Insulin resistance: a contributing factor to age-related muscle mass loss?

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SUMMARY
Structural and functional modifications occur in skeletal muscle during aging. These defects lead to impairment in muscle strength, contractile capacity and performance. Among factors implicated in this age-related loss of muscle mass, a dysregulation of protein synthesis and breakdown has frequently been reported. Insulin plays a major role in regulating muscle protein metabolism, since its action contributes to increase net gain of muscle protein in animal and humans. More recently, specific actions of insulin on various muscle proteins, notably mitochondrial proteins, have been demonstrated, suggesting that insulin is also a major regulating factor of mitochondrial oxidative phosphorylation in human skeletal muscle. Insulin resistance develops with aging, classically involving changes in glucose tolerance. However, the effect of insulin on protein metabolism is less well documented, and insulin resistance could be involved in age-related muscle protein loss, progressively leading to sarcopenia. Therefore in a more general concept, insulin resistance found in many clinical settings, could be considered as a contributor to muscle wasting.

Key-words: Muscle · Protein · Mitochondria · Insulin resistance · Aging.


RéSUMÉ
La résistance à l’insuline : un facteur contribuant à la fonte protéique musculaire liée à l’âge ?
Au cours du vieillissement, des modifications structurelles et fonctionnelles des muscles squelettiques apparaissent. Ces modifications provoquent des altérations de la force musculaire, de la capacité contractile et des performances musculaires. Parmi les facteurs impliqués dans cette perte de masse musculaire observée avec l’âge, des altérations de la régulation de la synthèse et de la protéolyse des protéines sont fréquemment rapportées. L’insuline joue un rôle majeur dans la régulation du métabolisme protéique musculaire, en effet, son action contribue à augmenter la quantité de protéines musculaires, tant chez l’animal que chez l’homme. Plus récemment, il a été mis en évidence des actions spécifiques de l’insuline sur différentes protéines musculaires, en particulier sur les protéines mitochondriales, ce qui suggère que l’insuline serait également un facteur majeur de la régulation de la phosphorylation oxydative mitochondriale dans le muscle squelettique humain. L’insulinorésistance qui se développe avec le vieillissement, classiquement s’accompagne d’altérations de la tolérance au glucose. Cependant, les effets de l’insuline sur le métabolisme protéique sont moins bien documentés, et l’insulinorésistance pourrait être impliquée dans la perte de masse protéique musculaire observée avec l’âge, aboutissant progressivement à la sarcopénie. Ainsi, d’une manière générale, l’insulinorésistance rencontrée dans de nombreuses situations cliniques, peut être considérée comme contribuant à la perte musculaire.

Mots-clés : Muscle · Protéines · Mitochondries · Insulinorésistance · Vieillissement.
Normal aging in humans is associated with a physiological decline in skeletal muscle mass [1, 2], also defined as sarcopenia. This age-related loss is associated with numerous health consequences for elderly people such as alterations of muscle strength, quality and functionality leading to an increased prevalence of falls and fractures, greater morbidity and loss of autonomy. Sarcopenia is the consequence of complex multifactorial processes [3], but the mechanisms leading to it are still unclear. The reduction of muscle mass is explained by a loss of proteins resulting from an imbalance between the rates of protein synthesis and breakdown (or proteolysis). Both of these parameters are influenced by a variety of hormones, the nutritional status, and the level of muscular activity [4, 5].

In addition, a progressive resistance to insulin action on glucose metabolism is frequently reported in elderly people. However, insulin, the main post-prandial hormone, also regulates protein metabolism specifically in muscle. Its anabolic action is essential for protein gain and muscle growth. A lack of insulin, such as in type 1 diabetes, is associated with a rapid and major muscle protein mass wasting [6]. This review will focus on the possible involvement of insulin resistance developed during aging in muscle protein loss characterizing sarcopenia.

