Sports Med (2018) 48:1329–1343
https://doi.org/10.1007/s40279-018-0883-7

REVIEW ARTICLE

Protein Availability and Satellite Cell Dynamics in Skeletal Muscle

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Published online: 20 March 2018

Abstract Human skeletal muscle satellite cells are activated in response to both resistance and endurance exercise. It was initially proposed that satellite cell proliferation and differentiation were only required to support resistance exercise-induced hypertrophy. However, satellite cells may also play a role in muscle fibre remodelling after endurance-based exercise and extracellular matrix regulation. Given the importance of dietary protein, particularly branched chain amino acids, in supporting myofibrillar and mitochondrial adaptations to both resistance and endurance-based training, a greater understanding of how protein intake impacts satellite cell activity would provide further insight into the mechanisms governing skeletal muscle remodelling with exercise. While many studies have investigated the capacity for protein ingestion to increase post-exercise rates of muscle protein synthesis, few investigations have examined the role for protein ingestion to modulate satellite cell activity. Here we review the molecular mechanisms controlling the activation of satellite cells in response to mechanical stress and protein intake in both in vitro and in vivo models. We provide a mechanistic framework that describes how protein ingestion may enhance satellite activity and promote exercise adaptations in human skeletal muscle.

Key Points

The regenerative capacity of skeletal muscle is dependent on an undifferentiated niche of myogenic-specific precursor cells, referred to as satellite cells. The role of satellite cells in skeletal muscle remodelling following exercise has long been known. However, whether dietary protein ingestion can modulate satellite cell responses is less well understood.

In vitro literature indicates that amino acids improve satellite cell dynamics; however, results in vivo remain ambiguous. Findings from human trials suggests that dietary protein may have the most pronounced effect on satellite cell activity after unaccustomed exercise when most myocellular damage and structural repair occur, but may have diminishing returns with prolonged periods of training.

The potential for protein supplementation to accelerate satellite cell responses after acute muscle damage may be of clinical and economic significance by expediting skeletal muscle remodelling processes and recovery from injury.

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1 Introduction

The regenerative capacity of skeletal muscle is dependent on an undifferentiated niche of myogenic-specific precursor cells, referred to as satellite cells. In adult skeletal muscle, satellite cells exist in a quiescent state and are located between the sarcolemma and basal lamina [1]. Classically, they are activated in response to muscle damage, such as mechanical stress caused by exercise [2–7]. Once activated, satellite cells proliferate and differentiate in order to contribute to the repair of existing muscle fibres through the formation of new myonuclei, a process known as myogenesis [8]. In turn, the addition of new myonuclei increases the transcriptional capacity of the fibre to support further hypertrophy. However, evidence for the requirement for satellite cells in supporting overload hypertrophy is equivocal. McCarthy and colleagues [9] demonstrated that in a novel mouse strain developed to deplete $>90\%$ of satellite cells, short-term (2 weeks) mechanical overload-induced hypertrophy was not blunted compared to wild type mice, suggesting satellite cells are not required for load-induced hypertrophy. In contrast, results from other investigations show that satellite cell depletion effectively attenuates muscle fibre hypertrophy over both short-term (2 weeks) [10] and long-term (8 weeks) [11] overload. While the notion that satellite cells are required to facilitate muscle growth responses is a topic of considerable debate [12–21], current evidence indicates that the presence and activation of satellite cells are obligatory for supporting training-induced adaptations.

The activation of satellite cells is influenced by the delivery of growth factors to muscle such as insulin-like growth factor-1 (IGF-1), hepatocyte growth factor (HGF) and the myokine interleukin 6 (IL-6) [22–25]. Changes to the concentrations of circulating cytokines or growth factors can induce satellite cell activation [26–29]. However, information on the effect of nutrient delivery, specifically amino acids from dietary protein consumption, on satellite cell activation is lacking. This is surprising considering the numerous studies demonstrating the stimulatory effects of protein ingestion on muscle hypertrophy with exercise [30, 31] and the purported roles of satellite cells to promote muscle hypertrophy. Given in vitro findings showing that leucine availability can promote myocyte proliferation and differentiation [32–35], protein ingestion in conjunction with exercise may provide an additional stimulus to promote satellite cell activation in vivo. This review focuses on the role of protein availability to regulate satellite cell dynamics in both cell and animal models and in the adaptive response to both resistance- and endurance-based exercise in human skeletal muscle. We discuss studies that have determined the effects of protein ingestion on satellite cell activation following exercise and provide putative mechanistic insight into the regulation of exercise adaptation responses through increased satellite cell activity with protein availability.

2 The Role of Satellite Cells in Exercise Adaptations

Adaptations to exercise training are specific to the mode, intensity, frequency and loading pattern of activity being undertaken [36, 37]. For example, endurance-based exercise classically results in increased skeletal muscle oxidative capacity and improved whole-body maximal oxygen uptake (VO_{2max}) [38, 39]. This is predominantly due to an increase in mitochondrial proteins (e.g. energy-producing oxidative enzymes) to facilitate metabolic adaptations, leading to a more fatigue-resistant muscle [40]. Conversely, resistance-based exercise (i.e. weightlifting) is characterized by its ability to induce skeletal muscle hypertrophy and maximal force-generating capacity [41], particularly via the synthesis of contractile myofibrillar proteins (e.g. myosin heavy chain proteins). Though the specificity of training produces phenotypically divergent adaptations [36, 37], both endurance and resistance exercises stimulate the turnover of skeletal muscle tissue.

