Chapter 5
Anabolic Resistance

Jean-Paul Thissen

Abstract Muscle atrophy is the hallmark of several catabolic conditions. Whatever the cause, the skeletal muscle loss is associated with comorbidities and poor survival. The mass of the skeletal muscle is maintained normally by equilibrium between protein synthesis and breakdown. Rate of synthesis in particular is positively regulated by nutrition and exercise. Anabolic resistance can be defined as a situation where the skeletal muscle is unable to respond appropriately to these anabolic stimuli by stimulating protein synthesis. Anabolic resistance contributes to muscle mass loss in elderly, during immobilization as well as in response to inflammation and cancer. The mechanisms responsible for this blunted response to anabolic stimuli are still under investigation. Several strategies may serve to compensate for anabolic resistance. Optimization of protein intake, resistance exercise, and anti-inflammatory agents appear promising to override this anabolic resistance and mitigate its consequence, the skeletal muscle mass loss.

5.1 Background

Muscle atrophy is the hallmark of several catabolic conditions. Whatever the cause, the skeletal muscle loss is associated with comorbidities and poor survival [1].

Muscle proteins are in dynamic equilibrium between their respective rates of synthesis and breakdown. A net gain of muscle mass is only possible if muscle protein synthesis [MPS] exceeds muscle protein breakdown [MPB]. Rate of MPS is positively regulated by nutrient availability and physical activity and negatively regulated by disuse, aging, and muscle wasting-related diseases [2].

J.-P. Thissen, MD, PhD
Pôle Endocrinologie, Diabétologie et Nutrition,
Institut de Recherches Expérimentales et Cliniques,
Université Catholique de Louvain,
Avenue Hippocrate 55 bte B1.55.06,
Bruxelles B-1200, Belgium
e-mail: jeanpaul.thissen@uclouvain.be

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The mass of the skeletal muscle is maintained normally by ingestion of *protein-containing meals*, which results in systemic hyperaminoacidemia. With feeding, MPS is stimulated, and, to a lesser extent, MPB is decreased, causing the protein balance to become positive. During the postprandial period, the balance of muscle protein becomes negative, due to decreased MPS and increased MPB. Therefore, postprandial stimulation of MPS is an important factor in muscle mass maintenance [3].

The stimulation of MPS by protein intake is *transient and dose dependent*. Indeed, increase in MPS after feeding is of finite duration despite enduring substrate availability. MPS increases after an oral protein bolus with a peak at 2 h before returning to baseline levels, despite elevated plasma amino acid (AA) concentrations [4]. Thus, the muscle becomes refractory to persistent elevations of circulating AA concentrations ("muscle full" concept). The relationship between the dose of protein intake and its resulting stimulation of MPS is also well described. With increasing the dose of protein, MPS increases fast to reach a plateau at approximately 20 g of high-quality protein, with higher-protein intake stimulating AA oxidation rather than MPS. In contrast, MPB decreases slightly but continuously with increasing protein intake. Therefore, protein intake higher than known to stimulate MPS maximally might lead to a greater anabolic response via suppression of MPB [5, 6].

While most AAs are contained in proteins, it appears only to be the essential or indispensable AAs (EAAs), which represent 40–50 % of the total AAs of a high-quality protein, that stimulate MPS. Of the EAAs, the branched chain AAs (BCAAs) are primarily responsible for stimulating MPS [7]. In this regard, *leucine* is important as it comprises about one-fifth of EAAs needs, and apart from serving as a substrate, it directly activates the signaling pathway stimulating protein synthesis. Leucine on its own appears to stimulate MPS to nearly the same level as a mixture of AAs [8].

Although *insulin* is of great importance in inhibiting MPB, it plays only a permissive role in stimulating MPS [9]. Although the postprandial rise in circulating insulin might modulate the MPS response through its vasodilatory properties resulting in greater nutritive blood flow to muscle tissue, the basal levels of circulating insulin seem sufficient for maximally stimulating MPS in humans [10]. Systemic administration of insulin alone causes hypoaminoacidemia by inhibiting protein degradation which may in turn inhibit MPS [11]. However, when hypoaminoacidemia is prevented by exogenous AAs replacement, insulin co-administration effectively increases MPS, but this effect is due to the hyperaminoacidemia [12]. The primary effect of insulin on the skeletal muscle is therefore to decrease MPB without a significant effect on MPS [13].

