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HORMONAL REGULATION OF HUMAN MUSCLE PROTEIN METABOLISM

Olav E. Rooyackers and K. Sreekumaran Nair
Endocrinology Division, Mayo Clinic and Foundation, Rochester, Minnesota 55905

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ABSTRACT

A continuous turnover of protein (synthesis and breakdown) maintains the functional integrity and quality of skeletal muscle. Hormones are important regulators of this remodeling process. Anabolic hormones stimulate human muscle growth mainly by increasing protein synthesis (growth hormone, insulin-like growth factors, and testosterone) or by decreasing protein breakdown (insulin). Unlike in growing animals, insulin's main anabolic effect on muscle protein in adult humans is an inhibition of protein breakdown. Protein synthesis is stimulated only in the presence of a high amino acid supply. A combination of the stress hormones (glucagon, glucocorticoids, and catecholamines) cause muscle catabolism, but the effects of the individual hormones on human muscle and their mechanisms of action remain to be clearly defined. Although thyroid hormone is essential during growth, both an excess and a deficiency cause muscle wasting by yet unknown mechanisms. A greater understanding of the regulation of human muscle protein metabolism is essential to elucidate mechanisms of muscle wasting.

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INTRODUCTION

Skeletal muscle is the largest tissue of the human body. Its mass comprises approximately 40–45% of the total body weight. However, it accounts for about 70–80% of the cell mass, since adipose tissue, bone, and the extracellular fluid compartment make up approximately 55% of body weight, with the rest of the cell mass compromising only about 10% of body weight (104). The main function of skeletal muscle is to provide power for locomotion. In addition, it has an important metabolic role. Skeletal muscle is the major repository of protein (±50% of total body protein) and free amino acids in the body. Muscle protein is a source of precursors for glucose (via gluconeogenesis) and for conditionally essential protein synthesis in other tissues. During starvation, gluconeogenic amino acids (mainly glutamine and alanine) are released from muscle for glucose production in the liver and kidneys and, subsequently, to provide fuel for the brain (28). Muscle wasting during catabolic diseases, such as cancer, severe trauma, and sepsis, may have the beneficial purpose of supplying amino acids for gluconeogenesis, wound healing, and synthesis of antibodies and acute phase proteins (127).

Net loss or gain of muscle protein is determined by the balance between protein synthesis and protein breakdown. There is a continuous turnover (synthesis and breakdown) of proteins in the human body for the purpose of repair and adaptation. During gain of muscle protein (e.g. growth and after feeding), synthesis exceeds breakdown, whereas during loss of muscle protein (e.g. starvation and disease), breakdown exceeds synthesis.

Muscle turnover rates are affected by many physiological conditions such as fasting (33), feeding (132), disease (130), and aging (159, 168). Hormones are likely to be major regulators of protein turnover in these conditions. Many of the endocrinopathies are characterized by muscle wasting and loss of muscle function (138). The influence of hormones on skeletal muscle protein metabolism has been studied extensively in animals (reviewed in 87, 146), incubated muscles (49), and cell cultures (49, 50). Advances in tracer technology and molecular biology have enabled the study of human protein metabolism over the last

15 years and have enhanced our knowledge of the hormonal regulation of human skeletal muscle protein metabolism. From these studies it appears that the hormonal regulation of protein metabolism varies between species. A classic example is the effect of insulin on skeletal muscle protein synthesis rates.

In this review, we focus on the action of hormones on human skeletal muscle protein metabolism. We discuss the limitations of the different techniques utilized to measure muscle protein metabolism in humans, since many of the controversies may be related to methodological issues.

METHODS TO STUDY SKELETAL MUSCLE PROTEIN METABOLISM IN HUMANS

Measurement of both muscle protein mass and protein dynamics is an important aspect when studying muscle protein metabolism. Because of methodological limitations, information on muscle mass is scarce. In vivo protein dynamics (protein synthesis and breakdown) regulate both the quantity and quality of muscle protein, and their measurement is of great importance to elucidate mechanisms involved in changes of muscle mass and function.

Muscle Mass and Protein

Methods to quantify total body skeletal muscle mass remain limited. Although muscle makes up a large part of the fat-free mass or lean body mass, body composition measurements (52, 83, 164) can approximate muscle mass only roughly.

The technique that measures muscle mass more directly is use of urinary creatinine excretion (78). Creatine is almost exclusively confined to muscle (>98% of the total body pool), and after its irreversible conversion to creatinine, it is excreted quantitatively in urine. However, for a reliable measurement, the subjects have to take a meat-free diet for 5 days (95) and 72-h urine should be collected. During renal failure, acute infections, and muscle injury, creatinine excretion measurements should be interpreted with caution (78). Dual energy X-ray absoptiometry allows measurement of whole body bone, fat, and fat-free mass (FFM) but not specifically muscle mass. However, since FFM (minus bone) in arms and legs is almost exclusively muscle, whole body muscle mass can be estimated based on the assumption that 75% of the muscle is located in the four extremities (79). Although not a direct measurement of muscle mass, it is a technique easily done (10–30 min) and highly reproducible (coefficient of variation, <5%).

Muscle protein content can be measured only by analyzing the protein in muscle biopsies and by assuming that the protein content in the entire muscle is the same as in the biopsy. On average, 18–20% of muscle mass is protein.

Balance Studies

Amino acid balance across a leg or a forearm may provide estimates on net gain or loss of protein in muscle tissue. Amino acid balance across an organ is derived by multiplying the arteriovenous difference of the amino acid concentrations by the blood flow. For amino acids such as phenylalanine, tyrosine, and lysine, which have no other fates than protein synthesis and breakdown in skeletal muscle, the net uptake or release reflects net gain or loss of muscle protein. However, alterations in protein turnover (synthesis or breakdown) rates cannot be defined with this method.

Protein Turnover

Protein turnover is the overall rate at which protein is synthesized or degraded. Whole body protein turnover (18, 155, 156) represents the average turnover rates of all proteins of all the individual tissues in the body (Figure 1). Therefore, to study muscle protein metabolism, it is important to apply techniques specifically designed to measure skeletal muscle protein synthesis or breakdown.

AMINO ACID INCORPORATION TECHNIQUES Amino acid incorporation techniques measure fractional synthesis rates (percentage of protein renewed per hour) of specific proteins or tissue protein based on the incorporation rate of an isotopically labeled amino acid from the precursor pool into the protein(s) of interest. Details and limitations of this technique are discussed elsewhere (65, 113, 134, 156).

Two approaches to administration of the tracer and to achieve an isotopic equilibrium in the precursor pool are used: the primed continuous infusion and the flooding dose. The continuous infusion technique involves an intravenous infusion of the tracer, which is often preceded by a priming dose of the tracer to reach an early plateau (98, 132). The limitation of this approach is that the precursor pool for protein synthesis (amino acyl-tRNA) is technically difficult to measure, and therefore, various surrogate measures are used to calculate fractional synthesis rates (97, 142). Studies addressing this issue in a swine model (10, 157) and in human subjects during surgical anesthesia (158) provide conflicting results on the validity of plasma measurements of the keto acid of leucine (α-ketoisocaproic acid) as a surrogate measure of leucyl-tRNA in muscle. Experimental validation of this surrogate measure in a human subject in the normal physiological state has not been reported.

The flooding dose technique has been introduced to eliminate the problem of the precursor pool enrichments (65, 66, 101). The rationale is that all the precursor pools are flooded with the large dose of tracee injected with the tracer and that they all achieve the same isotopic enrichment within a couple of

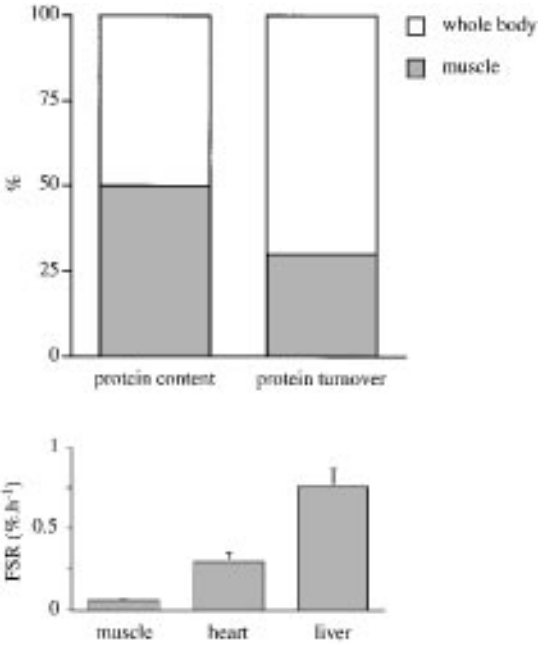

Figure 1 (*top*) Percentage contribution of skeletal muscle to whole body protein content and turnover (adapted from 113). Although skeletal muscle determines a large part of the whole body protein mass (>50%), its contribution to whole body protein turnover is less than 30%. (*bottom*) The low contribution of skeletal muscle to whole body protein turnover is the result of other tissues having much higher protein turnover rates, as is illustrated by the protein synthesis rates of different pig tissue (adapted from 10). The fractional synthesis rates (FSR) were obtained utilizing incorporation of [^{13}C]leucine in tissue proteins using leucyl-tRNA enrichment as precursor pool.

minutes. However, it has been noted that the flooding dose of amino acids itself or the concomitant metabolic changes may stimulate protein synthesis, since higher rates have been measured in comparison with the continuous infusion technique (134, 144).

ARTERIOVENOUS BALANCE TECHNIQUE Simultaneous measurements of arteriovenous fluxes of tracee and tracer across a tissue allow estimation of both protein breakdown and synthesis rates (9, 32, 67, 111, 115). For an amino acid with a constant intracellular concentration, the net uptake or release is the difference between production and utilization. For amino acids, such as phenylalanine, tyrosine, and lysine, which are neither synthesized nor catabolized in skeletal muscle, the rate of disappearance in the leg or forearm represents protein synthesis and the rate of appearance represents protein breakdown. Leucine

has also been used for these measurements, but interpretation of the results is more complicated, since transamination and oxidation of leucine should be subtracted from the rate of disappearance to obtain protein synthesis rates (32).

These calculations do not take into account the reutilization of amino acids derived from protein breakdown, and therefore, synthesis and breakdown rates are minimal estimations. Recently, a modification of this method has been described that also takes the reutilization of amino acids into account (19).

