The Regulation of Body and Skeletal Muscle Protein Metabolism by Hormones and Amino Acids

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ABSTRACT For many decades, it has been recognized that insulin, growth hormone, glucocorticoids, insulin-like growth factor 1, thyroid hormones, and other hormones regulate body protein metabolism. It has been more recently recognized, but not understood, that humor factors present in states of acute and chronic inflammation could have a strong impact on protein turnover. Most recently, the role of amino acids, acting as signaling molecules, has become increasingly clarified. In aggregate, these factors (together with neuromuscular activity) determine the balance of body protein mass. We will review some of these data, particularly focusing on amino acids, insulin, and the growth hormone axis and their actions in muscle and how these relate to whole-body protein metabolism. J. Nutr. 136: 212S–217S, 2006.

KEY WORDS: • insulin • growth hormone • IGF-I • amino acids • protein degradation • protein synthesis • skeletal muscle

Muscle, as the largest organ in the body, with a mass of ~28 kg in the average 70-kg human, and being ~20% protein by weight, is a major contributor to total body protein dynamics. Insulin (1), insulin-like growth factor 1 (IGF-I) (2), and growth hormone (GH) (3) each has acute, anabolic actions on skeletal muscle protein. Each also has distinct effects on glucose and fat metabolism in muscle. In recent years, more has been learned regarding the mechanisms by which these agents exert their biological effects. In this review, we will compare and contrast actions of each of these hormones on skeletal muscle in humans. Where necessary, we will draw upon mechanistic studies done in experimental animals. We will take the approach of considering specifically the acute actions of these agents as opposed to their chronic effects, which may differ from their acute actions. In addition, prior to discussing their actions on skeletal muscle protein metabolism, we will briefly summarize their effects on nutrient/hormone delivery to skeletal muscle because this may be another mechanism by which these hormones regulate the metabolic response.

Effects of GH, IGF-I, and insulin on nutrient delivery to muscle

Resting blood flow to skeletal muscle is slow (~3–4 mL min⁻¹ 100 g⁻¹) relative to tissues like liver, kidney, brain, and heart, which have basal blood flows 20- to 40-fold greater (4–6). In contrast, skeletal muscle has an enormous capacity to increase its blood flow (e.g., with intense exercise this might rise by 20-fold) (7). Whereas exercise is the most dramatic and best-studied stimulus to muscle blood flow, in recent years it has been appreciated that insulin (8), IGF-I (9), and GH (3) each can also augment skeletal muscle blood flow in humans.

Figure 1 provides a general protocol that we have used (with minor variations) to study the effects of these three hormones on forearm hemodynamics and glucose and protein metabolism. All subjects were healthy young humans of normal body weight and on no chronic medications and were studied after an overnight fast. Brachial artery and median deep anterior cubital vein were cannulated and a 3H-phenylalanine infusion was based upon its prior extensive use in the perfused rat heart (11) and hindlimb (12). After 2 h of a basal tracer equilibration period, blood flow was measured and both arterial and venous samples were collected for later measurements of glucose and
illustrates the time course of acute changes in GH, IGF-I, and insulin effects on forearm blood flow. Figure 2 shows the effect of GH, IGF-I, and insulin on forearm blood flow, which is lower than in plasma (30). This is consistent with a barrier function of the endothelium. Whether amino acid concentrations in muscle interstitium bear a similar relation to plasma concentrations in muscle interstitium has not been studied to our knowledge.

