Decrease in human quadriceps muscle protein turnover consequent upon leg immobilization


Departments of Physiology, Orthopaedic Surgery, Medicine and Anatomy, The University, Dundee, and Division of Clinical Sciences, Clinical Research Centre, Northwick Park Hospital, Harrow, Middlesex, U.K.

(Received 15 July/17 September 1986; accepted 20 November 1986)

Summary

1. Quadriceps muscle protein turnover was assessed in the post-absorptive state in six men immediately after the end of unilateral leg immobilization (37 ± 4 days) in a plaster cast after tibial fracture. A primed-constant intravenous infusion of L-[1-13C]leucine was administered over 7 h. Quadriceps needle biopsies, taken bilaterally at the end of the infusion, were analysed for muscle protein leucine enrichment with 13C.

2. Quadriceps muscle protein synthetic rate, calculated from the fractional incorporation of [13C]leucine into protein compared with the average enrichment of blood α-ketoisocaproate, was 0.046 ± 0.012%/h in the uninjured leg, but was only 0.034 ± 0.007%/h in the quadriceps of the previously fractured leg (P < 0.05, means ± SD).

3. Muscle RNA activity (i.e. protein synthetic rate per RNA) fell from 0.27 ± 0.08 µg of protein synthesized h⁻¹ µg⁻¹ of RNA in the control leg to 0.14 ± 0.03 µg of protein synthesized h⁻¹ µg⁻¹ of RNA in the immobilized leg (P < 0.02).

4. Immobilization was associated with a significant atrophy of type I muscle fibres (mean diameter 69.5 ± 21 µm immobilized, 81.1 ± 18 µm control, P < 0.05), but no significant change occurred in type II fibre diameter. Mean quadriceps fibre volume calculated from the values for fibre diameter and percentage of each fibre type, was smaller in the injured leg by 10.6%; this value was near to the calculated difference in muscle thigh volume (calculated from thigh circumference and skin-fold thickness) which was less by 8.3%.

5. From estimated mean daily values for quadriceps protein synthetic rate (1.65 ± 0.44%/day in the control legs and 1.22 ± 0.28%/day in the injured legs) and change in fibre volume, mean daily muscle protein breakdown rates were calculated as 1.65%/day and 1.53%/day respectively, suggesting that muscle protein breakdown was not enhanced and may have fallen.

6. The results suggest a decrease in muscle protein turnover during limb immobilization in man, with the decrement in muscle mass being due mainly to a substantial (25%) depression of muscle protein synthesis.

Key words: fractured tibia, immobilization, protein breakdown, protein synthesis, skeletal muscle.

Abbreviations: APE, atoms percent excess; α-KIC, α-ketoisocaproate.

Introduction

Quadriceps wasting occurs during knee joint immobilization with a consequent loss of muscle force on remobilization. Histological analysis of muscle from human leg immobilization has shown no evidence for a change in the number of muscle fibres per unit cross-sectional area, but there is a significant decrease in muscle fibre diameter after leg immobilization [1, 2]. The muscle atrophy must be the result of an alteration in the balance between muscle protein synthesis and breakdown, but no information on the pattern of the changes presently exists.

We have some information from animal studies on the relative contribution of muscle protein synthesis and breakdown to muscle atrophy, by
measurement of the rate of incorporation of radioactive tracers into muscle, and the rate of loss of protein from muscle. Seider and co-workers concluded from a series of studies on immobilized rat gastrocnemius muscle in vivo [3, 4] that a fall in the rate of protein synthesis, rather than an increase in the rate of protein breakdown, was the predominant mechanism of atrophy of the muscle during the first week of immobilization. This finding was consistent with earlier studies in vitro [5] which revealed a significant decline in protein synthesis and unchanged breakdown during early soleus immobilization in a shortened position (less than 2 days), although breakdown rate was increased when reviewed after more than 2 days of immobilization. The reliability of the soleus preparation in vitro for measurement of absolute and relative rates of protein turnover has, however, been questioned [6]. No data concerning protein turnover are available from immobilization of human leg muscle.

Most of our current information regarding the control of skeletal muscle turnover has come from measurement of turnover rates in animals (e.g. [7–9]) and two studies in man [10, 11]. These studies provide considerable evidence that in skeletal muscle protein synthesis is, physiologically, the primarily controlled variable; it is facilitative of the observed changes in protein mass and muscle protein breakdown is adaptive to changes in synthesis, since the breakdown changes largely limit the extent of the effects of changes in synthesis alone. Furthermore, the mechanisms also seem to operate, at least in chronic wasting, pathophysiological [12].