Myonuclei are post-mitotic, and therefore the addition of new myonuclei to support fibre adaptations is ultimately dependent on satellite cell differentiation. Accretion of myonuclei with exercise training is assumed to accommodate the increased demands for transcriptional activity and synthesis of new proteins to support hypertrophy. It has been suggested that a single myonucleus only has control over a limited volume of cytoplasm, known as the myonuclear domain [42]. During robust hypertrophy, expansion of the myofibre volume places strain on the myonuclear domain. Accordingly, additional myonuclei are hypothesised to permit muscle fibre hypertrophy beyond a definite extent ($\sim 2250\ \mu m^2$), a postulate referred to as the 'ceiling theory' [12, 13]. Similarly, it has been speculated that only when the relative magnitude of fibre hypertrophy exceeds a certain threshold ($\geq \sim 25\%$ of cross-sectional area) are additional myonuclei required to sustain growth [43]. However, myonuclear accretion has been observed during periods of hypertrophy ($\sim 18\%$ of cross-sectional area) where this threshold is not met [44]. Furthermore, myonuclear content and fibre size are linearly related, whereas myonuclear domain and fibre size share a logarithmic relationship, with smaller fibres possessing disproportionately smaller myonuclear domains [20]. Though the reason for this relationship is unclear, it indicates that myonuclear domains may be different between smaller and larger fibres, and raises questions as to the scope of satellite cell behaviour dictated by previously established thresholds.

In human skeletal muscle, the activation of satellite cells following resistance exercise is well accepted [4, 6, 7, 13, 14, 16, 18, 23, 43–50]. Following a single bout of resistance exercise, increases in satellite cell proliferation are typically detectable after 24 h, with these responses peaking 72 h post–exercise [51]. However, the precise timing of initial satellite cell proliferation is equivocal, and early ($\leq$ 24 h) increases in satellite cell number may likely be due to an increased cell size prior to division, as suggested by ex vivo data [52, 53], which may increase the likelihood of detection though immunohistochemistry. Nevertheless, a positive correlation exists between satellite cell-mediated myonuclear accumulation and muscle fibre hypertrophy [12, 13, 15, 16, 18, 54], which has led some [13, 16] to hypothesise that an individual's 'responsiveness' to resistance exercise may be based on satellite cell activation. Indeed, Petrella and colleagues [13] reported that individuals with the highest basal quantity of satellite cells achieved the greatest magnitude of myonuclear addition and hypertrophy after 16 weeks of resistance training. Bellamy and associates [16] also demonstrated that an acute expansion of the satellite cell pool, rather than basal number, after a single bout of resistance exercise was associated with the magnitude of hypertrophy achieved over 16 weeks of resistance training. However, it should be noted that Petrella and colleagues [13] used the membrane-bound satellite cell marker neural cell adhesion molecule (NCAM), while Bellamy and colleagues [16] used the paired-box transcription factor Pax7, which is confined to the satellite cell nucleus. As a result of their cellular locations, staining of successive 7-µm cryosections shows that the same satellite cell is detectable through only two sections for Pax7, whereas NCAM is detectable through four or five sections [55]. Thus, discrepancies regarding baseline satellite cell enumeration between studies may be attributable to inherent differences in staining profiles of satellite cell markers and thickness of cryosections. Irrespective of the marker used, these data collectively suggest that satellite cell activation and muscle fibre size may be closely related over chronic periods of resistance training.

While the majority of investigations that have determined the role of satellite cells in adaptations to exercise have focused on muscle hypertrophy, less is known regarding the role of satellite cells during less 'anabolic' stimuli, such as endurance exercise and high-intensity interval training [4, 5, 45, 56–60]. However, recent evidence suggests a contribution of satellite cells to muscle fibre remodelling in the absence of hypertrophy [5, 57]. Following 6 weeks of sprint cycle interval training (10×60 s at $\sim$ 90% of maximal heart rate, three times per week) in untrained women, the number of satellite cells associated with hybrid fibre types (type I/II myosin heavy chain isoforms) increased as a mechanism hypothesised to assist in fibre type remodelling [5]. Similarly, both continuous moderate-intensity and high-intensity sprint interval cycle training have been shown to increase the number of activated and differentiating satellite cells post-exercise without an expansion of the satellite cell pool or myonuclear content [57]. Though less predominant, unaccustomed aerobic training can result in muscle hypertrophy that is accompanied by increases in both satellite cell and myonuclear content in type I fibres [45, 60]. While discrepancies regarding the increase in myonuclear content may have been driven by the robust hypertrophy seen in the latter investigation, these studies collectively demonstrate the recruitment of satellite cells in response to endurance-based exercise stimuli. Though the heightened activity and constant turnover of satellite cells in the absence of hypertrophy remains ambiguous, it may be required for myonuclear turnover during enucleation processes [61] or regulation of the extracellular matrix required during myofibre remodelling [62].

The molecular basis for this response with both endurance and sprint interval exercise may centre on the activation of the transcriptional coactivator peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α) (Fig. 1). As a key regulator in endurance exercise adaptations through its co-activation of several DNA binding transcription factors including the nuclear respiratory factors (NRF-1 and NRF-2) [63] and peroxisome proliferator activated receptors (PPARs) [64], PGC-1α may play a role in regulating satellite cell activation by increasing both the mitochondrial content and activity of satellite cells [52]. Additionally, PGC-1α may also be involved in remodelling the extracellular matrix composition, thereby improving the propensity for satellite cells to proliferate [65]. However, several isoforms of PGC-1α are known to exist and are differentially activated based on the mode of exercise performed [66]. For example, PGC-1α4 becomes activated only after resistance or combined resistance and endurance exercise (termed concurrent exercise) and promotes muscle fibre hypertrophy [66]. Whether the effects of PGC-1α on satellite cell regulation are isoform-specific is currently unknown. Similarly, the transcription factor prospero-related homeobox-1 (Prox1) has been proposed as a critical regulator of satellite cell differentiation in slow-twitch type I fibres, while also being responsible for fast- to slow-fibre type gene programming through modulation of the nuclear factor of activated T-cells (NFAT) signalling pathway [67]. Whether endurance exercise modifies Prox1 activity has yet to be determined. Indeed, the precise role(s) of satellite cells during adaptation to endurance training requires further investigation. Notably, an acute bout of concurrent exercise impairs satellite cell proliferation [4]. While this

