



TOPICAL REVIEW

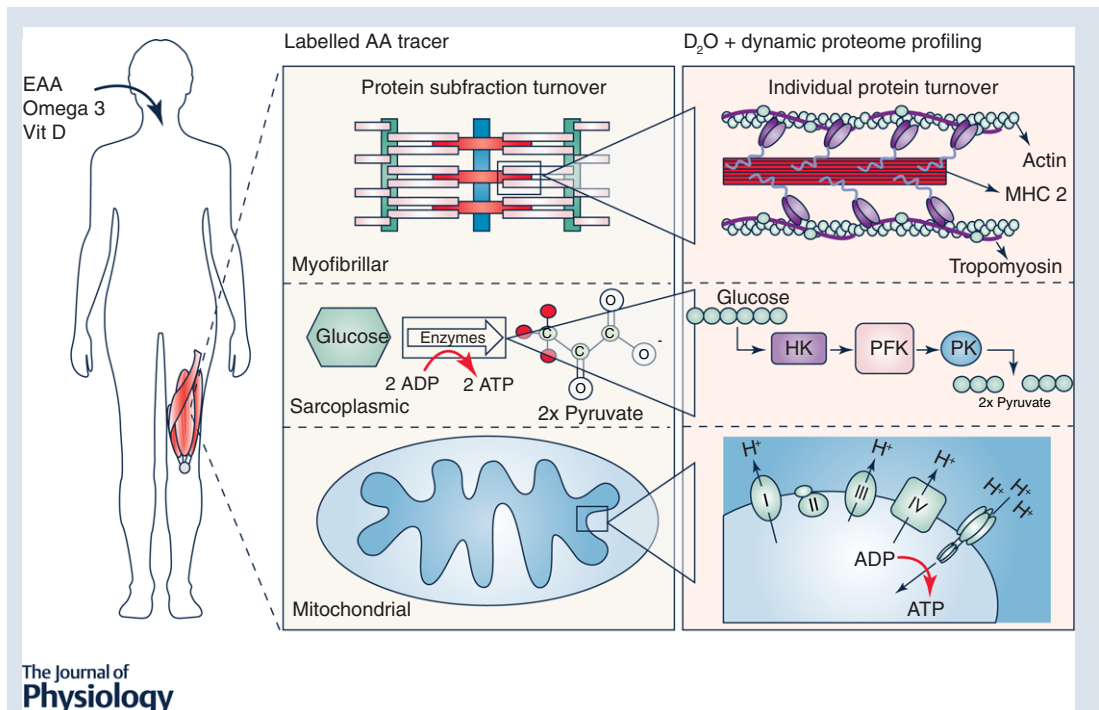
The impact of exercise and nutrition on the regulation of skeletal muscle mass

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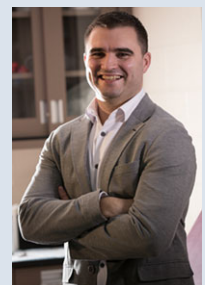
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Abstract The maintenance of skeletal muscle mass and strength throughout life is a key determinant of human health and well-being. There is a gradual loss of both skeletal muscle mass and strength with ageing (a process termed sarcopenia) that increases the risk of functional dependence, morbidity and mortality. Understanding the factors that regulate the size of human

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muscle mass, particularly during the later years of life, has therefore become an area of intense scientific inquiry. The amount of muscle mass is determined by coordinated changes in muscle protein synthesis (MPS) and muscle protein breakdown (MPB). In this review, we assess both classical and contemporary work that has examined how resistance exercise and nutrition impact on MPS and MPB. Special consideration is given to the role of different sources of dietary protein (food vs. supplements) and non-protein nutrients such as omega-3 fatty acids in regulating MPS. We also critically evaluate recent studies that have employed novel 'omic' technologies such as dynamic protein profiling to probe for changes in rates of MPS and MPB at the individual protein level following exercise. Finally, we provide suggestions for future research that we hope will yield important information for the development of exercise and nutritional strategies to counteract muscle loss in a variety of clinical settings.

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Abstract figure legend Schematic illustration of the measurement of changes in the muscle proteome in response to nutritional intervention using labelled amino acid/deuterium and dynamic proteome profiling.

