THE RENIN-ANGIOTENSIN SYSTEM AND THE BIOLOGY OF SKELETAL MUSCLE: MECHANISMS OF MUSCLE WASTING IN CHRONIC DISEASE STATES

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ABSTRACT

Sarcopenia and cachexia are muscle-wasting syndromes associated with aging and with many chronic diseases such as congestive heart failure, diabetes, cancer, chronic obstructive pulmonary disease, and renal failure. While mechanisms are complex, these conditions are often accompanied by elevated angiotensin II (Ang II). We found that Ang II infusion in rodents leads to skeletal muscle wasting via alterations in insulin-like growth factor-1 signaling, increased apoptosis, enhanced muscle protein breakdown via the ubiquitin-proteasome system, and decreased appetite resulting from downregulation of hypothalamic orexigenic neuropeptides orexin and neuropeptide Y. Furthermore, Ang II inhibits skeletal muscle stem cell proliferation, leading to lowered muscle regenerative capacity. Distinct stem cell Ang II receptor subtypes are critical for regulation of muscle regeneration. In ischemic mouse congestive heart failure model skeletal muscle wasting and attenuated muscle regeneration are Ang II dependent. These data suggest that the renin-angiotensin system plays a critical role in mechanisms underlying cachexia in chronic disease states.

INTRODUCTION

Cachexia is a severe medical complication of many chronic disease conditions and considered to be a significant cause of morbidity and mortality affecting more than 5 million people in the United States (1). Compared to non-cachectic patients, the median duration of hospital stay for cachectic patients is twice as long (3 days vs. 6 days), and the median cost is ~70% higher ($6,000 vs. $10,000) (2). Although cachectic patients experience greater loss of function than those admitted with other diagnoses, cachexia is not always well recognized and

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adequately managed by healthcare professionals (3). Weight loss and reduced muscle mass in patients with cachexia are associated with a reduction in quality of life and increased mortality. However, cachexia is a complex multifactorial syndrome, and current therapies are limited to the treatment of underlying illness. Thus, the development of interventions to block or attenuate this process would have significant therapeutic benefits in a wide array of chronic diseases. During the past 2 decades, we have been studying the effect of the renin-angiotensin system (RAS), a hormone system that regulates blood pressure and fluid balance, on cachexia development in chronic disease conditions. These finding are discussed in this article.

REGULATION OF MUSCLE MASS BY ANGIOTENSIN II AND IGF-1

In an attempt to study the effect of angiotensin II (Ang II) on the circulating insulin-like growth factor-1 (IGF-1) system in vivo, we infused rats with Ang II through osmotic minipumps for up to 2 weeks (4). In addition to increasing systolic blood pressure, Ang II decreased systemic IGF-1 levels by 56% at 1 week and 41% at 2 weeks. Interestingly, we found that these animals lost weight (18% reduction at 1 week and sustained up to 2 weeks) and daily food intake (22.7 ± 0.7 g in sham vs. 5.0 ± 0.6 g in Ang II). Although the Ang II type 1 receptor (AT1R) antagonist losartan and the vasodilator hydralazine had comparable effects to blunt the Ang II-induced hypertension, only losartan blocked the changes in circulating IGF-1 and body weight, indicating that Ang II produces weight loss through a pressor-independent mechanism. To determine whether Ang II–induced reduction in body weight was secondary to reduced food intake, we performed pair-feeding experiments, in which sham-operated animals' food intake was limited to the exact amount eaten by the Ang II–infused animals. While pair-fed animals lost ~ 18% body weight compared to ad-lib fed controls, Ang II infused animals lost significantly more weight (25%), indicating that Ang II-induced weight loss is attributable to both a reduction in food intake and a catabolic effect. In addition to a systemic decrease of IGF-1, we found that Ang II infusion caused significant reduction in skeletal muscle IGF-1, IGF-1 binding protein 3 and IGF-1 binding protein 5 levels. IGF-1 signaling is the main anabolic pathway in skeletal muscle (5,6). Ang II caused an increase of muscle protein breakdown via the ubiquitin-proteasome system (UPS). Ang II also activated caspase-3 in skeletal muscle, leading to cleavage of actin, an important component of muscle

