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ScienceDirect
Journal of Nutritional Biochemistry 21 (2010) 325–331
Journal of Nutritional Biochemistry

Presence of low-grade inflammation impaired postprandial stimulation of muscle protein synthesis in old rats☆

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Received 1 August 2008; received in revised form 8 January 2009; accepted 9 January 2009

Abstract

Aging is characterized by a decline in muscle mass that could be explained by a defect in the regulation of postprandial muscle protein metabolism. This study was undertaken to examine a possible link between the development of low-grade inflammation (LGI) in elderly and the resistance of muscle protein synthesis and degradation pathways to food intake. Fifty-five 20-month-old-rats were studied for 5 months; blood was withdrawn once a month to assess plasma fibrinogen and α2-macroglobulin. Animals were then separated into two groups at 25 months old according to their inflammation status: a control non-inflamed (NI, $n=24$) and a low-grade inflamed group (LGI, $n=23$). The day of the experiment, rats received no food or a meal. Muscle protein synthesis was assessed in vivo using the flooding dose method ([1-^{13}C]phenylalanine) and muscle phosphorylation of protein S6 kinase, and protein S6 was measured in gastrocnemius muscle. Muscle proteolysis was assessed in vitro using the epitrochlearis muscle. Postabsorptive muscle protein synthesis and proteolysis were similar in NI and LGI. After food intake, muscle protein synthesis was significantly stimulated in NI but remained unresponsive in LGI. Muscle proteolysis was similar in both groups whatever the inflammation and/or the nutritional status. In conclusion, we showed that development of LGI during aging may be responsible, at least in part, for the defect in muscle protein synthesis stimulation induced by food intake in rats. Our results suggested that the control of LGI development in elderly improve meal effect on muscle protein synthesis and consequently slow down sarcopenia.

Keywords: Aging; Muscle; Protein synthesis; Nutrition; Low-grade inflammation; Rat

1. Introduction

Aging is characterized by a gradual loss of muscle proteins (sarcopenia), which is ultimately responsible for decreased mobility and autonomy. Sarcopenia also reduces the ability of the elderly to cope with nutritional, infectious or traumatic stresses, whose incidence increases in aging [1]. Proteins in skeletal muscle, as in other mammalian tissues, undergo a continuous process of synthesis and degradation. Thus, sarcopenia should be due to an imbalance between rates of protein turnover. Skeletal muscle protein synthesis decreases in the postabsorptive (PA) state and increases in the postprandial (PP) state, while protein breakdown follows the inverse pattern. In adults, net positive protein balance in the PP state and net negative protein balance in the PA state cancel each other and allow maintaining muscle mass. There is strong evidence that the stimulatory effect of food intake on protein synthesis and its inhibitory effect on proteolysis is blunted in old muscles from both animals [2–5] and humans [6–8]. It has been hypothesized that this impairment may be responsible for muscle wasting during aging. However, the origin of this resistance to the stimulatory effect of food intake (i.e., dietary amino acids) is unknown. Aging may be also characterized by the development of a low-grade inflammatory state. Age-associated inflammation may affect skeletal muscle protein metabolism based on the well-established negative effect of acute and chronic inflammation on skeletal muscle mass and protein metabolism. For example, sepsis induces a dramatic muscle mass loss associated with a reduction in protein synthesis rate in adult rats [9]. TNF-α, which is one of the main inducers of the acute-phase response [10], plays an important role in alterations of muscle protein metabolism in this model of sepsis [11]. Cytokines and particularly TNF-α impair skeletal muscle protein synthesis by decreasing the stimulation of the mTOR signaling pathways [12]. Interestingly, this

☆ This project was supported by a grant from the French Ministry of Research (UNH1019).
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doi:10.1016/j.jnutbio.2009.01.005

signaling pathway has been shown to be responsible for muscle protein synthesis stimulation by food intake and amino acids and shown to be altered during aging [13,14].

Levels of inflammatory markers, such as interleukin-6 (IL-6) and C-reactive protein (CRP), increase slightly with aging, and these higher levels are correlated with disability and mortality in humans [15–18]. Even if the increase is moderate, higher levels of cytokines and CRP increase the risk of muscle strength loss [19] and are correlated to lower muscle mass in healthy older persons [20]. We have recently shown that old rats also present a moderate increase in plasma inflammatory markers such as α2-macroglobulin and fibrinogen concentrations [21]. These observations raise the question of a possible link between low-grade inflammation (LGI) and sarcopenia in elderly. To support this observation, a recent study including young and old volunteers shows that levels of CRP, IL-6 and TNF-α RII were negatively correlated with skeletal muscle protein synthesis rate [22]. Based on these data, we hypothesized that the development of LGI during aging may be involved in the impairment of PP muscle protein metabolism.

2. Materials and methods

2.1. Animals and experimental design

The experiments were conducted in accordance with the National Research Council's Guidelines for the Care and Use of Laboratory Animals. Old Wistar rats (n=55, 20 months old) were housed under controlled environmental conditions (temperature, 22°C; 12-h dark period starting at 0800 h). They were given free access to water and commercial laboratory chow (U.A.R. A04, Usine d'Alimentation Rationnelle, Villemoisson, France) containing 19% protein, 3% fat, 59% carbohydrates, water, fibers, vitamins and minerals. Old rats were studied for 5 months. Animals were weighed each week and food intake was recorded as well. Once a month, a blood sample was withdrawn from a lateral tail vein to assess plasma fibrinogen, α2-macroglobulin and albumin levels. We have recently shown that α2-macroglobulin levels are strong predictors of persistent low-grade inflammatory status in old rats when levels are above ≈100 mg/l [23]. According to this plasma parameter, rats were separated into two groups: a group of rats showing low levels of α2-macroglobulin (≤100 mg/l) that was considered as the non-inflamed group (NI) and a group in which α2-macroglobulin levels increased (100≤mg/l≤800) was considered as the LGI group [23]. Rats that showed α2-macroglobulin concentrations higher than 800 mg/l have been considered highly inflamed according to our previous work [23] and were eliminated of the study. They represented only eight rats.

