counted in each biopsy from a minimum of 200 fibres. The overall mean fibre area (MFA) was then calculated for each quadriceps of each patient. Previous work in this laboratory, with paired biopsies from 30 quadriceps, has shown that the 'within-quadriceps' coefficient of variation for MFA is 16%. Very similar figures have been reported by Thorstensson *et al.* (16–17%) and by Halkjaer-Kristensen & Ingemann-Hansen (15–20%) [11, 12].

All muscle fibre area measurements were made without knowledge of the limb from which they had been taken and without knowledge of

the corresponding whole muscle CSA measurements.

Results

Quadriceps cross-sectional area

The male patients were similar to the normal men in respect of the absolute values for the CSA of the quadriceps of their better limbs (Table 2) (i.e. their uninjured and stronger limbs respectively). The female patients and the normal women were also similar in this respect, although one of the patients had a smaller quadriceps CSA in her uninjured limb than any of the normal women had in their stronger limbs.

In the 25 normal subjects, the difference in quadriceps CSA was usually small (median value for weaker/stronger = 100%; range = 90–108%). The CSA of the quadriceps of the patients' injured limbs was always smaller than that of the contralateral muscle (median = 78%; range = 54–89%).

Muscle fibre cross-sectional area

None of the biopsies showed any abnormality apart from a reduction in fibre size. In particular, the muscle fibres retained their closely packed arrangement. In all 14 patients, the injured limb's quadriceps MFA was smaller than that for the uninjured limb (median = 72%; range = 49–96%). Therefore, in each case, the injured limb's reduced quadriceps CSA was associated with a reduced mean fibre area (Fig. 1).

The uninjured limbs' MFA values cover a similar range to those of similarly aged, normal men and normal women previously studied in our laboratory (Table 3). The normal men had a greater mean MFA but, of course, had not been matched with the patients for body size or for habitual physical activity.

Biopsies from the patients' wasted quadriceps had a slightly lower percentage of Type I fibres ($x = 39\%$) than those from the contralateral

TABLE 2. Quadriceps cross-sectional area at mid-thigh in normal men and women and in the uninjured limb of male and female patients with unilateral thigh muscle wasting

	Cross-sectional area (cm ²)		
	Mean	Range	n
Normal men (stronger limb)	77.2	64–89	12
Male patients (uninjured limb)	80.0	51–104	10
Normal women (stronger limb)	59.1	49–69	13
Female patients (uninjured limb)	52.8	43–57	4

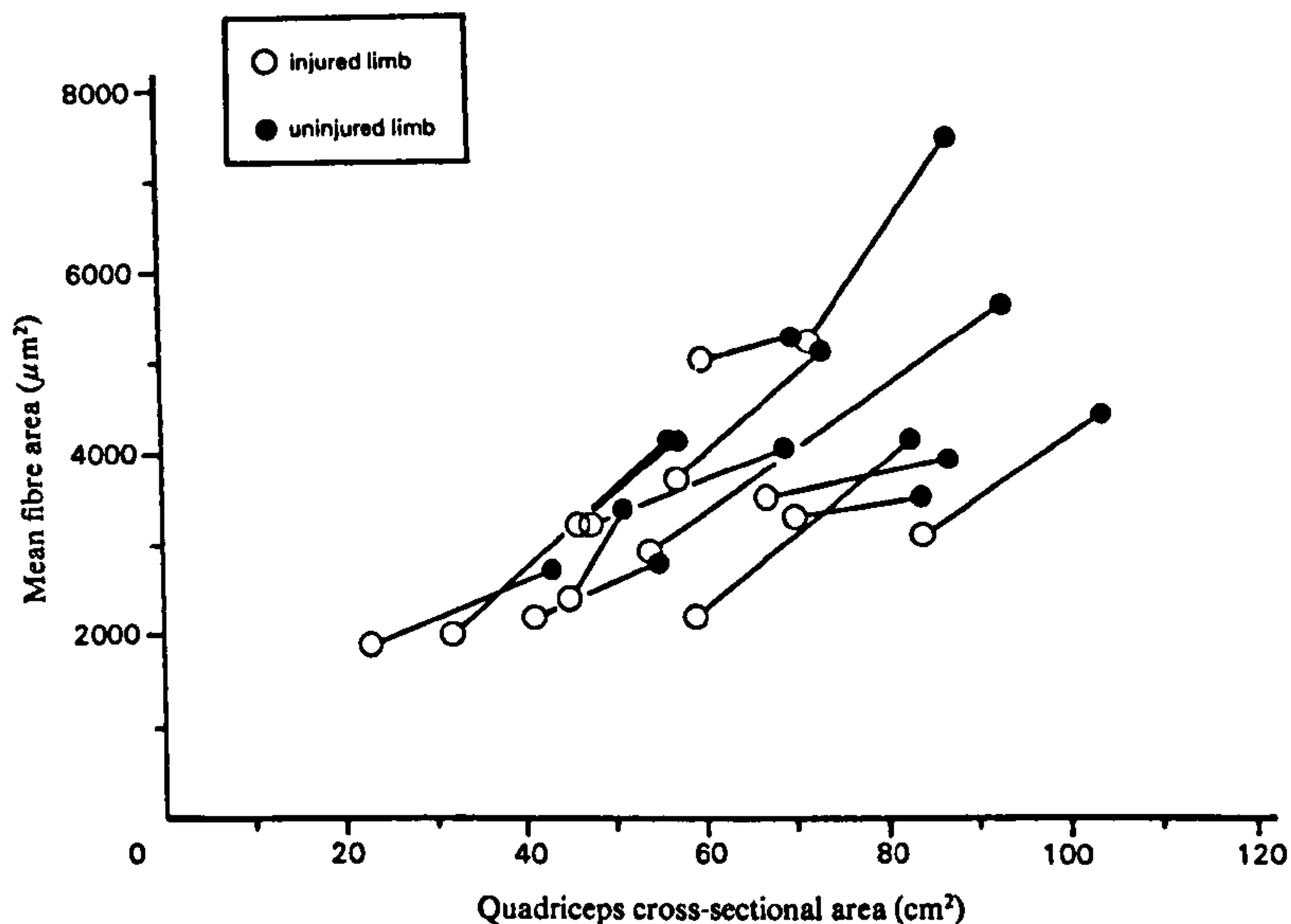


FIG. 1. Bilateral measurements of quadriceps cross-sectional area and quadriceps myofibre cross-sectional area, all made at mid-thigh, in 14 patients with unilateral thigh muscle wasting.

TABLE 3. Mean fibre cross-sectional area in needle biopsy specimens taken at mid-thigh from the lateral quadriceps of normal men and women and from the uninjured limbs of male and female patients with unilateral thigh muscle wasting

	Mean fibre area (μm^2)		
	Mean	Range	n
Normal men (either limb)	5621	3680–7220	21
Male patients (uninjured limb)	4669	3370–7400	10
Normal women (either limb)	3490	1900–5130	14
Female patients (uninjured limb)	3411	2780–4100	4

muscles ($x = 46\%$). This small, but statistically significant difference ($P = 0.03$, two-tailed Wilcoxon signed-ranks test) is now the subject of further investigation.

