Effects of Nutrition on Muscle Metabolism

Michael Tisdale, PhD, Aston University, Birmingham, UK

Atrophy of skeletal muscle is common in a number of conditions including cancer sepsis, metabolic acidosis, weightlessness, immobility, diabetes, and AIDS, and can lead to weakness (asthenia) and death through respiratory failure. In cancer patients, an inverse relationship exists between weight loss and survival time. Various nutrients have the ability to attenuate this condition by increasing protein synthesis and/or decreasing protein degradation in skeletal muscle.

Eicosapentaenoic Acid (EPA)

EPA is a 20-carbon (n-3) polyunsaturated fatty acid found in oily fish. EPA was first recognized as a potential treatment for cachexia because of its ability to attenuate weight loss in mice bearing the MAC16 tumor. EPA preserves muscle mass by reducing the increased protein degradation seen in the skeletal muscle of cachectic mice, but it has no effect on the depression of protein synthesis. The increased protein degradation seen in skeletal muscle of both mice and humans with cancer cachexia is due to an increased expression and activity of the ubiquitin proteasome pathway. In this process, myofibrillar proteins, such as myosin, are marked for degradation by proteolytic enzymes in the 20S proteasome by the attachment of a polyubiquitin chain (Fig 1).[1]

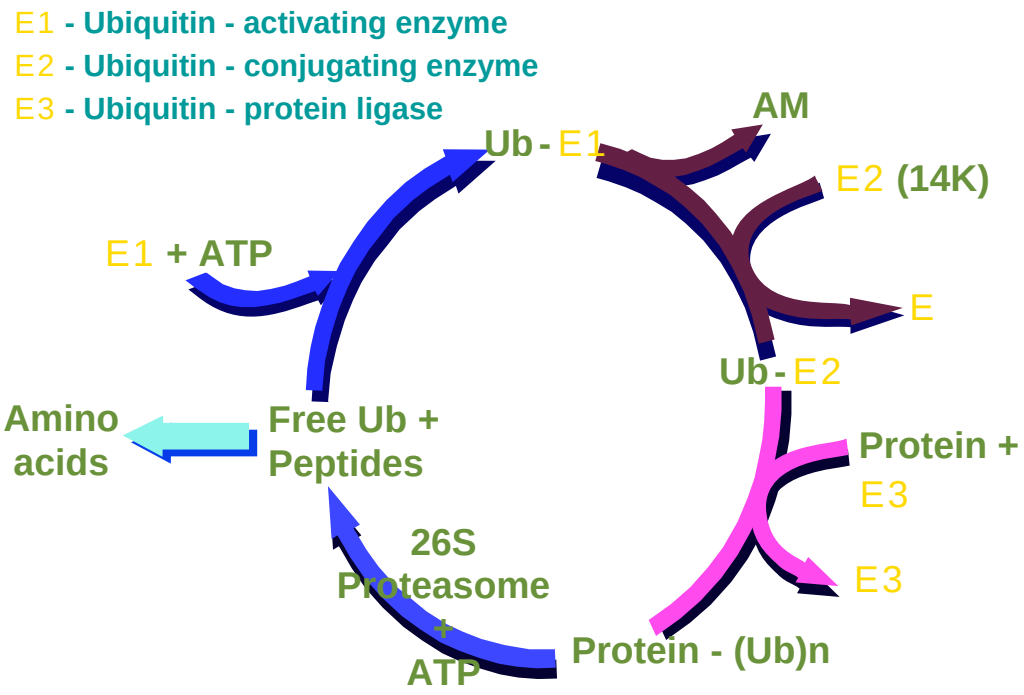

Fig 1. Ubiquitin-proteasome pathway for breakdown of intracellular proteins in skeletal muscle.[1]

From "Loss of skeletal muscle in cancer: Biochemical mechanisms" by Tisdale et al. *Frontiers in Bioscience* 2001;6:164. © 2001 by Frontiers in Biocscience Publications. Reprinted with permission.

