nuclear domain ceiling" exists such that additional increases in myofiber cross-sectional area require myonuclear addition. Accordingly, satellite cell-independent and -dependent phases of myofiber growth occur as shown by analyzing irradiated soleus muscles at multiple time points after a growth stimulus (17). As distinct phases of muscle growth exist, multiple time points are necessary to conclusively determine the role of satellite cells in any hypertrophic model. Although some studies concluded that satellite cells are not required for hypertrophy (6, 7, 16), their analyses were limited only to early times points after the application of a growth stimulus.

What about human muscle hypertrophy? Mechanistic studies can only be performed in animal models where anti-proliferative treatments can be administered or in transgenic models displaying defects in satellite number or function. Thus human studies are limited to demonstrating correlative changes in myonuclear number with increases in myofiber size (5, 11, 19, 25). Myofiber hypertrophy in humans independent of myonuclear addition (10, 12, 19) may be due to not having reached a set myonuclear domain ceiling. A theoretical myonuclear domain ceiling of ~2,000 μm^2 has been recently proposed beyond which myonuclear addition occurs (19).

In summary, we believe satellite cell myogenesis is necessary for muscle hypertrophy at later phases of growth to maintain a constant myonuclear domain.

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COUNTERPOINT: SATELLITE CELL ADDITION IS NOT OBLIGATORY FOR SKELETAL MUSCLE HYPERTROPHY

The stated point for this debate is that satellite cell addition is obligatory for skeletal muscle hypertrophy. The definition of the word "obligatory" is important for our argument and we

interpret it to mean mandatory, necessary, or essential. Thus, as the counterpoint to this position, we will provide evidence of skeletal muscle hypertrophy in the absence of satellite cell addition.

Our position that skeletal muscle is capable of hypertrophy without the addition of satellite cells is supported by a large number of studies investigating the effect of β-adrenergic agonists, such as clenbuterol and cimaterol, on skeletal muscle mass (1, 3, 6–9, 12, 14, 15, 17, 19, 20, 22–25, 29). These studies have been performed in a number of species, including mice, chicken, and sheep and have convincingly shown administration of β-adrenergic agonists can induce skeletal muscle hypertrophy in the absence of any increase in DNA content or increases in myonuclear number (1, 6, 8, 9, 15, 19, 20, 23, 25). Thus one can look at muscle mass/DNA, protein content/DNA, or fiber diameter/DNA and the result for all of these variables is that there is a significant increase in the ratio following β-adrenergic agonist treatment.

Early studies by Maltin and Delday (13) treated rats with clenbuterol and analyzed protein content, total DNA content, and the number of myonuclei per fiber in the soleus muscle. They reported a 15% increase in protein content with no change in total DNA or myonuclear number/fiber. Rehfeldt et al. (23) found that clenbuterol treatment in rats resulted in a 24–28% decrease in the ratio of nuclei to cytoplasmic in the EDL muscle. More recently, Sharma et al. (25), reported that mice administered clenbuterol showed a 26% increase in muscle mass with no change in total DNA content. They also documented that clenbuterol treatment induced hypertrophy with increases in muscle fiber cross sectional area in both red (i.e., type I or IIA) and white (i.e., type IIX or IIB) fibers. Many of these studies showed that while total DNA content does not increase with β-adrenergic agonist treatment, there is a robust increase in total RNA and this likely reflects an increase in ribosomal RNA and protein synthetic capacity (5, 18). In fact, several studies have shown that rates of protein synthesis are increased in skeletal muscle, whereas rates of degradation are decreased in response to β-adrenergic agonist treatment (2, 7, 10, 12, 14, 19, 26). Collectively, these studies provide strong evidence that β-adrenergic agonists promote skeletal muscle hypertrophy by enhancing protein synthesis with no requirement for the addition of satellite cells.