In order to provide information about the possible mechanisms of atrophy during limb immobilization in man, we have measured quadriceps muscle protein synthetic rate in patients with unilateral tibial fracture, using stable-isotope labelled leucine as a tracer. From the relative differences in muscle fibre volume and protein synthetic rate and protein concentration between the control and immobilized legs, we have made estimates of rates of muscle protein breakdown.

Some of the data reported in this paper appeared in a communication presented to the British Orthopaedic Research Society in Bradford on 16 September 1985.

Methods

Patients

Six men (mean age 36 years, range 19–57 years, 71 ± 10 kg body weight, means ± SD) presented to the Orthopaedic Unit, Dundee Royal Infirmary, with a simple tibial fracture were recruited for study. Three patients had fractured their dominant leg and three the non-dominant. The fractures were manipulated and immobilized in a long-leg plaster cast for a period of 5 weeks (37 ± 4 days). Each patient was supplied with crutches and instructed not to apply weight on the injured leg until the study had been performed. Ethical approval was granted by local University and Health Board Committees according to the Helsinki Declaration and each patient gave informed consent.

Procedures

All patients were studied at rest after a 12 h overnight fast and remained fasting during the study. t-[1-13C]leucine (1 mg/kg) and NaH13CO3 (0.16 mg/kg) both 99% 13C (Tracer Technologies Incorporated, Newton, MA, U.S.A.) were administered as a priming bolus followed by an infusion of t-[1-13C]leucine at the rate of 1 mg h–1 kg–1 [13]. A cannula in the contralateral forearm, maintained patent with a slow saline (150 mmol/l NaCl) infusion, was used for blood sampling. Muscle biopsies were taken after 7 h of infusion (using the 5 mm Allendale needle; Northern Hospital Supplies, Edinburgh, U.K.), from the vastus lateralis of both quadriceps muscles, 15 cm proximal to the knee joint and at a standard depth of 5 cm [14].

Expired gas was collected over 7 min into 100 litre Douglas bags on three occasions at 2 h intervals. The first ventilation was used to accustom the patient to the technique. Expired volumes were measured with a dry gas meter (Harvard Apparatus Ltd, Edenbridge, U.K.) and production of CO2 estimated from the CO2 concentration (measured by infra-red absorbance gas analyser; Grubb Parsons Ltd, Newcastle, U.K.). Variability of the expired CO2 values between the second and third bags was 6.1 ± 5.0% (mean ± SD, n = 6). The enrichment of expired CO2 with 13C was measured from samples collected hourly into evacuated glass tubes (Vacutainer, Slough, U.K.) by isotope-ratio mass spectrometry [15] using the Delta D instrument (Finnigan MAT, Hemel Hempstead, U.K.). Thigh muscle volume was calculated in each patient from measurements of thigh length, circumference at various points and skinfold thickness [16], assuming a bone volume equal to 11% of total bone and muscle volume in the control leg, and identical bone volumes on each side [17].

Biochemical analysis

Plasma α-ketoisocaprate (α-KIC) labelling is more likely to represent the labelling of the intracellular leucine pool, from which precursor leucine is removed by protein synthesis, than plasma leucine [18, 19]. From the α-trimethylsilyl-
quinoxalinol derivatives of α-KIC [20] the level of enrichment with 13C was determined after high resolution capillary gas chromatography, by electron-impact selected-ion monitoring mass spectrometry (GCMS 10/20B, Finnigan MAT, Hemel Hempstead, U.K.) [21]. Alkali soluble muscle protein from fat-free (60% petroleum ether extracted) freeze-dried biopsy samples was hydrolysed and leucine was separated by preparative gas chromatography (W. Read & D. Halliday, unpublished work). Leucine-CO2 was liberated by ninhydrin at pH2, dried and its enrichment measured in an isotope-ratio mass spectrometer (VG micromass 602D) after trapping of solid CO2 in a cold finger cooled with liquid nitrogen [22]. Whole body protein synthesis and breakdown rates were calculated from the mean values of plasma leucine flux, oxidation and dietary input [11, 13] over the last 5 h of the study. Muscle protein synthetic rates were calculated [11] from the increase in incorporation of 13C into the leucine of the muscle protein from an assumed basal level of enrichment of 0.0054±0.0005 atom percent excess (APE). The basal value was that measured in muscle samples taken from four age-matched non-infused control patients.

