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Presence of low-grade inflammation in old rats does not worsen skeletal muscle loss under an endotoxemic and dietary stress

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Running title: Response to a stress in inflamed or not old rats

Keywords: Ageing; Inflammation; Lipopolysaccharide; α_2 -macroglobulin; Fibrinogen; Albumin.

Abbreviations used: CRP: C-reactive protein, BW: body weight, EDL: *extensor digitorum longus*, IL-6: interleukin-6, LGI-Con: low-grade inflamed rats submitted to the control treatment, LGI-Str: low-grade inflamed rats submitted to the stress, NI-Con: non-inflamed

- 25 rats submitted to the control treatment, NI-Str: non-inflamed rats submitted to the stress,
26 TNF- α : tumor necrosis factor-alpha.

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27 **Abstract**

28 The study aimed to determine if age-associated low-grade inflammation aggravates the
29 response to a stress, especially regarding to sarcopenia. Initial inflammatory status in 22
30 month-old rats was based on plasma α_2 -macroglobulin and fibrinogen concentrations. The
31 stress applied was a single intra-peritoneal injection of lipopolysaccharide followed by a 23-
32 day period of malnutrition, *i.e.* a 4% casein diet distributed in quantity limited to 50% of
33 spontaneous food intake. The response to the stress was analyzed in non-inflamed and low-
34 grade inflamed rats and compared to non-inflamed and low-grade inflamed rats, which
35 received the control treatment (*i.e.* no lipopolysaccharide injection and an 18% casein diet).
36 The stress-induced body weight loss was higher in inflamed than non-inflamed rats, but the
37 decrease in muscle weight was not worsened. Muscle protein turnover was not affected by the
38 stress. Plasma α_2 -macroglobulin levels increased after the stress, whatever the initial
39 inflammatory status. However, fibrinogen levels decreased more in inflamed than non-
40 inflamed rats and albumin levels were not affected by the stress. Independently of the initial
41 inflammatory status, the liver glutathione content was strongly depleted by the stress. These
42 results extend and support our previous findings by demonstrating that age-associated low-
43 grade inflammation does not aggravate sarcopenia in old rats.

1. Introduction

Frailty syndrome is an age-associated physiological state of vulnerability, resulting from impaired homeostatic reserves and a reduced capacity to withstand stress (Fried and Walston, 2003). Frailty consists of multi-system decline and is considered to be a consequence of changes in neuromuscular, endocrine and immune functions that occur as people progress in age (Fried and Walston, 2003; Vanitallie, 2003; Ferrucci et al., 2005; Paganelli et al., 2006). Frailty often results in several “vicious loops”: neuromuscular impairment induces increased rate of falls and fractures in old age; the act of falling causes itself hospitalization and immobilization, which aggravates sarcopenia; sarcopenia leads to deficit in continuous neuromuscular training which brings about neuromuscular impairment (Bales and Ritchie, 2002; Fried and Walston, 2003; Mühlberg and Sieber, 2004). The unintentional body weight loss, which is a frailty criterion (Fried et al., 2001), usually results from poor responses to successive various stresses (Vellas et al., 1992).

Frail subjects exhibit evidence of increased inflammation revealed by modified acute phase protein concentrations (Walston et al., 2002; Puts et al., 2005). Age-associated increase in the levels of inflammatory markers, such as interleukin-6 (IL-6) and CRP, are associated with disability and mortality (Ferrucci et al., 1999; Harris et al., 1999; Ferrucci et al., 2002; Cohen et al., 2003). Since age-associated inflammation appears to be an important component of frailty (De Martinis et al., 2006), it could worsen the aged subjects’ response to a stress. In the same way, acute phase proteins, such as α_2 -macroglobulin and fibrinogen have been recently identified as markers of frailty in old rats (Mayot et al., 2007).

CRP, IL-6 and tumor necrosis factor- α (TNF- α) receptor-2 levels were negatively correlated with the rate of skeletal muscle protein synthesis (Toth et al., 2005). Similarly, in frail human skeletal muscle, TNF- α mRNA and protein levels decreased in concert with increases in strength and protein synthesis after 3 months of resistance exercise (Greiwe et al.,

2001). These results support the idea that age-associated low-grade inflammation affects skeletal muscle metabolism and could participate in sarcopenia development. However, they do not prove the causal relationship within the old population. Indeed, inflamed/frail subjects are often older than control groups (Walston et al., 2002; Barbieri et al., 2003). Thus, these observations may be independent of inflammatory status and more directly related to age. Indeed we observed that low-grade inflammation, lasting at least one month, does not enhance sarcopenia in old rats (Mayot et al., 2006). Since this investigation was performed in rats kept in conditions minimizing any kind of stress, it is not excluded that age-associated low-grade inflammation could impact the old rats' response to a stress.