Besides protein intake, *resistance exercise* is a potent stimulator of MPS and appears to synergistically enhance the gain stimulated by feeding. Even though the rate of MPB increases following exercise, the corresponding increase in MPS is three- to fivefold greater [14]. While the feeding-mediated increase in MPS lasts only few hours at most, the resistance exercise-induced MPS is sustained for about 24–48 h. The synergistic effect of resistance exercise and protein ingestion on muscle protein anabolism is well established. Indeed, the effect of protein intake on muscle protein accretion can further be stimulated by prior exercise training [15],

thereby permitting muscle hypertrophy when practiced frequently over time. This effect appears to be mediated by an exercise-induced improvement in nutrient-stimulated vasodilatation and nutrient delivery to muscle rather than potentiation of insulin signaling [16]. Food combined to exercise elevates net muscle protein balance for many hours after exercise [17].

5.2 Anabolic Resistance Concept

In many situations, MPS in response to feeding or exercise is blunted. This abnormal response of the skeletal muscle to previously well-established anabolic stimuli is known as anabolic resistance [18, 19]. In these situations, the MPS is refractory to EAA provision, irrespective of the availability of insulin. Therefore, anabolic resistance can be defined as a situation where the skeletal muscle is unable to respond appropriately to anabolic stimuli by stimulating protein synthesis and less importantly by inhibiting proteolysis. Indeed, MPB has a minor role in the protein anabolic response to EAAs [20]. The mechanisms responsible for this blunted response of MPS to anabolic stimuli are still under investigation. However, it is highly probable that alterations in protein digestion, AA absorption, hormonal response, microvascular blood flow, AA uptake into the muscle, and intramuscular signaling may play a role.

5.3 Methodological Considerations

Although the skeletal muscle accounts for 40 % of the body weight and constitutes between 50 and 75 % of all proteins, it represents only 30 % of whole-body protein synthesis. Furthermore, muscle proteins turn over at a rate that is significantly less [about 20-fold] than splanchnic or plasma proteins. Therefore, whole-body protein turnover does not reflect changes which take place into the skeletal muscle [21].

In terms of mechanisms underlying atrophy, the data on rodent and human muscles are difficult to compare for several reasons. Among many, it is worth noting that most of the rodent work has been carried out with young and not adult animals. Moreover, while muscle atrophy in humans mainly involves reduced MPS, increased MPB seems the predominant feature in rodents [22, 23].

Studies focusing on acute changes in MPS cannot predict the net changes in muscle protein balance as opposite changes in MPB may damper the muscle anabolic effect expected based on changes in MPS, especially when long-term changes on the skeletal muscle mass are considered.

Finally, from a functional perspective, changes in muscle function are probably more important than changes in the skeletal muscle mass and should be the most clinically relevant end point to study. This point has not been investigated however in the context of anabolic resistance.

5.4 Clinical Situations of Anabolic Resistance

5.4.1 Aging

Skeletal muscle mass begins to decline in the fourth or fifth decade of life, at a rate of 0.5–1.5 %/year between 50 and 80 years old. The debilitating effects of muscle loss include declines in physical function and quality of life and increases in morbidity and mortality. Loss of muscle mass with aging is thus a major public health concern. The age-related muscle mass wasting is known as sarcopenia. The loss of muscle mass with aging is probably not due to chronic changes in MPS or MPB but to a blunted feeding-induced rise in MPS [24]. Indeed, muscles of the elderly are resistant to normally robust anabolic stimuli. This anabolic resistance may be a key factor in the development and progression of sarcopenia. Elderly are characterized by a resistance to the three main anabolic stimuli (exercise, AAs, and insulin). Indeed, aging reduces the anabolic response to resistance exercise [25, 26], despite equal circulating and muscle AA concentrations. Furthermore, the stimulation of the muscle anabolic signaling pathways and MPS by AA infusion is significantly blunted in elderly [27, 28]. Finally, aging is associated with reduced inhibition of proteolysis in response to insulin [29]. This anabolic resistance may result from gradual decline in physical activity or to low-grade inflammation (cf. infra).

