Is disruption of skeletal muscle molecular clocks the fundamental basis for the development of metabolic syndrome in spinal cord injured individuals?

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ABSTRACT
Here we consider the inter-connectiveness between the clinical presentation, metabolic changes associated with spinal cord injury and the relationship to skeletal molecular clocks. There is incontrovertible evidence that significant metabolic complications can appear over time with spinal cord injuries, which are associated with significant pathologies, and in many cases, reduced life expectancy. The current biophysical and metabolic data only confirms the presence of pathology but fails to identify the underlying cause at the cellular level. Based on current published experimental evidence on muscle molecular clock function, we propose a hypothesis that the underlying cellular mechanism for the metabolic disruption is that following spinal cord injuries, the skeletal muscle metabolic clock uncouples and fails to provide the correct signaling cues for normal skeletal muscle metabolism. The consequence of stopping or slowing the muscle molecular clock results in the metabolic chaos and comorbidities seen in people with spinal cord injury. Key words: Spinal cord injury, disrupted metabolism, skeletal metabolic clocks.

INTRODUCTION
Spinal cord injury resulting in paralysis below the lesion is associated with extensive changes in body composition (1-3). In addition to the primary injury there is a strong association with the increased incidence of heart disease and type 2 diabetes (4-6) which results in the progressive loss of muscle mass and increased adiposity below the lesion (2,7,8). These changes are strongly associated with significant metabolic disruption, thereby increasing the risk factors for metabolic syndrome, such as insulin resistance and dyslipidaemia (5). Previous research has identified alterations in the desaturases and elongase in spinal cord injury that are linked with the development of type 2 diabetes (9). More recently it has been reported that both plasma glycerol and free fatty acids were significantly higher in spinal cord injury than in comparable controls, providing additional evidence for a mechanism to develop insulin resistance (10). We present a hypothesis linking dysfunctional skeletal muscle molecular clocks as a primary causative factor for the development of metabolic syndrome in spinal cord injury.

Muscle metabolic changes consequent to spinal cord injury
The transition from lean tissue muscle mass to adipose tissue is associated with progressive denervation leading to a decrease in the capillary circulation and progressive loss of mitochondria (11,12). These changes result in a shift of normal metabolic response away from muscle to adipose tissue. However, while such metabolic changes occur, other tissue and metabolic changes may precede these and provide an underpinning cellular mechanism for the metabolic changes.

Although there are diverse reasons for general muscle wasting, spinal cord injury and the subsequent muscle wasting below the lesion represents a unique physiological and biochemical situation. Following spinal cord injury there is a progressive loss of the balance between anabolic and catabolic states (1,5) with the injury resulting in changes in muscle fibre and micro-vasculature (12). As disuse atrophy progresses there is a shift from type I (slow) fibre type, to type II (fast) fibre type, with a significant shift to type II by 4.7 months post-injury (11,12). Additionally, there is a progressive increase in sarcoplasm lipid content and denervation (12). The former being considered a function (in part) of decreased mitochondrial function (12,13).

Of note, with the shift from type 1 to type II fibres, an increase in myostatin has been observed (14,15). Previous research has demonstrated increased myostatin production from myotubes from both obese and type 2 diabetes, both of whom had increased type II fibres, which correlated with impaired glucose metabolism and poor fitness (14,16). An imbalance of muscle metabolic homeostasis will result in increased proteolysis and muscle wasting. In addition, increases in myostatin synthesis are compounded in spinal cord injury with both loss of activity below the lesion and the loss of neuronal signaling, which communicates between muscle and bone. The change in muscle-bone interactions leads to the decalcification commonly observed in spinal cord injury (17,18). This interaction between muscle, bone and whole-body metabolism is compromised in spinal cord injury; in particular glucose metabolism where normally up to 80% of post-prandial glucose is utilized by skeletal muscle (19), as well as the utilisation of free fatty acids as an alternative energy source. Therefore, maintaining muscle mass is a key factor in maintaining metabolic homeostasis.

