

# Skeletal muscle atrophy

**Assistant Professor Andrew Judge** focuses on skeletal muscle atrophy. Here, he explains how understanding the forkhead boxO pathway could lead to novel treatments

ASSISTANT PROFESSOR ANDREW JUDGE



## Could you begin by elaborating on your work investigating molecular signalling pathways which lead to skeletal muscle atrophy?

Skeletal muscle wasting can be a consequence of a multitude of conditions, but unfortunately there are currently no effective drugs to counter it. This is partly due to the limited understanding of the molecular signalling pathways that cause muscle wasting. However, in recent years this understanding has significantly improved due to the hard work of several research labs. Our lab has contributed to this by focusing on the forkhead boxO (FoxO) pathway, which is a key determinant of muscle wasting in multiple conditions.

## How could the FoxO pathway be utilised to counteract muscle wastage seen as a consequence of disease?

Although the upstream triggers that initiate the signalling pathways and drive muscle wasting are unique to each condition, there are common signalling pathways across multiple conditions. The FoxO pathway is one example, and is implicated in muscle wasting associated with muscle disuse, sepsis, cancer, starvation, diabetes, ageing and heart failure. Thus, FoxO is an attractive therapeutic target.

However, FoxO proteins are transcription factors that regulate the expression of a whole spectrum of genes, not just those related to atrophy. The challenge is to identify therapeutic targets which regulate FoxO's ability to transcribe atrophy-related genes

without compromising the expression of other necessary FoxO target genes.

## Have you faced any particular problems or challenges during the course of your work?

There are always challenges when conducting experiments. When we face problems that we feel we cannot resolve by ourselves, we reach out to others. Our investigation has benefited from the generosity of several fine investigators, sharing research tools and advice. I have also been fortunate to consistently have an excellent research team that understands the challenges of this profession.

## What plans do you have for the future of your lab research?

It is hard to predict the future direction of our lab; we follow data and see where it takes us. Six years ago, we began studying the role of heat shock proteins in regulating skeletal muscle atrophy and discovered that they regulate FoxO signalling. In trying to determine the mechanism of this regulation, it became apparent that our understanding of FoxO signalling in skeletal muscle was in its infancy. We therefore began trying to understand the regulation of FoxO in skeletal muscle, which led us to our current work on FoxO acetylation.

We would never have predicted that today we would be studying FoxO acetylation. Envisaging the specific direction of our research in the future is impossible, though it will certainly remain focused on FoxO signalling in skeletal muscle.

## Has collaboration been important to your work? Who are your main collaborators?

We have been incredibly fortunate in collaborating with several outstanding scientists, which has allowed us to expand our work into areas that would otherwise have been impossible. Dr Susan Kandarian, Professor at Boston University, has and continues to be an invaluable collaborator. Her expertise and knowledge base in the use of cellular and molecular approaches to studying pathways that mediate skeletal muscle atrophy are unparalleled.

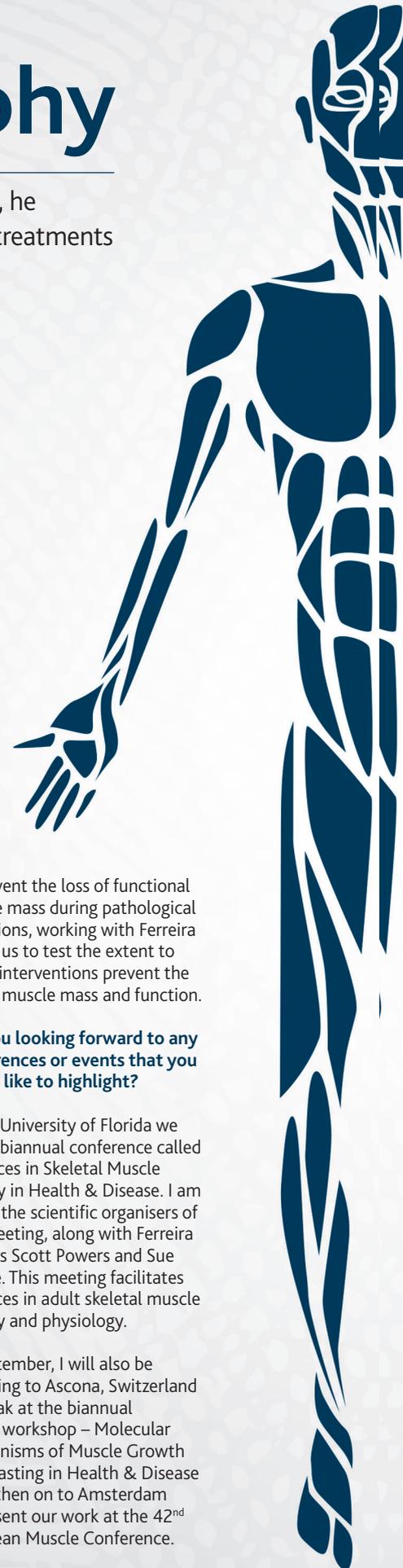
I am also actively collaborating with Dr Leo Ferreira, Assistant Professor at the University of Florida. Ferreira has expertise assessing skeletal muscle contractile properties in isolated muscle and single fibres. Since the goal of our work is

to prevent the loss of functional muscle mass during pathological conditions, working with Ferreira allows us to test the extent to which interventions prevent the loss of muscle mass and function.

