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Nutrition and Muscle Catabolism in Maintenance Hemodialysis: Does Feeding Make Muscle Cells Selective Self-Eaters?

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Abstract

Efforts to build muscle by increased protein feeding in hemodialysis patients have been thwarted by parallel increases in both muscle protein synthesis and degradation. The evidence suggests that muscle cells replace older proteins in response to feeding rather than using new proteins to drive muscle cell hypertrophy. This review presents the hypothesis that protein feeding provides an opportunity for muscle to accelerate proteolysis of proteins which have been damaged by oxidation, nitrosylation and/or glycosylation and to replace damaged mitochondria that contribute to oxidative stress. Increases in proteolysis with feeding are driven by insulin resistance and the increased oxidative stress of mitochondrial respiration. Oxidized proteins and organelles are excellent substrates for degradation by the proteasome, macroautophagy, and chaperone-mediated autophagy: these systems of proteolysis seem to be activated by oxydative stress. Replacement of oxidized and other damaged proteins may be a benefit of protein feeding in hemodialysis, but alternative strategies, including exercise, will be required to build muscle.

Introduction

The loss of lean body mass in patients on maintenance hemodialysis is strongly associated with increased mortality (8). Unintentional loss of as little as 5 % of body weight carries significant morbidity and mortality. For this reason, nutritional recommendations for this population provide ample protein (1.0–1.2 g/kg/day) and calories (30–35 g/kg/day) in the hope of maintaining or building muscle mass (1). Results of this strategy have been mixed: younger patients with fewer co-morbid conditions maintain muscle mass, while many others continue to suffer from protein energy wasting (7). Newer approaches with intensive oral or intradialytic parenteral feeding have also been employed. These regimens have been successful both in increasing serum albumin among other markers of visceral protein and BMI (4). Because physical activity increased in these studies, it is difficult to know if the apparent increases in muscle mass are do to exercise or a direct effect of feeding. Careful radiotracer studies on whole body and muscle protein synthesis made during feeding regimens show mixed results. Although whole body synthesis is clearly increased with feeding in this population, muscle protein synthesis increases more variably. Even when muscle protein synthesis increases, measurements of muscle protein and nitrogen balance do not increase, because muscle degradation increases match the rise in protein synthesis (13;20). It is not immediately clear why muscle protein breakdown should rise to nullify the

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increase in protein synthesis with feeding. If the muscle cell cannot grow, why does it take up newly infused amino acids? If the muscle cell is going to destroy proteins to preserve its size, then why does it synthesize so much new protein?

A preference for eating its old

One possible answer for these questions comes from radiotracer studies where carbon labeling of the infused amino acids allows measurement of labeled CO₂ as a marker of specific amino acid oxidation (6). Because amino acids are not stored in the body except as part of protein chains, the labeled CO₂ appearance can detect both amino acids that are not used for synthesis and amino acids incorporated into proteins which are quickly destroyed. If feeding strategies cause newly synthesized protein to be subsequently destroyed, one would expect an increase in the labeled CO₂. In general this is not observed. For example, Raj and his co-workers, measured amino acid oxidation in dialysis patients receiving intradialytic parenteral nutrition (20). Although this treatment induced robust increases in both protein synthesis and protein degradation (including increased appearance of the 14 kDa actin fragment that serves as a marker of caspase 3-mediated myofibrillary degradation (5)), there was no change in labeled CO₂ appearance. The proteins being destroyed were not the ones being synthesized. Rather older proteins were being selectively degraded.

How does this occur? At first glance, it is not clear which direct amino acid cell signaling mechanisms could explain this phenomena. Feeding induces insulin which activates type 1 Phosphatidylinositol 3 Kinase (PI 3-kinase) and allows the activation of Akt. Akt suppresses the forkhead family transcription factors (FoxOs) which no longer are able to drive the transcription of muscle specific catabolic factors MuRF1 (Atrogin1) and MAFbx (24;28;32). These factors are important in driving ubiquitin/proteasome and macroautophagic pathways of proteolysis. Thus amino acids should suppress proteolysis rather than stimulate it. However, amino acid entry into C2 C12 myocytes also activates the VPS34 type 3 PI 3 and the products of type 3 PI 3 kinase can activate macroautophagy (3). Thus it is possible that direct amino acid signaling could stimulate only macroautophagy, but this pathway accounts for only a relatively small percentage of proteolysis in muscle cells (18).