Diseases, such as infections, and periods of malnutrition, which are frequent in elderly patients, are stressor events that may accelerate frailty (Fried and Walston, 2003). Sarcopenia is associated with a doubled risk of nosocomial infection (Cosqueric et al., 2006). Poor daily energy intake and protein intake adjusted for energy intake are related to frailty (Bartali et al., 2006). Four to 31% of free-living and up to 60% of geriatrics patients on admission in hospitals or nursing homes have shown evidence of malnutrition (Seiler, 2001). Most of these deficits affecting ill elderly subjects are related to proteins and micronutrients. A low protein diet (0.45 vs. 0.92 g/kg/d) reduced muscle mass and function in elderly women (Castaneda et al., 1995). Acute diseases and malnutrition decrease skeletal muscle mass, which provides amino acids for most vital functions (Heymsfield et al., 1982; Obled et al., 2002).

The aim of the present study was to evaluate whether age-associated low-grade inflammation impacts the old rats' response to a stress. The chosen stress combines a short-lasting inflammation (endotoxin injection) and protein-energy malnutrition. This double stress was used to mimic a situation in elderly subjects, who frequently face any kind of transient febrile episode followed by a period of protein-energy malnutrition. The investigation was primarily focused on body weight change, skeletal muscle weight and protein metabolism.

The acute phase protein response, weights of various organs and the glutathione liver status were considered as complementary targets.

2. Material and methods

2.1. Animals

Wistar male rats were produced and bred in our conventional, non specific-pathogen-free, animal facility (Unité Expérimentale de Nutrition Comparée, INRA, Theix, France). Before the experiment, rats were maintained in collective cages (3 to 4 per cage) under controlled conditions (temperature 21°C, relative humidity 55%, 12-h dark period starting at 2000 h). They had free access to water and standard pellets (A04 from SAFE (Scientific Animal Food and Engineering), Villemoisson-sur-orge, France). The composition of the diet was 16% protein, 3% fat, 60% carbohydrates, 12% water, fibers, vitamins and minerals. The study was performed according to the current legislation on animal experiments in France.

2.2. Experimental design

2.2.1. Pre-experimental period

At the age of 22 months, rats which were apparently ill and those exhibiting acute phase proteins levels that are predictive of old rat mortality (Mayot et al., 2007) [i.e. α_2 -macroglobulin and fibrinogen concentrations higher than 500 mg/l and 5.4 g/l, respectively] were excluded. Thirty-four apparently healthy rats were housed in individual cages and acclimatized to their new environment for 3 weeks. They received a diet containing 18% casein over 4 daily periods of 3 hours each, regularly spaced. The quantity of food distributed during the light and dark periods were adjusted to represent about 30 and 70% of the *ad libitum* daily intake, respectively. Four rats died during this period and two rats, which did not

eat, were excluded. Blood was withdrawn from a lateral tail vein 7 days before the end of the pre-experimental period.

2.2.2. Main experiment

Rats were distributed into 2 classes (non-inflamed (NI) and low-grade inflamed (LGI), $n = 14$, each) based on their inflammatory status that was analyzed by hierarchical clustering using Ward distance on α_2 -macroglobulin and fibrinogen variables (Mayot et al., 2007). At day 0, each class was sub-divided into two groups receiving either the control (Con) or the stress (Str) treatment. The control treatment, consisting in maintaining rats in the pre-experimental conditions, was applied to 6 non-inflamed rats (NI-Con group) and 5 low-grade inflamed rats (LGI-Con group). The stress consisted in a single intra-peritoneal injection of lipopolysaccharide from *Escherichia coli* 0127:B8 (0.4 mg/kg) followed by a 23-day period of protein-energy malnutrition, during which rats received a diet containing 4% casein in quantity individually limited to 50% of spontaneous food intake (distributed in four meals as for control treatment). It was applied to 8 non-inflamed rats (NI-Str group) and 9 low-grade inflamed rats (LGI-Str group). Rats were weighed at day 0 then three times per week. Blood was sampled at day 8 at day 21 for plasma analyses. At day 23, all surviving rats ($n = 5$ for NI-Con, LGI-Con and NI-Str groups and $n = 7$ for LGI-Str group) were anaesthetized with an intra-peritoneal injection of ketamine (150 mg/kg). *Epitrochlearis* muscle was quickly removed and incubated to measure *in vitro* protein metabolism, as already described (Combaret et al., 2003). Rats were then killed by aortic blood withdraw. Posterior leg skeletal muscles, liver, spleen, kidneys, epididimal adipose tissue, mesentery, small intestine, colon were dissected and weighted. Liver was frozen at -80°C until glutathione analysis.