3-METHYLHISTIDINE MEASUREMENTS 3-Methylhistidine (MeHis) is a constituent of actin and myosin. After release from protein breakdown it is neither reutilized for protein synthesis nor catabolized, and it is rapidly excreted in the urine (171). Since >90% of the total human body MeHis is located in skeletal muscle, urinary MeHis has been used to estimate skeletal muscle myofibrillar protein breakdown. However, smooth muscle in the gut may also contribute to excretion of MeHis, especially since the gut has a high turnover (133). Sjölin and coworkers (143) found a relatively small release of MeHis from the splanchnic region in patients during infection, but this may vary under different study conditions. Measurements of arteriovenous differences of MeHis across the leg or forearm may be used to measure skeletal muscle myofibrillar protein breakdown (126, 131), but the low plasma MeHis concentrations and especially arteriovenous concentration differences result in a low accuracy using the current techniques.

HORMONES

Hormones are important regulators of metabolism. Some obvious examples are growth, reproduction, and the handling of nutrients. Hormones also play a pivotal role in the metabolic derangements during disease. Some hormones have well-known anabolic effects (e.g. insulin, growth hormone, insulin-like growth factor-1, and testosterone), while others are considered as catabolic (glucagon, glucocorticoids, and catecholamines). The role of other hormones is less clearly defined. Thyroid hormones, for instance, are essential for growth of children, but hyperthyroidism is characterized by muscle wasting and weakness. Although not considered classical hormones, both prostaglandins and cytokines are discussed because of their hormone-like role in the regulation of protein metabolism, especially during many pathological conditions. Unlike the controlled environment in in vitro studies, levels of various hormones change simultaneously in many in vivo situations. Interactions of these various hormones make investigation of hormonal effects in human subjects complex.

ANABOLIC HORMONES

Insulin

A large number of studies of insulin's effects on muscle protein metabolism have been performed with cell cultures, muscle incubations, perfused muscle preparations, and animal models. These studies showed an anabolic effect of insulin on muscle by stimulating amino acid uptake and protein synthesis and by inhibiting protein breakdown (for reviews see 49, 63, 86, 87, 146).

That there is muscle wasting in insulin-deficient diabetic patients has been known for centuries, and reversal of it with insulin treatment is a clear demonstration of the anabolic effect of insulin. Initial studies on insulin's regulation of human protein metabolism have been performed in type 1 diabetic patients (110, 112, 136, 150, 151). These studies demonstrated that during insulin-deprivation both whole body protein breakdown and synthesis were elevated. Net whole body protein loss occurred because protein breakdown exceeded protein synthesis. Later studies also failed to demonstrate any effect of insulin treatment on fractional synthesis rates of muscle protein in type 1 diabetic patients (Table 1).

The first study performed with human skeletal muscle showed a decreased amino acid release from the forearm when insulin was infused intra-arterially during the postabsorptive state (122). This confirmed that the anabolic effect of insulin on skeletal muscle in man was the same as was found in animals, but the mechanism of the effect remained unclear. In vitro experiments, using human skeletal muscle, showed an increase in protein synthesis with no effect on protein breakdown when incubated with insulin (93, 94).

In contrast to the data based on animal and in vitro studies, most in vivo human studies using tracer methodology showed an inhibition of muscle protein breakdown but no effect on muscle protein synthesis by insulin (Table 1). These latter experiments are complicated by insulin-induced hypoaminoacidemia, which may influence protein metabolism. To overcome this problem, experiments have been performed using intra-arterial infusion of insulin, so as to prevent any decline in systemic amino acid concentrations. Alternatively, hypoaminoacidemia was prevented by a simultaneous infusion of amino acids.

Most studies performed in both healthy volunteers (5, 13, 20, 43, 44, 58, 61, 67, 77, 91, 102, 105, 116, 162) and type 1 diabetic patients (12, 13, 110, 111, 117, 119, 147) showed an insulin-induced decrease in protein breakdown irrespective of any change in amino acid concentrations. In most of the other studies, the decrease in protein breakdown failed to reach statistical significance (Table 1). In healthy human subjects, insulin did not change MeHis fluxes across either the leg or the forearm (5, 105). These studies suggest that the insulin-induced reduction in skeletal muscle protein breakdown occurs in

Table 1 Effect of insulin in combination with amino acids on human skeletal muscle protein synthesis and breakdown in normal subjects

References	Insulin infusion	Insulin conc (μU/ml)	Amino acid infusion	Amino acid conc	Tissue/method	Protein synthesis	Protein breakdown
Normal control							
Gelfand et al '87 (67)	Local	124	no	↔	Forearm/Leu, Phe balance	↔	↓
Arfvidsson et al '91 (5)	Systemic	100–120	no	↓	Leg/Tyr balance	↔	↔
Bennet et al '90 (13)	Systemic	597[a]	yes[b]	↑	Leg/Leu, Phe balance	↔ or ↑	↓ or ↔
Fryburg et al '90 (58)	Local	±30	no	↔	Forearm/Leu, Phe balance	↔	↓
Denne et al '91 (44)	Systemic	2614	no	↓	Leg/Phe balance	↔	↓
Tessari et al '91 (149)	Systemic	75	no	↓	Forearm/Leu, Phe balance	↔	↔
Heslin et al '92 (77)	Systemic	71	yes	↔ or ↑	Forearm/Leu balance		↓
Louard et al '92 (91)	Local	30–124	no	↔	Forearm/Leu, Phe balance	↔	↓
Wolf et al '92 (162)	Systemic	60	yes	↑	Forearm/Phe balance	↑	↔
McNurlan et al '94 (102)	Systemic	40	no	↔ or ↓	V. lateralis/flooding dose	↔	
Möller-Loswick et al '94 (105)	Systemic	100	yes	↓[c]	Leg/Tyr, Phe balance	↔	↓
					Forearm/Tyr, Phe balance	↓	↓
Newman et al '94 (116)	Systemic	70	yes	↑	Forearm/Leu, Phe balance	↑	↓ or ↔
Biolo et al '95 (20)	Local	77	no	↔ or ↓	V. lateralis/cont. infusion	↑	
					Leg/Leu, Phe balance	↑	↔
Denne et al '95 (43)	Systemic	19,000[a]	no	↓	Leg/Phe balance		↓
Fryburg et al '95 (61)	Local	444–606[a]	yes	↑	Forearm/Phe balance	↑[d]	↓

Type 1 diabetes							
Nair et al ’84 (110)	Systemic		no	↓	V. lateralis/cont. infusion	↔	
Pacy et al ’89 (119)	Systemic	8 vs 15	no	↔ or ↓	V. lateralis/cont. infusion	↔	
Bennet et al ’90 (14)	Systemic	5 vs 63	yes[b]	↑ or ↓[e]	T. anterior/cont. infusion	↔	
Tessari et al ’90 (147)	Systemic	6 vs 44	no	↓	Forearm/Leu balance		↔
Bennet et al ’91 (12)	Systemic	37 vs 529[a]	no	↓	Leg/Leu, Phe balance	↔	↓
	Systemic	61 vs 561[a]	yes[b]	↑	Leg/Leu, Phe balance	↔	↓
Pacy et al ’91 (117)	Systemic		no	↓	Forearm/Leu balance	↔	↔
Nair et al ’95 (111)	Systemic	3 vs 12	no	↔ or ↓	Leg/Leu, Phe balance	↔	↓
Type 2 diabetes							
Denne et al ’95 (43)	Systemic	200 vs 12,000[a]	no	↓	Leg/Phe balance	↔	↔

[a] pM.

[b] Amino acids were infused during both the control and the insulin study, with a higher infusion rate during the insulin study.

[c] Amino acids were infused during both the control and the insulin study. During the insulin infusion the amino acid concentration decreased in comparison with the control.

[d] Same increase as with amino acid infusion alone.

[e] Despite an increase in plasma amino acid concentration, intracellular concentrations of several amino acids were decreased.

nonmyofibrillar proteins. Against the above observation, an increase in urinary MeHis excretion was observed in diabetic patients (both type 1 and type 2) in a poorly controlled state (96). This discrepancy may be related to the lack of a reliable technique to measure MeHis concentrations in blood and urine.

Skeletal muscle protein synthesis seems to be insensitive to insulin in humans. The few studies that show an increase are those with substantial increases in amino acid concentrations following amino acid infusion (Table 1). However, these studies do not confirm or exclude the possibility that normal levels of intracellular amino acid levels limit insulin's ability to stimulate protein synthesis. Local infusion of insulin is likely to decrease intracellular amino acid concentrations (4) despite normal systemic levels. Also, the decrease in protein breakdown reduces the supply of amino acids, and this may influence protein synthesis and its sensitivity to insulin. Even during simultaneous infusion of insulin and amino acids, intracellular amino acid levels may fall (14) and thereby modulate the insulin effect on protein synthesis. One study reported an increased protein synthesis and unchanged breakdown rate during a local insulin infusion (20). In this study, a modified mathematical model was used, taking into account the amino acids coming from breakdown and reutilized for protein synthesis. The authors explain the different results in their study by a possible larger reutilization of amino acids during insulin infusion. However, they also reported increased synthesis and unchanged protein breakdown when the traditional model was used. The difference between this and other studies remains to be resolved

In type 1 diabetic patients, muscle protein synthesis seems to be relatively insensitive to both insulin and increased amino acid concentrations. In one study, insulin administration with a profound increase in systemic amino acid concentrations failed to stimulate muscle protein synthesis in type 1 diabetics (12). Also, when synthesis rates of the main contractile protein in skeletal muscle—myosin heavy chain—were studied, no stimulatory effect of insulin was observed in type 1 diabetic patients (31). Muscle protein metabolism in type 2 diabetic patients has been investigated in only one study (43). Despite intensive insulin treatment, no effect on skeletal muscle protein metabolism was observed.

The increased whole body protein synthesis rates observed during insulin deprivation in type 1 diabetic patients do not occur in skeletal muscle but in the splanchnic bed (Figure 2) (111). However, the decreased whole body protein breakdown is largely the result of the decrease in skeletal muscle protein breakdown. The relative contribution of muscle to whole body protein synthesis is higher during insulin treatment than during insulin deprivation, although the absolute protein synthesis did not change.

The discrepancy between the insulin effect on skeletal muscle protein synthesis between humans and animals could be related to differences in age (63).

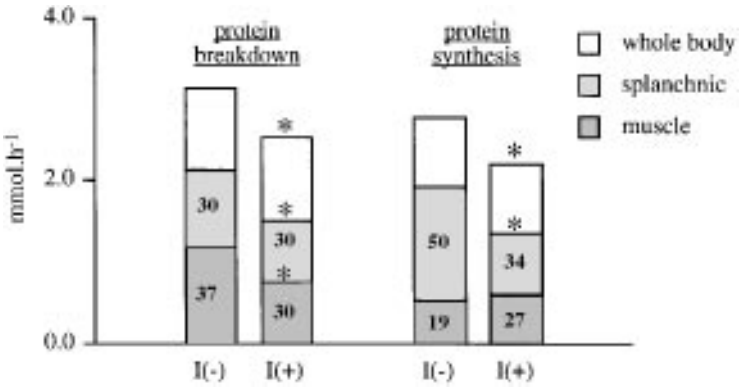

Figure 2 Changes in whole body, splanchnic region, and skeletal muscle protein metabolism in type 1 diabetic patients during insulin deprivation [I(−)] and insulin treatment [I(+)] (adapted from 111). The numbers represent the percentage contribution to whole body protein synthesis and breakdown. Whole body skeletal muscle protein kinetics are estimated using the assumption that one leg is 25% of the whole body skeletal muscle mass. The entire decrease in whole body protein synthesis is accounted for by the reduction in splanchnic region protein synthesis. A decline in protein breakdown in the splanchnic region with insulin treatment is matched by a similar decline in protein synthesis. The reduction in whole body protein breakdown with insulin treatment is largely the result of a decrease in skeletal muscle protein breakdown. *, Significantly different from insulin-deprived state.

