Long-term training induces a healthy inflammatory and endocrine emergent biomarker profile in elderly men

David de Gonzalo-Calvo · Benjamín Fernández-García ·
Beatriz de Luxán-Delgado · Susana Rodríguez-González ·
Marina García-Macia · Francisco Manuel Suárez · Juan José Solano ·
María Josefa Rodríguez-Colunga · Ana Coto-Montes

Received: 27 January 2011 / Accepted: 5 May 2011 / Published online: 4 June 2011
© American Aging Association 2011

Abstract The objective of the present study was to investigate the changes in a large panel of emergent geriatric biomarkers in long-term trained elderly men to analyze the effects of long-term exercise on an aged population. We collected blood samples from two groups of male volunteers older than 65 years who maintain a measure of functional independence: one group of sedentary subjects without a history of regular physical activity and the other of subjects who have sustained training, starting during adulthood (mean training time=49±8 years). We studied morbidity, polypharmacy, cellular and serological inflammatory parameters, and endocrine mediators. After adjusting for confounding factors, we observed reduced medication intake per subject and lower

number of diseases per subject with statistical differences nearly significant in the long-term exercise group. We showed that long-term training was associated with lower levels of white blood cell counts, neutrophil counts, interleukin-6, interleukin-10, interleukin-1 receptor antagonist, and soluble TNF receptor-I. Furthermore, we noted an increase in the concentrations of insulin-like growth factor-1 and dehydroepiandrosterone in the long-term training group. We concluded that long-term exercise training from adulthood to old age is clearly associated with a healthy profile of emergent geriatric biomarkers. Long-term training could improve the inflammatory–endocrine imbalance associated with disease, frailty, functional decline, and mortality in elderly men. Our results point to the benefits of prolonged exercise from adulthood to old age.

D. de Gonzalo-Calvo (✉) · B. Fernández-García ·
B. de Luxán-Delgado · S. Rodríguez-González ·
M. García-Macia · M. J. Rodríguez-Colunga ·
A. Coto-Montes (✉)
Department of Morphology and Cellular Biology, Faculty
of Medicine, University of Oviedo,
Julián Clavería s/n,
33006 Oviedo, Spain
e-mail: ddgonzalo@hotmail.com
e-mail: acoto@uniovi.es

F. M. Suárez
Regional Health Ministry of Principado de Asturias,
33001 Oviedo, Spain

J. J. Solano
Geriatrics Service, Monte Naranco Hospital,
33012 Oviedo, Spain

Keywords Aging · Long-term training · Exercise ·
Inflammatory biomarkers · Endocrine biomarkers

Introduction

Aging consists of a multisystem decline that is considered to be a consequence of redox, neuromuscular, endocrine, and immune changes that occur as people age (Chung et al. 2009; Maggio et al. 2010; de Gonzalo-Calvo et al. 2011). It is currently believed that the anabolic–catabolic imbalance that favors catabolism is a key factor underlying the deleterious

consequences of aging (Cappola et al. 2003; Juge-Aubry et al. 2003). This imbalance, mostly related to abnormalities in endocrine and inflammatory mediator levels, might facilitate several medical conditions that, in turn, induce morbidity, disability, frailty, and mortality in the elderly population (Joseph et al. 2005).

Both the increase in proinflammatory mediators and the decline in growth factors have been described as important contributors to age-related decline. Low levels of gonadal hormones, serum 25-hydroxyvitamin D, growth hormone (GH), and insulin-like growth factor-1 (IGF-1) have been implicated in atherosclerosis, sarcopenia, osteoporosis, metabolic syndrome, and disability (Maggio et al. 2005; O'Connor et al. 2008; Aldred et al. 2009). Several studies have proposed subclinical chronic inflammation, defined by a two- to fourfold increase in proinflammatory mediators, such as tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), interleukin-1 β (IL-1 β), interferon- γ (IFN- γ), or white blood cell counts (WBC), as an underlying biological mechanism responsible for decline in the elderly (De Martinis et al. 2006; de Gonzalo-Calvo et al. 2010). Geriatric syndromes, such as frailty and age-related diseases like Alzheimer's disease, Parkinson's disease, hypertension, type 2 diabetes, and cognitive decline, are initiated or worsened by chronic inflammation (Bruunsgaard et al. 2003; Chung et al. 2009). Chronic inflammation is also a robust predictor of both disability and mortality even in the absence of clinical disease (Beavers et al. 2010a). Moreover, there are several reasons to expect that chronic low-grade inflammation and endocrine decline are intimately associated. Proinflammatory cytokines often act as negative regulatory signals that temper the action of hormones and growth factors (O'Connor et al. 2008). The circulating and intramuscular concentration of IGF-1 and the responsiveness of muscle to IGF-1 stimulation are reduced in most medical conditions that are characterized by high circulating levels of proinflammatory cytokines (Frost and Lang 2003).