## Are you looking forward to any conferences or events that you would like to highlight?

At the University of Florida we host a biannual conference called Advances in Skeletal Muscle Biology in Health & Disease. I am one of the scientific organisers of this meeting, along with Ferreira and Drs Scott Powers and Sue Bodine. This meeting facilitates advances in adult skeletal muscle biology and physiology.

In September, I will also be travelling to Ascona, Switzerland to speak at the biannual EMBO workshop – Molecular Mechanisms of Muscle Growth and Wasting in Health & Disease – and then on to Amsterdam to present our work at the 42<sup>nd</sup> European Muscle Conference.



# Pathway to impact

Scientists at the **University of Florida** are conducting novel studies into muscle atrophy and how to prevent, reduce and even reverse it. By researching molecular signalling pathways and protein families that affect muscle wastage, the team is uncovering groundbreaking results

**MUSCLE ATROPHY, OR** wasting, refers to a general decrease in muscle mass characterised by a reduction in muscular size and strength. Skeletal muscle atrophy can be caused by a number of factors which range from involuntary disuse – due to bed-rest or spinal cord injury – to diseases such as cancer, AIDS, heart failure and peripheral arterial disease. Muscle wasting is also commonly associated with ageing. Sufferers experience functional deterioration, loss of independence, reduced tolerance to treatments, prolonged hospital stays and, ultimately, increased mortality. Such serious consequences make the search for effective drug treatments for this condition all the more imperative.

Before specific work towards the development of pharmacological treatments for muscle atrophy can begin, better comprehension of the molecular signalling pathways that cause muscle wasting is necessary. It is this vital niche of knowledge that scientists at the University of Florida's Department of Physical Therapy are focusing their attention. Led by Assistant Professor Andrew Judge, the team is conducting research into the role of specific proteins in driving muscle atrophy. Driving this research are a number of related sub-projects. Current work includes endeavours to better understand the forkhead boxO (FoxO) signalling pathway and its relationship with skeletal muscle mass, heat shock proteins (HSPs) and the mechanisms by which the latter regulate signalling pathways affecting muscle mass.

The repercussions of Judge's research could stretch beyond the bounds of medicine. Indeed, at a time where sedentary lifestyles are becoming evermore common, a solution for preventing muscle wastage (or one which can even promote muscle growth) could have wider societal impacts. Perhaps in the long term, this research could solve the problem of how to maintain muscle mass in the face of inactivity – involuntary or otherwise.

## WHY FOXO?

A central part of the Florida group's work is understanding factors that regulate FoxO activity. FoxO is a family of protein transcription factors which plays a significant role in the regulation of skeletal muscle mass. FoxO proteins, and/or their targets, may provide a

focus for therapies aimed at reducing muscle wasting and thus could improve the quality of life and survival rates for patients with many associated diseases.

This line of enquiry could illuminate how therapeutics could potentially target the regulation of forkhead boxO acetylation

In 2004, scientists in Boston discovered that the activation of FoxO could cause skeletal muscle fibre atrophy. Following on from these important findings, the team in Florida distinguished the role of FoxO in driving muscle wasting associated with certain physiological conditions. In 2010, Dr Sarah Senf genetically blocked FoxO transcriptional activity during muscle disuse, and demonstrated that FoxO activation drives a significant proportion of muscle atrophy during disuse. Since this discovery, Judge's team has dedicated time to further researching FoxO. Results have shown that the protein is also an integral part of muscle wasting associated with cancer and sepsis.

## ESTABLISHING THE ROLE

In making this discovery, the team genetically inhibited the activity of FoxO proteins in the skeletal muscle of rodents. Such genetic approaches are a key part of the investigation, as they allow for the establishment of cause and effect. The researchers utilise both overexpression and loss of function approaches, primarily using direct gene transfer to skeletal muscle, to identify the role of specific proteins in the muscle atrophy phenotype. This approach has been tremendously successful, not only in identifying the importance of FoxO in the muscle atrophy programme, but also in the identification of proteins which promote FoxO nuclear localisation and activation during atrophy. Furthermore, the ability to conduct these genetic manipulations in whole muscle, immediately prior to and during a wasting condition, is especially powerful as it

## INTELLIGENCE

### FOXO SIGNALING AND SKELETAL MUSCLE ATROPHY

#### OBJECTIVES

To contribute to understanding the molecular signalling pathways that cause muscle wasting by focusing on the forkhead boxO (FoxO) pathway, which is activated and required for atrophy in several different conditions.

