Could you provide a brief synopsis of your research career to date?

I’ve always been fascinated by skeletal muscle. It’s one of the most highly differentiated tissue in the body and it forms about 40 per cent of total body mass. In the early stages of my career I primarily studied mechanisms of electrical signalling in the muscle membrane and transverse tubules, but about 10 years ago I became interested in the role of the sodium/potassium-ATPase in skeletal muscle.

Why does skeletal muscle fatigue occur, even where there is adequate energy supplies?

Metabolic changes and energy depletion can cause fatigue, but it’s not the only cause. Skeletal muscles have a good supply of energy stores and it takes time to deplete them – it’s actually quite difficult to do so in some muscle types. Sometimes fatigue occurs before the energy stores are depleted or before other biochemical events that trigger fatigue.

For instance, fatigue may result if electrical excitation fails. As the action potential or signal that propagates along the membrane and into the transverse tubules, electrical excitation is generated by an influx of sodium and an efflux of potassium. So, as soon as a muscle begins propagating these action potentials, it starts to accumulate sodium while also losing potassium. In a single action potential, these amounts of sodium and potassium are insignificant, but in repetitive action potentials the concentrations can accumulate and become significant. If potassium concentrations get high enough outside the cell, this will depolarise the membrane and cause excitation to fail, which will consequently result in fatigue as the muscle will be unable to contract.

What role does the alpha 2 isoform of the sodium/potassium-ATPase play in skeletal muscle?

In most cells, there is a ubiquitous isoform called alpha 1, which is either the predominant or only isoform. In skeletal muscles, however, both alpha 1 and alpha 2 are present, with alpha 2 being the predominant isoform (comprising up to 85-90 per cent of the sodium/potassium-ATPase). Importantly, alpha 2 is the only isoform localised to the transverse tubules. Given that transverse tubules have a specific job to do with propagating the electrical signal that triggers muscle contraction, I am conducting investigations into alpha 2’s activation and role in skeletal muscle fatigue.

How do you hope to see your research develop in the future?

I would like to initiate investigation into the role of alpha 2 in sympathetic stimulation (for example, the fight or flight response). We know that when the sympathetic system is activated, epinephrine and norepinephrine stimulate the ATPase – but we don’t know its isoform target in the skeletal muscles. I want to find out whether one of the isoforms is a specific target of sympathetic adrenergic stimulation, and to what extent each of them are activated. I am especially interested in this because beta adrenergic drugs are commonly used clinically, and I think it’s important to know what they are doing to the skeletal muscle systems.

Can you discuss the possible translation applications for your research?

As the inability to produce force even though the motor nerve is giving a command, skeletal muscle fatigue is a healthy and protective physiological reaction in many cases. However, there are some situations where muscle fatigue is abnormal – and this is an area with significant potential for translational applications.

For example, skeletal muscle fatigue is almost a diagnostic criteria for heart failure. It’s been shown that you can have a normal oxygen supply, yet the skeletal muscles still become fatigued earlier. Exactly why that happens is not yet known. While treating this would not cure the underlying heart problem, it would reduce one of the most debilitating aspects of heart failure.

Another example is muscular dystrophy in which skeletal muscle fatigue is diagnostic and debilitating. Again, reducing the muscle fatigue ability in some of these dystrophies will not cure the disease but it will alleviate some of the symptoms and significantly improve quality of life.
At the University of Cincinnati, researchers are working to understand the role of two sodium/potassium-ATPase isoforms in the development of skeletal muscle fatigue, with the hope of identifying novel therapeutic targets.

**Muscle Makes up** approximately 40 per cent of the human body. It falls within three categories: cardiac muscle, smooth muscle and skeletal muscle. Of these, only the skeletal muscle can be controlled voluntarily. All conscious human movements, from running to speaking, rely on the successful control of skeletal muscle contraction by the somatic nervous system.

However, these muscles cannot perform indefinitely – as anyone who has ever pushed their physical limits during a period of intense exercise can attest. Under certain conditions, all skeletal muscles will eventually reach a point where they are unable to contract any further, in spite of neurological instructions to continue. This phenomenon is known as skeletal muscle fatigue.

In many instances, skeletal muscle fatigue is a normal, healthy reaction to physical exertion. However, there are certain conditions in which it can behave abnormally, with debilitating consequences. For example, elevated skeletal muscle fatigue is associated with heart failure, age-related frailty and a number of muscular dystrophies. The development of a therapy that could reduce skeletal muscle fatigue could have a significant impact on quality of life. In addition, strategies that reduce or delay muscle fatigue are of major interest to the athletics industry.