Muscle RNA, DNA and alkali soluble protein concentrations were determined by standard methods [23–25]. The rate of protein synthesis per unit of RNA [26] was determined as an index of the "RNA activity" or efficiency of protein synthesis.

Histology

Biopsied muscle samples were orientated, mounted on cork discs (OCT Compound; Miles Laboratories, Slough, U.K.) and rapidly quenched in melting isopentane (cooled in liquid nitrogen). Unfixed cryostat sections (10 μm) of each sample were processed to reveal actomyosin ATPase activity at pH 9.4 using the technique of Mabuchi & Sreter [27]. The muscle fibres were classified by the intensity of their ATPase reactivity into type I (weak staining) and type II fibres (moderate to strong staining). From photomicrographs of each ATPase-stained specimen, the mean lesser diameter of about 100 transversely cut fibres of each type was determined using an Imagan semi-automatic image analyser (Graphics Information Systems, Blairgowrie, Perthshire, Scotland, U.K.). A ruled graticule was used to calibrate the system. Elliptical and distorted fibres were excluded from the measurements.

Statistical analysis

Mean values ± sd are given. The values from immobilized legs were compared with those from the normal legs by using Student’s t-test for paired data.

Results

To allow calculation of the results the assumption was made that both the free and protein bound amino acid pools remain constant in size during the infusion. Mean plasma leucine concentration was 112±6.5 μmol/l, and individual plasma enrichments of α-KIC with 13C varied by less than 1.0 APE at plateaux of 4.4–6.4 APE over the period of the study, confirming that the assumption was reasonable. Plasma leucine enrichment with 13C was not routinely measured, but in single samples taken at the end of the infusion leucine enrichment was on average 18% greater than that of α-KIC. Values for components of whole body leucine turnover, i.e. plasma flux, leucine oxidation and leucine removed by whole body protein synthesis (Table 1), were similar to those from previous studies for post-absorptive men [11, 13].

Measurement of total thigh volume showed a mean difference between the control and immobilized legs of 7.5±2% (6594±903 vs 6108±824 ml, P<0.01), after the period of immobilization. Calculated muscle volume differed by 8.3±3.1% (4841±521 vs 4436±500 ml, P<0.01). There was no significant change in muscle volumes of the thighs of control legs over the period of immobilization (4931±476 vs 4841±521 ml), suggesting that no hypertrophy due to increased weight-bearing occurred. Histological analysis of the muscle biopsies (Table 2) showed a significant (P<0.05) difference of 14.3% in mean type I fibre diameter between the two legs. There was no significant change in type II fibre diameter with immobilization, nor in the relative fibre proportions (about 53% type I).

Muscle protein concentration (Table 3) was identical in the control and immobilized legs at the time of study. Muscle DNA concentration

### Table 1. Whole body leucine turnover in six patients studied after 37 days' leg immobilization

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucine flux (Q) (μmol h-1 kg-1)</td>
<td>132±20</td>
</tr>
<tr>
<td>Leucine oxidation (O) (μmol h-1 kg-1)</td>
<td>47±16</td>
</tr>
<tr>
<td>Leucine removed by synthesis (S) (μmol h-1 kg-1)</td>
<td>84±23</td>
</tr>
<tr>
<td>Dietary intake (D) (μmol h-1 kg-1)</td>
<td>0</td>
</tr>
<tr>
<td>Leucine from breakdown (B)/(Q–D) (μmol h-1 kg-1)</td>
<td>132±20</td>
</tr>
<tr>
<td>O/Q (%)</td>
<td>36±11</td>
</tr>
<tr>
<td>S/Q (%)</td>
<td>64±11</td>
</tr>
</tbody>
</table>
(expressed per mg of protein) was significantly higher in the injured legs (Table 3). Muscle RNA concentration (also expressed per mg of protein) was slightly greater (P<0.1) in muscle from the immobilized leg with no significant change in RNA/DNA ratio.

