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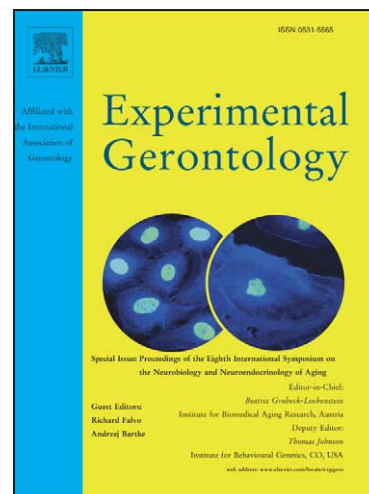
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**Prognostic values of  $\alpha_2$ -macroglobulin, fibrinogen and albumin in regards to mortality and frailty in old rats**

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Running title: Mortality and frailty in inflamed old rat

*Keywords:* Frailty; Inflammation; Mortality;  $\alpha_2$ -macroglobulin; Fibrinogen; Albumin.

Abbreviations used: APP: acute phase protein, CI: confidence interval, CRP: C-reactive protein, IL-6: interleukin-6, LBP: lipopolysaccharide binding protein, TNF- $\alpha$ : tumor necrosis factor- $\alpha$ , sTNFR-1: soluble TNF- $\alpha$  receptor-1.

## 1 Abstract

2 The study aimed to determine if acute phase proteins (APP) are markers of frailty in  
3 old rats. We evaluated in male Wistar rats at 96 weeks of age (n=72) whether single  
4 measurements of  $\alpha_2$ -macroglobulin, fibrinogen and albumin are predictive of mortality, body  
5 weight loss and inflammatory status during a 10-week follow-up period. Rats were clustered  
6 depending on levels of these APP at baseline. Rats with extremely high levels of  $\alpha_2$ -  
7 macroglobulin or fibrinogen (upper quartiles), or extremely low level of albumin (lower  
8 quartile), had an 11.6, 8.1 and 5.3-fold higher risk of mortality, respectively, than other rats.  
9 Body weight loss was negatively correlated with  $\alpha_2$ -macroglobulin, a trend was observed with  
10 fibrinogen ( $P = 0.08$ ) but not with albumin. Rats with fibrinogen levels  $> 4.0$  g/L or  $\alpha_2$ -  
11 macroglobulin levels  $> 91$  mg/L (respective top halves) at 96 weeks of age had higher levels  
12 of  $\alpha_2$ -macroglobulin and fibrinogen and lower levels of albumin throughout the follow-up  
13 period and higher levels of sTNFR-1 and lipopolysaccharide-binding protein at 106 weeks of  
14 age. Highest levels of  $\alpha_2$ -macroglobulin, fibrinogen and lowest albumin were predictive of  
15 mortality, whereas moderate levels of  $\alpha_2$ -macroglobulin and fibrinogen were, according to  
16 body weight loss and inflammatory status, markers of frailty in old rats.

17

## 18 1. Introduction

19 Frailty syndrome is a physiological state of susceptibility that places older individuals  
20 at high risk for adverse outcomes such as falls, disability, morbidity and institutionalization  
21 (Dayhoff et al., 1998; Fried et al., 2001; Ferrucci et al., 2002a; Walston, 2004). Frailty  
22 ultimately leads to death. Frailty consists of multi-system decline and is considered to be a  
23 consequence of changes in neuromuscular, endocrine and immune functions that occur as  
24 people progress in age (Ferrucci et al., 2002a; Walston, 2004). Frailty can and does result in  
25 several “vicious loops”: neuromuscular impairment induces increased rate of falls and  
26 fractures in old age; the act of falling causes itself hospitalisation and immobilisation, which

1   aggravates sarcopenia; sarcopenia leads to deficit in continuous neuromuscular training which  
2   brings about neuromuscular impairment (Muhlberg and Sieber, 2004; Walston, 2004).  
3   Moreover, frailty has nutritional and metabolic implications which have themselves  
4   deleterious implications (Chevalier et al., 2003; Muhlberg and Sieber, 2004; Puts et al., 2005).

5       Early screening of frailty in the elderly population is essential to reduce the adverse  
6   outcomes described above. Despite the growing awareness of this problem over the past two  
7   decades, there is no clear guidelines for identifying and describing older adults as frail  
8   (Markle-Reid and Browne, 2003; Walston, 2004). Screening for frailty syndrome in humans  
9   is often based on the measurement of parameters related to mobility and motor performance.  
10   These include weakness (grip strength), weight loss, fatigue, slow walking speed and low  
11   levels of physical activity (Fried et al., 2001). Subjects meeting three or more of these criteria  
12   are categorized as frail (Fried et al., 2001). Several researchers have sought to find sensitive  
13   biomarkers of frailty (Ferrucci et al., 2002a). Among the candidate biomarkers are  
14   compounds associated with the inflammatory process (Ferrucci et al., 2002a; De Martinis et  
15   al., 2006). Frail subjects have evidence of increased inflammation (Leng et al., 2002; Puts et  
16   al., 2005). Increased levels of certain acute phase reactants, including C-reactive protein  
17   (CRP), clotting factors such as fibrinogen and factor VIII, and decreased level of albumin,  
18   were associated with increasing frailty level in elderly (Walston et al., 2002). In accordance,  
19   the levels of inflammatory markers, such as interleukin-6 (IL-6) and CRP, increase with  
20   ageing, and that elevated levels are associated with disability and mortality (Harris et al.,  
21   1999; Ferrucci et al., 2002b; Cohen et al., 2003; Bautmans et al., 2005).