In addition to the proteasome, two ubiquitin ligases (E3), muscle-specific RING finger-1 (MuRF-1) and muscle-specific F-box protein (MAFbx/atrogin-1) are important, both in recognition of the target protein and in the transfer of ubiquitin from the ubiquitin-conjugating enzyme (E2). EPA attenuates the enhanced protein degradation seen in skeletal muscle of cachectic mice by decreasing expression of the 20S proteasome and other key components of the pathway down to levels found in noncachectic animals.[2] Thus, EPA is not a proteasome inhibitor but normalizes expression down to basal levels. This is significant because the proteasome has an important function in cellular

homeostasis, degrading mutated, misfolded, or oxidized proteins. Proteasome inhibition, therefore, can lead to toxicity, but this is not seen with EPA.

EPA reduces expression of the ubiquitin-proteasome pathway by interfering with the signaling pathway involved in its upregulation. EPA works by blocking the action of a tumor catabolic factor, proteolysis-inducing factor (PIF), which acts specifically on skeletal muscle to depress protein synthesis and increase degradation. EPA blocks the action of PIF-induced phospholipase A_2, which causes the release of arachidonic acid from membrane phospholipids, and its conversion to prostaglandins and hydroxy-eicosatetraenoic acids (HETE). Only one of these metabolites, 15-HETE, is found to be directly catabolic on muscle by activating nuclear factor-kappaB (NF-κB), which increases expression of both the 20S proteasome and MuRF-1.[3] NF-κB is held as an inactive complex in the cytosol with an inhibitory protein, IκB. On activation of an upstream kinase (IKK) by PIF, possibly through protein kinase C (PKC) or reactive oxygen species (ROS), IκB is phosphorylated and degraded, releasing free NF-κB, which migrates into the nucleus and causes increased gene transcription by binding to its specific sites on DNA (Fig 2). This effect is not seen in the presence of EPA, and NF-κB remains as an inactive complex in the cytosol with IκB.

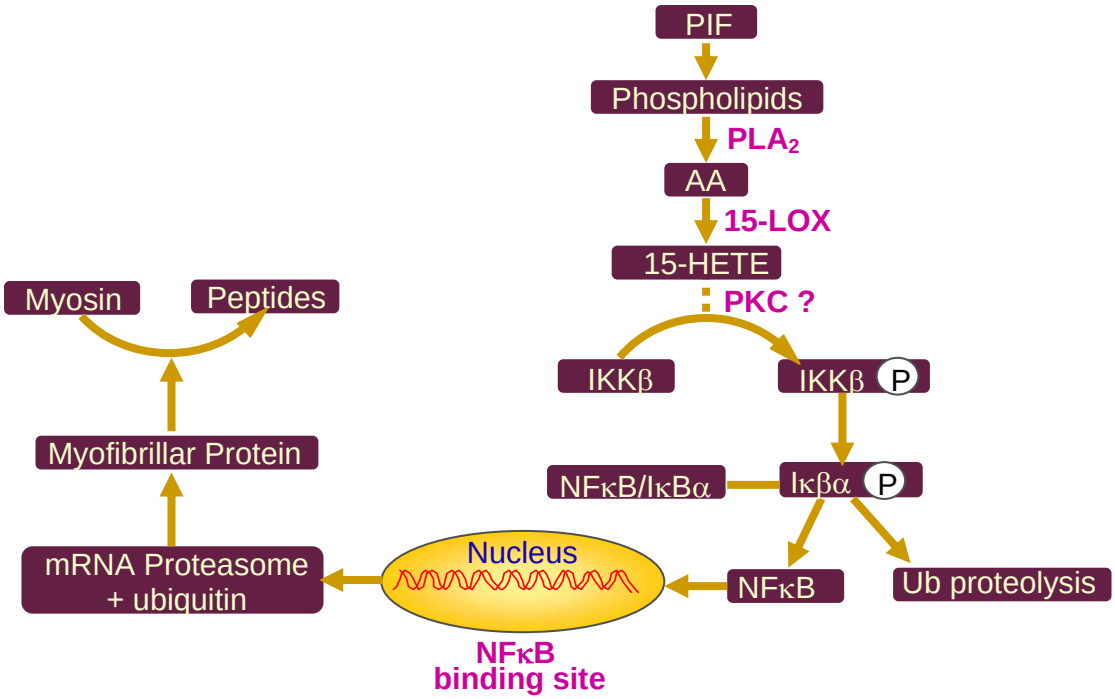

Fig 2. Potential intracellular events in skeletal muscle involved in PIF-induced proteasome activation. PIF=proteolysis-inducing factor, AA=arachidonic acid, PLA_2= phospholipase A2, 15-LOX= 15-lipoxygenase, HETE= hydroxy-eicosatetraenoic acids, PKC= protein kinase C, IKK(beta)=IkB kinase, NF-κB=nuclear factor kappa B, Ub= ubiquitin