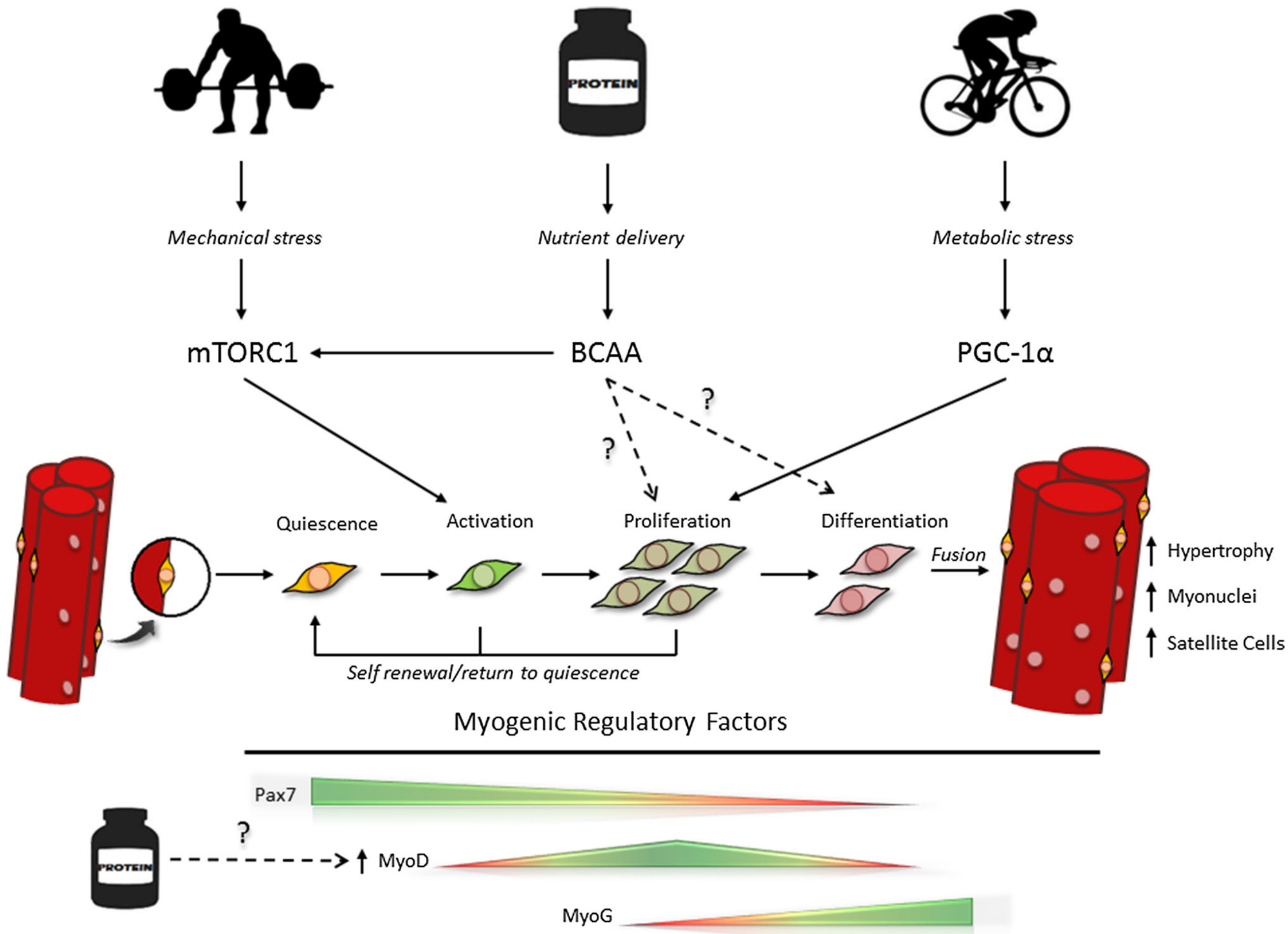

Fig. 1 Graphical representation of the potential mechanistic underpinning for satellite cell stimulation by resistance exercise, endurance exercise, and protein ingestion, as well as the expression pattern of associated transcription factors based on evidence presented from in vitro and murine models. Following a bout of resistance exercise, mechanical stress results in the activation of the mechanistic target of rapamycin complex 1 (mTORC1), which, in turn, assists in the transition of satellite cells from a quiescent state into an active state. Upon activation, satellite cells can either continue along the path of myogenic commitment to proliferate into myoblasts, or return to quiescence and self-renew to maintain the satellite cell pool. Metabolic stress caused by endurance exercise stimulates the activity of the transcriptional coactivator peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α), which can promote the proliferation of satellite cells. Protein/branch chained amino acid (BCAA) supplementation may enhance both proliferation and differentiation of satellite cells. Though the mechanisms are not fully understood, potential pathways of satellite cell modulation through protein/BCAA supplementation have been included as dashed arrows. Myogenic regulatory factor expressions are present in higher levels (green) through specific stages and become suppressed (red) as the myogenic process advances as depicted by the shift from green to red in representative expression bars. Solid black arrows indicate increases/activation of downstream target proteins/ processes

attenuated response may be linked to the ‘interference’ in muscle hypertrophy typically observed when resistance and endurance exercise are performed concurrently over several months, the precise mechanisms directing this response and whether this phenomenon manifests after a chronic concurrent training program (i.e. 12–16 weeks) is unknown.

3 The Impact of Protein Ingestion on Satellite Cell Responses to Exercise

Dietary protein is a critical substrate for providing amino acids to facilitate skeletal muscle repair and regeneration during recovery from exercise. Accordingly, sufficient protein needs to be consumed to facilitate the synthesis of new proteins during the immediate (2–3 h) post-exercise recovery period, which provides the basis for both resistance and endurance training-induced adaptations in skeletal muscle [68–70]. Moreover, the addition of new satellite cell-derived nuclei through exercise-induced myonuclear turnover is essential to the continued contribution of genetic information for protein synthesis [71]. Several interrelated factors including the dose [72], type [73], timing [74] and distribution [75, 76] of protein ingestion directly impact the anabolic effects of post-exercise protein ingestion. An in-depth discussion on these factors is beyond the scope of this review and readers are referred to several comprehensive reviews on this topic [77–79].