Introduction

In the last century, the study of human health has shifted from one focused on the extension of lifespan to the protraction of healthspan. We loosely define healthspan as the amount of time spent in a state of functional independence and free of major disease. Critical to the sustenance of healthspan is the maintenance of skeletal muscle mass and strength throughout life. In particular, skeletal muscle provides the requisite contractile force for locomotive activities, which enables independent living and can enhance key aspects of health such as insulin sensitivity and joint mobility. Skeletal muscle also plays an important role in the recovery from major disease and it is now well established that possessing a greater proportion of skeletal muscle to total body mass is associated with increased survival and recovery rates from several disease states (van Venrooij *et al.* 2012; Cho *et al.* 2016; Shachar *et al.* 2016; Landi *et al.* 2017). However, as we age, there is a natural loss of skeletal muscle mass and strength, termed sarcopenia (Rosenberg, 1997), that is associated with a 2- to 3-fold increased risk of falls, frailty, disability, loss of independence, and mortality (Cao & Morley, 2016). Sarcopenia affects ~10–30% of independently living older adults without major illness, and is even more prevalent in those with chronic diseases and/or institutionalized older adults (Cao & Morley, 2016). Despite continued debate as to the cellular mechanisms and the exact definitions of what constitutes sarcopenia (Fielding *et al.* 2011), the negative health effects associated with sarcopenia have become such a world-wide health concern (Drew, 2018) that it has recently been recognized as an independent condition having its own International Classification of Disease (Cao & Morley, 2016).

Regulation of skeletal muscle mass by exercise and feeding

The study of the biological mechanisms regulating the size of human muscle mass has, and continues to be, one of intense scientific inquiry. Over 30 years ago, Professor Mike Rennie and colleagues (Rennie *et al.* 1982) demonstrated for the first time that the incorporation rate of an intravenously infused stable isotope-labelled amino acid tracer into skeletal muscle protein is stimulated by feeding. Compared to overnight fasting conditions, the muscle protein synthesis (MPS) rate doubled postprandially, and the postprandial increase in MPS accounted for the majority of the positive net protein balance at the whole-body level (Rennie *et al.* 1982). Since then, a series of studies by Professor Rennie's group (e.g. Rennie *et al.* 2004; Atherton *et al.* 2010) and others (e.g. Biolo *et al.* 1995, 1997; Reidy *et al.* 2017) have explored the diurnal regulation of muscle protein turnover (e.g. the balance between synthesis and breakdown). From this work we have learned that during basal, postabsorptive conditions, the rate of muscle protein breakdown (MPB) exceeds the rate of MPS, causing net loss of protein (Rennie *et al.* 2004), and meal intake compensates for these losses because dietary protein-derived amino acids stimulate MPS and insulin suppresses MPB (Rennie *et al.* 2004). The postprandial net protein gain is largely determined by the amount of protein ingested, because the resulting increase in essential amino acids in plasma stimulates MPS in a dose-dependent manner up to ~30 g of dietary protein (or an equivalent amount of amino acids) (Cuthbertson *et al.* 2005; Moore *et al.* 2015) whereas the concentration of insulin necessary to achieve maximal suppression of MPB (~15–30 mU/L) already occurs after consuming a

small amount of protein or carbohydrate (Bohe *et al.* 2001; Greenhaff *et al.* 2008; Moore *et al.* 2009). Accordingly, simply eating more protein results in increased rates of amino acid oxidation without a further increase in MPS (Moore *et al.* 2015).

Besides protein/amino acid feeding, exercise serves as the other main anabolic stimulus to skeletal muscle. Classic work by Rennie *et al.* (1981) revealed that an acute bout of exercise resulted in an improved post-exercise whole body net protein balance through a rise of whole body protein synthesis in excess of protein breakdown. Over a decade later, similar findings were made at the level of the skeletal muscle showing that a single bout of resistance exercise increased both MPS and MPB for up to 48 h, but while the relative stimulation of MPS was greater than MPB, MPB still exceeded MPS in the fasted state, resulting in no net muscle protein accretion (Phillips *et al.* 1997). Importantly, the effects of exercise and protein ingestion are additive (Witard *et al.* 2009; Pennings *et al.* 2011). Exercise training therefore increases muscle mass largely because of an increase in MPS rather than suppression of MPB.

