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Hormonal Regulation of Regional and Tissue Protein Turnover

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Abstract

Hormones are major regulators of protein synthesis and breakdown in human and other animal species. Two key hormones that change in the circulation in response to meal are insulin and glucagon. When insulin levels are low (fasted state), muscle protein breakdown increases releasing amino acids into the circulation which will ensure the maintenance of the production rates in liver of essential proteins such as clotting factors and tissue remodeling. Following a mixed meal, the gut via portal vein provides amino acids to liver. The increased insulin levels, in combination with increased circulating amino acids, decrease muscle protein breakdown and enhance muscle protein synthesis, thus resulting in a net accretion of muscle proteins. Insulin effect on protein synthesis appears to be tissue specific and protein specific. For example, insulin deficiency results in an increase of fibrinogen synthesis rate and inhibition of albumin synthesis rate. In contrast, increasing insulin level has no effect on liver tissue protein or albumin synthesis. Insulin effect on muscle protein depends upon the specific proteins and the availability to amino acids. Glucagon accelerates the catabolism of amino acids. Other hormones such as IGF-I and GH complement insulin's effect. Testosterone is a major stimulant of muscle myosin heavy chain but its effects on non-muscle proteins remains to be determined. Other hormones such as thyroid hormones, epi- and nor-epinephrine have variable effects on protein turnover and may be determined by the physiological state of the body. Alterations in hormones of any type substantially alter protein balance in the body, and this change in protein balance in specific tissues depends upon the specific hormonal changes.

Introduction

This review will focus on regulation of protein dynamics across major regional tissues such as splanchnic and muscle beds. The exchange of amino acids across various organ beds has a major impact on production of various essential proteins. The timely production of proteins for specific body functions is essential to maintain life. For example, the liver is an organ producing many circulating proteins, including clotting factors, albumin, fibrinogen, many acute reaction proteins, and many carrier proteins. Continuous amino acid supply to liver is essential to maintain the production of these proteins. Liver also is the site of glucose production, and gluconeogenesis is a major source of glucose in the circulation. Amino acids are important precursors of gluconeogenesis, and continuous provision of the amino acids to liver is essential for maintaining the production of glucose, the sole fuel of brain in normal conditions. Peripheral tissues, especially skeletal muscle, are the major location of glutamine production, and continuous supply of glutamine to the gut is essential for its metabolic needs. Based on many *in vivo* studies, it is clear that insulin plays a key role in maintaining the amino acid levels in the blood in addition to its role in regulating breakdown and synthesis of many proteins. Recent evidence indicates that insulin's effect is tissue-specific, which allows insulin to play the key role of regulating the concentrations of circulating amino acids so as to maintain regional protein synthesis to ensure the welfare of the species.

The balance between protein synthesis and breakdown determines the size of the protein pool in the body. The protein pool in the body consists of thousands of proteins with various rates of turnover (breakdown, and synthesis) (1,2). For example, there are proteins such as muscle structural proteins (example: myosin heavy chain) which are synthesized at a rate of 1 to

1.5% per day (3). In contrast, liver protein synthesis occurs at about 7-8 times faster than that of muscle (4,5), and gut mucosal protein synthesis occurs over 30 times faster than that of skeletal muscle (6). Therefore, the whole body protein turnover is determined not only by large protein pools such as muscle proteins but also by small protein pools, which turn over at a faster rate. The protein net balance in the body, as determined by urinary nitrogen balance studies, does not give information about the type of protein within the body that changes with a specific intervention. Whole body protein turnover studies based on isotope tracer approaches provide information about the average values of protein breakdown and protein synthesis. These measurements are not sufficiently sensitive to detect changes occurring in certain individual protein pools, such as that of muscle myosin heavy chain, because of its slow turnover rate. It is, therefore, important to study regional protein dynamics to understand the regulation of amino acid exchange across tissue beds and synthesis of specific tissue proteins to understand the mechanism of functional changes attributed to the specific proteins.

