Every day, in adequately nourished adult humans 3–4% of whole-body protein is remodeled through a continuous process of degradation and synthesis. When compared to glucose and lipid turnover, protein turnover in terms of energy costs is much more expensive, accounting alone for 10–25% of the resting metabolic rate (1, 2). Periodic protein breakdown is required to prevent the accumulation of abnormal and potentially harmful proteins or peptides. The turnover rates of individual peptides and proteins are very different, ranging from half-lives of seconds for peptides and enzymes to several days for structural proteins. These differences in turnover rates are the result of the different functions of individual proteins. For instance, enzymes must have a fast turnover rate in order that their concentrations can be rapidly changed by factors that modulate their rates of synthesis or degradation. Among the potential factors that play a role in the regulation of protein metabolism, a number of substrates (3) and hormones have been identified. Hormones, by affecting the turnover rates of key proteins, can modulate cell differentiation and growth and direct the flux of substrates through specific metabolic pathways.

Most of the present knowledge on the effects of hormones on protein metabolism in humans derives from studies employing the technique of infusing healthy subjects or endocrine patients with labeled amino acids. This technique is based on the isotope dilution and the precursor/product approaches and allows the kinetics of whole-body protein, mixed proteins of specific tissues and single proteins to be traced. Readers interested in the advantages and the methodological limitations intrinsic in the use of labeled amino acid infusions in humans should refer to recent reviews (4, 5). In this article only the basic principles of the methodology are described.

The isotope dilution approach is used to calculate the rate of release of a given amino acid from endogenous protein. During the intravenous infusion of a strategically labeled amino acid (tracer), blood samples are collected to determine the dilution of the tracer by the endogenous amino acid (tracee). When radioactive isotopes are used, this tracer/tracee ratio is defined by the term "specific activity". The flux of the tracee (Ra, rate of appearance) is calculated using the formula:

\[
Ra(\mu mol/min) = \frac{i}{\text{Plasma amino acid SA}}
\]

where \(i\) is the tracer infusion rate (dpm/min) and \(\text{SA}\) is the specific activity of the amino acid (dpm/\(\mu\)mol). In the post-absorptive state, the Ra of essential amino acids
reflects their rate of release from endogenous protein, as supported by the fact that the rates of appearance of essential amino acids are proportional to their content in muscle protein (5). Among the essential amino acids, leucine is most commonly used to estimate whole-body protein kinetics because it offers several methodological advantages (4, 5). A most important fact is that leucine, through a reversible transamination reaction, is in equilibrium with its ketoacid (KIC, α-ketosocaproate). Because this reaction takes place only intracellularly, plasma KIC SA, during the infusion of labelled leucine, allows a close estimate of the intracellular leucine SA (4, 5). Considering that protein metabolism (protein breakdown, synthesis, amino acid oxidation) occurs inside the cells, leucine Ra can be better estimated by substituting plasma leucine with plasma KIC SA:

$$Ra(\mu\text{mol/min}) = \frac{i(\text{leucine})}{\text{Plasma KIC SA}}$$

The precursor–product model is used to calculate the rate of leucine oxidation. Leucine can be utilized in two different ways: oxidation or incorporation into nascent proteins. To be oxidized, leucine must be converted into KIC and when KIC undergoes irreversible oxidation its first carbon atom generates CO₂. Thus, when the first carbon atom of the leucine tracer is labeled, the fractional rate of leucine oxidation can be calculated by dividing the rate of labeled CO₂ (dpm/min) by the tracer infusion rate(dpm/min). The absolute rate of leucine oxidation (μmol/min) is obtained by multiplying this fractional oxidative rate by leucine Ra. Under steady-state conditions, the rate of appearance of leucine will be equal to the rate of leucine disappearance (Rd). Consequently, the rate of non-oxidative leucine disposal (NOLD, μmol·kg⁻¹·min⁻¹), i.e. the rate of leucine incorporation into body protein, can be estimated as follows:

$$\text{NOLD} = \text{Total Ra} - \text{Ox}$$

The precursor–product model is also used to calculate the fractional synthesis rates (FSR, %/h) of mixed tissue proteins and of individual plasma proteins using the following formula:

$$\text{FSR} = \frac{\Delta S_{\text{leu protein}}/\Delta t}{S_{\text{KIC plasma}}} \times 100 \times 60$$

where ΔSAleu protein/Δt is the incorporation rate of labeled leucine into proteins during the sampling period, and SAKIC plasma is the mean plasma KIC SA over the same time period. The validity of plasma KIC SA as an index of the precursor pool SA for protein synthesis of different tissues in the post-absorptive and the absorptive states remains to be clearly established (4).