Ageing results in characteristic changes in muscle protein turnover, referred to as age-associated 'anabolic resistance' (Moore *et al.* 2015), which is characterized by a blunted response of both MPS and MPB to the anabolic effects of amino acids and exercise and the anti-proteolytic effect of insulin, which leads to a gradual loss of muscle mass. The first evidence for this phenomenon was provided by Professor Rennie and colleagues (Cuthbertson *et al.* 2005; Wilkes *et al.* 2009; Kumar *et al.* 2009a), who demonstrated that, although the basal rates of MPS and MPB are not different in young and older adults, the increase in MPS to amino acid ingestion is diminished in older adults. Later, they demonstrated that the insulin-mediated suppression of MPB (Wilkes *et al.* 2009) and the exercise-induced increase in MPS (Kumar *et al.* 2009b) are also reduced in older compared to young adults. The presence of anabolic resistance in older adults has since been confirmed by several other investigators (Moore *et al.* 2015). In addition, this anabolic resistance affects older women more than older men (Smith *et al.* 2012). Strategies to prevent and treat age-associated sarcopenia therefore focus on overcoming this anabolic resistance (Bauer *et al.* 2013). These efforts have led to novel discoveries related to the potential importance of non-protein food components for the regulation of muscle protein turnover.

Muscle protein remodelling after ingestion of protein-rich foods

Although we have learned a lot about the regulation of muscle protein turnover from studies that administered isolated protein or amino acids, food is what people eat and

it has become evident that although protein/amino acids are the primary anabolic stimulus for muscle, the source of dietary protein and interactions with the food matrix could have important effects on the biological activity of protein/amino acids. While ingestion of protein-rich lean meat was found to stimulate MPS in a dose-dependent curvilinear manner in both young and older adults (Symons *et al.* 2009; Robinson *et al.* 2013), and the MPS response to lean beef ingestion was maximal with a serving size of ~113–170 g (4–6 oz), which provides ~30–35 g of protein. Studies of whole food proteins are rare, but have been made. For example, ingestion of isonitrogenous skimmed milk *vs.* lean (97% fat-free) beef resulted in greater availability of circulating dietary amino acids after ingestion of beef than milk in the early postprandial phase (0–2 h), yet paradoxically the MPS response was greater after milk than beef during this time (Burd *et al.* 2015). When assessed over a 5 h period, no differences were observed in MPS rates between lean beef and skimmed milk, despite a trend towards higher dietary amino acid availability after beef ingestion. A recent comparison of egg white *vs.* whole egg (both providing 18 g of dietary protein) consumption during post-exercise recovery in healthy young men revealed a superior 5 h MPS response after whole egg ingestion, despite similar postprandial amino acid profiles after ingestion of these two protein sources (van Vliet *et al.* 2017). These findings confirmed the results of a previous study in which whole milk consumption resulted in a greater net uptake of amino acids by skeletal muscle (presumably for use in protein synthesis) compared to ingestion of skimmed milk matched for protein content (Elliot *et al.* 2006).

Besides amino acids, protein-rich foods often also provide a wide spectrum of lipids, vitamins, minerals and other bioactive compounds (growth factors, peptides, miRNAs, etc.; Moller *et al.* 2008; Zanovec *et al.* 2010; Phillips *et al.* 2015) that appear capable of modulating the MPS response upon ingestion. For instance, in animal models it has been demonstrated that the provision of oleate (Tardif *et al.* 2011), the vitamins A, D and E, and the minerals selenium and zinc can also directly influence the muscle anabolic response (Zhao *et al.* 2016; Chanet *et al.* 2017). Moreover, Smith *et al.* demonstrated that 8 weeks of fish oil-derived omega-3 fatty acid supplementation increased the EPA and DHA content in skeletal muscle phospholipids and enhanced MPS under hyperaminoacidaemic-hyperinsulinaemic conditions in both older (Smith *et al.* 2011a) and younger adults (Smith *et al.* 2011b). The same authors extended this work by demonstrating that supplementation with fish oil-derived omega-3 fatty acids increased skeletal muscle mass and function in healthy older adults (Smith *et al.* 2015). This report corroborated findings by other investigators who have shown that dietary supplementation with omega-3 fatty acids promoted gains in skeletal muscle strength

