Studying Muscle Protein Turnover in CKD

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It has been known for many years that inflammation, acidosis, and insulin resistance stimulate the loss of muscle proteins and contribute to CKD-induced morbidity and mortality (1). To understand how CKD and its complications might predispose to loss of lean body mass, it is important to recognize that body protein is in a dynamic state, with a daily turnover of approximately 250–300 g (of which 100–120 g derives from skeletal muscle) in a 60-kg man (2). The efficiency by which amino acids are recycled from protein breakdown into protein synthesis is so high that only 50–80 g protein/d escape from protein turnover and are catabolized, leading to urea formation (2). Given the great rates of protein turnover in the whole body and muscle, even small imbalances between protein synthesis and degradation can lead to substantial protein loss.

The simultaneous measurement of mixed muscle protein synthesis and degradation is possible using primed constant infusions of isotopically labeled amino acids associated with the arteriovenous catheterization technique across the forearm, which is mainly made of muscle (3). By the use of this technique, our understanding on whether changes in protein loss are caused primarily by a change in synthesis or breakdown has greatly improved in the last two decades.

A variety of *in vitro* and *in vivo* studies in rodents have shown that acidosis, insulin resistance, and inflammation cause an increase in protein degradation by activating the ubiquitin-proteasome system, lysosomes, and myostatin (a negative regulator of skeletal muscle growth) in experimental uremia (4,5). Several research groups, including our own group, have used amino acid tracer techniques to examine whether protein turnover in patients with CKD is imbalanced because of changes in protein synthesis or degradation. The results from these studies have shown a remarkable concordance, showing increased muscle protein degradation as a major determinant of wasting (6–9) in CKD. In addition, selective alterations in the synthesis rate of myosin heavy chain, the main contractile protein responsible for the conversion of ATP to mechanical energy, have been observed to occur early in the course of CKD (10).

In a substudy of the Omega-3 Fatty Acid Administration in Dialysis Patients Study, now reported in this issue of the *Clinical Journal of the American Society of Nephrology*, Deger *et al.* (11) tested the hypothesis that fish oil supplementation would improve the chronic uremic inflammation and decrease muscle protein degradation. The trial enrolled 20 participants for the study of muscle protein synthesis and degradation, and tests were performed at the enrollment and after 12 weeks of fish oil supplementation or placebo. The hypothesis in the work by Deger *et al.* (11) was formulated, because patients with CKD have lower levels of ω3-fatty acids in plasma and cells compared with patients without CKD; also, they often have very low consumption of ω3-fatty acids (12). In retrospective studies in patients on hemodialysis, a higher dietary ω6-to-ω3 ratio is associated with both worsening inflammation over time and a trend toward higher death risk (13). There is also is growing evidence that ω3-fatty acids are positively correlated with insulin sensitivity and also, have intrinsic anabolic/anticatabolic properties in skeletal muscle (14), mechanisms that could be explained through the reduction in inflammatory markers by the activation of peroxisome proliferator-activated receptor γ, which suppress NF-κB activity (15,16). In a previous randomized, controlled trial in elderly individuals, it was observed that ω3-fatty acids potentiated the muscle protein synthesis response to simulated feeding after an 8-week supplementation period (17).

In a previous report of the study, Hung *et al.* (18) showed that fish oil supplementation decreased the levels of endothelial chemokines (regulated upon activation, normal T cell expressed and secreted and monocyte chemoattractant protein 1) but had no significant effect on serum inflammatory markers (C-reactive protein, IL-6, and procalcitonin). As a new finding, in the substudy reported here, Deger *et al.* (11) observed that, compared with placebo, ω3-supplementation was significantly associated with decreased muscle protein breakdown at 12 weeks of treatment, which remained significant after multivariate adjustment. This finding *per se* is important, because no reliable method to prevent CKD–induced muscle wasting currently exist; also, inflammation seems to be a new target for preventive and therapeutic interventions. However, ω3-fatty acid supplementation resulted in decreased forearm muscle protein synthesis, whereas the rate in the placebo group increased. Even if there was no longer a statistically significant difference in skeletal muscle protein synthesis after multivariate adjustment, net protein balance (the difference between synthesis and degradation) was not affected by treatment. Overall, the data presented here by Deger *et al.* (11) show that high–dose ω3-supplementation over 12 weeks in patients on hemodialysis with systemic inflammation was associated with attenuation of forearm muscle protein degradation but did not influence skeletal

muscle protein synthesis, skeletal muscle net protein balance, or any component of the whole–body protein balance.

Why did the study fail to show an effect of high–dose ω3-supplementation on muscle protein net balance? This kind of study is difficult, and variability between subjects and changes in forearm blood flow and nutrient intakes may have hindered possible meaningful effects of ω3-supplementation. The placebo group showed higher fat mass, inflammation, and insulin resistance baseline than the treated group. In addition, it is not clear if nutrient intake (which is a major determinant of protein metabolism) was stable in the treatment/placebo periods. Also, the study was small and therefore, underpowered. The imbalance in baseline characteristics between the two groups may have had an effect and probably illustrates the difficulty of study stratification for different variables that can influence protein metabolism. Because of the imbalance in the groups for several characteristics, Deger *et al.* (11) needed to adjust their model for a propensity score that was derived from age, sex, race, baseline high sensitivity C-Reactive Protein, diabetes mellitus, and fat mass.

The data, albeit in pilot nature, can leave us speculating if fish oil can actually modify favorably protein turnover. However, the results in the work by Deger *et al.* (11) are not sufficient to allow extrapolation of the responses into treatment strategies for patients with CKD. Given the observed neutral effects of fish oil supplementation on muscle protein net balance and the methodologic limitations of this study, the hypothesis that fish oil is anabolic could be tested again. In addition, studies of larger sample size and longer duration are required to further evaluate effects of ω3-fatty acids on systemic markers of inflammation, other metabolic parameters, and clinical outcomes, particularly cardiovascular outcomes, in patients with CKD. Even with these considerations, the study by Deger *et al.* (11) seems to still be inconclusive on the effects of ω3-fatty acids on muscle protein metabolism. The tempting results shown by the study are to be considered preliminary with regard to influencing the treatment of patients with CKD.

Disclosures

None.

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