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The effects of injury and joint disease on muscle mass and protein turnover

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It is a great compliment to be invited by the Clinical Metabolism and Support Group of the Nutrition Society to give the first lecture in memory of Sir David Paton Cuthbertson. Over a 60-year period Cuthbertson pursued many different lines of investigation into the changes in animal and human nutrition induced by disease, producing numerous research papers and reviews. His first contributions arose from an interest in the disturbances of metabolism after orthopaedic injury and it is perhaps appropriate that this, the first Cuthbertson lecture to the Society, again addresses this important subject.

Loss of skeletal muscle protein and consequent muscle atrophy is readily observed clinically following skeletal injury and subsequent immobility, reflecting the major contribution of skeletal muscle to whole-body protein turnover. At the outset, Cuthbertson appears to have been inspired by observations of Bauer (1873) and Bernard (1877) who had noted an increased excretion of urinary nitrogen following haemorrhage, and Wertheimer & Clogne (1919) who observed rises in blood non-protein N and urinary urea following war trauma. From careful measurements of urinary catabolites, in patients immobilized after fracturing a long bone, he was able to report on the time course of N, sulphur and phosphorus loss from the body. The increments observed suggested an increased net breakdown of protein (Cuthbertson, 1930). In addition, Cuthbertson (1930) confirmed in his early studies that changes in metabolic rate correlated with losses in muscle mass and suggested that these changes could be considered in two phases following trauma. Initially (the ‘ebb phase’) an early decrement occurred in the patient’s oxygen consumption, proportional to the severity of shock, followed by a rise (the ‘flow phase’) which paralleled the increased N excretion (for review, see Cuthbertson, 1980*a*,*b*). It is only recently, with the development of more sophisticated biochemical techniques, that we have been able to delineate the relative contributions of different lean tissues to the metabolism of different amino acids, and the importance of local and systemic influences on protein metabolism required for energy production (Waterlow *et al.* 1978).

In many medical conditions, such as occur in patients with cancer cachexia, cardiac, renal and pulmonary disease, muscle atrophy is very apparent clinically. In order to define the mechanics of muscle wasting in a given condition, however, it is necessary to measure the contribution of skeletal muscle protein turnover to changes in whole-body

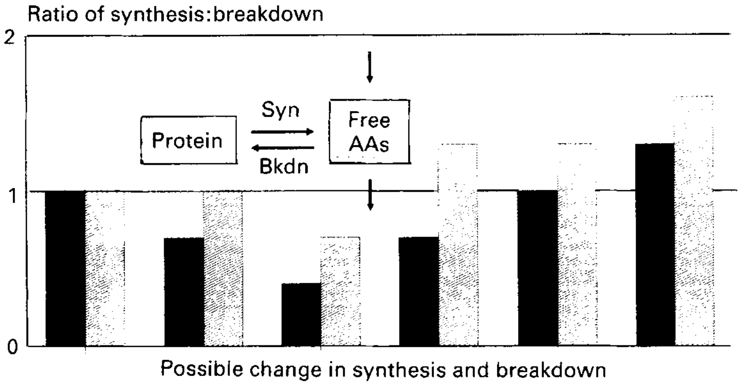

Fig. 1. Possible mechanisms of muscle protein wasting. (■), Synthesis; (▨), breakdown; AAs, amino acids.

protein metabolism (Rennie & Halliday, 1984) and specifically to determine whether muscle wasting is due to a depression in rate of muscle protein synthesis, increment in breakdown or some combination of the two (Fig. 1). Millward (1980) considers changes in muscle protein synthesis and breakdown to be either facilitative or adaptive to loss in muscle mass, depending on which was primarily either the physiologically or pathologically controlled variable. In most pathological conditions, it would appear that change in synthetic rate is the primary influence on change in muscle mass (facilitative), with changes in breakdown limiting (adaptive to) this (Rennie, 1985; Morrison *et al.* 1988, 1990). The answers to similar questions concerning muscle protein turnover in patients presenting with gross muscle atrophy after skeletal injury or joint disease is the subject of the present article.