Albeit time-consuming, the measurement of protein kinetics has several advantages when compared to the simpler determination of the plasma amino acid and protein concentrations or to nitrogen balance studies (4, 5). It allows determination of the mechanisms through which hormones, substrates or other stimuli affect protein metabolism. For instance, it is possible that an “x” stimulus affects the metabolism of an “x” plasma protein by increasing its rate of synthesis and degradation to the same extent. In this case, the plasma protein concentration will not change, thus masking the effect of that stimulus. More commonly, under the effect of different physiological or pathological stimuli, plasma amino acid and protein concentrations, as well as nitrogen balance, change but, unless kinetic studies are performed, it is not possible to establish the mechanism by which this change occurred. A knowledge of the mechanisms of the action of hormones and substrates on the rates of protein breakdown, synthesis and amino acid oxidation is critical in defining the basis for rationale therapeutic interventions.

The aim of this article is to serve as a critical review of the results of the studies designed to establish the effects of hormones on protein metabolism in human subjects. The initial analysis of the possible physiological role played by individual hormones and the pathological consequences of hormonal excess or deficiency on protein metabolism will be based on the results of the studies conducted in vivo in humans. Available data on the effects of multiple hormonal infusions on protein metabolism will be discussed later.

On the basis of their net effect on whole-body protein balance (protein synthesis minus protein breakdown), hormones can be differentiated into two groups: those having a prevalent anabolic action and those with a prevalent catabolic action (Table 1). This classification is proposed to stress the concept that hormones considered to be catabolic, because they counteract insulin action on glucose and lipid metabolism (6–9), do not necessarily have a catabolic effect on protein metabolism. For instance, growth hormone and adrenalineline, which are two classic counter-regulatory hormones, in concentrations similar to those observed during stress conditions promote protein anabolism in healthy humans.

**Insulin**

Muscle wasting complicating the fatal course of diabetes mellitus before the introduction of insulin therapy is

<table>
<thead>
<tr>
<th>Classification of hormones on the basis of their net effect on protein balance (the difference between the rates of whole-body protein synthesis and breakdown) in humans.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anabolic action</td>
</tr>
<tr>
<td>Insulin</td>
</tr>
<tr>
<td>GH</td>
</tr>
<tr>
<td>IGF-1</td>
</tr>
<tr>
<td>Adrenaline</td>
</tr>
<tr>
<td>Androgens</td>
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</tbody>
</table>
clinical proof of the essential role played by insulin in the regulation of protein metabolism. Credit for the first
description of the effects of uncontrolled diabetes on
body protein stores can perhaps be ascribed to Areteus
Cappadocceus (Rome, ca. AD 30–90) who emphasized
this concept in his book on the Therapeutics of Chronic
Disease: "Diabetes is a wonderful affection, not very
frequent among men, being a melting down of the flesh
and limbs into urine" (10).
The mechanisms through which insulin deficiency
reduces muscle or lean body mass in humans have only
recently been elucidated. By infusing labeled leucine,
several groups of investigators (11–18) compared the
effects of insulin deficiency and treatment on the rates of
protein breakdown, synthesis and amino acid oxidation
in type 1 diabetic subjects (Table 2). The results of these
studies (11–18) show that basal insulin concentration
is required to maintain a normal rate of whole-body
proteinynthesis. In fact, insulin deficiency in the post-
absorptive state leads to an approximately 30% increase
in the rate of protein breakdown (Table 2), which in
turn increases the intracellular and plasma concentra-
tions of most amino acids (16). The resultant greater
amino acid availability stimulates amino acid oxidation
(11–18) and, as found in some studies (11, 14–16, 18),
whole-body protein synthesis. However, the increment
of protein synthesis, even when detectable (11, 14–16,
18), cannot counterbalance the stimulation of amino
acid release and oxidation, and as a result body protein
wasting occurs.
The above-cited studies, carried out in the post-
absorptive state in insulin-deficient diabetic patients,
demonstrate that basal insulin concentration plays an
important role in reducing protein catabolism caused by
the fasting state. During the absorptive period insulin
deficiency results in a defective suppression of endo-
genous proteinolysis (19) restraining, in this case meal-
induced protein anabolism. There is indirect evidence that
post-prandial hyperinsulinemia induces protein
anabolism, other than through the suppression of
whole-body proteinolysis, also by facilitating the incor-
poration of dietary amino acids into new proteins.
In fact, when post-prandial hyperinsulinemia and
hyperaminoacidemia are reproduced in normal subjects
by a combined intravenous infusion of insulin and
amino acids, the estimates of whole-body protein
synthesis increase more than after amino acids alone
(20). The stimulatory effect of hyperinsulinemia on
whole-body protein synthesis cannot be demonstrated
when insulin alone is infused (20–25). In this case,
by reducing the rate of protein breakdown, hyperinsu-
linemia decreased the intracellular concentrations of
most amino acids (26), limiting their utilization for
protein synthesis (27). Branched-chain amino acids are
particularly sensitive to hyperinsulinemia (28) and it
has been shown the insulin-induced suppression of
plasma isoleucine concentration (29), i.e. of a single
essential amino acid, is sufficient to decrease whole-
body protein synthesis.
The results of recent studies demonstrate that the
effects of insulin on whole-body protein kinetics
represent the mean results of differential effects of the
hormone on the rates of protein breakdown and
synthesis of individual proteins. For instance, despite
the rate of whole-body proteinolysis being decreased by
insulin (20–25), the rate of muscle proteinolysis is
not affected by local hyperinsulinemia (30). Such a
differential effect can be explained by the fact that
insulin decreases the proteolytic activity of lysosomes
but does not control the ubiquitin system (31) that is
responsible for the bulk of muscle proteinolysis (31).
Differential effects of insulin on the synthetic rates of
individual hepatic plasma proteins have been reported
during either hyperinsulinemia in normal subjects (25)
or insulin deficiency in type 1 diabetic patients (32).
The studies in vitro demonstrate that insulin can modulate
protein synthesis, affecting the steps of transcription
(33–36) and translation (37, 38). In fact, insulin can
alter gene expression for specific proteins by increasing
the synthesis of their mRNAs (36–39) and can
influence the step of translation by modulating
the activity of initiation factors (40–42), promoting
ribosomal synthesis or reducing ribosomal degradation
(43, 44). Recent results, demonstrating that in cultured
hepatocytes insulin differentially affects the concentra-
tions of a number of mRNAs, suggest that the effect at