5.4.2 Immobilization

During disuse, the skeletal muscle loss occurs at a rate of 0.5 % of muscle mass per day, which translates in 150 g of muscle tissue lost per day [30]. Prolonged disuse (more than 10 days) leads to a decline in basal and postprandial rates of MPS without apparent changes in muscle MPB, except maybe at the early stage (−5 days) [30, 31]. Protein ingestion or AA infusion, even at high rate, increased MPS less in the immobilized leg than in the control one [30, 32]. Therefore, this anabolic resistance can account for the immobilization-induced muscle atrophy. Older adults are more susceptible than young persons to muscle loss after short-term bed rest [23].

5.4.3 Inflammation

The inflammation associated with sepsis is often responsible for a state of anabolic resistance. In animal models, sepsis blunts the MPS response caused by leucine, exercise, and insulin, the three main anabolic stimuli [33, 34]. The inability of these anabolic stimuli to stimulate muscle protein synthesis during sepsis seems to be related to a defect in signaling to step in translation initiation [34, 35]. This

sepsis-induced anabolic resistance in the skeletal muscle results from the cooperative interaction of both cytokines such as TNFα and glucocorticoids [36]. Anabolic resistance in aging may also be related to inflammation, as suggested by the increase in muscle NFkB activity. Indeed, low-grade inflammation as observed in old animals impairs the MPS response to feeding [37].

5.4.4 Cancer

Cancer cachexia, a metabolic condition caused by tumor burden, is characterized by muscle atrophy and sometimes fat loss. It is observed in 85 % of cancer patients and is implicated in 25 % of deaths. Cancer patients display reduced postprandial MPS compatible with a state of anabolic resistance. Interestingly, tumor resection is able to restore the normal postprandial MPS response [38]. However, a state of anabolic resistance does not seem to be always observed in cancer patients. Several authors showed indeed that cancer patients and controls show comparable protein anabolism during feeding or AAs ingestion, at least at high dose [39–42]. These observations suggest that a window of anabolic potential exists early on in cachexia development [43]. However, in some of these studies, protein anabolism was assessed at the whole-body level and not at the skeletal muscle level.

5.4.5 Obesity

The MPS in response to AAs appears to be negatively correlated to whole-body fat mass and insulin sensitivity [44]. This suggests that insulin resistance caused by lipid excess due to intracellular accumulation of lipid in muscle or to increased free fatty acid availability may lead to anabolic resistance. In animals, chronic lipid accumulation in muscles as observed in high-fat diet-induced obesity is associated with a concomitant reduction of MPS in response to feeding [45]. Muscle ectopic fat deposition may contribute to anabolic resistance through increase in muscle ceramides. Intramuscular accumulation of ceramides has been previously involved in the insulin resistance for glucose metabolism observed in obesity. Interestingly, ceramides are also able to reduce intracellular AAs availability and protein synthesis in muscle cells [46]. The MPS response to exercise is also blunted in obese animals compared to the lean ones [47]. In humans, lipid infusion to cause reduced whole-body glucose disposal impairs the MPS in response to AAs ingestion [48]. This anabolic resistance to AAs caused by lipid infusion is associated with repression of translation initiation. In obese subjects, the net protein anabolic response to insulin was also blunted compared to healthy subjects [49]. Therefore, excess lipid availability can induce insulin resistance of the skeletal muscle glucose metabolism but also anabolic resistance of AA metabolism. This is not unexpected as many conditions of anabolic resistance such as aging, disuse, and critical illness are

characterized by the inability of the skeletal glucose metabolism to respond adequately to insulin.

5.4.6 *Chronic Kidney Disease*

In animal models, acute uremia causes a severe resistance to leucine-induced activation of the MPS [50]. Metabolic acidosis by itself impairs leucine-stimulated MPS [51]. Therefore, acidosis may account for the anabolic resistance and ultimately contribute to the muscle wasting which develops in uremia. In human subjects, besides blunting muscle glucose uptake in response to insulin, chronic renal failure and acidosis interfere with the normal suppression of MPB in response to insulin [52]. This state of insulin resistance is also observed in dialysis patients where it is associated with increased MPB [53].

5.4.7 *Other Conditions of Anabolic Resistance*

Normobaric hypoxia blunts the increased MPS response to acute resistance exercise [54]. This may contribute to muscle atrophy and blunt the hypertrophic response to resistance exercise in hypoxic conditions.