However, proteasomal proteolysis could be stimulated by secondary insulin resistance induced by protein feeding of an adequate calorie diet (31). In normal subjects, chronic protein feeding of an isocaloric diet induces increased insulin plasma levels for a given glucose load, indicating insulin resistance (14). Part of this effect may be due to the higher fat content of the high protein diets because free fatty acids are known to induce insulin resistance, however, acute intravenous infusion of amino acids or bathing isolated muscle in a high concentration of amino acids increases the insulin requirement for glucose entry into cells (31). At low levels, amino acids increase Akt phosphorylation in isolated muscle cells and improve insulin sensitivity, but at higher concentrations amino acids strongly activate p70 S6 kinase 1 which directly phosphorylates IRS1 on inhibitory serine residues 312 and 636/639 and blunts Akt phosphorylation.(30). Just as with protein synthesis, the various individual amino acids had different degrees of effect with leucine inducing less insulin resistance (31). Thus, insulin resistance could play a role in a non-selective increase in proteolysis with intensive feeding. Thus, it appears that increases in protein degradation are an integral part of the muscle's response to increased amino acid delivery. However, it is not clear from the cell signaling how selective destruction of older proteins occurs.

A hypothesis on selective degradation of older proteins

Unlike epithelia, muscle cells do not have significant cell turnover. Hypertrophy and atrophy occur largely through changes in the diameter of the individual muscle fibers (23). The

problem with not replacing whole cells is that proteins that get damaged or misfolded must be replaced. Covalent modification of proteins by glycosylation, oxidation, and (in CKD or hemodialysis patients) nitrosylation occurs over time in muscle cells. These post-translational modifications reduce protein function or destabilize the three dimensional conformation which leads to protein unfolding (16). Ultimately these proteins can aggregate and form deposits which further destabilize cellular function. The cell defends itself against these adverse modifications by increasing the removal of misfolded proteins via the ubiquitin-proteasome and chaperone-mediated autophagy systems while protein aggregates are removed by macroautophagy (15). Because the insulin resistance induced by protein feeding up-regulates proteolytic capacity, there could be increased clearance of these proteins if there was a mechanism for their specific removal. Thus, a hypothesis can be formulated arguing that a selective turnover of older proteins could occur because these proteins are marked by oxidative or other modifications that preferentially accelerate their proteolysis. This hypothesis would predict that adequate protein feeding would be beneficial to the hemodialysis patient by increasing the turnover of damaged proteins and replacing them with new ones.

Oxidation as a model of selective proteolysis

While there is little data showing selective clearance of nitrosylated or glycosylated proteins before they form aggregates, extensive data exists that oxidized proteins are selectively destroyed (2;21;25). This is even more significant because there is increased protein oxidation in hemodialysis patients (19). Oxidative stress is increased in hemodialysis patients by inflammation, the dialysis procedure itself, and by other co-morbid conditions (10). Dietary protein intake modulates mitochondrial oxidant production. When protein intake is inadequate, oxidation occurs due to a deficiency of sulfur containing anti-oxidants including GSH (17). With increasing protein intake, there is increased deamination of amino acids which provides increased substrate for mitochondrial respiration. Mitochondrial respiration creates oxygen free radicals that are dissipated by anti-oxidants until excessive feeding overwhelms anti-oxidant capacity (26). Moreover, damaged mitochondria have less efficient respiration and produce more oxidants. This is significant because oxidant damage to mitochondria can lead to cytochrome c release, caspase activation and the cleavage of myofibrillary protein into fragments which can serve as substrate for the ubiquitin-proteasome system. Thus oxidation can provide a critical step to cleave the insoluble myofibrillary protein a required step for proteasomal proteolysis.

Seen in this light, the direct stimulation of macroautophagy by amino acids makes teleologic sense. Macroautophagy, with its unique ability to destroy entire organelles, plays a unique role in regulating mitochondria. Respiration with its generation of oxygen free radicals damages mitochondria necessitating replacement of the organelles every 10–25 days (29). Oxidized mitochondria are more likely to release cytochrome C which in turn triggers the caspase 3 pathway and leads to disassembly of myofibrillary protein. Because older mitochondria are more likely to oxidatively damage surrounding myofibrillary protein, this process could target entire damaged sections of the muscle fiber. Damaged mitochondria are also selectively chosen for destruction by macroautophagy. The process differs from normal macroautophagy in that it occurs independently of type 3 PI 3 kinase, bypassing the normal signal required for macroautophagy (29). Thus older, oxidatively damaged mitochondria are uniquely capable of triggering a reaction that not only allows their own destruction but also the destruction of surrounding myofibrillary protein (Figure 1).