Dramatically selective type II fibre atrophy was seen in one biopsy: the ratio MFA II/MFA I was only 0.37 for the biopsy from the wasted quadriceps of patient no. 7. A lesser degree of selectivity was evident in 10 other patients: in seven the ratio MFA II/MFA I was less in the injured limb by ≥ 0.1 (implying predominantly type II atrophy) and in three the ratio was greater in the injured limb by ≥ 0.1 (implying predominantly type I atrophy).

Number of muscle fibres

If the CSA of a whole muscle is divided by its MFA, the quotient is a function of the number of

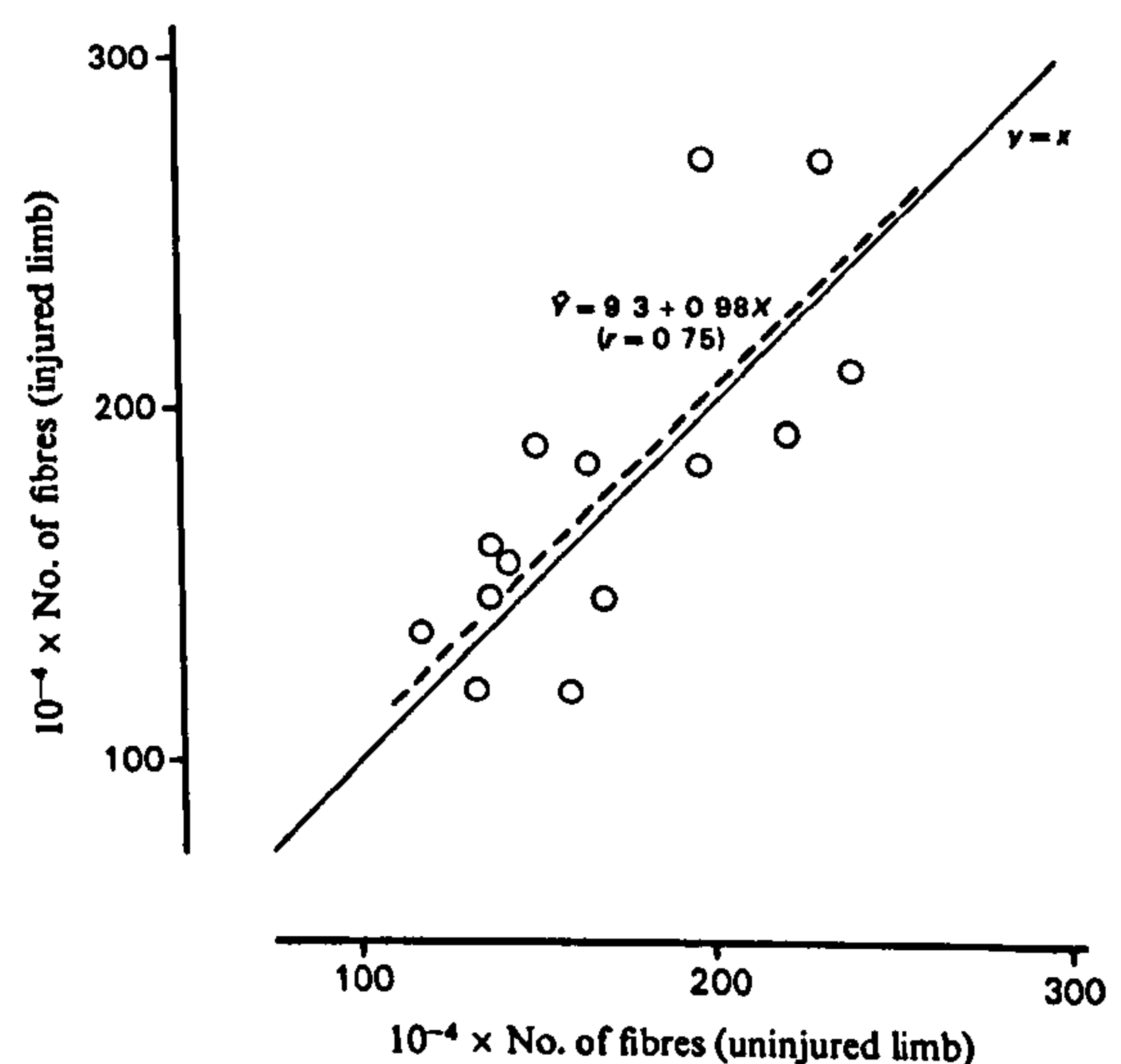


FIG. 2. Comparison of the 'number of fibres' (CSA/MFA) in each quadriceps of patients with unilateral thigh muscle wasting. ----, Regression line calculated by Bartlett's three-group method for model II regression [13].

fibres it contains. It is therefore possible to compare the number of fibres in the quadriceps of each injured limb with the number in the corresponding uninjured limb (Fig. 2).

The ratio CSA/MFA varied considerably from patient to patient (mean \pm SD: uninjured limb = $(173 \pm 40) \times 10^4$; injured limb = $(178 \pm 48) \times 10^4$). There is a close correlation, however,

between the values of CSA/MFA obtained for the two limbs of each patient ($r = 0.75$, $P < 0.005$). Moreover, the regression of CSA/MFA_{Injured} on CSA/MFA_{unInjured} (viz. $\hat{Y} = 9.3 + 0.98 X$, calculated by Bartlett's three-group method for model II regression [13]), is virtually indistinguishable from the line of identity. The 95% confidence limits for the regression coefficient are quite wide (0.44 – 1.61) but it is extremely unlikely ($\beta < 0.01$) that a 'type II' error is concealing a between-limbs difference in fibre number big enough to account for the observed between-limbs difference in quadriceps CSA.

Discussion

Our patients had quadriceps muscle wasting which was much more severe than might have been expected from thigh circumference measurements alone. This is partly explained by subcutaneous fat concealing the severity of wasting, but it is largely due to localization of the wasting to the quadriceps itself and relative sparing of the other thigh muscles [1]. The microscopic findings suggest that myofibre shrinkage can account for all of the quadriceps wasting demonstrated by the scans: there is no need to invoke hypoplasia as a contributing factor.

The number of fibres in a muscle may not be fixed. Under some circumstances, it seems that immobilization may alter the number of myofibres in a muscle. Cast-immobilization of both hind limbs of rats for 4 weeks reduced the number of fibres in soleus [14]. In contrast, another study with rats showed that cast-immobilization of the ankle for 6 weeks reduced the myofibre size in soleus by more than it decreased the weight of the muscle [15]. Since the joint was immobilized in the neutral position, the discrepancy between the change in muscle weight and that in MFA seems more likely to be the results of an increased fibre number than an increased fibre length.