2.2. Plasma acute-phase protein measurement and cytokine assay

Plasma fibrinogen was measured by turbidimetry according to Ref. [24] on a Cobas Mira analyzer (ABX Diagnostics, Montpellier, France). Concentration is expressed as grams of human equivalent per liter since human fibrinogen was used as reference (Ingen, Rungis, France). Plasma albumin and α2-macroglobulin were measured by single radial immunodiffusion [9]. Plasma TNF-α, IL-1β, IL-6, monocyte chemoattractant protein-1 (MCP-1) and plasminogen activator inhibitor-1 (PAI-1) were assayed by ELISA (Linco, LincoPlex, MO, USA).

2.3. Protein synthesis measurement

Rats were studied in the PA or PP state (90–120 min after the meal intake). Protein synthesis was assessed with the flooding dose method. As described and validated by Dardevet et al. [3], each rat was injected intravenously with L-[1-^{13}C] phenylalanine (99%) (50 μmol/100 g body weight), 45 min before the time of sacrifice. Animals were then anesthetized with pentobarbital sodium (6 mg/100g body weight); blood was rapidly collected and centrifuged at 3000×g for 10 min. Tibialis anterior muscles, liver and spleen were excised, weighed, frozen in liquid nitrogen and stored at −80°C until analysis.

Free and bound phenylalanine enrichments were determined and measured as previously described [3]. Measurement of free phenylalanine enrichment was done as its t-butyldimethylsilyl derivative by gas chromatography electron impact mass spectrometry, using a HP-5890 gas chromatograph coupled to a HP-5972 organic mass spectrometer quadrupole (Hewlett-Packard, Paris, France). The ions m/z 336 and 337 were monitored. Enrichment of [1-^{13}C]phenylalanine into proteins (muscle proteins and albumin) was measured as its N-acetyl-propyl derivatives by gas chromatography–combustion-isotope ratio mass spectrometry (GC-C-IRMS, Micromass Isochrom II, Fisons Instruments, Middlewitch, UK).

2.4. Calculations of protein synthesis rates

Protein fractional synthesis rate (FSR, in %/d) was calculated from the formula (24):

$$FSR = Sb \times 100/Sa \times t$$

Where Sb is the enrichment at time t (minus natural basal enrichment of protein) of the protein-bound phenylalanine, t is the incorporation time in d and Sa is the mean enrichment of free tissue phenylalanine between Time 0 and Time t. The mean Sa enrichment was the Sa (t1/2) value calculated from the linear regression obtained in tissue between the Time 0 and Time t. For albumin synthesis, the mean Sa enrichment was the Sa (t1/2) minus the excretion time of albumin from liver estimated at 20 min [25].

2.5. Amino acid measurements

An aliquot of plasma (700 μl) was homogenized in 7 volumes of trichloroacetic acid 0.6 mol/l containing 2.5% of thiodiglycol. Norleucine (2.9 mmol/l) was added as internal standard. Samples were incubated on ice for 20 min and centrifuged at 8000×g for 15 min at 4°C. This procedure was repeated one time, and pooled supernatants were passed through columns of cation exchange resin (AG 50W-X8, 100–200 mesh, Bio-rad, Richmond, CA). Purified amino acids eluted from the column by 4 mol/l NH_4OH were dried and reconstituted in 1 ml of 0.1 mol/l lithium acetate buffer pH 2.2. Amino acid concentrations were then determined using an automated amino acid analyzer (HPLC System, BioTEK Instruments).

2.6. In vitro rates of muscle proteolysis

Epitrochlearis muscles from rats were quickly excised and rinsed in Krebs–Henselheit bicarbonate buffer [(mM) NaCl 120, KCl 4.8, $NaHCO_3$ 25, KH_2PO_4 1.2 and $MgSO_4$ 1.2, pH 7.4], supplemented with 5 mM Hepes, 5 mM glucose and 0.1% BSA [14]. Muscles were then transferred to plastic tubes containing 1.5 ml of fresh buffer saturated with a 95% O_2–5% CO_2 gas mixture. After 30 min of preincubation, muscles were transferred to a fresh medium of identical composition and further incubated for 1 h. Rates of protein breakdown were measured by following the rates of tyrosine release into the medium in the presence of 0.5 mM cycloheximide, which blocks protein synthesis [5]. Nonlysosomal and Ca^{2+}-independent proteolysis was measured in a Ca^{2+}-deprived medium supplemented with 50 μM leupeptin and 10 mM methylamine [5]. Protein degradation was expressed in nanomoles of tyrosine released in the medium per milligram protein per hour. Muscle protein content was measured according to the bicinchoninic acid procedure.