The insulin-induced increase in protein synthesis rates in growing rats is reported to be attenuated by age (7), indicating that adult rats responded similarly to adult humans.

In addition, human heart muscle seems to respond to insulin treatment the same as skeletal muscle does. Hyperinsulinemia in patients with cardiovascular disease and insulin resistance reduced myocardial protein breakdown rates by 80% and had no effect on protein synthesis rates (100).

Not only an insulin deficiency but also insulin resistance may influence skeletal muscle protein metabolism. It has been suggested that insulin resistance during severe catabolic diseases (e.g. severe trauma, major surgery, sepsis, burn injury, cancer), which occurs mainly in the peripheral tissues, may contribute to the muscle wasting observed in these conditions (22). The increased levels of the catabolic hormones in these conditions and their potential effect on muscle proteins (161) make it difficult to draw a definitive conclusion. Two studies reported a beneficial effect of insulin infusion in catabolic patients sufficiently fed with formulas, including amino acids (121, 139). In both studies, the predominant results were a positive protein balance from increased muscle protein synthesis.

In conclusion, it seems that in adult human subjects insulin reduces muscle protein breakdown without any effect on protein synthesis. The latter may be related to lack of a sufficient amino acid supply for increased protein synthesis. An additional problem with these measurements is that none of these techniques utilizes the true precursor pool for protein synthesis. Insulin and the related changes in amino acid levels may influence the tracer enrichment in the tRNA pool. With the recent improvements in tRNA extraction and sensitivity of mass spectrometers (10, 157, 158), it is possible to measure tRNA enrichments in human muscle samples and determine the effect of insulin on muscle protein synthesis using the most reliable approach.

Growth Hormone and Insulin-Like Growth Factor-1

The anabolic effect of growth hormone (GH) replacement in GH-deficient children and adults is well established (34, 40, 84, 85, 140). All these studies showed an increase in lean body mass or muscle mass and a decrease in fat mass. In addition to the increase in muscle mass, an improvement in muscle function has also been reported (40, 84, 85). This improved muscle function involved both muscle strength and exercise capacity. In the elderly, in whom GH secretion is diminished, increases in lean body mass and muscle mass have been reported during GH supplementation (141).

In vitro and animal studies have demonstrated that GH increases protein synthesis rates and, in most instances, decreases protein breakdown in muscle tissue (49, 50, 57, 87, 146). These results are much more pronounced in GH-deficient than in normal animals.

Human whole body protein synthesis rates, in general, are increased with GH administration (15, 35, 81, 166). The main anabolic effect on whole body protein metabolism seems to be a diminished amino acid oxidation (15, 36, 81). A recent study showed a stimulating effect of GH on skeletal muscle protein synthesis with none or only a minor influence on breakdown, if administered to the artery supplying the muscle bed (Table 2). The effect of systemic infusion of GH on muscle protein metabolism remains controversial, mainly because of a wide variety of study designs and subject groups (Table 2). However, all the studies measuring both protein synthesis and breakdown rates reported a positive protein balance in skeletal muscle. Since all these studies were performed in the postabsorptive state, the effect of GH during a meal remains to be investigated.

Three studies that investigated the influence of local insulin-like growth factor-1 (IGF-1) infusion on human skeletal muscle protein metabolism showed an increase in protein synthesis (Table 2). This result is in contrast with the lack of stimulation of whole body protein synthesis when IGF-1 was systemically infused (45). The stimulatory effect of IGF-1 on muscle protein synthesis can be

Table 2 Effect of GH and IGF in combination with amino acids on human skeletal muscle protein synthesis and breakdown

References	Hormone infusion/ time	Hormone conc. (ng/ml)	Amino acid infusion	Amino acid conc.	Tissue/method	Protein synthesis	Protein breakdown
GH							
Fryburg et al '91 (59)	Local/6 h	35	No	↔	Forearm/Leu, Phe balance	↑	↔
Wolf et al '92 (162)	Systemic/3 d	39–59	No	↓	Forearm/Phe balance	↑	↑
Yarasheski et al '92 (166)[a]	Systemic/12 wk	52	No		V. lateralis/cont. infusion	↔	
Fryburg et al '93 (56)	Systemic/6 h	30–34	No	↔ or ↓	Forearm/Leu, Phe balance	↑	↔
Copeland et al '94 (35)	Systemic/3.5 h[b]	±20	No	↔	Leg/Leu, Phe balance	↔	↓
Yarasheski et al '95 (169)[a]	Systemic/16 wk		No		V. lateralis/cont. infusion	↔	
Welle et al '96 (160)[c]	Systemic/shot[d]	7–18	No		V. lateralis/cont. infusion	↔	
	Systemic/12 wk		No		V. lateralis/cont. infusion	↔	
IGF							
Fryburg et al '94 (54)	Local/6 h	403	No	↔	Forearm/Phe balance	↑	↔
		577	No	↓		↑	↓
		878	No	↓		↑	↓
Fryburg et al '95 (61)	Local/6 h	763–815	Yes	↑	Forearm/Phe balance	↑	↔
Fryburg et al '96 (55)	Local/4 h	604	No	↓	Forearm/Phe balance	↑	↔

[a] Both the control and the growth hormone (GH)–supplemented subjects also participated in an extensive resistance exercise program. IGF, Insulin-like growth factor.

[b] Simultaneous infusion of somatostatin, replacement doses of insulin and glucagon, and a replacement or high dose of GH.

[c] Subjects were over 60 years old.

[d] GH was given as a single injection; measurements were started 4 h later.

attenuated by inhibiting the concomitant increase in blood flow by a nitric oxide inhibitor. This indicates that IGF-1 effect on human muscle protein synthesis depends on nitric oxide production for an increased blood flow or possibly for a direct effect (55). Muscle protein breakdown seems to be inhibited by IGF-1 only with the higher doses and with a simultaneous decrease in systemic amino acid concentration (Table 2).

The metabolic action of GH may, in part or fully, be secondary to the production of IGF-1. However, this GH-IGF axis is far from understood. Most likely, GH acts both directly and indirectly via IGF-1, depending on the metabolic action and tissue (1, 165). IGF-1 seems to affect human skeletal muscle protein metabolism mainly by increasing protein synthesis. To eliminate an indirect effect on protein metabolism, GH has been infused locally in the brachial artery (59). No increase in systemic and deep venous IGF concentrations was measured with this approach. Skeletal muscle protein synthesis rates were increased to a greater extent in this study than when systemic infusion was used (56). GH is capable of stimulating IGF-1 gene expression in skeletal muscle of rats (92). Since human muscle expresses IGF-1 and some of the IGF-binding proteins (152), a possibility of an intramuscular IGF-1 increase following local GH infusion cannot be excluded in humans. The biological activity of IGF-1 is determined by its interaction with IGF receptors and IGF-binding proteins (IGFBPs) (6, 165). Systemic concentration measurements, therefore, are of limited use in interpreting these results. Our knowledge of the GH-IGF axis is rapidly increasing, making it possible to study the regulation of skeletal muscle growth in more detail.

The results of four studies contradict the other studies (Table 2; 35, 160, 166, 169). None of these studies found an effect of GH on skeletal muscle protein synthesis in humans. However, comparison with the other studies is difficult since treatment was superimposed on a program of intensive resistance exercise training (166, 169) or was conducted in human subjects over 60 years of age (160, 169). Copeland & Nair (35) infused GH for 3.5 h, whereas in the other acute studies the administration lasted for at least 6 h (56, 59). If the effect of GH on skeletal muscle is regulated via IGF-1 and includes a lag time, 3.5 h may have been too short to observe an effect. In the studies by Fryburg and coworkers, the increased synthesis rates were observed only after 6 h, not after 3 h, of GH infusion (56, 59). It is possible that IGF-1 production in skeletal muscle was stimulated by GH during this 6-h infusion. In the study by Copeland & Nair (35), the confounding effects of other hormones, such as insulin, were eliminated. By infusing somatostatin and replacement doses for insulin and glucagon, any additional effects of one of these hormones were excluded from this study. It is known that insulin-like growth factor binding proteins (IGFBPs) are secreted under the influence of various physiological

stimuli (GH, IGF, insulin, feeding). Since the IGFBPs can either potentiate or inhibit IGF action, different levels of various hormones are likely to influence GH and IGF-1 action on muscle protein metabolism. Little is known about the interaction between various hormones and growth factors and possible additive effects.

The effect of GH on muscle mass in addition to resistance training has been studied by Yarasheski and coworkers (166, 167, 169). In young men, older men, and experienced weight lifters, GH supplementation had no additional effect on skeletal muscle protein synthesis over training alone. In the young and older men, both fat-free mass and whole body protein synthesis rates were, however, increased (166, 169). This study also supports the data from acute administration of GH (35) in young subjects showing that tissues other than muscle may be more sensitive to GH.

When GH and insulin is infused simultaneously into the forearm, increased protein synthesis and unchanged protein breakdown have been observed (62). These data indicate that the normally observed insulin-induced decrease in muscle protein breakdown is blunted by GH.

Although GH levels are either unchanged or slightly increased in catabolic patients (including the critically ill), most GH intervention studies show an increased nitrogen balance, indicating preservation of body protein (for review see 154). Gore and coworkers observed increased skeletal muscle protein synthesis in burn patients supplied with GH (72). In contrast, no effect of GH has been observed on skeletal muscle protein synthesis and breakdown rates in catabolic cancer patients (162). Obviously more research is needed, especially since these patients seem to be GH resistant, which could only be overcome by high doses of GH (39). Because of several side effects (negative mineral balances, water retention and edema, hyperglycemia, carpel-tunnel syndrome), high doses of GH are not desirable. A combination of GH and IGF with nutritional support has potential for treatment, but well-controlled studies are needed to confirm this (39). In myotonic dystrophy patients, GH supplementation is reported to have a positive effect on muscle strength, indicating a possible beneficial effect on protein metabolism (153).