Myofibre hyperplasia may occur in other circumstances. Cross-sectional studies have demonstrated that the number of fibres in a cross-section of the semi-tendinosus muscle of dogs and horses [16] and in the gastrocnemius of the rat [17] increases during growth. In a longitudinal study with cats, Gonyea [18] showed that a 44% increase in the weight of flexor carpi radialis, produced in response to high resistance, weight-lifting exercise, was due to approximately equal increases in fibre size and fibre number. Gonyea's technique for estimating fibre number has, however, been severely criticized by Gollnick

et al. [19]; their painstaking study failed to show any evidence of hyperplasia in rat muscles hypertrophied by synergist ablation, with and without exercise. Gollnick's criticisms of Gonyea's technique do not invalidate the approach used in our study to examine fibre number.

Only three comparisons of fibre size and whole muscle size *in vivo* have been reported for man [20–22]; all have used computed axial tomography. Biceps brachii was the muscle used in one study [20] because its fibres run parallel to the long axis of the muscle. The ratio of muscle size to fibre size therefore represented the true number of fibres per muscle cross-section, and the values obtained were similar to those previously reported for actual fibre counts made on stillborn infants.

The only full-length report [21] demonstrated a close linear relationship between the CSA of vastus medialis plus vastus intermedius and the MFA of fibres taken from the same mass of muscle. The authors concluded that this indicated a surprisingly constant number of fibres in their nine male subjects, chosen for their widely differing habitual levels of physical activity. Their between-subjects coefficient of variation for 'number of fibres' was 12%, much lower than in the present study but the same as our between-legs/within-subjects variation. The discrepancy is hard to explain, but suggests that the contribution of vastus lateralis plus vastus intermedius to the CSA of the whole quadriceps varies from person to person. It also emphasizes that in studies such as this it is important that the experimental design should allow each subject to act as his own control. This principle was applied in a study of two patients undergoing minor knee surgery, studied before and after a period of rehabilitation exercise [22]. The relationship between MFA (vastus lateralis) and CSA (whole quadriceps) apparently remained constant for both legs.

Our conclusions from the present study depend on four important assumptions:

- (1) that the relationship between quadriceps CSA and MFA in the uninjured limbs at the time of the study is similar to that in the other limb before it was injured;
- (2) that the biopsied fibres are representative of those in all four heads of the quadriceps;
- (3) that measurements at mid-thigh are representative of the whole length of the muscle;
- (4) that both quadriceps have the same myofibre length.

We shall discuss these assumptions in turn.

The male patients' uninjured limbs had quadriceps muscles with CSA similar to those obser-

ved in normal men and, as expected, the women had rather smaller muscles. The two quadriceps of each normal man had approximately equal CSA. Similarly, fibre sizes observed in the patients' uninjured limbs are similar to those of normal men and normal women previously studied in our laboratory (Table 3). Previous work in our laboratory has also shown that normal young men are nearly symmetrical in respect of quadriceps MFA [23]. It is impossible to know the pre-morbid condition of the uninjured limbs without measurements made at or before the time of injury, and adequate matching of the patients' uninjured limbs with the limbs of normal subjects seems impractical. Nevertheless, the evidence suggests that it was reasonable for us to treat the patients' uninjured limbs as being approximately representative of the pre-morbid condition of their injured limbs.

How justifiable is our second assumption, that the biopsied fibres are representative of those in the rest of the quadriceps, at the same level? The variability of MFA measurements obtained from duplicate biopsies taken through the same skin incision is high (see the Methods section). This suggests that there is inhomogeneity of fibre size distribution within a relatively small volume of muscle in the deep part of the lateral quadriceps. This variability must be considered when evaluating the results of a study such as this, as must the possibility of a systematic difference in MFA between the biopsied region and the rest of the quadriceps at the same level. Very little information is available to answer this question.

Polgar *et al.* studied six cadavers and measured the lesser diameters of muscle fibres from seven different sites in the quadriceps [24]. Unfortunately, they give no indication of the levels in the length of the quadriceps at which the specimens were taken. Calculations of MFA based on their data in both this and a companion paper [25] show the seven values of MFA to be very similar in four sites (deep and superficial vastus lateralis and medialis) and slightly higher in the other three sites (all in rectus femoris). Altogether, the seven values range only from 89 to 114% of their average value.

The same authors also demonstrated a clear tendency for vastus lateralis, vastus medialis and the lateral part of rectus femoris to have a higher percentage of type II fibres in their surface layers than in their deep layers [25]. Measurements made on a biopsy from deep in the quadriceps could, therefore, result in an overestimate of the MFA for the whole muscle in a patient with selective type II fibre atrophy (or an underestimate in a patient with type I atrophy). This

would mean under- and over-estimates respectively of the number of fibres in the muscle. This will be a significant source of error in the present analysis only if a patient shows a difference between the two quadriceps in respect of the relative sizes of the two fibre types. In eight patients the ratio MFA II/MFA I is greater in the uninjured limb by ≥ 0.1 and in three patients it is less by ≥ 0.1 . These two subgroups of patients, however, cannot be differentiated in respect of the distribution of their points in Fig. 2. Allowance for this potential source of error would tend to increase the similarity in 'fibre number' between the limbs of six patients and reduce the similarity in five.

The third assumption on which our conclusions depend is that differences measured at mid-thigh are associated with similar differences along the whole length of the muscle. This was tested in only one patient (no. 11): the injured/uninjured limb ratios for quadriceps CSA at mid-thigh, 7.5 cm proximally and 7.5 cm distally were 71, 72 and 76% respectively. Another patient, similar to those in this study, has proved to have injured/uninjured limb ratios of 59% and 56% at mid-thigh and 9 cm distally respectively.

The relative contribution of each head of the quadriceps to the CSA of the whole muscle varies along the length of the thigh. Preferential wasting of one head could therefore result in scans at different levels varying in the extent to which they show a reduction in CSA of the whole quadriceps.

It was only occasionally possible to define the complete boundary of individual heads of the quadriceps in the scans of both legs of a patient. It is therefore very difficult to say whether the overall wasting was equally severe in each head. Nevertheless, we were able to compare the injured/uninjured limb ratio for the CSA of rectus femoris with that for the CSA of the rest of the quadriceps at the same level in two patients. The ratios were 65 and 81% for patient no. 6 (mid-thigh) and 82 and 70% for patient no. 11 (7.5 cm proximal to mid-thigh). Halkjaer-Kristensen *et al.* reported that in two patients who had undergone minor knee surgery the lateral portion of the quadriceps showed 'about the same extent' of atrophy as the rest of the muscle [22]. They also reported elsewhere that rectus femoris showed the same degree of 'hypotrophy' as the rest of the muscle [2].

Insofar as it is possible to draw any general conclusions from these very limited data, it seems that measurements of changes in quadriceps CSA at mid-thigh may be representative of events over the whole length of muscle.

