Discussion

Leader: Jeffrey Baxter, PhD, Abbott Nutrition, Columbus, OH

Dr Baxter: I think everyone in this room would agree that skeletal muscle is important for a lot of reasons that have nothing to do with moving around. Since skeletal muscle status plays a role in the pathology, morbidity, and mortality of a number of different diseases, it is a key target for Abbott Nutrition. That is why we sponsored this conference, and why we invited you to be here. Skeletal muscle status is probably amenable to alterations of diet as well as exercise, and so I will open the floor to any questions or comments on this topic.

Dr Supinski: Dr Guttridge, two hypotheses were proposed to account for force loss in muscle in response to activation of proteolytic pathways. The first hypothesis is that caspase and calpain are initially activated and play a key role in dissembling the myofibrillar lattice. The second proposal, from Goldberg's lab, is that E3 components of the proteasomal system (eg, atrogin) enter the myofibrillar lattice and attach to contractile proteins, including myosin, initiating lattice disruption.

Do you think that, if the Goldberg hypothesis is true, it would be consistent with the finding that you do not see much loss of actin in your model? On the other hand, other researchers report that there is actin degradation in a variety of common chronic diseases, including diabetes and uremia. Which of these theories is correct? Is it also possible that these two processes work in combination? How do you fit all of this together?

Dr Guttridge: First, back to your point about actin. I am often confused about actin because I see the 14 kilodalton cleavage, but that does not really explain its total degradation. That could explain a dysfunction of actin. That is why I ask, what about the other sarcomeric proteins? Does actin get degraded later on in either a denervation model or a cancer cachexia model? We do not know. As in Goldberg's model, perhaps you first degrade the thick filament proteins, and then over time, maybe out of default, actin, tropomyosin, and troponin also become substrates and become degraded. But I do not consider that complete actin degradation in those models you suggested. I consider it as a cleavage product that may cause actin dysfunction but does not completely clear actin out of the cell.

I hope that your research group and other groups can continue on with this because I was fascinated by the work of Goldberg's lab. I think it opens up the door to understanding that both play a role. Or perhaps there is a greater role for the calpain system in sepsis, or for the caspase system versus a denervation model.

Dr Supinski: My research team is interested in this. We find that at 2 days after the induction of sepsis, diaphragm muscle force has gone down a great deal. Administration of an inhibitor of calpain or caspase prevents force loss, but we have found that administration of proteasomal inhibitor does not at all prevent reductions in muscle-specific force generation. We have tested virtually every proteasomal inhibitor we could get our hands on, and none of them have prevented loss of specific muscle force generation. One limitation to our measurements is that they are performed early after the

induction of sepsis, at which point calpain and caspase activation may be the major factors influencing contractile-protein-lattice degradation.

It is possible that in more chronic inflammatory states (eg, prolonged infections or other inflammatory stimuli) that selective myosin loss may become progressively a more important issue, and this latter phenomenon may represent a targeted proteasomally mediated selective degradation of myosin.

Dr Guttridge: Right. The reason I brought this up is because of examples from your publication, because I do think we are trying to understand whether there are common themes in different types of catabolic states. It would be nice if these mechanisms overlapped. Then we could find the nodal points, target those, and maybe have an effect on sepsis, cancer, or diabetes. However, I think your paper points out that specificities may exist such that, in a very acute condition, this may be regulated better by the caspase activity, and that in that short window, a role for the proteasome may not exist in a denervation model over 10 to 14 days. The Goldberg study shows that there may be a role for the ubiquitin-proteasome system, or they may be acting in concert in some way.

Dr Supinski: I should mention that we recently have found that caspase does degrade myosin and degrades it fairly well. I do not think anyone else has reported that.

Dr Baracos: I would like to throw out a couple of thoughts. One is that the animal models some of us use could be described as caricatural, or at least very specific

scenarios. One person here prefers to work in cell culture because these models are so complicated. I wonder how we can get from that generalized view to where we might predict the clinical efficacy of a new nutritional formulation. I see us as being a great distance from that.