#### KEY COLLABORATORS

**Dr Susan Kandarian**, Boston University, Massachusetts, USA

**Dr Leo Ferreira**, University of Florida, USA

**Dr Sarah Senf**, University of Florida, USA

**Dr Scott Powers**, University of Florida, USA

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#### CONTACT

**Andrew Judge, PhD**

Assistant Professor

Department of Physical Therapy  
University of Florida  
Box 100154, UFHSC  
Gainesville, FL 32610-0154  
USA

T +1 352 273 9220

F +1 352 273 6109

E arjudge@phhp.ufl.edu

<http://pt.phhp.ufl.edu/about-us/faculty/judge-laboratory>

**ANDREW JUDGE** is Assistant Professor in the Department of Physical Therapy at the University of Florida. He received his BS from Loughborough University in the UK, his Master's from McNeese State University in Louisiana and his PhD from the University of Florida. Judge completed his postdoctoral training with Dr Susan Kandarian at Boston University.

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and Health Professions  
Physical Therapy  
UNIVERSITY of FLORIDA

means bypassing compensatory effects that may be activated in muscle, due to lifelong genetic manipulation.

Despite the success enjoyed by Judge and his collaborators in elucidating the role of FoxO, the team still has more work to do before the protein is fully understood. For example, the regulation of FoxO is complex – the family of proteins can be phosphorylated, acetylated and ubiquitinated. Understanding such post-translational factors requires more detailed studies as modifications could regulate FoxO's stability, cellular localisation and transcriptional activity. Therefore, the team currently focuses upon understanding the role of acetylation in regulating FoxO signalling in muscle wasting. It has already been identified that decreased acetylation of FoxO3a, one of the FoxO family members, is an important mechanism driving muscle atrophy.

#### HEAT SHOCK PROTEINS

HSPs have also formed the basis of much of this research due to their role in skeletal muscle plasticity. These proteins are constitutively expressed in cells, but their expression is further induced under various types of stress – including mechanical injury, physical activity, oxygen or nutrient deprivation and exposure to reactive oxygen species. Importantly, elevated HSPs during such conditions can promote cell survival; helping return cells to their normal homeostatic state, thus maintaining cellular function.

HSP70, a key member of the HSP family, has been one focus of attention. Judge's group has shown that HSP70 is downregulated during periods of skeletal muscle disuse, and that restoration of HSP70 levels during disuse can prevent associated muscle fibre atrophy. Furthermore, it has been demonstrated that the knockout of HSP70 can decrease the skeletal muscle fibre cross-sectional area and reduce skeletal muscle force production. Following these discoveries, Senf's work has also shown that HSP70 is necessary for normal muscle fibre regeneration and regrowth following injury. This evidence supports the overarching point that endogenous HSP70 upregulation is a vital mechanism in supporting muscle fibre size maintenance, promoting muscle fibre growth and preserving muscle function.

#### HISTONE DEACETYLASES AND FUTURE PROSPECTS

Another focus of Judge's dynamic programme of research considers the role of protein acetylation in the regulation of skeletal muscle mass. This line of enquiry is interlinked with their work on FoxO and could illustrate how therapeutics could potentially target the regulation of FoxO acetylation. FoxO



Up to 80 per cent of advanced stage cancer patients suffer from profound muscle wasting, referred to as cachexia, which significantly affects patient morbidity and mortality. When images of Steve Jobs appeared on the internet with advanced stage pancreatic cancer, the world saw the devastating effects of muscle wasting on an iconic figure. By Matthew YoheMatt Yohe at en.wikipedia [CC-BY-3.0 (<http://creativecommons.org/licenses/by/3.0/>)], from Wikimedia Commons.

acetylation is regulated by opposing enzymatic activities – histone acetyltransferases (HATs) and histone deacetylases (HDACs). The former process adds acetyl-coA to lysine residues, while HDACs remove acetyl groups.

As Judge's team has already shown, decreased acetylation of FoxO3a is an important mechanism driving muscle atrophy. Therefore, it is conceivable that inhibiting FoxO3a deacetylation could be a potential means to reduce muscle atrophy. Judge believes that this could be achieved by treatment with HDAC inhibitors. To date, HDAC inhibitors have been used therapeutically in experimental models of muscular dystrophy and have shown great promise in increasing muscle fibre size and function. Other signalling pathways that drive muscle atrophy – such as myostatin signalling – can also be negatively regulated by HDAC inhibitors. With this in mind, HDAC inhibitors may well prove beneficial during muscle wasting conditions through targeting not only FoxO, but multiple pathways that regulate skeletal muscle size and function.

This hypothesis will form the basis of the team's future work. Inhibiting all HDACs may not be optimal, and could even diminish the effectiveness of inhibiting those involved in the atrophy phenotype. Therefore, the lab's ongoing aim is to understand the specific HDAC or HDACs which regulate FoxO signalling and muscle atrophy. This means that future, targeted countermeasures can be tested and perfected. So far, findings are very promising.