Recent advancements in stable-isotope methodology have enabled the study of the normal and patho-physiological control of muscle mass in vivo in man. These depend on the measurement of the incorporation of infused labelled tracer into the free amino acid and protein pools, using gas–liquid chromatography–mass spectrometry and isotope-ratio mass spectrometry respectively (Halliday & McKeran, 1975; Rennie *et al.* 1982). Using [^{13}C]leucine as tracer, I, together with my colleagues, aimed initially to apply these techniques to answer the following questions:

(1) What is the mechanism of quadriceps muscle atrophy associated with complete knee immobilization?

(2) In patients with degenerative osteoarthritis of the knee who have restricted and painful knee movement, is the mechanism of quadriceps atrophy identical to that occurring with complete joint immobilization?

(3) What is the effect of a transcutaneous electrical stimulus on muscle protein turnover?

(4) In the presence of increased tonic muscle contractile activity induced by muscle stretch, is there an increment in muscle protein synthesis in vivo?

(5) Is there a pattern of change in muscle protein turnover characteristic of loss of muscle mass in patients with rheumatoid arthritis? Does the change in muscle protein synthesis directly relate to the change in muscle mass, severity of myositis and drug therapy?

GENERAL PLAN OF INVESTIGATIONS

The ability to measure muscle protein synthesis in vivo in man has been radically improved by the development of stable-isotope techniques. We can now reject the use of radioactive tracers for most clinical studies and indeed their use would be unethical in any study of the growing child (see p. 505). In the studies summarized here we have used primed, constant infusions of L-[1–^{13}C]leucine to achieve steady-state labelling of tracer in plasma, from which uptake of label occurs via the intramuscular free pool into tissue protein. To measure the muscle protein incorporation muscle biopsies were taken by open or percutaneous techniques (Coakley *et al.* 1987) after 6–10 h of tracer infusion. The incorporation of tracer (from which synthetic rate (k_s) is derived) is then calculated from an assumed starting isotopic enrichment (E; measured in control, non-infused patients) as

$$k_s = \frac{\triangle\, E_{\text{muscle}}}{E_{\text{plasma}}} \times \frac{1}{t_1 - t_0} \times 100.$$

Basal enrichments are approximately 0·005 (SD 0·0005) atoms % excess (means and SD; comparisons against our routine carbon dioxide gas standards) compared with post-infusion enrichments of 0·01–0·04 atoms % excess after 8 h infusion of [^{13}C]leucine at the rate of 1 mg/kg per h.

Some authors have underlined the need to obtain accurate information on the isotopic content of the immediate precursor pool when measuring absolute or even relative rates of muscle protein synthesis (Schneible *et al.* 1981). In the studies reported below we have routinely used the isotopic enrichment of the leucine transamination product α-ketoisocaproate to accurately reflect the labelling of the precursor pool (Matthews *et al.* 1982; Rennie & Halliday, 1984; Bennet *et al.* 1989). Certainly, rates of protein synthesis derived by this method compare well with values derived using the labelling of the intermediate muscle leucyl-tRNA pool (Watt *et al.* 1990*b*). Although measurements of leucyl-tRNA labelling have been shown not to be influenced significantly by delays in either sample freezing or the processing for preparation of labelled tRNA, suggesting that its use for subsequent calculation of protein synthesis based on it are probably robust (Watt *et al.* 1990*a*), further investigations to delineate the compartmentation of precursor leucine are required.