Table 2. Effects of insulin deficiency on the rates of protein kinetics in insulin-dependent diabetic subjects.

<table>
<thead>
<tr>
<th>Study</th>
<th>Protein breakdown</th>
<th>Protein synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nair et al. (Diabetologia 1983)</td>
<td>↑ 27%</td>
<td>↑ 20%</td>
</tr>
<tr>
<td>Robert et al. (Diabetes 1985)</td>
<td>↑ 19%</td>
<td>=</td>
</tr>
<tr>
<td>Umpleby et al. (Diabetologia 1986)</td>
<td>↑ 25%</td>
<td>=</td>
</tr>
<tr>
<td>Nair et al. (Metabolism 1987)</td>
<td>↑ 29%</td>
<td>↑ 18%</td>
</tr>
<tr>
<td>Pacy et al. (Diabetes 1989)</td>
<td>↑ 19%</td>
<td>↑ 12%</td>
</tr>
<tr>
<td>Bennet et al. (Diabetologia 1990)</td>
<td>↑ 34%</td>
<td>↑ 20%</td>
</tr>
<tr>
<td>Pacy et al. (Clin Sci 1991)</td>
<td>↑ 21%</td>
<td>=</td>
</tr>
<tr>
<td>Bennet et al. (Diabetes 1991)</td>
<td>↑ 13%</td>
<td>↑ 20%</td>
</tr>
<tr>
<td>De Foe et al. (J Clin Invest 1991)</td>
<td>↑ 35%</td>
<td>=</td>
</tr>
<tr>
<td>Darnaun et al. (Am J Physiol 1991)</td>
<td>↑ 14%</td>
<td>=</td>
</tr>
</tbody>
</table>
transcriptional level should account for the selective effects of insulin on the synthesis of individual proteins (45). In humans, it has been demonstrated that hyperinsulinemia increases the hepatic secretory rates of albumin (25) but decreases those of fibrinogen (25), antithrombin III (25) and VLDL apoB-100 (46); conversely, short-term insulin withdrawal in type 1 diabetic patients is associated with decreased albumin and increased fibrinogen fractional secretory rates (32). In extrahepatic tissues, mixed muscle protein synthesis is increased significantly by local hyperinsulinemia (30), whereas the synthesis of plasma immunoglobulin G is stimulated by dietary amino acids and not by postprandial hyperinsulinemia (47). On the basis of these data, it is clear that a complete understanding of the role played by insulin in the regulation of protein metabolism in humans requires identification of the effects of the hormone on all its potential target proteins.

Growth hormone

In analogy to insulin, the anabolic role of growth hormone (GH) in whole-body protein metabolism in humans can be argued through the consequences of the lack of GH replacement therapy in adults with growth hormone deficiency (GHD) (48). Chronic GHD, similarly to insulin deficiency, leads to a loss of lean body mass and muscle strength that is reversed by rhGH administration (48–50). However, from a quantitative point of view, the effects of GHD are less dramatic than those due to insulin deficiency and are compatible with survival.