Another thought is that I have heard about three classes of essential nutrients just now as implicated in muscle wasting. One of them is n-3 polyunsaturated fatty acids, which are required in the dietary supply. Another one is branched-chain amino acids, which are essential nutrients of which leucine is prominently discussed. The last one is antioxidant nutrients. I do not see a convenient way to go forward, because I do not believe I have an answer to the question of which one of these nutrients is likely to be first-limiting. If I am going to start feeding someone n-3 polyunsaturated fatty acids, do I know whether that person has sufficient branched-chain amino acids and antioxidants to permit the anabolism to happen? Is there a credible basis for tossing all three of those things into a cocktail and saying, one is good, two are good, three are good, but all three of them are better.

Dr Tisdale: Without doing the requisite experiments, it is difficult to say, but the n-3 fatty acids only inhibit protein degradation in skeletal muscle and have no effect on protein synthesis. If you want a combination that promotes protein synthesis, you have to have something like a branched-chain amino acid combination. You already have two of your three.

Regarding antioxidants, we found that production of reactive oxygen species (ROS) is part of the signaling cascade leading to activation of nuclear factor kappa B (NF-kB) and, therefore, protein degradation. Eicosapentaenoic acid (EPA) may have the same effect. We may be able to have an antioxidant that is superior to that. We need either two or three in various combinations. But until we do the requisite experiments using the most appropriate animal model for the condition we are studying, then it is impossible to say.

Dr Johnson: Dr Tisdale, you described some nice data on reversal of muscle wasting with EPA and beta-hydroxy-beta-methylbutyrate (HMB). Can you hypothesize what the effect of those nutrients on the model of sarcopenia, disuse, or injury would be?

Dr Tisdale: The catabolic stimuli we have used—tumor necrosis factor (TNF), lipopolysaccharide (LPS), angiotensin II, and proteolysis-inducing factor (PIF)—all seem to go down the same pathway. If you can tell me which of those four stimuli are involved in the catabolic stimulus in those disease models, I can answer your question. At the moment, however, I do not know which, if any, of them are important in the conditions you mentioned. Do you know?

Dr Johnson: I do not know right now, but we will hear more about sarcopenia and some of its etiology later.

Dr Tisdale: The more I work in the film field, the more I think that one central pathway is involved in all catabolic stimuli. Therefore, agents that are effective in one type of

muscle wasting will probably be effective in all of them. At the moment, that is more of a hypothesis than a fact. I can say that we have never done any experiments that showed a signaling pathway that was not inhibited by the same type of inhibitors that we had previously used. That is not to say that we will not find one.

Dr Supinski: A previous report said that the researchers used a proteasome inhibitor in an animal with heart failure, and it improved the animal's condition. We used the same agent in sepsis, and it made things worse.

Dr Tisdale: The problem with proteasome inhibitors is that they tend to be toxic. Therefore, if you are comparing two conditions, you should compare doses and agents, because if you knock out all of the proteasome you are likely to get a very toxic effect.

Dr Supinski: We used a variety of doses. None of our doses were beneficial, and the higher the dose the more toxicity we observed. In contrast, the team that demonstrated a beneficial effect of proteasomal inhibitor used relatively high doses and found they were beneficial.

Ultimately, I think we may come up with a variety of treatments, such as antioxidants, EPA, and other drugs, that we could reasonably give to people when they are first sick to try to stop muscle damage. I wonder, however, whether the same agents will reverse this damage after it has occurred, or whether other agents will be needed to reverse dysfunction. So I wonder whether in the long run, we will have two classes of therapeutic

agents—one that prevents dysfunction, such as EPA in an inflammatory state, and another that restores muscle function.