2.3. Biochemical measurements

Plasma α_2 -macroglobulin, fibrinogen and albumin were measured as already described (Mayot et al., 2007). Total glutathione was quantified spectrophotometrically using a standard

enzymatic recycling procedure and 5,5'-dithio-bis-2-nitrobenzoic acid as oxidant (Malmezat et al., 1998).

2.4. Statistical analysis

Values are given as means $\pm$ SE. The significance of difference between non-inflamed and low-grade inflamed rats during the pre-experimental period was analyzed with Student's t test for unpaired data. The significance of the initial inflammatory status and the treatment effects was analyzed with 2-way ANOVA. For parameters measured before and at various times after stress induction ANOVA for repeated measurements was performed with the initial inflammatory status and the treatment as the between-rat factors and the time as the within-rat factor. The significance of differences among the four groups was further analyzed by Fisher's PLSD test, when appropriate. Linear regression was used to determine the relationship between acute phase protein variation and body weight change. P values ≤ 0.05 were considered significant. Statistical analyses were performed using StatView for Windows, version 5 software (SAS Institute, Cary, NC), excepted the hierarchical clustering using Ward distance, which was performed with XLSTAT, version 7.5 software (Addinsoft, Paris, France).

3. Results

3.1. Old rats' characteristics before the experimental period

According to the experimental design, low-grade inflamed rats exhibited 287 and 41% higher initial plasma concentrations of α_2 -macroglobulin (147 ± 21 vs. 38 ± 6 mg/l, $P = 0.0002$) and fibrinogen (4.5 ± 0.2 vs. 3.2 ± 0.1 g/l, $P = 0.0002$), respectively (Table 1). As expected, low-grade inflamed rats exhibited 13% lower initial concentration of albumin than non-inflamed rats (12.1 ± 0.6 vs. 13.9 ± 0.6 g/l, $P = 0.055$); however this difference was only a trend. Maximal initial concentrations of α_2 -macroglobulin and fibrinogen were 307 mg/l

and 5.6 g/l, respectively, and minimal initial concentration of albumin was 8.3 g/l. The relative body weight loss occurring over the pre-experimental period (day -22 to day 0) was 74% higher in low-grade inflamed than non-inflamed rats (-6.7 ± 1.3 vs. -3.9 ± 1.2 %, $P = 0.134$) without change in food intake; however the effect of the initial inflammatory status did not attain statistical significance. Based on 2-way ANOVA, groups did not differ with respect to mean body weights at days -22 and 0, and food intake before day 0 (Table 1). No interaction between treatment and initial inflammatory status was significant for any measured parameter (Table 1) indicating the absence of any bias related to the configuration of the groups.

3.2. Stress-induced mortality and body weight change in low-grade inflamed and non-inflamed old rats

The stress (endotoxin injection followed by protein-energy malnutrition) applied to the old rats induced 37 and 22% of mortality in NI-Str and LGI-Str groups, respectively. Indeed, three rats from NI-Str group deceased at days 1, 2, and 6 and two rats from LGI-Str group deceased at days 1 and 22. No mortality was observed in non-stressed rats, but one rat from NI-Con group was euthanized at day 17 due to its huge body weight loss. As expected, mean relative body weight losses (%) over 23 days were very much higher in stress groups (NI-Str: 21.0 ± 1.0 and LGI-Str: 24.0 ± 1.3) than in control groups (NI-Con: 2.2 ± 0.6 and LGI-Con: 2.2 ± 1.4). Based on the ANOVA for repeated measurements, cumulative body weight loss was dependent on the treatment and the time but not on the initial inflammatory status of rats (Fig. 1). The significant interaction between the treatment, the initial inflammatory status and the time likely reflects the fact that relative body weight losses over time were higher in LGI-Str than NI-Str, whereas they were similar in NI-Con and LGI-Con groups. Comparison of results from LGI-Str and LGI-Con groups with the ANOVA for repeated measurements indicated that the relative body weight losses over time was higher in LGI-Str than in NI-Str

(there was a significant interaction between the initial inflammatory status and time). It is worthwhile to note that stress-induced body weight loss (NI-Str and LGI-Str groups) was correlated with initial concentration of either α_2 -macroglobulin ($r^2 = 0.545$, $P = 0.006$) or fibrinogen ($r^2 = 0.331$, $P = 0.050$) and inversely correlated with the initial albumin concentration ($r^2 = 0.340$, $P = 0.047$).