Amino Acids

Certain amino acids, namely the branched chain amino acids (BCAA) leucine, isoleucine, and valine are not only substrates for protein synthesis, but also stimulate the process and reduce protein degradation. For this reason, EPA has been combined with a nutritional supplement enriched with both calories and protein that helps prevent muscle atrophy in cachexia, not only by depressing protein degradation, but also by increasing protein synthesis.[4]

Protein synthesis is mainly regulated at the initiation and elongation steps of translation. There are two control points in initiation:

(i) Binding of methionyl tRNA to the 40S ribosomal subunit. This process is regulated by eukaryotic initiation factor 2 (eIF2) and inhibited when eIF2 is phosphorylated on the α-subunit.

(ii) Binding of mRNA to the 43S subunit. This process is stimulated by activation of the mammalian target of rapamycin (mTOR), which phosphorylates the eIF4E binding protein (4EBP1) allowing dissociation of eIF4E, which can then complex with eIF4G to form the active eIF4F complex, allowing binding of the 5'-cap of mRNA.

Leucine and valine, when administered to mice bearing the cachexia-inducing MAC16 tumor, attenuate loss of body weight and increase skeletal muscle mass.[5] Leucine and valine also produce a significant increase in protein synthesis, whereas only leucine produces a decrease in protein degradation in skeletal muscle.[5] Growth of the MAC16 tumor is associated with a significant increase in phosphorylation of eIF2α in gastrocnemius muscle, which reduces protein synthesis. Activation (phosphorylation) of the eIF2α kinase, the dsRNA-dependent protein kinase, PKR, also increases. Treatment with leucine attenuates the increased phosphorylation of both PKR and eIF2α to levels found in nontumor-bearing animals, without affecting total levels.[5] The decreased phosphorylation of PKR is probably due to a 2.5-fold increase in protein phosphatase 1 (PP1). Leucine treatment also increases expression of phospho mTOR, causes hyperphosphorylation of 4E-BP1, allowing the eIF4E to associate with eIF4G to form the active eIF4F complex, which stimulates translation initiation and thus global protein

synthesis.[5] Levels of phosphorylation of the eukaryotic elongation factor (eEF2) also increase in skeletal muscle of mice bearing the MAC16 tumor, which results in an inhibition of elongation by decreasing its affinity for the ribosome 10-100 times.[5]

The ability of leucine to attenuate phosphorylation of PKR also explains its ability to reduce protein degradation, since PKR also induces protein degradation through activation of NF-κB by activation of IKK and the subsequent phosphorylation and degradation of I-κB (Fig 3).[6] Increased phosphorylation of both PKR and eIF2α is seen not only in muscles of mice bearing the MAC16 tumor, but also in cancer patients, increasing with increasing weight loss.