Targeted interventions might slow the age-associated decline of functional capacity and performance and prolong the disability-free lifespan in the elderly population (Topinkova 2008). One of the possible strategies that experts propose to attenuate or to slow aged-related decline is exercise. Regular and moderate physical activity is a relatively side-effect-free strategy to improve significantly the quality of life of the elderly. There is evidence that regular

exercise can minimize the deleterious effects of the modern sedentary lifestyle and limit the development and progression of chronic disease and frailty conditions (Ji et al. 2010), so long as it is practiced according to the current exercise guidelines (Chodzko-Zajko et al. 2009). It is believed that many of the benefits of exercise are associated with the improvement in the balance of inflammatory-related cytokines and anabolic hormones. Cotman and colleagues (Cotman et al. 2007) proposed that the effects of exercise on brain health (protection from neurodegenerative disorders and depression) are related to reduced inflammation impairment of growth factor signaling. Tissandier and colleagues (Tissandier et al. 2001) reported that regular physical activity could maintain higher dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEA-S), IGF-1, and lean body mass levels in elderly men. Furthermore, several investigations suggested that higher levels of physical activity are inversely correlated with circulating levels of inflammatory mediators in elderly humans (Jankord and Jemioło 2004; Petersen and Pedersen 2005).

The mechanisms underlying these proanabolic and anti-inflammatory effects of training are complex and not fully elucidated. Because a single bout of exercise induces an inflammatory response that is similar to that induced by infection or trauma, there is controversy surrounding whether increasing physical activity may be effective for reducing chronic inflammation in the long term, especially in older individuals (Nicklas and Brinkley 2009). Currently, there are very limited data from clinical trials to conclude definitively that long-term exercise training reduces chronic inflammation (Beavers et al. 2010b). The literature published on training and aging is generally associated with the topics of exercise programs or short-term training (8–12 weeks) (Polak et al. 2006; Karolkiewicz et al. 2009; Michishita et al. 2010); literature about long-term training (e.g., decades) is scarce, which limits knowledge about the substantial physiological adaptations associated with exercise. Because the physiological effects of exercise are dose-dependent (Urso et al. 2009), the duration of the training protocol seems to alter the metabolic, immune, and endocrine consequences of exercise. Indeed, Bar-Shai and colleagues (Bar-Shai et al. 2008) proposed that if physical training is initiated prior to a critical physiological threshold in younger

rodents, it may prove beneficial for the muscles and bones of older animals, but not in short-term trained elderly animals. Moreover, a recent population-based cohort study of 2,205 men with follow-up over 35 years concluded that there is a graded reduction in the total mortality risk at old ages with increasing physical activity levels during middle age (Byberg et al. 2009). This situation emphasizes the importance of investigating long-term training in elderly humans who began training during adulthood.

Therefore, the purpose of this study was to determine several emergent endocrine and inflammatory biomarker levels in the blood of elderly men who have had sustained exercise since adulthood (mean training time = 49 ± 8 years). The study of the different endocrine and inflammatory factors in this group would enable us to investigate the effects of long-term exercise on the elderly population.

Methods

Participants

The study included two groups of men older than 65 years who maintain a measure of independence: one group of 13 sedentary subjects without a history of regular physical activity (SE group) and one group of 13 subjects who had a history of long-term training since adulthood (TE group).

To form the two groups, 13 community-dwelling men over 65 years who practiced regular physical activity were recruited from the Sports Service of Oviedo University (Oviedo, Spain), and 29 institutionalized subjects from Santa Teresa nursing home (Oviedo, Spain) were screened for inclusion. Initial evaluations were performed by experienced geriatricians and sport clinicians and included a medical and pharmacology history review, a physical examination, and a questionnaire to determine the characteristics of training. Our questionnaire is based on participants recall; therefore, we tried to make a questionnaire as simple as possible. The questionnaire only required that the participant provide data of past and current physical activities. Diseases included in the current analysis were cognitive impairment, dementia, osteoporosis, hypertension, chronic obstructive pulmonary disease, depression, heart failure, ischemic heart disease, rheumatoid arthritis, cancer, liver disease,

hyperthyroidism, dyslipidemia, and diabetes. Only subjects with a Barthel Index (BI) above 85 (functional independence) were enrolled. BI is a widely used tool for the assessment of a patient's level of independence regarding the basic activities of daily life (Tiainen et al. 2010). BI is a 10-item scale with the following items: feeding, grooming, bathing, toilet use, dressing, walking, transfers, climbing stairs, fecal incontinence, and urinary incontinence. The highest score is 100 (independence), and the lowest is 0 (total dependence) (Mahoney and Barthel 1965). Exclusion criteria were recent or current infection, malignant disease, malnutrition, and pharmacological interference (steroids, nonsteroidal anti-inflammatory agents, immunosuppressive and antineoplastic drugs, and testosterone).