Both GH and IGF-1 have a stimulating effect on human skeletal muscle protein synthesis when administered directly, although this effect is not universally observed when administered systemically. Systemically administered IGF-1 inhibits whole body protein breakdown (45) and possibly skeletal muscle protein breakdown at high doses. GH seems not to affect protein breakdown. IGF-1 alone may cause hypoglycemia, which limits its systemic administration. Combing with GH may overcome this problem. The metabolic action and interaction of these hormones is not fully understood, and therefore, more studies are needed.

Gonadal Steroids (Testosterone)

Little is known about the effect of estrogen and progesterone on protein metabolism. Menopause in women is associated with accelerated muscle loss (53). However, it is unclear whether estrogen replacement enhances muscle mass or not. Because of the lack of information on estrogen and progesterone, only testosterone is discussed.

From animal studies using castrated rats, an anabolic effect of testosterone on skeletal muscle involving predominantly hypertrophy is apparent (106). This anabolic effect was mainly accomplished by increased protein synthesis rates (48, 106). In normal animals, the effect of testosterone was less clear, but some positive effects on protein metabolism have been described (29).

In human subjects, testosterone increases lean body mass in a dose-dependent manner (51). Testosterone is undoubtedly able to increase muscle mass in hypogonadal humans (29). Anabolic steroids have been studied in athletes to improve muscle gain with training. However, the results are inconsistent (29). Recently it has been demonstrated that supraphysiological doses of testosterone, especially in combination with strength training, increase muscle mass and strength in normal men (17).

Several studies measured the effect of testosterone on human skeletal muscle protein metabolism. A pharmacological dose of testosterone given to healthy subject for 12 weeks increased muscle mass (both creatinine excretion and whole body potassium) and increased muscle protein synthesis rates by 27% (75). Also, in myotonic dystrophy patients, a pharmacological dose of testosterone increased muscle mass and protein synthesis rates (74). There was no improvement in muscle strength in either of the above studies. A recent study showed a 20% increase in muscle mass and a 56% increase in muscle protein synthesis in hypogonadal men treated with testosterone for 6 months (Figure 3). In addition, this study showed a nonsignificant increase in myosin heavy-chain synthesis rates in skeletal muscle as a result of the testosterone treatment (25). Testosterone replacement in elderly men to the level of young men resulted in an almost twofold increase in skeletal muscle protein synthesis (152). In addition, muscle strength but not endurance capacity was increased in this last study. Unfortunately, none of these studies measured muscle protein breakdown. The latter study did not observe an increase in thigh muscle volume, from which an additional increase in protein breakdown was concluded (152). However, it appears that the muscles of the pectoral and shoulder regions are more androgen sensitive in humans. The other studies estimated whole body muscle mass by creatinine excretion or whole body potassium. The observed increase in muscle mass in these studies indicates that breakdown is not increased. However, direct measurements are needed to clarify this issue.

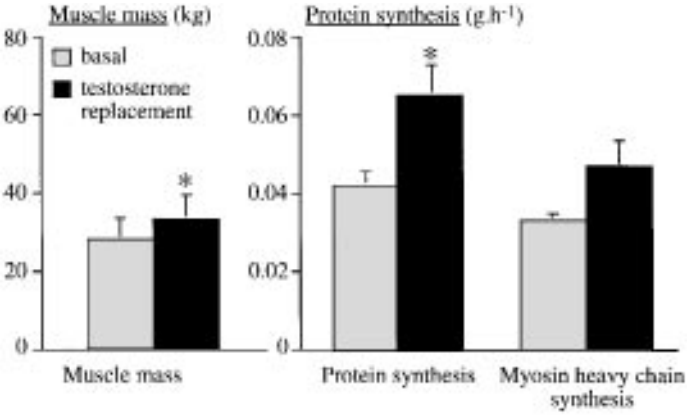

Figure 3 Effect of a 6-month testosterone replacement on muscle mass (*left*) and muscle protein synthesis rates (*right*) in hypogonadal men (adapted from 25). Synthesis rates were measured using the incorporation method with a primed continuous L-[^{13}C]leucine infusion. Muscle mass was assessed by creatinine excretion. *, Significantly different from baseline.

Testosterone administration increases IGF-1 levels in normal men (80). It has been suggested that testosterone may have its anabolic effect mediated via IGF-1, since IGF-1 increases muscle protein synthesis (see above). The testosterone replacement study in the elderly men did indeed show increased mRNA levels of IGF-1 in skeletal muscle (152). This, together with decreased concentrations of IGFBP-4 mRNA, suggests an enhanced intracellular IGF-1 availability with testosterone administration.

Few studies have been performed using testosterone to improve muscle wasting in catabolic patients. Some studies showed an increased nitrogen balance, while others failed to confirm this (64).

CATABOLIC HORMONES

Glucagon, glucocorticoids, and catecholamines are considered the most important catabolic hormones. These stress or counter-regulatory hormones are elevated in clinically stressful situations and are thought to be involved in the net protein catabolism seen in these situations (3). The elevated levels of these hormones may counteract the hyperinsulinemia, also present in these situations, thereby inducing a relative insulin resistance (161). Infusing normal volunteers with a mixture of these hormones increased nitrogen and MeHis excretion (69), as well as net release of amino acids from muscle tissue (16, 26). However, the net amino acid release was smaller than that observed in burn patients (27),

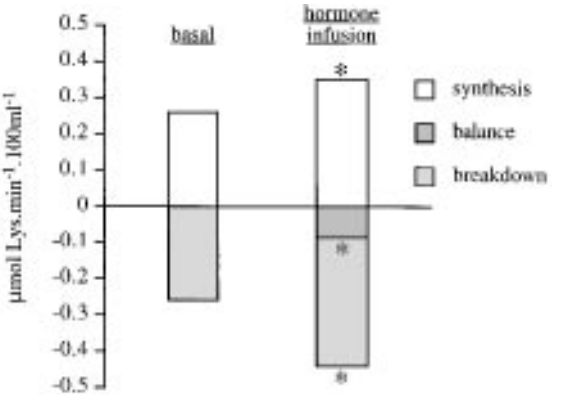

Figure 4 Effect of a 4-h catabolic hormone infusion (glucagon, 3 ng kg^{-1} min^{-1}; cortisol, 6 ng kg^{-1} min^{-1}; epinephrine, 15 ng kg^{-1} min^{-1}) on leg protein metabolism in healthy volunteers using [^{15}N]lysine kinetics (adapted from 73). *, Significantly different from basal.

showing that the counter-regulatory hormones contribute, but are not the sole mediators of, the catabolic response during disease. The net protein loss seems to be the result of increased muscle protein breakdown in excess of an increased synthesis (Figure 4).

Glucagon

Glucagon's effect has been considered to be mainly in the liver. However, incubating rat muscles with high concentrations of glucagon and infusing rats with a pharmacological dose of glucagon resulted in decreased skeletal muscle protein synthesis rates (123, 124).

From whole body studies in humans it appeared that glucagon stimulates protein breakdown in the insulin-deficient state, although the main effect was a stimulation of leucine oxidation (114). Insulin, with its known inhibitory effect on protein breakdown, eliminated the glucagon-induced enhancement of protein breakdown when present at normal concentrations (37). In addition, glucagon's catabolic effect on whole body level was clearly demonstrated in a recent study where glucagon infused with amino acids attenuated the amino acid–induced increase in whole body protein synthesis (30).

Glucagon appears to have no effect on human skeletal muscle protein, based on MeHis excretion and net amino acid balance across the forearm (46, 163). Pacy and coworkers showed a reduced leucine balance across the forearm, but no changes in either muscle protein synthesis or breakdown were observed (118). This latter study was performed during insulin deficiency, and since

insulin has a major effect on muscle protein metabolism, these results are difficult to interpret in the context of normal human physiology. The catabolic effect of glucagon is mostly relevant in type 1 diabetic patients during insulin deficiency.

Glucocorticoids

Animal studies showed a loss of muscle protein induced by decreased protein synthesis rates and possibly increased protein breakdown rates in response to glucocorticoid treatment (49, 64, 146).

That glucocorticoids have an effect on human muscle is known from marked muscle wasting and weakness in Cushing's syndrome (increased glucocorticoid production) and during intensive cortisol administration in a variety of clinical situations (82). Whole body protein breakdown is increased in healthy human subjects infused with high physiological and pharmacological doses of glucocorticoid (11, 41, 81, 90). It is intriguing that an increased protein breakdown was associated with a smaller increase in protein synthesis with cortisol administration (24). This increased whole body protein turnover may reflect an increased breakdown in skeletal muscle to supply amino acids for an increased synthesis of proteins in more vital organs. In patients with Cushing's syndrome, the results are conflicting: decreased whole body protein synthesis and increased protein breakdown (23) in contrast to no changes (148). However, if the results are expressed per kilogram of lean body mass, then no change in protein breakdown was found in this first study.

Dexamethasone (a synthetic glucocorticoid) had no effect on skeletal muscle protein synthesis or breakdown if infused in healthy subjects. It did, however, blunt the insulin-induced anticatabolic effect during hyperinsulinemia (90). This may lead to a daily net loss of muscle protein if the insulin-induced anabolism after a meal is blunted as well. Glucocorticoid treatment in patients with rheumatoid arthritis decreased skeletal muscle protein synthesis (70). No information on protein breakdown was available from this study. In contrast to this is an increased muscle mass with no changes in skeletal muscle protein synthesis rates in Duchenne dystrophy patients treated with a synthetic glucocorticoids (prednisone) for 6–8 weeks (135). The increase in muscle mass is likely to be related to a glucocorticoid-induced decrease in muscle protein breakdown, as indicated by a decreased excretion of MeHis. These results are in agreement with the known effect of prednisone on improving muscle strength and function in Duchenne dystrophy (135).

Insufficient information is available to draw a definitive conclusion on the effect of glucocorticoids on human muscle protein metabolism. Part of the problem may arise from concomitant changes in other hormones, especially hyperinsulinemia, occurring during glucocorticoids administration.

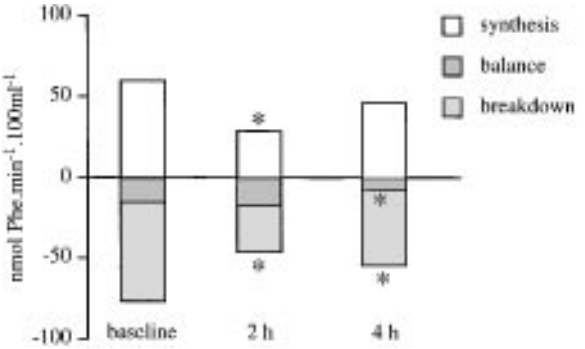

Figure 5 Effect of local intra-arterial epinephrine infusion (0.45 ng kg^{-1} min^{-1}) on forearm protein metabolism in healthy volunteers as estimated using [^{3}H]phenylalanine kinetics (adapted from 60). Protein synthesis, breakdown, and balance were measured at baseline and at 2 and 4 h during the epinephrine infusion. *, Significantly different from baseline.