In a pennate muscle it is theoretically possible for changes in whole muscle cross-sectional area to result from changes in fibre length without any change in fibre area. Since the quadriceps is pennate, the fourth major assumption implicit in our calculations is that each wasted muscle has retained the same fibre length as its fellow. This may not be the case: a muscle can gain or lose sarcomeres if it is immobilized in a stretched or shortened position [26, 27]. At the time of the study six of the 14 patients lacked more than 20° of knee flexion on the injured side, by comparison with the uninjured joint. If this had been associated with a significant shortening of the muscle fibres in the wasted muscle, these patients' wasted quadriceps might have been expected to have had lower values for CSA/MFA than their contralateral muscles. In fact, five of these six patients had a small 'number of fibres' in both quadriceps, but there was no evidence of any tendency in these patients for the wasted muscle to have the smaller 'number of fibres'. Therefore, although it is possible that our fourth assumption may not have been entirely accurate, it seems unlikely that any small loss of sarcomeres has significantly affected our results.

Under the conditions of this study it seems that the quadriceps wasting produced by knee injury is largely (perhaps entirely) due to the reduction in quadriceps MFA. There is no evidence for a change in the number of fibres in the muscle. The clinical significance of these findings is that the appropriate therapeutic exercise for such patients can be chosen simply on the basis of its ability to increase muscle fibre size, and there is no need to look for a form of exercise that will increase muscle fibre number in man.

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THE EFFECT OF HIGH-RESISTANCE TRAINING ON THE STRENGTH AND
CROSS-SECTIONAL AREA OF THE HUMAN QUADRICEPS

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Running head: Quadriceps hypertrophy and strength

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ABSTRACT

Seventeen volunteers performed unilateral strength-training of the quadriceps with high-resistance, low-repetition, dynamic exercise, thrice weekly for an average of 5 weeks. Both before and after the training period, bilateral measurements were made of isometric quadriceps strength, quadriceps cross-sectional area (by ultrasound scanning), and thigh circumference.

There were no significant changes in the untrained thighs. The trained quadriceps increased their isometric strength by more than they changed their cross-sectional area (mean increments = 15% and 6% respectively). Quadriceps hypertrophy was underestimated by measurements of thigh circumference and could not be predicted from them.

We conclude that studies of localized muscle growth require direct measurements of the size of the muscle(s) concerned. Nevertheless, these may still underestimate the improvements in strength produced by high-resistance training.

Key words: muscle, hypertrophy, training, strength, ultrasonography, growth, cross-education

INTRODUCTION

Studies of the growth of human muscle in response to strength-training have been limited by their dependence on limb circumference measurements as an index of muscle growth. We have previously demonstrated the inadequacy of this approach for the evaluation of quadriceps atrophy [1] and there is good reason to believe that the same is true for studies of quadriceps hypertrophy [2]. Changes in the cross-sectional area of an individual muscle may be obscured by the presence in the limb of fat and of other muscles whose size may not be changing or could even be changing in the opposite direction.

With modern grey-scale ultrasonography it is not difficult to obtain an image of a transverse section through the quadriceps from which the cross-sectional area of the whole muscle (CSA) can be accurately measured [1]. We have used the technique to compare the changes in the isometric strength and the CSA of the quadriceps femoris muscle of normal volunteers performing high-resistance training ("strength-training"). Some of the results of this work have already been described in a preliminary communication [3].

SUBJECTS

Seventeen normal adults (including two of the authors) volunteered for this study. They comprised six men and eleven women (Table 1). All except one were members of staff of the Oxford hospitals or were students in the School of Radiography. The best pre-training strength values for fifteen of the subjects lay within the previously described normal range [4]. One female subject was just below the normal range and one male subject (active in recreational cycling and endurance running) was substantially above the normal range for his body weight. Four other subjects participated regularly in recreational or

TABLE 1

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competitive exercise but the pattern of their results did not distinguish them from other subjects. All subjects were asked not to alter their habitual level of physical activity in any way other than the prescribed training exercise.

None of the subjects had suffered an injury to either lower limb necessitating immobilization of a joint for more than a week within the preceding two years.

ETHICS

The study was carried out with the approval of the Nuffield Sector Ethics Committee, Oxford. Subjects undergoing needle biopsy of muscle signed an explanatory form of consent.

METHODS

Isometric strength

The isometric strength of each quadriceps was measured with the subject seated in an adjustable, straight-backed chair, the pelvis secured by a broad strap, the lower leg dependent, and the knee at 90° . On each occasion that the strength of the quadriceps was measured, it was recorded as the best of at least three maximal, voluntary contractions (MVCs). The force of an MVC was measured at the ankle and was defined as the maximal force maintained for at least one second. This procedure has been described and discussed in detail elsewhere [4,5].

The strength of each quadriceps of each subject was measured in this way on 3 occasions before the beginning of training and again on 2 occasions after the end of training. Two subjects had their second and third pre-training measurements taken on the same day and 3 subjects made both sets of post-training measurements on the same day. Otherwise, the measurements were made on consecu-

tive days. The change in strength over the training period was calculated for each quadriceps as the difference between the greatest pre-training MVC and the greatest post-training MVC.

At least 24 hours (usually 48) elapsed between the last training session and the first set of post-training strength measurements.

Whole muscle cross-sectional area

Bilateral transverse ultrasound scans were made through the quadriceps muscle group at "mid-thigh" - i.e. half-way between the greater trochanter and the lateral joint line of the knee. Scanning was performed with a conventional diagnostic ultrasound scanner with grey-scale attachment (Nuclear Enterprises, "Diasonograph NE 4200"). Subjects lay supine, with the thigh to be scanned supported at an angle of 5 degrees and the knee extended [1].

The scan image was photographed, giving an ultrasonogram which was about half life-size. Four transverse ultrasonograms were made at the mid-thigh level of both lower limbs of each subject on one occasion before training and on one occasion after training. The pre-training scans were done on the same day as the third set of pre-training strength measurements and the post-training scans were performed between the two sets of post-training strength measurements. The outline of the quadriceps group was traced from each ultrasonogram and the area of each tracing was then measured with the aid of a MOP planimeter (Reichert-Jung). The CSA of each quadriceps group was taken to be the mean of the four values obtained on that occasion, subject to a calibration factor derived from equivalent photographic records of different separations of the scanner's electronic calipers.

For the pre-training scans, mid-thigh was identified with the aid of a stadiometer. The mid-thigh level was marked and its position with respect to naevi, scars or other skin blemishes was recorded on a transparent sheet held against the thigh. This sheet was then used to re-locate the mid-thigh level for the post-training scans [6]. This procedure has reduced the coefficient of variation for measurements of quadriceps CSA made on consecutive days from 4% [1] to < 2% (Stokes & Young, unpublished work).

The scans were all measured without knowledge of whether they were obtained before or after the training and without knowledge of the corresponding strength measurements.

