HMB supplementation can increase growth hormone (GH) and insulin-like growth factor 1 (IGF-1) levels [29], it may also stimulate protein synthesis via GH/IGF-1 axis signaling, though HMB-induced increases in these hormones have not been directly linked to protein synthesis. Some studies have proposed that HMB stimulation of muscle protein synthesis involves activating AKT [43–45]. However, others have shown *in vivo* [46,47] that HMB enhances muscle protein synthesis without activation of AKT. Additionally, HMB has been shown to activate AMP activated protein kinase (AMPK) *in vitro* [48].

Preclinical evidence shows that HMB also decreases muscle protein breakdown through multiple pathways, including the suppression of the ubiquitin-proteasome pathway [49–51], inhibition of myonuclear apoptosis via mitochondrial-associated caspase signaling [52], and suppression of lysosomal autophagy pathways [53]. Clinical studies show that 3 g of HMB significantly decreases muscle protein breakdown independent of HMB form (i.e. calcium vs free acid) [28,42]. HMB-Ca supplementation during resistance training also results in dose-dependent decreases in muscle protein breakdown as measured by urinary 3-methylhistidine, a byproduct of muscle contractile protein breakdown that is not metabolized or re-used for protein synthesis [6]. Though muscle protein breakdown is typically regulated through an insulin-dependent process [54], HMB-FA suppresses muscle protein breakdown in human muscle via an insulin-independent process [28].

The specific mechanisms by which HMB suppresses muscle protein breakdown in humans remain to be elucidated. The primary clinical evidence of HMB's mechanisms of action on protein synthesis and protein breakdown, though methodologically rigorous, is limited by the inclusion of only young, healthy men in these studies. No changes in the expression of atrophy-associated proteins (e.g. MuRF1, MAFbx) were observed in human muscle during clinical studies, though the researchers noted that peak signaling events may have occurred outside the single timepoint evaluated [28,42]. Baier et al. [22] evaluated rates of whole-body protein turnover by using primed/intermittent oral doses of ^{15}N-glycine. In older adults, a combination of HMB-Ca with arginine and glutamine increased rates of protein turnover at 3 and 12 months of supplementation, respectively. A more recent study examined muscle protein synthesis during 6 weeks of single-leg resistance exercise training with or without HMB in free-living older men using deuterium oxide methods [55]. While no main effects of HMB supplementation alone on muscle protein synthesis were observed, during the first two weeks of training, a significant increase in muscle protein synthesis with exercise was observed in the HMB group. The meaningfulness of these findings (or lack thereof) from this study is undermined by the limited muscle mass exercised and the inability to measure muscle protein breakdown. To our knowledge, other potential mechanisms for reduced muscle protein breakdown have not been evaluated in clinical studies. Additional research in women and older populations will add valuable information to our understanding of HMB's effects across different populations.

In summary, the primary mode of action of HMB appears to be through its dual mechanism to enhance muscle protein synthesis and suppress muscle protein breakdown.