Catecholamines

Although catecholamines (epinephrine and norepinephrine) are listed as catabolic hormones, most in vivo human studies show an opposite effect. Whole body protein metabolic studies showed that epinephrine infusion for a short duration decreased whole body protein breakdown (89, 103) and had no effect if infused for a longer period (99).

Net release of amino acids from muscle has been found to be either increased (89) or decreased (42) in similar epinephrine infusion protocols. The effect of epinephrine on human skeletal muscle protein metabolism is time dependent (60). An acute decline in protein breakdown is compensated by a similar decrease in protein synthesis, while a longer infusion period leads to an improvement in net balance due to decreased breakdown in combination with normal synthesis (Figure 5). This anabolic, rather than the expected catabolic, effect is supported by a potent effect of β-agonists, especially clenbuterol, on muscle growth in farm animals, used to improve meat production (11a).

OTHER HORMONES AND MEDIATORS

Thyroid Hormone

Thyroid hormone is essential for growth but has, on the other hand, a profound catabolic effect on skeletal muscle during both hypo- and hyperthyroidism (88, 125). Both hypothyroidism and hyperthyroidism are also characterized by muscle weakness. The main reason for this muscle weakness is a decrease in muscle efficiency rather than loss of muscle mass (172).

In animals, thyrotoxic myopathy is associated with muscle atrophy as a result of increased protein breakdown rates exceeding increased synthesis rates (8, 108). Thyroidectomy in animals generally induces muscle atrophy by both decreasing protein synthesis and increasing breakdown (64).

MeHis excretion studies suggested that muscle myofibrillar protein breakdown is increased in thyrotoxic patients and that this is reversed with treatment (2, 137, 170). Both hyperthyroidism and hypothyroidism reduced whole body protein mass by a greater decrease in protein synthesis than in protein breakdown (38, 107). However, increased whole body protein breakdown has also been reported in hyperthyroid patients (68). Only small or no changes in MeHis release from human muscle observed in the same study suggested a decreased synthesis rate as the most important mechanism for thyroid hormone–related muscle wasting. However, more direct estimations of protein turnover, e.g. in vitro measurements using muscle biopsies from hyperthyroid patients, showed a significant correlation between serum thyroid hormone levels and increasing protein breakdown rates (76).

Prostaglandins and Cytokines

Both prostaglandins and cytokine are important regulators of muscle protein metabolism in animals (21, 120). Prostaglandins are reported to play an important role in the muscle hypertrophy or atrophy related to muscle activity (64). In rats, prostaglandins seem to modulate the insulin-induced increase in muscle protein synthesis (128, 129) and the influence of glucocorticoids on muscle protein metabolism (120). However, the influence on insulin action could be different in humans, since insulin has a different effect in humans and animals. Distinct prostaglandins have different effects in rats, e.g. prostaglandin $F_{2\alpha}$ seems to be anabolic (increased synthesis and no effect on breakdown) while prostaglandin E_2 has a catabolic effect (increases breakdown and no effect on synthesis) (64).

Cytokines (e.g. tumor necrosis factor and interleukins) are most likely important mediators in the muscle wasting observed during severe illness (21). In rats, they generally induce muscle wasting by increasing muscle protein breakdown (47, 71, 87).

In humans, prostaglandin E_1 seems to have an anabolic effect on skeletal muscle (145). The direct effects of prostaglandins and cytokines and their interaction with, or modulation of, hormone actions on muscle protein metabolism in humans still have to be established.

SUMMARY

Although there is a substantial interaction among hormones in normal physiology, our knowledge of the hormonal regulation of human protein metabolism

Table 3 Summary: Hormonal regulation of human skeletal muscle protein metabolism

Hormone	Protein synthesis	Protein breakdown	Protein balance
Insulin	↔	↓	↑
Insulin + amino acids	↑	↓	↑↑
GH[a]	↑ or ↔	?[b]	↑
IGF	↑	↔ or ↓	↑
Testosterone	↑	?	↑
Stress hormones[c]	↑	↑↑	↓
Glucagon	↔	↔	↔
Glucocorticoids	?	?	↓
Epinephrine	↔	↓	↑
Thyroid hormone-hypothyroidism	?	?	↓
Thyroid hormone-hyperthyroidism	↓?	↑?	↓

[a] Possibly mediated through insulin-like growth factor-1. GH, Growth hormone.
[b] No or not sufficient evidence available.
[c] Glucagon, glucocorticoids, and catecholamines.

has been enhanced considerably by studying the effects of single hormones. However, the hormonal regulation of protein metabolism may also be modulated by secondary events. The insulin-induced hypoaminoacidemia and its impact on protein synthesis rates is a classic example. Insulin levels usually increase after a meal, but most of the human studies are performed in the postabsorptive state. Although postprandial studies are fraught with problems because of the non-steady state for several hours after a meal, carefully designed studies may provide information of hormonal regulations in more physiological conditions.

For most of the hormones, however, our knowledge is limited with regard to skeletal muscle protein metabolism (Table 3). Many controversies on the hormonal regulation of muscle protein turnover might be resolved if we could measure the enrichment of the amino acyl-tRNA and the intracellular pool into which the amino acids from protein breakdown appear. Much more is known from animal than from human studies, but the results from animals cannot always be directly translated to humans because of the species differences in hormonal effects on protein metabolism.

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Literature Cited

1. Adamo ML. 1995. Regulation of insulin-like growth factor 1 gene expression. *Diabetes Rev.* 3:2–27
2. Adlerberth A, Angeras U, Jagenberg R, Lindstedt G, Stenstr_m G, Hasselgren P. 1987. Urinary excretion of 3-methylhistidine and creatinine and plasma concentrations of amino acids in hyperthyroid patients following preoperative treatment with antithyroid drug or β-blocking agent: results from a prospective, randomized study. *Metabolism* 36: 637–42
3. Alberti KGMM, Batstone GF, Foster KJ, Johnston DG. 1980. Relative role of various hormones in mediating the metabolic response to injury. *J. Parenter. Enter. Nutr.* 4:141–46
4. Alvestrand A, Defronzo RA, Smith D, Wahren J. 1988. Influence of hyperinsulinaemia on intracellular amino acid levels and amino acid exchange across splanchnic and leg tissues in uraemia. *Clin. Sci.* 74:155–63
5. Arfvidsson B, Zachrisson H, Möller-Loswick AC, Hyltander A, Sandström R, Lundholm K. 1991. Effect of systemic hyperinsulinemia on amino acid flux across human legs in postabsorptive state. *Am. J. Physiol.* 260:E46–52
6. Bach LA, Rechler MM. 1995. Insulin-like growth factor binding proteins. *Diabetes Rev.* 3:38–61
7. Baillie AGS, Garlick PJ. 1991. Attenuated responses of muscle protein synthesis to fasting and insulin in adult female rats. *Am. J. Physiol.* 262:E1–5
8. Barrett EJ, Gelfand RA. 1989. The in vivo study of cardiac and skeletal muscle protein turnover. *Diabetes Metab. Rev.* 5:133–48
9. Barrett EJ, Revkin JH, Young LH, Zaret BL, Jacob R, Gelfand RA. 1987. An isotopic method for measurement of muscle protein synthesis and degradation in vivo. *Biochem. J.* 245:223–28
10. Baumann PQ, Stirewalt WS, O'Rourke BD, Howard D, Nair KS. 1994. Precursor pools of protein synthesis: a stable isotope study in a swine model. *Am. J. Physiol.* 267:E203–9
11. Beaufrere B, Horber FF, Schwenk WF, March HM, Matthews D, et al. 1989. Glucocorticosteroids increase leucine oxidation and impair leucine balance in humans. *Am. J. Physiol.* 257:E712–21

11a. Beckett AH. 1992. Muscling in on clenbuterol. *Lancet* 340:403 (Lett.)

12. Bennet WM, Connacher AA, Jung RT, Stehle P, Rennie MJ. 1991. Effects of insulin and amino acids on leg protein turnover in IDDM patients. *Diabetes* 40:499–508
13. Bennet WM, Connacher AA, Scrimgeour CM, Jung RT, Rennie MJ. 1990. Euglycemic hyperinsulinemia augments amino acid uptake by human leg tissue during hyperaminoacidemia. *Am. J. Physiol.* 259:E185–94
14. Bennet WM, Connacher AA, Smith K, Jung RT, Rennie MJ. 1990. Inability to stimulate skeletal muscle or whole body protein synthesis in type 1 (insulin-dependent) diabetic patients by insulin-plus-glucose during amino acid infusion: studies of incorporation and turnover of tracer L-[1-^{13}C]leucine. *Diabetologia* 33: 43–51
15. Berneis K, Keller U. 1996. Metabolic actions of growth hormone: direct and indirect. *Bailliere Clin. Endocrinol.* 10:337–52
16. Bessey PQ, Lowe KA. 1993. Early hormonal changes affect the catabolic response to trauma. *Ann. Surg.* 218:476–91
17. Bhasin S, Storer T, Berman N, Callegari C, Clevenger B, et al. 1996. The effects of supraphysiological doses of testosterone on muscle size and strength in normal men. *New Engl. J. Med.* 335:1–7
18. Bier DM. 1989. Intrinsically difficult problems: the kinetics of body proteins and amino acids in man. *Diabetes Metab. Rev.* 5:111–32
19. Biolo G, Chinkes D, Zhang X-J, Wolfe RR. 1992. A new method to determine in vivo the relationship between amino acid transmebrane transport and protein kinetics in muscle. *J. Parenter. Enter. Nutr.* 16:305–15
20. Biolo G, Fleming DRY, Wolfe RR. 1995. Physiologic hyperinsulinemia stimulates protein synthesis and enhances transport of selected amino acids in human skeletal muscle. *J. Clin. Invest.* 95:811–19
21. Bistrian BR, Schwartz J, Istfan NW. 1992. Cytokines, muscle proteolysis, and the catabolic response to infection and inflammation. *Proc. Soc. Exp. Biol. Med.* 200:233–39
22. Black PR, Brooks DC, Bessey PQ, Wolfe RR, Wilmore DW. 1982. Mechanisms of insulin resistance following injury. *Ann. Surg.* 196:420–35
23. Bowes SB, Benn JJ, Scobie IN, Umpleby AM, Lowy C, Sönksen PH. 1993. Leucine