22       In spite of the fact that rat is a common model for human nutritional and metabolic  
23   studies, to our knowledge, no investigation has assessed frailty in old rats. Frail rats are  
24   expected to exhibit the human frailty phenotype, including vulnerability to mortality, body  
25   weight loss and inflammation. Physical performance which is classically assessed in human  
26   clinical settings cannot be easily evaluated in old rats. So, there is a need to identify plasma

1 biomarkers of rat frailty. Measurements of inflammatory markers, that correlate with frailty,  
2 has recently been proposed for identification of frail animals (Walston et al., 2006). In the  
3 present study, we investigated whether acute phase proteins (APP) are potential biomarkers of  
4 frailty in old rats.

5 The APP  $\alpha_1$ -acid glycoprotein,  $\alpha_2$ -macroglobulin and fibrinogen are known to  
6 increase with inflammation in rats (Fuller, G. M., 1993; Roberts, R. C., 1993; Breuillé et al.,  
7 1998; Ruot et al., 2002). CRP is commonly used as a marker of inflammation in human  
8 clinical trials and research settings (Kushner, I. and Maciewicz, A., 1993). But CRP is a poor  
9 marker of inflammation compared to fibrinogen in the rat (Giffen et al., 2003). By contrast,  
10  $\alpha_2$ -macroglobulin in rats is an APP characterized by a kinetic relatively similar to human CRP  
11 kinetic since both of them peak very quickly after inflammatory stress and thus  $\alpha_2$ -  
12 macroglobulin is a good marker of inflammation in the rat. The APP albumin is known to  
13 decline with inflammation in rats (Aldred, A. R. and Schreiber, G., 1993; Breuillé et al., 1998;  
14 Ruot et al., 2002). We have recently shown that  $\alpha_2$ -macroglobulin and fibrinogen  
15 concentrations were higher and albumin concentration was lower in old rats (95 week-old)  
16 compared to adults (35 week-old), whereas plasma  $\alpha_1$ -acid glycoprotein concentration was  
17 similar in both groups (Papet et al., 2003). In addition, greater variability was observed in the  
18 APP in the old rats compared to their adult rats counterparts. Taken together,  $\alpha_2$ -  
19 macroglobulin, fibrinogen and albumin are potential candidates for assessing frailty in old  
20 rats.

21 The purpose of the study was to determine if the APP,  $\alpha_2$ -macroglobulin, fibrinogen  
22 and albumin, are biomarkers of frailty in old rats. The parameters assessed included mortality,  
23 body weight loss and persistent inflammatory status evaluated in a 10-week longitudinal study  
24 on a cohort of 96-week old rats. Inflammatory status was assessed by measuring the APP  
25 throughout the follow -up period and IL-6, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), soluble TNF- $\alpha$   
26 receptor-1 (sTNFR-1) and lipopolysaccharide binding protein (LBP) at the end of the study.

1

## 2 **2. Materials and methods**

### 3 *2.1. Animals*

4 Male Wistar rats were produced and bred in our conventional (not specific-pathogen-free)  
5 animal facility (Unité Expérimentale de Nutrition Comparée, INRA, Saint-Genès-  
6 Champanelle, France). They were maintained in collective cages (3 to 4 rats per cage) under  
7 controlled conditions (temperature 21°C, relative humidity 55 %, 12-h light/dark cycles with  
8 free access to water and standard pelleted food (A04 from SAFE (Scientific Animal Food and  
9 Engineering), Villemoisson-sur-orge, France). The composition of the diet was 16 % protein,  
10 3% fat, 60 % carbohydrates, 12 % water, fibers, vitamins and minerals. The study was  
11 performed according to the current legislation on animal experiments in France.