QUADRICEPS ATROPHY FOLLOWING JOINT IMMOBILIZATION

Initially I set out to determine the mechanisms of muscle atrophy in normal, disease-free muscle, choosing as a model patients who had an enforced period of leg immobilization in a plaster cast following unilateral tibial fracture. In the presence of muscle atrophy equivalent to a diminution of quadriceps fibre volume over 6 weeks of 10·6% relative to the uninjured leg (mainly type I fibre atrophy), we were able to demonstrate a fall in the muscle protein synthetic rate of up to 25%. It was also possible to calculate from changes in fibre diameters over the 6 weeks (negative growth), by assuming a constant fraction process (i.e. $y_t = y_0 e^{-kt}$, where y is the fibre volume at times 0 and t, k is the fractional rate of loss and e is the base of the natural logarithm) that breakdown fell by 8% (synthesis − breakdown = ± growth; Table 1, Gibson *et al.* 1987).

Table 1. *Effects of 36 d leg immobilization on quadriceps muscle protein turnover*†

(Mean values and standard deviations)

	Control leg		Immobilized leg		
	Mean	SD	Mean	SD	Difference (%)
Synthesis (%/d)	1·65	0·44	1·22**	0·28	–25
Wasting (%/d)	0·00		0·30	0·05	
Calculated breakdown (%/d)	1·65	0·44	1·52**	0·35	–8

Mean values were significantly different from control values (Student's paired *t* test): $^{**}P<0·01$.
† From Gibson *et al.* (1987).

WASTING ASSOCIATED WITH DEGENERATIVE JOINT DISEASE

After completing studies of patients with immobilization-induced wasting of muscle, it seemed appropriate to consider whether muscle disuse secondary to joint immobilization would also explain the severe quadriceps atrophy apparent in patients with knee osteoarthritis. Patients presenting to orthopaedic clinics with unilateral degenerative joint disease may be subdivided into those in whom joint destruction is such that joint replacement is required, and those with predominantly medical compartment disease, for whom a lateral valgizing realignment osteotomy of the tibia is appropriate. After osteotomy, a 6-week period of complete knee immobilization in a plaster of Paris cylinder is necessary.

We studied seven overnight fasted patients (mean age 75 (range 68–82) years) during the 8 h preceding total knee arthroplasty, comparing the results with those from seven fasted patients (mean age 58 (range 43–76) years) 40 (SD 6) d following upper tibial osteotomy and subsequent plaster immobilization of the knee. The two patient groups had a similar range of knee flexion before surgery (83 (SD 29) ° arthroplasty, 92 (SD 13) ° osteotomy). A depression in joint movement and consequently quadriceps motor activity is expected in patients with osteoarthritis, due to reflex inhibition (secondary to joint pain) and also increased mechanoreceptor discharge (due to joint effusion). The expected reduction in muscle protein synthesis was, however, only present in the group of patients following osteotomy; paradoxically we observed an increase in muscle from the side of arthroplasty (Table 2; Gibson *et al.* 1986). In explanation of the unexpected results we postulated that the patients awaiting arthroplasty are walking on an unstable malaligned joint with consequent quadriceps muscle stretch acting to maintain the rate of synthesis; muscle atrophy must consequently have been due to increased muscle protein breakdown. Presumably, in the osteotomy patients the complete immobilization of the knee by the cast removes the intermittent muscle stretch which normally occurs during walking.

TRANSCUTANEOUS ELECTRICAL STIMULATION

Following these findings, an attempt was made to determine whether the depression in quadriceps protein synthesis expected with knee immobilization, might be prevented by low levels of electrical stimulation. Electrical stimulation of muscle is not a new concept

Table 2. *Quadriceps protein synthetic rates* (k_s) *in patients with knee osteoarthritis*

(Mean values and standard deviations)

	Muscle protein/ DNA (μg/μg)		k_s (%/h)	
	Mean	SD	Mean	SD
Osteotomy + plaster				
Control leg	1379	857	0·032	0·01
Arthritic leg	768**	263	0·026**	0·01
Arthroplasty				
Control leg	665	350	0·043	0·03
Arthritic leg	246**	94	0·068**	0·04

Mean values were significantly different from control values (Student's *t* test): $^{**}P<0·01$.

(Forster & Palastanga, 1981), having been used for many years by physiotherapists to increase muscle strength during rehabilitation. There has, however, been little published information on the effects of stimulation on muscle mass and none on muscle protein turnover.