metabolism in patients with Cushing's syndrome before and after successful treatment. *Clin. Endocrinol.* 39:591–98
24. Brillon DJ, Zheng B, Campbell RG, Matthews DE. 1995. Effect of cortisol on energy expenditure and amino acid metabolism in humans. *Am. J. Physiol.* 268:E501–13
25. Brodsky IG, Balagopal P, Nair KS. 1996. Effects of testosterone replacement on muscle mass and muscle protein synthesis in hypogonadal men. *J. Clin. Endo. Metab.* 81:3469–75
26. Brown J, Gore DC, Lee R. 1993. Dichloroacetate inhibits peripheral efflux of pyruvate and alanine during hormonally simulated catabolic stress. *J. Surg. Res.* 54:592–96
27. Brown JA, Gore DC, Jahoor F. 1994. Catabolic hormones alone fail to reproduce the stress-induced efflux of amino acids. *Arch. Surg.* 129:819–24
28. Cahill GF. 1970. Starvation in man. *New Engl. J. Med.* 282:668–75
29. Celotti F, Cesi PN. 1992. Anabolic steroids: a review of their effects on the muscles, of their possible mechanism of action and of their use in athletics. *J. Steroid Biochem. Molec. Biol.* 43:469–77
30. Charlton MR, Adey DB, Nair KS. 1996. Evidence for a catabolic role of glucagon during an amino acid load. *J. Clin. Invest.* 98:90–99
31. Charlton MR, Balagopal P, Nair KS. 1996. Skeletal muscle myosin heavy chain synthesis rate in type I diabetic patients. *Diabetes* 45 (Suppl. 2):98A (Abstr.)
32. Cheng KN, Dworzak F, Ford GC, Rennie MJ, Halliday D. 1985. Direct determination of leucine metabolism and protein breakdown in humans using the L-[1-^{13}C, ^{15}N]leucine and the forearm model. *Eur. J. Clin. Invest.* 15:349–54
33. Cheng KN, Pacy PJ, Dworzak F, Ford GC, Halliday D. 1987. Influence of fasting on leucine and muscle protein metabolism across the human forearm determined using L-[1-^{13}C, ^{15}N]leucine as a tracer. *Clin. Sci.* 73:241–46
34. Collipp PJ, Curti V, Thomas J, Sharma RK, Maddaiah VT, Cohn SH. 1973. Body composition changes in children receiving human growth hormone. *Metabolism* 22:589–95
35. Copeland KC, Nair KS. 1994. Acute growth hormone effects on amino acid and lipid metabolism. *J. Clin. Endocrinol. Metab.* 78:1040–47
36. Copeland KC, Nair KS. 1995. Effects of rhGH on glucose, lipid, and amino acid metabolism. In *GHRH, GH and IGF-1,* ed. MR Blackman, SM Harman, J Roth, JR Shapiro, pp. 185–94. New York: Springer
37. Couet C, Fukagawa NK, Matthews DE, Bier DM, Young VR. 1990. Plasma amino acid kinetics during acute states of glucagon deficiency and excess in healthy adults. *Am. J. Physiol.* 258:E78–85
38. Crispell KR, Parson W, Hollifield G. 1955. A study of the rate of protein synthesis before and during the administration of L-triiodothyronine to patients with myxedema and healthy volunteers using N-15 glycine. *J. Clin. Invest.* 35:164–69
39. Crown AL, Holly JMP. 1995. The insulin-like growth factor system in critical illness—pathophysiology and therapeutic potential. *Clin. Nutr.* 14:321–28
40. Cuneo RC, Salomon F, Wiles CM, Hesp R, Sönksen PH. 1991. Growth hormone treatment in growth hormone-deficient adults. I. Effects on muscle mass and strength. *J. Appl. Physiol.* 70:688–94
41. Darmaun D, Matthews DE, Bier DM. 1988. Physiological hypercortisolemia increases proteolysis, glutamine, and alanine production. *Am. J. Physiol.* 255:E366–73
42. Del Prato S, DeFronzo RA, Castellino P, Wahren J, Alvestrand A. 1990. Regulation of amino acid metabolism by epinephrine. *Am. J. Physiol.* 258:E878–87
43. Denne SC, Brechtel G, Johnson A, Liechty EA, Baron AD. 1995. Skeletal muscle proteolysis is reduced in noninsulin-dependent diabetes mellitus and is unaltered by euglycemic hyperinsulinemia or intensive insulin therapy. *J. Clin. Endocrinol. Metab.* 80:2371–77
44. Denne SC, Liechty EA, Liu YM, Brechtel G, Baron AD. 1991. Proteolysis in skeletal muscle and whole body in response to euglycemic hyperinsulinemia in normal adults. *Am. J. Physiol.* 261:E809–14
45. Elahi D, McAloon-Dyke M, Fukagawa NK, Sclater AL, Wong GA, et al. 1993. Effects of recombinant human IGF-I on glucose and leucine kinetics in men. *Am. J. Physiol.* 265:E831–38
46. Fitzpatrick GF, Meguid MM, Gitlitz PH, Brennan MF. 1977. Glucagon infusion in normal man: effects on 3-methylhistidine excretion and plasma amino acids. *Metabolism* 26:477–85
47. Flores EA, Bistrian BR, Pomposelli JJ, Dinarello CA, Blackburn GL, Istfan NW. 1989. Infusion of tumor necrosis factor/cachectin promotes muscle catabolism in the rat. A synergistic effect with interleukin 1. *J. Clin. Invest.* 83:1614–22

48. Florini JR. 1970. Effects of testosterone on qualitative pattern of protein synthesis in skeletal muscle. *Biochemistry* 9:909–12
49. Florini JR. 1987. Hormonal control of muscle growth. *Muscle Nerve* 10:577–98
50. Florini JR, Ewton DZ, McWade FJ. 1995. IGFs, muscle growth, and myogenesis. *Diabetes Rev.* 3:73–92
51. Forbes GB. 1985. The effect of anabolic steroids on lean body mass: the dose response curve. *Metabolism* 34:571–73
52. Forbes GB. 1987. *Human Body Composition. Growth, Aging, Nutrition, and Activity.* New York: Springer. 350 pp.
53. Forbes GB. 1987. Influence of hormones. See Reference 52, pp. 267–81
54. Fryburg DA. 1994. Insulin-like growth factor 1 exerts growth hormone- and insulin-like actions on human muscle protein metabolism. *Am. J. Physiol.* 267:E331–36
55. Fryburg DA. 1996. NG-monomethyl-L-arginine inhibits the blood flow but not the insulin-like response of forearm muscle to Igf-I. Possible role of nitric oxide in muscle protein synthesis. *J. Clin. Invest.* 97:1319–28
56. Fryburg DA, Barrett EJ. 1993. Growth hormone acutely stimulates skeletal muscle but not whole-body protein synthesis in humans. *Metabolism* 42:1223–27
57. Fryburg DA, Barrett EJ. 1995. Insulin, growth hormone, and IGF-1 regulation of protein metabolism. *Diabetes Rev.* 3:93–112
58. Fryburg DA, Barrett EJ, Louard RJ, Gelfand RA. 1990. Effect of starvation on human muscle protein metabolism and its response to insulin. *Am. J. Physiol.* 259:E477–82
59. Fryburg DA, Gelfand RA, Barrett EJ. 1991. Growth hormone acutely stimulates forearm muscle protein synthesis in normal humans. *Am. J. Physiol.* 260:E499–504
60. Fryburg DA, Gelfand RA, Jahn LA, Oliveras D, Sherwin RS, et al. 1995. Effects of epinephrine on human muscle glucose and protein metabolism. *Am. J. Physiol.* 268:E55–59
61. Fryburg DA, Jahn LA, Hill SA, Oliveras DM, Barrett EJ. 1995. Insulin and insulin-like growth factor-1 enhance human skeletal muscle protein anabolism during hyperaminoacidemia by different mechanisms. *J. Clin. Invest.* 96:1722–29
62. Fryburg DA, Louard RJ, Gerow KE, Gelfand RA, Barrett EJ. 1992. Growth hormone stimulates skeletal muscle protein synthesis and antagonizes insulin's antiproteolytic action in humans. *Diabetes* 41:424–29
63. Garlick PJ, Baillie AGS, Grant I, McNurlan MA. 1992. In-vivo action of insulin on protein metabolism in experimental animals. In *Protein Metabolism in Diabetes Mellitus*, ed. KS Nair, pp. 15–25. London: Smith-Gordon
64. Garlick PJ, Burns HJ, Palmer RM. 1988. Regulation of muscle protein turnover: possible implications for modifying the responses to trauma and nutrient intake. *Bailliere Clin. Gastroenterolog.* 2:915–40
65. Garlick PJ, Mcnurlan MA, Essén P, Wernerman J. 1994. Measurement of tissue protein synthesis rates in vivo: a critical analysis of contrasting methods. *Am. J. Physiol.* 266:E287–97
66. Garlick PJ, Wernerman J, McNurlan MA, Essén P, Lobley GE, et al. 1989. Measurement of the rate of protein synthesis in muscle of postabsorptive young men by injection of a 'flooding dose' of [1-^{13}C]leucine. *Clin. Sci.* 77:329–36
67. Gelfand RA, Barret EJ. 1987. Effect of physiologic hyperinsulinemia on skeletal muscle protein synthesis and breakdown in man. *J. Clin. Invest.* 80:1–6
68. Gelfand RA, Hutchinson-Williams KA, Bonde AA, Castellino P, Sherwin RS. 1987. Catabolic effect of thyroid hormone excess: the contribution of adrenergic activity to hypermetabolism and protein breakdown. *Metabolism* 36:562–69
69. Gelfand RA, Matthews DE, Bier DM, Sherwin RS. 1984. Role of counterregulatory hormones in the catabolic response to stress. *J. Clin. Invest.* 74:2238–48
70. Gibson JN, Poyser NL, Morrison WL, Scrimgeour CM, Rennie MJ. 1991. Muscle protein synthesis in patients with rheumatoid arthritis: effect of chronic corticosteroid therapy on prostaglandin $F_{2\alpha}$ availability. Eur. *J. Clin. Invest.* 21:406–12
71. Goodman MN. 1991. Tumor necrosis factor induces skeletal protein breakdown in rats. *Am. J. Physiol.* 260:E727–30
72. Gore DC, Honeycutt D, Jahoor F, Wolfe RR, Herndon DN. 1991. Effect of exogenous growth hormone on whole-body and isolated-limb protein kinetics in burned patients. *Arch. Surg.* 126:38–43
73. Gore DC, Jahoor F, Wolfe RR, Herndon DN. 1993. Acute response of human muscle protein to catabolic hormones. *Ann. Surg.* 218:679–84
74. Griggs RC, Halliday D, Kingston W, Moxley RT. 1986. Effect of testosterone on muscle protein synthesis in myotonic