3.3. Stress effects on skeletal muscles in low-grade inflamed and non-inflamed old rats

Based on two-way ANOVA, the stress (endotoxin injection followed by protein-energy malnutrition) applied to the old rats decreased absolute and relative *gastrocnemius* muscle weights by 12% (2.02 ± 0.07 vs. 2.31 ± 0.06 g) and 15% (323 ± 11 vs. 379 ± 17 mg/100g body weight at day 0 (BW)), respectively (Table 2). The same trend was observed for the absolute and relative weight of *tibialis anterior* (-9% (744 ± 26 vs. 815 ± 24 g) and -11% (119 ± 5 vs. 134 ± 5 mg/100 g BW)). The stress had no effect on *extensor digitorum longus* (EDL) and *soleus*. The initial inflammatory status did not affect the muscle weights, excepted for the relative weight of *soleus*, which was 18% higher in inflamed (30.1 ± 0.8 mg/100 mg BW) than in control rats (25.5 ± 1.9 mg/100 mg BW). Neither protein synthesis, nor protein degradation was affected by the treatment or the initial inflammatory status (Fig. 2).

3.4. Stress effects on organs and adipose tissues in low-grade inflamed and non-inflamed old rats

The stress (endotoxin injection followed by protein-energy malnutrition) applied to old rats decreased absolute (and relative) weights of spleen, liver, kidneys and small intestine by about 33 (34), 27 (28), 26 (28) and 10% (12%), respectively (Table 3). The significance was not reached for the stress-induced decreases in absolute and relative weights of colon, mesentery and epididymal adipose tissue respectively. Inflamed rats exhibited higher absolute (42%) and relative (48%) weights of kidneys and 11% higher relative weight of liver than

non-inflamed rats. Interaction between stress and initial inflammatory status was significant for absolute weight of kidneys and relative weights of kidneys and liver, indicating that stress-induced decreases in these organ weights were higher in inflamed than non-inflamed rats. The initial inflammatory status had no significant effect on absolute or relative weights of colon, mesentery and epididymal adipose tissue.

3.5. Stress effects on acute phase proteins and liver glutathione in low-grade inflamed and non-inflamed old rats

The stress, the initial inflammatory status and the time had significant effects on both α_2 -macroglobulin and fibrinogen plasma concentrations (Fig. 3), and the interaction between the stress and the time was significant for fibrinogen. Only initial inflammatory status had a significant effect on albumin plasma concentration. As expected, α_2 -macroglobulin, fibrinogen and albumin were stable over time in non-stressed rats (NI-Con and LGI-Con groups). In stressed rats (NI-Str and LGI-Str groups), α_2 -macroglobulin was higher at days 8 and 21 than day -7. In NI-Str group, it was higher at day 8 than day 21. However, due to high variability, differences were not significant in LGI-Str group. The decrease in fibrinogen at days 8 and 21 was higher in LGI-Str than NI-Str group to reach similar values as in NI-Con group. Albumin was stable in stressed rats.

The stress, but not the initial inflammatory status, had a significant effect on glutathione status (Fig. 4). Glutathione concentration and content were 59% (3.04 ± 0.6 vs. 7.50 ± 0.21 $\mu\text{mol/g}$) and 70% (37 ± 7 vs. 126 ± 8 μmol) lower in stressed than non-stressed rats, respectively.

4. Discussion

The present data indicate that age-associated low-grade inflammation renders old rats more vulnerable to stress-induced body weight loss, although without further decrease in

skeletal muscle weight. Stress-induced decreases in some abdominal organ weights and in fibrinogen plasma concentrations were enhanced by the initial low-grade inflammatory status, but responses of the other acute phase proteins to the stress and stress-induced decreases in liver glutathione concentration and content were independent of the initial inflammatory status of old rats.