We, therefore, went back to the model of disuse atrophy following tibial fracture, to study the effects of transcutaneous electrical stimulation on quadriceps mass, composition and rate of protein synthesis. A surprisingly mild stimulus (amplitude 70 V, 300 μs square wave pulses at 30 Hz in 2 s on–9 s off cycles), applied via two electrodes placed through 'windows' in the plaster cast for 1 h each day prevented muscle atrophy in legs immobilized for 6 weeks (Gibson *et al.* 1988*b*).

The decrements in muscle cross-sectional area and protein synthesis expected with immobilization were also abolished (Fig. 2). If stimulation were to affect the rate of muscle protein synthesis alone, then lesser benefits would have been expected to accrue from its use in patients with osteoarthritis awaiting knee arthroplasty, since their quadriceps synthetic rate was already increased above normal. It was, however, possible to increase both muscle mass and muscle cross-sectional area in quadriceps on the side of prospective surgery by a similar regimen of stimulator use (Fig. 2; Gibson *et al.* 1989). Rate of muscle growth, calculated from change in fibre diameters, was similar to the rate of muscle protein synthesis at the end of stimulation, suggesting that muscle protein breakdown had fallen to a value approaching zero. So far as we are aware, this is the only condition yet reported in man in which muscle growth has been the result, not of an increase in protein synthesis, but a decrease in protein breakdown.

MULTIFIDUS MUSCLE PROTEIN TURNOVER IN ADOLESCENT IDIOPATHIC SCOLIOSIS

It is well known from studies in vitro in animals that muscle stretch is associated with increments in muscle protein synthetic rate (Buresova *et al.* 1969). In contrast, an increment in rate of muscle protein breakdown and decrement in rate of synthesis has been demonstrated in animal muscle following immobilization in a shortened position (Goldspink, 1977). It is virtually impossible to construct a satisfactory experimental

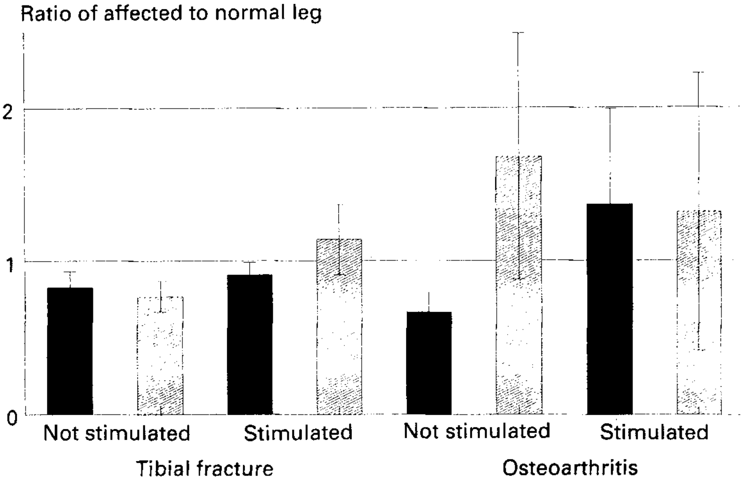

Fig. 2. Effect of 6 weeks electrical stimulation on muscle cross-sectional area (CSA; ■) and protein synthetic rate (k_S; ▨) in patients with complete knee immobilization following tibial fracture and partial knee immobilization secondary to degenerative arthritis: Measurements from the affected side are expressed as a proportion of those from the contralateral (normal) leg. Values are means and standard deviations represented by vertical bars and are calculated from those reported by Gibson *et al.* (1988*b*, 1989).