proteolysis, and to increased apoptosis (7). We found that restoration of circulating IGF-1 levels by infusion failed to restore muscle mass in Ang II–infused animals, suggesting that a reduction in autocrine IGF-1 signaling in skeletal muscle is responsible for Ang II–induced muscle wasting. To study the skeletal muscle IGF-1 signaling pathways involved in Ang II–induced muscle wasting, we utilized myosin light chain promoter-driven skeletal muscle IGF-1 (MLC/mIgf-1) mice, a transgenic mouse strain in which IGF-1 is overexpressed under the control of a skeletal muscle specific promoter (7). In MLC/mIgf-1 mice, Ang II–induced muscle weight loss was completely prevented compared to pair-fed wild-type controls. In MLC/mIgf-1 mouse muscle, the reduction in IGF-1 signaling induced by Ang II, as evidenced by reduced levels of phospho-Akt, phospho-mTOR (mechanistic target of rapamycin) and phospho-p70S6K (p70 S6 kinase), was restored. Consequently, activation of UPS, caspase-3–mediated actin cleavage and apoptosis were prevented in MLC/mIgf-1 mice (7,8). In summary, Ang II and IGF-1 have opposing roles in regulating muscle protein synthesis and degradation. Disruption of IGF-1 signaling by Ang II plays a critical role in Ang II–induced atrophy, and local activation of IGF-1 signaling can prevent Ang II–induced muscle wasting.

There have been conflicting reports regarding the actions of Ang II on skeletal muscle that potentially lead to wasting. Sanders et al. and Russell et al. showed that Ang II acts directly on cultured muscle cells and induces proteolysis via the UPS pathway (9,10). On the other hand, we have demonstrated that multiple circulating hormones and cytokines mediate Ang II's action on skeletal muscle. Glucocorticoids are required for activation of the UPS in acidosis and diabetes, and glucocorticoid inhibition significantly restored Ang II–induced loss of muscle mass (7). Furthermore, we found that there is an increase of circulating interleukin 6 (IL-6) and serum amyloid A (SAA) after Ang II infusion. IL-6 and SAA coordinately act on muscle cells to cause wasting, and blockade of IL-6/SAA prevented Ang II–induced wasting in vivo (11) These data indicate that IL-6/SAA act as critical intermediates of Ang II–induced muscle wasting.

ANG II CAUSES MUSCLE WASTING THROUGH GENERATION OF REACTIVE OXYGEN SPECIES

Reactive oxygen species (ROS) play an important role in Ang II–induced signaling in different cell types, contributing to cardiac myocyte and vascular smooth muscle cell hypertrophy, endothelial dysfunction,

hypertension, and insulin resistance (12–14). Since nicotinamide adenine dinucleotide phosphate (NAPDH) oxidase and mitochondria are major sources of ROS in atrophied skeletal muscles (15,16), we blocked NADPH and mitochondria-derived ROS generation after Ang II infusion to study the involvement of ROS signaling in Ang II–induced muscle wasting. We infused $p47^{phox-/-}$ mice, in which the NADPH oxidase subunit $p47^{phox}$ gene is deleted, with Ang II and analyzed skeletal muscle wasting (17). Superoxide production was increased 2.4-fold in the wild-type skeletal muscle after 7 days of Ang II infusion, and this increase was prevented in $p47^{phox-/-}$ mice. Furthermore, the Ang II–induced decrease in body weight and muscle mass was significantly attenuated in $p47^{phox-/-}$ mice. The UPS is an important protein quality-control mechanism, and the proteasome is responsible for selective degradation of oxidized proteins. Ang II infusion caused an increase in 20S proteasome activity (32% increase compared to pair-fed controls), and this effect was completely inhibited in $p47^{phox-/-}$ mice. Although Ang II infusion did not alter mitochondrial content in skeletal muscle, mitochondrial cytochrome C oxidase activity was decreased by 47% after Ang II infusion (18). Ang II also increased mitochondrial-derived superoxide, consistent with Ang II–induced mitochondrial dysfunction. However, blockade of mitochondrial-derived superoxide by MitoTEMPO (Sigma-Aldrich, St. Louis, Missouri, USA), a specific scavenger of mitochondrial superoxide, did not prevent Ang II–induced muscle wasting. These data show that ROS derived from NADPH oxidase, but not from mitochondria, play a critical role downstream of Ang II to cause muscle wasting. It is suggested that specific targeting of ROS and NADPH oxidase could be a beneficial, novel therapy to treat Ang II–induced wasting.

