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REVIEW ARTICLE

Regulation of protein turnover in skeletal and cardiac muscle

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INTRODUCTION

Background

The aim of this review is to assess the current understanding of the regulation of protein synthesis and degradation in striated muscle, a tissue which constitutes the largest single element of the protein-bound nitrogen pool in the mammalian organism. Numerous experimental interventions alter protein turnover rates *in vitro* and we will discuss whether or not these might fulfil regulatory functions *in vivo*. We will also consider the molecular mechanisms that may be involved in the regulation of protein turnover.

Protein turnover is a cyclical process with synthesis of protein being opposed by its concomitant degradation. Because of this, rates of protein synthesis and degradation are considerably greater than the net flux (k_g) through the protein turnover cycle. Amino acids derived from intracellular protein degradation are reutilized for protein synthesis to a considerable extent. Thus the rate of whole body protein synthesis can be much greater than the rate of dietary influx of amino acids or the rate of nitrogen excretion. Because of the cyclical nature of protein turnover, any imbalance between k_s and k_d will lead to a change in the size of the tissue protein pool. Thus a loss of tissue protein does not necessarily entail an increase in k_d nor does a gain necessarily entail an increase in k_s.

k_s is expressed as the rate of amino acid incorporation into protein relative to tissue or protein weight, or as a fractional synthesis rate. Most measurements of k_s cited in this review refer to the overall synthesis rates of total cell protein, although the same techniques may be used to measure the synthesis rates of individual proteins when combined with suitable separation methods. k_d is expressed in a manner analogous to k_s. Since individual proteins turn over at different rates, overall values of k_s and k_d are weighted averages of the turnover rates of individual proteins. Under a given physiological condition, k_s values in the various tissues in an organism are largely proportional to the total RNA concentrations (which are expressed as RNA: tissue protein ratios, C_s). Because of this proportionality, C_s indicates the capacity of a tissue for protein synthesis. The largest pool of cellular RNA is rRNA (85% of total RNA) with tRNA and mRNA constituting about 10% and 3% of the total RNA, respectively. Division of k_s by C_s gives an estimate of the efficiency of translation (or 'RNA activity', k_{RNA}), which is essentially a measure of the mean protein synthesis rate per ribosome. This is a composite term and may be influenced by any of the many reactions involved in the translational phase of protein synthesis.

Some *in vivo* values of k_s in muscle tissues of mature female rats are shown in Table 1, emphasizing that even in fully-grown animals, rates of protein turnover are considerable. A recent detailed study has shown that there is a correlation between protein synthesis and the content of slow oxidative fibres in skeletal muscles [1]. As shown in Table 1, k_s is modulated by changing k_{RNA} and/or C_s. Because changes in k_{RNA} are rapid, they are readily amenable to investigation both *in vivo* and *in vitro*. k_{RNA} is primarily dependent on the rates of peptide-chain initiation and peptide chain elongation/termination (reviewed in [2,3]). Although rates of amino acid transport and amino

Table 1. Protein-synthesis rates in various muscles of fed or fasted mature female rats

Fully-grown mature (8–9 weeks old) female rats weighing about 200 g were used in this study [22]. The period of fasting was 48 h. The values in parentheses are the fasted values expressed as a percentage of the control values. At this age, the soleus consists of about 60% slow oxidative fibres and about 40% fast oxidative glycolytic fibres. The plantaris and gastrocnemius mainly consist of fast glycolytic and fast oxidative glycolytic fibres (about 40% of each), data taken from [367].

	k_s (%/day)		C_s (mg of RNA/g of protein)		k_{RNA} (g of protein/day per g of RNA)	
Muscle	Fed	Fasted	Fed	Fasted	Fed	Fasted
Heart	8.58	6.48 (76%)	7.24	6.09 (84%)	12.30	10.66 (87%)
Diaphragm	5.74	3.93 (68%)	5.16	4.21 (82%)	11.90	9.29 (78%)
Soleus	6.74	5.72 (85%)	4.32	3.37 (78%)	16.80	16.89 (101%)
Plantaris	4.59	2.51 (55%)	3.01	2.19 (73%)	16.34	11.61 (71%)
Gastrocnemius	4.69	2.42 (52%)	2.68	2.02 (75%)	18.81	12.15 (65%)

Abbreviations used: k_s, rate of protein synthesis; k_d, rate of protein degradation; k_g, rate of protein accumulation; k_{RNA}, efficiency of protein synthesis; C_s, capacity for protein synthesis (i.e. RNA/protein ratio); S_A, specific (radio)activity; eIF, eukaryotic initiation factor; IGF, insulin-like growth factor; 3-MeHis, N^{τ}-methylhistidine; PCr, phosphocreatine; PG, prostaglandin; T_3, tri-iodothyronine; TNF, tumour necrosis factor; TPA, 12-tetradecanoylphorbol 13-acetate; $tRNA_i$, initiator tRNA; for pH or ions, the subscript i indicates an intracellular value and the subscript o indicates an extracellular value.

acylation of tRNA could theoretically influence k_{RNA}, there is little evidence that these processes are ever rate-limiting. Analysis of ribosomal profiles on sucrose density gradients can reveal whether it is peptide-chain initiation or elongation/termination that is primarily affected by a transition in k_{RNA} [2]. Care must be taken in interpreting k_{RNA}, since a constancy in the relative proportions of the individual RNA species is often assumed. Thus, a selective alteration in mRNA content brought about by changes in RNA turnover will not result in any detectable change in C_s since mRNA is quantitatively a minor RNA species. However, k_s may change and this change could be interpreted incorrectly as an alteration in k_{RNA}. Suitable control experiments can clarify the situation. Although changes in C_s are seen *in vivo*, the duration of experiments with incubated whole muscle *in vitro* is too short for any changes in C_s (or in total protein pools) to be detectable. Reliable measurement of RNA turnover would reveal the potential for a change in C_s, but suitable techniques have not yet been fully developed. It is perhaps here that cultured cells may prove particularly useful since the culture period is often sufficiently long for changes in C_s to become detectable.

Methodological considerations

Background. The study of protein turnover is fraught with methodological difficulties. This review of methodology is relatively short but more detailed reviews have been published elsewhere [4–10]. The most important general problem is a consequence of the cycling of amino acids into and out of the amino acid and protein pools. Thus, reincorporation of amino acids derived from intracellular protein degradation may lead to underestimation of k_d, especially *in vivo*. Similarly, preferential degradation of newly-synthesized protein may lead to underestimation of k_s.

Measurement of k_s *in vitro*. Protein synthesis *in vitro* is invariably measured by the incorporation of isotopically-labelled amino acids. Phenylalanine or tyrosine is the amino acid of choice for use in muscle since neither is appreciably metabolized [11]. The use of [*side-chain*-2,3-^{3}H]-labelled phenylalanine or tyrosine should be avoided because of the rapid loss of tritium through exchange reactions [11]. Leucine has also been used. For measurements to be reliable and simple, there must be rapid and complete isotopic equilibration between the radiolabelled precursor amino acid and the aminoacyl-tRNA pool. In the heart, this can be achieved by perfusion with [^{14}C]phenylalanine at about five times the plasma concentration [12]. The S_A of the radiolabel can then be used to calculate k_s directly. At lower concentrations, the S_A of the aminoacyl-tRNA pool in muscles may rapidly attain a plateau value but this may be less than the S_A of the administered amino acid either because of transport limitations or because of preferential tRNA charging with amino acids provided by intracellular protein degradation [12–14]. In this case, k_s can only be quantified reliably if the S_A of the aminoacyl-tRNA is monitored throughout the experiment. This is difficult. Similar problems of isotopic equilibration arise in cultured cells, but these can be circumvented using a dual-labelling protocol [10,15]. The dual-labelling technique is not easily applicable to whole muscles because of the limited duration of viability.

Measurement of k_s *in vivo*. Problems of isotopic equilibration are potentially greater here than *in vitro* and measurements of the S_A of aminoacyl-tRNA are sparse. In the heart, the S_A of leucyl-tRNA and plasma leucine rapidly equilibrate (but both exceed the intracellular S_A of leucine) [16]. Two principal methods have been used to administer isotopically-labelled amino acids to man or laboratory animals, namely the constant infusion technique and the large-dose technique. With both, it is necessary to define the S_A of the plasma and tissue amino acid pools as far as possible over the course of the experiment. In the constant infusion technique, a tracer quantity of isotope is infused intravenously over a matter of hours so that the plasma amino acid pool attains isotopic equilibrium. An allowance for the pre-equilibrium state is made in the calculation of k_s. In man, a priming bolus of isotope is given to decrease the length of the approach-to-equilibrium phase. The method is relatively difficult technically and, in prolonged infusion, isotope reutilization can present a considerable problem [17]. In the large-dose technique, a large quantity of isotope is rapidly administered intravenously (preferred route) or intraperitoneally to flood the body pools [18–21]. The tissue S_A of the administered amino acid rapidly equilibrates with the plasma S_A and, at least in muscles, remains constant over the course of the experiment (usually 10–20 min) [19,22]. The large-dose method gives consistently higher values of k_s than the constant infusion method in both rat and man [20,23]. This cannot be attributed to stimulation of k_s by the high tissue concentrations of amino acid injected [19] and probably results from the more rapid equilibration of the S_A of the injectant throughout the body pools. It is difficult to assess whether the higher k_s values obtained by the large-dose method result from preferential weighting in favour of rapidly-synthesized short-lived proteins. The synthesis rate obtained by the large-dose method may also depend on the amino acid used [24]. Thus, although leucine and phenylalanine give similar results for whole body k_s (31 %/day), threonine gives 41 %/day and lysine gives 26 %/day. These results could be interpreted in terms of differential rates of equilibration of each amino acid with its aminoacyl-tRNA, although the explanation preferred by the authors is either that threonine-rich proteins turn over more rapidly than lysine-rich proteins or that the proteins in tissues which turn over rapidly are rich in threonine compared with lysine. Given these provisos, the large-dose technique is generally preferred to the constant infusion technique. It is technically simpler and, in laboratory animals, it avoids the need for prolonged restraint, a manoeuvre which itself can decrease k_s [25].