That oxidatively damaged proteins are destroyed in the absence of normal signals applies to both the ubiquitin-proteasome system and to chaperone-mediated autophagy. In the ubiquitin-proteasome system, regulation of target proteins occurs by ubiquitin E3 ligases

which recognize and attach polyubiquitin chains to the target protein. This polyubiquitin chain is then recognized by the 26S cap on the proteasome where unfolding and insertion into the 20S proteasome core particle occurs. Proteases in the 20S particle then cut the polypeptide chain completing proteolysis. Oxidized proteins bypass ubiquitination and the 26S cap by being able to be recognized and inserted in the 20S core particle directly without processing. It is likely that oxidation destabilizes the protein structure creating enough unfolding to allow entry into the 20S particle (27). In chaperone-mediated autophagy, proteins that display on their surface a sequence of highly charged proteins near bulky hydrophobic residues and an ASN (the KFERQ motif) are recognized by the 73 kDa heat shock cognate protein (hsc73). Through recruitment of co-chaperones the protein is unfolded (9). This complex recognizes LAMP2a on the surface of the lysosome, and the protein is imported into the lysosome for destruction. Oxidative modification may create new KFERQ motifs by adding negative charges near bulky hydrophobic and ASN residues or may unfold a protein exposing a previously hidden KFERQ motif allowing lysosomal import (11). In both cases, the normal mechanism of proteolytic regulation is bypassed to allow efficient destruction of the modified proteins.

Besides creation of new substrate for proteolytic systems, oxidation also increases the capacity of at least one of these proteolytic pathways. Kifflen and her co-workers showed increased synthesis and decreased intramembrane degradation of the CMA acceptor protein LAMP2a during oxidative stress (12). This increased capacity resulted in much more rapid degradation of the oxidized substrates. The cause of this increase in capacity is unclear, but we have found that it correlates with regulation of FoxO1 protein levels in kidney cells (9). In NRK-52E cells, growth factors reduce the amount of proteolysis via CMA via a FoxO1 sensitive pathway. Growth factors also inhibit FoxO1 signaling by phosphorylating it which makes it rapidly destroyed by the ubiquitin-proteasome system. We found that adding an oxidant stress (hydrogen peroxide) does not block the phosphorylation of FoxO1, but does prevent a reduction in FoxO1 protein (Brown and Franch, unpublished data). Thus, peroxide inhibits a mechanism that turns off chaperone-mediated autophagy downstream of PI 3 kinase/Akt. If this pathway is similar in muscle cells where FoxO3 regulates the ubiquitin-proteasome system and macroautophagy (24;28), this finding could explain increased capacity for proteolysis under oxidative stress.

An integrated strategy for muscle growth

If the muscles response to feeding is this remodeling and restoring of its proteins by the selective degradation of substituted or misfolded proteins and damaged mitochondria (figure 1), the clinical benefit of adequate protein intake in hemodialysis patients is self-evident. Dietary protein helps muscle restoration by removing oxidized (and likely nitrosylated and glycosylated proteins). Increased protein synthesis replaces damaged protein which has been destroyed. If this hypothesis is correct, feeding refreshes muscle protein and improves muscle function, leading to increased activity. Of course, feeding also benefits by maintaining visceral protein stores. Overfeeding of protein, however, does not provide any additional benefit to muscle because of increased insulin resistance, protein nitrosylation and mitochondrial oxidative stress beyond the muscles capacity to increase synthesis.

The disappointing implication of increased proteolysis with increased feeding is that muscle cannot be built by food alone. Exercise efficiently allows muscle cell hypertrophy by both increasing muscle protein synthesis and decreases muscle protein breakdown (22). It also reduces insulin resistance which improves muscle mitochondrial function to reduce tissue oxidation. The different biology of exercise is beyond the scope of this review, but involves recruitment of satellite cells to increase the number of nuclei in the skeletal muscle fibers, an effect that has not yet been seen by feeding strategies alone. Nevertheless improvements in

muscle function with increased protein turnover may allow increased exercise. Further work with exercise training used in conjunction with feeding strategies may create an integrated approach to improve muscle function and mass even in severe protein energy wasting.

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Figure 1.

A cartoon illustrates important features of the hypothesis concerning selective proteolysis with feeding. A muscle fiber has areas served by healthy mitochondria (L) or mitochondria damaged either by age or by external oxidation (R). The fibers surrounding the damaged mitochondria have become oxidized either by an external source or by reactive oxygen species (ROS) from the mitochondria. Feeding does not injure the healthy mitochondria and stimulates protein synthesis, but injures the damaged mitochondria so that caspase 3 becomes activated. The caspase 3 cleaves the myofibrillary protein into fragments capable of proteasomal proteolysis, while inhibition of insulin signaling and activation of type 3 PI3K increase the capacity of the proteasomal and lysosomal systems. The damaged mitochondria is destroyed by macroautophagy while the myofibrillary fragments are destroyed by the proteasome. At the same time, new synthesis and mitochondrial biogenesis replace the destroyed proteins, repairing the damaged area. Thus, feeding induces a repair process to remove damaged myofibrillary protein and organelles and replace them with new protein.