Thigh circumference

Bilateral measurements of thigh circumference were made at the mid-thigh level on one occasion before training and on one occasion after training. A flexible tape measure was used and measurements were made with the subject standing in a relaxed position with the feet 9 to 12 inches apart. Post-training measurements were made in ignorance of the equivalent pre-training values.

Muscle fibre composition

Seven of the subjects (4 men, 3 women) agreed to undergo needle biopsy of the quadriceps before and after the training. Biopsies were only taken after all the other pre- and post-training measurements had been obtained. Duplicate biopsies were taken on 11 occasions and single biopsies on the other 3 occasions.

Muscle biopsies were taken, at the mid-thigh level, from the lateral part of the quadriceps of the training leg. A UCH muscle biopsy needle of outside diameter 4.5mm was used [7,8]. The direction and depth of insertion of the biopsy needle was such that biopsies were taken from deep in the lateral mass of the muscle, in the same coronal plane as the femur. It seems likely, therefore, that the samples were usually taken from vastus intermedius [1,9].

After preliminary orientation under a dissecting microscope, specimens were rapidly frozen in isopentane cooled to its melting point by liquid nitrogen. Transverse cryostat sections were prepared from each biopsy and stained to show myosin adenosine triphosphatase activity at pH 9.4 [10] to identify type I and type II muscle fibres. Three specimens were omitted from further analysis because of ice artefact.

The mean cross-sectional area of the muscle fibres in each biopsy was calculated from measurements of the mean cross-sectional area of each type of fibre and the relative frequency of the two fibre types. The former were obtained by measuring 100 fibres of each type with the MOPPET planimetry system [11] and the latter by counting a minimum of 200 fibres.

All muscle fibre area measurements were made without knowledge of the subject from whom they had been taken.

Training

Each subject trained one quadriceps three times a week for a total of 11-20 sessions (mean = 16). The training exercise comprised knee extension against resistance, from 90 degrees of flexion to full extension and a controlled return to 90 degrees of flexion (Fig. 1). Each repetition therefore included both a concentric and an eccentric component. Each exercise session comprised three sets of

FIG. 1

the training weight was kept as heavy as possible. Whenever a subject was successful in completing three sets of 8 repetitions, the training weight was increased and he/she returned to attempting three sets of six repetitions.

The quadriceps training apparatus(Fig.1) was such that the knee was not subject to distraction (as occurs with a de Lorme boot) nor was there any risk of excessive forced flexion (as may occur with "squat" or "leg press" exercises). This was to ensure that the post-training strength measurements were not limited by knee discomfort precipitated by the training exercise; the problem had arisen in a pilot study where vigorous knee-extension training has been performed without the use of such an apparatus .

During the performance of the training exercise, the untrained thigh was supported by the training bench, with the knee flexed and the lower leg suspended freely (Fig. 1). During a maximal effort there was a tendency for subjects to flex the knee of the untrained side.

Whenever possible, the object was that subjects should train their dominant limb in order to reduce the likelihood of increased strength measurements resulting from the acquisition of skill. Fifteen of the subjects considered themselves to be right dominant. In the event, 13 subjects trained the dominant limb and 2 trained the stronger but non-dominant limb. The other 2 subjects trained the weaker and non-dominant limb because, during the initial strength-testing, they experienced hip or knee discomfort in the dominant limb.

Statistics

Comparisons were evaluated by the Wilcoxon matched-pairs signed-ranks test (two-tailed).

RESULTS

Pre-training measurements - both limbs

TABLE 2

The results for quadriceps strength, quadriceps CSA, and thigh circumference before the start of training are summarized in Table 2.

Evidence of an early increase in the recorded MVC was sought by comparing the best MVC recorded for each quadriceps during each of the three pre-training strength tests. There was no significant difference in strength over the three days for either the leg which was to be trained or for the leg which was to remain untrained.

Post-training measurements - untrained limb

TABLE 3

There was no significant change in the size or strength of the untrained quadriceps nor in the circumference-squared of the untrained thigh (Table 3).

Post-training measurements - trained limb

On the trained side, there were highly significant increases ($P < 0.001$) in quadriceps strength, quadriceps CSA and thigh circumference-squared (Table 3).

FIG. 2

The training-induced changes in quadriceps CSA were under-estimated by the circumference measurements ($P \neq 0.001$) and could not be predicted from them (Fig. 2). The changes in quadriceps CSA were insufficient to account for the increases in knee-extension strength ($P < 0.001$) and it was not possible to predict the magnitude of the strength gain from the degree of quadriceps hypertrophy (Fig. 3).

FIG. 3

The trained muscles of the 7 subjects who underwent needle biopsy showed changes in strength and CSA which were similar to those of the group as a whole. Their mean change in muscle fibre cross-sectional area (6%) was

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similar to their mean change in CSA (8%) but the variability was considerable (range = -31% to + 37%). Changes in mean fibre area were due to equal changes in the mean areas of type I and type II fibres (% increase in type II fibre size = $0.1 + 1.0 \times$ % increase in type I fibre size; $r = 0.92$).

DISCUSSION

Although one study [6] failed to show any change in isometric knee-extension strength after training thrice weekly for 7 weeks, most other authors [e.g. 12-15] have reported isometric strength gains similar to those in the present study (Table 3), after roughly comparable training periods.

The striking finding of this study was the confirmation that the changes in quadriceps CSA were insufficient to account for its increased isometric strength. Numerous other authors [e.g. 16-20] have claimed that appropriate training can increase strength by more than it increases muscle bulk but the evidence upon which such claims have been based is open to considerable criticism. In most cases, the size of the individual muscle groups concerned was not measured, limb circumference being recorded instead.

Only two previous studies have actually attempted to compare training-induced changes in muscle strength with directly measured changes in the cross-sectional of the muscle group concerned. Using ultrasonography, Ikai and Fukunaga [21] demonstrated a 23% increase in the cross-sectional area of the anterior compartment of the upper arm in five men whose elbow-flexion strength had increased by 92%. The other study [6] tried to use ultrasound scanning to examine the effect of strengthening exercise on the quadriceps. Quadriceps CSA was not measured directly but was estimated by measuring skin to bone thickness at 33° intervals around a 100° sector of the

antero-lateral thigh. This study failed to demonstrate any increase in the CSA of the trained muscle or, indeed, in its isometric strength. The claimed increase in dynamic knee-extension strength is very questionable since it was based on an improved performance in the "squat" - a weight-lifting manoeuvre which requires a considerable contribution from the plantarflexors of the foot and the extensors of the hip and spine. The timing of these contributions can make a large difference to the weight lifted - a fact well known to weight-lifters and remarked on by Tanner [22].