ANG II, MUSCLE METABOLISM, ENERGY STORES, AND MUSCLE WASTING

Muscle contractions are fueled by adenosine triphosphate (ATP), and three sources supply the muscle's ATP pool: 1) creatine phosphate, 2) glycogen, and 3) mitochondrial respiration. This ATP pool needs to be quickly replenished for full muscle contraction and sustained exercise. We hypothesized that Ang II alters muscle metabolism and energy stores, leading to muscle wasting. After 4 days of Ang II infusion, skeletal muscle ATP content was reduced by 74% in a food intake-independent manner (18). When the cellular energy status is low (high adenosine monophosphate [AMP]:ATP ratio), AMP-activated protein kinase (AMPK) is activated. AMPK is a metabolic master switch and

regulates several intracellular systems including the cellular uptake of glucose, the β-oxidation of fatty acids, and the biogenesis of glucose transporter 4 and mitochondria. However, in Ang II–infused muscle, AMPK activity is suppressed despite the low ATP level. Importantly, the AMPK activator 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) reversed Ang II–induced inhibition of AMPK, leading to restoration of ATP levels and inhibition of the Ang II–induced muscle wasting. These data indicate that Ang II causes muscle wasting in part by preventing skeletal muscle homeostatic capacity to maintain energy balance. However, precise mechanisms whereby Ang II reduces AMPK activity and causes muscle wasting remain to be determined. For instance, our data showed that AICAR blocked Ang II–induced upregulation of E3 ubiquitin ligases atrogin-1 and MuRF-1, well characterized markers of muscle atrophy. On the other hand, it has been shown that S6 kinase-1–deficient mice showed skeletal muscle atrophy with increased AMP levels and AMPK activity (19). In these mice, AMPK inhibition restored muscle cell growth and sensitivity to nutrient signals. In another report, AMPK-mediated phosphorylation of FoxO activated E3 ubiquitin ligase expression in muscle cell culture in vitro (20). Future studies are required to understand the mechanisms whereby AMPK activation by AICAR prevented the increase in E3 ubiquitin ligases in Ang II–infused animals. However, it is of note that our data showed that AICAR treatment resulted in activation of Akt and inhibitory phosphorylation of FoxO1, which is the upstream signaling pathway that inhibits atrogin-1. One of the most important findings from these studies is that Ang II increased expression of PPC2α, an upstream phosphatase that inactivates AMPK. We hypothesized that PP2Cα (Protein phosphatase 2C alpha) is the key regulator of AMPK signaling in Ang II–induced muscle wasting. For this purpose, we knocked down PP2Cα in skeletal muscle via electroporation-mediated small interfering RNA delivery in skeletal muscle in vivo (21). Consistent with our hypothesis, PP2Cα knockdown restored AMPK activity and blocked Ang II–induced muscle wasting. We also found that Ang II infusion impaired muscle mitochondrial biogenesis [reduced PGC-1α (Peroxisome proliferator-activated receptor gamma, coactivator 1 alpha) and TFAM (Mitochondrial transcription factor A)] and mitophagy [reduced ULK1 (Unc-51 Like Autophagy Activating Kinase 1)], leading to mitochondrial dysfunction. Knockdown of PP2Cα normalized PGC-1α, TFAM and ULK1 expression and ATP levels, suggesting the restoration of mitochondrial function. Although the precise mechanism whereby Ang II inhibits AMPK via upregulation of PP2Cα remains to be elucidated, these data suggest

a therapeutic potential of targeting PP2Cα in chronic wasting conditions with increased Ang II levels.