3.1 In Vitro and Animal Models of Satellite Cell Activity in Response to Amino Acids

Work from as early as the 1970s reported the branched-chain amino acid (BCAA) leucine accelerates muscle regeneration in crushed animal skeletal muscle [80]. In vitro-based models demonstrate C2C12 myoblast proliferation and differentiation enhanced with BCAAs [35] or leucine supplementation alone [34]. Leucine treatment has also been shown to promote myotube formation and increase MyoD and myogenin (MyoG) expression in primary preterm rat satellite cells [33], while leucine withdrawal from culture media blunts C2C12 myoblast and primary satellite cell differentiation [32]. Kornasio and colleagues [81] investigated the effects of adding various concentrations of the leucine metabolite β-hydroxy-β-methylbutyrate (HMB) on serum–starved myoblasts and observed enhanced proliferation, differentiation and accelerated fusion, indicating a capacity for HMB to drive quiescent adult myoblasts into the cell cycle. Similarly, HMB supplementation in neonatal pigs results in increased satellite cell proliferation and protein synthesis [82] during a period of rapid growth that is accompanied by myonuclear addition [83], and may serve as an effective strategy to increase muscle mass in clinical settings such as low-birth-weight or preterm births.

Leucine induces hypertrophy on tissue engineered skeletal muscle as evidenced by increases in myotube width in supplemented constructs compared to a rapamycin control [84]. With regard to animal models, Alway and co-workers [85] reported enhanced muscle stem cell proliferation exclusively in type II skeletal muscle of aged rats during recovery from disuse with HMB supplementation. Leucine ingestion has also been shown to improve muscle force production and increase the number of proliferating satellite cells of regenerating young and old skeletal muscles after cryolesion independent of modulating rates of muscle protein synthesis [86]. Collectively, these findings provide strong evidence for a beneficial effect of leucine supplementation on muscle regenerative processes.

Recently, Rodgers and colleagues [52] demonstrated that the leucine-sensitive mechanistic target of rapamycin complex 1 (mTORC1) controls the transition of satellite cells between a quiescent and an initial 'alert' phase of the cell cycle in mice. This finding is noteworthy as subsequent investigations have demonstrated that mTORC1 signalling is rapidly activated during skeletal muscle regeneration [87] and is not only required for the adaptive transition of cell cycle phases, but necessary for satellite cell proliferation, differentiation and overall skeletal muscle regeneration [88, 89]. Given the ability of leucine to both activate mTORC1 directly [90] and promote proliferation and differentiation in vitro through an mTORC1-MyoD cascade [33], protein ingestion in conjunction with an appropriate exercise stimulus may provide an additional signal to promote satellite cell activation in vivo (Fig. 1).

3.2 Satellite Cell Activity in Response to Protein Availability in Human Skeletal Muscle

To date, few studies have investigated the interaction between protein supplementation and satellite cell activity in human skeletal muscle. In the following section, we discuss the acute (defined here as a single exercise session), short-term (<2 weeks exercise training), and chronic (>2 weeks training intervention) exercise protocols that have determined the effects of protein ingestion/supplementation on markers of satellite cells activity in human skeletal muscle. We also review studies that have investigated how acute and short–term protein restriction can impact satellite cells activity.

3.2.1 Acute and Short-term Exercise

Following a single bout of resistance exercise in elderly men, Hulmi and colleagues [91] reported that the ingestion of 15 g of whey protein immediately before and after exercise increased the gene expression of myogenic regulatory factors and cell cycle regulators in the 48 h post-exercise (Table 1). Likewise, in elderly men, ingesting 10 g of essential amino acids after a single bout of resistance exercise increased the number of proliferating satellite cells during 24 h of post-exercise recovery compared to a non-caloric placebo beverage [92]. Specifically, an increase in the number of MyoD^{+} cells was observed only in the essential amino acid supplemented condition at 24 h post-exercise. Likewise, only essential amino acid supplementation resulted in an increase in Pax7^{+}/Ki67^{+} cells post-exercise, which was significantly greater than the placebo condition. Though an increase in type I satellite cell content was observed with essential amino acid supplementation at 24 h, there was no difference in satellite cell content of type II fibres between groups. Similarly, when all myofibre types were pooled, no significant difference in satellite cell content was apparent between groups. There may be several explanations for these

Table 1 Change in myogenic gene expression in human skeletal muscle in response to acute and short-term high and low protein intakes with exercise

Publication [Refs.]	*N* (Sex)	Age	Exercise mode	Daily protein intake ($g \cdot kg^{-1} \cdot day^{-1}$)	Protein bolus (g)	Satellite cell gene marker	0.5 h	1 h	2 h	4 h	6 h	12 h	24 h	48 h	72 h
Hulmi et al. [91]	18 (M)	62 ± 4	RES	1.2	30	MyoD		↔					↔	↔	
						MyoG		↔					↔	↔	
						cdk2		↔						↑ ~ 250%	
						MSTN		↔					↔	↔	
Roberts et al. [96]	10 (M)	22 ± 4	RES	Not specified	25	MyoD			↔		↔				
						p21			↔		↔				
Snijders et al. [7]	12 (M)	21 ± 2	RES	0.1	N/A	MyoD						↔	↔	↔	↔
						MyoG						↔	↔	↔	
						MSTN						↔	↔	↔	
Rowlands et al. [95]	12 (M)	30 ± 7	END	Not specified	70	MyoD1	↑ ~ 120%								
						MyoG				↑ ~ 120%					
Reidy et al. [92]	19 (M)	72 ± 2	RES	Not specified	10 (EAA)	Pax7							↔		
						MyoD		↑ ~ 64%							