2.7. Measurement of muscle protein S6 kinase phosphorylation

Gastrocnemius muscle was homogenized at 4°C in 7 volumes of lysis buffer: 20 mM Hepes (pH 7.4), 100 mM potassium chloride, 0.2 mM EDTA, 2 mM EGTA, 1 mM DTT, 50 mM NaF, 50 mM glycerophosphate, 0.1 mM PMSF, 1 mM benzamidine and 0.5 mM sodium vanadate using a Polytron homogenizer. The homogenate was centrifuged at 10,000×g at 4°C for 10 min. The supernatant containing 50-μg proteins was separated by SDS-PAGE (8.5% polyacrylamide) prior to being transferred on PVDF membrane (Amersham). Western blots were performed using antibody against S6 kinase-1 (S6K1) and phosphorylated S6K1 (Thr421/Ser424) or ribosomal S6 protein and phosphorylated S6 (Ser240/244) (Cell Signaling Technology, Danvers MA, USA).

2.8. Statistical analysis

Data are expressed as means±S.E.M. and analyzed by XLStat (Addinsoft NY, USA, version 7.5.2). Differences in fibrinogen, albumin and α2-macroglobulin levels during the 5 months of the experimental period were assessed by a one-way repeated-measures analysis of variance to test the time effect on these parameters. For protein synthesis and inflammation markers at 25 months old, statistical evaluation of the data was performed by one-way ANOVA to analyze the inflammation status effect or two-way ANOVA to analyze the inflammation and nutritional status. When a significant overall effect was detected, differences among individual means were assessed with Fisher test to determine significant differences. A Student's t test was performed when appropriate. The level of significance was set at P<.05 for all statistical tests.

3. Results

3.1. Animal characteristics

As previously observed, food intake did not stimulate muscle protein synthesis in old rats (4.11±0.18%/day vs. 4.50±0.28%/day in PA and PP state, respectively; not significant). When taken together (n=47), rats exhibited a slight increase in fibrinogen levels associated with a significant increase in α2-macroglobulin (×3)

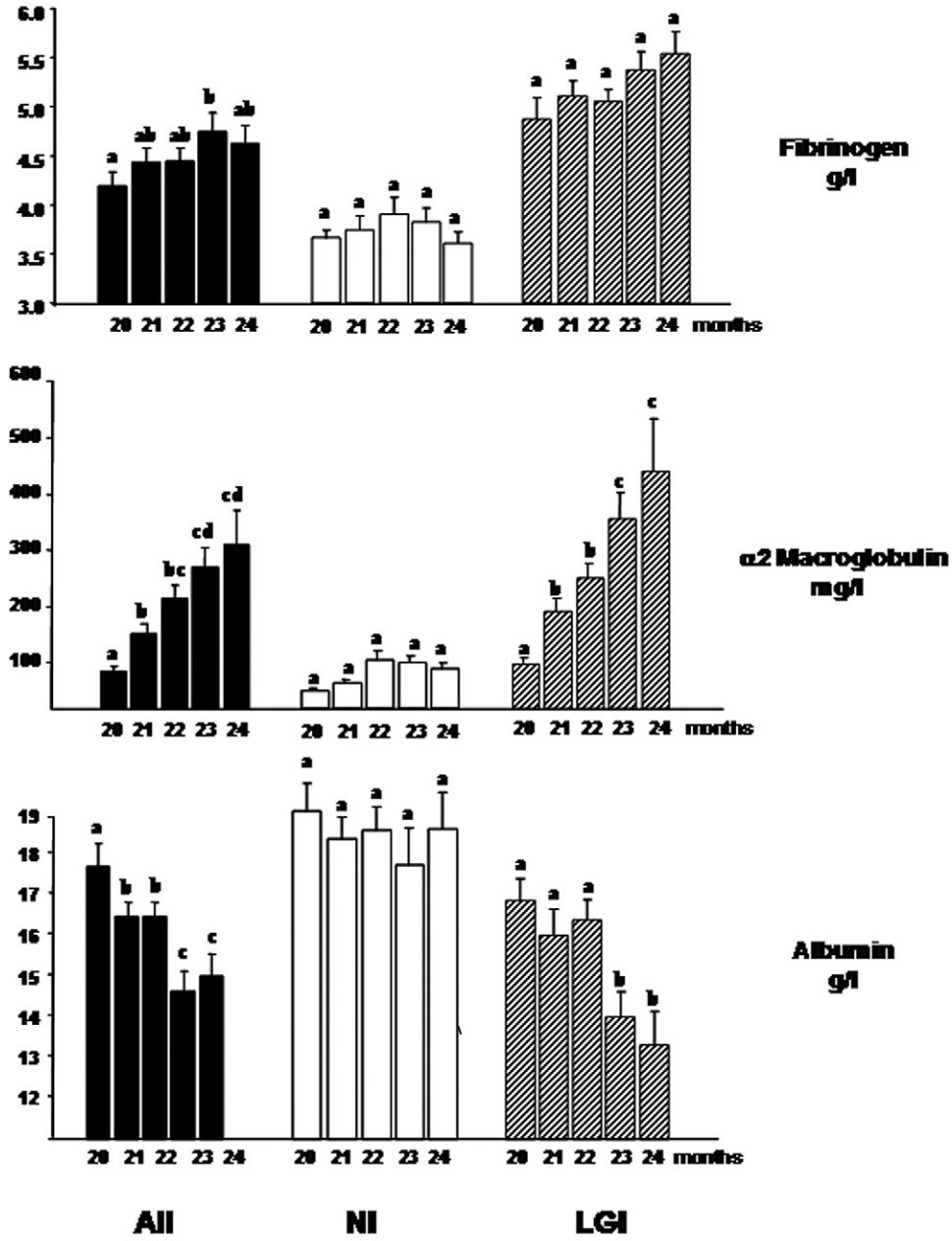

Fig. 1. Evolution of fibrinogen, a2-macroglobulin and albumin levels in old rats for 5 months. Old rats were studied for 5 months. Once a month, a blood sample was withdrawn from a lateral tail vein to assess plasma fibrinogen, a2-macroglobulin and albumin levels. Values are means±S.E.M.; n=47 (group "All"), n=24 (NI) and n=23 (LGI). Values with different letters are significantly different within the same group ($P<.05$).