**Regulation of muscle protein metabolism by insulin**

Roles of insulin in muscle protein metabolism regulation have been explored since the 70’s with various experimental models. *In vitro* experiments, in isolated muscles, reported that protein synthesis is stimulated by insulin [7, 8]. However, most of the studies performed *in vivo* in humans, demonstrated that insulin inhibited muscle protein degradation without any effect on protein synthesis [9-12]. Only one author combining an isotopic approach together with a new mathematical model reported a stimulation of muscle protein synthesis in human during physiological hyperinsulinemia [13]. Among anabolic factors regulating protein metabolism, amino acids are known to stimulate protein synthesis in skeletal muscle [14, 15]. Thus, the inhibition of proteolysis induced by insulin, leading to a concomitant decrease in amino acid plasma concentration during insulin infusion [16, 17], may reduce the amino acid availability for protein synthesis, which could explain the apparent lack of effect of insulin on muscle protein synthesis reported *in vivo*. Several studies have demonstrated the relevant combined action of insulin and amino acids in stimulating protein synthesis in skeletal muscle [18-20]. In addition, respective roles of these two factors on protein synthesis have been explored in rat epitrochlearis muscle after meal ingestion containing either 25% or 0% of amino acids [21]. Postprandial insulin levels were either maintained or blocked with diazoxide injections [22]. The meal containing 25% amino acids in the presence of a higher plasma insulin stimulated protein synthesis and inhibited proteolysis in skeletal muscle in comparison with the postabsorptive state. Together these data indicated that both insulin and amino acids are required to stimulate muscle protein synthesis. In humans, simultaneous insulin and amino acid infusions induced an increase in muscle protein synthesis associated with an inhibition of proteolysis [23, 24]. In addition, Volpi *et al.* observed that after the administration of an amino acid-glucose mixture, protein synthesis was stimulated in young subjects’ vastus lateralis muscles [25]. These observations confirm that stimulation of muscle protein synthesis by insulin implicates a concomitant elevation of amino acids.

**Influence of age on insulin action on muscle protein metabolism**

During aging, a progressive impairment of insulin action on glucose metabolism develops progressively both in animals [26] and in humans [27]. Indeed, it is usually reported that peripheral glucose utilization mediated by insulin is reduced in elderly humans with a normal suppression of hepatic glucose production [27-29]. Nevertheless, age-related changes in insulin action on body protein homeostasis have been less documented. Whether the age-related defect in insulin action on glucose metabolism extends to amino acid metabolism is controversial. Previous studies interested in this question observed a lower glucose disposal rate in elderly subjects, but a normal reduction of whole body protein breakdown under different insulin infusion rates [30, 31]. However, the effect of insulin on protein breakdown observed in elderly subjects was obtained with higher insulin concentrations than in young subjects, suggesting a reduced insulin clearance with age as previously reported [32]. Considering this discrepancy, we clearly observed a lower inhibition of whole body protein breakdown by insulin in elderly subjects [33, 34]. Therefore, these latter studies demonstrated that a resistance to insulin action developed with age for both glucose and protein metabolism at whole body level. At the muscle level, the response of protein to insulin seems to be also affected by senescence. In old rats, a lower effect of insulin on muscle protein synthesis has been previously observed in comparison with young rats [35]. In humans, impairment in muscle protein anabolism in response to the intake of an amino acid-glucose mixture, increasing plasma insulin and amino acid concentrations [25], or to the simultaneous infusion of insulin and amino acid [36], has been reported in healthy elderly subjects. These data suggest that the combined action of insulin and amino acids to stimulate muscle protein synthesis is impaired in this population. Therefore, loss of control of the anabolic action of insulin on aging muscle is a key factor favoring sarcopenia.
Defect in intracellular insulin signaling pathway