Glucocorticoids cause the skeletal muscle atrophy by themselves but also by contributing to muscle atrophy observed in many catabolic conditions such as cancer, diabetes, and acute inflammation [55]. Furthermore, glucocorticoids amplify the muscle atrophy caused by immobilization [56].

Most patients who are critically ill combine several causes of anabolic resistance. In addition to the procatabolic hormonal and cytokine milieu, age, immobilization, and hypoxia contribute to a blunted MPS response [18]. Poor delivery of hormones and nutrients caused by compromised microvascular blood flow may also play a role.

5.5 Cellular Mechanisms

Protein synthesis in the skeletal muscle is regulated through a number of signaling pathways that control the mRNA translation. The protein kinase mTORC1 [mechanistic target of rapamycin complex 1] plays a crucial role in this process by serving as a critical point of integration of most of the anabolic stimuli for the skeletal muscle [57]. Furthermore, blocking mTORC1 activity with the rapamycin drug blocks the contraction and the EAAs-mediated increase in MPS [58, 59].

In response to anabolic stimuli, mTORC1 targets and activates downstream kinases such as S6kinase1 (S6K1) or binding proteins like eIF4E-binding protein1

(4E-BP1). The action of mTORC1 and its downstream mediators heightens the efficiency of ribosomal biogenesis and ultimately translation. Indeed, phosphorylation of 4E-BP1 by mTORC1 prevents its association with eIF4E, allowing eIF4E to bind eIF4G to form the active mRNA cap-binding complex, eIF4F. Thus, mTORC1 stimulates mRNA joining to the 43S preinitiation complex [60].

Growth factors such as insulin and IGF-I activate mTORC1 by stimulating the PI3kinase-Akt/PKB pathway. AAs promote mTORC1 signaling independently of changes in growth factors or mechanic load. The mechanism of activation of mTORC1 by AAs, in particular leucine, involves several molecular actors, in particular a family of GTPases called Rags which act as AA sensors. The lysosomal membrane represents the site at which the AA- and growth factor-sensing machineries converge to stimulate mTORC1. Compared with the regulation of mTORC1 signaling by growth factors and AAs, much less is known about the pathway through which resistance exercise stimulates mTORC1 activity. A specific form of PGC-1α, PGC-1α4, which results from alternative promoter usage and splicing of the primary transcript PGC-1α, is preferentially induced in mouse and human muscle during resistance exercise. This isoform specifically induces IGF-I and represses myostatin [61]. Therefore, PGC1α4 may be a major player in the muscle hypertrophy induced by exercise. However, how this factor stimulates MPS activity is still unknown [62].

REDD1 has been recently identified as an inhibitor of mTORC1. Therefore, REDD1 induction by immobilization [63], glucocorticoids [55], hypoxia, or sepsis [33] may limit MPS by inhibiting mTORC1 signaling and contribute to the anabolic resistance observed in these conditions [64]. AMP-activated kinase (AMPK) functions as a fuel sensor. This enzyme plays an important role in regulating the muscle response to negative energy balance. More specifically, it inhibits mTORC1 signaling when cellular ATP levels are decreased and AMP levels increase in response to limited energy availability. Therefore, insufficient energy availability may limit MPS by inhibiting mTORC1 through activation of AMPK.

Many observations support the role of alterations in the mTOR pathway and its downstream effectors in the muscle resistance to anabolic stimuli.

In elderly, the phosphorylation of mTORC1 and its downstream targets, S6K1 and 4E-BP1, is dampened in the skeletal muscle as compared with the young in response to AAs infusion and exercise [25, 28, 65, 66]. Interestingly, this decline in MPS in response to AAs occurs in association with a reduction of AA transporter content [67].

Interestingly, immobilization which causes a decrease in the global rates of MPS has been associated with a decrease [68] as well an increase in mTOR [69] signaling. In the latter case, the mTOR activation might help to alleviate the immobilization-induced decrease in MPS and muscle mass, as blocking mTOR signaling with rapamycin exacerbates the decline in MPS and muscle mass [69]. Nevertheless, in human immobilization studies, decreases in mTOR have not been observed [10].