### 12 *2.2. Experimental design*

13 The experimental cohort consisted of 119 rats at the age of 13 weeks, born over a two  
14 week period. Blood samples were withdrawn from the lateral vein of 32 rats at 35 week-old in  
15 order to estimate the adult cohort values for plasma levels of the three APP:  $\alpha_2$ -  
16 macroglobulin, fibrinogen and albumin. The longitudinal study was initiated when the rats  
17 were 96 week-old (n = 72) and lasted for an additional 10 weeks. Body weight was measured  
18 at weeks 96 and 106 and sample collection from a lateral tail vein was performed at weeks 96,  
19 100, 104 and 106. Mortality was recorded weekly. Samples collected throughout the study  
20 were assayed for  $\alpha_2$ -macroglobulin, fibrinogen and albumin. Additionally, samples collected  
21 at 106 weeks of age were also assayed for IL-6, TNF- $\alpha$ , sTNFR-1 and LBP. All samples were  
22 stored at -80°C until subjected to analyses.

### 23 *2.3. Acute-phase protein concentrations*

24 Plasma fibrinogen level was measured by turbidimetry (Macart et al., 1989) on a  
25 Cobas Mira analyzer (ABX Diagnostics, Montpellier, France). Concentration is expressed as  
26 g human equivalent/L since human fibrinogen was used as reference (Ingen, Rungis, France).

1 Plasma levels of  $\alpha_2$ -macroglobulin and albumin were measured by single radial  
2 immunodiffusion (Breuillé et al., 1998). Serum concentration of LBP was measured by  
3 Enzyme Linked ImmunoSorbent Assay (ELISA) that detects free LBP (Biometec,  
4 Greifswald, Germany); its detection limit was 5 ng/mL.

#### 5 *2.4. Serum levels of cytokines and cytokine receptor*

6 Serum levels of IL-6, TNF- $\alpha$  and sTNFR-1 were quantified with specific ELISA kits  
7 (R6000B, RTA00 and MRT10, respectively, R&D Systems, Abingdon, UK). All  
8 quantifications were realized according to the manufacturer. Detection limits were 21, 12 and  
9 16 pg/mL for IL-6, TNF- $\alpha$  and sTNFR-1, respectively. All samples and standards were  
10 measured in duplicate and the means were used in statistical analyses.

#### 11 *2.5. Statistical analysis*

12 All values are given as means  $\pm$  SD. Statistical analyses were performed using  
13 StatView for Windows, version 5 software (SAS Institute, Cary, NC). Values of  $P < 0.05$  are  
14 considered significant for all analyses.

15 The effect of age was analyzed using one-way ANOVA and Fisher's PLSD test and  
16 Student's  $t$  test for unpaired data was used to compare alive and dead old rats. Linear  
17 regression was used to determine the relationship among APP themselves and between the  
18 percentage of body weight change and each APP.

19 Several statistical tests were used to analyse mortality data obtained for all rats ( $n =$   
20 72). Rats were divided into quartile categories of either  $\alpha_2$ -macroglobulin, fibrinogen or  
21 albumin plasma levels at the age of 96 weeks. Survival curves were generated by the Kaplan-  
22 Meier method and compared by the log-rank test in order to analyze the impact of APP  
23 plasma levels measured in 96 week-old rats on mortality across the 10 weeks of follow-up.  
24 The relative risk of death was calculated using the Cox proportional hazards model for  
25 categorical variables. Univariate Cox and multivariate Cox regressions for continuous  
26 variables were used to analyze how APP were predictive of mortality.



1 Changes in APP during the follow-up period and the values of sTNFR-1 and LBP at  
2 the age of 106 weeks were analyzed on rats which were still alive at 106 weeks (n = 48). For  
3 each APP, data from alive rats from quartiles 1 and 2 (bottom half) were compared to those of  
4 alive rats from quartiles 3 and 4 (top half). Two-way ANOVA for repeated measurements  
5 with group as the between-rat factor and time as the within-rat factor was performed to  
6 analyze APP changes. When significant effects were observed for group and time, Fisher's  
7 PLSD test and Student's *t* test for unpaired data were performed as post ANOVA tests.  
8 Student's *t* test for unpaired data was also used to analyze the group effect for sTNFR-1 and  
9 LBP at the age of 106 weeks.

10

### 11 **3. Results**

#### 12 *3.1. Rat characteristics*

13 The three APP,  $\alpha_2$ -macroglobulin, fibrinogen and albumin, and body weight were  
14 significantly affected by age (Table 1).  $\alpha_2$ -macroglobulin and fibrinogen plasma levels were  
15 higher and albumin plasma level lower at the age of 96 and 106 weeks than at the age of 35  
16 weeks. In 96 week-old rats,  $\alpha_2$ -macroglobulin and fibrinogen were highly positively  
17 correlated to each other ( $r = 0.73$ ,  $P < 0.001$ ,  $n = 72$ ) and each of them was negatively  
18 correlated with albumin ( $r = -0.71$  and  $-0.67$ , respectively,  $P < 0.001$ ,  $n = 72$ ). Mean body  
19 weight was higher in 96 week-old rats than in 35 week-old rats. At 96 weeks of age, rat body  
20 weight was negatively correlated with  $\alpha_2$ -macroglobulin and fibrinogen ( $r = -0.46$  and  $-0.29$ ,  
21 respectively,  $P < 0.05$ ,  $n = 72$ ) and was positively correlated with albumin ( $r = 0.46$ ,  $P <$   
22  $0.001$ ,  $n = 72$ ).