Measurement of k_d *in vitro*. Measurement of the rates of release of phenylalanine or tyrosine in the presence of cycloheximide or, less frequently, puromycin (to prevent re-incorporation of the amino acids into protein) is the soundest and simplest method for the determination of k_d in incubated muscles. Alternatively, techniques are available to measure k_s and k_d simultaneously [26–28]. Neither of these methods provides any information about the degradation of individual proteins. For this, loss of enzymic activity is potentially useful although it is dangerous to assume that loss of activity is equivalent to degradation (covalent modification, for example, could complicate interpretation). In any case, only a very few enzymes of short half-life (for example, those involved in polyamine metabolism) are amenable to this type of investigation [29]. Degradation of myofibrillar protein can be conveniently measured from release of 3-MeHis. 3-MeHis is present in actin and the myosin heavy chains of fast skeletal muscle (but not of slow-skeletal or cardiac muscle) and is formed by post-translational methylation of single specific histidinyl residues in each protein [30]. 3-MeHis is neither metabolized nor reutilized for protein synthesis in muscle. Because actin is present in a seven-fold molar excess over myosin heavy chain and because the fractional turnover of the latter is only about two-fold greater than actin, release of 3-MeHis predominantly measures actin degradation in all muscles. Additionally, two points should be made. First, care must be taken to allow for the intracellular free 3-MeHis pool which can vary in size depending

Table 2. Contributions of myofibrillar and non-myofibrillar protein degradation to overall protein degradation

Data are taken from [32], Table 4, experimental group B. Incubations were carried out in the presence of cycloheximide. The contribution of myofibrillar protein degradation to the overall k_d was calculated by multiplying the rate of 3-MeHis release by the molar ratio of tyrosine: 3-MeHis in actin (16 mol/mol) making the assumption that the degradation of myosin heavy chain does not contribute significantly to 3-MeHis release (see the text). The contribution of non-myofibrillar protein degradation to the overall k_d was calculated by subtraction of the rate of myofibrillar protein degradation from the overall k_d.

	Amino acid release from protein degradation (nmol/h per g wet wt.)			
	Overall k_d (Tyr release)	Myofibrillar k_d (3-MeHis release)	Myofibrillar k_d (Tyr release)	Non-myofibrillar k_d (Tyr release)
1-day fasted control	144	2.03	32	112
Refed complete diet (24% casein)	137	0.73	12	125
Refed protein-free diet	127	2.13	34	93

on the physiological state of the animal and which can contribute significantly to 3-MeHis release [31]. Secondly, the contribution of tyrosine or phenylalanine derived from myofibrillar degradation to the overall release of these amino acids from total protein degradation should be calculated from the known amino acid sequence of actin since this consideration can affect conclusions concerning the degradation of the non-myofibrillar protein pool. In the heart, the contribution is < 10% [31]. However, using the data of Goodman & del Pilar Lopez [32], the contribution of myofibrillar protein degradation to the overall k_d may be > 25% in skeletal muscle (Table 2).

Measurement of k_d *in vivo*. It is difficult to measure overall k_d reliably *in vivo*. Three methods have been used. The simplest approach is to measure the difference between k_s and k_g. The difficulty is that changes in k_g are small and need to be measured over a matter of days whereas k_s is often measured over a matter of minutes. The calculation rests on the assumption that k_s is constant over the time that k_g is measured. A second method measures the loss of radiolabel (for example, [^{14}C]leucine) from prelabelled proteins (reviewed in [33]). Amino acid reutilization complicates such experiments and a correction needs to be made by exposure to a second isotope (for example, [^{3}H]leucine) for a short period. The third method involves selective catherization and infusion of an isotopically-labelled amino acid across a muscle bed and is based on the isotopic dilution of infused amino acid by that derived from intracellular protein degradation [4,34–38]. Phenylalanine is particularly suitable since its only fate in muscle is incorporation into protein or release from the tissue. k_d and k_s are calculated from measuring the S_A and concentration of phenylalanine in the arterial and venous blood and, additionally, blood flow. As discussed in [34], rates obtained by this method are still minimal estimates since no correction is made for reutilization of amino acids. Furthermore, the method may be readily applicable only to larger animals because of the catheterization and blood flow measurements.

In rats and man (but not in some other species [39]), 3-MeHis (or its hepatically-acetylated derivative) derived from the degradation of myofibrillar protein is quantitatively and rapidly excreted in the urine [30]. Since skeletal muscle constitutes the largest protein-bound pool of 3-MeHis, the urinary excretion of 3-MeHis (or better the 3-MeHis/creatinine ratio to control for muscle protein pool size) has been used to measure the k_d of skeletal muscle protein *in vivo*. A problem with this approach is that the rapidly-turning-over pools of 3-MeHis in the gut (and other tissues such as skin) probably contribute to 3-MeHis excretion in a manner that is disproportionate for their size [40–44]. This is still debated [45,46]. Some of these problems can be avoided by measurement of the arteriovenous differences of 3-MeHis across limbs, but the differences are small and again the turnover of the 3-MeHis pools in the limb may be heterogeneous. Thus, overall, the problems associated with the reliable measurement of k_d frequently impede an understanding of its regulation.

Validity of measurements of protein turnover *in vitro*. Measurements of muscle protein turnover *in vitro* generally involve the use of superfused or perfused tissues, although a more novel and particularly promising approach involves the use of cultured cells. How well do superfused and perfused tissue preparations simulate the situation *in vivo*? A major problem with superfused muscles taken from growing animals is that they are in net negative nitrogen balance [26,47]. This is obviously not true *in vivo*, where k_{RNA} is generally higher and k_d is generally lower than *in vitro* [26,47]. These differences may be partly associated with the removal of humoral and physical influences present *in vivo* (see, for example, [48]). Many experiments *in vitro* are therefore 'replacement' experiments in which protein turnover rates are restored towards their *in vivo* values. One problem is that even small muscles develop hypoxic cores *in vitro* in which k_s is reduced [49–51]. Cutting muscles can also decrease k_s and increase k_d [52]. Muscles perfused through an intact circulation may fare better. In the rat heart perfused in the presence of insulin, k_s, k_{RNA} and k_d resemble the values for fed animals *in vivo* [53]. In the gastrocnemius of the perfused hemicorpus, k_s and k_{RNA} are also close to *in vivo* values [54]. Attempts to improve flow [55] or oxygenation [56] in skeletal muscle preparations have generally not been beneficial. All muscle tissues and perfused limbs contain many cell types in addition to striated myocytes (for example fibroblasts, cells of the vascular system, etc.) whose rates of protein synthesis or degradation may contribute disproportionately to the overall k_s or k_d. Caution should thus be exercised in interpreting results obtained in *in vitro* systems.

INSULIN AND THE REGULATION OF DIURNAL PROTEIN TURNOVER

Effects on protein turnover *in vitro*

Many studies have shown that insulin acutely stimulates protein synthesis and inhibits protein degradation in skeletal and cardiac muscles preparations incubated under basal conditions with glucose as a fuel. The maximal stimulation of synthesis is usually about 50–100% and maximal inhibition of degradation is about 30–50%. These responses are elicited by physiological concentrations (upper limit: 100 μunits/ml, 0.67 nM-insulin monomer) of the hormone (see, for example, [57,58]). The effects of insulin on protein turnover in muscle cell cultures are similar

to those in incubated muscles although hyperphysiological concentrations of the hormone may be required for maximal responses [59–61]. Compared with mature cells, these embryonically-derived cells express only low numbers of insulin receptors [60,62]. Also, they may not contract. These properties may affect their responses to insulin.

Insulin increases k_s at the level of translation (i.e. it increases k_{RNA}) by enhancing peptide-chain initiation (reviewed in [63,64]). Stimulation is unaffected by actinomycin D (see, for example, [65]) and is independent of the effects of the hormone on amino acid transport (see, for example, [28]). Although insulin probably stimulates the synthesis of most or all muscle proteins, it is not known whether the stimulation is uniform. Unequal enhancement has been detected in cultured NIH 3T3 cells, suggesting that the effects of insulin may be selective [66].

The mechanism by which insulin inhibits protein degradation is not understood. In the heart, it may do so by decreasing formation of autophagosomes [28]. Myofibrillar protein degradation (measured by 3-MeHis release) may not be susceptible to inhibition by insulin although there is still disagreement about this [31,67–69]. Since the overall k_d is certainly inhibited by insulin, insensitivity of myofibrillar k_d would suggest that myofibrillar and non-myofibrillar proteins are degraded by distinct pathways.

Fasting and diabetes *in vivo* and *in vitro*

Protein turnover has been extensively studied in these insulin-deficient states. It must be recognized that fasting or chemically-induced diabetes cause complex metabolic and hormonal disturbances (Table 3) and it is therefore simplistic to attribute all of the observed disturbances in protein turnover solely to the absence of insulin. *In vitro*, k_s values are depressed in muscles taken from insulin-deficient animals and they are least partly restored towards fed values by addition of insulin (see, for example, [47,70,71]). Protein synthesis *in vivo* is inhibited by insulin-deficiency induced by fasting (Table 1 and [22]) or by diabetes [72–75]. Synthesis of myofibrillar protein is especially sensitive [72,73]. Fast-twitch muscles (plantaris, gastrocnemius) are more severely-affected than slow-twitch muscles (soleus); see Table 1. In the former, inhibition is mediated by decreases in k_{RNA} through inhibition of peptide-chain initiation (which is relieved by insulin) in combination with decreases in C_s. In the latter, decreases in C_s are primarily responsible [22,70,74–77].