and a significant decrease of plasma albumin levels (−15%) between 20 and 25 months (Fig. 1), suggesting the development of a spontaneous low-grade inflammatory state in old rats. However, when α2-macroglobulin concentration between the 20th and 25th month was analyzed individually, old rats may be separated into two subgroups (Fig. 1): one group showing no change in α2-macroglobulin level and considered as NI rats ($n=24$) and one group exhibiting a significant increase in α2-macroglobulin level and considered as LGI rats ($n=23$). This increase has been observed spontaneously in only 50% of the old rats studied. Moreover, the LGI group presented an increased fibrinogen concentration ($P<.05$) associated with a decrease of albumin levels over time when compared to the NI group (Fig. 1). Food intake was not affected by LGI development (29.02±0.66 and 29.64±1.04 g/day in NI and LGI, respectively). However, LGI rats lost significantly more body weight than the NI rats (−128±12 vs. −79±10g, $P<.05$).

In order to further characterize the inflammation state of each group, other inflammatory markers were measured at 25 months old. Albumin levels were significantly lower in the LGI group compared to the NI group [14.5±0.3 vs. 17.9±0.4 g/L, respectively (−18%, $P<.05$)].

Table 1
Plasma levels of albumin, fibrinogen, IL-6, IL-1β, TNF-α, MCP-1 and PAI-1 in NI ($n=24$) and LGI ($n=23$) old rats at 25 months of age

	NI	LGI	P
Albumin (mg/ml)	17.90±0.4	14.50±0.3	<.05
Fibrinogen (mg/ml)	3.45±0.11	5.53±0.21	<.05
IL-6 (pg/ml)	25.85±4.59	90.50±21.35	<.05
IL-1β (pg/ml)	60.26±20.58	57.33±18.22	NS
TNF-α (pg/ml)	ND (<4.88)	ND (<4.88)	
MCP-1 (pg/ml)	56.66±13.93	97.04±20.25	<.1
PAI 1 (pg/ml)	77.01±22.75	170.18±47.44	<.1

ND, nondetectable; NS, nonsignificant.

Table 2
Postprandial amino acid concentration (μM) following 90–120 min feeding in NI and LGI old rats

	NI		LGI	
	Mean	S.E.M.	Mean	S.E.M.
Methionine	10.128	0.702	12.322	1.879
Isoleucine	40.158	1.789	47.597	3.309
Leucine	61.413	3.394	69.601	5.996
Tyrosine	170.939	9.120	211.281	26.150
Phenylalanine	238.684	9.163	251.380	12.578
Histidine	54.135	4.449	50.410	2.376
Aspartic cid	18.807	1.455	17.402	2.392
Threonine	209.346	8.299	203.228	20.167
Serine	194.218	7.704	217.767	13.088
Asparagine	30.222	2.842	29.377	1.473
Glutamic acid	285.680	26.460	317.764	25.944
Glutamine	212.119	13.119	210.975	16.784
Glycine	197.008	8.675	214.086	18.382
Proline	158.471	10.797	165.096	14.125
Lysine	256.175	7.651	311.200	20.000
Valine	107.857	6.788	114.397	7.185
Alanine	503.444	37.335	590.997	61.661

LGI rats showed an increase in plasma fibrinogen (1.6 times, $P<.05$) and IL-6 levels (3.5 times; $P<.05$) compared to the NI rats and a trend in higher MCP-1 and PAI-1 levels ($P<.1$) with no modification of circulating IL-1β levels (Table 1). TNF-α levels remained not detectable in both groups (i.e., levels <4.88 pg/ml). Liver weight was significantly higher in the LGI rats by 11% (16.2±0.4 vs. 14.5±0.5 g, $P<.05$). Spleen weight was also higher, but the difference (+16%) did not reach the statistical significance.

3.2. Plasma amino acid concentration

Food consumption during 1 hour of feeding was not different between the NI and the LGI groups (10.14±0.69 vs. 9.30±0.86 g, respectively). The resulting PP plasma amino acid concentrations observed at the sacrifice (90–120 min after feeding) was not different between the NI and the LGI groups (Table 2). Any difference in protein metabolism recorded could not be attributed to different amino acid availability between the NI and LGI old rats.

3.3. Protein synthesis and muscle proteolysis

Muscle protein synthesis was unchanged at the PA state between the two groups (4.06±0.16%/day vs. 4.17±0.2%/day in NI and LGI rats, respectively) (Fig. 2). Food intake increased significantly muscle protein synthesis by 25% in NI rats, whereas it had no effect in LGI rats (Fig. 2). Albumin synthesis was significantly increased by 30% in LGI rats compared to NI but was not sensitive to food intake (Fig. 2). Total amount of skeletal muscle S6K1 protein as well as its phosphorylation was not different in NI and LGI groups at the PA state. S6K1 phosphorylation on threonine 421/serine 424 did not increase after feeding in either NI or LGI old rats (Fig. 3). Amount of the ribosomal protein S6 was similar between the NI and the LGI groups. Food intake increased significantly the phosphorylation of S6 on serine 240/244 to the same extent in both groups (Fig. 3).