#### 23 *3.2. APP measurements at 96 weeks of age are predictive of the mortality*

24 Between 96 and 106 weeks of age, 24 rats died which represented a 33 % of mortality  
25 rate. The rats that died prior to 106 weeks had higher  $\alpha_2$ -macroglobulin (851 %) and

1 fibrinogen (47 %) and lower albumin (24 %) plasma levels than alive rats (Table 2). In  
2 addition, the mean body weight of deceased rats was 12 % lower than their alive counterparts.

3 Kaplan-Meier analyses show that each APP measured at 96 weeks of age was  
4 associated with 10-week mortality (Figure 1).  $\alpha_2$ -macroglobulin plasma level in the top  
5 quartile ( $\geq 506$  mg/L) was the strongest predictor of mortality (relative risk = 11.6; 95 %  
6 confidence interval (CI) = 4.8 to 27.7;  $P < 0.0001$ ). Rats belonging to the top fibrinogen  
7 quartile had an 8.1-fold higher risk of mortality (95 % CI = 3.5 to 18.8;  $P < 0.0001$ ) than  
8 those of the other quartiles ( $< 5.4$  g/L). The mortality risk of rats in the bottom albumin  
9 quartile ( $\leq 10$  g/L) relative to the three other quartiles was 5.3 (95 % CI = 2.3 to 11.9;  $P <$   
10  $0.0001$ ). Univariate Cox analyses show that each of the three APP measured at the age of 96  
11 weeks was predictive of mortality. The multivariable Cox regression model using forward  
12 stepwise selection method conducted with the three APP show that  $\alpha_2$ -macroglobulin alone  
13 was sufficient to explain rat mortality during the 10-week follow-up.

### 14 3.3. APP levels at 96 weeks of age and body weight change

15 Percentage of body weight loss throughout 10-week follow-up in rats alive at 106  
16 weeks ( $n = 48$ ) was negatively correlated with  $\alpha_2$ -macroglobulin levels measured at 96 weeks  
17 old ( $P < 0.01$ ) (Table 3). A similar correlation with fibrinogen level was observed, but did not  
18 attain statistical significance ( $P = 0.08$ ). However, no associations between body weight  
19 changes and albumin levels were observed.

### 20 3.4. APP levels at 96 weeks of age and inflammatory status throughout 10-week follow-up

21 To determine if single measurements of APP were predictive of the consecutive  
22 inflammatory status, changes in the APP throughout the follow-up period and values of other  
23 inflammatory markers at 106 weeks of age were assessed in surviving rats. This analysis was  
24 performed on the top half and the bottom half groups constituted on the basis of each plasma  
25 APP measured in all rats at 96 weeks of age (Table 4). Rats with  $\alpha_2$ -macroglobulin levels  $>$

1 91 mg/L at 96 weeks of age exhibited higher levels of plasma  $\alpha_2$ -macroglobulin (~ 450 %)  
2 and fibrinogen (~ 34 %) and lower levels of plasma albumin (~ 20 %) at all tested times  
3 during the follow-up period than the other rats, excepted for albumin at the age of 96 weeks.  
4 Interestingly serum sTNFR-1, detected in all rats at the age of 106 weeks, had a 66 % higher  
5 level in rats having the highest  $\alpha_2$ -macroglobulin level. No difference was observed in levels  
6 of serum LBP. No detectable level of serum IL-6 and TNF-a was observed. Rats with  
7 fibrinogen levels > 4 g/L at 96 weeks of age exhibited higher levels of plasma  $\alpha_2$ -  
8 macroglobulin (~ 280 %) and fibrinogen (~ 47 %) and lower levels of plasma albumin (~ 17  
9 %) at all tested times during the follow-up than other rats, excepted for albumin at the age of  
10 96 weeks and for  $\alpha_2$ -macroglobulin at the age of 100 weeks. The levels of both serum  
11 sTNFR-1 and LBP at the age of 106 weeks were higher in rats having the highest fibrinogen  
12 level (70 and 130 %, respectively). Rats with albumin levels lower than 12 g/L at 96 weeks of  
13 age exhibited similar values for the three APP throughout 10-week period (except albumin at  
14 the age of 96 weeks) and similar levels of sTNFR-1 and LBP at the age of 106 weeks than  
15 other rats.

16

#### 17 4. Discussion

18 In this paper, we report that highest plasma levels of  $\alpha_2$ -macroglobulin, fibrinogen and  
19 lowest albumin are predictive of mortality. According to body weight loss and persistent  
20 inflammatory status, moderate levels of  $\alpha_2$ -macroglobulin and fibrinogen are biomarkers of  
21 frailty in old rats, with  $\alpha_2$ -macroglobulin being slightly more powerful than fibrinogen.