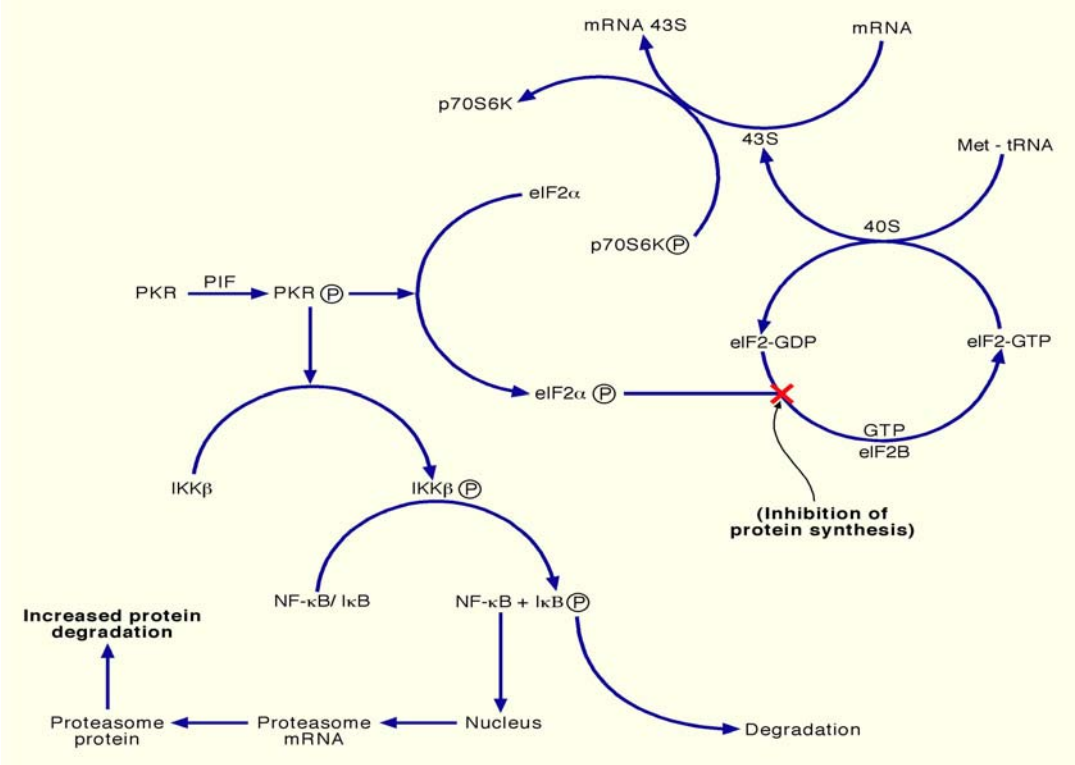

Fig 3. Central role of PKR in the control of protein synthesis and degradation in skeletal muscle.[6] PKR=protein kinase R, PIF=proteolysis-inducing factor, IKKβ=IkB

kinase, NF-κB=nuclear factor kappa B, eIF2=eukaryotic initiation factor 2, GTP=guanosine-5'-triphosphate

β-Hydroxy-β-Methylbutyrate (HMB)

HMB is a metabolite of leucine formed by transamination to α-ketoisocaproate (KIC) in the muscle followed by oxidation of KIC to HMB in the cytosol of the liver (Fig 4).

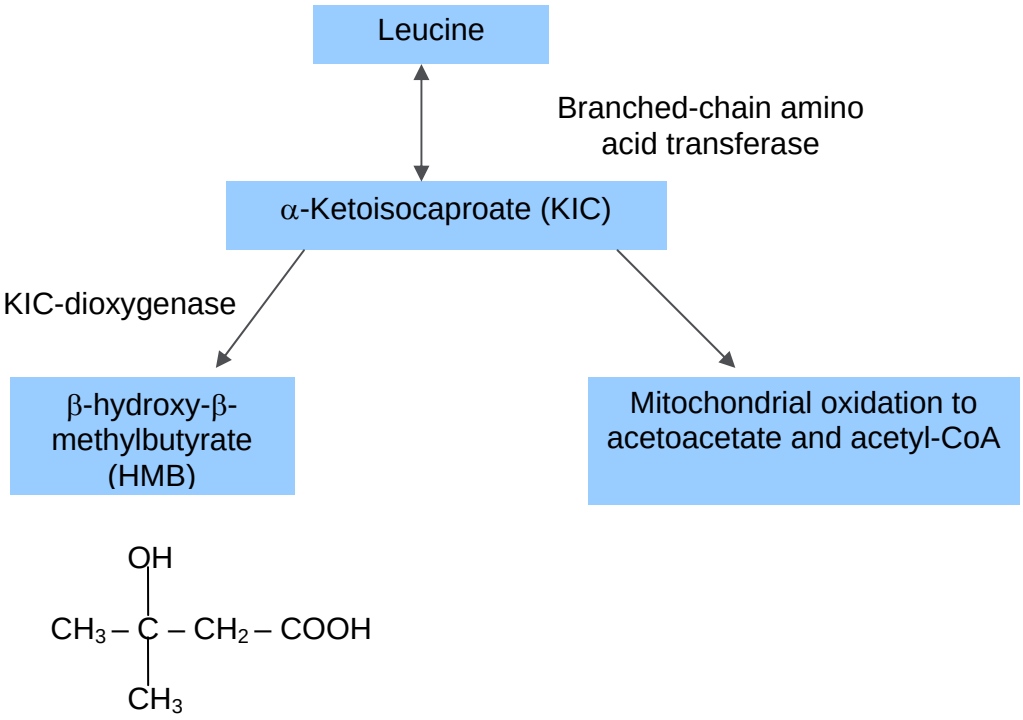

Fig 4. Formation of HMB.