In agreement with our previous characterization of a cohort of old rats regarding their inflammatory status (Mayot et al., 2007), rats belonging to the non-inflamed groups NI-Con and NI-Str were clearly non-inflamed and those belonging to the inflamed groups LGI-Con and LGI-Str exhibited low-grade inflammation at the beginning of the experiment. Indeed for the formers, their initial plasma α_2 -macroglobulin and fibrinogen levels did not differ from mean values previously observed for each corresponding first quartile in old rats or for adult rats (8 months). Conversely, the others groups exhibited α_2 -macroglobulin and fibrinogen levels similar to mean values previously observed in third quartiles in old rats. Of note, these concentrations reflect low-grade inflammation since they are much lower than those reported in acute (Breuillé et al., 1998; Breuillé et al., 1999) and chronic (Mercier et al., 2002) inflammation. In addition, initial plasma concentrations of acute phase proteins in inflamed old rats are consistent with those observed with age in human. Indeed, in old (66-76 years) healthy subjects fibrinogen level was ~ 47% greater and albumin level ~ 12% lower than in young (22-26 years) healthy subjects (El Yousfi et al., 2005). We have recently shown that increased fibrinogen and α_2 -macroglobulin levels to the range observed in present inflamed rats were associated with increased levels of soluble TNF- α receptor-1 (Mayot et al., 2007). An activation of the TNF- α signaling is likely to occur in inflamed rats and might be active on skeletal muscle protein metabolism, as recently suggested in human (Toth et al., 2005).

Interestingly, old rats submitted to the control treatment (NI-Con and LGI-Con groups) exhibited stable levels of plasma acute phase protein concentrations over time. Thus,

the inflammatory status defined at the beginning of the experiment was maintained all along the experiment in non-stressed rats. In parallel, final relative weights of liver, kidney and spleen were higher in inflamed than non-inflamed old rats submitted to control treatment. These organs play key role in the control of inflammation and immunity. Taken together, our results support the fact that the spontaneous low-grade inflammation observed in old rats is a chronic inflammation, which could affect the immune system and potentially weaken old rats. This is further supported by the observation that body weight loss observed after the stress was higher in inflamed than non-inflamed old rats.

In this context, the response of skeletal muscle to the stress regarding its mass and protein turnover was interesting to compare between initially inflamed and non-inflamed old rats. We recently showed that low-grade inflammation did not aggravate sarcopenia in control conditions (Mayot et al., 2006). In the present study, we confirmed our precedent results since skeletal muscle masses and protein turnover rates were not different between the two control groups NI-Con and LGI-Con. Moreover, present results are going one step further, showing that age-associated low-grade inflammation does not induce any negative effect in regards to skeletal muscle mass and protein metabolism in case of additional stress.

The stress applied to old rats, consisting in a single endotoxin injection followed by a 23-day period of protein-energy malnutrition, induced severe metabolic disturbances leading to increased mortality rate, loss of body weight and decreased abdominal organ weights. The effect of the stress on acute phase protein response was protein-dependent. Indeed, plasma levels of α_2 -macroglobulin increased, whereas fibrinogen levels decreased and albumin levels did not change in stressed old rats. The increase in α_2 -macroglobulin is consistent with the fact that sepsis and protein-deficient diet are known to stimulate its synthesis in young rats (Breuillé et al., 1998; Lyoumi et al., 1998; Breuillé et al., 1999). If the stress would have been only an inflammatory stress, an increase in fibrinogen would

have been also expected. The fact that its plasma concentration decreased under the stress suggests that either its utilization increased or its synthesis was limited by the malnutrition in these old rats. The decrease in fibrinogen concentration is in agreement with data obtained in piglets that were fed with a protein-deficient diet (Jahoor et al., 1999). Taken together, these observations suggest that the transient inflammatory stress (*i.e.* endotoxin injection) and the following protein-energy malnutrition stress used in the present study in old rats may have had opposite effects on some metabolic aspects. Albumin levels were not affected by the stress applied to old rats. By contrast, sepsis and protein-deficient diet are known to decrease it in young rats (Lyouni et al., 1998; Ruot et al., 2002). Thus, as we already suggested (Mayot et al., 2007), albumin may not be a good nutritional marker in old rats. Glutathione which is known to be very sensitive to protein intake (Hum et al., 1992) and to decrease during infection (Breuillé et al., 1994) was highly depleted in the stressed old rats. The overall effect of the present stress applied in old rats on plasma levels of acute phase proteins and liver glutathione status was largely consistent with known effects of protein-deficient nutrition in young animals, albumin excepted.

Unfortunately, the effect of low-grade inflammation on stress-induced mortality in old rats could not be statistically analyzed because of too low number of animals. Low-grade inflammation increased the susceptibility of old rats to body weight loss. Indeed, low-grade inflamed rats lost almost 30 g more body weight under the stress than non-inflamed rats. The higher stress-induced body weight loss in inflamed vs. non-inflamed rats could not be explained by differences in adipose tissue or skeletal muscle weights. Part of the higher body weight loss can be explained by higher decreases in liver, spleen kidneys and colon weights, (Table 3). However, the results obtained from longitudinal observations (*i.e.* body weights measured in the same rats before and during the treatment) are difficult to explain by the transversal observations concerning mean skeletal muscles and organs weights from different

groups of rats. Indeed, transversal observations are generally associated with higher variability than measurements performed in longitudinal observations. Finally, tissues and organs that have not been dissected such as the skin, whose weight contributes to ~ 20% of body weight (Obled and Arnal, 1992), might have been more affected in inflamed than non-inflamed old rats.