22 Regardless of the type of statistical analysis performed,  $\alpha_2$ -macroglobulin, fibrinogen  
23 and albumin measured in 96 week-old rats were associated with 10-week mortality. Rats that  
24 died during the 10-week follow-up had higher concentrations of  $\alpha_2$ -macroglobulin and  
25 fibrinogen, and lower concentration of albumin (Student's *t* test). Kaplan and univariate Cox

1 analyses were significant for each APP. The multivariate Cox regression model showed that  
2  $\alpha_2$ -macroglobulin alone was sufficient, likely due to the fact that the three APP are correlated  
3 to each other. This is in agreement with the highest log rank test for  $\alpha_2$ -macroglobulin alone.

4 The mortality was significantly enhanced in rats exhibiting extremely high  
5 concentrations of  $\alpha_2$ -macroglobulin (506 to 3390 mg/L) and fibrinogen (5.4 to 10.6 g/L), and  
6 extremely low concentrations of albumin (10 to 4 g/L). These values correspond to those  
7 observed during acute inflammation, such as sepsis (Breuillé et al., 1998; Breuillé et al., 1999;  
8 Ruot et al., 2002). In addition, these rats had the lowest body weight. This inflammation could  
9 be due to aging *per se*; however, age-associated inflammation in humans is a much lower  
10 grade inflammation (Bruunsgaard and Pedersen, 2003). These rats may have been infected by  
11 chronic pathogen since they were bred in none specific-pathogen-free animal facility.  
12 Moreover, these rats may have had some acute or chronic illness that activated inflammatory  
13 pathways such as chronic nephropathy, cardiomyopathy, and neoplasia which are the major  
14 age-related pathologies in rats (Maeda et al., 1985). Unfortunately, no data were collected on  
15 any illnesses and death causes in the present study. Even if the etiology of this inflammation  
16 is not known, it definitively enhanced mortality rate. It has been shown in elderly that an  
17 increase in CRP level, corresponding to either the third tertile or the fourth quartile in most  
18 human studies, was associated with increased risk of mortality (Harris et al., 1999; Reuben et  
19 al., 2002; Tice et al., 2003; Kritchevsky et al., 2005). In a recent meta-analysis, the age-  
20 adjusted relative risk per 1 g/L increase in usual fibrinogen level was 2.06-2.76 for cardio-  
21 vascular disease mortality and 2.03 for nonvascular mortality in healthy middle-aged adults  
22 (Danesh et al., 2005). In addition, lowest albumin levels were associated with an increased  
23 mortality risk in community-dwelling elderly subjects (Corti et al., 1994; Sahyoun et al.,  
24 1996; Reuben et al., 2002). Altogether, our results obtained in rats are in line with data known  
25 in humans and give a prognostic value of some acute phase proteins in regards to mortality.

1           Body weight loss is both a characteristic and an outcome of frailty in humans (Fried et  
2 al., 2001). In the survivors of our cohort of rats, among the three APP tested, only  $\alpha_2$ -  
3 macroglobulin was significantly associated with 10-week body weight loss. A similar trend  
4 was observed for fibrinogen, but not with albumin. In the elderly, the influence of  
5 inflammatory status on body weight loss has not been directly assessed, but acute  
6 inflammation plays a major role in the pathophysiological of cachexia (Morley et al., 2006).  
7 Albumin decreases in inflammatory and malnutrition states (Don and Kaysen, 2004).  
8 Considering that old rats, which died were the ones that lost the most weight, it is surprising  
9 that low level of albumin was only associated with high mortality but not with body weight  
10 loss. This questions the validity of albumin as a nutritional marker in old rats. Unfortunately,  
11 measurement of food intake or other nutritional markers were not assessed in this study.

12           Single measurements of  $\alpha_2$ -macroglobulin and fibrinogen, but not albumin, were  
13 predictive of consecutive inflammatory status in survivors, assessed not only by these APP  
14 but also with additional inflammatory markers, including LBP and sTNFR-1. Plasma level of  
15 LBP, which is recognized as an APP (Tobias et al., 1986), rises dramatically after  
16 inflammatory challenges (infectious or not) (Gallay et al., 1994; Myc et al., 1997; Fenton and  
17 Golenbock, 1998). It was higher in old rats characterized as inflamed from repartitions based  
18 on fibrinogen. It is well-known that TNF- $\alpha$  plays an important pathophysiological role in a  
19 wide range of chronic disorders, but sTNFRs are stronger markers of severity and clinical  
20 outcome than TNF- $\alpha$  itself (Aderka, 1996) and are essential for evaluation of the TNF system  
21 (Ferrari et al., 1995). Unfortunately, the pro-inflammatory cytokines TNF- $\alpha$  and IL-6 were  
22 undetectable in the sera of our old rats, probably due to the low sensitivity of the ELISA.  
23 Interestingly, sTNFR-1 levels were consistently higher in rats characterized as inflamed based  
24 on  $\alpha_2$ -macroglobulin or fibrinogen levels. Inflammatory status in old rats, assessed with  $\alpha_2$ -  
25 macroglobulin or fibrinogen was not transient but persistent for at least 10 weeks.