model which could be applied to measure the effects of stretch on human muscle protein turnover in vivo. However, in adolescents with an idiopathic scoliosis there is a discrepancy of tonic contractile activity in muscle of the two sides of the spine during development of the progressive lateral spinal curvature. Although initiating factors and reasons explaining curve progression appear to be multifactorial (Enneking & Harrington, 1969; Ponseti *et al.* 1976), myopathic aetiologies for curve development have been postulated (Yarom & Robin, 1979; Reuber *et al.* 1983; Ford *et al.* 1984). It is reasonable to assume that the muscle on the convexity of the spinal curve, particularly during changes in posture, is stretched relative to that on the side of curve concavity. Differences in muscle morphometry between the two sides, notably a predominance of slow-twitch fibres on the convexity (Ford *et al.* 1984) have been observed, but whether these occur primarily or occur as an adaptation to the nature and application of the deforming force is uncertain. The finding of type I fibre atrophy in deltoid muscle on the side of the concavity might less readily be explained as a secondary adaptive change (Yarom *et al.* 1982).

We found previously that in quadriceps muscle after knee immobilization, type I fibre atrophy occurred secondary to muscle disuse, with an associated diminution in rate of muscle protein synthesis. It was, therefore, a reasonable hypothesis that the converse might be true in muscle from the convex side of an idiopathic curve, i.e. in the presence of an increased proportion of type I muscle fibres and greater contractile activity a greater synthetic rate would be present. The results from the studies using stable isotopes to measure multifidus muscle protein synthesis on both sides of idiopathic scolioses in nine children (mean age 14 years 6 months, mean weight 48 (range 34–63) kg) are shown later. Table 3 shows the differences in muscle morphometry observed, which were similar to those reported elsewhere (Ford *et al.* 1984). Figs 3 and 4 show the measured

Table 3. *Multifidus muscle morphometry*†

(Mean values and standard deviations for nine determinations)

	Type I fibres			
	Diameter (μm)		Percentage	
	Mean	SD	Mean	SD
Top: Convex	49·7	10·1	51	6
Concave	56·4	9·8	47	8
Apex: Convex	50·9	8·5	63	12
Concave	38·3*	2·4	49*	9
Bottom: Convex	51·0	5·2	54	8
Concave	49·8	7·4	53	3

Mean concave values were significantly different from convex values (Student's paired *t* test): *$P<0·05$.
† Values from Gibson *et al.* (1988*a*).

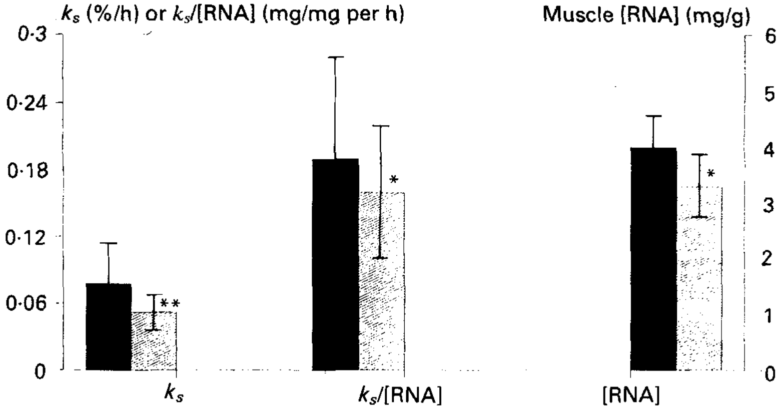

Fig. 3. Rates of multifidus muscle protein synthesis from opposite sides of scoliotic spine at apex (+/–stretch). (■), Convex side (+); (▨), concave side (–); k_s, protein synthetic rate. Values are means and standard deviations are represented by vertical bars **$P<0·01$,*$P<0·05$: (Student's paired *t* test). From Gibson *et al.* (1988*a*).

values of muscle protein synthesis in graph form and the relative rates on the two sides of the spinal curve (Gibson *et al.* 1988*a*). These results show a 50% greater synthetic rate on the convexity of the curve. Presumably the growth adaptation and strength of the muscle at the apex of the curve on the convex side is not sufficient to prevent curve deterioration and, therefore, the curve continues to progress until skeletal maturity is reached as shown in Fig. 5.