What possible mechanisms are there whereby strength might be increased more than cross-sectional area? Previous authors [e.g. 14,20] have suggested that strength-training may result in the ability to increase motor-unit recruitment in a maximal voluntary contraction. They have based their argument on an increase in the integrated surface EMG signal recorded during a maximal voluntary contraction. This interpretation presupposes that we understand the effect of increased muscle bulk and decreased subcutaneous fat on the surface EMG signal which is recorded. This is not the case. Also against this interpretation of their findings is the conclusion drawn by Merton [23] for adductor pollicis and by Edwards and his colleagues [4,24,25] for the quadriceps femoris from their studies of force generation and/or metabolic heat production during both voluntary contractions and contractions produced by supramaximal electrical stimulation. These studies indicated that, in an isometric contraction, the well-motivated subjects' maximal, voluntary effort will activate a muscle as fully as supramaximal, electrical stimulation of its motor nerve.

As was emphasised by Ikai and Steinhaus [26], a good level of motivation is critical. While no tangible rewards were offered to our subjects, we made every effort to elicit their maximal cooperation. Testing sessions

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took on a competitive atmosphere. Nevertheless, it is clear that we cannot ignore the possibility that our subjects acquired the ability to increase their maximum voluntary level of motor-unit activation. It seems likely, however, that they would have learned such a skill early in the study; indeed, Moritani & de Vries [20] claim that this is the case. It was with this in mind that we measured strength on 3 consecutive days prior to the start of the training programme. There was no detectable increase in strength over these three test occasions. Warshal's demonstration of an increase in isometric strength over three consecutive test days [27] is easily explained by her definition of "strength" as the mean of the 3 MVCs in each test. The present subjects made some very tentative MVC efforts the first time they were tested but their best MVC did not change over the three days.

Might it be that our mid-thigh measurements of quadriceps CSA have failed to detect hypertrophy elsewhere in the thigh which was sufficient to account for the 15% mean increase in strength? Vastus medialis does not run the length of the whole muscle and has its greatest relative bulk (45-50% of local CSA) at a level distal to mid-thigh (where it is 20% of CSA). Could the discrepancy between the mean change in quadriceps strength and the mean change in its mid-thigh CSA be explained by relatively selective vastus medialis hypertrophy, or by selective hypertrophy of the other three heads of the muscle? (The other three heads are each well represented at mid-thigh and run most of the length of the muscle; for the purposes of this argument they can therefore be considered together.) At the distal level where vastus medialis has its greatest relative bulk, total quadriceps CSA is about 80% of its value at mid-thigh. For a 6% increase in mid-thigh CSA to be compatible with a 15% increase in total quadriceps mass, it is necessary to postulate a 60-90% hypertrophy of vastus medialis plus a 5-15% atrophy of the other three heads, or vice versa. Neither postulated explanation seems likely. Indeed, in the eight subjects where the scan definition allowed measurement of the amount of hypertrophy of vastus medialis, there was no significant change in its contribution to quadriceps CSA (\bar{x} = 19.95%, SD = 3.12 before training and 19.71%, 1.52 after training; P = 0.7, paired t test).

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There is a suggestion from animal studies [28], that type II fibres may be able to generate more force/cross-sectional area than some type I fibres. If this is also true for the human quadriceps, preferential hypertrophy of type II fibres would result in an increment in strength greater than that in muscle CSA. Cross-sectional studies of established athletes [29,30] certainly suggest that strength-training should produce a predominantly type II fibre hypertrophy but there is only slender evidence of this in human longitudinal studies [15,31,32]. The biopsy data from the present study showed no evidence of selective hypertrophy of either fibre type. Indeed, the biopsy data merely demonstrated the variability inevitable when measuring fibres in repeated biopsies. (Even when paired biopsies are taken through the same incision and processed together, the coefficient of variation for measurements of mean fibre area is 16% [33].)

The observed discrepancy between the increments in strength and CSA might be explained if there had been a selective increase in the proportion of the muscle CSA comprising myofibrils. We have no data on this point and there is evidence in the literature both to support and to challenge such a suggestion. Some animal studies provide biochemical [34] and morphological [35] evidence of an increased concentration of myofibrillar material. This has not been seen in man; in contrast, 6 men who performed strength-training for 6 months, producing a 91% increase in elbow-extension strength with only a 31% increase in mean fibre area in triceps brachii, had the same myofibrillar volume-density in pre-and post-training electron micrographs [36]. Penman [19] went a step further and suggested that increased strength might be accompanied by a closer packing of myosin filaments within each fibril. This explanation also remains a possibility although the data on which it rests were derived from only 3 subjects and could also be explained by a processing artefact.

Finally, the disparity between the mean gains in quadriceps strength and its CSA might be explained if the initial effect of training was "not to produce hypertrophy in the muscle as a whole but to consolidate the tissue". This was the explanation proposed by Goldspink [37] when he found that weight-lifting increased

the weight of mouse muscles more than it increased the girth of the muscle fibres. The myofibres within a fascicle of untrained muscle was already very closely packed but fibre hypertrophy might conceivably result in compression of interfascicular connective tissue so that the degree of hypertrophy was not reflected by the change in whole muscle CSA. Goldspink's finding, however, is unusual. For example, in rat muscles hypertrophied by a combination of synergist-ablation and running, the changes in myofibre dry weight and in whole muscle dry weight were very similar to the changes in whole muscle wet weight [38]. Goldspink's interpretation therefore does not seem a likely explanation for our observation. The biopsy data from our study might have helped resolve this question. The mean fibre hypertrophy was very similar to the mean change in whole muscle CSA but the individual figures were too variable to permit complete rejection of Goldspink's suggestion.

'Cross-education'

The untrained limbs of our 17 subjects did not show a significant change in their isometric strength, quadriceps CSA or mid-thigh circumference. There was therefore no evidence of a 'cross-education' effect, whereby unilateral strength-training is said to enhance the strength of the contralateral limb [e.g. 14,16,39,40]. A large and carefully conducted study, however, has also failed to demonstrate any 'cross-education' effect on isometric strength [41]. The absence of a 'cross-education' effect in the present study may simply reflect the relatively short training period (and therefore the relatively small increments in strength observed even in the trained limb). More probably, it is because our subjects were not permitted any support under the foot of the untrained limb while performing the training exercise; we had previously found that subjects given such support tended to contract the 'untrained' quadriceps while straining with the opposite muscle. Indeed, one of the studies which reported a cross-education effect [39] described the subjects "gripping the table with the leg of the contralateral side".

Conclusions

Our findings have important implications for both clinical and experimental studies of muscle strength and muscle growth. First, the training-induced changes in quadriceps bulk were underestimated by, and could not be predicted from, the measurements of thigh circumference. Just as for quadriceps wasting [1], the extent of quadriceps growth cannot be inferred from thigh circumference measurements. Studies of muscle growth must include a direct measurement of the size of the muscle(s) concerned. Second, strength-training can increase the isometric strength of a muscle by more than can be explained by the increase in its cross-sectional area - a conclusion often suspected but previously demonstrated in only a single study of 5 subjects [21]. The mechanism for this 'extra' gain in strength is uncertain but the practical implication is clear. In order to monitor the treatment of a patient with muscle weakness, there is no completely adequate substitute for measurements of strength as an index of progress. When strength measurements are impossible (e.g. following injury), measurements of muscle mass may be useful but they may underestimate the strength gains which result from appropriate exercise.