In vitro, overall k_d is greater in skeletal muscles taken from fasted animals [47,67,78–81]. Rapidly-growing animals show the greatest increase [67] possibly because for them a given period of starvation is more detrimental than for older animals. The overall k_d is reduced when amino acids and insulin are included in the incubation medium [47]. In contrast, fasting may cause a small decrease in overall k_d in the perfused heart [31,82] but diabetes may increase it [77,83,84]. The increase in cardiac k_d in diabetes is associated with a decrease in lysosomal enzyme latency [83,84]. There is less agreement about the effects of insulin-deficiency on muscle k_d *in vivo*. Some experiments have suggested that short-term diabetes increases overall k_d values in all skeletal muscles [69,75] whereas chronic diabetes decreases it [75]. Short-term and long-term fasting may have an analogous effect (reviewed in [85]). In contrast, other experiments have failed to detect any significant overall change in k_d during insulin deficiency [86,87]. Of the five muscle tissues studied (see Table 1) in mature female rats, we were able to detect changes in protein content only in the heart after a short-term (48h) fast [22]. This finding suggests that k_d was in fact decreased in the skeletal muscles. The inconsistencies in these various studies are probably related to the severity of the insulin deficiency, the age of the animal and the time of sampling in addition to the problem of the reliable measurement of k_d.

In vitro, the increases in myofibrillar k_d in muscles taken from insulin-deficient animals are proportionally greater than any increase in overall k_d [31,32,67,68,80,88] (but see also [87] for a contrasting view). It is difficult to assess whether insulin deficiency affects myofibrillar k_d *in vivo* and hence whether the *in vitro* observations have any physiological relevance. In an early study, dietary insufficiency decreased 3-MeHis excretion, suggesting an inhibition of myofibrillar protein breakdown [89]. In contrast, others have shown that mild diabetes increases 3-MeHis excretion [87]. Nor is it clear whether myofibrillar k_d is acutely inhibited by insulin *in vivo*. In diabetic rats, treatment with insulin decreases 3-MeHis excretion by only 10% [90]. If 3-MeHis excretion is further raised by administration of corticosterone, then insulin decreases 3-MeHis excretion by about 30%. As explained above, 3-MeHis excretion may not provide a valid measure of striated muscle protein degradation *in vivo* and results must be interpreted with this qualification in mind.

A protein pool which is preferentially degraded during insulin-deficiency is that associated with muscle ribosomes. In perfused normal hearts, insulin stimulates synthesis of both ribosomal and total proteins equally [91]. During diabetes *in vivo*, there is a selective loss of skeletal and cardiac muscle ribosomes as shown by decreases in C_s, for although synthesis of ribosomal proteins and total protein synthesis decrease in concert *in vivo* [86], there is a dramatic enhancement of ribosomal degradation in heart and gastrocnemius [86]. On administration of insulin, ribosome degradation quickly ceases and there is a slower stimulation of ribosomal and overall protein synthesis [92]. Thus although there may be little change in overall k_d in this particular model of diabetes [86], the degradation of certain species of protein may be enhanced. Since ribosomal protein degradation represents only a small fraction of the overall rate of protein degradation, its increased degradation does not contribute significantly to the overall k_d but the loss of ribosomes would significantly affect the capacity for protein synthesis.

Table 3. Changes in the plasma concentrations of hormones and fuel during insulin deficiency

Insulin	Decreased
IGF-1	Decreased
Free T_3	Decreased
Glucocorticoids	Increased
Glucagon	Increased
Lipid-derived fuels	Increased
Branched-chain amino acids	Increased

Interdependence between insulin-status and the plasma concentrations of other hormones and fuels: consequences for protein turnover

Background. Insulin stimulates k_{RNA} and inhibits k_d *in vitro*. There is good evidence that insulin-deficiency inhibits protein synthesis *in vivo*. However, there are alterations in the plasma concentrations of several hormones and fuels in insulin-deficient states (Table 3). It is thus necessary to assess the roles of these other factors in the regulation of protein turnover before the relative importance of insulin and other factors in the regulation of diurnal protein turnover *in vivo* can be considered.

Thyroid hormones. Plasma insulin and free T_3 concentrations are positively correlated and interdependent [93,94]. Thyroid hormone stimulates protein synthesis, an effect which probably is mediated by a pretranslational action of the hormone (or, less

likely, a post-translational action at the level of RNA degradation) [95–98]. Either will result in an increase in C_s. Although restoration of the euthyroid state after thyroidectomy may increase k_{RNA} [95], the effect is probably indirectly mediated through increases in insulin concentrations [94,99]. There is general agreement that thyroid hormones increase muscle k_d [97] and 3-MeHis excretion (reviewed in [4]). Regulation at the levels of lysosomal [97,100,101] and Ca^{2+}-dependent [102] proteolysis has been implicated.

Glucocorticoids. Glucocorticoid concentrations increase in insulin-deficient states. There is general agreement that glucocorticoids decrease protein synthesis. However, glucocorticoids cause insulin resistance [103] and hence effectively cause an operational deficiency of insulin. Indeed, many of the effects of glucocorticoids are analogous to those seen during insulin deficiency. Hence, pretreatment of normal or adrenalectomized rats with glucocorticoids lowers k_s, C_s and k_{RNA} in fast-twitch muscles of subsequently-perfused hemicorpora or hind-quarters [104]. Peptide-chain initiation is inhibited. Slow-twitch skeletal and cardiac muscles are less affected than fast-twitch skeletal muscles [104]. Since these experiments involved pretreatment with glucocorticoids, a direct or indirect chronic change is presumably involved. In addition, acute inhibition of protein synthesis in soleus muscle by glucocorticoids has also been reported [105] suggesting that some of the effects of glucocorticoids are direct. *In vivo*, results [106–109] are broadly consistent with the *in vitro* observations but differ in detail. Goldspink and colleagues have concluded that the primary effect of glucocorticoids on protein synthesis is mediated through a decrease in C_s [106,107] whereas others have shown that glucocorticoids decrease k_{RNA} in the face of elevated insulin concentrations [103]. Myofibrillar protein synthesis is particularly sensitive to inhibition by glucocorticoids [109].

The effects of glucocorticoids on skeletal muscle k_d are controversial. Stimulation [107,110–112], lack of effect [104,106] or inhibition [105,113] has been variously reported. Differences may be attributable in part to experimental design. Some studies in which a stimulation of k_d has been demonstrated have rested on the urinary excretion of 3-MeHis [110–112]. A synthesis of results in a number of papers [108,109,114,115] leads us to conclude that the effects of glucocorticoids on muscle k_d are transient, may affect primarily the degradation of myofibrillar protein and may be observed only by using pharmacological dose regimes. The physiological relevance of such effects is unclear.

In spite of the above, anabolic effects of glucocorticoids have been described under some specialized circumstances [106,107,110]. Assessment of the relevance of these is complicated by the fact that, in addition to causing insulin resistance, glucocorticoids exert a number of other indirect effects. Because they increase glycaemia, they may indirectly elevate insulin secretion [108,110]. Their anabolic action on the heart [106,107] may result from their hypertensive effect [106]. The predominating effects of the glucocorticoids are thus probably critically dependent on experimental design.

Amino acids. Amino acids modulate protein turnover in incubated rodent muscles (reviewed in [116]). Whether infusion of amino acid mixtures modulates protein turnover *in vivo* has not been unambiguously established. Increases in skeletal muscle k_s have been observed in human subjects [35,117] but not in rats [118]. The ability of amino acids to stimulate protein synthesis may be independent of their substrate function since the general opinion is that tRNA is normally fully charged. If this is true, one or more amino acids must either have a regulatory role or else must act indirectly by, for example, stimulating insulin secretion [117], reviewed in [98]. However, since most of the work on tRNA charging has been carried out in the liver (see, for example, [119]), it may not be strictly applicable to muscle.

Addition of amino acids decreases overall k_d in incubated skeletal muscles taken from either fed or starved animals (see, for example, [47]). In contrast, there is no change in human skeletal muscle k_d following infusion of amino acids [35]. Because of the considerations concerning the relative contributions of tyrosine derived from degradation of myofibrillar and non-myofibrillar protein that were outlined above, the re-feeding experiments of Goodman & del Pilar Lopez [32] are difficult to interpret and have, in fact, been misinterpreted by other commentators [83]. Here, refeeding a complete diet containing protein or a protein-free diet does not alter overall k_d in the subsequently-perfused rat hindquarter. However, although the complete diet suppresses myofibrillar k_d compared with a protein-free diet, the protein-free diet inhibits non-myofibrillar k_d more effectively than the complete diet (Table 2). This suggests that dietary protein *stimulates* non-myofibrillar k_d, a finding which seems improbable. To summarize, the suspicion is that amino acids have a role in modulating muscle protein turnover but their role is currently difficult to define.

Regulatory functions of amino acids. Two amino acids in particular may have important regulatory functions. These are glutamine [120,121] and leucine (and possibly the other branched-chain amino acids valine and isoleucine) [122,123]. In skeletal muscle *in vitro*, raised intracellular glutamine concentrations increase protein synthesis [124,125] and inhibit overall (but not myofibrillar) k_d [126]. A correlation exists between k_s, C_s or k_{RNA} and skeletal muscle glutamine concentrations in a number of catabolic states [127,128]. This relationship could be fortuitous with all variables being influenced by a common third factor such as insulin or T_3. The experiments *in vitro* [124] indeed suggest a direct link. The molecular mechanism(s) responsible for the effects of glutamine is ill-defined but may involve modification of protein phosphatase activity [129] and hence the phosphorylation state of protein factors involved in translation (see below).

Infusion of glutamine into post-absorptive rats *in vivo* did not stimulate protein synthesis [130] but the rise in plasma glutamine concentration achieved was modest. Administration of glutamine to patients is not productive because of its chemical instability and rapid metabolism by the viscera especially during stress. Instead, Stehle *et al.* [131] administered the dipeptide Ala-Gln to surgical patients with resultant improvements in nitrogen balance and maintenance of muscle glutamine concentrations (see [132,133] for reviews on the use of peptides in parenteral nutrition). There is a need for more work on the effects of glutamine or suitable dipeptides on protein turnover *in vivo*.