It is interesting that in clinical practice electrical stimulation has been administered to the muscle of the convexity posteriorly or laterally via surface or implanted electrodes (Axelgaard & Brown, 1983). It is difficult to conceive that electrical stimulation would have much effect on modulation of muscle protein synthetic rate since this is already increased, and indeed its therapeutic value is now questioned (Akbarnia *et al.* 1985).

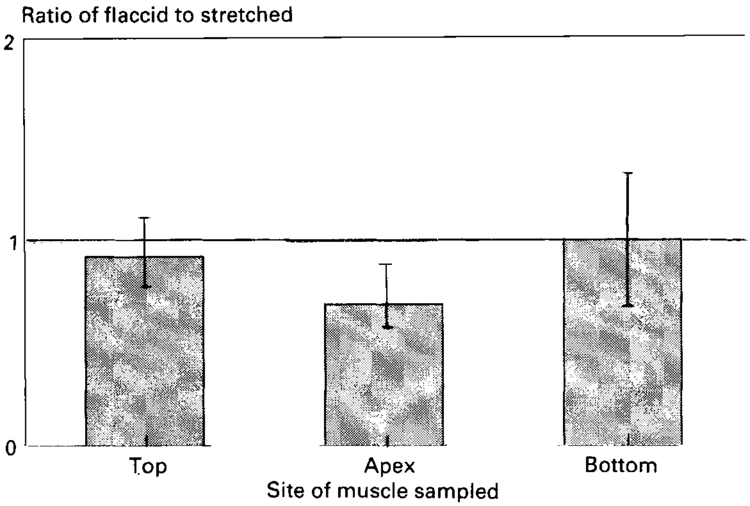

Fig. 4. Effect of degree of spinal curvature on protein synthesis in muscle. Values are means and standard deviations are represented by vertical bars and calculated from those reported by Gibson *et al.* (1988*a*).

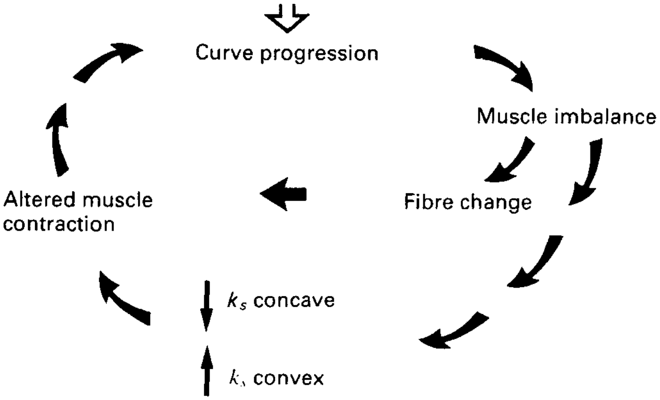

Fig. 5. Cycle of scoliosis progression. k_s, Protein synthetic rate.

STUDIES IN PATIENTS WITH RHEUMATOID ARTHRITIS

Although clinical studies relating changes in muscle protein turnover to muscle activity have extended the concepts and premises formulated by Cuthbertson, several important questions remain to be answered when alterations in muscle protein turnover in diseased muscle are considered: (1) are there differences in muscle protein turnover in the presence of inflammatory myositis or in association with inflammatory arthropathy? (2) what is the effect of the mediators of inflammation, such as interleukin-1 and prostaglandins on protein synthesis? (3) what are the dose–response relationships of amino