Growth hormone promotes protein anabolism with mechanisms different from insulin. It does not affect the rates of whole-body proteolysis but decreases those of amino acid oxidation (51, 52). The sparing effect on amino acid oxidation results in a greater rate of their incorporation into proteins (51–53), with a net protein anabolic effect. This mechanism of action, described after GH administration to normal humans, has also been confirmed in GHD adults after GH replacement therapy (54). In the case of insulin, the target proteins whose synthesis is promoted by GH are not yet fully established. The results of two studies have, accordingly, demonstrated that hepatic protein synthesis is not promoted by GH (55, 56), whereas the studies assessing the effects of the hormone on muscle protein synthesis (52, 53, 57–59) are contradictory, despite the clinical evidence for an important effect of the hormone (48–50). Some studies have shown that subcutaneous GH administration (57) and systemic (58) or local (59) GH infusions increase muscle protein synthesis; in contrast, two other studies were unable to demonstrate any stimulatory effect of GH on muscle protein synthesis (52, 53).

Considering the pulsatile nature of GH secretion, characterized by peaks occurring approximately 3 h after meals and at bed-time (60), it is conceivable that the largest part of the anabolic role of this hormone on protein metabolism is exerted in the post-absorptive state, especially during the night. However, it is also possible that basal circulating GH has a constant anabolic effect because the infusion of an amount of GH, to replace basal concentrations in GHD adult patients, was sufficient to reduce urinary nitrogen excretion (61). At present it is unclear whether part of the anabolic effects of GH on protein metabolism could be mediated through the hormone-induced increase of IGF-I production. Contrary to this hypothesis is the evidence that, in analogy to glucose metabolism, the effects of IGF-I on protein metabolism (62–65) are different from those of GH (see below) and are not reproduced by GH administration (51, 52, 54).

Insulin-like growth factor I

The isolated effects of IGF-I on intermediate metabolism are difficult to demonstrate in vivo because its administration is followed by significant modifications in circulating concentrations of other hormones known to affect protein metabolism, namely insulin and GH (66). The data available in humans indicate that IGF-I has a mechanism of action similar to insulin on protein metabolism (62–65) because IGF-I administration also reduces the rates of whole-body protein breakdown and synthesis. When compared on a molar basis, the action of IGF-I is ~14 times less potent than that of insulin (65), which raises some concerns about the potential physiological role played by IGF-I in the regulation of whole-body protein metabolism. Accordingly, it has also been observed that, after GH administration, the rates of whole-body proteolysis do not change, in spite of the significant increase in circulating IGF-I concentrations (51–58). More research is definitely required in this field. For instance, it remains to be established whether IGF-I might affect protein metabolism only in selected tissues through a paracrine action on whether IGF-I, due to its longer half-life (67) could influence whole-body protein metabolism when plasma GH concentrations decline; and the role played by IGF-I binding proteins in the modulation of the endocrine action of IGF-I on protein metabolism needs to be established.

Adrenaline

In contrast to the counter-regulatory (anti-insulin) effects on glucose and lipid metabolism (9, 68, 69), the increase in plasma adrenaline concentration has a net anabolic effect on protein metabolism. The intravenous infusion of adrenaline in healthy humans, in an amount that simulates the plasma increase occurring during stress, results in an ~20% decrement in the rate
of whole-body proteolysis (70–72) and this effect is still
evident when endogenous insulin secretion is blocked
by somatostatin infusion (71, 72). The reduction of
protein breakdown is probably responsible for the fall in
the plasma concentration of most amino acids (73) and
for the resultant lower rate of amino acid oxidation
(71). In contrast to insulin (20–25), adrenaline
infusion does not decrease the rate of non-oxidative
amino acid disposal, despite the reduction of whole-
body proteolysis; therefore, net protein balance
improves after adrenaline administration (71, 72).
The effects of adrenaline on protein metabolism
should be mediated through β-adrenergic receptors
because it has been shown that they can be prevented
by the concomitant administration of propranolol (71).
Adrenaline and insulin appear to have an additive effect
on protein anabolism. In fact, their combined infusion
results in a greater suppression of the rates of proteolysis
and of amino acid oxidation (72).

At present, it is not known whether the basal
catecholamine concentrations play a role in the
regulation of whole-body or tissue protein metabolism.
Furthermore, the role played by adrenaline during
catabolic diseases, in which the plasma concentrations
of adrenaline increase together with those of other
counter-regulatory hormones (74), remains to be
established. According to the above-cited results (70–
72), during stress illness the increase in plasma
adrenaline, as well as that of GH (51–53), should
mitigate protein wasting induced by hormones with
a demonstrated catabolic action like cortisol and
glucagon (see below). However, it is also possible that
the effects of a single hormone on protein metabolism
could change when its response is associated with that
of other counter-regulatory hormones. This hypothesis
is supported indirectly by the experiments assessing
protein kinetics during multiple hormonal infusions (see
below). Despite increased adrenergic activity (74, 75),
the fact is that protein wasting progresses during stress
illness or uncontrolled diabetes mellitus. This suggests
that the anabolic action of catecholamines is negligible
when compared to the effects of catabolic hormones
and/or insulin deficiency.