The importance of branched chain amino acids in the regulation of muscle protein turnover is still not clear. Early studies suggested that, in addition to their substrate function, branched-chain amino acids (especially leucine) or, in some cases, their metabolites stimulated k_s and inhibited k_d in incubated muscle preparations (reviewed in [122,123]). Based mainly on these findings, many attempts have been made to improve nitrogen balance in patients in hypercatabolic states by administration of branched-chain amino acids but with little overall success (reviewed in [123,134]). In the rat, administration of leucine *in vivo* does not alter k_s in gastrocnemius or heart [135]. In man, branched-chain amino acids had no effect on muscle amino acid balance [136] or ribosomal profiles [137]. Furthermore, the plasma concentrations of the branched-chain amino acids rise in catabolic states (see, for example, [77]) and it has been argued that a fall in their concentrations would have been anticipated if they

were of prime importance in the regulation of nitrogen balance. It could equally be argued that the rise in branched-chain amino acids limits what would otherwise be a much greater loss of nitrogen. Indeed, as discussed below, recent work has suggested a synergy between the branched-chain amino acids and insulin [118,130] and hence a regulatory role cannot yet be excluded.

Glucagon. Elevated plasma glucagon concentrations and glucagon/insulin ratios are seen during catabolic states. Glucagon acutely inhibits k_s *in vivo* and *in vitro* although hyperphysiological concentrations are required [138,139]. Hyperglucagonaemia may accelerate whole-body k_d in insulin-deficient states *in vivo* [140]. The current feeling is that any physiological role for glucagon in the regulation of protein turnover is relatively limited.

Lipid-derived and non-carbohydrate fuels. Protein synthesis rates are relatively well maintained in the heart and slow-twitch skeletal muscles during insulin-deficient states as compared with those in fast twitch skeletal muscles. In the heart, this may be attributable to the elevated plasma concentrations of lipid-derived fuels (long-chain fatty acids and ketone bodies) which increase cardiac protein synthesis to the same extent as insulin *in vitro* by stimulating peptide-chain initiation [141,142]. Other non-carbohydrate fuels (lactate, pyruvate and acetate) also stimulate k_s *in vitro* [71,141,142]. Their mechanism of action is unclear. Although some of these fuels and their metabolites have been reported to inhibit cardiac k_d [29,143], we have been unable to confirm this [58]. In man, β-hydroxybutyrate infusion fails to decrease skeletal muscle k_d [144] but may increase k_s [145]. Thus any role of lipid-derived fuels in protein conservation is likely to be exerted through their action on protein synthesis.

Insulin-like growth factors. IGF-1 and IGF-2 are potential paracrine and autocrine regulators of growth [146,146a]. Their effects on muscle protein turnover have only been studied in detail in cultured skeletal muscle cells. Here, physiological concentrations of IGF-1 or IGF-2 stimulate k_s and inhibit k_d [59–61,147]. Since the cell lines are derived from embryonic tissue, the IGF family may be more important than insulin in the regulation of protein turnover here. In addition, the IGF family may also be important in the regulation of protein turnover in the mature animal. A positive correlation between plasma concentrations of insulin and IGF-1 in rats fed protein-deficient diets has been demonstrated [148,149]. These results should be interpreted cautiously since the actions of IGF-1 are probably influenced by several IGF-1 binding proteins. We anticipate that interest in the regulation of protein turnover by the IGF family will grow in the future. Indeed, a preliminary report [85] suggests that IGF-1 stimulates k_s and inhibits k_d in fully-differentiated muscles. We have also recently shown that physiological concentrations of IGF-1 stimulate k_s in cardiomyocytes isolated from adult rats (S. J. Fuller & P. H. Sugden, unpublished work).

Role of insulin in the regulation of protein turnover *in vivo*

In fed animals, administration of insulin does not stimulate muscle k_s [150] presumably because its pre-existing plasma concentrations (and possibly those of other modulators of protein turnover) are high enough to maintain a maximal rate. How important is the contribution of insulin to the regulation of protein turnover *in vivo* compared with the contribution of these other factors (Fig. 1)? The experimental design involves replacement of insulin (preferably to within the physiological concentration range) in insulin-deficient animals either directly by infusion or indirectly by refeeding. The two methods yield rather different results. Although direct replacement of insulin in

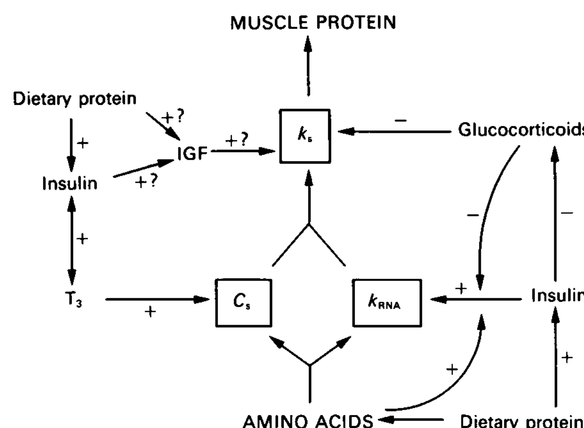

Fig. 1. Regulatory interactions between hormonal status and protein synthesis

A positive sign indicates a stimulation and a negative sign indicates an inhibition. When a hormone is known to affect either C_s or k_s, that interaction is shown. If the point of action is not known, the overall effect on k_s is shown.

fasted rats stimulates protein synthesis in skeletal and cardiac muscle by increasing k_{RNA}, k_{RNA} is not increased to fed values [74,103,150]. In fasted rats, plasma insulin concentrations have been moderately raised to similar values either by refeeding or by insulin infusion. Only refeeding raises k_s [151]. The increase in k_s on refeeding or on intragastric administration of amino acids + glucose is however attenuated by anti-insulin serum [118,151]. These findings suggest that insulin is important in the response of k_s to refeeding (and, by implication, in the diurnal regulation of k_s) but other factors are additionally involved.

What are the other factors? T_3 is apparently not involved in the acute response of k_s to refeeding [94] but may be important in the longer-term response that leads to an increase in C_s [98]. The fall in glucocorticoids following insulin repletion could be important, but conclusions differ [152,153]. A combined role for amino acids and insulin is suggested by the findings that, in the fasted rat, intravenous infusion of amino acids (especially the branched-chain amino acids) increases the 'sensitivity' of muscle k_s to insulin [118,130]. The importance of amino acid supply in the regulation of protein turnover has recently been emphasized by Millward and his colleagues [98] who have shown that dietary protein (but not dietary energy) have both direct and indirect roles in the regulation of protein turnover *in vivo*. They suggest that dietary protein increases protein accretion by increasing C_s (mediated by T_3 and possibly the IGF family) and by inhibiting k_d (possibly mediated by branched-chain amino acids or their metabolites). Additionally, dietary protein increases insulin concentrations which then stimulate k_{RNA} acutely. In some species (man and sheep), insulin replacement fails to raise k_s [154–156]. Infusion of insulin (+glucose) is known to decrease plasma and muscle amino acid concentrations [156–158] which may limit any stimulation of k_s by insulin [159]. Indeed, recent evidence suggests that insulin stimulates k_s in human skeletal muscle only if amino acid supply is adequate [159a].

It is not clear whether insulin affects muscle k_d *in vivo*. In man, it has been suggested that the major effect of insulin on protein turnover is to inhibit degradation rather than to stimulate synthesis [154,156], see also [159a]. In contrast, Millward and colleagues have recently suggested that insulin *increases* k_d in the rat [98]. There have been occasional reports that insulin stimulates k_d *in vitro* [26] but the majority opinion is that the hormone inhibits k_d. Thus the general conclusions are that insulin is important in the acute regulation of protein turnover especially

through its stimulation of k_s but that other factors such as amino acids are certainly important in addition.

Molecular mechanisms involved in the action of insulin on peptide-chain initiation

Protein phosphorylation and peptide-chain initiation. The pathway of peptide-chain initiation is shown in Fig. 2. Many of the proteins of the translational machinery exist as phosphoproteins (reviewed in [160]). In rabbit reticulocyte lysates, Ser-51 of the α-subunit of eIF-2 undergoes a phosphorylation/dephosphorylation cycle, the phosphorylation being catalysed by two distinct protein kinases [the haem-controlled eIF-2(α) kinase and the double-stranded RNA-activated eIF-2(α) kinase] with a loss of peptide-chain initiation activity (reviewed in [3,161]). Phosphorylation of Ser-48 may also be important in the inhibition of eIF-2 activity [162] although this is controversial. Phosphorylation of only 30 % of the total eIF-2 present leads to complete inhibition of translation because (i) the affinity of the guanine nucleotide exchange factor eIF-2B for eIF-2(α-*P*) is much greater than for the dephosphorylated protein, (ii) the molar ratio of eIF-2:eIF-2B is about 5:1, and (iii) the eIF-2(α-*P*)·eIF-2B complex does not catalyse guanine nucleotide exchange. Thus eIF-2B is sequestered in an inactive form. In a number of cultured cell lines, there is an inverse correlation between eIF-2(α-*P*) levels and the rate of protein synthesis [161]. Site-directed mutagenesis/transfection experiments show that mutation of eIF-2(α, Ser-51) to eIF-2(α, Ala-51) (now unavailable for phosphorylation) increases protein synthesis in COS-1 cells whereas mutation to eIF-2(α, Asp-51) [now an eIF-2(α-*P*) analogue] inhibits [160,163]. There is thus good evidence for a regulatory role for eIF-2 phosphorylation in some cell lines. In addition, the activities of other initiation factors may be regulated by phosphorylation. Thus, eIF-2B is phosphorylated *in vitro* by casein kinase II, increasing its guanine nucleotide exchange activity [164]. eIF-4F (which is involved in mRNA selection/binding) is also a potential candidate for regulation by phosphorylation. Exposure of certain non-muscle cells to TPA increases the phosphorylation of eIF-4F (and other initiation factors) [165–167] and this may stimulate peptide-chain initiation.