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	n	median	(range)
<hr/>			
AGE (yr)			
men	6	30	(24-48)
women	11	23	(19-28)
WEIGHT (kg)			
men	6	76	(65-85)
women	11	63	(50-73)

TABLE 1. Experimental subjects

	Trained			Untrained	
	n	median	(range)	median	(range)
<hr/>					
Strength (N)					
men	6	592	(451-761)	558	(527-695)
women	11	378	(347-511)	363	(321-505)
Quadriceps CSA (cm ²)					
men	6	81.4	(76.0-88.5)	77.9	(71.3-88.9)
women	11	59.4	(52.5-69.3)	60.9	(50.5-69.0)
Thigh circumference (cm)					
men	6	51.3	(48.8-55.0)	50.9	(49.6-54.5)
women	11	48.9	(45.0-56.4)	49.2	(45.6-58.7)

TABLE 2. Pre-training measurements of isometric knee-extension strength, quadriceps cross-sectional area (at mid-thigh), and thigh circumference (at mid-thigh)

	Trained			Untrained	
	n	median	(range)	median	(range)
Strength	17	14.8	(4.7 to 39.2)	0.0	(-14.3 to 11.3)
Quadriceps CSA	17	5.6	(1.7 to 12.9)	0.0	(- 5.0 to 4.9)
(Thigh circumference) ²	17	3.0	(0.4 to 11.6)	1.3	(- 6.4 to 6.0)

TABLE 3.

Percentage increments in isometric knee-extension strength, quadriceps cross-sectional area and thigh circumference-squared following a period of unilateral strength-training

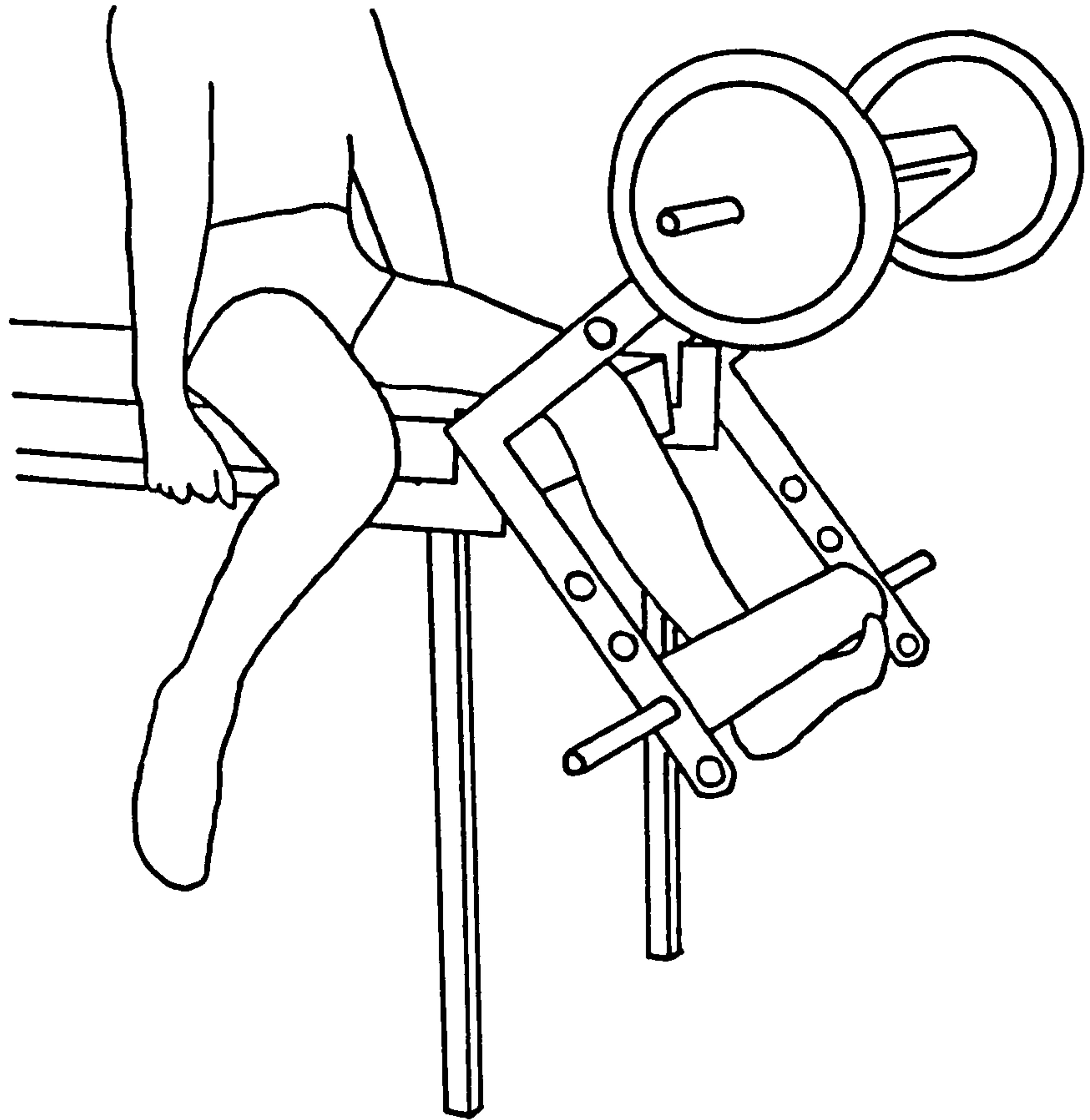


FIG. 1. Apparatus used for unilateral strength-training of the quadriceps.