To examine the potential mechanisms leading to these alterations, we have investigated some components of the insulin signal transduction pathway in human muscle. Insulin and amino acids stimulate protein synthesis through molecular mechanisms (Fig. 1) specified in vitro and in animal or humans [37]. Gene transcription, ARN stability and ARN messenger of initiation translation are activated by insulin [38, 39]. Insulin binding on its receptor located on muscle cells induces receptor autophosphorylation and activation of IRS-proteins initiating the biochemical pathway of phosphatidylinositol 3-kinase (PI 3-kinase). This protein plays a major role in both glucose and protein metabolism, since it activates glucose uptake, and regulates translation initiation through rapamycin-sensitive signaling intracellular pathway: the mammalian target of rapamycin (mTOR) pathway (Fig. 1) [40]. This pathway involves two key regulatory proteins, 70-kDa ribosomal protein S6 kinase (S6K1) and eukaryotic initiation factor 4E binding protein-1 (4E-BP1). The increase of the 4E-BP1 phosphorylation state, probably induced by mTOR activation, results in dissociation of the 4E-BP1·eIF4E complex. Modification of the complex allows the formation of an active component, which induces one of the first steps of the translation initiation. In addition, the activation of this pathway is associated with an increase in the S6K1 activity and subsequently the phosphorylation of ribosomal protein S6. S6 phosphorylation plays an important role in regulating the synthesis of proteins involved in the production of the transnational apparatus (ribosomal proteins, translation initiation and elongation factors). To our knowledge, possible changes in insulin transduction signal during aging have not been fully investigated. In old rat muscle, phosphorylation of S6K1 appeared not to be altered in basal state, but was less sensitive to the stimulating action of leucine compared to young and adult rats [41]. This modification was correlated with a lower response of muscle protein synthesis to leucine in old rats. In elderly subjects a defect in 70-kDa ribosomal protein S6 kinase (S6K1) activation was found, whereas other translation initiation factors were normally activated by insulin and amino acids [36]. These results obtained in our laboratory and confirmed recently by another group [42] could explain the lower response of muscle protein to anabolic factors (insulin and amino acids) and represent a mechanistic basis for the understanding of muscle protein loss occurring during aging. In addition, several recent findings suggest that decreased activity of the insulin/insulin-like growth factor 1 (IGF-1) intracellular signaling pathway (IGF-PI3K-AKT) can lead to muscle atrophy [43]. Atrophying muscles are characterized by an increased rate of protein degradation primarily through activation of a specific proteolytic system, the ubiquitin-proteasome pathway [44]. The protein induced most dramatically during atrophy is a muscle-specific ubiquitin-ligase, atrogin-1 [45]. Knockout animals lacking atrogin-1 gene show a
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reduced rate of muscle atrophy after denervation [46]. A recent study has observed in normal, growing myotubes and adult muscle fibers, that the IGF-P3K-AKT pathway suppresses the expression of atrogin-1 by inactivating FOXO (Forkhead box O) transcription factors [47]. By contrast, in catabolic conditions, where insulin and IGF-1 are low (during fasting or diabetes), AKT is dephosphorylated and its activity reduced below control levels, leading to activation of FOXO and to transcription of atrogin-1 and other genes that promote wasting [47] (Fig. 2). Consequently, all these states should provoke a reduced protein synthesis and an increased protein breakdown which leads to an important decrease in muscle cell protein content. The FOXO proteins also seem to play a role in the loss of muscle mass occurring with age. Indeed, increased expression and amount of FOXO were reported in aged muscle both in animals [48] and in humans [49, 50]. Moreover, a study performed in aged rats, showed increased atrogin mRNA expression [51]. Interestingly, it is also important to mention that FOXO factors are implicated in the development of insulin resistance in type 2 diabetes [52, 53].

Taken together, all these data suggest that during aging, defects in activation of the intracellular signaling pathway of insulin may promote activation of FOXO proteins and thus increase transcription of “atrogens” progressively leading to muscle protein loss.

Skeletal muscle mitochondria and insulin resistance

Skeletal muscle is composed of several groups of proteins that can be globally separated according to their physiological functions into myofibrillar proteins, implicated in contractile movements, mitochondrial proteins involved in energy production, and sarcoplasmic proteins contributing to anaerobic energy production, intracellular transport, and numerous other cell functions. Synthesis rate measurement of mixed muscle proteins represents the average of many proteins present in different amounts and with different turnover rates [54, 55]. Thus, when regarding the regulation of muscle proteins, changes observed in mixed muscle synthesis rate may not reflect the changes occurring in specific protein fractions synthesis rates. Interestingly, these various muscle proteins may be differently affected by insulin. Indeed, a stimulation of mitochondrial protein synthesis has been recently shown after insulin infusion in animals [56] and in humans [36, 57]. On the contrary, insulin has no effect on the synthesis rate of the myosin heavy chain [56]. Therefore, the lack of stimulation of mixed muscle protein synthesis by insulin observed previously could be due to different action of insulin on various protein fractions in skeletal muscle. The increase in mitochondrial protein synthesis by insulin is a crucial factor for the maintenance of mitochondria proteins and their functional activity. Indeed, citrate synthase, cytochrome c oxidase activities and ATP production were increased after insulin infusion whereas ATP production was blunted in insulin-resistant type 2 diabetic patients [57]. Thus, a defect of insulin action on mitochondrial proteins can have direct consequences on mitochondrial function and integrity. Interestingly, in type 2 diabetic patients, insulin seemed to have no effect on mitochondrial protein synthesis and on enzyme activity [58]. In the same way, we recently observed a reduced effect of insulin on mitochondrial protein synthesis and enzyme activities in insulin-resistant elderly subjects [59]. In sedentary elderly subjects, insulin resistance was also associated with mitochondrial oxidative capacities, suggesting that mitochondrial integrity is linked to insulin sensitivity [60].