Investigations into progressive loss of muscle mass in humans is limited; however, the major proteolytic pathway to cause loss of muscle mass is the ubiquitin proteasome pathway and is considered to be the major non-lysosomal pathway for intracellular protein degradation (20,21). Whilst a number of activators of the ubiquitin proteasome pathway have been described (22) evidence indicates that in muscle the ubiquitin proteasome pathway can be activated by increased myostatin (23). As indicated earlier in this paper there is a strong association with the transition to type II fibres and the increase in myostatin in spinal cord injury. It is interesting to speculate that alongside this physiological transition, the associated myostatin increase acts as an activating factor (at least in part) to initiate the ubiquitin proteasome pathway and the muscle wasting sequela. In their review of skeletal muscle atrophy, Jackman and Kandarian indicated that myostatin receptor activin IIB (ACTIIB) is up regulated during muscle atrophy (24). In addition, myostatin inhibits GLUT 4 mRNA and protein expression in-vitro (25), providing a potential mechanism for insulin resistance in muscle following spinal cord injury.
Finding the ‘key’ to muscle wasting.

The question now arises whether other metabolic mechanisms are disrupted following spinal cord injury and whether there is a disrupted “controller”? Is there a key to the biochemical transitions leading to the development of insulin resistance, type 2 diabetes, and the pathogenesis of the metabolic syndrome? Results from Bmal1 knock-out mice provides evidence that the molecular clock is directly involved in the transition to type II muscle fibres and disruption of glucose homeostasis (26). More recently Perrin et al. (27) confirmed the findings of Harffmann using in-vitro cultures of human myotubes and suppression of BMAL1. Additionally, Perrin et al. disrupted lipid diurnal oscillations confirming earlier work of Loizides-Mangold et al. (28) who identified that both in vitro cultured human myotubes and muscle biopsies demonstrated lipid oscillations, which were disrupted when siRNA mediated clock disruption was introduced to the myotubes.

Is it all about loss of ‘timing’?

The circadian clock is a well-defined gene regulatory network that controls the expression of transcriptional-translational regulatory networks. These exist in all cells of the body, including muscle, and are expressed in a cyclical manner over an approximate period of 24 hours to synchronize and ‘fine tune’ local tissue metabolism. Skeletal muscle is entrained by the suprachiasmatic nucleus, which is cued by environmental signals such as light and in turn can modulate muscle response via a number of signaling systems such as neurohormonal, temperature, and nutrition control (29). Whilst the mode of suprachiasmatic nucleus signaling is not completely understood, muscle molecular clocks respond in a prescribed manner using the well-established molecular clock regulating system thereby facilitating synchrony with other body organs and tissues (30). This autonomous molecular clock regulates muscle insulin sensitivity via a diurnal rhythm as well as a diurnal rhythm in mitochondrial oxidative capacity (31). In addition, skeletal muscle lipid and amino acid metabolism has been strongly linked to regulation by muscle molecular clocks (32). The mechanism for the cellular metabolic synchronization is linked via the BMAL1:CLOCK transcriptional activation. When the BMAL1:CLOCK heterodimer form a complex in the nucleus they can activate a regulatory loop involving Rev-erb-a, which is highly expressed in oxidative skeletal muscle (33,34). Inactivation or deficiency of Rev-erb-a has been demonstrated to reduce mitochondrial content and the consequential decrease in oxidative function, as well as upregulating autophagy (33,34). Similarly MYOD1, which is involved in skeletal muscle lineage, has been shown to be under the control of the BMAL1:CLOCK complex and it is considered that this control is necessary for maintaining muscle phenotypes by acting as a muscle molecular clock amplifier for down-stream genes (35,36). Taken collectively, disruption of signaling either to or from the suprachiasmatic nucleus will disrupt the integrity of the muscle molecular clock, which in turn will modify the response of the BMAL1:CLOCK complex. Failure or downregulation of BMAL1 activation and the consequent down regulation of Rev-erb-a will disrupt the ability of skeletal muscle to respond to insulin via the GLUT4 receptor and consequently disrupt glucose homeostasis (30). This has been further confirmed using Bmal1 muscle–mice that demonstrated impaired skeletal muscle glucose uptake and metabolism (37). In addition, disruption of CLOCK in human skeletal muscle biopsies in tissue culture also had a similar effect on disrupting skeletal diurnal lipid response as well as a glucose response as identified with Bmal1 disruption (27).

In conclusion, we consider that following a spinal cord injury the fundamental mechanism for mediating the metabolic consequences and development of metabolic syndrome is linked to the disruption of the molecular clock in skeletal muscle. The potential uncoupling of the muscle molecular clock not only provides evidence for the development of insulin resistance, but also provides the basis for the transition of muscle fibres from type I to type II and the disruption of muscle myotube function via the down-regulation of MYOD1 function and the consequential loss of signaling to other down-stream genes and gene products.

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REFERENCES