Moreover, in states of native or experimentally induced insulin resistance (19–21), the vascular action of insulin in skeletal muscle appears to be impaired along with the metabolic response. For insulin, it is thought that the vascular response is important for the delivery of both insulin and glucose to the muscle interstitium (22). Kinetic studies of the time course for insulin action in skeletal muscle tissue suggests that insulin’s access to muscle interstitium is a rate-limiting step (23,24) in overall insulin action in vivo. Inasmuch as GH and IGF-I (which circulates complexed to binding proteins) are larger than insulin, access of these hormones to the muscle interstitium would also likely be dependent upon the rate of their delivery to the microvasculature and their rate of passage through the endothelium. Little is known about the rate at which these processes occur in vivo. We have recently observed, using confocal microscopy and fluorescein-labeled insulin, that insulin is concentrated within the endothelial cell and crosses the endothelium via a trans-cellular, receptor-mediated pathway in vivo (H. Wang, Z. Liu, G. Li, and E. Barrett, unpublished results). For IGF-I, addressing this issue is greatly complicated by the fact that many somatic tissues, including skeletal muscle, can make IGF-I (25). This local production contributes to a paracrine/autocrine regulatory system. The transfer of GH from the vasculature to the muscle interstitium has not been studied to our knowledge.

It must also be considered that the vascular actions of these hormones could have an impact on the delivery of nutrients, especially amino acids, to myocytes. Numerous recent studies (both in vitro and in vivo) have demonstrated that amino acids per se have a profound regulatory effect on whole-body and skeletal muscle protein synthesis (2,26–29). In careful microdialysis studies, investigators have shown that under steady-state conditions glucose concentration in the muscle interstitium is lower than in plasma (30). This is consistent with a barrier function of the endothelium. Whether amino acid concentrations in muscle interstitium bear a similar relation to plasma is not known. Such information would be helpful in assessing the impact of changes in blood flow, blood flow distribution, and transcapillary transport on nutrient delivery to muscle. This would predictably also have an impact on our understanding of the validity of our use of tracer methods to estimate protein turnover in vivo. These methods rely on assumptions regarding the steady-state relation between tracers in plasma and in remote compartments. To the extent that the vasculature serves as a barrier to the access of either tracer, tracee, or, in the case of hormones, the provocative stimulus being studied, the assumptions of steady-state kinetic models may be compromised. As an example of this, we have observed that, in human skeletal muscle, infusion of BCAAs markedly diminishes the degradation of skeletal muscle protein (27,31). In contrast, in human heart muscle, BCAA infusion stimulates protein synthesis (32). Whether these differing kinetic behaviors reflect differences in the delivery of either BCAAs or differences in the behavior of the tracer used to quantify protein synthesis and degradation is uncertain.

Effects of GH, IGF-I, and insulin on forearm glucose and protein metabolism

Figure 3 shows the effect of GH, IGF-I, and insulin on forearm glucose balance in healthy young adults. Clearly, at the concentrations used, GH has little or no effect. However, in other studies we have observed that GH at these concentrations can inhibit insulin-stimulated glucose disposal (33). IGF-I has a small stimulatory effect on muscle glucose uptake, whereas insulin has a major stimulatory effect on glucose disposal.
Because GH, IGF-I, and insulin were each infused locally into the brachial artery, the increases in concentration of the infused hormones were essentially localized to the forearm. These infusions did not perturb systemic concentrations of glucose, insulin, or amino acids. Thus the differences in glucose uptake seen are essentially under eumetabolic conditions. However, as noted above, even under these conditions and even with local hormone infusion into the brachial artery, some time will be required before the infused hormone equilibrates with the interstitial space in muscle and before any metabolic effects ensue. In addition, given that the hormone may perturb the rate of protein synthesis or degradation, non-steady-state conditions may ensue that compromise the use of the tracer kinetic methods that we have applied (34).

Turning to the effects of these agents on forearm skeletal muscle protein metabolism, a somewhat different picture emerges. All three hormones significantly reduce the net negative forearm phenylalanine balance that typifies the postabsorptive state in humans. Indeed, with both insulin and IGF-I, the net balance becomes positive (Fig. 4). Equally interesting is that the shift in forearm protein (phenylalanine balance) appears to be due to different processes occurring among the different hormones. Thus, GH and IGF-I had a significant action to increase the uptake of the radiolabeled phenylalanine into skeletal muscle (an index of protein synthesis), whereas insulin did not affect this variable at all. However, insulin did significantly blunt the release of unlabeled phenylalanine from skeletal muscle protein (an index of protein degradation).

