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CLASSICAL PERSPECTIVES

Skeletal muscle: not simply an organ for locomotion and energy storage

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From Little Things Big Things Grow is a rock protest song recorded by Australian artists Paul Kelly and The Messengers in 1991. The song is based on the story of The Gurindji Strike and Vincent Lingiari as part of the Indigenous Australian struggle for land rights and reconciliation. However, a decade later this title could, in the scientific sense, apply to the paper published in *The Journal of Physiology* by Bente Klarlund Pedersen and colleagues entitled 'Evidence that interleukin-6 is produced in skeletal muscle during prolonged running' (Ostrowski *et al.* 1998).

For nearly half a century, researchers had hypothesized that skeletal muscle cells possessed a 'humoral' factor that was released in response to increased glucose demand during contraction (Goldstein, 1961). Interestingly, it had been known for many years that physical exercise resulted in a marked increase in the circulating concentration of the multifunctional, immune modulating cytokine interleukin-6 (IL-6), yet the cellular origin and functional role of this cytokine was unclear. The paper by Ostrowski *et al.* (1998) was a harbinger for the numerous subsequent studies that have elucidated the nature of the exercise–IL-6 relationship and have initiated a paradigm shift in our understanding of the role of skeletal muscle in the aetiology of tissue cross-talk in health and disease. This was highlighted earlier this year in a review by Handschin & Spiegelman (2008) in *Nature* who commented, 'The recent discovery of myokines – that is, cytokines that are produced and secreted by skeletal muscle cells – sheds light on the association between exercise and inflammation'.

As with many paradigm shifting studies, the major finding in the paper by Ostrowski *et al.* (1998) was somewhat serendipitous. The authors hypothesized that eccentric exercise, which causes muscle damage, may also lead to cytokine production by muscle cells or the phagocytic inflammatory cells that are recruited to damaged muscle. The authors collected skeletal muscle biopsy and blood samples from human subjects who took part in the 1996 Copenhagen Marathon. They observed an approximate 75-fold increase in the concentration of IL-6 in the blood when comparing samples immediately post-exercise with those pre-exercise. Using conventional RT-PCR techniques, they could not detect IL-6 mRNA in either blood mononuclear cells (BMNCs) or skeletal muscle samples prior to exercise. However, while they still could not detect IL-6 mRNA levels in BMNCs after the run, they were able to detect IL-6 mRNA in 5 out of 8 muscle biopsy samples following the marathon. The authors tentatively concluded that IL-6 was produced locally in the skeletal muscle in response to prolonged exercise with a large 'eccentric component'. There were several questions raised by this study. Were muscle cells the site of IL-6 production? If so, was this production related to muscle damage or muscle contraction *per se*?

At the turn of the millennium two studies were published that confirmed, in part, the findings of Ostrowski *et al.* (1998). A study from the same group, using the arterio-venous balance technique was able to show that a contracting limb released IL-6 into the circulation and that this could account for the systemic rise in IL-6 (Steensberg *et al.* 2000). Meanwhile, using the then relatively novel technique of real time RT-PCR, work from another group was able to show that IL-6 mRNA was in fact detectable in non-contracting muscle samples and, moreover, as little as 60 min of either running or cycling exercise was sufficient to increase the mRNA levels of IL-6 20- to 30-fold in skeletal muscle (Starkie *et al.* 2001). Importantly, as these studies adopted exercise protocols such as dynamic knee extensor and cycling, they both demonstrated that IL-6 production by muscle was not related to muscle damage as first thought. It took some time to definitively confirm that muscle cells were in fact the cells producing IL-6 during contraction, achieved using techniques such as *in situ* hybridization (Hiscock *et al.* 2004) and immunohistochemistry (Penkowa *et al.* 2003; Hiscock *et al.* 2004).

If IL-6 production by muscle cells was not related to an inflammatory, acute phase response, what was the stimulus that promoted such rapid release of IL-6 from contracting muscle? In an editorial published in *The Journal of Physiology* in 2000, Gleeson suggested that IL-6 might be acting as a hormone, being released by the muscle to signal to the liver to liberate glucose when required, e.g. in times of carbohydrate depletion such as during prolonged exercise (Gleeson, 2000). Indeed, evidence began to accumulate to suggest that IL-6 was related to metabolism and energy deprivation rather than inflammation. When two-legged knee-extensor exercise was performed in individuals who had one leg previously depleted of glycogen, this leg released more IL-6 compared with the contralateral limb (Steensberg *et al.* 2000), while ingesting carbohydrate during exercise completely suppressed leg IL-6 release compared with the ingestion of a placebo (Febbraio *et al.* 2003). Importantly, the hypothesis that IL-6 may be released from contracting muscle to stimulate liver glucose production during exercise was proven to be correct in 2004, when it was shown that recombinant human IL-6 infusion during low intensity exercise, to mimic systemic IL-6 levels observed during higher intensity exercise, resulted in an increase in endogenous glucose production to levels comparable to that seen during high intensity exercise (Febbraio *et al.* 2004). This study provided new insights into factors that mediate glucose production and implicated IL-6 in the so-called 'humoral' factor that was postulated many years before (Goldstein, 1961).

It has subsequently been shown that IL-6 has diverse metabolic effects such as being able to activate the energy sensing enzyme AMP-activated protein kinase and in doing so increasing lipolysis, fat oxidation and muscle glucose uptake (for review see Pedersen & Febbraio, 2008), and ligands that target the IL-6 family receptor complex are now the focus of anti-obesity therapy (Febbraio, 2007). All of this research in the past decade had its origins from the participants in the Copenhagen Marathon published by Ostrowski *et al.* (1998).

From little things big things grow.

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