Type 1 Diabetes Mellitus

Patients with type 1 diabetes mellitus provide an ideal model to study the effect of insulin action. During the pre-insulin era, type 1 diabetic patients were known to be cachexic with substantial muscle wasting. Withdrawal of insulin treatment in type 1 diabetes patients resulted in increased urinary nitrogen loss (7) and also an increased circulating amino acid levels (7,8). Whole body studies performed in patients with type I diabetes mellitus showed that insulin deprivation is associated with increased whole body protein breakdown and oxidation of amino acids such as leucine (9-11). The net protein loss, as measured by urinary nitrogen loss, occurred

because the magnitude of increase in whole body protein breakdown exceeds that of synthesis rate.

Recent studies have demonstrated that there is a regional difference in which insulin deprivation caused changes in protein balance (10). Insulin deprivation is associated with an increase in muscle protein breakdown with no significant effect on muscle protein synthesis (Figure 1). In contrast, both the protein breakdown and synthesis increases in the splanchnic tissue, resulting in a net increase in protein accretion in the splanchnic bed. This was confirmed by the net uptake of amino acids such as leucine, lysine, etc (Figure 2). Measurement of gut mucosal protein synthesis indicated that this increase in splanchnic protein synthesis does not occur in the gut mucosa (Figure 3) (6). It has also been shown that insulin deprivation in people with type 1 diabetes mellitus is associated with an inhibition of albumin synthesis rate, whereas fibrinogen synthesis rate increases (Figure 3) (12). All of these results clearly demonstrate that, even within a tissue bed, synthesis rate of individual proteins is differentially regulated by insulin.

Some of the changes that occur in type I diabetes mellitus during insulin deprivation are not the direct effect on insulin deficiency per se but are related to many secondary events. It has been shown that increase in leucine oxidation that occurs following insulin deprivation is largely related to increased circulating glucagon (13). It has also been shown that glucagon selectively increases synthesis of fibrinogen (14). Since glucagon levels increase during insulin deprivation, it is; therefore, likely that the increased oxidation of amino acids and increased fibrinogen synthesis are related to high circulating glucagon levels. Another effect of high circulating glucagon in people with type 1 diabetes is increased energy expenditure (13,15,16).

Insulin deprivation resulting in increases in β -hydroxybutyrate (17), circulating amino acids (18), and fatty acids (19) may impact turnover of proteins that occurs during insulin deprivation.

Nondiabetic People (Figure 4)

Studies performed in nondiabetic people demonstrated that there is a net efflux of amino acid from muscle bed during the postabsorptive state (20). This increased efflux of amino acids occurs because muscle protein breakdown exceeds that of muscle protein synthesis (Figure 4). Insulin has a dose-dependent effect of inhibiting muscle protein breakdown and reducing the efflux of amino acid from the muscle bed. During the postabsorptive state, splanchnic tissue takes up the amino acids from the circulation, presumably to maintain the synthesis of various essential proteins that are synthesized in the liver. Insulin administration inhibits muscle protein breakdown and reduces the uptake of amino acid by the splanchnic tissue. It is unclear whether the reduced uptake of amino acids by the splanchnic tissue during insulin infusion is due to direct effects of insulin or secondary to the decrease in circulating amino acids due to inhibition of muscle protein breakdown. Direct measurement of liver protein synthesis in a pig model demonstrated that infusion of insulin with or without amino acids has little effect on the overall synthesis of liver proteins. This suggests that liver protein synthesis is maintained at a constant rate if sufficient amino acid supply is available (21). The preliminary data indicates that amino acid replacement may prevent the decline in liver uptake of amino acids that occurs during insulin infusion alone (22). Further studies are needed to understand the effect of insulin with or

without amino acid on synthesis rate of specific liver proteins. At whole body level, preventing the circulating amino acids (Figure 5) enhances insulin effect on protein breakdown.

Based on these studies, it is concluded that in the postabsorptive state amino acids for the synthesis of essential protein in the liver are made available by increasing muscle protein breakdown (Figure 5). In contrast, during the postprandial state, the gut provides the amino acid necessary for synthesis of liver proteins and there is an increased accretion of protein in the muscle proteins.