Arrows pointed upwards (↑) indicate a significant increase relative to non-protein control at each time point. Arrows pointed left–right (↔) indicate no difference between protein supplement and controls at each time point. Exercise modalities are abbreviated as RES (resistance training) or END (endurance training). Essential amino acid supplementation is abbreviated as EAA findings. First, the timing of analysis may have been too early to detect new satellite cells which typically occurs later (i.e. 48–72 h) in human skeletal muscle [51]. Second, two separate essential amino acid supplements were used and were not matched for amino acid composition, particularly leucine (1.85 g, $n = 4$ vs. 3.5 g, $n = 7$). Third, immunohistochemistry was only performed on nine participants in the essential amino acid group and five control participants and thus may have underpowered the analysis. Nevertheless, it appears that essential amino acids can accelerate proliferation compared to a placebo. In line with these findings, consumption of 28 g of protein during the post-exercise recovery period increased satellite cell content compared to a placebo control for up to 48 h in healthy young men [6] (Table 2). Notably, exercise alone was unable to stimulate a satellite cell response in the placebo group. This is surprising given previous investigations have shown robust satellite cell proliferation within 48 h of completing exercise in the absence of protein supplementation [25, 93]. However, it may be that a delayed response occurred in the placebo group as others have shown satellite cells to accumulate as late as 4–8 days after exercise [2, 94]. When considering the homogeneity in number of satellite cells between the protein and placebo group at 168 h, it is possible that the satellite cell response was not completely captured across the selected sampling time points, highlighting the difficulty with biopsy sampling collection for timing of satellite cell proliferation.

Consuming a 70-g bolus of milk protein (providing 15 g of leucine) following prolonged endurance exercise upregulates MyoD and MyoG gene signalling networks in the first 4 h of post-exercise recovery compared to a lower protein intake (23 g, providing 5 g of leucine) or an isoenergetic carbohydrate placebo [95]. Though not a direct measure of satellite cell content, the augmented gene expression of these myogenic regulatory factors may be indicative of a greater propensity for satellite cell proliferation and differentiation. Collectively, the results from these studies suggest that protein ingestion may accentuate myogenic regulatory factor gene expression and promote satellite cell activation and proliferation following single bouts of resistance and endurance–based exercises.

Not all findings have been analogous amongst all studies. For example, in a crossover study in which healthy young men completed three separate bouts of lower-body resistance exercise before ingesting 25 g of whey protein isolate or placebo (maltodextrin or artificial sweetener) beverages, no differences in MyoD mRNA expression were observed between conditions [96]. While not directly measured, the isolated gene expression of MyoD suggests an absence of satellite cell proliferation. Similarly, results from a crossover trial in a cohort of well-trained cyclists (VO_{2max} $\sim$ 63 ml·kg^{-1}·min^{-1}) performing 10 days of intensified cycle training (120% of average daily training volume) followed by a period of reduced volume training ($\sim$ 60% of average daily training volume) in combination with intra-session (38 g) and post-session (29 g) whey protein or carbohydrate placebo supplementation have shown limited effects of protein availability on satellite cell function [60]. Specifically, following intensified training with protein supplementation, an increase in the number of satellite cells associated with type I fibres in the absence of myonuclear addition was observed, whereas the carbohydrate placebo condition elicited a rapid increase in both type I satellite cells and myonuclear density [60]. Additionally, both satellite cell and myonuclear number

Table 2 Change in satellite cell content measured through immunohistochemistry (IHC) in human skeletal muscle in response to acute and short-term high and low protein intakes with exercise

Publication [Refs.]	N (Sex)	Age	Exercise mode	Daily protein intake (g·kg^{-1}·day^{-1})	Protein bolus (g)	Satellite cell IHC marker	12 h	24 h	48 h	72 h	168 h
Farup et al. [6]	24 (M)	23 ± 1	RES	1.2	28	Pax7$^+$		↔	↑ ~ 67%		↔
Snijders et al. [7]	12 (M)	21 ± 2	RES	0.1	N/A	Pax7$^+$	↔	↔	↔	↔	
McKenzie et al. [60]	6 (M) / 2 (F)	25 ± 7	END	2.6	67	Pax7$^+$			↔		
Reidy et al. [92]	19 (M)	72 ± 2	RES	Not specified	10 (EAA)	Pax7$^+$		↑ ~ 100% (type I)			
						Pax7$^+$/Ki67$^+$		↑ ~ 300%			
						MyoD$^+$		↔			

Arrows pointed upwards (↑) indicate a significant increase relative to non-protein control at each time point. Arrows pointed left–right (↔) indicate no significant difference between protein supplement and control at each time point. Values are presented for mixed muscle fibers, unless specified. Exercise modalities are abbreviated as RES (resistance training) or END (endurance training). Essential amino acid supplementation is abbreviated as EAA increased following reduced volume training in the carbohydrate placebo condition. Whether a similar response is also apparent in the protein condition was not determined due to insufficient tissue yield from the small sample size ($n = 8$). Nonetheless, protein supplementation resulted in type I and II myofibre hypertrophy [97] following intensified training. Collectively, the increase in satellite cell content and myofibre hypertrophy suggests that protein supplementation may be beneficial for skeletal muscle during periods of heavy endurance training.