In some cell lines, the 40 S ribosomal protein S6 is phosphorylated on up to five seryl residues in response to insulin or mitogens (reviewed in [160]). Several S6 kinases have been identified. The current opinion is that there is a complex network of interacting phosphorylations and dephosphorylations. One phosphorylation cascade is activated by insulin and may involve the microtubule-associated-protein 2 protein kinase [168,169]. A second may involve protein kinase C [170]. However, there is very little evidence that the extent of S6 phosphorylation affects translational activity in any readily discernible way. A physiological role for S6 phosphorylation has therefore yet to be established.

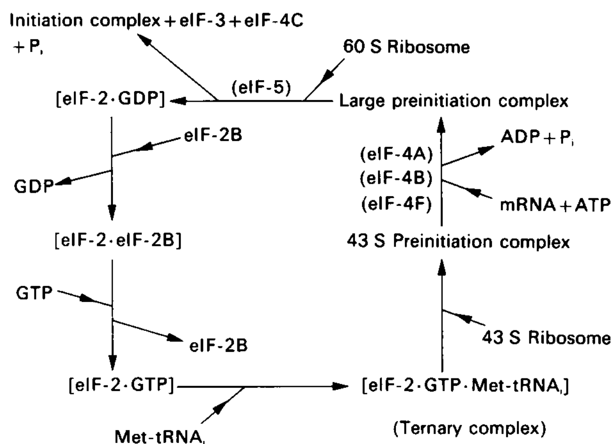

Fig. 2. Peptide chain initiation

In the first step, the GDP present in the [eIF-2·GDP] complex is exchanged for GTP in a reaction that requires the guanine nucleotide exchange factor, eIF-2B. The [eIF-2B·GTP] complex binds Met-tRNA$_i$ to form the [eIF-2·GTP·Met-tRNA$_i$] ternary complex. The ternary complex binds to the 43 S ribosome, which is a 40 S ribosome complexed with eIF-3 and eIF-4C, to form the 43 S preinitiation complex. mRNA is then bound in a reaction which is catalysed by eIF-4A, eIF-4B and eIF-4F and which requires the hydrolysis of ATP to form the large preinitiation complex. In reticulocytes with globin mRNA as message, the sedimentation coefficient of the large preinitiation complex is 48 S. Its sedimentation coefficient will depend on the size of the mRNA bound. The 60 S ribosome is then bound in a reaction catalyzed by eIF-5 which involves GTP hydrolysis to form the complete 80 S initiation complex. [eIF-2·GDP], eIF-3 and eIF-4C are released at this stage and the cycle restarts.

Effects of diabetes and food deprivation on initiation factor activity. Postmitochondrial supernatant fractions from gastrocnemius muscles of fasted or diabetic rats show a 30–40 % decrease in the rate of 43 S pre-initiation complex formation (as measured by [^{35}S]Met-tRNA$_i$ incorporation) which is reversed by administration of insulin before death or, importantly, by addition of exogenous eIF-2 to the assay system [171]. In related studies using perfused hemicorpora, alloxan-diabetes or fasting increases the number of 80 S ribosome monomers and decreases the amount of [^{35}S]methionine complexed with the 40 S ribosomal subunit peak (presumably as [^{35}S]Met-tRNA$_i$) [172]. Insulin reverses these changes. Furthermore, insulin decreases the level of eIF-2(α-*P*) in chondrocytes [173]. These observations suggest that insulin stimulates peptide-chain initiation by enhancing 43 S pre-initiation complex formation whereas diabetes or fasting inhibits it. These findings have been interpreted as showing that insulin possibly acts through regulation at the level of eIF-2/eIF-2B.

Before the concept of regulation of peptide-chain initiation through eIF-2 phosphorylation is extended to muscle, two considerations should be mentioned. First, the eIF-2:eIF-2B molar ratio in muscle is unknown. It is thus difficult to assess the change in the level of eIF-2(α-*P*) that is necessary to sequester eIF-2B. If the eIF-2:eIF-2B ratio were high, a proportionately small increase in eIF-2(α-*P*) may be sufficient to sequester a large proportion of eIF-2B, rendering changes in eIF-2(α-*P*) levels difficult to detect. Secondly, unlike the reticulocyte lysate system, protein synthesis is never inhibited by more than about 50 % by any manipulations *in vivo*. This implies that eIF-2B (or eIF-2) cannot be completely sequestered in an inactive form.

In post-mitochondrial supernatant fractions of gastrocnemius muscles from 48h-fasted rats, careful study by Cox *et al.* [174] could not find any evidence of a change in either eIF-2(α-*P*) levels [which were always < 5 % of total eIF-2(α) levels] or in eIF-2B activity. These results are difficult to reconcile with those of Kimball & Jefferson [175]. In alloxan-diabetic rats, they showed that eIF-2B activities are depressed in post-mitochondrial supernatant fractions of fast-twitch muscles but not in those from slow-twitch muscle or heart. Brief (2 h) exposure of diabetic rats to insulin reversed the effects of diabetes on eIF-2B activities in fast-twitch muscles but paradoxically decreased eIF-2B activities in soleus and heart. The authors suggest that k_s values correlate with eIF-2B activities in all four tissues from diabetic or control animals and that only in those tissues where k_{RNA} is especially sensitive to diabetes (fast-twitch muscles) do eIF-2B

activities change. If they do indeed occur, how might the changes in eIF-2B activities be mediated? Two mechanisms have been suggested [175]. First, reticulocyte eIF-2B activity is stimulated by a casein kinase II-catalysed phosphorylation [164]. The activity of this kinase is stimulated by insulin in adipocytes and hepatoma cells [176]. Secondly, redox state may be important in the maintenance of eIF-2B activities [177]. To us it seems likely that phosphorylation and dephosphorylation of the components of the protein synthesis machinery regulate translational activity and that this may be a point of action of insulin. Resolution of the problem will require a greater understanding of the phosphorylation cycles involved, especially in intact muscles.

Do prostaglandins have any role in the mediation of insulin action? Although differing in detail, early *in vitro* experiments showed that muscle k_s is stimulated by $PGF_{2\alpha}$ and k_d is stimulated by PGE_2 [178–180]. (It should be noted that some of this work has proved difficult to repeat [181,181a].) Further work suggested that $PGF_{2\alpha}$ may be involved directly in the initiation (but not the maintenance) of insulin action [182,183]. The stimulation of k_s by insulin in isolated skeletal muscle is correlated with $PGF_{2\alpha}$ release and is abolished by cyclo-oxygenase inhibitors. Furthermore, prostaglandins may mediate the stimulation of rRNA synthesis by insulin in muscle cell cultures [184]. *In vivo*, the stimulation of rat muscle k_s and prostaglandin synthesis by insulin infusion is prevented by the cyclo-oxygenase inhibitor indomethacin [185]. Indomethacin also prevented the increase in muscle k_s following refeeding in rats but did not affect the response of whole-body k_s in man [186]. It is not clear whether these effects indicate a direct interference of indomethacin with the synthesis of a prostaglandin mediator of insulin action or whether they indicate an indirect action by, for example, inhibiting insulin secretion or decreasing insulin sensitivity. In contrast to these studies, no role for prostaglandins could be established in the mediation of the effects of insulin on protein turnover in the perfused heart [187]. Our personal view is that an involvement of prostaglandins in the mediation of insulin-action has not yet been proved unequivocally.

PROTEIN TURNOVER AND OTHER HORMONE AND SECOND MESSENGER SYSTEMS

The sympathoadrenal system

Effects of adrenergic agonists in skeletal muscle. The acute effects of catecholamines on protein turnover in skeletal muscle are inconsistent and no consensus view has been established [81,188–192]. Lowering the basal k_s may be necessary to allow detection of effects [193]. Permeant analogues of cyclic AMP or cyclic GMP have been reported to stimulate both k_s and k_d [194] but these results are difficult to interpret. Catecholamines inhibit alanine and glutamine release from skeletal muscle *in vitro* [195,196] (but see also [197]). This has been interpreted as an inhibition of k_d by catecholamines. This interpretation is erroneous since (i) these amino acids are both synthesized and degraded by muscle and (ii) amino acid reutilization for protein synthesis was not prevented in these experiments.

Dietary administration or injection of the β_2-adrenergic agonist clenbuterol into rats stimulates skeletal muscle protein and RNA accretion *in vivo* and induces muscle fibre hypertrophy ([198–200], reviewed in [192]). Denervated muscles respond to clenbuterol suggesting that the drug may be useful in reducing atrophy [201,202]. The hypertrophic effects of stretch and clenbuterol are additive [203] suggesting parallel pathways of stimulation of protein accretion. Blockade by β- or β_1-antagonists does not affect the accretion of protein or RNA by clenbuterol in the soleus [204–206], raising the possibility that some actions of clenbuterol are not mediated through the β-adrenoceptor although the efficacy of β-blockade in these experiments was not independently established. The point of action of clenbuterol is still disputed (reviewed in [207]). Its effect to increase C_s suggests a pretranslational action or an action at the level of RNA degradation. This view is reinforced by the demonstration that the drug prevents the loss of α-actin and cytochrome *c* mRNA species (as well as total RNA) during muscle disuse [208]. It is still unclear whether there is also control at the levels of translation and protein degradation. These questions are important to answer because of the potential usefulness of clenbuterol in increasing muscle mass.

Effects of adrenergic agonists in cardiac muscle. Recent studies have taken two main directions emphasizing either the α- or the β-adrenoceptor-mediated responses to the hormones. Administration of adrenaline or the β-agonist isoprenaline at doses which are low enough to avoid necrotic side-effects causes cardiac hypertrophy *in vivo* [209–211]. An increase in C_s is soon apparent [212]. The number (but not the proportion) of translating ribosomes is increased [212]. It has been difficult to establish that the hypertrophy induced by catecholamines is a direct effect and is not mediated by the concomitant increase in cardiac work. The perfused beating heart has not yet proved particularly useful and results have not always been consistent, partly because catecholamines cause ATP depletion *in vitro* owing to a mismatch between O_2 supply and demand [213,214]. In hearts arrested with tetrodotoxin (to prevent adenine nucleotide depletion), increases in cyclic AMP stimulate k_s [215] and isoprenaline stimulates the synthesis of non-contractile proteins in cultured myocytes [216]. These experiments suggest a direct action of β-adrenoceptor stimulation on protein synthesis.