The effect of HMB on muscle metabolism closely resembles that of leucine. Thus, HMB at doses >0.125 g/kg in mice bearing the MAC16 tumor causes a significant reduction in weight loss caused by increased lean body mass without an effect on adipose mass.[7] The increase in muscle mass is caused by both a depression in protein degradation and a significant increase in protein synthesis. As with leucine, HMB attenuates the increased phosphorylation of both PKR and eIF2α in skeletal muscle, supporting its ability to

suppress protein degradation, and at the same time increasing protein synthesis. In vitro studies using murine myotubes show that HMB attenuates both the depression of protein synthesis and an increase in protein degradation in response to a number of catabolic stimuli including PIF, angiotensin II (ang II), tumor necrosis factor-α (TNF-α), and lipopolysaccharide (LPS), essentially by the same mechanism.[8-12] As observed in vivo, all stimuli increase phosphorylation of both PKR and eIF2α, which is completely attenuated by HMB.[8-12] This process is shown to be responsible for the depression of protein synthesis using myotubes transfected with a catalytically inactive variant of PKR called PKRΔ6, which lacks 6 amino acids (361-366) between catalytic domains IV and V. Thus, while LPS and TNF-α depresses protein synthesis on myotubes transfected with empty plasmid (pcDNA) and wild-type PKR, there is no effect in myotubes transfected with PKRΔ6. Protein degradation by all catabolic stimuli also is attenuated by HMB. This is shown to involve the caspase-3 and caspase-8 activation of PKR and the subsequent formation of ROS, which leads to activation of NF-κB (Fig 5).[11]

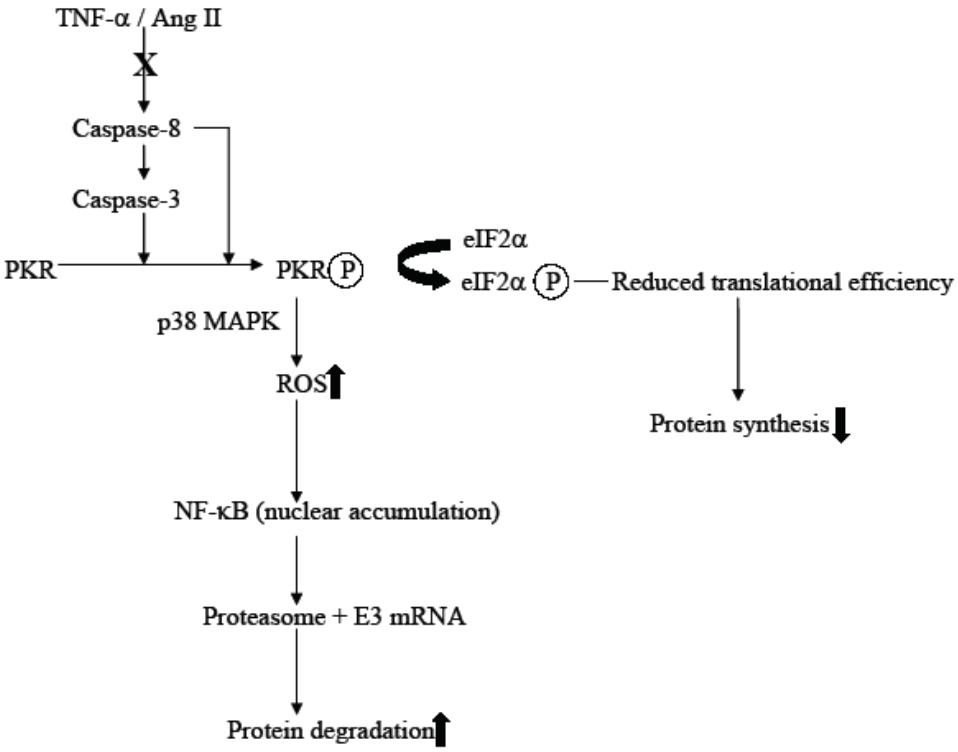

Fig 5. Signaling cascade initiated by LPS/TNF-α/ang II and the effect of HMB on this process.[11] TNF=tumor necrosis factor, ang II, angiotensin II, PKR=protein kinase R, eIF2=eukaryotic initiation factor 2, MAPK=mitogen-activated protein kinase, ROS= reactive oxygen species, NF-κB=nuclear factor kappa B, LPS= lipopolysaccharide.