dystrophy. *Ann. Neurol.* 20:590–96
75. Griggs RC, Kingston W, Jozefowicz RF, Herr BE, Forbes G, Halliday D. 1989. Effect of testosterone on muscle mass and muscle protein synthesis. *J. Appl. Physiol.* 66:498–503
76. Hasselgren PO, Adlerberth A, Angeras U, Stenstrom G. 1984. Protein metabolism in skeletal muscle tissue from hyperthyroid patients after preoperative treatment with antithyroid drug or selective β-blocking agent. Results from a prospective, randomized study. *J. Clin. Endocrinol. Metab.* 59:835–39
77. Heslin MJ, Newman E, Wolf RF, Pisters PWT, Brennan MF. 1992. Effect of hyperinsulinemia on whole body and skeletal muscle leucine carbon kinetics in humans. *Am. J. Physiol.* 262:E911–18
78. Heymsfield SB, Arteaga C, McManus C, Smith J, Moffitt S. 1983. Measurement of muscle mass in humans: validity of the 24-hour urinary creatinine method. *Am. J. Clin. Nutr.* 37:478–94
79. Heymsfield SB, Smith R, Aulet M, Bensen B, Lichtman S, et al. 1990. Appendicular skeletal muscle mass: measurement by duaphoton absorptiometry. *Am. J. Clin. Nutr.* 52:214–18
80. Hobbs CJ, Plymate SR, Rosen CJ, Adler RA. 1993. Testosterone administration increases insulin-like growth factor-1 levels in normal men. *J. Clin. Endocrinol. Metab.* 77:776–79
81. Horber FF, Haymond MW. 1990. Human growth hormone prevents the protein catabolic side effects of prednisone in humans. *J. Clin. Invest.* 86:265–72
82. Horber FF, Scheidegger JR, Grünig BE, Frey FJ. 1985. Thigh muscle mass and function in patients treated with glucocorticoids. *Eur. J. Clin. Invest.* 15:302–7
83. Jensen MD, Kanaley JA, Roust LR, O'Brien PC, Braun JS, et al. 1993. Assessment of body composition with use of dual-energy X-ray absorptiometry: evaluation and comparison with other methods. *Mayo Clin. Proc.* 68:867–73
84. Jorgensen JO, Pedersen SA, Thuesen L, Jorgensen J, Ingemann-Hansen T, et al. 1989. Beneficial effects of growth hormone treatment in GH-deficient adults. *Lancet* 1:1221–25
85. Jorgensen JO, Thuesen L, Müller J, Ovesen P, Skakkebaek NE, Christiansen JS. 1994. Three years of growth hormone treatment in growth hormone-deficient adults: near normalization of body composition and physical performance. *Eur. J. Endocrinol.* 130:224–28
86. Karinch AM, Kimball SR, Antonetti DA, Jefferson LS. 1992. Mechanism of insulin action on protein synthsis: translational regulation. In *Protein Metabolism in Diabetes Mellitus*, ed. KS Nair, pp. 139–48. London: Smith-Gordon
87. Kettelhut IC, Wing SS, Goldberg AL. 1988. Endocrine regulation of protein breakdown in skeletal muscle. *Diabetes Metab. Rev.* 4:751–72
88. Kissel JT, Mendell JR. 1992. The endocrine myopathies. *Myopathies* 18(62): 527–51
89. Kraenzlin ME, Keller U, Keller A, Thélin A, Arnaud MJ, Stauffacher W. 1989. Elevation of plasma epinephrine concentrations inhibits proteolysis and leucine oxidation in man via β-adrenergic mechanisms. *J. Clin. Invest.* 84:388–93
90. Louard RJ, Bhushan R, Gelfand RA, Barrett EJ, Sherwin RS. 1994. Glucocorticoids antagonize insulin's antiproteolytic action on skeletal muscle in humans. *J. Clin. Endocrinol. Metab.* 79:278–84
91. Louard RJ, Fryburg DA, Gelfand RA, Barrett EJ. 1992. Insulin sensitivity of protein and glucose metabolism in human forearm skeletal muscle. *J. Clin. Invest.* 90:2348–54
92. Loughna PT, Mason P, Bates PC. 1992. Regulation of insulin-like growth factor 1 gene expression in skeletal muscle. *Symp. Soc. Exp. Biol.* 46:319–30
93. Lundholm K, Edström S, Ekman L, Karlberg I, Walker P, Schersten T. 1981. Protein degradation in human skeletal muscle tissue: the effect of insulin, leucine, amino acids and ions. *Clin. Sci.* 60:319–26
94. Lundholm K, Schersten T. 1977. Protein synthesis in human skeletal muscle tissue: influence of insulin and amino acids. *Eur. J. Clin. Invest.* 7:531–36
95. Lykken GI, Jacob RA, Munoz JM, Sandstead HH. 1980. A mathematical model of creatine metabolism in normal males—comparison between theory and experiment. *Am. J. Clin. Nutr.* 33:2674–85
96. Marchesini G, Forlani G, Zoli M, Vannini P, Pisi E. 1982. Muscle protein breakdown in uncontrolled diabetes as assessed by urinary 3-methylhistidine excretion. *Diabetologia* 23:456–58
97. Matthews DE, Bier DM, Rennie MJ, Edwards RHT, Halliday D, et al. 1981. Regulation of leucine metabolism in man: a stable isotope study. *Science* 214:1129–31
98. Matthews DE, Motil KJ, Rohrbaugh DK, Burke JF, Young VR, Bier DM. 1980. Measurements of leucine metabolism in man from a primed, continuous infusion

of L-[1-^{13}C]leucine. *Am. J. Physiol.* 238:E473–79
99. Matthews DE, Pesola G, Campbell RG. 1990. Effect of epinephrine on amino acid and energy metabolism in humans. *Am. J. Physiol.* 258:E948–56
100. McNulty PH, Louard RJ, Deckelbaum LI, Zaret BL, Young LH. 1995. Hyperinsulinemia inhibits myocardial protein degradation in patients with cardiovascular disease and insulin resistance. *Circulation* 92:2151–56
101. McNurlan MA, Essén P, Heys SD, Buchan V, Garlick PJ, Wernerman J. 1991. Measurement of protein synthesis in human skeletal muscle: further investigation of the flooding technique. *Clin. Sci.* 81:557–64
102. McNurlan MA, Essén P, Thorell A, Calder AG, Anderson SE, et al. 1994. Response of protein synthesis in human skeletal muscle to insulin: an investigation with L-[$^{2}H_5$]phenylalanine. *Am. J. Physiol.* 267:E102–8
103. Miles JM, Nissen SL, Gerich JE, Haymond MW. 1984. Effects of epinephrine infusion on leucine and alanine kinetics in humans. *Am. J. Physiol.* 247:E166–72
104. Millward DJ, Halliday D, Rennie MJ. 1984. The extent of protein synthesis in muscle in man. *Hum. Nutr. Clin. Nutr.* 38C:151–54 (Reply)
105. Möller-Loswick AC, Zachrisson H, Hyltander A, Körner U, Matthews DE, Lundholm K. 1994. Insulin selectively attenuates breakdown of nonmyofibrillar proteins in peripheral tissues of normal men. *Am. J. Physiol.* 266:E645–52
106. Mooradian AD, Morley JE, Korenman SG. 1987. Biological actions of androgens. *Endocrinol. Rev.* 8:1–28
107. Morrison WL, Gibson JNA, Jung RT, Rennie MJ. 1988. Skeletal muscle and whole body protein turnover in thyroid disease. *Eur. J. Clin. Invest.* 18:62–68
108. Müller MJ, Seitz HJ. 1984. Thyroid hormone action on intermediary metabolism. Part III. Protein metabolism in hyper- and hypothyroidism. *Klin. Wochenschr.* 62:97–102
109. Beckett AH. 1992. Comments: Muscling in on clenbuterol. *Lancet* 340:403
110. Nair KS. 1984. *Energy and Protein Metabolism in Diabetes and Obesity.* London: Council of National Academic Awards. 190 pp.
111. Nair KS, Ford GC, Ekberg K, Fernqvist-Forbes E, Wahren J. 1995. Protein dynamics in whole body and in splanchnic and leg tissues in type 1 diabetic patients. *J. Clin. Invest.* 95:2926–37
112. Nair KS, Garrow JS, Ford C, Mahler RF, Halliday D. 1983. Effect of poor diabetic control and obesity on whole body protein metabolism in man. *Diabetologia* 25:400–3
113. Nair KS, Halliday D, Griggs RC. 1988. Leucine incorporation into mixed skeletal muscle protein in humans. *Am. J. Physiol.* 254:E208–13
114. Nair KS, Halliday D, Matthews DE, Welle SL. 1987. Hyperglucagonemia during insulin deficiency accelerates protein catabolism. *Am. J. Physiol.* 253:E208–13
115. Nair KS, Schwartz RG, Welle S. 1992. Leucine as a regulator of whole body and skeletal muscle protein metabolism in humans. *Am. J. Physiol.* 263:E928–34
116. Newman E, Heslin MJ, Wolf RF, Pisters PW, Brennan MF. 1994. The effect of systemic hyperinsulinemia with concomitant amino acid infusion on skeletal muscle protein turnover in the human forearm. *Metabolism* 43:70–78
117. Pacy PJ, Bannister PA, Halliday D. 1991. Influence of insulin on leucine kinetics in the whole body and across the forearm in post-absorptive insulin dependent diabetic (type 1) patients. *Diabetes Res.* 18:155–62
118. Pacy PJ, Cheng KN, Ford GC, Halliday D. 1990. Influence of glucagon on protein and leucine metabolism: a study in fasting man with induced insulin resistance. *Br. J. Surg.* 77:791–94
119. Pacy PJ, Nair KS, Ford C, Halliday D. 1989. Failure of insulin infusion to stimulate fractional muscle protein synthesis in type 1 diabetic patients. Anabolic effect of insulin and decreased proteolysis. *Diabetes* 38:618–24
120. Palmer RM. 1990. Prostaglandins and the control of muscle protein synthesis and degradation. *Prostaglandin Leuk. Essent. Fatty Acids* 39:95–104
121. Pearlstone DB, Wolf RF, Berman RS, Burt M, Brennan MF. 1994. Effect of systemic insulin on protein kinetics in postoperative cancer patients. *Ann. Surg. Oncol.* 1:321–32
122. Pozefsky T, Felig P, Tobin JD, Soeldner JS, Cahill GF. 1969. Amino acid balance across tissue of forearm in postabsorptive man. Effects of insulin at two dose levels. *J. Clin. Invest.* 48:2273–82
123. Preedy VR, Garlick PJ. 1985. The effect of glucagon administration on protein synthesis in skeletal muscles, heart and liver in vivo. *Biochem. J.* 228:575–81
124. Preedy VR, Garlick PJ. 1988. Inhibition of protein synthesis by glucagon in different rat muscles and protein fractions in