There is disagreement about whether isoprenaline directly inhibits k_d in the perfused heart. Using a protocol in which protein in the beating perfused heart was prelabelled with [^{3}H]leucine, Lockwood showed that isoprenaline inhibits k_d by a mechanism which involves a non-lysosomal pathway [217,218]. In contrast, Morgan and co-workers were unable to establish any effect of isoprenaline on k_d in the arrested perfused heart using phenylalanine release in the presence of cycloheximide to measure k_d [219]. These differences may result from the fact that the degradation of different protein species is being measured or from problems related to ATP depletion in beating hearts perfused with isoprenaline. The overall conclusion is that the β-adrenoceptor may play a direct role in the regulation of cardiac protein synthesis and degradation but that its role is still poorly defined.

In cardiomyocyte cultures and in the heart *in vivo*, α_1-adrenergic agonists stimulate protein accretion and the transcription of proto-oncogenes and other genes [220–222]. Contractile activity is not necessary for the expression of this effect suggesting mediation through an independent pathway [223,224]. Protein synthesis is stimulated in the absence of any effect on protein degradation [223,225]. Since these effects involve RNA accretion and are blocked by actinomycin D, they are probably exerted at the level of gene expression [222,223]. As in other tissues, α_1-adrenergic stimulation in cardiomyocytes causes hydrolysis of membrane phosphatidylinositols through phosphoinositidase activation [226–228] and hence potentially results in the formation of inositol phosphates and the protein kinase C activator, diacylglycerol. Indeed, some of the effects of α_1-adrenergic stimulation on protein accretion were mimicked by TPA, suggesting the involvement of protein kinase C [225,229]. In addition to these transcriptional effects, α_1-adrenergic agonists or TPA acutely stimulate translation in cardiac myocytes and perfused hearts [65,230]. In the perfused heart, α_1-adrenergic

agonists increase pH_i and PCr concentrations [231] which may be important in the mediation of the effects of these agonists (see below). The simplest interpretation of these experiments is that α_1-adrenoceptor occupancy or TPA stimulates both transcription and translation by activating protein kinase C. This interpretation may be facile in view of the finding that these two treatments have quite distinct actions on the redistribution of protein kinase C in cardiomyocytes [232]. We feel that the effects of α_1-adrenergic agonists on muscle protein turnover are worthy of further study, especially since skeletal muscle also contains the necessary α_1-adrenoceptors [233].

Calcium ions

Although a role for $Ca^{2+}{}_i$ concentrations in coupling increased contractile activity to increased protein accretion is attractive, there is very little evidence for such a function. In skeletal muscles, there is general agreement that removal of $Ca^{2+}{}_o$ in fact decreases overall k_d and the Ca^{2+}-ionophore A23187 (in the presence of $Ca^{2+}{}_o$) enhances it [194,234–238]. These findings suggest that an increase in $Ca^{2+}{}_i$ could result in a loss of muscle protein and could be relevant to the muscle atrophy that occurs during catabolic states (see, for example, [239]). However, degradation of myofibrillar protein is unaffected by Ca^{2+} [239,240]. This type of approach is relatively simplistic especially since $Ca^{2+}{}_i$ was not measured in any study. The mechanisms responsible for the effects of Ca^{2+} on k_d are unclear. Involvement of the calpains is possible [238]. Other work suggested mediation by PGE_2 (reportedly a stimulator of k_d [179]) through a Ca^{2+}-dependent activation of phospholipase A_2 [237]. Subsequent experiments from the same laboratory were unable to repeat many of the original findings, which must therefore be regarded with scepticism [234].

Ca^{2+} stimulates k_s in some non-muscle mammalian cell lines (reviewed in [241]). In perfused heart, there is very little evidence that Ca^{2+} concentrations have any consistent effect on k_s or k_d over the range that they can be varied (0.5–5 mM) without seriously compromising heart function [161,242–244]. It remains possible that Ca^{2+} could stimulate k_s if it were to act synergistically with diacylglycerol to stimulate protein kinase C. This has not yet been examined. The effects of Ca^{2+} on skeletal muscle protein synthesis are inconsistent. One group has shown stimulation of k_s by A23187 [236], another inhibition [194] and a third no change [237]. The effects of removal of $Ca^{2+}{}_o$ on skeletal muscle k_s have not been examined in detail. One problem is that removal of $Ca^{2+}{}_o$ or addition of A23187 in the presence of $Ca^{2+}{}_o$ decreases muscle PCr and ATP contents [238], which would be expected to inhibit both k_s and k_d. Thus, from the point of view of energy metabolism, the stimulation of k_s or k_d by Ca^{2+}/A23187 is unexpected and is therefore more probably explicable in terms of mediation through Ca^{2+} uptake.

Protein kinase C

A number of preliminary studies have suggested the involvement of the Ca^{2+}/phospholipid-activated protein kinase C family in the regulation of protein turnover. Phorbol esters stimulate k_s in pituitary cell cultures (reviewed in [241]). The stimulation of the phosphorylation of components of the protein synthesis machinery by TPA in non-muscle cells has been mentioned above. In isolated cardiomyocytes, TPA stimulates transcription [229,245,245a] and translation [65]. In skeletal muscle, TPA restricts the degradation of myofibrillar protein but not overall protein degradation [246]. The transmembrane Na^+ flux may be partly under the control of protein kinase C since the Na^+/H^+ exchanger is activated by a protein kinase C-catalysed phosphorylation (reviewed in [247,248]). A number of other experiments imply that trans-sarcolemmal Na^+ flux may interact with myofibrillar protein degradation [246], suggesting a mechanism by which protein kinase C could influence the degradation of this protein pool. Clearly, study of the involvement of protein kinase C in the regulation of protein turnover is at a very early stage and much work is yet required.

EFFECTS OF PHYSICAL FACTORS

Stretch and contractile activity

Stretch and skeletal muscle protein turnover. Numerous studies in many model systems have shown that imposition of stretch or increased workload on skeletal muscle *in vitro* or *in vivo* improves nitrogen balance and may be involved in the hypertrophic response. Removal of tension or unweighting [249] has the opposite effect. Additionally, Grinstein and co-workers have speculated that an increase in cell volume, which may stretch the cell, might be an essential prelude to macromolecular synthesis [248]. The current feeling is that the response of protein turnover to stretch involves control at both the translational/post-translational level (including regulation at the level of protein degradation) and at the pretranslational level [250–256].

A particularly promising approach to this problem involves using cultured muscle cells. In chick myotube cultures, total protein synthesis and accumulation are not dependent on contractile activity but contraction does increase myosin accumulation [257] and is also required for the expression of neonatal (as opposed to embryonic) myosin heavy chain [258]. In contrast to spontaneous contraction, intermittent mechanical stretch induces hypertrophy and hyperplasia, and stimulates k_s in chick myotubes cultures [257,259]. This effect is much reduced in unsupplemented media. The mechanisms responsible for these effects may include the production of endogenous growth factors and the removal of contact inhibition (see [259] for references).

Tension and contractile activity are common factors linking muscle protein turnover to a number of interventions. An obvious example is exercise, the molecular and cellular physiology of which has been recently reviewed [260–262]. It has been surprisingly difficult to establish any consensus on the acute effects of exercise on skeletal muscle protein turnover *in vivo* (reviewed in [263,264]). The general opinion is that acute exercise either has no effect or inhibits k_s and its effects on overall or myofibrillar k_d are also inconsistent [190,264–269]. The reasons for the inconsistencies are obscure but we will make four comments. First, exercise may affect turnover only at some time point after its termination. Secondly, inappropriate exercise regimes may have been used. Endurance (aerobic) exercise such as the prolonged running frequently used in studies on protein turnover does not significantly increase skeletal muscle mass. In contrast, anaerobic exercise such as weight-training does increase skeletal muscle mass. A weight-lifting exercise model has recently been developed for the rat [270] and this model may be more useful. Thirdly, exercise-induced changes in muscle mass occur only slowly and acute effects on protein turnover may be scarcely detectable. Fourthly, exercise (especially endurance exercise) induces many changes in hormonal status and sensitivity/responsiveness, and changes in fuel metabolism [265,271]. These alterations present interpretative complications.

Tension and cardiac protein turnover. *In vivo*, the heart adapts to an increased workload by increasing its muscle mass (reviewed in [272–274]). Increases in both C_s and k_{RNA} are probably involved [275], although there is a view that pretranslational control is the more important [273,274]. Transcription of proto-oncogenes, heat shock protein genes and transitions in the transcription of other cardiomyocyte-specific genes are detectable in response to increased workload *in vivo* (reviewed in

[221,276–279]). *In vitro*, the acute imposition of stretch or of raised aortic pressure on cardiac muscle increases k_{RNA} by stimulating peptide-chain initiation [187,280–285] and also decreases k_d [187,244,286]. The effects of raised aortic pressure are probably mediated by the increased coronary vessel volume causing stretching of the ventricular wall (the garden hose effect) [244,281,285]. Synthesis of ribosomal proteins is stimulated preferentially over synthesis of total protein [287,288]. These combined effects of workload on RNA accretion and protein turnover lead to the increase in cardiac mass.

An interesting example of differential cardiac growth associated with workload occurs in the heart of the mammalian neonate. Here, normal circulatory changes result in the imposition of a raised aortic pressure on the left ventricle whereas the pressure-load on the right ventricle remains relatively low. In the pig, the left ventricular wall grows about three times more quickly than the right wall probably as a result of both hypertrophy and hyperplasia [289]. In the left ventricular wall, C_s and mRNA content are greater than in the right ventricular wall. When neonatal pig hearts are perfused *in vitro*, k_s and ribosome formation are more rapid in the left ventricular wall than in the right. In the absence of insulin, this was attributable to increases in both C_s and k_{RNA} whereas in the presence of insulin increases in C_s alone were responsible for increased k_s [289,290]. Calculated k_d values showed no differences between the compartments, suggesting that the controls were exerted at the level of protein synthesis and that the increase in C_s was probably the predominant influence [289].