Dr Tisdale: Clinical data support what you are saying because bortezomib has been tested in patients with cancer cachexia and shown to have no beneficial effect. With regard to regaining muscle mass, we do not know what happens to the anabolic signaling pathways, because those can be switched off mainly by phosphorylation and switched on by dephosphorylation. We do not know in these patients what the state regulation of these pathways is. Until we know that, we do not know whether we can build up muscle mass. But we could consider a process of catabolism to start, followed by defective anabolism. We may need to use agents that can stimulate anabolism in patients, whether they be branched-chain amino acids, exercise, or something else. It would be interesting to have biopsies from these patients to see the state of regulation of the pathways and whether this is a permanent or self-perpetuating change.

Dr Supinski: From what we know, it seems that it is self-perpetuating, but I have no idea what that mechanism could be. We hope that mechanism will be determined soon. I think this is a major issue. I know that ARDSnet, which is a national organization looking at outcomes in patients with acute respiratory distress syndrome (ARDS), has come to the same conclusion. The association is trying to figure out what to do next. ARDSnet thinks mortality from the acute events is reduced fairly well, but the problem is that the long-term morbidity in weak patients is tremendous. We can understand how TNF (tumor

necrosis factor), LPS (lipopolysaccharides), or PIF (proteolysis-inducing factor) acutely injures a muscle, but we do not understand why these people are still weak 1 year later.

Dr Reid: Dr Baracos, I would like to go back to your question about the systems and experimental approaches we use and how we recruit patients. It worries me because we live by the mantra that we do mechanistic research in culture and in vitro, and then we take it to animal models. If all the results are consistent and we have a probe that can go to humans, then we try human trials. It sounds as though that general gestalt is not satisfactory for you. Do you think the animal models are inadequate or that they are inconsistent with what you are seeing in patients?

Dr Baracos: The patients at my center and others are heterogeneous. Their ages range from 40 to 90 years. They have solid tumors and other kinds of malignancies at different sites. They have something that people often ignore—a whole host of comorbid conditions. Their nutritional status is all over the map. This is because of their disease condition or conditions, as well as their lifelong previous dietary patterns and the dietary patterns they may have adopted after cancer diagnosis when they start to eat some unusual things.

I am disappointed by a number of randomized clinical trials of nutritional support and drug therapy directed at cancer cachexia, in which the clinical entity was not clearly defined and which generated results that were negative, inconclusive, or equivocal. These results seem to offer an opportunity for people to conclude that nutrition does not work or

that nutrition does work. I am aware that a great deal of money, time, and energy of researchers and participation of patients goes into these clinical trials. They are onerous to conduct, and I would like to be able to better predict the right therapies to apply.

Dr Reid: In the 1980s, there was a lot of interest in free radical biology and free radicals as mediators of fatigue. People did exactly what you described—conducted well-intended, carefully controlled trials with nutritional antioxidants in a variety of subject populations under a variety of conditions, and it never altered performance. By the early 1990s, researchers had generally concluded that it was an epiphenomenon, a result of fatiguing exercise, not a cause of fatigue. Then the seminal experiment came from Dr Supinski's lab. Instead of using vitamins and nutritional supplements, they used a drug, N-acetyl-cysteine, and they inhibited fatigue dramatically. Then other people were able to reproduce those findings, which opened the door for the field. Sometimes a seminal experiment in the right animal model can be what it takes to move us forward. That is not an answer to your question, but it is a tiny ray of hope from another field.

Dr Phillips: I want to make one comment about the animal model. If you compare animal model data on simple muscle disuse with human data, you find a drastic difference. If you hind-limb suspend a rat for just 12 hours, it will lose about 5% of its muscle mass. Suspended for a couple of days, it will lose 15%. There is no human equivalent of that. We see drastic upregulation of the proteasomal genes in the rat, and yet no evidence of that in humans. We also do not see measured increase in protein

breakdown when we measure it in vivo in humans. So some important differences should be considered in these models of disease when there is a component of disuse.

In answer to one of Dr Tisdale's questions, I will share data later showing that fish oil supplementation actually stimulates protein synthesis in humans. We can see the effects.