Fig. 4 shows that muscle total proteolysis, measured in vitro, was similar in NI and LGI rats at the PA state. Moreover, food intake has no effect on total muscle proteolysis in both groups. Nonlysosomal and Ca^{2+}-independent proteolysis was assessed in both fasted and postprandial states in each group of rats, and no effect of LGI or food intake has been shown on these muscle proteolysis pathways (Fig. 4).

4. Discussion

Aging is characterized by a decrease of muscle mass named *sarcopenia*. Several studies have shown that impairment in the regulation of muscle protein synthesis and proteolysis after food intake may in part explain this phenomenon [2,5]. However, our data clearly showed that the stimulatory effect of food intake on muscle protein synthesis was only impaired in old rats that developed LGI, whereas it was maintained in NI rats. Moreover, LGI increased body weight loss. Postabsorptive muscle protein synthesis as well as muscle proteolysis were not affected by the spontaneous inflammatory state of the elderly animals studied.

Indeed, LGI but not pathological inflammation is clearly present in the LGI group compared to the NI group as shown by the increase in plasma fibrinogen, α2-macroglobulin levels [acute-phase proteins (APPs)], increased liver and spleen weights and decreased albumin levels. The variation of these blood parameters has been identified as downstream indicators of inflammation [26–28]. The hepatic production of these APPs is closely dependent of the circulating levels of pro-inflammatory cytokines such as IL-6 and TNF-α that have been shown to increase during aging in humans [15,29]. However, the levels of APP reached in the LGI group were far from values which are predictive of mortality [23] and remain at much lower levels than in acute inflammation [9,11] or chronic pathological inflammation [30]. Moreover, TNF-α was not detectable in our study (<4.88 pg/ml), whereas IL-6 was moderately increased by 3.5 times with no modification of IL-1β levels or significant increase in plasma PAI-1 and MCP-1. This avoids the presence of acute or pathological

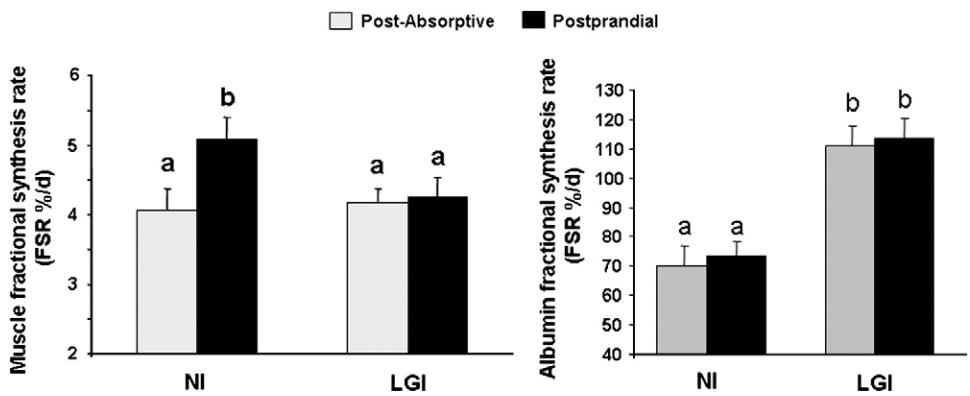

Fig. 2. Effect of food intake on muscle and albumin synthesis in NI and LGI old rats that were food-deprived (PA) or refed (PP). Values are means±S.E.M.; $n=11–12$. Values with different letters are significantly different ($P<.05$).