Androgens

Androgenic hormones have an anabolic effect in
hypogonadal and GH deficient subjects (76). Phar-
macological doses of androgens increase lean body mass in
normal men (77) and muscle sized in trained athletes
(78). The mechanisms responsible for the anabolic
effects of testosterone have been explained by Griggs
et al. (79). In a group of healthy volunteers, a 12-week
administration of a pharmacological dose of testo-
sterone enanthate increased mixed muscle protein
synthesis (muscle biopsy during the infusion of labeled
leucine) by 27% did not significantly affect leucine
estimates of the whole-body protein breakdown and
synthesis but decreased the rate of leucine oxidation
(79). The authors suggest that testosterone also
stimulated muscle proteolysis because muscle mass
increased less than expected based on the measured
increment of protein synthesis (79). The results on the
effects of androgens on whole-body protein kinetics
were only partially confirmed by Mauras et al. in
prepubertal boys (80). In this study, after a 4–6-week
administration of testosterone enanthate, the inhibitory
effect on the rate of leucine oxidation was observed but
both leucine estimates of whole-body protein break-
down and synthesis were significantly increased (80).
The discrepancy between the two studies might be the
result of possible differences in the sensitivity of
androgen receptors that would be exaggerated by the
preceding long-term exposure to very low levels of
hormones in prepubertal boys.

Thus, taken together, the results of these two studies
(79, 80) suggest that androgens promote protein
anabolism by sparing amino acids from oxidation and
increasing their incorporation into proteins, especially
muscle proteins. However, at present, a positive conclu-
sion on the effects of androgens on protein metabolism
is not possible because testosterone administration
increases circulating GH concentrations (80). Thus,
part of the effects attributed to androgens, namely the
suppression of leucine oxidation (51, 52) and the
stimulation of whole-body (53–54) and muscle (57–
59) protein synthesis, might be mediated by GH. The
use of a systemic somatostatin infusion with replace-
ment amounts of GH, glucagon and insulin (81) and the
intra-arterial infusion of androgenic hormones to
measure regional muscle protein kinetics is necessary
to address this question.

Estrogens and progesterone

The present understanding on the effects of ovarian
hormones on protein metabolism is very limited; only
indirect data can be extrapolated from the results of
the studies comparing the effects of gender and/or
menstrual cycle on protein kinetics, on nitrogen excretion
and on basal or 24-h energy expenditure.

Resting metabolic rate and 24-h energy expenditure
adjusted for age, body composition and physical activity
are significantly lower in women than men (82). Because
protein metabolism significantly contributes
to body energy expenditure (1, 2), one could hypothe-
size that the sex differences in the hormonal milieu
might account for the lower turnover rate of body
protein in women. However, the gender difference in
energy expenditure also occurs when postmenopausal
women are compared with age-matched males (82),
thus a potential role for sex hormones should be
excluded.

After a meticulous review of the literature, Borel and
Flakoll concluded that no difference exist in the rates of
protein breakdown and synthesis under fasting and fed
conditions between young women and men (83). This conclusion is weakened by the fact that the studies included in this review were made for other purposes and not to address the question of possible gender differences in protein metabolism. If such a difference exists, it is expected to be of small magnitude and to lie near the limits of detection of the techniques employed to measure protein kinetics and body composition; therefore, a definite conclusion will only be possible when a carefully designed study, involving a large number of subjects, is carried out.

Some studies examined the possible effects of the changes in hormonal environment associated with the menstrual cycle on nitrogen excretion (84) and on leucine estimates of whole-body protein kinetics (85). When the follicular and the luteal periods were compared in the same female subjects, nitrogen excretion (84) and the rates of whole-body proteolysis and amino acid oxidation (85) were increased by ~10–20% during the follicular phase. It remains to be determined whether the greater protein catabolism occurring in the follicular period is explained by the fall of estrogen or the increase of progesterone concentrations, or by other hormonal changes observed in the luteal phase, like the increases in plasma concentrations of cortisol (86) and T₃ (85). Certainly, further research is warranted to define better our understanding of hormonal regulation of protein metabolism in women.