Because of its relevance to clinical conditions, the regulation of growth in cultured cardiomyocytes is being actively studied. In the absence of serum, neonatal rat cardiomyocytes grow only if they are both contracting and attached to a substratum of laminin [291]. Growth is enhanced by serum [291]. In related experiments, McDermott & Morgan showed that K^+-arrested neonatal rat cardiomyocytes maintain a stable size but growth occurs when contraction is initiated [223]. These studies suggest that contraction is a prerequisite for growth. In contrast, neonatal cat heart myocytes grow when attached to a substratum but quiescent [292]. These different findings could result from species differences or differences in technique. In rat cardiomyocytes, total protein synthesis and myosin synthesis increase without any large change in k_d [223,293]. The increase in k_s is the result of an increase in C_s with the increase in RNA synthesis exceeding a concomitant increase in RNA degradation [223,294].

Thyroid hormones and cardiac hypertrophy. Administration of thyroid hormone causes cardiac hypertrophy and can also alter the expression of certain cardiac genes (especially those for myosin heavy chain [295]). The current view is that the hypertrophy induced by thyroid hormone is secondary to increased cardiac work [296] whereas transitions in myosin heavy chain gene expression are directly attributable to thyroid status. The best evidence comes from studies in which a second heart is transplanted into a recipient rat [297,298]. Only the original *in situ* heart carries a haemodynamic load. Although myosin heavy chain transitions occur in both hearts on treatment with thyroid hormone, only the *in situ* heart hypertrophies. *In vivo*, thyroid hormone-induced hypertrophy involves increases in C_s, k_s, k_d [101,299] and mRNA concentrations [300]. *In vitro*, k_s values in perfused hearts taken from rats pretreated with thyroid hormone are 25% greater than those in control hearts [91]. This increase is attributable solely to the increase in C_s. An early event is the stimulation of ribosomal protein synthesis. In contrast to the *in vivo* studies, no change in k_d is detectable. In line with the evidence described above, these results suggest that the principal effect of thyroid hormone is at the level of RNA turnover.

Cellular mechanisms involved in the coupling of the stretch stimulus to macromolecular turnover. Very little is known about the mechanisms by which stretch produces changes in protein turnover rates. As already discussed, there is little evidence as yet of a role for Ca^{2+}. Several alternative hypotheses have been proposed. These include mediation by prostaglandins [301,301a], by cyclic AMP [215,288] and by modulation of Na^+ transport [302]. We do not think that the evidence with respect to a role for prostaglandins is unequivocal. Although the stretch-induced stimulation of k_s and $PGF_{2\alpha}$ release in rabbit skeletal muscles *in vitro* is antagonized by cyclo-oxygenase inhibitors [301], fenbufen (a well-tolerated cyclo-oxygenase inhibitor pro-drug) does not diminish the degree of hypertrophy of the plantaris *in vivo* in response to tenotomy of the ipsilateral gastrocnemius [303]. In the perfused heart, the effects of aortic pressure on k_s or k_d are not prevented by cyclo-oxygenase inhibitors [187,244]. The recent finding that acute pressure overload increases cyclic AMP concentrations and cyclic AMP-dependent protein kinase activity ratios in concert with increasing k_s in the perfused heart, and that these effects are prevented by muscarinic inhibition of adenylate cyclase [215,288], deserves further study. A problem is that, *in vivo*, pressure overloading of the heart apparently causes only small non-significant increases in cyclic AMP content [211].

Stretch-activated (mechanotransducer) ion channels (reviewed in [304]) are present in skeletal and cardiac muscle [305,306]. Stretch stimulates a load-responsive Na^+ influx in cardiac papillary muscles and the stimulation of k_s by tension development is prevented by streptomycin (an inhibitor of mechanotransducer ion channels) [302]. Additionally, other agents which stimulate Na^+ influx (the Na^+ ionophore, monensin or the fast-Na^+ channel stimulator, veratridine) also stimulate k_s. Any role for mechanotransducer channels in the regulation of protein turnover is still ill-defined.

Our overall conclusion is that the mechanisms involved in the tension/stretch activated-transduction pathway remain poorly-understood. Probably the most promising general area of research involves the role of stretch in stimulating endogenous growth factor release and proto-oncogene expression, although whether these processes respond quickly enough to account for the acute changes in protein turnover is debatable. There is a need for more work on the relationship between ion transport and protein turnover. In view of the finding that activation of protein kinase C may acutely stimulate cardiac protein synthesis [65], we are particularly interested in the recent observation that stretch stimulates the hydrolysis of cardiac phosphatidylinositols [307]. We feel that investigations into these three areas (growth factors, ion transport and phosphatidylinositol turnover) currently offer the greatest potential for understanding the coupling of stretch to protein turnover.

Extracellular and intracellular pH

A number of anabolic factors and hormones (including insulin) increase pH_i in certain cells by stimulation of Na^+/H^+ exchange (reviewed in [247,308,309]). *In vitro*, pH_i in muscle can be conveniently altered by varying pH_o. In the perfused heart, increasing pH_o above the physiological value of 7.4 progressively stimulates k_s and inhibits k_d without compromising heart function or changing adenine nucleotide concentrations [310]. The effects of pH_o on k_s are similar in isolated cardiomyocytes [311]. Effects of raised pH_o on k_s and k_d can be as great as those of insulin [310]. A more thorough examination of this effect has shown that k_s is significantly correlated with pH_i over a range between pH 7.25 and 7.65 (i.e., the slightly acidotic to the alkalinotic) [312]. In addition, k_s is positively correlated with PCr concentrations which also increase progressively as pH_i rises [310,312]. Since

creatine kinase catalyses a near-equilibrium reaction in the perfused heart [313] and the synthesis of PCr from ATP and creatine involves production of a proton [314], we suggest that the alterations in pH_i are responsible both for the changes in PCr contents (through a mass action effect) and for the changes in protein turnover [310,312]. However, we have not excluded the possibility that PCr itself (rather than pH_i) or even some unrecognized factor modulates protein turnover. *In vivo*, pH_o remains relatively constant (except in some pathological conditions) and is therefore unimportant in regulating protein turnover. We do however think that pH_i might be important in the mediation of some of the effects of hormones (such as α-adrenergic agonists, as discussed above) on protein turnover.

Oxygen tension and redox status

In vivo or *in vitro*, hypoxia or ischaemia decreases k_{RNA} and k_d in both heart and skeletal muscles [29,264,315–318]. It is unclear which stage of translation is primarily affected. In the perfused heart, ischaemia preferentially inhibits peptide-chain elongation/termination whereas hypoxia additionally inhibits peptide-chain initiation [315]. In contrast, in the hindlimb *in vivo*, ischaemia inhibits peptide-chain initiation [318]. It is not clear whether decreases in purine nucleoside triphosphate concentrations influence protein turnover in these circumstances, although this is a strong possibility. A positive correlation between k_s and PCr concentrations and an inverse correlation between k_s and lactate concentrations have been detected [29,264,318]. We speculate that lactate may decrease pH_i and that the changes in protein turnover and PCr concentrations result directly from this decrease (see above for a description of the effects of pH on PCr concentrations).

There is some evidence that the most damaging phase of cardiac ischaemia *in vivo* is during reoxygenation/reperfusion because of free-radical formation. To our knowledge, the effects of increased free-radical production on muscle protein turnover have not been studied in detail. Exposure of erythrocytes or their lysates to free radicals stimulates proteolysis of endogenous protein [319,320]. This is not particularly surprising since oxidative disruption of protein secondary structure should increase peptide bond hydrolysis. Two comments can be made. First, probably all cells produce free radicals. Secondly, it is possible that diurnal oxidative damage to proteins may partly underpin the physiological necessity for protein turnover.

Exposure of rats to hyperoxia (100 % O_2) *in vivo* inhibits k_s in the heart [321]. In cultured cells, k_s is decreased by hyperoxia (reviewed in [321]). The mechanisms by which hyperoxia inhibits k_s are not understood. It is difficult to see how cardiac k_s could be directly affected since haemoglobin is saturated with O_2 at its normal atmospheric partial pressure (unless there is a direct effect of increased O_2 dissolved in the plasma). Inhibition could result from exposure of the heart to oxygen-derived radicals and H_2O_2 formed in the lungs.

It has been suggested that redox state may be of primary importance in controlling protein turnover since increases in the [NADH]/[NAD^+] ratio have been reported to stimulate k_s [322] and inhibit k_d ([323,324], reviewed in [325]). For degradation, a mechanism involving the oxidation of protein thiol groups by GSSG to form a protein–SSG conjugate + GSH has been proposed, the [GSH]/[GSSG] ratio being related to the [NAD(P)H]/[$NAD(P)^+$] ratio. The conjugate might then be preferentially degraded. This is an attractive hypothesis but others have failed to establish a correlation between k_d and redox state [326,327]. For protein synthesis, redox state might maintain a reduced state of the thiol groups in eIF-2 [322] but there is no firm evidence to support this.

PROTEIN TURNOVER IN PATHOLOGICAL CONDITIONS

Effects of pathological conditions on protein turnover

There are changes in protein turnover in many pathological conditions (reviewed in [134,328–331]). We will limit our discussion mainly to selected studies in animal models (see Fig. 3). There is still little agreement about the relative changes in protein turnover that occur during these complex conditions. In general, skeletal muscle k_s values decrease and hepatic k_s values increase. To what extent changes in protein turnover can be ascribed to the myriad hormonal, metabolic and behavioural changes that occur is still not understood.