Other Hormones

Hormones such as glucagon have been shown to increase catabolism of many amino acids, especially that of glycolytic amino acids (23). As a result, when glucagon levels are high, the provision of amino acids is associated with a smaller increase in synthesis rate of whole body protein than when glucagon levels are low. This has also been demonstrated in studies done in type 1 diabetes patients (13). Although the whole body and regional protein turnover is acutely regulated by insulin and glucagon, other hormones such as cortisol, thyroid hormones, growth hormone, IGF-I, IGF-II, epinephrine, and androgens also affect protein turnover. The summary of these hormonal effects is given in Table 1 (24). The effect of thyroid hormone depends on whether replacement or excessive circulating thyroid hormones are present (24,25).

There is increasing evidence that the effect of hormones and other factors may be specific to specific proteins or specific protein fractions. For example, with aging, there is a reduction in synthesis rate of myosin heavy chain and mitochondrial protein in skeletal muscle without any effect on sarcoplasmic proteins (26,27). Age also has specific effect on the gene transcript level

of specific muscle proteins such as isoforms of myosin heavy chain I, II, and III (28). These effects may be also partly related to age-related changes in various hormonal levels. These are under investigation, especially the age-related changes in testosterone and DHEA levels.

Testosterone has been shown to increase the synthesis rate of myosin heavy chain and mixed muscle protein synthesis in hypogonadal men (29). Testosterone has also been shown to have a dose effect on muscle mass in people who undergo a resistance training program (30). It remains to be determined whether this dose effect is seen on muscle protein synthesis and breakdown.

Summary and Conclusion

Hormones are major regulators of whole body protein turnover. Hormonal effects on protein synthesis are tissue-specific. It is likely that hormones such as insulin may enhance synthesis rate of a protein by increasing the translation of mRNA (31) as well as increasing the gene transcription. Insulin also may stimulate or inhibit the transcription of many other genes, as a result causing decrease or increase in synthesis of specific proteins (32). If studies are focused entirely on measurement of mixed tissue protein synthesis, this important differential effect of insulin on specific proteins will not be evident. Future studies should focus on measuring synthesis rate of specific proteins and how hormones regulate the process. With the recent elucidation of the human genome sequence, it becomes increasingly clear that there are more proteins than genes, suggesting alternative splicing and posttranslational changes may occur. Hormones may be the major modulators of these changes and open up new areas of study, which may further enhance our understanding of the hormonal regulation of protein metabolism in humans and animals.

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Figure Legends

Figure 1. Protein \rightarrow phenylalanine represents protein breakdown and phenylalanine \rightarrow protein represents protein synthesis (ref #10). This shows that during insulin deprivation (I-) type 1 diabetic patients had increased protein breakdown in all tissues, although protein synthesis increased only in splanchnic tissue. Insulin treatment had no effect on muscle protein synthesis (increased the percentage contribution of muscle to whole body), although decreased muscle protein breakdown reduced net protein loss.

Figure 2. Amino acid balance (ref #10). While leucine, phenylalanine (Phe), tyrosine (Tyr), and lysine balances were negative during insulin deprivation across the leg although balances of these amino acids were positive across splanchnic bed. Insulin treatment reduced the negative balance in muscle and positive balances across splanchnic bed.

Figure 3. During insulin deprivation albumin fractional synthesis rate was lower, while that of fibrinogen was higher (ref 12) in type 1 diabetic patients. Gut mucosal protein fractional synthesis rate was lower during insulin deprivation than during insulin treatment in type 1 diabetic patients and the nondiabetic control subjects (ref 23).

Figure 4. Protein breakdown and synthesis in leg and splanchnic regions during saline infusion (0) and insulin infusion (0.5 mU/kg/min and 1.0 mU/kg/min) in nondiabetic control subjects. Insulin in a dose-dependent manner reduced protein muscle breakdown and splanchnic protein synthesis achieving net zero balance in these two tissue beds (ref 20).

Figure 5. Progressive decline in leucine flux occurred following replacement of amino acids during insulin infusion. Insulin infusion alone was less effective in reducing leucine flux (ref 33). This indicates a cumulative effect of amino acids and insulin on protein breakdown or development of insulin resistance when amino acid levels decline.

Table 1. 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	↓?	↑?	↓

^aPossibly mediated through insulin-like growth factor-1. GH, Growth hormone.

^bNo or not sufficient evidence available.

^cGlucagon, glucocorticoids, and catecholamines combination.