Muscle protein synthetic rate was 0.046±0.012%/h in the control leg but only 0.034±0.007%/h in the fractured leg (P<0.05). The difference apparently resulted mainly from a decrease in muscle RNA activity (µg of protein synthesized h⁻¹ µg⁻¹ of RNA) with the value in the control leg double the value in the immobilized leg (P<0.02) (Table 3).

Calculation of muscle protein breakdown

From the alteration in mean fibre diameters and proportion of each fibre type, if we assume that the fibres maintained a constant length (a robust assumption for muscles immobilized at resting length), we can calculate a mean decrease in fibre volume with immobilization of 10.6±3.0% over 37 days. Thus the average rate of loss of fibre volume, assuming a constant fraction process (i.e. \[ y = y_0 e^{-kt} \] where \( y \) is the fibre volume at times 0 and \( t \), \( k \) is the fractional rate of loss and \( e \) is the base of natural logarithm) was 0.30%/day. Since protein concentration was identical in the two legs, alteration in fibre volume must have been directly proportional to changes in muscle protein mass.

In the steady state, muscle protein synthesis must be balanced by breakdown. During immobilization the rate of muscle atrophy or wasting is equal to the rate of muscle protein synthesis less the rate of loss by breakdown. Our previous studies in man have shown that muscle protein synthesis in the fed state is double that 18 h after feeding [11], and we have recently confirmed this (unpublished work). The present patients were studied after a 12 h fast and therefore the average daily muscle protein synthetic rates are likely to be no more than 36 times (i.e. average of 1 plus 2 times 24) the hourly rates, i.e. average daily values of 1.65±0.44%/day in the control leg and 1.22±0.28%/day in the injured leg. From the calculated values in the immobilized leg of muscle protein synthetic rate (1.22%/day) and the measured rate of net muscle wasting (0.30%/day) breakdown was estimated to have decreased from 1.65%/day (i.e. the same as in the uninjured leg) to 1.52%/day in the immobilized leg, a fall over the period of 37 days of approximately 8%.

**Table 2. Morphometric values from quadriceps muscle biopsies in six men after 37 days' leg immobilization**

Results are means ± s.d. Statistical significance: *P<0.05.

<table>
<thead>
<tr>
<th>Fibre type (%)</th>
<th>Control leg</th>
<th>Immobilized leg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td>53.8±15.3</td>
<td>52.8±12.7</td>
</tr>
<tr>
<td>Type II</td>
<td>46.2±15.3</td>
<td>47.2±15.3</td>
</tr>
<tr>
<td>Mean fibre diameter (µm)</td>
<td>81.1±18.0</td>
<td>69.5±20.6*</td>
</tr>
<tr>
<td>Type I</td>
<td>68.0±10.6</td>
<td>73.1±13.5</td>
</tr>
<tr>
<td>Type II</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 3. Quadriceps muscle protein synthesis**

Abbreviations: Con., control leg; Imm., immobilized leg. Statistical significance: *P<0.05 (Student's paired t-test).