From "Mechanism of attenuation of muscle protein degradation by tumour necosis factor-a and angiotensin II by β-hydroxy-β-methylbutyrate" by Eley et al. *American Journal of Physiology – Endocrinology and Metabolism* 2008;295(6): E1417. © 2008 by American Physiological Society. Reprinted with permission.

Thus, all stimuli increase activity of both caspase-3 and caspase-8, and this is attenuated by HMB. This attenuation is important in protein degradation because both the caspase-3 and caspase-8 inhibitors attenuate protein degradation and autophosphorylation of PKR. Activation of PKR is also important in protein degradation, because, as with protein synthesis, it is not seen in myotubes transfected with PKRΔ6. Thus, HMB blocks the depression of protein synthesis and increased protein degradation induced by catabolic stimuli by inhibiting an upstream signaling pathway (activation of caspase-3/-8) leading

to activation of PKR. The similarity in the signaling pathway employed by a range of catabolic stimuli suggests that EPA, BCAAs, and HMB may be effective in the treatment of other catabolic disorders in addition to cancer cachexia.

References

1. Tisdale MJ: Loss of skeletal muscle in cancer: Biochemical mechanisms. *Front Biosci* 2001;6:164-174.
2. Whitehouse AS, Smith HJ, Drake JL, Tisdale MJ: Mechanism of attenuation of skeletal muscle protein catabolism in cancer cachexia by eicosapentaenoic acid. *Cancer Res* 2001;61:3604-3609.
3. Cai D, Frantz JD, Tawa NE Jr, et al: IKKβ/ NF-κB activation causes severe muscle wasting in mice. *Cell* 2004;119:285-298.
4. Smith HJ, Greenberg NA, Tisdale MJ: Effect of eicosapentaenoic acid, protein and amino acids on protein synthesis and degradation in skeletal muscle of cachectic mice. *Br J Cancer* 2004;91:408-412.
5. Eley HL, Russell ST, Tisdale MJ: Effect of branched-chain amino acids on muscle atrophy in cancer cachexia. *Biochem J* 2007;407:113-120.
6. Eley HL, Skipworth RJE, Deans DAC, et al: Increased expression of phosphorylated forms of RNA-dependent protein kinase and eukaryotic initiation factor 2alpha may signal skeletal muscle atrophy in weight-losing cancer patients. *Br J Cancer* 2008;98:443-449.
7. Smith HJ, Mukherji P, Tisdale MJ: Attenuation of proteasome-induced proteolysis in skeletal muscle by β-hydroxy-β-methyl-butyrate in cancer-induced muscle loss. *Cancer Res* 2005;65:277-283.
8. Eley HL, Russell ST, Baxter JH, et al: Signaling pathways initiated by β-hydroxy-β–methylbutyrate to attenuate the depression of protein synthesis in skeletal muscle in response to cachectic stimuli. *Am J Physiol Endocrinol Metab* 2007;293:E923-E931.

9. Smith HJ, Wyke SM, Tisdale MJ: Mechanism of the attenuation of proteolysis-inducing factor stimulated protein degradation in muscle by β-hydroxy-β-methylbutyrate. *Cancer Res* 2004;64:8731-8735.
10. Eley HL, Russell ST, Tisdale MJ: Attenuation of depression of muscle protein synthesis induced by lipopolysaccharide, tumor necrosis factor, and angiotensin II by β-hydroxy-β-methylbutyrate of muscle protein degradation induced by lipopolysaccharide. *Am J Physiol Endocrinol Metab* 2008;295:E1409-E1416.
11. Eley HL, Russell ST, Tisdale MJ: Mechanism of attenuation of muscle protein degradation by tumour necrosis factor-a and angiotensin II by β-hydroxy-β-methylbutyrate. *Am J Physiol Endocrinol Metab* 2008;295:E1417-E1426.
12. Russell ST, Tisdale MJ: Mechanism of attenuation by β-hydroxy-β-methylbutyrate of muscle protein degradation induced by lipopolysaccharide. *Mol Cell Biochem* 2009;330:171-179.
13. Barber MD, Ross JA, Voss AC, et al: The effect of an oral nutritional supplement enriched with fish oil on weight loss in patients with pancreatic cancer. *Br J Cancer* 1991;81:80-86.
14. Fearon KCH, von Meyenfeldt MF, Moses AGW, et al: Effect of a protein and energy dense n-3 fatty acid enriched oral supplement on loss of weight and lean tissue in cancer cachexia: A randomized double-blind trial. *Gut* 2003;52:1479-1486.
15. Tisdale MJ, Beck SA: Inhibition of tumour-induced lipolysis in vitro and cachexia and tumour growth in vivo by eicosapentaenoic acid. *Biochem Pharmacol* 1991;41: 103-107.
16. Wigmore SJ, Barber MD, Ross JA, et al: Effect of oral eicosapentaenoic acid on weight loss in patients with pancreatic cancer. *Nutr Cancer* 2000;36:177-184.