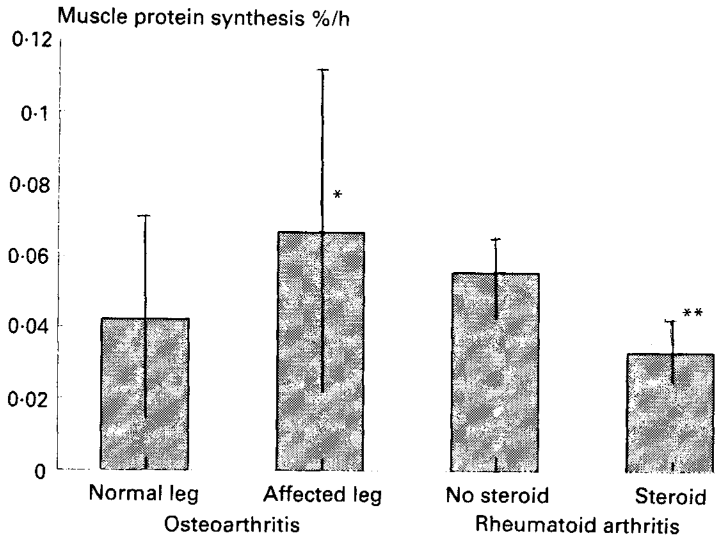

Fig. 6. Rates of muscle protein synthesis in patients with degenerative (osteo-) and inflammatory (rheumatoid) arthritis. Values are means and standard deviations are represented by vertical bars. Values for normal and affected legs were significantly different (Student's *t* test). Mean values for steroid treated and untreated rheumatoid patients were significantly different (Mann-Whitney *U* test): $^{*}P<0.5$, $^{**}P<0.01$. From Gibson *et al.* 1989, 1990.

acids, anabolic hormones and paracrine factors such as insulin-like growth factor in regulating muscle protein synthesis and breakdown?

Recent work (Gibson *et al.* 1990) has been directed at answering some of these questions. We felt it to be appropriate to study patients with classical rheumatoid arthritis (Ropes *et al.* 1958), who had been receiving corticosteroids over a prolonged period, and compare quadriceps muscle protein synthesis in these patients with that from patients who had never received corticosteroids. In patients with rheumatoid arthritis the problems of mobilization following total knee replacement are particularly acute due to the severity of myositis occurring independently of muscle disuse (Haslock *et al.* 1970). In addition, corticosteroids prescribed as immuno-suppressants are known to depress muscle protein turnover (Wool & Weinshelbaum, 1959; Odedra & Millward, 1982). Our results suggest that in patients not receiving steroids who have muscle atrophy, protein synthetic rate is maintained; this finding is similar to that observed in the patients with degenerative joint disease (Fig. 6).

Cytokine concentration is increased in tissue and synovial fluid of patients with rheumatoid arthritis (Mizel *et al.* 1981; Wood *et al.* 1983) along with an increased prostaglandin production (Kantrowitz, 1975). In addition it has been suggested that prostaglandin concentration may modulate the rate of muscle protein turnover. Certainly in vitro incubation of muscle with prostaglandin E_2 has been shown to be associated with an increase in protein breakdown and prostaglandin $F_{2\alpha}$ with an increment in synthesis (Rodemann & Goldberg, 1982). It was, therefore, interesting to note that intramuscular prostaglandin E_2 concentration was up to 100% greater in patients not taking steroids (increased breakdown) than in normal controls. In contrast patients taking corticosteroids had a markedly depressed rate of muscle protein synthesis and a 56% reduction in intramuscular prostaglandin $F_{2\alpha}$ concentration (Gibson *et al.* 1990). The exact inter-relationship between individual cytokines, prostaglandin concentration and muscle protein turnover requires further elucidation.

FUTURE INVESTIGATIONS

The development and application of tracer arterio–venous difference techniques, measurement of stable-isotope tracer incorporation and more recently micro-methods for the measurement of labelling of individual protein fractions in muscle using electrophoresis and fast protein liquid chromatography, should continue to extend our present knowledge. It is anticipated that the technology developed over the last 5 years will, after some modification, be directly applicable to the measurement of collagen turnover in human tissues, probably using a ^{15}N stable-isotope label on proline. Development of such techniques would at last allow the calculation of turnover of bone matrix in man as an indicator of osteoporosis and the assessment of the effects on turnover of prescribed oestrogen and growth hormone.

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