**Isoform Investigations**

To reduce or delay skeletal muscle fatigue, scientists must first understand the precise mechanisms involved in the complex, multistep physiological process that leads to it. It is this drive for knowledge that underpins research into the sodium/potassium-ATPase in skeletal muscle at Professor Judith A Heiny’s laboratory in the University of Cincinnati, USA. Sodium/potassium-ATPase is an enzyme that is responsible for maintaining cellular concentration gradients for sodium and potassium ions that play a vital role in many biological processes.

Within skeletal muscles, two specific isoforms of sodium/potassium-ATPase are crucial: alpha 1 and alpha 2. The Heiny lab is working to elucidate the physiological roles of these two isoforms within the context of skeletal muscle fatigue. They initially attracted the research team’s interest because, the sodium/potassium-ATPase in skeletal muscle is predominantly alpha 2 rather than alpha 1. Moreover, alpha 2 is the only sodium/potassium-ATPase isoform found in transverse-tubule membranes – a specialised membrane system essential for initiating contraction in skeletal muscles. This led the researchers to hypothesise that the roles of alpha 1 and alpha 2 are specialised in skeletal muscle.

**Of Mice and Muscles**

Much of the research carried out in the Heiny lab has been aimed at elucidating the mechanisms of alpha 2. Working alongside a University of Cincinnati colleague, Professor Jerry Lingrel, the researchers developed genetic models to assist them in these endeavours. Most notable was a mouse model in which the alpha 2 isoform was only removed from the skeletal muscles. The scientists used it to gain an insight into the role of alpha 2 in muscle fatigue. Initially, Heiny and her colleagues measured the model’s exercise capacity by running them on treadmills and comparing them with controls. The difference was dramatic: while healthy mice can achieve speeds of 20-30 metres per minute for prolonged periods of time, the modified mice only managed speeds of 6-8 metres per minute before succumbing to skeletal muscle fatigue.
MOLECULAR MECHANISMS OF MUSCLE FATIGUE

OBJECTIVE
To elucidate both the physiological roles of the sodium/potassium-ATPase alpha isoforms in skeletal muscle.

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The development of a therapy that could reduce skeletal muscle fatigue. Could have a significant impact on quality of life

This led the scientists to conclude that alpha 2 plays an important role in reducing and delaying such fatigue.

Heiny and her collaborators also conducted experiments on the surgically removed muscles from these mouse models, using a tension transducer to test their ability to produce force and resist fatigue. “We found that, once stimulated, the muscles without alpha 2 would start to experience fatigue more rapidly than a normal muscle,” reveals Heiny. “This provided us with evidence that the alpha 2 isoform plays a special role in resisting muscle fatigue.”

A FUNCTIONAL INSIGHT
The Heiny lab has generated a number of important insights into the functional roles played by the alpha 1 and alpha 2 isoforms in skeletal muscle. Highlights include the demonstration that alpha 2, despite its majority content, is not highly active in non-contracting skeletal muscles, nor is it required to set resting sodium/potassium concentrations or resting membrane potential; rather, the minor alpha 1 isoform serves these functions. Further investigations have revealed that alpha 2 plays a crucial role in working muscles – it is activated immediately upon muscle contraction, and is required both to maintain contraction and resist fatigue.

Deving deeper, Heiny and her colleagues found that alpha 2’s ability to stave off skeletal muscle fatigue is rooted in its capacity to safeguard membrane excitation, via the removal of extracellular potassium produced during intense exercise as the result of multiple action potentials. If potassium is not returned to the muscle, this can lead to membrane depolarisation and subsequent excitation failure – a very early mechanism by which fatigue can occur, even before metabolic changes and energy loss. Most recently, the researchers discovered that the alpha 2 isoform is activated in part because of its low binding affinity for potassium, and that it is matched to extracellular potassium increases that occur in working muscles.

AN INTERNAL TURBOCHARGER
All of these findings led Heiny and her colleagues to conclude that alpha 2 functions as a ‘turbocharger’, enabling sodium extrusion and potassium re-uptake – both of which are needed for the effective maintenance of membrane excitation, and hence muscle contraction. “It seems that alpha 1 has evolved to be specialised for handling the potassium and intracellular sodium changes that occur in a resting muscle, while alpha 2 is specialised to handle the range of potassium concentration changes that occur in working muscles,” Heiny explains.

Yet Heiny’s team has plenty of questions it still hopes to answer. “Lingrel’s lab have made other models with different contents of alpha 1 and alpha 2; we’re only just beginning to use these with the aim of separating the different contributions of the different isoforms,” Heiny reveals. The researchers also plan to initiate studies into the role of alpha 2 in sympathetic stimulation, with a view to assessing the possible impact of widely prescribed beta adrenergic drugs.

Development of a therapy that could reduce skeletal muscle fatigue. Could have a significant impact on quality of life.