Dr Morley: One problem I have when I look at animal scientists and cell scientists—I am basically a human doctor in the end—is that people study only one thing. There is this concept that if you study one thing and you study it often and to excess, it miraculously will solve your problem. If I want a grant, I have to write it that way because that is how we get grants. But if we look at normal physiology in animals or anywhere else, we see homeotic mechanisms in which some doses will produce one effect and other doses will produce a different effect within the system. Dr Reid, you have shown us wonderful data indicating that more or less nitric oxide is bad for you. In muscular dystrophy, we see that if there is no nitric oxide, muscles will fatigue quickly.

Our problem is that we all are looking at different parts of the elephant. Until we put the elephant together and realize that the tail and the trunk are not the same part of that elephant, we will continue not knowing where to go. In the end, we will have to find combination treatment for people who have severe disease, whether it is cachexia or end-stage sarcopenia. This is why I push for diagnosing and treating early.

I will add that we do not have nutritional studies worth anything, to be quite honest, because the numbers of subjects are too small. Scientists, including myself, believe that if

we study 12 or 14 people in each group, the study is pretty good. We expect a nutritional study of 20 or 30 people to be able to show a major effect. Then we do statistics and the results are not significant. Even studies of cholesterol-lowering agents involving thousands of people have found only a small change. Weiskopf's research, for instance, showed no change in mortality at 6 years. I am trying to say to big nutrition companies that it is time to do some reasonable studies of 1000, 2000, or more people using the agents we think will work, because otherwise we are going to keep on doing what Dr Baracos says—rejecting results. I think we have to be more realistic about what we are doing if we want to go forward in this field. The big studies in humans will finally answer questions about where we should be with nutrition.

Dr Bosaeus: We also should think about the role of muscle in this. Could that role be different in different situations? In inflammatory disease, for instance, is muscle the innocent bystander in the "crossfire" between immune systems and other things, or is it the taxpayer that should contribute to the war effort? The role also depends on the person's nutritional status from the beginning or during the course of the disease. I am a bit doubtful that it helps to multiply the trial size before sorting that out.

Dr Refaat [Abbott Nutrition]: Dr Morley, I agree that nutrition research has a way to go, but let me comment on the effect size and the sample size based on the effect size. If we have a homogenous sample and the effect size is tremendous, we do not need a thousand patients, correct? Cholesterol-lowering studies need a lot of people because of heterogeneity of the studied patients and a smaller anticipated effect size.

Dr Reid, I want to ask about TNF. You showed it looks selective in the pathogenesis of cachexia. Do you think biological therapy will prevent or at least ameliorate the anti-TNF?

Dr Reid: Our philosophical approach to the problem is to not inhibit TNF per se. Arthritis patients are already getting soluble TNF receptors or monoclonal antibodies to TNF as a therapy. It is possible to give TNF both to animals and humans to try to block cachexia. To my knowledge, that has not been very successful because TNF is part of an integrated inflammatory response that may be important, as Dr Bosaeus points out, for other processes going on elsewhere in the body. We do not want to compromise the inflammatory response. For that reason, we may not want to give antioxidants either. However, if we can identify a receptor-mediated response causing the cachexia and then a muscle-specific target in the cell to inhibit, we may be able to preserve the muscle and allow the inflammation to proceed for the greater benefit of the body.

Dr Baxter: I think we all agree on one thing: This is an extremely complicated set of conditions. We have all kinds of stressors that can affect lean muscle mass and functionality, and once the functionality or mass is gone, it is difficult to replace it. Nutrition can play a dramatic role, not only in prevention, but also in the restoration of not only muscle mass but also muscle functionality. However, if a cachectic, fatigued patient is cured of cancer, he or she could stay in that state for years.

Dr Tisdale: If you cure the cancer, the muscle mass comes back.

Dr Baxter: If we do the nutritional interventions necessary to replete the energetic systems and make sure that the patient has plenty of protein to rebuild muscle and so on, the patient may not stay in that state. For whatever reason, however, the nutritional follow-up may not take place.