The inability of the major anabolic stimuli to stimulate MPS during sepsis seems to be related to a defect in signaling to step in translation initiation, with the redistribution of eIF4E from the active eIF4E.eIF4G complex to the inactive eIF4E.4E-BP1 complex [34, 70], impairing the formation of eIF4F.

Muscle ectopic fat deposition may contribute to anabolic resistance through increase in eIF2α activation [71]. The anabolic resistance to AAs caused by lipid infusion is associated with repression of translation initiation at the level of 4E-BP1 [48].

In animal models, acute uremia causes a severe resistance to leucine-induced activation of the MPS [51] and the mTOR anabolic signaling pathway [50]. Metabolic acidosis impairs leucine-stimulated MPS and activation of the signaling downstream of mTOR at the level of S6K1.

5.6 Tissular Mechanisms

5.6.1 Splanchnic Sequestration

About 95 % of the AAs are absorbed through the intestine and released into the portal vein or used by the gut, with only 50–60 % of the ingested dietary protein being released into the systemic circulation. An increase in hepatic/splanchnic uptake of AAs will reduce the amount of AAs available to stimulate the MPS and may blunt the MPS response to protein feeding [72, 73]. This mechanism may therefore contribute to anabolic resistance.

5.6.2 Microvascular Blood Flow Alterations

Nutritive blood flow is a very important determinant of the anabolic response to food. Poor delivery of nutrients at the sites of microvascular perfusion may contribute to anabolic resistance. Changes in MPS induced by insulin are correlated with changes in AA delivery and blood flow. Alterations in microvascular blood flow associated with reduced AA delivery are observed in critically ill patients, after immobilization, and in elderly. This mechanism may therefore contribute to anabolic resistance. However, although resistance exercise enhances muscle microvascular blood flow in older individuals, it does not restore muscle anabolic response to nutrition [74].

5.7 How to Override Anabolic Resistance?

5.7.1 Optimization of Protein Intake

Several nutrition-based strategies may serve to compensate for anabolic resistance. Optimization of protein intake in particular can be applied to maximize the skeletal muscle protein anabolism. The MPS in response to protein intake may be improved

by controlling the amount of dietary protein, the nature of the protein, the content in specific AAs, the timing of administration, and the co-ingested macronutrients [3].

In contrast to young adults, in whom postexercise rates of MPS are saturated with 20 g of protein, elderly subjects may need as much as 35–40 g to maximally stimulate MPS [75–79]. Therefore, the elderly may require more protein to acutely increase rates of MPS than the young. This fits with the hypothesis of an increased AA threshold that must be surpassed after protein ingestion to stimulate MPS above rest in elderly [24]. It remains to be determined whether more protein is required to maximize MPS in other situations of anabolic resistance such immobilization and inflammation.

Besides the amount of protein, the nature of the protein ingested may also determine the degree of MPS. The MPS response to a specific protein depends on its digestibility and absorption as well as its AA composition. Both milk and beef ingestion augments the postexercise MPS response, with a stronger stimulation of MPS during the early postprandial stage after milk ingestion [80]. Dairy proteins seem to offer some advantage for muscle anabolism over other protein sources, in particular plant-based soy protein. The two major types of milk protein, casein and whey, markedly stimulate MPS. The more rapidly the protein is digested and absorbed, the greater the postprandial MPS is. Keeping with this, hydrolyzed casein is more potent than intact or micellar casein to stimulate MPS [81]. Whey, a fast digestible soluble protein, increases MPS even more than casein [82]. While casein is converted to a solid clot in the gastric acid environment, whey remains soluble in the stomach, allowing rapid digestion. Differences in anabolic properties of various protein sources are also attributable to differences in AA composition. The higher leucine content of whey protein versus casein may contribute to the greater anabolic properties of whey protein compared to casein. Indeed, there is a good correlation between the rise in circulating leucine concentrations and the postprandial MPS after whey and casein ingestion [83].

Supplementation with BCAAs has been shown to attenuate the loss of muscle mass caused by disuse [84–86] or aging [87]. Supplementation of a suboptimal protein intake with leucine is as effective as a complete protein intake in stimulating postprandial MPS [88]. Leucine-enriched EAAs ingestion after resistance exercise seems to prolong the anabolic response of the skeletal muscle to AAs in older adults [88]. Leucine-rich AA mixtures or proteins appear more efficient than leucine alone to improve muscle mass and performance. However, until now, there is no evidence that chronic free leucine supplementation promotes muscle mass or prevents protein loss during states of anabolic resistance [89, 90]. This may be due to desynchronization between leucine signal and the rise in all AAs, decline in other circulating BCAAs valine, and isoleucine or parallel stimulation of MPB [91].