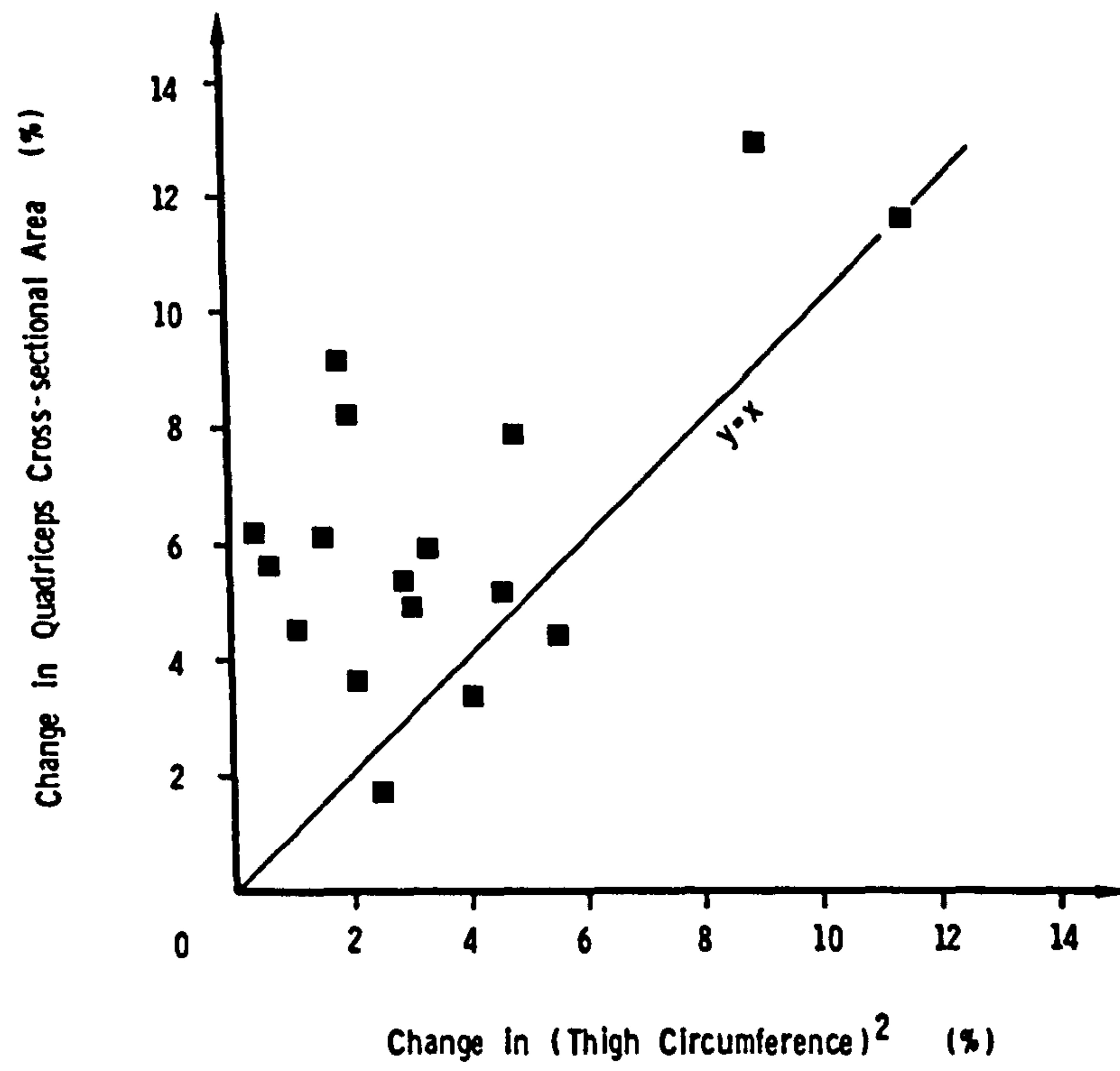


FIG. 2. Training-induced changes in quadriceps cross-sectional area and thigh circumference-squared (both measured at mid-thigh).

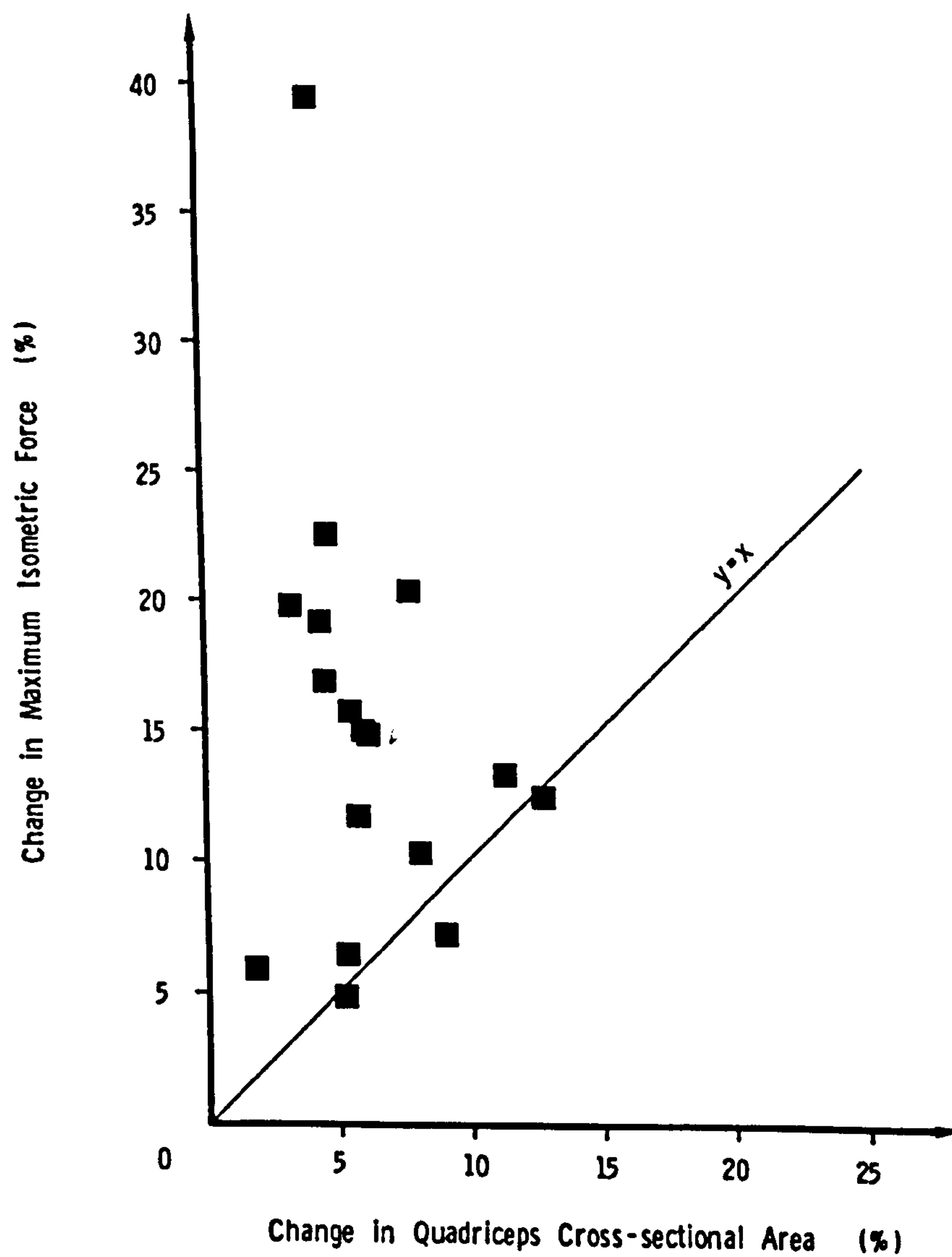


FIG. 3. Training-induced changes in quadriceps strength and cross-sectional area.