While the factor(s) responsible for discrepant outcomes between acute exercise trials is unclear, they may partially be explained by inherent differences in study design and methodology (i.e. type and volume of exercises performed, training status of participants, sex of participants, biopsy timing, analytical measurements, etc.). In particular, several investigations conducted satellite cell analyses as secondary measures and selected biopsy time points around separate primary outcomes (such as cell signalling and gene expression, as well as muscle protein synthesis analyses). As a result, measurement time points across the aforementioned studies ranged from 0 to 144 h post-exercise (Table 2). Likewise, the use of different satellite cell markers (i.e. Pax7 vs. NCAM, gene vs. protein, etc.) between studies can also introduce considerable variability due to potential issues with differences in antibody sensitivity and detection between markers. Furthermore, satellite cell populations are heterogeneous in their expression of different molecular markers and using a single molecular marker for their identification may underestimate total satellite cell content [98, 99]. Therefore, multiple labelling methods should be implemented to improve detection of subpopulations of satellite cells progressing through terminal differentiation (i.e. $Pax7^{-}/NCAM^{+}$) [98]. Although multiple labeling will most likely provide a more comprehensive identification of the total satellite cell pool, use of multiple markers in immunohistochemistry can be cumbersome, especially when combining multiple nuclear markers with surface proteins for activation status and myosin heavy chain isoforms for fibre type-specific analysis. Such inconsistencies in methodological approaches highlight the need to design studies with satellite cell dynamics as primary outcomes and establish consistent analytical techniques between investigations in order to accurately evaluate satellite cell responses to exercise.

3.2.2 *Chronic Training*

Olsen and colleagues [50] were the first to demonstrate that chronic protein supplementation in combination with strength-based resistance training amplifies the expansion of satellite cell and myonuclei numbers in human skeletal muscle compared to a placebo control (Table 3). In that study, healthy young male participants performed lower body periodised strength training (external loads corresponding to 6–12 repetition maximum) three times per week and consumed 20 g of cow milk protein in close proximity to each training session (10 g pre- and 10 g post-exercise) and once daily on non-training days. Whilst robust increases in muscle fibre cross-sectional area were observed both with and without protein supplementation, the increase in number of satellite cells per fibre was significantly greater with protein supplementation. However, data on habitual dietary intake for participants was not provided, making it unclear whether the larger expansion in satellite cell content was a result of a greater daily protein intake or due to protein availability in close proximity to exercise. Furthermore, whether protein feeding influenced satellite cells in a fibre-type-specific manner was not determined. Nonetheless, findings from this study provided the first evidence that consuming a bolus amount of additional protein around resistance exercise bouts could augment long-term training-induced satellite cell expansion and yield concomitant increases in myonuclear accretion and fibre hypertrophy.

To further explore how increased protein availability may influence satellite cell numbers in response to chronic resistance training, Farup and colleagues [100] investigated the effect of contraction mode (i.e. concentric vs. eccentric) on fibre type specific satellite cell response in the presence of a protein supplement. Using a within-subject design, healthy young male participants undertook 12 weeks of unilateral resistance training of the knee extensors, three times per week, with one leg performing eccentric (lengthening) contractions only and the contralateral leg performing concentric (shortening) contractions only. For the duration of the training program, participants were randomised into either a protein supplement ($\sim$ 20 g of whey protein) or a control group (isocaloric carbohydrate placebo). On all training days, participants ingested half of their supplement before and the remaining half after training (providing $\sim$ 10 g of protein pre- and $\sim$ 10 g of protein post-exercise). Though both protein and placebo supplementation resulted in equivalent increases to type I fibre cross-sectional area and number of satellite cells per unit of fibre cross-sectional area, protein supplementation elicited a significantly greater satellite cell expansion compared to the placebo group. Though not directly measured, the greater increase in satellite cell content with concentric contractions was hypothesised to be caused by the larger metabolic demands and greater transcription of IGF-1 with concentric versus eccentric contractions. Additionally, concentric contractions combined with protein supplementation lead to increases in type II fibre cross-sectional area with parallel myonuclear accretion. Notably, a similar degree of myonuclear addition was also observed

Table 3 Change in satellite cell and myonuclear content measured through immunohistochemistry in human skeletal muscle in response to chronic resistance exercise with protein supplementation

Publication [Refs.]	*N* (Sex)	Age	Intervention length (weeks)	Daily protein intake ($g \cdot kg^{-1} \cdot day^{-1}$)	Protein bolus (g)	Biopsy time (h)	Satellite cell marker	Satellite cell density	Myonuclear density
Olsen et al. [50]	32 (M)	24 ± 2	16	Not specified	20	N/A	NCAM	↑ ~ 39%	↔
Molsted et al. [105]	16 (M) / 13 (F)	55 ± 14	16	1.3	9.4	48–72	Pax7	↔	↔
Mobley et al. [101]	75 (M)	21 ± 1	12	1.95	26	72	Pax7	↑ ~ 67%	↔
Reidy et al. [18]	54 (M)	25 ± 1	12	1.6	22	72	Pax7	↔	↔
Dirks et al. [17]	12 (M) / 22 (F)	77 ± 1	24	1.3	30	72	Pax7	↔	↔
Reidy et al. [103]	9 (M) / 14 (F)	23 ± 1 and 66 ± 1	8	Not specified	17	72–120	Pax7	↔	↔
Farup et al. [100]	22 (M)	24 ± 1	12	Not specified	19.5	72–144	Pax7	↑ ~ 25% (type I)	↔

Arrows pointed upwards (↑) indicate a significant increase relative to non-protein control at each time point. Arrows pointed left–right (↔) indicate no significant difference between protein supplement and control at each time point. Values are presented for mixed muscle fibres, unless otherwise specified

in type II fibres in the absence of hypertrophy with eccentric training in the placebo group. While it is unclear why nutrient intake resulted in contraction mode specific changes to myonuclear content, the similar increase in type II fibre myonuclei suggests any potential ergogenic effects of protein to drive hypertrophy may not have been responsible for myonuclear addition. However, information regarding changes to myonuclear domain were not presented and therefore cannot be ruled out as a possible explanation for expansion of myonuclear number. Nevertheless, the results provide further evidence for the consideration of protein supplementation to augment satellite cell content with chronic training.

The findings of Farup and colleagues [100] raise the possibility that increasing supplemental protein availability around concentric-based exercise could amplify long-term training-induced increases in satellite cell and myonuclei numbers and promote fibre hypertrophy. It has previously been reported that an increase in myogenic gene expression and satellite cells associated with type I fibres manifests after acute bouts of cycling exercises [60, 95]. Given the reliance upon type I fibres for aerobic-based contractile activity, consumption of additional protein after endurance exercise may be a useful strategy to promote type I fibre hypertrophy and myonuclear turnover to support tissue repair through increased satellite cell proliferation. To date, no investigation has assessed the effects of chronic endurance training with protein supplementation on satellite cell function, and is an area that deserves further attention.