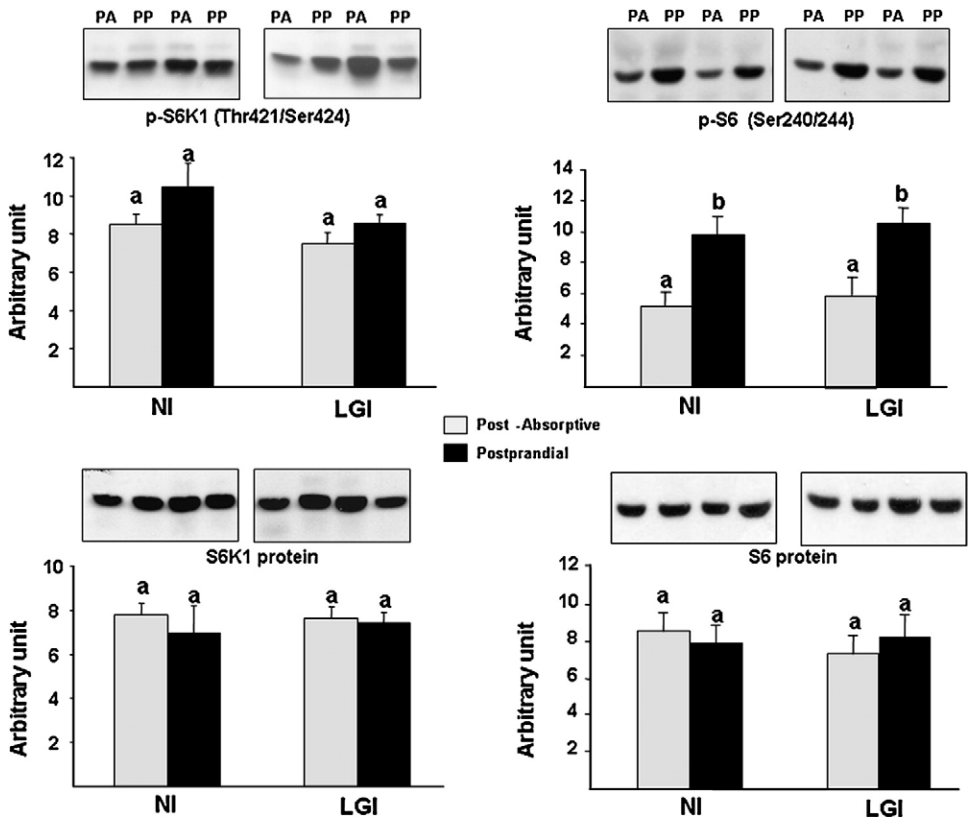

Fig. 3. Amount and phosphorylation state of skeletal muscle protein S6K1 and ribosomal protein S6 in the PA and the PP states from NI and LGI old rats. Values are means±S.E.M. and representative Western blots are shown. Values with different letters are significantly different ($P<.05$).

inflammation in our LGI group. Even if TNF-α was undetectable, we have previously demonstrated that sera TNF-receptor 1 levels were consistently higher in old rats characterized as "low-grade inflamed" based on fibrinogen and α2-macroglobulin levels [23]. The present study clearly showed that all old rats did not exhibit these LGI characteristics, but it is important to note that it was progressively seen spontaneously in about 50% of the animals over a period of 5 months without any apparent pathology, excessive body weight loss or decreased food intake.

The mechanisms leading to sarcopenia are still unclear but result from an imbalance between rates of protein synthesis and degradation. This imbalance is not obvious when basal rates of protein turnover are measured [2,31,32] but is detected in the PP state. An apparent defect in the stimulation of muscle protein synthesis has been shown in old rats [2] and elderly humans [33] after the ingestion of a normal protein meal. Similarly, muscle protein breakdown has been shown to be unresponsive to food intake in aged rats [5]. This defect results in a daily small muscle protein loss, leading in the long term to muscle wasting in elderly. The origin of this alteration remains obscure, but levels of inflammatory markers, such as IL-6 and CRP that increase with aging, have been correlated to lower muscle mass in healthy older persons [20]. Cuthbertson et al. [7] showed in human skeletal muscle that the expression of NF-κB that mediates TNF-α signal was significantly increased in elderly compared to young adults and was associated with a decreased sensitivity and responsiveness of muscle protein synthesis to amino acids. These studies suggested a

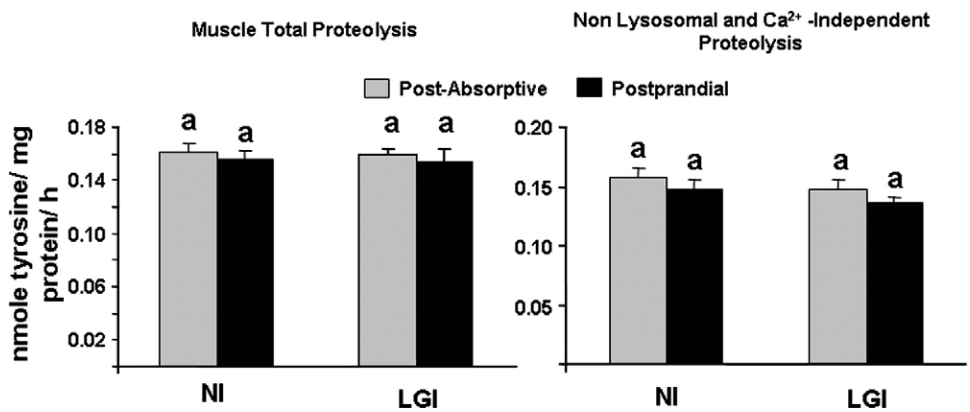

Fig. 4. Effect of food intake on muscle proteolysis in NI and LGI old rats that were food-deprived (PA) or refed for 1 h (PP). Values are means±S.E.M.; $n=11–12$.

possible link between LGI and sarcopenia in elderly. Our study showed that a defect in muscle protein synthesis after food intake occurred only in rats which developed LGI. These observations suggest that LGI which develops during aging challenged anabolic responses associated to food intake, at least in rats.

Muscle protein synthesis is under the control of factors and protein kinases (mTOR, S6K1, eIF4E) involved in the signaling pathways leading to the translation initiation of proteins. Interestingly, this signaling pathway has been shown to be correlated with muscle protein synthesis stimulation by food intake and amino acids. We may hypothesize that LGI even with a slight increased cytokine production renders this signaling pathways within skeletal muscle insensitive to food intake. In the present study, we showed that muscle S6K1 and S6 protein levels were not affected by LGI. S6K1 phosphorylation was not increased by food intake in aged muscle, but its downstream target, that is, the ribosomal protein S6 was similarly phosphorylated with food intake whatever the inflammation state. Reiter et al. showed that the phosphorylation of S6K1 was maximally increased 60 min after refeeding fasted rats and returned toward control values within 180 min [34]. In contrast, phosphorylation of ribosomal protein S6 was maintained through the 180-min time point. Therefore, because muscle samples in the current study were collected 90–120 min after food intake, ribosomal protein S6 phosphorylation should be a good indicator of mTOR/S6K1 activation. However, a specific experimental design to assess the activation muscle signaling pathway should be performed in order to conclude definitively on the effect of LGI on the mTOR/S6K1/S6 activation.

