JOURNAL CLUB

FoxO transcription factors and endurance training: a role for FoxO1 and FoxO3 in exercise-induced angiogenesis

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Physical exercise is a stress that modulates cellular signalling pathways involved in morphological and metabolic adjustments. Chronic exercise affects phenotypic properties and the size of muscle cells, as well as the rate of protein and organelle turnover. Endurance training is assumed to promote a shift to a slower muscle phenotype by improving oxidative protein content and activity, as well as mitochondrial biogenesis and angiogenesis (i.e. the growth of new capillaries). In contrast, strength and resistance training are more related to skeletal muscle hypertrophy. For these reasons, exercise, especially endurance training, is highly recommended as a countermeasure against a broad spectrum of muscle and metabolic diseases, for example chronic obstructive pulmonary disease (COPD). This disease progressively leads to a dramatic decrease in exercise tolerance partially explained by a reduced skeletal muscle capillarization. In recent years, a plethora of studies have been conducted to identify molecular pathways underlying skeletal muscle adaptations in response to exercise. Among them, the role of forkhead box class 'other' O (FoxO) transcription factors in skeletal muscle adaptation to exercise has been increasingly considered but remained descriptive. Several studies found that a single bout of exercise induces FoxO1 and FoxO3a expression and modification of their phosphorylation levels, suggesting an increase in their activities. FoxO factors play a significant role in the regulation of muscle energy homeostasis during catabolic conditions (Sanchez *et al.* 2014*b*, for review). Thus, FoxO1 was shown to reduce carbohydrate catabolism during fasting by increasing the expression of the pyruvate dehydrogenase kinase 4, resulting in the conservation of glucose and gluconeogenic substrates (lactate, pyruvate and alanine), and a decrease in glycolytic flux by the inactivation of the pyruvate dehydrogenase complex. FoxO1 also regulates the expression of several factors involved in fatty acid oxidation (i.e. lipoprotein lipase, adiponectin receptors, and fatty acid translocase/cluster of differentiation 36), indicating that FoxO1 acts as a switch for a shift toward the use of lipids. Regarding FoxO3a, it has been recently established that, under conditions of energy stress, the mitochondrial histone deacetylase sirtuin 3 mediates FoxO3a binding to the mitochondrial DNA-regulatory regions to activate the mitochondrial genome and increase mitochondrial respiration. Among FoxO targets, FoxO1 and 3 also regulate the two main proteolytic systems, the ubiquitin–proteasome and the autophagy–lysosome pathways. It has been well documented that both acute and chronic exercise affect the activity of these systems, especially when exercise intensity or duration becomes high (Sanchez *et al.* 2014*a*, for review). In addition to these roles related to muscle energetics and protein turnover, studies performed in ischaemic muscle showed that FoxO1 and 3 promote an angiostatic phenotype through the regulation of the matrix protein thrombospondin 1 (THBS1) expression (Roudier *et al.* 2013). These data revealed a new role for FoxO factors in the process of angiogenesis. However, the importance of FoxO proteins in angiogenesis had never been studied in the context of exercise. During such a stress, angiogenesis occurs only after several weeks of repeated bouts of endurance exercise. Paradoxically, important increases in pro-angiogenic factors (i.e. vascular endothelial growth factor) take place in response to acute exercise. Taking into account that FoxO factors may be involved in the angiogenesis process, this paradox suggests that acute and chronic exercise differentially regulate the activity of FoxO proteins.

In a recent study published in *The Journal of Physiology*, Slopack and coworkers (Slopack *et al.* 2014) investigated the involvement of FoxO factors in skeletal muscle angiogenesis during exercise. The authors hypothesized that FoxO proteins would be involved in restricting the angiogenic response to endurance exercise, especially during the early days. For this purpose, mice (9 weeks old) were subjected to 7, 10 or 14 days of endurance training. Mice ran on a treadmill for 60 min per day at a speed of 25 m min^{-1}. Other mice were subjected to a single bout of the same endurance exercise. By using quantitative PCR analysis, the authors first demonstrated that acute exercise increases FoxO1 and 3 mRNA levels immediately after exercise and at 2 h of recovery. The protein levels were more elevated only at 2 h of recovery for both FoxO1 and FoxO3a. Importantly, FoxO1 phosphorylation at Thr-24, a protein kinase B (PKB/Akt)-linked inhibiting phosphorylation, was decreased at 2 h after exercise, suggesting an increase in FoxO1 activity. In agreement with this, the authors found that acute exercise increased the expression of the angiostatic protein THBS1 concomitantly to FoxO activation. It has been previously demonstrated that FoxO1 binds to THBS1 promoter to induce its expression and THBS1 induction mediates a repression of capillary growth in skeletal muscle (Roudier *et al.* 2013). Another group previously reported that THBS1 mRNA levels are elevated for 3 days after a single bout of endurance exercise (Olfert *et al.* 2006). Collectively, these data support the idea that the FoxO/THBS1 axis plays a role in restricting angiogenesis in the early days of endurance training.

Importantly, this study also showed that chronic exercise leads to an abolishment in the exercise-induced rise in FoxO protein levels from 10 days of training. This decrease in total FoxO levels was accompanied at the same time point by a decrease in nuclear content and an increase in cytosolic level of both FoxO1 and FoxO3a, indicating that chronic exercise also leads to an exclusion of FoxO proteins from the nucleus. Even if FoxO transcriptional activity was not measured, these results strongly suggest that chronic exercise progressively mediates a decrease in the functional role of FoxO proteins. Accordingly, THBS1 protein induction was abolished from 10 days of training. In order to confirm the role of FoxO proteins in THBS1 regulation and muscle capillarization during exercise, the authors used a model of mice that express reduced levels of endothelial FoxO1 and FoxO3. They found by immunoblot analysis that

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these mice present no THBS1 modulation after exercise. More importantly, mice lacking FoxO proteins showed a faster angiogenic response to endurance training. A significant increase in the number of capillaries was observed from 7 days of training whereas wild-type mice presented an increase in the angiogenic response from 14 days. Altogether, these results highlight the fact that FoxO inhibition is essential to support skeletal muscle angiogenesis in response to chronic exercise. Consistent with the model in which FoxO inhibition is important for skeletal muscle adaptation to endurance training, FoxO repression by chronic exercise may also participate in the enhancement of muscle oxidative capacity. Indeed, FoxO1 has been shown to decrease the expression of type I (slow oxidative) fibre-related genes and proteins (i.e. troponin I (slow) and myoglobin) and was associated with a diminished number of type I fibres, leading to an alteration of skeletal muscle oxidative capacity in part through inhibition of the calcineurin pathway (Sanchez *et al.* 2014*b*, for review). It would be very helpful to test if FoxO deletion affects these different pathways in the context of exercise in order to discover if FoxO inhibition affects further mechanisms related to endurance phenotype.

In conclusion, Slopack and coworkers are the first to highlight a role for FoxO transcription factors in skeletal muscle angiogenesis in response to endurance training. While a single bout of endurance exercise increases both the mRNA and protein levels of FoxO1 and 3, chronic exercise leads to a decrease in the exercise-induced rise in FoxO expression. The authors clearly demonstrated that FoxO repression is critical for training-induced angiogenesis, supporting a new role for FoxO factors in adaptation to training. As regular exercise is associated with an improved quality of life and is assumed to be one of the most effective ways to maintain muscle mass and limit metabolic disorders, these research topics are of major interest in the battle against a wide range of diseases, including COPD that presents systemic problems such as loss in muscle mass and decreased angiogenesis.

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