during exercise training in older women (Rodacki *et al.* 2012; Da Boit *et al.* 2017). The biological mechanisms by which these fatty acids might regulate skeletal muscle anabolism remain unknown. Incorporation of the highly unsaturated fatty acids EPA and DHA into the diet has been associated with enhanced mechanistic target of rapamycin complex-1 signalling (Smith *et al.* 2011a; Kamolrat *et al.* 2013; McGlory *et al.* 2014), which provides one potential mechanism through which anabolism might be augmented. It is also possible that omega-3 fatty acid incorporation into skeletal muscle alters lipid raft formation (Hou *et al.* 2016) and the transmission of mechanical and nutritional cues to the translational machinery. Thus, omega-3 fatty acids represent a promising area of research for the development of nutritional strategies to counteract anabolic resistance in older adults. The next logical step would be to investigate whether ingestion of fatty acids such as omega-3s can offset skeletal muscle atrophy during periods of bed rest/muscle disuse in ageing persons. Such data would inevitably have an important clinical impact, given the pervasive metabolic consequences of periodic muscle disuse in older adults (English & Paddon-Jones, 2010; McGlory *et al.* 2017).

In summary, we have only recently begun to understand, and perhaps appreciate, the complexity of various food sources and their role in stimulating remodelling of the muscle proteome. Clearly much more work is needed to understand how ingestion of food, as opposed to protein/amino acids alone, may affect daily protein requirements, particularly in older individuals. However, current evidence would suggest that the presence of vitamins, minerals, lipids and other bioactive nutrients in food work in concert with amino acids/protein to support the postprandial rise in MPS. Elucidating the active biological non-protein components of food, such as specific lipid species, may yield important data relevant to the development of novel nutraceutical therapies to promote musculoskeletal health in older adults.

Proteomic/phosphoproteomic analysis of muscle protein turnover

Traditional methods of assessing protein turnover in humans rely on the infusion of a stable isotope-labelled amino acids coupled with serial muscle biopsies. The muscle biopsy samples are analysed for tracer incorporation into mixed or specific muscle protein fractions (i.e. myofibrillar, sarcoplasmic, mitochondrial) or a few select individual proteins (e.g. actin or myosin heavy chain). How resistance exercise and nutrition affect turnover rates of the numerous individual proteins that constitute skeletal muscle has remained unknown until lately. In an attempt to address this knowledge gap, Murphy and colleagues (2018) administered deuterated

water (D₂O) to their study participants and used tandem-mass spectrometric proteomic analysis of muscle biopsy samples (Shankaran *et al.* 2016b) to identify changes in the synthetic rates of > 150 individual muscle proteins contained in the myofibrillar, mitochondrial and sarcoplasmic protein fractions. The primary finding from this study (Murphy *et al.* 2018) was that older adults performing resistance exercise during an energy restricted state enhanced the synthesis of 175 out of 195 measured individual skeletal muscle proteins in each of the specific protein fractions. Besides upregulation of individual skeletal muscle proteins in the myofibrillar (i.e. contractile) category, the synthesis of proteins involved in the regulation of numerous metabolic processes such as glycolysis (i.e. 6-phosphofruktokinase) and the electron transport chain (i.e. ATP synthase subunits) were also increased after resistance exercise.