The type of protein ingested has also recently received attention with regard to satellite cell response to chronic resistance training in young healthy men [101]. In a study involving 12 weeks of periodised whole-body resistance training, participants were randomly allocated to either a leucine supplementation (~ 3 g), one of two leucine-matched protein supplements (whey protein: ~ 26 g, or soy protein: ~ 39 g), or a carbohydrate placebo supplement (~ 44 g) condition to be consumed twice daily that resulted in habitual daily protein intakes of ~ 1.35, ~ 1.95, and ~ 1.3 $g \cdot kg^{-1} \cdot day^{-1}$, respectively, throughout the intervention. Regardless of dietary condition, all participants increased total lean body mass and muscle strength, as well as fibre cross- sectional area and myonuclear number in both type I and II fibres at the end of the 12 weeks. However, only participants consuming whey or soy protein supplements significantly increased satellite cell count (~ 94%) in mixed muscle fibre types. These results suggest that consumption of intact protein influences the satellite cell response to chronic training.

Not all studies, however, have reported added benefits of protein supplementation on satellite cell activity during periods of chronic resistance training in young men [18, 102, 103] or elderly men and women [17]. Work from Reidy and colleagues [18] found 12 weeks of resistance exercise training (including both concentric and eccentric contractions) in the presence of protein supplementation (22 g of either whey or a soy-dairy protein blend ingested immediately post-exercise on training days, and once between meals on non-training days) resulted in similar increases in mean fibre satellite cell content, proportion (percentage of satellite cells per myonuclei), and domain (satellite cells per mm^2) compared to an isocaloric maltodextrin placebo condition. Notably, habitual protein intake for participants across conditions in this study was $\sim$ 1.3 $g{\cdot}kg^{-1}{\cdot}day^{-1}$, and was increased to $\sim$ 1.6 $g{\cdot}kg^{-1}{\cdot}day^{-1}$ in the protein supplemented group. The authors concluded that habitual protein intake without supplementation was sufficient to promote skeletal muscle remodelling and satellite cell activity following chronic resistance training. Therefore, it appears that when adequate protein is available, additional protein supplementation is otherwise of negligible benefit. However, the authors did observe a trend for greater satellite cell content increases in myosin heavy chain type I fibres with protein supplementation compared to the placebo control. Thus, it is also plausible that these studies did not observe any added (or synergistic) benefits of protein supplementation due to the high individual variability in responses to protein ingestion and potential low effect size for protein supplementation to enhance muscle anabolism and associated satellite cell responses [104].

Recent work from Dirks and associates [17] examined whether protein supplementation over a 24-week whole-body resistance training program in frail elderly men and women modulates satellite cell content. Participants were randomly allocated to either a protein (30 g of milk protein) or placebo (non-protein-containing dairy beverage) supplement group and trained twice weekly over the course of the intervention. Baseline habitual protein intake for participants was 1.0 $g{\cdot}kg^{-1}{\cdot}day^{-1}$ and was increased to 1.3 $g{\cdot}kg^{-1}{\cdot}day^{-1}$ in the protein-supplemented group. While there was a trend for muscle fibre hypertrophy in the placebo group after training (no change in type I and $\sim$ 20% in type II; $P = 0.051$), only significant hypertrophy was observed in the protein-supplemented group ($\sim$ 23% in type I and $\sim$ 33% in type II, $P < 0.01$). Despite the marked increase in fibre cross-sectional area, no changes in satellite cell or myonuclear content were observed in either group. The authors attribute the lack of changes in satellite cell and myonuclear content to smaller baseline myonuclear domains, which may have allowed fibre hypertrophy to occur without the need for additional myonuclei. These findings are in contrast to previous reports in elderly individuals [14], whereby resistance training-induced hypertrophy is accompanied by an increase in satellite cell content.

Incorporating protein supplementation to chronic exercise rehabilitation programmes following short-term bed rest has also been equivocal [103]. Following 5 days of bed rest, both young and older adults completed 8 weeks of eccentric knee extensor training three times per week. During the rehabilitation programme, half of the young participants and all of the older participants were provided with 17 g of a BCAA-enriched (4.6 g leucine, 2.4 g isoleucine, 2.3 g valine) whey protein. Though only the older participants increased myofibre cross-sectional area from the cessation of bed rest to completion of training, all participants demonstrated significant increases in satellite cell density and number per fibre, regardless of protein supplementation. Despite no further benefit of protein supplementation to satellite cell content in the young cohort, these results are inconclusive with regard to older individuals as there was no non-supplemented older participant group to determine whether the satellite cell response in these older subjects was solely due to exercise training, protein supplementation or a combination of the two.

Protein supplementation in combination with 16 weeks of resistance exercise in clinical populations undergoing dialysis has also shown no effect on satellite cell content, myonuclear number, or myonuclear domain compared to a placebo condition [105]. In this study, habitual protein intake for participants was 1.3 $g{\cdot}kg^{-1}{\cdot}day^{-1}$ in both groups, and was unchanged with the additional 9.4 g of whey protein ingested by the protein supplement group. Considering the equivalent daily protein intake between conditions, it is possible that the protein supplement consumed may not have been an effective dose to elicit a meaningful change to satellite cell activity given the relatively low leucine content. However, a dose-response study has yet to be performed to determine if a protein threshold exists to stimulate satellite cell activity. Additionally, dialysis patients have reduced type I fibre satellite cell content, but not fibre area or myonuclear content, compared to healthy untrained men [106]. Consequently, the satellite cell pool of dialysis patients may be under excessive stress in order to maintain fibre size and myonuclear numbers. Accordingly, the results related to satellite cell content/activity from investigations involving disease-states must be interpreted with caution when making comparisons to healthy populations.