ANOREXIA CAUSED BY ANG II

Anorexia is frequently associated with wasting, and anorexia and loss of body fat is a powerful predictor of mortality in cancer cachexia patients (22). As mentioned above, a major part of Ang II–induced wasting is attributable to reduced food intake (4). Since food intake is regulated by actions of hypothalamic orexigenic/anorexigenic neuropeptides and circulating factors secreted from peripheral organs (e.g., adipose tissues and gastrointestinal tract), we hypothesized that Ang II alters these neuropeptides and/or circulating factors, leading to loss of appetite. We found that Ang II infusion rapidly reduced food intake, an effect that was measurable within 6 hours. At this time point, hypothalamic neuropeptide Y (Npy) and orexin expression were reduced, whereas peripheral leptin, ghrelin, adiponectin, glucagon-like peptide, peptide YY, or cholecystokinin levels were not altered (23). After prolonged infusion (4 days), fat mass was significantly reduced due to reduced food intake, and this was associated with reduced leptin levels and increased Npy and orexin. These changes at 4 days are secondary to reduced food intake initiated by Ang II within 6 hours of infusion. This rapid anorexigenic effect of Ang II led us to hypothesize that Ang II acts directly on hypothalamic neurons to reduce orexigenic neuropeptide expression. Indeed, intracerebroventricular infusion of Ang II caused reduced food intake and fat mass. Furthermore, Ang II reduced Npy and orexin expression through an AT1R-dependent manner in ex vivo hypothalamic cultures. AMPK is an important positive regulator of Npy expression in the hypothalamus (24), and peripheral Ang II infusion suppressed hypothalamic AMPK activity. Furthermore, Ang II–mediated reduction in Npy and orexin in hypothalamic ex vivo culture was restored by AMPK activation with AICAR. Consistent with our hypothesis and data, it has been shown that the AT1R is expressed in multiple hypothalamic neurons, including the lateral hypothalamic area, paraventricular nucleus, retrochiasmatic area and perifornical nucleus (25). Although angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor blockers (ARBs) are widely used in patients with cardiovascular and renovascular disease, their potential effects on food intake and lean body mass are very little known. Our data have important implications in conditions such as end-stage renal disease (ESRD) and congestive heart failure (CHF), in which the RAS

is activated and in which the anorexia-cachexia syndrome contributes significantly to poorer outcomes.

ANG II REGULATES SATELLITE CELLS AND MUSCLE REGENERATION

Skeletal muscle has a remarkable ability to maintain its homeostasis in the face of injury or wasting by activating a well-orchestrated regenerative response to repair damaged myofibers. This regenerative process is mainly mediated by muscle stem (satellite) cells, which are activated by muscle damage and differentiate to generate new myofibers and to repair damaged myofibers. Thus, we questioned why skeletal muscle repair processes did not seem to restore muscle mass in the setting of Ang II–induced muscle wasting. In cancer cachexia, it has been suggested that atrophying muscle has less regenerative capacity and possibly a reduction in satellite cell function (26–28). Also, aged satellite cells display reduced regenerative capacity and potentially contribute to the development of sarcopenia (29,30). Thus, we hypothesized that high Ang II levels, similar to cancer cachexia and aging, inhibit satellite cell regenerative capacity and muscle regeneration, further worsening the muscle wasting. We infused animals with Ang II in the setting of injury-induced muscle regeneration (31). In Ang II–infused animals, both number and size of regenerating myofibers (myofibers with centralized nuclei) were reduced. These reductions were associated with blunted increase in expression of myogenic transcription factors Pax7 (paired box transcription factor 7), MyoD (Myogenic differentiation antigen), and myogenin in isolated satellite cells. Furthermore, there was a reduction in satellite cell number in Ang II–infused animals. Importantly, we found that AT1R was expressed in satellite cells, whereas there was no detectable expression in mature myofibers, suggesting that Ang II's effect to reduce muscle regeneration is mediated through AT1R signaling in satellite cells. Indeed, Ang II inhibited the proliferative capacity of isolated satellite cells in vitro through an AT1R- and Notch-dependent mechanism. Although there is conflicting evidence on the potential involvement of Ang II on skeletal muscle regeneration (32–36), our study is the first to report that Ang II directly acts on satellite cells and inhibits proliferation, leading to a reduction in muscle regeneration. Our data and clinical studies strongly suggest that increased Ang II could be a cause of reduced muscle regenerative capacity in pathophysiological conditions with an activated RAS, such as CHF, ESRD, and aging, thereby contributing to the development of cachexia and sarcopenia. However, an important