At about same time as Murphy and colleagues (2018) reported their results, Camera and colleagues (2017a) employed the dynamic proteome profiling approach to evaluate the effect of resistance exercise during a high-fat, low-carbohydrate diet on the synthesis and breakdown of individual muscle proteins in young adults. They found the abundance of 28 out of 90 measured myofibrillar and sarcoplasmic proteins increased and that this was due to both increased synthesis and decreased breakdown rates of these proteins. Interestingly, the initial increase in protein abundance after resistance exercise occurred with little to no change in protein synthesis, thus highlighting the potential key role played by MPB in the remodelling of the muscle proteome with resistance exercise. Such findings would seem at odds with extant knowledge that changes in the proteome with resistance exercise are *predominantly* driven by changes in rates of MPS. However, it is important to interpret these findings with a degree of caution as the rate of breakdown was estimated from the difference between the change in protein abundance and synthesis over time. Thus, protein breakdown was not directly assessed and 11 proteins even demonstrated a physiologically impossible negative value for the estimated breakdown rate, highlighting the need for further improvement in 'omics' approaches used to study the role of MPB in the skeletal muscle adaptive response to physical activity and nutritional interventions. As the authors acknowledge, their findings related to MPB could be attributable to technical errors but it is also possible that amino acid recycling may have increased protein abundance to a greater extent than predicted by synthesis. Nevertheless, this study (Camera *et al.* 2017a) demonstrates the power of dynamic proteome profiling applied to human skeletal muscle research, which could be used to discover novel and clinically important interventions to improve musculoskeletal health. For instance, one finding of the study (Camera *et al.* 2017b) was that in response to resistance exercise there was a significant

increase in the synthesis of myosin heavy chain protein, but only that of fast-twitch 2a (MYHC2), which is a fascinating discovery given that sarcopenia is characterized by a reduction in fast twitch fibre expression (Lexell *et al.* 1988).

Although advances in molecular/cell biology techniques over the past 20 years have provided important insights into the regulation of muscle protein turnover, our understanding of the mechanisms responsible for muscle loss with advancing age remain elusive. There is evidence that failure to adequately activate mTORC-1 and subsequently translate genes that encode muscle proteins plays some role. However, such signalling networks are multifaceted and interrelated, and unravelling the complexity of these interactions is unlikely to be achieved by employing contemporary approaches such as immunoblotting or immunohistochemistry alone. One interesting avenue of research that may provide some answers is phosphoproteomics. Phosphoproteomics provides a global and unbiased approach to studying changes in phosphorylation networks in response to anabolic stimulation. As an example, two recent studies have identified novel phosphorylation sites on numerous protein kinases following endurance exercise in humans (Hoffman *et al.* 2015) and maximal eccentric contractions in rodents (Potts *et al.* 2017). One of these

two studies (Potts *et al.* 2017) discovered a striated muscle-specific serine/threonine protein kinase and obscurin as contraction-sensitive z-disk kinases that may sense mechanical cues for transmission to the translational machinery. Whether failure to fully activate these and/or other related kinases plays some role in the anabolic resistance of older adults to exercise and even nutrition remains unknown. Future research that combines proteomic (Shankaran *et al.* 2016b), phosphoproteomic (Potts *et al.* 2017) and, potentially, lipidomic (Jeromson *et al.* 2017) technologies to study skeletal muscle plasticity following exercise and feeding in both older and young adults would no doubt address this important gap in our understanding (Figure 1).

Future directions

Since the first studies of protein turnover, which relied on the arterio-venous balance approach and measuring labelled amino acid incorporation into muscle proteins, significant strides have been made in our understanding of the factors that regulate muscle protein turnover following exercise and nutrition. The next era of scientific advancement in this field will no doubt be underpinned by the use of ‘omic’ technologies (e.g. lipidomic, proteomic, metabolomic, transcriptomic). A primary advantage of