**Glucagon**

It is likely that glucagon plays a physiological role in everyday regulation of dietary amino acid disposal. This is indirectly supported by the observations that dietary amino acids stimulate glucagon secretion (87) and that glucagon infusion in healthy subjects reduces the plasma concentrations of most amino acids (88, 89). Studies in humans suggest that glucagon-induced hypoaminoacidemia is due to an increase in the rates of intercellular amino acid transport and of amino acid conversion into glucose (90). Accordingly, in dogs an intraportal infusion of glucagon that mimics physiological hyperglucagonemia enhances the hepatic extraction of amino acids and decreases their plasma concentrations in the absence of significant effects on leucine estimates of protein kinetics (91). In humans, despite several studies performed to establish whether glucagon affected protein kinetics (89, 92–95), it is difficult to make a clear conclusion on the physiological role of glucagon. The interpretation of these results is complicated by the fact that, in human studies, glucagon cannot be infused through the portal vein and that the infusion of glucagon must be combined with that of somatostatin (SRIF) because glucagon infusion significantly changes plasma insulin concentrations (96). Because SRIF inhibits insulin, glucagon and GH secretion, its administration should be associated with that of replacement amounts of these hormones to avoid additional experimental variables (81). However, neither insulin nor GH have been replaced in one study (92) and GH has not been replaced in the other two published studies (89, 93) exploring the effects of physiological hyperglucagonemia on protein metabolism. In one (89) of these two studies glucagon deficiency or excess did not affect leucine and lysine fluxes, suggesting that glucagon does not influence whole-body proteolysis. In contrast, in the other study (93), physiological hyperglucagonemia had a net catabolic effect because it decreased leucine estimates of whole-body protein synthesis and increased the rate of leucine oxidation. The results of the study in which insulin also was not replaced (92) indicate that the catabolic effect of glucagon is exacerbated by insulin deficiency, because, under this experimental condition, hyperglucagonemia increases both whole-body proteolysis and leucine oxidation. Two recent studies published in preliminary form (94, 95), in which SRIF infusion was associated with an infusion of replacement amounts of glucagon, insulin and GH, support the concept that glucagon has a net protein catabolic effect. In fact, physiological hyperglucagonemia increased the rates of phenylalanine (95) or of leucine oxidation (94) and reduced the stimulatory effect of exogenous amino acids on leucine estimates of whole-body protein synthesis (94).

**Glucocorticosteroids**

In healthy humans, acute glucocorticoid administration increases whole-body protein breakdown (51, 97–99) and the plasma concentrations of several amino acids, particularly of branched-chain amino acids (97–99). Because increased amino acid availability does not stimulate protein synthesis but only oxidative disposal (51, 99), a net catabolic effect results (51, 99). This mechanism of the catabolic action of glucocorticoids has been confirmed during both the post-absorptive and the absorptive states (51, 99) and occurs in spite of increased plasma insulin concentrations (97–99), suggesting that the protein wasting associated with Cushing’s syndrome (100) or prolonged glucocorticoid therapy (101) is in part the result of steroid-induced resistance to insulin (102). Surprisingly, the results of the studies involving these patients (103, 104) suggest different conclusions on the mechanisms mediating the catabolic action of cortisol. Tessari et al. measured leucine estimates of protein kinetics in five patients with Cushing’s syndrome and were unable to show any difference between the patients and the normal controls (103). More recently, Bowes et al. estimated protein kinetics in a group of patients with Cushing’s syndrome before and after surgical therapy (104). Hypercortisolism was associated with reduced rates of whole-body protein breakdown and synthesis, whereas amino acid oxidation was similar before and after treatment (104). Thus, the
authors proposed that muscle wasting related to Cushing's syndrome should be mediated primarily through a reduction in the rate of muscle protein synthesis and not through increased muscle proteolysis as suggested by acute glucocorticoid administration (51, 97–99). Data obtained in healthy subjects after short-term glucocorticoid administration and those reported in patients with Cushing's syndrome may be reconciled through several explanations. Foremost, it is important when comparing Cushing's patients with controls to take into account body composition, because Cushing's patients have a greater fat content and a lower lean body mass (LBM) (104). Because the contribution of adipose tissue to whole-body protein metabolism is negligible, to avoid an underestimate of leucine flux the results should be expressed as kilograms of LBM. For instance, when Bowes et al. determined LBM in a subset of patients, the rate of whole-body proteolysis was not reduced by hypercortisolism (104). Another variable that should be considered is that patients with Cushing's syndrome are chronically hyperinsulinemic. Because insulin decreases both rates of protein breakdown and oxidation, it is possible that the insulin resistance induced by short-term glucocorticoid administration (102) might be counterbalanced by the progressive development of greater plasma insulin concentrations over the course of the disease. Finally, increased muscle proteolysis cannot be ruled out even in the presence of a normal rate of whole-body proteolysis, because muscle contributes to less than half of whole-body protein turnover (105). Thus, increased muscle proteolysis could be counterbalanced by a concomitant decrease of proteolysis in tissues like gut. Data in rats indirectly suggested this hypothesis: corticosteroids had opposing effects because they promoted proteolysis in the muscle but decreased it in the gut (106). In conclusion, further studies are required to assess the sequence of events leading to the catabolic effect of glucocorticoid excess: in this regard, direct measurement of the rates of proteolysis and synthesis in muscle tissue is required.