question remained to be answered: what is the physiological role of RAS components in satellite cells? We hypothesized that Ang II and possibly other RAS components play an important role in regulating muscle regeneration in physiological conditions, and that an imbalance of the RAS in pathophysiological conditions deteriorates muscle regenerative capacity. To explore this hypothesis, we analyzed expression of RAS components in satellite cells during differentiation and muscle regeneration. Interestingly, we found that Ang II type 2 receptor (AT2R) was robustly increased during satellite cell differentiation, whereas quiescent and proliferating satellite cells did not express AT2R above detectable level (31,37). This expression pattern of AT2R clearly contrasted to that of AT1R, which is expressed only in quiescent and proliferative satellite cells. Since it has been shown that AT1R and AT2R counteract each other in different physiological settings (38), we hypothesized that AT2R has a distinct role in regulating satellite cell function and AT1R and AT2R coordinately regulate satellite cell physiology. In contrast to AT1R, AT2R activation by an AT2R-specific agonist (CGP42112) increased the size of regenerating myofibers. Whereas AT2R activation did not alter the number of regenerating myofibers, it increased the number of nuclei in the regenerating myofibers, suggesting that AT2R regulates satellite cell fusion and myofiber maturation processes. Consistent with these findings, AT2R inhibition using an AT2R-specific antagonist (PD123319) or small interfering RNA-mediated knockdown had the opposite effects on muscle regeneration. In isolated satellite cells cultured in vitro, AT2R knockdown prevented satellite cell fusion and differentiation to mature myofibers. We found that ERK1/2 (Extracellular signal-regulated kinase 1/2) signaling is suppressed downstream of AT2R, and possibly there is a negative feedback loop on AT2R expression. Although currently the role of RAS components other than AT1R and AT2R in regulating muscle function and regeneration is poorly understood, our data suggest that the different components of RAS coordinately regulate satellite cell function in physiological conditions. Thus, alterations in satellite cell RAS function in chronic diseases could be an underlying mechanism promoting worsening cachexia.

CLINICAL IMPLICATIONS AND FUTURE PROSPECTS

Substantial progress has been made in recent years in our understanding of underlying molecular mechanisms of cachexia in chronic diseases and several novel therapies are being tested in clinical trials (39). Among these potential targets, myostatin and activin A pathways

[mediated via the ActRIIB (activin receptor IIB) receptor] are currently the most promising. However, a major challenge underlying the development of cachexia treatment is the complex and multifactorial nature of the syndrome. Thus, identification and understanding of effective target pathways is critical since it is unlikely one therapeutic intervention could be effective in all the cachexia conditions associated with different chronic diseases. Indeed, our preliminary data showed that the expression of myostatin was not altered after Ang II infusion or in CHF (unpublished data), suggesting that ActRIIB blockade might not be as effective as in the case of cancer cachexia.