1 Accordingly, it has been shown in a human study that CRP values appear relatively tightly  
2 regulated over a 6-month period (Macy et al., 1997).

3 In the present study, moderate  $\alpha_2$ -macroglobulin and fibrinogen levels in old rats was  
4 clearly associated with worsening of body weight loss and persistent inflammation, which are  
5 strong criteria of frailty in humans. The etiology of this moderate inflammation is not known,  
6 it could be either associated to age *per se* or to subclinical diseases (Grimble, 2003). In this  
7 regard, a recent paper shows an association between chronic asymptomatic infection,  
8 inflammation and frailty in elderly (Schmaltz et al., 2005). Even if rats exhibiting moderate  
9 inflammation were not at high risk of mortality during the 10-week follow-up, it is not  
10 excluded that they would be in a longer follow-up study. Hence, moderate  $\alpha_2$ -macroglobulin  
11 and fibrinogen levels can be considered as markers of frailty in old rats.

12 Taken together, the results presented here demonstrate that the highest levels of  $\alpha_2$ -  
13 macroglobulin and fibrinogen and the lowest levels of albumin are predictive of mortality,  
14 whereas, moderate levels of  $\alpha_2$ -macroglobulin and fibrinogen are biomarkers of frailty in old  
15 rat (as evaluated by body weight loss and persistent inflammatory status). These data are  
16 consistent with a human study showing that elevated levels of CRP (> 5.5 mg/L) or fibrinogen  
17 (> 3.9 g/L) are associated with higher risks for prevalent frailty, the relation between CRP  
18 levels and frailty being higher than that between fibrinogen levels and frailty (Walston et al.,  
19 2002). Thus, in old rats  $\alpha_2$ -macroglobulin, as CRP in humans, is a more powerful biomarker  
20 of frailty than fibrinogen.

21

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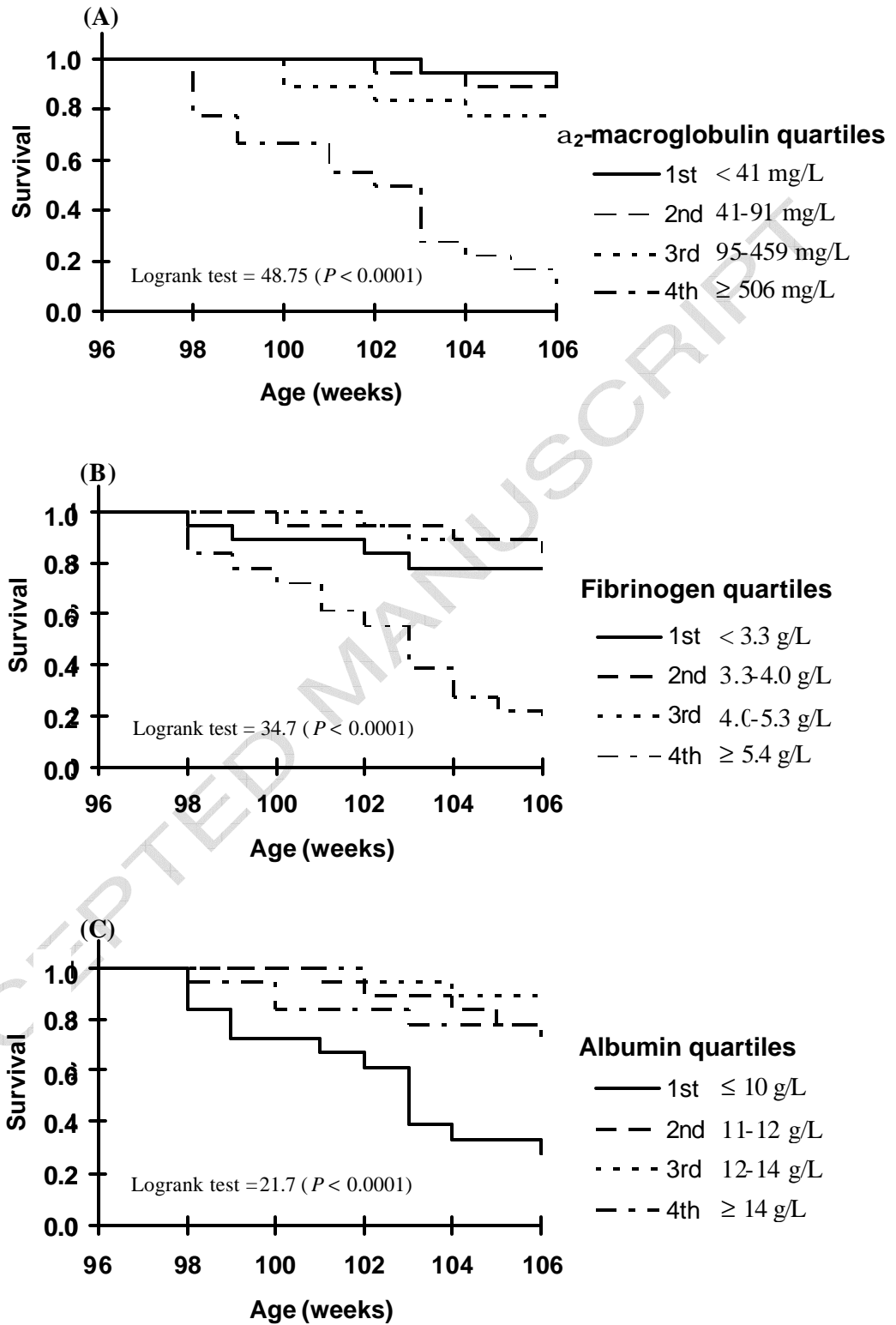
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**FIGURE LEGEND**