Finally, the timing of protein intake is an important parameter to consider for optimizing the anabolic response of AAs. The ingestion of protein in the hours just after exercise enhances the MPS. It even seems that this “anabolic window” lasts for at least 24 h following exercise. To reach a near-maximal postprandial MPS response, it is advised to provide 20–25 g dietary protein every 4–5 h and a further 25–40 g protein prior to sleep [30, 92]. Repeated ingestion of 20 g of protein every

3 h during 12 h after a bout of resistance exercise is superior for stimulating MPS than two bolus of 40 g or eight pulses of 10 g [93]. Similarly, emerging evidence suggests that the elderly may need to distribute protein intake evenly throughout the day across three or more daily meals [24], as long as each meal provides enough protein to reach the anabolic threshold (at least 20 g).

It could be suggested that the stimulation of insulin secretion by CHO may enhance the MPS response to protein intake. However, ingestion of CHO with sufficient amounts of protein does not further increase MPS following exercise [94]. Indeed, circulating insulin is more permissive than stimulatory on MPS response [10].

In critically ill patients, there is a lack of data on protein requirements necessary to override the anabolic resistance [95].

5.7.2 Resistance Exercise

Exercise in general appears to depress MPS, whereas MPB is probably increased, causing negative net muscle protein balance. However, the inhibition of MPS that occurs during muscle contraction is rapidly reversed during postexercise recovery. Therefore, positive net balance may be achieved after the exercise when AA availability is increased, thereby raising MPS markedly. Postexercise-increased AA availability is less crucial than insulin for inhibiting MPB.

Resistance exercise is an important countermeasure to disuse atrophy and to age-related declines in the skeletal muscle mass. What is less well understood is how the intensity and the volume of the resistance exercise stimulus are sufficient to result in rises in MPS. Evidence suggests that minimal resistance exercise preserves MPS throughout bed rest [96]. In older adults, frequent high-intensity weight lifting or alternatively low-intensity high-volume weight lifting increases muscle mass.

The synergistic anabolic effect of resistance exercise in combination with EAAs ingestion has been well documented in particular in the elderly. It appears that protein ingestion at doses at least 20 g and perhaps as high as 30–40 g in close proximity to resistance exercise may be able to elicit an anabolic response in the elderly. Resistance training has been shown to sensitize the skeletal muscle to feeding for up to 24 h [15, 97]. Therefore, resistance exercise training combined with appropriately timed protein (likely leucine-rich) ingestion may represent a highly effective treatment strategy to counteract the sarcopenia.

Older adults are more susceptible than young persons to muscle loss after short-term bed rest. Interestingly, exercise rehabilitation has been shown to restore bed rest-induced deficit in lean mass, strength, and nutrient-induced anabolism in older subjects [23].

There are substantial evidence that resistance training increases muscle mass and strength in older adults [98] with improvement in function and performance of activities of daily living [99].

5.7.3 *Anti-inflammatory Agents*

Inflammation has been shown to blunt the anabolic effect of feeding on MPS [100]. Reduction of low-grade inflammation with NSAID restores blunting of postprandial muscle anabolism in old rats and potentiates the exercise-induced increase in muscle mass and strength in humans [101, 102]. Similarly, omega or n-3 fatty acids which exert anti-inflammatory action enhance the sensitivity of MPS to co-infusion of AAs and insulin in older adults [103]. This effect was associated with increased phosphorylation of mTORC1 and S6K1. These observations fit with the hypothesis of an increased anabolic threshold caused by inflammation and an attenuation of anabolic resistance by n-3 fatty acids. It is also possible that n-3 FA have some intrinsic muscle protein anabolic effect, as this stimulation of MPS occurs to the same extent in healthy young subjects where inflammation is probably absent. However, it is not known whether the decrease in the anabolic threshold obtained with anti-inflammatory agents is large enough to preserve muscle mass in the long term in humans.

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