Regarding the acute phase reaction, the stress applied in old rats modified differently the fibrinogen kinetic in inflamed as compared to non-inflamed rats. The fibrinogen concentration was decreased by the stress in both groups, but to a greater extent in inflamed than non-inflamed rats. The stress-induced increase in α_2 -macroglobulin might also have been higher but variability was too high to observe a significant effect in LGI-Str group. Finally, the inflammatory status of the old rats did not impact the stress response regarding the glutathione status and the skeletal muscle protein turnover. Taken together, the initial inflammatory status of old rats did not have a strong impact on the metabolic response to the present stress and for sure did not enhance the stress-induced skeletal muscle loss.

The failure of low-grade inflammation to worsen the stress-induced skeletal muscle loss extends our previous observation made in non-stressed rats showing that low-grade inflammation does not aggravate sarcopenia (Mayot et al., 2006). The present stress applied in old rats had more effects on splanchnic organs than on skeletal muscles. This is consistent with decreases observed in splanchnic organ weights but not in skeletal muscle weights after a 50% dietary restriction in old rats (Chambon-Savanovitch et al., 1999). Similarly, higher decreases in liver (49%) than in *gastrocnemius* (25%) weights were observed after 10 days of starvation in old rats (Mosoni et al., 1999). Since muscle wasting and weakness are late symptoms of malnutrition (Seiler, 2001), a longer malnutrition period might be required to see differential reduction of skeletal muscle weight according to the initial inflammatory status. In young rats, when starvation is initiated, there is a period of “saving” before skeletal

muscle loss becomes significant (Chérel et al., 1991). However, there is a large decrease in skeletal muscle protein synthesis observed in fasting young rats, even during the early phase during which muscle weight is preserved. A decrease in skeletal muscle protein synthesis is also observed in piglets fed a protein-deficient diet (Jahoor et al., 1999). In contrast, skeletal muscle protein turnover was not affected in our stressed old rats. Taken together, these observations suggest that the present double stress (endotoxin injection combined with protein-energy malnutrition) might have induced some early metabolic effects that might have been counteracted by the 23-day period of malnutrition. This assumption seems to be emphasized by the observation of the decreased fibrinogen concentration under the stress, as opposed to the increase usually observed after an inflammatory stress. Thus, to definitively conclude that low-grade inflammation does not worsen sarcopenia in aged, it would be probably necessary to test the impact of another type of stress, for example a stress inducing a more marked skeletal muscle loss than the present stress or a stress for which no opposite metabolic effects are observed.

In conclusion, the present study clearly indicates that low-grade inflammation increases the body weight loss induced by the endotoxin injection followed by malnutrition, a stress that mimics a transient febrile episode followed by a period of protein-energy malnutrition, which frequently occurs in elderly. Stress-induced decreases in organ weights and fibrinogen plasma concentration were enhanced by the initial low-grade inflammatory status. The response of skeletal muscle (weight and protein turnover) was not worsened by the low-grade inflammation. This extends and supports our previous findings that age-associated low-grade inflammation does not aggravate sarcopenia.

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Figure legends

FIGURE 1 *Time-course of cumulative relative body weight changes in non-inflamed and low-grade inflamed old rats submitted to the control or the stress treatment.*

Means $\pm$ SE (n = 5 or 7). There are significant effects of the stress and the time on body weight changes and significant interactions between the stress and the time, between the initial inflammatory status and the time, and between the stress, the initial inflammatory status and the time (ANOVA for repeated measurements with the stress and the initial inflammatory status as the between-rat factors and the time as the within-rat factor). NI-Con: non-inflamed rats submitted to the control treatment, NI-Str: non-inflamed rat submitted to the stress treatment, LGI-Con: inflamed rats submitted to the control treatment, LGI-Str: inflamed rats submitted to the stress treatment.

FIGURE 2 *Skeletal muscle protein turnover in non-inflamed and low-grade inflamed old rats submitted to the control or the stress treatment.*

Means $\pm$ SE (n = 5 or 7). Two-way (initial inflammatory status and treatment) ANOVA: *epitrochlearis* protein synthesis and protein degradation: none significant effect, $P < 0.05$, NI-Con: non-inflamed rats submitted to the control treatment, NI-Str: non-inflamed rat submitted to the stress treatment, LGI-Con: inflamed rats submitted to the control treatment, LGI-Str: inflamed rats submitted to the stress treatment.