**FIGURE 1** Kaplan-Meier survival curves of old rats by quartiles of levels of each acute phase protein measured at 96 weeks of age. (A)  $\alpha_2$ -macroglobulin (B) Fibrinogen (C) Albumin. n = 18 for each quartile.

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FIGURE 1



**TABLE 1***Age-related changes in plasma levels of acute phase proteins and body weight in ageing rats*

	Age (weeks)		
	35 (n = 32)	96 (n = 72)	106 (n = 48)
$\alpha_2$ -macroglobulin (mg/L)	38 ± 22 <sup>a</sup>	469 ± 811 <sup>b</sup>	215 ± 257 <sup>a</sup>
Fibrinogen (g/L)	3.0 ± 0.3 <sup>a</sup>	4.4 ± 1.6 <sup>b</sup>	4.1 ± 1.5 <sup>b</sup>
Albumin (g/L)	18 ± 3 <sup>a</sup>	12 ± 3 <sup>b</sup>	12 ± 3 <sup>b</sup>
Body weight (g)	600 ± 24 <sup>a</sup>	648 ± 87 <sup>b</sup>	625 ± 101 <sup>ab</sup>

All rats of the cohort were included at 96 and 106 weeks of age, whereas 32 rats were chosen when they were 35 weeks old. Values are means ± SD. One-way ANOVA was significant for all parameters. Data not sharing a common letter within a row are significantly different (Fischer's PLSD).

TABLE 2

*Plasma levels of acute phase proteins and body weight in 96 week-old rats*

	All (n = 72)	Died before 106 weeks (n = 24)	Alive at 106 weeks (n = 48)
$\alpha_2$ -macroglobulin (mg/L)	469 $\pm$ 811	1162 $\pm$ 1110 **	122 $\pm$ 153
Fibrinogen (g/L)	4.4 $\pm$ 1.6	5.6 $\pm$ 2.1 **	3.8 $\pm$ 0.9
Albumin (g/L)	12 $\pm$ 3	10 $\pm$ 4 **	13 $\pm$ 2
Body weight (g)	648 $\pm$ 87	596 $\pm$ 86 *	674 $\pm$ 76

Values are means  $\pm$  SD. \*  $P < 0.001$ , \*\*  $P < 0.0001$  versus age-matched rats alive at 106 weeks (Student's  $t$  test for unpaired data).



**TABLE 3**

*Correlations between percentage of body weight change throughout 10-week follow-up and plasma levels of acute phase proteins measured at 96 weeks of age.*

Correlate of percentage of body weight change	r	P
$\alpha_2$ -macroglobulin	-0.448	0.0012
Fibrinogen	-0.251	0.0849
Albumin	0.074	0.6210

Linear regressions were performed between body weight change throughout the follow-up and plasma levels of each of the three APP measured at the age of 96 weeks in rats alive at 106 weeks (n = 48), r : Pearson correlation coefficient.