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Alkali soluble protein (% wet wt.)</th>
<th>RNA (µg/mg of protein)</th>
<th>DNA (µg/mg of protein)</th>
<th>RNA/DNA</th>
<th>Protein synthesis, ( k_p ) (%/h)</th>
<th>( k_p/RNA ) (µg of protein h⁻¹ µg⁻¹ of RNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Con.</td>
<td>8.4</td>
<td>1.4</td>
<td>2.8</td>
<td>0.50</td>
<td>0.057</td>
<td>0.41</td>
</tr>
<tr>
<td>1 Imm.</td>
<td>8.2</td>
<td>3.3</td>
<td>5.1</td>
<td>0.65</td>
<td>0.046</td>
<td>0.14</td>
</tr>
<tr>
<td>2 Con.</td>
<td>6.7</td>
<td>1.8</td>
<td>2.8</td>
<td>0.64</td>
<td>0.039</td>
<td>0.22</td>
</tr>
<tr>
<td>2 Imm.</td>
<td>11.5</td>
<td>2.0</td>
<td>3.3</td>
<td>0.61</td>
<td>0.032</td>
<td>0.16</td>
</tr>
<tr>
<td>3 Con.</td>
<td>11.1</td>
<td>1.6</td>
<td>2.0</td>
<td>0.80</td>
<td>0.033</td>
<td>0.21</td>
</tr>
<tr>
<td>3 Imm.</td>
<td>12.8</td>
<td>2.1</td>
<td>2.5</td>
<td>0.84</td>
<td>0.025</td>
<td>0.12</td>
</tr>
<tr>
<td>4 Con.</td>
<td>11.3</td>
<td>1.5</td>
<td>2.7</td>
<td>0.56</td>
<td>0.047</td>
<td>0.31</td>
</tr>
<tr>
<td>4 Imm.</td>
<td>11.6</td>
<td>2.5</td>
<td>4.1</td>
<td>0.61</td>
<td>0.041</td>
<td>0.16</td>
</tr>
<tr>
<td>5 Con.</td>
<td>11.3</td>
<td>2.9</td>
<td>3.2</td>
<td>0.91</td>
<td>0.064</td>
<td>0.22</td>
</tr>
<tr>
<td>5 Imm.</td>
<td>11.1</td>
<td>3.6</td>
<td>5.4</td>
<td>0.67</td>
<td>0.033</td>
<td>0.09</td>
</tr>
<tr>
<td>6 Con.</td>
<td>14.3</td>
<td>1.5</td>
<td>2.5</td>
<td>0.60</td>
<td>0.036</td>
<td>0.24</td>
</tr>
<tr>
<td>6 Imm.</td>
<td>11.9</td>
<td>1.6</td>
<td>2.8</td>
<td>0.57</td>
<td>0.027</td>
<td>0.17</td>
</tr>
<tr>
<td>Mean±s.d.</td>
<td>Con. 10.5±2.6</td>
<td>1.8±0.6</td>
<td>2.7±0.4</td>
<td>0.67±0.16</td>
<td>0.046±0.012</td>
<td>0.27±0.08</td>
</tr>
<tr>
<td></td>
<td>Imm. 11.2±1.6</td>
<td>2.5±0.8</td>
<td>3.8±1.2*</td>
<td>0.66±0.10</td>
<td>0.034±0.007*</td>
<td>0.14±0.03*</td>
</tr>
</tbody>
</table>
In the present work we have, as in our previous studies [11], used the enrichment of $\alpha$-KIC and not that of leucine to calculate the whole body flux and muscle protein synthetic rate, on the assumption that the amino acid pool from which protein is synthesized is in equilibrium with that from which leucine is deaminated. This assumption remains unchecked since there is no information available on the relative labelling of plasma leucine, $\alpha$-KIC and leucyl t-RNA in skeletal muscle in man. However, work on isolated rat muscle suggests that not only are the intra- and extra-cellular amino acid and amino acyl t-RNA pools closely related with no more than a variation of $\pm 15\%$ about the mean of the three, but that the relationship between them alters little with alteration of metabolic status that caused substantial changes in muscle protein turnover [28]. Thus, at most the absolute errors in the measurement of skeletal muscle protein synthesis in man, introduced by an incorrect assignment of precursor enrichment in our present work, would be the difference between the $\alpha$-KIC and the plasma leucine labelling, i.e. a difference of 18%. The relative difference between the rates of protein synthesis from the control and immobilized leg would of course be unaffected by this error, unless the t-RNA charging was different between them. This possibility cannot be confirmed or denied at present.

Absolute values of quadriceps muscle protein synthetic rate (Table 3) calculated from change in $^{13}$C enrichment of the muscle leucine were, at 0.03-0.05%/h, somewhat less than those previously recorded in muscle from post-absorptive men at 0.09%/h [11]. This, we believe, was mostly due to significant improvements of methodology (e.g. by use of preparative gas, rather than ion-exchange resin, chromatography for separation of hydrolysed muscle amino acids); it was also partly due to the unconscious erroneous use of racemic (i.e. DL-) rather than L-leucine as a tracer in the previous studies. This was only discovered after publication. The present results were produced by improved techniques used throughout the series presented here. The purity and optical rotatory properties of the tracer were checked by the supplier using chiral capillary column gas chromatography–mass spectrometry and certified as such.

The results confirm those of other workers showing muscle wasting in immobilization in man and animals after casting [1, 4–6]. The wasting appears to be mainly due to type I fibre atrophy and loss of fibre protein as indicated by the significant fall in protein/DNA.