In addition to the pharmacological studies, contractile activity has also been shown to induce hypertrophy of skeletal muscle in the absence of increases in DNA content. In a series of studies by Wong and Booth (27, 28), skeletal muscle hypertrophy in response to a controlled training paradigm was tested in both the tibialis anterior (TA) and gastrocnemius (GTN) muscles of rats. The eccentrically loaded TA muscle exhibited a 30% increase in muscle mass and 28% increase in protein content with no change in total DNA per muscle. Curiously enough, the concentrically loaded GTN muscle did exhibit an increase in total DNA content with no significant change in mass or total protein after 10 wk of training. Increases in total DNA and nuclear content without muscle growth have been documented previously in studies of skeletal muscle in response to low-frequency chronic stimulation (16, 21). Thus these results from the trained GTN muscle add to the literature demonstrating that increases in DNA/nuclear content are not *sufficient* to induce skeletal muscle growth.

The other set of studies that argue for skeletal muscle growth in the absence of satellite cell addition are studies in which DNA replication was inhibited either pharmacologically or by γ-irradiation. In these studies, the inhibition of DNA replication was not sufficient to prevent skeletal muscle growth. Fleckman et al. (4) treated male Sprague-Dawley rats with hydroxyurea and/or cytosine arabinoside to inhibit DNA synthesis following synergist ablation and found no difference in the magnitude of growth in the treated and untreated rats. They also demonstrated that the increase in muscle weight was the result of an increase in myofibrillar protein content. More recently, Lowe and Alway (11) exposed Japanese quails to γ-irradiation prior to applying stretch overload to the anterior latissimus dorsi (ALD) muscle. As reported in the paper, the ALD muscle in both the control and irradiated quails exhibited the same magnitude of growth with 100% increases in mass and protein content. In addition, the authors report that there was no increase in total DNA and no increase in BrdU incorporation in the hypertrophied ALD of the γ-irradiated quail. Zeman et al. (29) also found that γ-irradiation of the mdx mouse did not block growth of the EDL or GTN muscles in response to clenbuterol treatment. Thus we feel that these studies provide an additional line of support for our position that skeletal muscle can hypertrophy in the absence of satellite cell proliferation and/or addition.

In summary, we provided evidence from studies of pharmacological and contraction-induced skeletal muscle growth in which satellite cell addition does not occur as measured by DNA content or nuclear number. We also present studies in which inhibition of satellite cell proliferation does not inhibit skeletal muscle growth. We believe that the evidence we provide clearly makes our argument that satellite cell addition is not obligatory for skeletal muscle growth.

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REBUTTAL FROM DRS. O'CONNOR AND PAVLATH

We believe to critically evaluate the contribution of satellite cells to hypertrophy, the following conditions need to be fulfilled: *1*) analysis of a complete spectrum of time points (early and late) after application of a hypertrophic stimulus; *2*) sufficiently large increases in myofiber cross-sectional area or diameter; *3*) enumeration of myonuclear number using a method to clearly delineate the sarcolemma; and *4*) to show cause and effect rather than correlation, ablation of satellite cell activity. The papers cited by McCarthy and Esser in various models of hypertrophy fail to satisfy one or more of these criteria and, therefore, do not provide conclusive evidence against the necessity for satellite cells in hypertrophy. For example, the vast majority of the clenbuterol literature only analyzes muscle weight and content of total protein, RNA, and DNA. Muscle weight and total protein are non-specific measures including both muscle and non-muscle components of the tissue. Total DNA is an inaccurate measure of myonuclei, including fibroblasts, inflammatory cells, etc. Kim et al. (3) analyzed myonuclear number in response to clenbuterol but only at 14 days using inappropriate methods. In contrast, myonuclear number was appropriately analyzed in some studies (5, 6) but only at early time points before the myonuclear domain ceiling of the existing myonuclei was likely to be exceeded. The time points chosen for study in the synergist ablation (2) and stretch overload (4) studies were too early in the hypertrophic process for appropriate interpretations on the role of satellite cells. Not commented on by McCarthy and Esser is that one of their cited studies (1) demonstrated preferential increments in DNA content rather than RNA or protein accretion during the *later* stages of cimaterol-induced hypertrophy, thus suggesting a role for satellite cells. The limitations inherent in using DNA content as a measure of satellite cells must be kept in mind though.

In summary, we believe the evidence generated from various models of muscle growth in multiple species support our contention that muscle growth may be viewed as a continuum of temporally regulated responses. We believe that muscle growth consists of multiple phases, including accelerated transcriptional and translational responses followed by satellite cell addition during the *later* stages of hypertrophy. Satellite cell addition is necessary only if a certain threshold myofiber size is reached.

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