**TABLE 4**

*Plasma acute phase protein kinetics and serum levels of soluble tumor necrosis factor- $\alpha$  receptor 1 and lipopolysaccharide-binding protein in rats, alive at 106 weeks, in bottom half and top half categories based on plasma levels of acute phase proteins measured at 96 weeks of age.*

		APP levels at 96 weeks of age					
		$\alpha_2$ -macroglobulin (mg/L)		Fibrinogen g/L		Albumin g/L	
		< 91 (n = 32)	> 91 (n = 16)	< 4.0 (n = 29)	> 4.0 (n = 19)	<12 (n =19)	> 12 (n =29)
$\alpha_2$ -macroglobulin (mg/L)							
Age (weeks)	96	43 $\pm$ 21	281 $\pm$ 179 **	68 $\pm$ 99	205 $\pm$ 184 *	107 $\pm$ 114	132 $\pm$ 175
	100	107 $\pm$ 355	430 $\pm$ 479 *	141 $\pm$ 382	327 $\pm$ 470	247 $\pm$ 472	194 $\pm$ 397
	104	65 $\pm$ 55	513 $\pm$ 640 *	77 $\pm$ 94	424 $\pm$ 612 *	207 $\pm$ 348	219 $\pm$ 470
	106	115 $\pm$ 200	415 $\pm$ 245 **	91 $\pm$ 102	404 $\pm$ 306 **	212 $\pm$ 274	217 $\pm$ 250
Fibrinogen (g/L)							
Age (weeks)	96	3.4 $\pm$ 0.7 <sup>ab</sup>	4.6 $\pm$ 0.7 <sup>ab **</sup>	3.3 $\pm$ 0.5 <sup>a</sup>	4.7 $\pm$ 0.5 <sup>ab **</sup>	3.8 $\pm$ 0.9 <sup>a</sup>	3.8 $\pm$ 0.8 <sup>ab</sup>
	100	3.2 $\pm$ 0.8 <sup>a</sup>	4.2 $\pm$ 1.0 <sup>a *</sup>	3.1 $\pm$ 0.8 <sup>a</sup>	4.2 $\pm$ 0.9 <sup>a **</sup>	3.7 $\pm$ 1.2 <sup>a</sup>	3.4 $\pm$ 0.9 <sup>c</sup>
	104	3.3 $\pm$ 0.9 <sup>ab</sup>	4.5 $\pm$ 1.3 <sup>ab *</sup>	3.1 $\pm$ 0.5 <sup>a</sup>	4.7 $\pm$ 1.2 <sup>ab **</sup>	3.8 $\pm$ 1.2 <sup>a</sup>	3.7 $\pm$ 1.2 <sup>ac</sup>

	106	$3.6 \pm 1.3^b$	$5.0 \pm 1.4^{b*}$	$3.3 \pm 0.8^a$	$5.2 \pm 1.6^{b**}$	$4.0 \pm 1.5^a$	$4.1 \pm 1.5^b$
<hr/>							
Albumin (g/L)							
	96	$14 \pm 3^{ac}$	$12 \pm 2^a$	$14 \pm 2^{ac}$	$13 \pm 2^a$	$11 \pm 1^{ab}$	$15 \pm 2^{a**}$
Age (weeks)	100	$12 \pm 2^b$	$9 \pm 2^{b*}$	$12 \pm 2^b$	$10 \pm 2^{b*}$	$10 \pm 3^b$	$12 \pm 2^b$
	104	$14 \pm 3^a$	$10 \pm 3^{b**}$	$14 \pm 3^a$	$11 \pm 3^{b**}$	$12 \pm 3^a$	$13 \pm 3^c$
	106	$13 \pm 2^c$	$10 \pm 2^{b*}$	$13 \pm 2^c$	$11 \pm 2^{b*}$	$12 \pm 3^a$	$12 \pm 2^b$
<hr/>							
sTNFR-1 (pg/mL)							
Age (weeks)	106	$289 \pm 78$	$479 \pm 261^*$	$275 \pm 57$	$468 \pm 241^*$	$361 \pm 162$	$341 \pm 195$
<hr/>							
LBP ( $\mu$ g/mL)							
Age (weeks)	106	$7.8 \pm 7.8$	$12.5 \pm 11.8$	$6.2 \pm 4.6$	$14.2 \pm 12.7^*$	$11.0 \pm 12.8$	$8.1 \pm 6.2$

Values are means  $\pm$  SD. Groups corresponded to rats alive at 106 weeks from quartiles 1 and 2 in one hand and quartiles 2 and 3 in another hand for each of the three APP (see figure 1). Two-way ANOVA for repeated measurements with group as the between-rat factor and time as the within-rat factor:  $\alpha_2$ -macroglobulin groups: significant effect of group for the three APP and time for fibrinogen and albumin and significant interaction between group and time for albumin only, fibrinogen groups: significant effect of group for the three APP and time for fibrinogen and albumin and significant interaction between group and time for albumin only, albumin groups: significant effect of group for albumin only and

time for fibrinogen and albumin and significant interaction between group and time for albumin only. \* $P < 0.05$ , \*\* $P < 0.0001$  (group effect: Student's  $t$  test). Data not sharing a common letter during the follow-up are different (Fischer's PLSD). sTNFR-1: soluble tumor necrosis factor- $\alpha$  receptor 1, LBP: lipopolysaccharide binding protein

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