**Thyroid hormones**

The common occurrence of muscle wasting and weakness in hyperthyroid patients (107) suggests that thyroid hormones exert a net catabolic effect on protein metabolism. It remains to be established whether the negative protein balance complicating hyperthyroidism is the result of an increased rate of whole-body proteolysis or of a decreased rate of whole-body protein synthesis, because conflicting data have been published (108–110). In this regard, the studies in which 3-methylhistidine excretion was used to estimate myofibrillar degradation are not conclusive because whilst some reported normal values (108, 109), others (111, 112) reported increased values during active disease. The studies exploring the rate of whole-body proteolysis by means of labeled leucine infusion (108–110) suggest that net protein catabolism associated with hyperthyroidism is primarily due to a depressed rate of protein synthesis when protein kinetics are measured in hyperthyroid patients (109) or to an increased rate of proteolysis (110) when experimental hyperthyroidism is induced in healthy subjects. Two studies on protein kinetics made comparisons before and after successful treatment of hyperthyroidism (108, 109). The hyperthyroid state was associated with lower (109) or similar (108) rates of whole-body proteolysis, increased rates of leucine oxidation (108) and decreased rates of whole-body protein synthesis (109). In contrast, both rates of whole-body protein breakdown and synthesis are increased by the administration of T3 and T4 to normal subjects (110). Under these circumstances net protein catabolism occurs because the stimulation of protein synthesis is overcome by a greater stimulation of amino acid oxidation (110). Interestingly, in experimental hyperthyroidism, the catabolic effect of thyroid hormones appears to be mitigated by the development of a greater sensitivity to the anti-proteolytic action of insulin (110). The different conclusions deriving from clinical and experimental hyperthyroidism, in analogy to those discussed previously between clinical and experimental hypercortisolism, could be explained partially by the fact that the studies exploring hyperthyroid patients underestimated the rates of whole-body proteolysis because LBM in these patients was not determined. It is also possible that in the course of disease the elevated production thyroid hormones initially induces protein catabolism by increasing whole-body proteolysis and, later, once LBM has been depleted, by primarily reducing whole-body and muscle protein synthesis.

The data on the role played by normal thyroid hormone concentration in the physiological regulation of everyday protein metabolism in normal humans are very limited. In growing rats it has been suggested that thyroid hormones contribute to the increase in protein synthesis induced by meal absorption (113). This does not appear to be the case in humans, according to the evidence that meal-induced changes in protein kinetics occur in the absence of significant changes in the plasma concentrations of T3 and T4 (114). Indirect information on the potential physiological role played by thyroid hormones can be extrapolated from the studies performed in humans with hypothyroidism. Classical studies published more than 40 years ago have shown that in patients with hypothyroidism the rates of whole-body protein degradation and synthesis are decreased and the protein kinetics are corrected upon replacement of thyroid hormones (115, 116). These conclusions have been confirmed more recently using labeled leucine to estimate whole-body protein turnover (109). A greater reduction of protein breakdown in comparison with protein synthesis could be the cause of the accumulation in hypothyroidism of albumin (117)
and plasma lipoproteins (118), whereas the deposition of mucoproteinaceous material could be the result of a net increase in the synthesis of the carbohydrate components (119). The fact that after experimental hypothyroidism several cytosolic and mitochondrial enzymes decrease (120), whereas specific liver enzymes increase (121), suggests that basal concentrations of thyroid hormones have differential effects on individual protein kinetics and they play a role in the physiological regulation of protein metabolism of selectively modulating the synthetic or the catabolic rates of target proteins.