Protein overfeeding ($\sim$ 2.5 $g{\cdot}kg^{-1}{\cdot}day^{-1}$) has little effect on markers of satellite cell activity [102]. Following 8 weeks of whole-body resistance training in healthy young men supplemented daily with either a mixed protein-carbohydrate-fat beverage (94, 196 and 22 g of protein, carbohydrate and fat, respectively) or carbohydrate beverage (312 g) before and after each resistance exercise session, no changes to c-Met content, a proxy for satellite cell quantification, were observed in either condition. However, c-Met is expressed in several epithelial cell types and is not exclusive to satellite cells [99]. Thus, without having also directly measured satellite cell specific markers (i.e. Pax7), it is unclear whether the training stimulus or protein supplementation affected satellite cell content. Nevertheless, the findings from this study suggest that high protein intakes provide no benefit to satellite cell responses during chronic training.

3.2.3 Protein Restriction

Several studies have investigated the effects of protein restriction on satellite cell activity in human skeletal muscle. Four days of severe protein restriction (0.1 $g \cdot kg^{-1} \cdot day^{-1}$) in healthy young men had little impact on satellite cell content during post-exercise recovery following a single bout of resistance exercise compared to a protein intake of a 1.2 $g \cdot kg^{-1} \cdot day^{-1}$ [7] (Table 2). While there were no differences in satellite cell content or myogenic regulator factor gene expression between the low and higher protein diets over the 72-h post-exercise recovery period, a pronounced reduction in the number of satellite cells expressing myostatin protein was observed in the low protein group at 72 h. Myostatin is a member of the transformation growth factor-β (TGF-β) superfamily and is known to be a negative regulator of satellite cell activity [107, 108] as well as muscle protein synthesis [109]. The authors speculated that in the absence of dietary protein, the co-localization of myostatin with satellite cells remains repressed for a prolonged period as a compensatory mechanism to allow muscle remodelling to occur when an adequate concentration of amino acids becomes available. To conclude that protein has no effect on satellite cell activity based on studies in which protein intake has been severely restricted may be an over-simplification. Previous reports have indicated that short-term (7–18 days) protein restriction results in decreased transcription of genes associated with satellite cell proliferation and increased transcription profile of genes related to ubiquitin-dependent protein catabolism and apoptosis [110, 111]. Thus, it would appear that several protective mechanisms exists to allow for skeletal muscle remodelling during an acute period of protein deprivation, although these mechanisms appear to be down-regulated over time.

4 Conclusions and Future Directions

The role of satellite cells in skeletal muscle remodelling has long been known. However, the role for protein ingestion to modulate satellite cell activity is less well understood. Based on current in vitro literature, there are clear indications that amino acids improve satellite cell activity; however, results from in vivo work remain ambiguous. Data from human trials suggest that dietary protein has the most pronounced effect on satellite cell activity under acute exercise conditions, but may have diminishing returns with prolonged periods of training (i.e. months or years). One potential explanation for this is that acutely increasing dietary protein intake simply accelerates the myogenic response to exercise, likely through increasing MyoD gene expression, which will eventually be reached with adequate protein intake (Fig. 1). Further, the effects of protein on satellite cell response after initiating unaccustomed exercise training, when most myocellular damage occurs, is most pronounced during acute structural repair to combat unfamiliar stress and may not be predictive of long-term responses [112]. In this regard, these potential acute effects with protein supplementation may be of clinical and economic significance by enhancing skeletal muscle remodelling processes that reduce injury occurrence, muscle damage and soreness [113]. Macrophage activity is also closely tied to satellite cell activity and may be modulated with amino acid availability [95, 114]. Thus, the regulation of immunity pathways with protein ingestion following unaccustomed exercise stimuli may in part be responsible for accelerated satellite cell activity.

Little is currently known about the potential mechanistic bases that may govern enhanced satellite cell dynamics with protein ingestion following either resistance or endurance exercise. Therefore, an emphasis on designing studies in which satellite cell responses (i.e. time course of response) are primary outcome measures is essential to critically evaluate such mechanisms. Future studies in which diets are tightly monitored by daily food records in conjunction with supervised exercise training are also required to advance the current understanding of how nutrition (specifically protein) can stimulate satellite cell contribution to support exercise adaptations. Similarly, how variable protein intake affects satellite cell activity in response to divergent modes of exercise (e.g. resistance, endurance or combined resistance and endurance) is a topic that warrants further exploration. In this regard, we have previously shown protein ingestion following a bout of concurrent resistance and endurance exercise increases rates of muscle protein synthesis and attenuates markers of muscle catabolism compared to a placebo control [70]. Whether increased protein availability during a chronic concurrent training program can rescue the inhibition of satellite cell activity previously observed after a single bout is unknown. Thus, future investigations combining concurrent exercise and protein consumption with regards to satellite cell activity are needed to improve the translation from research to practice when prescribing exercise and dietary interventions to promote skeletal muscle health and quality with this training modality. Likewise, how the distribution of daily protein intake affects satellite cell activity after exercise is currently unknown. Low protein diets affect satellite cell activity and this has important implications for clinical populations. Accordingly, studies are needed to determine how changes to feeding patterns may impact the time course of satellite cell activity and skeletal muscle remodelling. Additionally, whether specific amino acids have potential regulatory roles in the return of satellite cells to quiescence is unknown and deserves consideration to improve our understanding of how satellite cells maintain regenerative capacity. Finally, a better understanding of the association, if any, between amino acid transporter expression/activation and satellite cell activity is warranted to determine whether the capacity for these transporters may be a limiting factor for the inward transport of amino acids to subsequently regulate satellite cell dynamics.

Acknowledgements D.M.C is supported by an Australian Catholic University Research Funding Grant (#36331).

Compliance with Ethical Standards

Funding No financial support was received for the preparation of this manuscript.

Conflict of interest Baubak Shamim, John A. Hawley and Donny M. Camera declare that they have no conflicts of interest.

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