FIGURE 3 *Time-course of plasma α_2 -macroglobulin, fibrinogen and albumin concentrations in non-inflamed and low-grade inflamed old rats receiving the control or the stress treatment.*

Bars represent means and SE for 5 or 7 rats in each group at each time. α_2 -macroglobulin: significant effects of the stress, the initial inflammatory status and the time, (interaction

between the stress and the time $P = 0.056$); fibrinogen: significant effects of the stress, the initial inflammatory status and the time, significant interaction between the stress and the time (interaction between the stress, the initial inflammatory status and the time $P = 0.072$); albumin: significant effect of the initial inflammatory status (ANOVA for repeated measurements with the stress and the initial inflammatory status as the between-rat factors and the time as the within-rat factor). Within a group, bars not sharing a common letter are different (ANOVA for repeated measurement, followed by Fisher's PLSD test). NI-Con: non-inflamed rats submitted to the control treatment, NI-Str: non-inflamed rat submitted to the stress treatment, LGI-Con: inflamed rats submitted to the control treatment, LGI-Str: inflamed rats submitted to the stress treatment.

FIGURE 4 *Liver glutathione status in non-inflamed and low-grade inflamed old rats submitted to the control or the stress treatment.*

Means $\pm$ SE (n = 5 or 7). Two-way (initial inflammatory status and treatment) ANOVA: significant effect of the treatment for both concentration and content, $P < 0.05$, NI-Con: non-inflamed rats submitted to the control treatment, NI-Str: non-inflamed rat submitted to the stress treatment, LGI-Con: inflamed rats submitted to the control treatment, LGI-Str: inflamed rats submitted to the stress treatment.

Table 1

Old rats' characteristics before the experimental period

Initial inflammatory status	Non-inflamed		<u>Low-grade</u> inflamed		Two-way ANOVA
Treatment	Control	Stress	Control	Stress	
Groups	<u>NI-Con</u>	<u>NI-Str</u>	<u>LGI-Con</u>	<u>LGI-Str</u>	
α_2 -macroglobulin at D-7(mg/l)	36 $\pm$ 4	40 $\pm$ 12	167 $\pm$ 37	133 $\pm$ 26	I
Fibrinogen at D-7 (g/l)	3.24 $\pm$ 0.18	3.12 $\pm$ 0.22	4.42 $\pm$ 0.39	4.51 $\pm$ 0.28	I
Albumin at D-7 (g/l)	14.6 $\pm$ 0.9	13.3 $\pm$ 0.7	12.2 $\pm$ 0.8	12.1 $\pm$ 1.0	I ($P = 0.066$)
Body weight at D-22 (g)	693 $\pm$ 68	627 $\pm$ 24	613 $\pm$ 33	704 $\pm$ 45	ns
Body weight at D0 (g)	663 $\pm$ 59	603 $\pm$ 22	576 $\pm$ 17	648 $\pm$ 35	ns
D-22 to D0 body weight change (%)	- 4.0 $\pm$ 1.9	- 3.8 $\pm$ 1.8	- 5.5 $\pm$ 2.9	- 7.6 $\pm$ 1.0	ns
Food intake before D0 (g/d)	22.0 $\pm$ 1.4	21.1 $\pm$ 1.2	21.3 $\pm$ 0.9	22.1 $\pm$ 0.7	ns

Means $\pm$ SE (n = 5 or 7). Two-way (initial inflammatory status and treatment) ANOVA: ns: none significant effect, I: significant effect of the initial inflammatory status, $P \leq 0.05$, P value is in parenthesis when a trend is observed. NI-Con: non-inflamed rats submitted to the control

treatment, NI-Str: non-inflamed rat submitted to the stress treatment, LGI-Con: low-grade inflamed rats submitted to the control treatment, LGI-Str: low-grade inflamed rats submitted to the stress treatment.

Table 2

Skeletal muscle weights in non-inflamed and low-grade inflamed old rats submitted to the control or the stress treatment.