We explored this further by varying the concentrations of insulin and IGF-I used. This was not done for GH because the concentrations we used were already at the upper end of the physiological range (~35 µg/L) and the effect on phenylalanine balance was less than for either of the other two hormones. Figures 5 and 6 show the effect of local infusions of IGF-I at low, medium, and high (1.8, 6.0, and 10 µg kg⁻¹ h⁻¹, respectively) concentrations (9). Likewise, for insulin, three different concentrations were explored, 0.01, 0.035, and 0.05 mU min⁻¹ kg⁻¹ given into the brachial artery (35). These doses raise plasma insulin by ~20, 60, and 110 µU/mL above fasting. The effect seen on glucose balance was greatest at the two higher doses of insulin. However, when considering phenylalanine balance or the rate of tracer uptake into the skeletal muscle, IGF-I had either comparable or greater effects.

Whereas in each case we used hormone concentrations at the upper physiological range, it remains possible that looking over a more extended range of hormone concentrations would reveal additional differences or similarities between these hormones. We have, in addition, studied forearm responses to insulin at doses as high as 5.0 mU min⁻¹ kg⁻¹ (36). In these experiments, insulin was infused directly into the brachial artery thereby allowing exaggerated local increases in insulin concentration. With these very high insulin doses, sufficient hormone recirculates to raise the systemic insulin concentration dramatically. As a result, it was necessary to systemically infuse both glucose and amino acids to prevent the decline in circulating concentrations of these substrates during the study. In these cases, as occurs when lower doses of insulin are infused locally into the forearm in the absence of increases in the systemic insulin concentration, the arterial amino acid and glucose concentrations did not change significantly with hormone infusion compared with the respective basal state.

Figure 5 shows the forearm glucose balance during the progressively incremented infusions of insulin or IGF-I. As can be seen even at high physiological concentrations of insulin, it is substantially more effective than IGF-I at incrementing skeletal muscle glucose uptake. At the highest insulin concentration used (with the plasma glucose maintained at basal using the insulin clamp method), glucose uptake was >10-fold above
basal. At these insulin concentrations, it is possible that insulin would bind to both the insulin and IGF-I receptors as well as to insulin/IGF-I hybrid receptors. However, as can be seen from the glucose dose response with IGF-I infusion, progressive increases in IGF-I doses over a 3.5-fold range (1.8–6 μg kg\(^{-1}\) h\(^{-1}\)) incremented forearm glucose uptake only modestly (2- to 3-fold) and further increase from 6–10 μg min\(^{-1}\) kg\(^{-1}\) had no additional effect on forearm glucose disposal. It must be cautioned that these increments in plasma IGF-I are difficult to relate to total IGF-I concentrations in the circulation. Total IGF-I increased from basal values of ~150 μg/L to >450 μg/L. However, the free IGF-I concentration was likely substantially higher than is typically seen under physiological conditions. This is illustrated by the observation that similar total IGF-I concentrations were seen in the contralateral arm as well as in the hormone-infused arm. However, there were no increments in glucose uptake (or changes in protein metabolism) in the arm contralateral to the hormone infusion.

Whereas insulin was strikingly more effective than IGF-I in promoting forearm glucose uptake, a different response was seen with regard to forearm phenylalanine balance (Fig. 6). Here, IGF-I was equally or more effective than insulin in stimulating a net uptake of phenylalanine. As mentioned above, the mechanism by which insulin and IGF-I appeared to influence forearm protein metabolism differed, with insulin’s dominant effect to inhibit muscle proteolysis, whereas IGF-I stimulated protein synthesis. Interestingly, when the plasma concentration of amino acids is raised (without raising either insulin or IGF-I), there is a modest rise in skeletal muscle protein synthesis and normally negative postabsorptive skeletal muscle protein balance becomes neutral or slightly positive. Adding either IGF-I or insulin to the amino acid infusion promotes a further positive protein balance. Mechanistically, these effects are again secondary to increases in protein synthesis (IGF-I) and decreases in degradation (insulin) (2). Thus addition of amino acids appears to allow a fuller expression of the normal actions of each of these hormones.