The present results indicate that during leg immobilization there occurs a 26% depression of quadiceps protein synthesis. The mean 26% difference between the muscles of the control and immobilized legs was reflected in the difference in rates of protein synthesized per unit RNA. This occurred in the absence of any significant change in RNA concentration (i.e. no differences in total ribosomal RNA content) and would indicate a decrease in transcription of DNA and/or in ribosomal activity, i.e. the efficiency of messenger RNA translation [26]. Calculations suggest there is no marked changes in muscle protein breakdown and there may actually be a fall of about 8% on average. The changes exist in the presence of normal rates of whole body protein synthesis and breakdown. The depression of synthesis is consistent with that occurring in animal models of immobilization [4] and during muscle loss in humans in other conditions of chronic muscle wasting [29].

To allow corresponding estimates of changes in net muscle wasting to be made it was necessary to assume that the control leg at 5 weeks had an equivalent protein turnover to that of the immobilized leg pre-injury. We believe this to be a reasonable assumption, but we cannot exclude the possibility of an increase in protein turnover of the muscle of the control leg stimulated by extramuscular work secondary to transmission of an increased proportion of the body weight through the leg when using crutches. Nevertheless, this was likely to have been largely offset by the negative effects of reduced mobility overall. Evidence for this comes from the fact that significant volume changes in the control leg were not recorded and there was no hypertrophy of the muscle fibres in comparison with those from other, healthy subjects of similar ages (P. J. Stoward & J. N. Gibson, unpublished work).

Calculated muscle protein breakdown rate fell slightly with immobilization. Unless protein synthesis had been underestimated by at least 40% or the degree of muscle wasting underestimated by at least 30%, the sizes of errors needed for breakdown to have actually risen, such a conclusion seems inescapable. It is quite likely that there were errors of $\pm 10\%$ in protein synthetic rate, but the estimates of muscle wasting are unlikely to have errors greater than $\pm 10\%$, making the conclusion of decreased turnover overall more secure. One factor with an unknown error bearing on the estimation of breakdown is the robustness of the relationship between protein synthesis in feeding and fasting; the factor of 1.5 used here may be in error, but this, in our experience, is not likely to be more than $\pm 20\%$. These estimates suggest that the overall relative error (i.e. root mean square of individual errors) in calculated muscle protein breakdown was unlikely to be more than $\pm 25\%$ of its calculated value.
The calculations of breakdown do require sampling of identical muscle for comparison between the two legs. Review of the reported studies on fibre type distribution in vastus lateralis [30-32], although the findings were not identical, would indicate a likely variation of the different fibre types as a function of biopsy depth, with a higher percentage of type II fibres in the surface than deep layers. In this study, muscle biopsies were taken at standard depth from the control and immobilized legs, and no significant difference in fibre proportions of muscle from the two legs was observed.

Sampling of vastus lateralis necessitated the assumption that equal atrophy occurred in all the heads of quadriceps. The limited information available from ultrasound and computerized tomography studies [2, 33] would indicate that this was true. Equal atrophy would be expected based on the knowledge of equal action in the normal leg of all the heads of quadriceps, through the whole range of knee extension [34].

The degree of muscle atrophy during immobilization has been shown in animal studies to be dependent on the length of the immobilized muscle, with atrophy occurring when muscles were fixed either at or less than resting length [35] with proportionately greater atrophy at lesser length. The standard method used to immobilize the tibial shaft fractures was with ten degrees of knee flexion, thus preventing the femur and tibia rotating and thus coming together at the fracture site [36]. Although quadriceps is marginally less than resting length in this position, resulting in probable slight underestimation of net wasting, it is difficult to accept that this alone could be as much as the 30% required to vitiate the conclusion of a fall in breakdown rate.

The present results indicate that depression of muscle protein synthesis is the dominant mechanism of quadriceps atrophy during knee immobilization after fracture of the tibia in man. Stable-isotope methods for measurement of protein synthesis may in the future be useful for monitoring the effects of different immobilization methods and remobilization regimens.

Acknowledgments

We are grateful to the consultant orthopaedic staff of the Royal Infirmary, Dundee, for permission to study their patients. Invaluable technical assistance was given by W. W. and M. Read, C. Scrimgeour and K. Smith. The work was generously supported by grants from Action Research, the Mason Medical Research Foundation, the Medical Research Council, Muscular Dystrophy Group of Great Britain, the Scottish Hospital Endowments Research Trust, the Scottish Home and Health Department, the Stanley Thomas Johnson Foundation, the Wellcome Trust and The White Top Foundation, Dundee.

References