Q&A

Q: You gave a good presentation using the mouse model. Have you used EPA in human cancer patients and if so, have you shown that protein degradation decreases?

Dr Tisdale: We have done several studies in collaboration with Abbott Nutrition in patients with cancer cachexia [Barber MD et al: *Br J Cancer* 1991;81:80-86; Fearon KCH et al: *Gut* 2003;52:1479-1486]. We know that lean body mass is directly related to the amount of EPA in the serum of the patient. There is a linear relationship suggesting that EPA increases lean body mass [Fearon KCH et al: *Gut* 2003;52:1479-1486]. However, we did not measure protein synthesis and degradation in cancer patients, mainly because this was a large multicenter study. It also was difficult to do those measurements on patients who do not have long to live. However, it would be good to use these agents for such a study in the future.

Q: You probably are aware that a major class of chemotherapy drugs targets mammalian target of rapamycin (mTOR). Sorafenib is the model compound. I am interested in the obvious prediction that this class of drug would cause muscle wasting because it has a powerful catabolic effect on muscle. I am writing up a randomized placebo-controlled study of nutritional intervention in patients with renal cell carcinoma who are on sorafenib. If mTOR is important, these patients are not going to respond to any intervention. They have a fundamental block in their ability to utilize those nutrients and to direct amino acids toward protein synthesis. I think this is a critical point: Do we not need to know whether there are instances in which these stimuli cannot produce muscle anabolism?

Dr Tisdale: I think you are correct. If you are treating a patient with cancer who is losing muscle but increasing tumor, then the two processes are diametrically opposite. Therefore, if you have an agent that prevents the tumor from growing, you might have an agent that also stimulates muscle loss because of a hypotrophy pathway. Treatment in that condition depends on several factors. Leucine, for instance, has an effect on PKR

phosphorylation, which would be part of the process. But we do not know the relative weightings of mTOR and PKR. If that pathway is still operative, it may be possible to treat these patients even when they are being treated with a drug that inhibits mTOR. I cannot guess whether it will work, but it might be worth looking into.

Q: Going back to the EPA question, do you think there is a specific effect on muscle mass from the EPA, or would you have a similar effect if you gave docosahexaenoic acid (DHA) instead? Do you think that the replacement of arachidonic acid produces that effect, or is it just an effect from EPA or omega-3 fatty acids?

Dr Tisdale: In our mouse studies [Tisdale MJ, Beck SA: *Biochem Pharmacol* 1991;41:103-107], we initially found that only EPA had that effect. DHA did not. Although there has not been a clinical study with DHA alone, a study with EPA alone showed it had the same anticachectic effect as fish oil [Wigmore SJ et al: *Nutr Cancer* 2000;36:177-184]. So either DHA did not have an effect on patients, or it did not have any more effect than you might expect from the EPA alone.

The effect of EPA is quite complex, and like any signaling pathway, there is crossover. I tried to make the story as simple as possible by saying that EPA inhibits formation of 15-HETE. However, it also inhibits the 15-HETE-induced activation of NF-kB, so it seems to act separately on the IKK cascade as well. So it probably has more than one point within the signaling pathway in which it can act. The signaling pathway is also a lot more complicated than the process I first described, so it is possible to connect by multiple

mechanisms, ie, between the NF-κB and FoxO pathways. This may explain the efficacy of EPA. If the effect was just the result of replacement of arachidonic acid, DHA would be equally effective, but in our studies it was not.