Importantly, it has been suggested that Ang II levels are elevated in many clinical situations with muscle wasting, such as CHF, ESRD, chronic obstructive pulmonary disease, cancer, and aging (9,40–43), and it has been shown that AT1R blockade has beneficial effects on aging-associated disease states (34,44–46). There are two main current pharmacological approaches to target the effects of Ang II: inhibiting the formation of Ang II using an ACE inhibitor (ACEi) and blocking the AT1R using an ARB. ACEi therapy has been shown to help maintain body weight and muscle mass in CHF or in patients with hypertension (47–49). A phase III clinical trial treating cancer cachexia patients with the ACEi imidapril has been completed. ACEi effects on muscle weight loss in cancer patients varied between different cancer types. Although imidapril did not have a statistically significant effect when all the patients' data were combined, it prevented weight loss in patients with non–small cell lung cancer and colorectal cancer (50,51). ARBs such as losartan could be effective in treating cachexia, but there are no planned clinical trials by the manufacturer (51). Thus, so far blockade of Ang II signaling to treat cachexia has not been extensively explored in clinical trials. Also, it is important to be cautious when interpreting data involving activation or inhibition of one part of the entire RAS system. The RAS includes multiple angiotensins and receptors, and it is not fully understood how different angiotensin ligands and receptors act in orchestration. For instance, inhibition of Ang II production by ACEi would result in an increase of its precursor Ang I, whereas blockade of Ang II signaling using an AT1R blocker would increase Ang II through a compensatory mechanism. Our studies analyzing the RAS in muscle regeneration revealed that AT1R and AT2R clearly have opposing roles in regulating satellite cell function, and possibly other RAS components are involved in different pathways leading to muscle wasting in chronic diseases (Figure 1). Future studies are required to fully understand the RAS-mediated regulation of skeletal muscle physiology, including muscle mass, food intake and muscle regeneration.