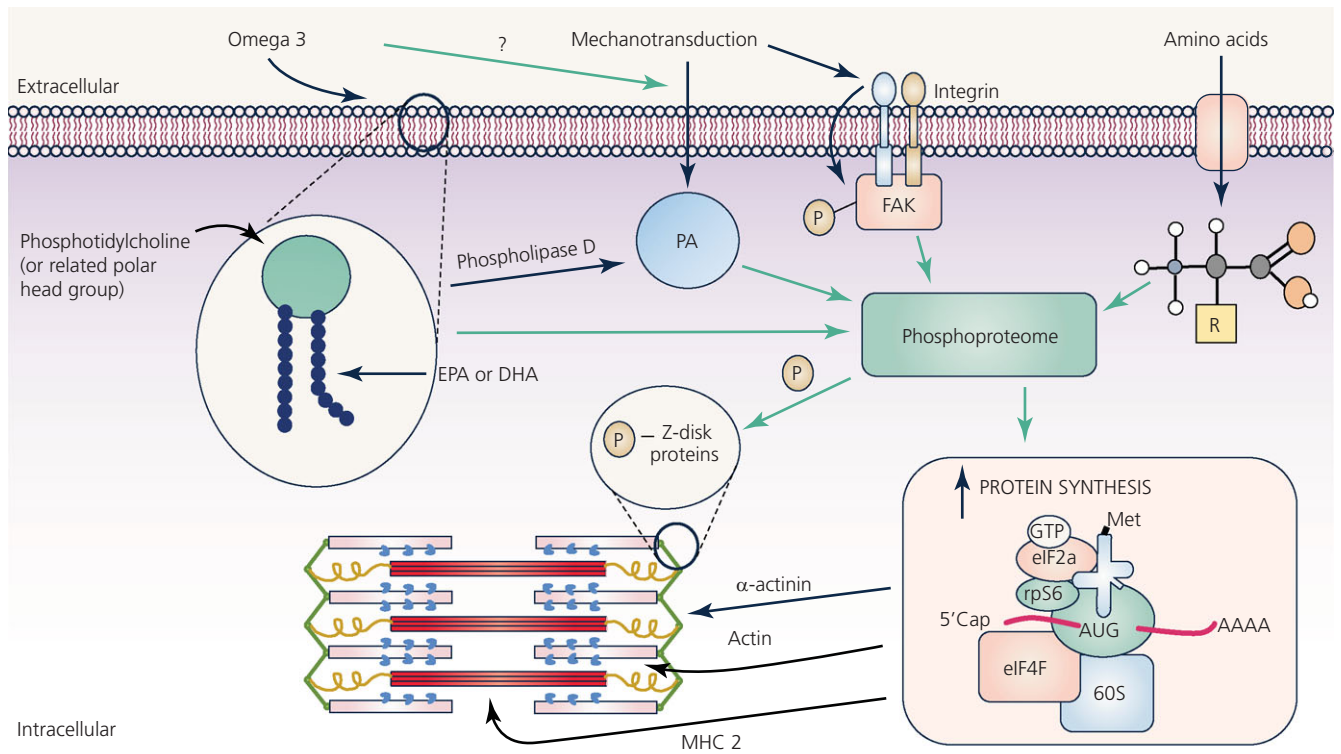


Figure 1. Illustration of lipidomic, proteomic, and phosphoproteomic interactions in skeletal muscle

applying 'omic' technologies to study muscle protein turnover and associated intracellular signalling networks is that the impact of nutritional and exercise strategies can be examined in greater scope and detail. Unbiased global analysis of changes in muscle protein kinetics and the molecular factors that regulate these changes will help pinpoint age- and disease-related alterations in entire biological networks. There is now also evidence that proteins assembled in muscle, such as creatine kinase M-type and carbonic anhydrase, escape into the circulation and that the fractional turnover rates of these proteins in plasma can be used as a surrogate marker of muscle protein turnover (Shankaran *et al.* 2016a; Murphy *et al.* 2018). Such data open up the exciting possibility of studying the impact of exercise and nutrition on muscle protein turnover with minimally invasive (blood draw) techniques. This is particularly important when dealing with compromised individuals, such as those in intensive care units or other similarly precarious clinical scenarios, for whom skeletal muscle biopsy sampling is not practical or possible. Another area worthy of further interrogation is the study of how changes in muscle-specific lipid species impact on muscle protein turnover. The discovery that omega-3 fatty acids render skeletal muscle more 'anabolically sensitive' was a first step in this direction and requires interrogation of the mechanisms responsible for their beneficial effect on muscle protein turnover, which could include improved membrane fluidity and/or alterations in lipid raft formation. Future work that manipulates phospholipid species content and composition in both pre-clinical and human models will no doubt help provide the answers.

To conclude, the ageing muscle proteome is an area of intense scientific investigation. By understanding the impact of physical activity and various nutrients sources on human muscle protein turnover at the individual protein level it will be possible to develop better strategies to counteract the normal age-associated loss of muscle loss and the loss of muscle caused by the numerous age-associated comorbidities. Achieving this aim will probably be expedited by employing a wide range of new and existing methodologies *pari passu*.

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Additional information

Competing interests

None.

Author contributions

C.M. and S.v.V. wrote the initial draft of the manuscript. All authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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