From a metabolic standpoint, insulin resistance is associated with decreased skeletal muscle oxidative capacity in type 2 diabetes [60] and in obesity [61, 62]. A reduction of muscle oxidative capacity has also been demonstrated during...
aging [63, 64], but this impairment seems to be more a result of physical inactivity than of aging per se [65]. Petersen et al. recently observed that this age-associated decline in mitochondrial function could contribute to insulin resistance in the elderly [66]. Actually in all these situations, the lower mitochondrial oxidative capacities result in accumulation of fat within the skeletal muscle which may lead to the development of insulin resistance [66, 67].

Thus, the relationship between insulin sensitivity and mitochondria function is a closed loop where reduced mitochondrial function is associated with insulin resistance which in turn may impair the stimulating action of insulin on mitochondrial protein synthesis and functions (Fig. 3). These findings raise the possibility that impaired mitochondrial function and protein synthesis is a pathogenic event associated with insulin resistance. Mitochondrial dysfunction therefore, may be a cause of or a result of insulin resistance, which is able to blunt the effect of insulin on mitochondrial protein synthesis. Data from our group revealed that in aged people, development of insulin resistance is associated with alterations in muscle oxidative capacity and with a reduced effect of insulin on mitochondrial protein synthesis [36]. Consequently, this defect may cause alteration in mitochondria function, which may contribute to impairment in muscle function with potential metabolic and contractile consequences in elderly people. The improvement in insulin sensitivity is thus of major interest not only to prevent metabolic or vascular risks associated with insulin resistance but also to preserve muscle metabolic capacity during aging, as well as in obesity and type 2 diabetes.

Inflammation and sarcopenia

An emerging issue in the past few years for sarcopenia research has been whether there is also an implication of catabolic signals which could be driven by systemic inflammation [68]. Indeed, chronic inflammation is frequently observed during aging and is considered as a major risk factor for age-related chronic diseases (Alzheimer’s disease, atherosclerosis, diabetes and cancer). This status is characterized by increased circulating levels of inflammatory markers, i.e. tumor necrosis factor α (TNF-α), interleukin-6 (IL-6) and C reactive protein (CRP). Systemic low-grade inflammation has been associated with decreased muscle mass as well as the development of functional disability in elderly populations [69-71]. TNF-α effects, including increased basal energy expenditure, anorexia, loss of muscle and bone mass, are associated with wasting/cachexia in chronic inflammatory disorders. These effects may directly contribute to sarcopenia. Consistent with these findings, muscle protein synthesis was inversely related to local levels of TNF-α protein in skeletal muscles in frail very old humans [72]. In a recent study of nursing home residents aged 85-96 years, systemic low-grade activation of the TNF-α system at baseline was inversely correlated to muscle strength after resistance training for 12 weeks, demonstrating that TNF-α could also be a limiting factor for training-induced improvement in muscle strength in very old people [73]. The role of IL-6 in sarcopenia is not clear. Epidemiological studies have reported that IL-6 is strongly associated with functional disability and loss of muscle mass but experimental studies have not been able to link IL-6 to sarcopenia [74].

Interestingly, close relationships exist between TNF-α and IL-6 expression levels and insulin-sensitivity [75, 76]. There is evidence favoring chronic inflammation as an inducer for insulin resistance, rather than the reverse situation [77-79]. Thus, during aging, elevation of inflammatory markers could be involved in the development of insulin resistance, resulting in impaired anabolic action of insulin on muscle protein metabolism.

![Figure 3](image-url) Relationship between impaired mitochondria oxidative capacities and insulin resistance in skeletal muscle.
Conclusion

During aging, insulin resistance seems to be involved in muscle protein loss. Its contribution in aged muscle is illustrated by a reduced response of protein metabolism to insulin at both whole body and muscle levels. The distinctive lower effect of insulin on skeletal muscle protein synthesis may involve a defect in the insulin signal transduction pathway. Moreover, development of insulin resistance during aging may induce mitochondrial alterations leading to a reduction in energy production required for muscle contraction. These novel concepts suggest that development of insulin resistance may be a potential worsening factor to consider in the progression of muscle wasting occurring with age and also in numerous clinical states (diabetes, cancer, HIV, obesity). Therefore, it is suggested that beyond glucose homeostasis, insulin treatment in elderly people is an appropriate therapeutic strategy to optimize muscle protein gain and limit the progression of sarcopenia.

References


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