In contrast to muscle protein synthesis, muscle proteolysis was not different in NI and LGI old rats and remained unresponsive to food intake independently of the inflammation status of the rats. It is surprising since cytokines (i.e., TNF-α and IL-6) have been shown to be involved in the increase of muscle proteolysis seen in different acute inflammation [35–37]. This suggests that higher level of cytokines than the ones recorded in our study are required to alter muscle proteolysis. LGI seems not to be the primary factor altering muscle proteolysis regulation during aging.

It is well established that the acute-phase response leads to important metabolic changes and particularly with liver protein and amino acid metabolism [38,39]. We showed that it was also the case during aging with an increase in the synthesis rate of fibrinogen and albumin that are exclusively produced by the liver [21]. In the present study, we demonstrated that albumin synthesis was higher (+30%) in the LGI group compared to the control group. Interestingly, the fractional synthesis rate recorded in the NI rats was similar to the fractional synthesis rate of albumin previously assessed in adult rats [21]. It may thus be hypothesized that the increased requirement of amino acids for the synthesis of these APPs in old LGI rats was provided by sparing amino acid utilization from peripheral tissues, that is, by blunting the postprandial stimulation of muscle protein synthesis.

In conclusion, we showed in the present study that the development of LGI during aging occurred in 50% of rats and was clearly correlated with impaired PP muscle protein synthesis stimulation without further altering muscle proteolysis response. Our results suggested that the control of LGI and/or its prevention in elderly may improve meal effect on muscle protein synthesis and consequently decrease and slow down the appearance of sarcopenia. However, further experiments were necessary to demonstrate that such an effect occurred in human.

Acknowledgments

We thank Arlette Cissoire and Christian Lafarge for their excellent assistance during animal experimentation and acknowledge grant UNH1019 from INRA Clermont-Ferrand-Theix, France. We also thank C. Sornet and V. Mahé for technical assistance.

References

[1] Young VR, Munro HN, Fukagawa N. Protein and functional consequences of deficiency. In: Horwitz A, MacFayden DM, Munro HN, Scrimshaw NS, Steen B, Williams TF, editors. Nutrition in the elderly. , New York: Oxford University; 1989. p. 65–84.
[2] Mosoni L, Valluy MC, Serrurier B, Prugnaud J, Obled C, Guezennec CY, et al. Altered response of protein synthesis to nutritional state and endurance training in old rats. Am J Physiol 1995;268:E328–35.
[3] Dardevet D, Sornet C, Bayle G, Prugnaud J, Pouyet C, Grizard J. Postprandial stimulation of muscle protein synthesis in old rats can be restored by a leucine-supplemented meal. J Nutr 2002;132:95–100.
[4] Arnal MA, Mosoni L, Dardevet D, Ribeyre MC, Bayle G, Prugnaud J, et al. Pulse protein feeding pattern restores stimulation of muscle protein synthesis during the feeding period in old rats. J Nutr 2002;132:1002–8.
[5] Combaret L, Dardevet D, Rieu I, Pouch MN, Bechet D, Taillandier D, et al. A leucine-supplemented diet restores the defective postprandial inhibition of proteasome-dependent proteolysis in aged rat skeletal muscle. J Physiol 2005; 569:489–99.
[6] Guillet C, Prod'homme M, Balage M, Gachon P, Giraudet C, Morin L, et al. Impaired anabolic response of muscle protein synthesis is associated with S6K1 dysregulation in elderly humans. FASEB J 2004;18:1586–7.
[7] Cuthbertson D, Smith K, Babraj J, Leese G, Waddell T, Atherton P, et al. Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle. FASEB J 2005;19:422–4.
[8] Rieu I, Balage M, Sornet C, Giraudet C, Pujos E, Grizard J, et al. Leucine supplementation improves muscle protein synthesis in elderly men independently of hyperaminoacidaemia. J Physiol 2006;575:305–15.
[9] Breuillé D, Arnal M, Rambourdin F, Bayle G, Levieux D, Obled C. Sustained modifications of protein metabolism in various tissues in a rat model of long-lasting sepsis. Clin Sci 1998;94:413–23.
[10] Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. N Engl J Med 1999;340:448–54.
[11] Breuillé D, Voisin L, Contrepois M, Arnal M, Obled C. A sustained rat model for studying long-lasting catabolic state of sepsis. Infect Immun 1999;67:1079–85.
[12] Lang CH, Frost RA, Nairn AC, MacLean DA, Vary TC. TNF-alpha impairs heart and skeletal muscle protein synthesis by altering translation initiation. Am J Physiol Endocrinol Metab 2002;282:E336–47.
[13] Kimball SR, Jefferson LS. Control of protein synthesis by amino acid availability. Curr Opin Clin Nutr Metab Care 2002;5:63–7.
[14] Dardevet D, Sornet C, Balage M, Grizard J. Stimulation of in vitro rat muscle protein synthesis by leucine decreases with age. J Nutr 2000;130:2630–5.
[15] Harris TB, Ferrucci L, Tracy RP, Corti MC, Wacholder S, Ettinger Jr WH, et al. Associations of elevated interleukin-6 and C-reactive protein levels with mortality in the elderly. Am J Med 1999;106:506–12.
[16] Ferrucci L, Penninx BW, Volpato S, Harris TB, Bandeen-Roche K, Balfour J, et al. Change in muscle strength explains accelerated decline of physical function in older women with high interleukin-6 serum levels. J Am Geriatr Soc 2002;50: 1947–54.
[17] Cohen HJ, Harris T, Pieper CF. Coagulation and activation of inflammatory pathways in the development of functional decline and mortality in the elderly. Am J Med 2003;114:180–7.
[18] Bautmans I, Njemini R, Lambert M, Demanet C, Mets T. Circulating acute phase mediators and skeletal muscle performance in hospitalized geriatric patients. J Gerontol A Biol Sci Med Sci 2005;60:361–7.
[19] Schaap LA, Pluijm SMF, Deeg DJH, Visser M. Inflammatory markers and loss of muscle mass (sarcopenia) and strength. Am J Med 2006;119:U82–90.
[20] Visser M, Pahor M, Taaffe DR, Goodpaster BH, Simonsick EM, Newman AB, et al. Relationship of interleukin-6 and tumor necrosis factor-alpha with muscle mass and muscle strength in elderly men and women: the health ABC study. J Gerontol A Biol Sci Med Sci 2002;57:M326–32.
[21] Papet I, Dardevet D, Sornet C, Bechereau F, Prugnaud J, Pouyet C, et al. Acute phase protein levels and thymus, spleen and plasma protein synthesis rates differ in adult and old rats. J Nutr 2003;133:215–9.
[22] Toth MJ, Matthews DE, Tracy RP, Previs MJ. Age-related differences in skeletal muscle protein synthesis: relation to markers of immune activation. Am J Physiol Endocrinol Metab 2005;288:E883–91.
[23] Mayot G, Vidal K, Martin JF, Breuille D, Blum S, Obled C, et al. Prognostic values of alpha2-macroglobulin, fibrinogen and albumin in regards to mortality and frailty in old rats. Exp Gerontol 2007;42:498–505.
[24] Macart M, Koffi A, Henocque G, Mathieu JF, Guilbaud JC. Optimized microturbidimetric assay for fibrinogen. Clin Chem 1989;35:211–4.
[25] Ruot B, Béchereau-Rambourdin F, Bayle G, Breuillé D, Obled C. The response of liver albumin synthesis to infection in rats varies with the phase of the inflammatory process. Clin Sci 2002;102:107–14.
[26] Ershler WB, Keller ET. Age-associated increased interleukin-6 gene expression, late-life diseases, and frailty. Annu Rev Med 2000;51:245–70.
[27] Danesh J, Collins R, Appleby P, Peto R. Association of fibrinogen, C-reactive protein, albumin, or leukocyte count with coronary heart disease: meta-analyses of prospective studies. JAMA 1998;279:1477–82.