Multiple hormonal infusion

Protein wasting complicating the course of critical illness is generally associated with significant increments in the plasma concentrations of glucagon, cortisol, growth hormone and catecholamines (74). For this reason and for their recognized gluco-counter-regulatory effects (6–9), these hormones are jointly considered as “catabolic hormones” despite some of them having proved to be anabolic (see Table 3). As discussed above in detail, a clear catabolic effect has been demonstrated only for cortisol and, under particular experimental conditions, for glucagon, whereas growth hormone and adrenaline resulted in a net anabolic effect. According to these results, among the “catabolic hormones” cortisol is the only one definitely contributing to protein wasting during critical illness, whereas hormones like GH and adrenaline should cooperate with insulin in restraining protein catabolism. This means that either the catabolic action of cortisol is powerful enough to overcome the effects of anabolic hormones, or, when counter-regulatory hormones increase simultaneously, their effects on protein metabolism differ from those predictable on the basis of their isolated administration. The results of the studies exploring the effects of multiple hormonal infusions on human protein metabolism are conflicting because they support both hypotheses (122, 123). Gelfand et al. infused cortisol, glucagon, adrenaline and noradrenaline in five obese patients for 72 h in order to mimic their circulating concentration during stress, and on another occasion infused only cortisol for four of the five patients (122). The urinary excretion of nitrogen was doubled and that of 3-methylhistidin increased by 25% by the multiple hormonal infusion; however, the authors failed to detect any significant change in leucine estimates of protein kinetics (122). When the isolated cortisol infusion was carried out, nitrogen loss was blunted on the first day but at the end of the study reached the same values as those by the multiple hormonal infusion (122). Thus, these results suggest that cortisol was the hormone responsible for the negative protein balance induced by the quadruple hormonal infusion and that catecholamines and glucagon could have contributed in an early period to nitrogen losses, presumably by stimulating gluconeogenesis (122). Different conclusions are indicated by the study of Bessey et al., who infused hydrocortisone, glucagon and adrenaline in healthy subjects for 72 h, and only adrenaline or hydrocortisone in a subset of subjects (123). The triple hormonal infusion resulted in a net protein catabolic effect as a consequence of the stimulation of protein breakdown not being compensated by an increase in the estimates of protein synthesis (123). During the isolated hormonal infusions only hydrocortisone, not adrenaline, induced a net nitrogen loss that was one-quarter of that caused by the triple hormonal infusion (123). This suggests that the three hormones when combined together have a synergistic catabolic action. At present, it is difficult to establish the reasons for the discrepancy between the two studies. However, one point can clearly be made: in both studies, multiple hormonal infusions increased nitrogen wasting by 3–4 g/day, i.e. to values well below those reported after severe traumatic injuries or burns (20–40 g/day) (124), indicating that other factors must give rise to the protein wasting observed during major injury or illness. The demonstration of a net protein catabolic effect of tumor necrosis factor (125) suggests an important role for cytokines and opens a new field for future investigations.

Because protein wasting associated with severe illness appears to derive largely from accelerated muscle protein catabolism, studies measuring the changes of protein kinetics in muscle tissue are particularly appropriate. The mechanism of the catabolic action exerted by cortisol, glucagon and adrenaline on muscle protein metabolism has been explored by Gore et al. (126). In this study, the hormones were infused directly into the femoral artery of healthy volunteers in order to assess leg muscle protein kinetics in the absence of changes in the circulating concentrations of insulin, other hormones or substrates. Under these experimental circumstances, the combined hormonal infusion increased the rates of muscle proteolysis and those of mixed muscle protein synthesis (126). Because muscle protein synthesis increased less than proteolysis, a net catabolic effect was demonstrable.

Table 3. Effects of hormonal administration on leucine estimates of whole-body protein synthesis, breakdown and the rates of leucine oxidation in humans.

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Synthesis</th>
<th>Breakdown</th>
<th>Oxidation</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin</td>
<td>=</td>
<td>=</td>
<td>=</td>
<td>20–25</td>
</tr>
<tr>
<td>Growth hormone</td>
<td>↑</td>
<td>=</td>
<td>↓</td>
<td>51–54</td>
</tr>
<tr>
<td>IGF-I</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>62–65</td>
</tr>
<tr>
<td>Adrenaline</td>
<td>=</td>
<td>=</td>
<td>↓</td>
<td>70–72</td>
</tr>
<tr>
<td>Androgens</td>
<td>=</td>
<td>=</td>
<td>↓</td>
<td>79–80</td>
</tr>
<tr>
<td>Glucagon</td>
<td>=</td>
<td>=</td>
<td>↑</td>
<td>89, 92–95</td>
</tr>
<tr>
<td>Glucocorticosteroids</td>
<td>=</td>
<td>=</td>
<td>↑</td>
<td>51, 97–99</td>
</tr>
<tr>
<td>Thyroid hormones</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>110</td>
</tr>
</tbody>
</table>
(126). Thus, whole-body (123) and muscle (126) protein catabolism induced by triple hormonal infusions appear to be mediated by a similar mechanism. The hormones, through the stimulation of protein breakdown, increase the intracellular availability of amino acids: the net catabolic effect results from the fact that hormonal action promotes the oxidative disposal of these amino acids more than their utilization for the synthesis of new proteins.

Conclusions

The available data on the hormonal regulation of human protein metabolism indicate that many hormones play a key role. However, the analysis of the physiological contribution of hormones to protein anabolism or catabolism is complicated by several factors. For instance, the effects of a single hormone are difficult to assess because they might be partially obscured by concomitant increments of other hormones, the same hormone can have opposite effects on the rates of protein breakdown and synthesis in the same organ and, finally, the combined effects of more hormones might be different from those expected on the basis of our knowledge of their individual effects. Certainly, further research will be required to establish the role played by the hormones in the stimulation of post-prandial protein anabolism, in the reduction of post-absorptive protein catabolism and in the promotion of protein catabolism during stress illness. The knowledge of these mechanisms will provide the necessary background for a better treatment of several endocrine diseases and help to design new therapeutic strategies able to successfully counteract protein wasting, often representing the final cause of death in several diseases.

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