Initial inflammatory status	Non-inflamed		Inflamed		Two-way ANOVA
Treatment	Control	Stress	Control	Stress	
Group	<u>NI-Con</u>	<u>NI-Str</u>	<u>LGI-Con</u>	<u>LGI-Str</u>	
<i>Gastrocnemius</i> (g)	2.31 ± 0.06	2.00 ± 0.09	2.29 ± 0.10	2.03 ± 0.11	T
mg/100g BW	360 ± 32	333 ± 19	399 ± 13	316 ± 15	T
<i>Tibialis anterior</i> (mg)	844 ± 40	719 ± 35	786 ± 23	762 ± 38	T (<i>P</i> =0.056)
mg/100g BW	130 ± 10	120 ± 8	137 ± 5	119 ± 6	T (<i>P</i> =0.069)
EDL (mg)	189 ± 18	179 ± 8	196 ± 9	190 ± 12	ns
mg/100g BW	29.3 ± 3.7	29.7 ± 1.1	34.2 ± 2.0	29.3 ± 1.1	ns
<i>Soleus</i> (mg)	156 ± 17	163 ± 17	172 ± 6	188 ± 6	ns
mg/100g BW	23.9 ± 2.6	27.1 ± 2.8	29.9 ± 0.9	30.3 ± 1.3	I

Means $\pm$ SE (n = 5 or 7). Two-way (initial inflammatory status and treatment) ANOVA: ns: none significant effect, I: significant effect of the initial inflammatory status, T: significant effect of the treatment, $P < 0.05$. P value is in parenthesis when a trend is observed. BW: body weight at day 0, NI-Con: non-inflamed rats submitted to the control treatment, NI-Str: non-inflamed rat submitted to the stress treatment, LGI-Con: low-grade inflamed rats submitted to the control treatment, LGI-Str: low-grade inflamed rats submitted to the stress treatment.

Table 3

Organ and adipose tissue weights in non-inflamed and low-grade inflamed old rats submitted to the control or the stress treatment.

Initial inflammatory status	Non-inflamed		Inflamed		Two-way ANOVA
Treatment	Control	Stress	Control	Stress	
Group	<u>NI-Con</u>	<u>NI-Str</u>	<u>LGI-Con</u>	<u>LGI-Str</u>	
Liver (g)	15.9 ± 1.3	11.8 ± 0.5	17.7 ± 1.3	12.5 ± 0.4	T
g/100g BW	2.41 ± 0.08 ^a	1.96 ± 0.02 ^b	3.06 ± 0.17 ^c	1.96 ± 0.09 ^b	T, I, TxI
Kidneys (g)	3.55 ± 0.17 ^{ab}	2.89 ± 0.14 ^a	5.66 ± 0.40 ^c	3.82 ± 0.25 ^b	T, I, TxI
mg/100g BW	548 ± 040 ^a	480 ± 19 ^a	986 ± 71 ^b	601 ± 50 ^a	T, I, TxI
Spleen (g)	1.43 ± 0.11	1.00 ± 0.02	1.62 ± 0.15	1.04 ± 0.08	T
mg/100g BW	220 ± 18	166 ± 4	280 ± 22	163 ± 14	T, I (<i>P</i> =0.085), TxI (<i>P</i> =0.063)
Small Intestine (g)	10.4 ± 0.5	8.5 ± 0.5	10.2 ± 0.3	9.7 ± 0.4	T
g/100g BW	1.60 ± 0.10	1.42 ± 0.03	1.78 ± 0.06	1.53 ± 0.11	T

Colon (g)	1.80 ± 0.10	1.78 ± 0.06	1.99 ± 0.22	1.67 ± 0.10	ns
mg/100g BW	279 ± 26	297 ± 16	344 ± 31	267 ± 28	TxI ($P=0.098$)
Mesentery (g)	7.36 ± 2.70	4.28 ± 0.87	6.78 ± 1.34	5.05 ± 1.28	ns
g/100g BW	1.07 ± 0.34	0.71 ± 0.14	1.18 ± 0.23	0.74 ± 0.17	T ($P=0.091$)
Epididymal adipose tissue (g)	12.1 ± 2.8	9.5 ± 2.1	9.1 ± 0.5	9.1 ± 1.3	ns
g/100g BW	1.77 ± 0.33	1.55 ± 0.32	1.58 ± 0.08	1.36 ± 0.17	ns

Means ± SE (n = 5 or 7). Two-way (initial inflammatory status and treatment) ANOVA: ns: none significant effect, I: significant effect of the initial inflammatory status, T: significant effect of the treatment, TxI: significant interaction between the two factors, $P < 0.05$. P value is in parenthesis when a trend is observed. ^{a,b,c}: values, within a row, not sharing a common letter are significantly different (Fisher's test). BW: body weight at day 0, NI-Con: non-inflamed rats submitted to the control treatment, NI-Str: non-inflamed rat submitted to the stress treatment, LGI-Con: low-grade inflamed rats submitted to the control treatment, LGI-Str: low-grade inflamed rats submitted to the stress treatment.

FIGURE 1

FIGURE 2

FIGURE 3

FIGURE 4