vivo and in the perfused rat hemicorpus. *Biochem. J.* 251:727–32
125. Ramsay ID. 1966. Muscle dysfunction in hyperthyroidism. *Lancet* 2:931–34
126. Rathmacher JA, Flakoll PJ, Nissen SL. 1995. A compartimental model of 3-methylhistidine metabolism in humans. *Am. J. Physiol.* 269:E193–98
127. Reeds PJ, Fjeld CR, Jahoor F. 1994. Do the differences between the amino acid compositions of acute-phase and muscle proteins have a bearing on nitrogen loss in traumatic states? *J. Nutr.* 124:906–10
128. Reeds PJ, Hay SM, Glennie RT, Mackie WS, Garlick PJ. 1985. The effect of indomethacin on the stimulation of protein synthesis by insulin in young post-absorptive rats. *Biochem. J.* 227:255–61
129. Reeds PJ, Palmer RM. 1983. The possible involvement of prostaglandin $F_{2\alpha}$ in the stimulation of muscle protein synthesis by insulin. *Biochem. Biophys. Res. Commun.* 116:1084–90
130. Rennie MJ. 1985. Muscle protein turnover and the wasting to injury and disease. *Br. Med. Bull.* 41:257–64
131. Rennie MJ, Bennegård K, Edén E, Emery PW, Lundholm K. 1984. Urinary excretion and efflux from the leg of 3-methylhistidine before and after major surgical operation. *Metabolism* 33:250–56
132. Rennie MJ, Edwards RHT, Halliday D, Matthews DE, Wolman SL, Millward DJ. 1982. Muscle protein synthesis measured by stable isotope techniques in man: the effect of feeding and starvation. *Clin. Sci.* 63:519–23
133. Rennie MJ, Millward DJ. 1983. 3-Methylhistidine excretion and the urinary 3-methylhistidine/creatinine ratio are poor indicators of skeletal muscle protein breakdown. *Clin. Sci.* 65:217–25
134. Rennie MJ, Smith K, Watt PW. 1994. Measurement of human tissue protein synthesis: an optimal approach. *Am. J. Physiol.* 266:E298–307
135. Rifai Z, Welle S, Moxley RT, Lorenson M, Griggs RC. 1995. Effect of prednisone on protein metabolism in Duchenne dystrophy. *Am. J. Physiol.* 268:E67–74
136. Robert JJ, Beaufrere B, Koziet J, Desjeux JF, Bier DM, et al. 1985. Whole body de novo amino acid synthesis in type I (insulin-dependent) diabetes studied with stable isotope-labeled leucine, alanine, and glycine. *Diabetes* 34:67–73
137. Rodier M, Richard JL, Bringer J, Cavalié G, Bellet H, Mirouze J. 1984. Thyroid status and muscle protein breakdown as assessed by urinary 3-methylhistidine excretion: study in thyrotoxic patients before and after treatment. *Metabolism* 33:97–100
138. Ruff RL, Weissmann J. 1988. Endocrine myopathies. *Neurol. Clin.* 6:575–92
139. Sakurai Y, Aarsland A, Herndon DN, Chinkes DL, Pierre E, et al. 1995. Stimulation of muscle protein synthesis by long-term insulin infusion in severely burned patients. *Ann. Surg.* 222:283–97
140. Salomon F, Cuneo RC, Hesp R, Sönksen PH. 1989. The effects of treatment with recombinant human growth hormone on body composition and metabolism in adults with growth hormone deficiency. *New Engl. J. Med.* 321:1797–803
141. Schwartz RS. 1995. Throphic factor supplementation: effect on the age-associated changes in body composition. *J. Gerontol.* 50A:151–56 (Special issue)
142. Schwenk WF, Beaufrere B, Haymond MW. 1985. Use of reciprocal pool specific activities to model leucine metabolism in humans. *Am. J. Physiol.* 249:E646–50
143. Sjölin J, Stjernström H, Arturson G, Andersson E, Friman G, Larsson J. 1989. Exchange of 3-methylhistidine in the splanchnic region in human infection. *Am. J. Clin. Nutr.* 50:1407–14
144. Smith K, Barua JM, Watt PW, Scrimgeour CM, Rennie MJ. 1992. Flooding dose L-[1-^{13}C]leucine stimulates human muscle protein incorporation of continuously infused L-[1-^{13}C]valine. *Am. J. Physiol.* 262:E372–76
145. Stiegler H, Rett K, Wicklmayr M, Mehnert H. 1989. Metabolic effects of prostaglandin E1 on human skeletal muscle with special regard to the amino acid metabolism. *Vasa* 28 (Suppl.):14–18
146. Sugden PH, Fuller SJ. 1991. Review article. Regulation of protein turnover in skeletal and cardiac muscle. *Biochem. J.* 273:21–37
147. Tessari P, Biolo G, Inchiostro S, Sacca L, Nosadini R, et al. 1990. Effects of insulin on whole body and forearm leucine and KIC metabolism in type 1 diabetes. *Am. J. Physiol.* 259:E96–103
148. Tessari P, Inchiostro S, Biolo G, Marescotti MC, Fantin G, et al. 1989. Leucine kinetics and the effects of hyperinsulinemia in patients with Cushing's syndrome. *J. Clin. Endocrinol. Metab.* 68:256–62
149. Tessari P, Inchiostro S, Biolo G, Vincenti E, Sabadin L. 1991. Effects of acute systemic hyperinsulinemia on forearm muscle proteolysis in healthy man. *J. Clin. Invest.* 88:27–33

150. Tessari P, Nosadini R, Trevisan R, De Kreutzenberg SV, Inchiostro S, et al. 1986. Defective suppression by insulin of leucine-carbon appearance and oxidation in type 1, insulin-dependent diabetes mellitus. Evidence for insulin resistance involving glucose and amino acid metabolism. *J. Clin. Invest.* 77:1797–804
151. Umpleby AM, Boroujerdi MA, Brown PM, Carson ER, Sönksen PH. 1986. The effect of metabolic control on leucine metabolism in type 1 (insulin-dependent) diabetic patients. *Diabetologia* 29:131–41
152. Urban RJ, Bodenburg YH, Gilkison C, Foxworth J, Coggan AR, et al. 1995. Testosterone administration to elderly men increases skeletal muscle strength and protein synthesis. *Am. J. Physiol.* 269:E820–26
153. Vlachopapadopoulou E, Zachwieja JJ, Gertner JM, Manzione D, Bier DM, et al. 1995. Metabolic and clinical response to recombinant human insulin-like growth factor 1 in myotonic dystrophy—a clinical research center study. *J. Clin. Endocrinol. Metab.* 80:3715–23
154. Voerman HJ, Strack van Schijndel RJM, De Boer H, Van der Veen EA, Thijs LG. 1992. Growth hormone: secretion and administration in catabolic adult patients, with emphasis on the critically ill patient. *Neth. J. Med.* 41:229–44
155. Waterlow JC. 1984. Protein turnover with special reference to man. *Q. J. Exp. Physiol.* 69:409–38
156. Waterlow JC, Garlick PJ, Millward DJ. 1978. *Protein Turnover in Mammalian Tissues and in the Whole Body.* Amsterdam: Elsevier. 804 pp.
157. Watt PW, Corbett ME, Rennie MJ. 1992. Stimulation of protein synthesis in pig skeletal muscle by infusion of amino acids during constant insulin availability. *Am. J. Physiol.* 263:E453–60
158. Watt PW, Lindsay Y, Scrimgeour CM, Chien PAF, Gibson JNA, et al. 1991. Isolation of aminoacyl-tRNA and its labeling with stable-isotope tracers: use in studies of human tissue protein synthesis. *Proc. Natl. Acad. Sci. USA* 88:5892–96
159. Welle S, Thornton C, Jozefowicz R, Statt M. 1993. Myofibrillar protein synthesis in young and old men. *Am. J. Physiol.* 264:E693–98
160. Welle S, Thornton C, Statt M, McHenry B. 1996. Growth hormone increases muscle mass and strength but does not rejuvenate myofibrillar protein synthesis in healthy subjects over 60 years old. *J. Clin. Endocrinol. Metab.* 81:3239–43
161. Wilmore DW, Black PR, Muhlbacher F. 1983. Injured man: trauma and sepsis. In *Nutritional Support of the Seriously Ill Patients*, ed. RW Winters, HL Greene, pp. 33–52. London: Acadamic
162. Wolf RF, Heslin MJ, Newman E, Pearlstone DB, Gonenne A, Brennan MF. 1992. Growth hormone and insulin combine to improve whole-body and skeletal muscle protein kinetics. *Surgery* 112:284–92
163. Wolfe BR, Culebas JM, Aoki TT, O'Conner NE, Finley RJ, et al. 1979. The effect of glucagon on protein metabolism in normal man. *Surgery* 86:248–57
164. Wolfe RR. 1992. Assessment of body composition. In *Radioactive and Stable Isotope Tracers in Biomedicine. Principles and Practice of Kinetic Analysis*, pp. 189–97. New York: Wiley-Liss
165. Yarasheski KE. 1994. Growth hormone effects on metabolism, body composition, muscle mass, and strength. *Exerc. Sport Sci. Rev.* 22:285–312
166. Yarasheski KE, Campbell JA, Smith K, Rennie MJ, Holloszy JO, Bier DM. 1992. Effect of growth hormone and resistance exercise on muscle growth in young men. *Am. J. Physiol.* 262:E261–67
167. Yarasheski KE, Zachweija JJ, Angelopoulos TJ, Bier DM. 1993. Short-term growth hormone treatment does not increase muscle protein synthesis in experienced weight lifters. *J. Appl. Physiol.* 74:3073–76
168. Yarasheski KE, Zachwieja JJ, Bier DM. 1993. Acute effects of resistance exercise on muscle protein synthesis rate in young and elderly men and women. *Am. J. Physiol.* 265:E210–14
169. Yarasheski KE, Zachwieja JJ, Campbell JA, Bier DM. 1995. Effect of growth hormone and resistance exercise on muscle growth and strength in older men. *Am. J. Physiol.* 268:E268–76
170. Yates RO, Connor H, Woods HF. 1981. Muscle protein breakdown in thyrotoxicosis assessed by urinary 3-methylhistidine excretion. *Ann. Nutr. Metab.* 25:262–67
171. Young VR, Alexis SD, Baliga BS, Munro HN, Muecke W. 1972. Metabolism of administered 3-methylhistidine. Lack of muscle transfer ribonucleic acid charging and quantitative excretion as 3-methylhistidine and its N-acetyl derivative. *J. Biol. Chem.* 247:3592–600
172. Zürcher RM, Horber FF, Grünig BE, Frey FJ. 1989. Effect of thyroid dysfunction on thigh muscle efficiency. J. *Clin. Endocrinol. Met.* 69:1082–86