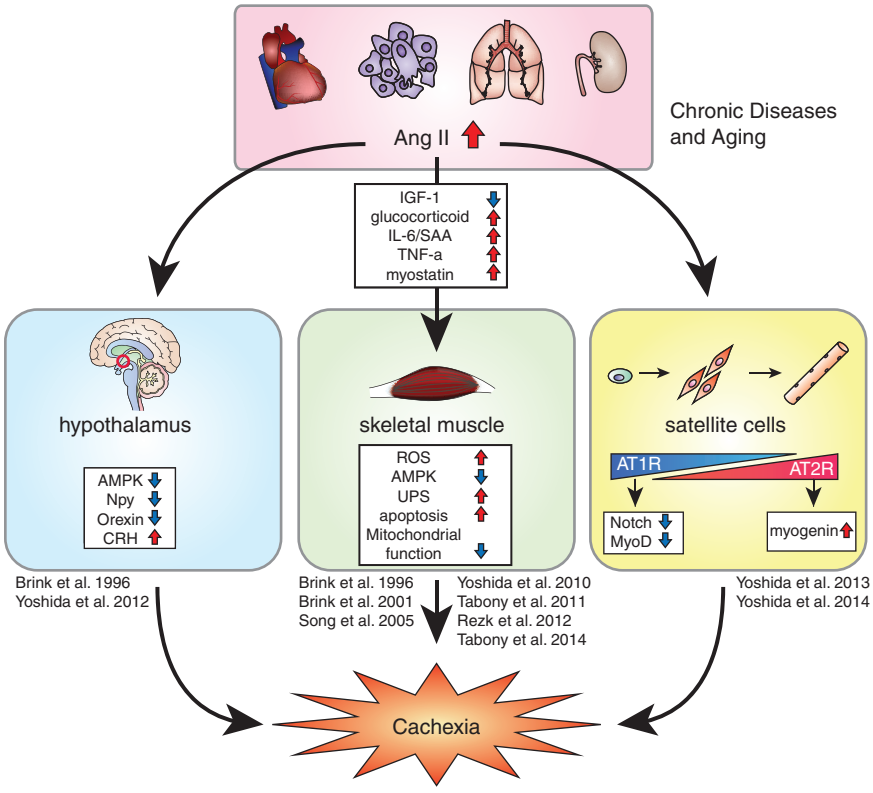

Fig. 1. Angiotensin II (Ang II) – induced muscle wasting: potential mechanisms of cachexia in chronic diseases. In chronic disease conditions there is an increase in circulating Ang II. Increased Ang II causes a reduction of insulin-like growth factor 1 (IGF-1) and increased glucocorticoids and interleukin 6 (IL-6)/serum amyloid A (SAA), which result in muscle wasting. In skeletal muscle, there is an increase of reactive oxygen species (ROS), reduction of adenosine monophosphate-activated protein kinase (AMPK) and increased ubiquitin proteasome system (UPS), all of which result in muscle proteolysis. Ang II also acts on hypothalamic neurons to reduce appetite via alterations of orexigenic/anorexigenic neuropeptide expression. Reduced appetite leads to muscle wasting due to insufficient energy intake to maintain muscle mass. Ang II prevents satellite cell proliferation and skeletal muscle regeneration via inhibition of Notch signaling. Contrary to Ang II type 1 receptor (AT1R), Ang II type 2 receptor (AT2R) signaling potentiates satellite cell differentiation. It is of note that potentially other renin-angiotensin system (RAS) components are involved in this process. The net effect of Ang II infusion in vivo is reduced muscle regeneration. The combination of Ang II-induced muscle wasting, reduced food intake, and lower muscle regeneration leads to the development of cachexia. Abbreviations: Npy, neuropeptide Y; CRH, corticotropin-releasing hormone; MyoD, Myogenic differentiation antigen.

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DISCUSSION

Howley, Boston: Thank you. Perhaps I can start with the first question. You mentioned that angiotensin increases protein degradation within the cells and show the upregulation

of several ubiquitin ligases. Is it known what the specific substrates might be for those ligases that are mediating this degradation?

Delafontaine, Columbia, MO: Yes, we haven't done those studies but the contractile proteins in skeletal muscle are the substrates for those ligases including myosin for instance. Alfred Goldberg, among others, has done that work in Boston.

Zeidel, Boston: Terrific work and very provocative. I noticed that in some of the studies you showed at the end, relate to sarcopenia in the elderly, and I would ask if you want to speculate a bit. Do people who are older have higher levels of circulating angiotensin, in part because of difficulty with auto regulation of blood pressure, and clearly there is a limit to how much you can give these inhibitors if they are depending on angiotensin to maintain adequate circulating blood volume or blood flow to the brain among other things. I wonder if maybe we should be tolerating higher blood pressures so we can give them angiotensin receptor antagonists so they won't have so much sarcopenia. Can you speculate on that?

Delafontaine, Columbia, MO: That's a very interesting question. There are clearly some data, well not a lot of published data, but there are some data that angiotensin may actually be increased in skeletal muscle in the aging population. Clearly that raises the issue you are talking about – impairment of autoregulation in the elderly and what should we be doing about it. I think it's ripe for investigation. We haven't done much work on that, although we are now looking at aging in animal models. There are some data showing a depletion of stem cells in aged muscles, similar to what we found in the LAD ligation model.

Mackowiak, Baltimore: I was a little surprised in your introduction that you suggested that cytokines have not been shown to be involved in cachexia, at least I think that is what you said. It sort of takes me back to many lectures that Bruce Beutler, and I think his father gave about cachexin which looked pretty convincing in terms of its role as a causative factor in tumor cachexia. I wonder if you could elaborate on that and I wonder if you have looked to see if there is any synergistic relationship between angiotensin and the cytokines?

Delafontaine, Columbia, MO: I didn't mean to suggest that cytokines are not involved. I think what I mentioned is that the clinical studies using inhibitors of TNF-alpha for instance have not resulted in better outcomes in patients looking specifically at cachexia. They have been looked at in cancer cachexia so they have been disappointing. It is likely that multiple cytokines acting synergistically do have an important role and certainly if one looks at the angiotensin model one can inhibit Interleukin-6 for instance and get significant inhibition of the wasting effect of angiotensin II.

Oates, Nashville: I wonder what you are thinking would be the involvement of the lymphocytes and the syndrome. You mentioned the hypertension that occurs with the angiotensin infusion and that's been shown to be dependent upon T-cells and with interleukin-17 playing a role and I wonder if a similar mechanism might also be functioning in the skeletal muscle?