It is of interest to consider how these differing responses of forearm muscle to insulin and IGF-I for glucose and protein metabolism might be viewed vis-à-vis the known pathways by which these hormones regulate protein and glucose metabolism within skeletal muscle. As illustrated in Figure 7, insulin and IGF-I each bind to plasma membrane receptor with intrinsic tyrosine kinase activity (37). Ligand binding enhances this intrinsic kinase resulting in the tyrosine phosphorylation of both the insulin and IGF-I receptors as well as other proteins [e.g., insulin-receptor substrate (IRS)-1, 2, etc.]. For both hormones, this triggers a cascade of first tyrosine followed by serine phosphorylation reactions that alter the activity of a variety of enzymes. Considering glucose transport, the pathway that traverses the lipid kinase phosphatidylinositol 3-kinase (PI-3-K), phosphoinositide-dependent kinase (PDK)-1 and 2, and protein kinase B (Akt), appears essential for the stimulation of glucose disposal. Activation of these signaling proteins is also involved in the early steps toward the regulation of mRNA translation initiation. It is at this step that insulin (based upon extensive studies in isolated tissues and cells) is believed to have its major action on protein synthesis (38,39). Thus, these early steps of postreceptor cellular activation appear to be common to both insulin and IGF-I signaling. How, then, can we explain the seemingly differential responses of protein synthesis and glucose uptake in forearm muscle? At this time, answers to this seeming paradox are not available. We have begun to examine the differential effects of IGF-I and insulin on the phosphorylation of individual kinases as well as on the activity of some of these enzymes within rat skeletal muscle in response to insulin or IGF-I. In general terms, our findings confirm in rats what we have observed in humans (i.e., the differential effects
of insulin on glucose disposal and inhibition of proteolysis with a greater effect of IGF-I on protein synthesis. We have not been successful in defining sites where these signaling pathways diverge or are alternatively regulated that might explain these phenomenological observations.

Our current working hypothesis is that IGF-I likely exerts effects by a pathway other than the PI-3-K, PDK, or Akt that crosses over at or downstream of the mammalian target of rapamycin (mTOR) protein to provide a stronger stimulus to translation initiation while having less effect on the early pathways of glucose uptake. Equally likely, insulin may be exerting additional effects via pathways other than through PI-3-K, PDK, and Akt that augments its effects on glucose disposal. Recent studies in adipocytes indicate that insulin action on glucose disposal in these cells also requires a stimulation of TC10 through insulin receptors that appear to be localized to caveolae and this does not involve the PI-3-K pathway. Whether this dual pathway for stimulation of glucose uptake also exists in skeletal muscle is not certain. If so, and if IGF-I were much less effective than insulin at this site, such an effect could equally well explain our findings.

It is instructive here to consider the effects of raising the plasma concentrations of amino acids. We have observed that this intervention alone, with little or no rise in the circulating insulin concentration, stimulates protein synthesis in human muscle. This effect could equally well explain our findings. Studies in adipocytes indicate that insulin action on glucose disposal in these cells also requires a stimulation of TC10 through insulin receptors that appear to be localized to caveolae and this does not involve the PI-3-K pathway. Whether this dual pathway for stimulation of glucose uptake also exists in skeletal muscle is not certain. If so, and if IGF-I were much less effective than insulin at this site, such an effect could equally well explain our findings.

Conclusion

In conclusion, we would emphasize that observations to date suggest that, at physiological concentrations, insulin has a greater effect on glucose disposal but a lesser effect on muscle protein synthesis compared with IGF-I. Resolving the cellular mechanisms involved in the differential effects of these two hormones requires studies beyond the use of the tracer kinetic approach and will be more amenable to resolution with the increasing availability of phospho-specific antibodies for signaling molecules involved in insulin and IGF-I cellular activation. The extent to which these signaling pathways influence not only the myocyte response to these hormones but also the vascular response within muscle will determine the overall tissue metabolic response to hormone stimulation and nutrient supply.

LITERATURE CITED