In fed rats rendered endotoxaemic by a bolus injection of endotoxin, k_s in fast-twitch skeletal muscle decreases (Fig. 3), initially because of a decrease in apparent k_{RNA} and, later, because of a decrease in C_s [18]. Muscle k_g declines initially and then returns to control values. Thus k_d increases initially but then decreases to less than control values. The response of liver protein turnover is almost the mirror image. Anorexia is probably not responsible for these changes. The lack of involvement of anorexia (or pyrexia) is confirmed by the findings of Ash & Griffin, who combined infusion of low doses of endotoxin with total parenteral nutrition [332]. In malaria-infected rats, the pattern of changes is somewhat different [333]. During the overt phase of infection and anorexia, C_s increases in heart, soleus and gastrocnemius whereas k_s and k_{RNA} decrease in skeletal muscles (Fig. 3) but increase in heart. However, before the onset of overt infection and anorexia, C_s *decreases* in the heart and gastrocnemius, and cardiac k_{RNA} increases. These recent examples [18,322,333] which all used reliable methods of k_s measurement, demonstrate the difficulty in producing any coherent model

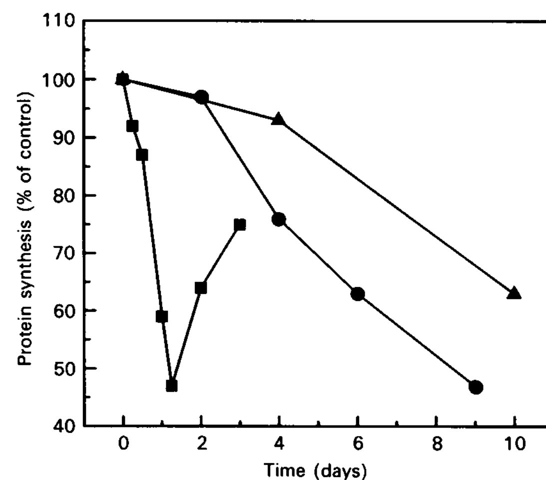

Fig. 3. Time-courses of changes in gastrocnemius k_s occurring during endotoxaemia, malaria and malignancy

Results were taken from [18] (endotoxaemia, ■), [333] (malaria, ▲) and [334] (Ehrlich ascites tumour, ●). Results are expressed as percentages of the most appropriate controls described by the authors. For endotoxaemia, fed rats were injected with *Escherichia coli* lipopolysaccharide every 24 h. Food consumption decreased by about 20 % on the first day following the first administration of endotoxin but then returned to normal. Controls were fed *ad libitum*. The muscle sampled was the combined gastrocnemius + plantaris. For malaria, rats were infected with *Plasmodium berghei* and controls were pair-fed. Food consumption decreased on the sixth day. For the Ehrlich ascites tumour model, the tumour was visible by 4–5 days after the intraperitoneal injection of the tumour cells. Food consumption was decreased by 10 % after 1 day and by 30 % after 4 days. Controls were fed *ad libitum*.

relating the response of protein turnover to such a complex condition as infection.

As recently reviewed [334], advanced malignancy in humans [335] or rodents [336] depresses skeletal muscle k_s and may be responsible for the severe muscle wasting seen in this condition. It is difficult to assess the extent to which wasting can be attributed to a specific effect of the tumour on muscle k_s as compared with the contributions of anorexia and tumour substrate requirements. The time-course of muscle loss may vary between different tumour models [337]. In mice carrying an implanted human hypernephroma, anorexia could not entirely account for the decreases observed in muscle k_s [336]. However, in Ehrlich ascites tumour bearing-mice, Pain and her colleagues have shown that anorexia and increased splenic k_s precede decreased skeletal muscle k_s (Fig. 3) and tumour visibility [334]. A small fall in cardiac k_s and large increases in skeletal muscle k_d and hepatic k_s are apparent later. The metabolic demands of the tumour and the liver are thus unlikely to account for decreased muscle k_s and the role of anorexia could not be easily discerned. Pain and her colleagues suggest that the initial effects of the tumour on appetite and muscle k_s were mediated by unidentified humoral factors.

The metabolic response to surgery and trauma is associated with a range of disturbances including negative nitrogen balance, insulin resistance, changes in the secretion of insulin, glucagon and catecholamines, and a shift towards oxidation of fatty fuels (reviewed in [338]). There does not appear to be a clear consensus on the effects of trauma on muscle protein turnover (reviewed in [22]). In the rat, we were unable to detect any alterations in k_s in leg muscles after laparotomy although we detected an increase in the diaphragm [22]. In burn-trauma, changes in muscle protein turnover have been detected, although results have again been inconsistent [78,339–341]. The reasons for differing results depend, *inter alia*, on the model of trauma used, the time-points examined and the state of nutrition of the animal.

Possible mechanisms

The mechanisms underlying changes in protein turnover in pathological conditions are unclear. A possible role for glutamine has been suggested [127,128]. In endotoxaemia [18], plasma insulin concentrations are unchanged or increased. Corticosterone concentrations are moderately raised. T_3 concentrations are decreased and these decreases could have caused the falls in muscle C_s. There are increases in V_{O_2} and decreases in muscle blood flow and O_2 extraction which might affect protein turnover [342]. Endotoxin itself (or possibly an unidentified cytokine) might affect k_{RNA} directly [18]. In a number of catabolic states, k_d and PGE_2 release are increased in skeletal muscles removed from animals and incubated *in vitro* [343–345]. Although this stimulation of k_d has been reported to be sensitive to indomethacin [343], others were unable to confirm this observation [344–346]. Similarly, inhibition of muscle loss by cyclo-oxygenase inhibitors has been observed [337,347,348] but such observations have not always been reproducible [127]. Increases in temperature *in vitro* exacerbate the negative nitrogen balance in normal muscles, suggesting that fever itself may be important in regulating protein loss [349]. It is difficult to extrapolate the results of these relatively unsophisticated experiments to the situation *in vivo*.

The possibility that cytokines (interleukins, TNF and other factors) released by monocytes in response to infection may directly affect muscle protein turnover has been extensively investigated (reviewed in [85,350]). Plasma from septic or traumatized animals contains a factor that stimulates net amino acid release from rat soleus muscles *in vitro* without any detectable change in k_s, implying a direct effect on k_d [351,352]. Monocytes challenged directly with heat-killed staphylococci also release a factor(s) which stimulates k_d in rat skeletal muscles *in vitro*, possibly through a PGE_2-dependent mechanism [352,353]. The factor does not affect k_s *in vitro* or *in vivo* [354]. Although the factor was initially thought to be interleukin-1, the results of a number of experiments have concluded that this is not the case [352,354]. An involvement of TNF has been sought. Apart from some dubious studies *in vivo* [355], the best evidence for a role for TNF comes from studies in nude mice where symptoms of severe cachexia have been induced by implanting tumour cells expressing the transfected TNF-α gene ([356], reviewed in [357]). In contrast, other studies have not detected any acute effect of administered TNF-α on protein turnover [352,354,358].

The conclusion from these studies is that sera from infected animals contain an unknown factor(s) which may affect protein turnover by primarily affecting k_d although the importance of these observations is difficult to assess since the work has concentrated on regulation at the level of degradation rather than at the level of synthesis. Much of the work *in vivo* has indicated that decreases in k_{RNA} and C_s are probably more important in protein loss in pathological states.

GENERAL POINTS

Space limitations have precluded discussion of many important areas. We have omitted discussion of normal growth on protein turnover and the decreases in protein turnover rates that occur with aging (reviewed briefly in [4]). We have also omitted discussion of the enzymology of intracellular protein degradation (reviewed in [85,359,360]) which still remains ill-understood partly because of the difficulty in detection of the peptide intermediates. Here, there has been much recent interest in the high-molecular-mass ATP-dependent multicatalytic protease (reviewed in [361,362]). Equally, there are other proteolytic pathways which are potentially operative in eukaryotic cells. These include the ubiquitin pathway [363], the calpain pathway [364] in which proteins are preferentially hydrolysed at 'PEST'-(Pro-Glu-Ser-Thr)-rich regions and the lysosomal pathways. In the lysosomal pathway, in addition to the relatively non-specific autophagosomal mechanisms, the KFERQ (Lys-Phe-Glu-Arg-Gln) sequence in the substrate may be important in a more selective transfer of protein into lysosomes [365] in an ATP-dependent process requiring a heat shock protein [366].

Much in the protein turnover field is still unclear. Indeed, the relative importance of regulation at the levels of protein synthesis and degradation is still disputed. Much of the work (especially that *in vivo*) is still largely phenomenological with little indication of the responsible intracellular regulatory mechanisms. Experiments *in vitro* are useful but may be difficult to apply to the *in vivo* situation. *In vivo*, interpretation of results may be difficult because of the complex changes in the hormonal and metabolic milieux that any intervention entails. It is hence often difficult to identify any single factor as being responsible for a change in protein turnover, nor should it be expected that this should be possible since it is probable that regulation is multifactorial. In some cases, there is still much debate about the relative contribution of events at the pretranslational level (transcription, RNA processing and degradation, etc.), the translational level and at the post-translational level (protein and RNA degradation, including ribosomal degradation). There are methodological problems, particularly for studies *in vivo*. Because changes in muscle mass occur relatively slowly, the changes in protein turnover responsible are often small and difficult to detect reliably. The timing of the sampling point and age, sex and nutritional state of animals used may critically affect the direction and magnitude of the response observed.

In spite of these problems, we feel that the following conclusions can be made. Much of this review has been devoted to a discussion of the effects of insulin, which we feel plays a fundamental role in synergy with other factors in regulating k_{RNA}. Additionally, the role of the IGF family in the regulation of protein turnover in adult animals needs further investigation. With respect to effects of other interventions, a problem arises because, in the fed animal, translation in muscle appears to be operating at a maximal rate with the majority (90 %) of ribosomes actively translating mRNA. This would limit the potential for enhancement of translation by other interventions to periods when insulin concentrations are reduced in between meals. *In vitro*, the effects of potential stimulators of k_{RNA} are rarely studied in the presence of maximally-effective concentrations of insulin. Such investigations might be productive. It would therefore seem that increases in C_s might be potentially the more important in enhancing k_s in the fed animal. One factor that is critically involved in modulating C_s is probably thyroid hormone status. The regulation of protein degradation remains poorly understood *in vivo*. This is undoubtedly related to the problem of reliable measurement of k_d and to a lack of understanding of the enzymology of protein degradation *in vivo*.

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