[28] Danesh J, Whincup P, Walker M, Lennon L, Thomson A, Appleby P, et al. Low grade inflammation and coronary heart disease: prospective study and updated meta-analyses. Br Med J 2000;321:199–204.
[29] Daynes RA, Araneo BA, Ershler WB, Maloney C, Li GZ, Ryu SY. Altered regulation of IL-6 production with normal aging. Possible linkage to the age-associated decline in dehydroepiandrosterone and its sulfated derivative. J Immunol 1993;150: 5219–30.
[30] Mercier S, Breuillé D, Mosoni L, Obled C, Patureau Mirand P. Chronic inflammation alters protein metabolism in several organs of adult rats. J Nutr 2002;132:1921–8.
[31] Dardevet D, Sornet C, Attaix D, Baracos VE, Grizard J. Insulin-like growth factor-1 and insulin resistance in skeletal muscles of adult and old rats. Endocrinology 1994;134:1475–84.
[32] Volpi E, Sheffield Moore M, Rasmussen BB, Wolfe RR. Basal muscle amino acid kinetics and protein synthesis in healthy young and older men. JAMA 2001;286: 1206–12.
[33] Arnal MA, Mosoni L, Boirie Y, Houlier ML, Morin L, Verdier E, et al. Protein pulse feeding improves protein retention in elderly women. Am J Clin Nutr 1999;69:1202–8.
[34] Reiter AK, Crozier SJ, Kimball SR, Jefferson LS. Meal feeding alters translational control of gene expression in rat liver. J Nutr 2005;135:367–75.
[35] Breuillé D, Farges MC, Rosé F, Arnal M, Attaix D, Obled C. Pentoxifylline decreases the body weight loss and muscle protein wasting characteristics of sepsis. Am J Physiol 1993;265:E660–6.
[36] Voisin L, Breuillé D, Ruot B, Rallière C, Rambourdin F, Dalle M, et al. Cytokine modulation by PX differently affects specific acute phase proteins during sepsis in rats. Am J Physiol 1998;275:R1412–9.
[37] Spate U, Schulze PC. Proinflammatory cytokines and skeletal muscle. Curr Opin Clin Nutr Metab Care 2004;7:265–9.
[38] Biolo G, Toigo G, Ciocchi B, Situlin R, Iscra F, Gullo A, et al. Metabolic response to injury and sepsis: changes in protein metabolism. Nutrition 1997;13:S52–7.
[39] Reeds PJ, Jahoor F. The amino acid requirements of disease. Clin Nutr 2001 (Suppl 1):15–22.