All subjects recruited from the Sports Service of Oviedo University satisfied the inclusion and exclusion criteria. These subjects trained for at least 60 min per session, three sessions per week, for the previous 40 years, and they were still practicing regular physical activity at the time of the study; therefore, they were assigned to the TE group. Training was self-directed and combined endurance (exercises in which the body's large muscles move in a rhythmic manner for sustained periods) and resistance (exercise that causes muscles to work or hold against an applied force or weight) activities (Chodzko-Zajko et al. 2009). No breaks in the training period were reported. Of the participants from Santa Teresa nursing home, 13 subjects satisfied the inclusion and exclusion criteria. These subjects neither had a history of regular physical activity nor practiced regular exercise at the time of the study; therefore, they were assigned to the SE group.

All participants received information about the purposes and objectives of the study and signed informed consent documents. The study was approved by the Hospital Central de Asturias (Oviedo, Spain) Ethics Committee.

Blood collection

Blood samples were obtained by venipuncture following an overnight fast and 15-min rest in the morning. To analyze the usual biomarker levels in the TE group, blood was collected at least 24 h after the last exercise session. Blood samples were drawn into vacutainer tubes with gel separator for serum or containing EDTA for plasma (BD, NJ, USA). After

processing, serum and plasma were divided into aliquots and stored at -80°C pending analysis.

Biochemical analysis

Total WBC, neutrophil (NEU), lymphocyte (LINF), and monocyte (MONO) counts were measured by an automated analyzer SYSMEX SF-3000 (GMI Inc., MI, USA) in a well-standardized hematology laboratory at Monte Naranco Hospital (Oviedo, Spain). All endocrine and inflammatory biomarkers were measured in duplicate using commercially available enzyme-linked immunosorbent assays according to the manufacturer's recommendations from frozen plasma and serum samples. The average of the two measures was used in the analysis. Levels of IL-6 (Gen-Probe Diaclone SAS, Besancon Cedex, France), IL-10 and soluble IL-6 receptor (sIL-6R) (Invitrogen Corp., CA, USA), soluble gp130 (sgp130), IGF-1 and GH (R&D Systems Inc., MN, USA), and monocyte chemoattractant protein-1 (MCP-1) (Bender MedSystems Inc. VI, AUT) were assayed in plasma. IL-1 receptor antagonist (IL-1ra) and TNF- α (Invitrogen Corp., CA, USA), soluble TNF receptor-I (sTNF-RI), and IL-1 β (Bender MedSystems Inc. VI, AUT) levels were measured in serum samples. Plasma DHEA was measured in duplicate using an enzyme-linked immunosorbent assay based on the principle of competitive binding (IBL International, Hamburg, Germany).

Statistical analysis

Variables are expressed as the mean and the standard deviation of the mean (SD). The statistical software package, SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA), was employed for all statistical analyses.

Descriptive statistics were used to characterize the study population and to analyze age and BI differences. Categorical variables were compared between groups using chi-square test. Continuous variables were compared between groups with analysis of variance (ANOVA). The normality of the data was analyzed using the Kolmogorov–Smirnov test. Because IL-6 and GH were not normally distributed, the analyses were performed using their log values. For clarity, original values are used to describe the results.

Total number of diagnoses per subject, total number of medications per subject, and biological markers were analyzed by multivariate analysis of variance (MANOVA). This study was designed to evaluate associations between inflammatory and endocrine emergent biomarkers and long-term training, and their subsequent association with morbidity and polypharmacy. Therefore, all data presented were adjusted using age and functional status as covariates. However, in order to establish whether the association of emergent biomarkers levels and long-term training could be influenced by morbid conditions, the analysis was repeated using age, Barthel Index, and total number of diseases per subject as covariates. Differences were considered statistically significant when $p < 0.05$.

Results

Characteristics of the study subjects

Table 1 summarizes the demographic, clinical, and training characteristics of the study groups. Thirteen sedentary and 13 long-term exercised subjects participated in the study. The mean age was 79 ± 4 years for the sedentary group and 74 ± 5 years for the trained

Table 1 Demographic, clinical, and training characteristics of the study groups

	Sedentary ($N=13$)	Long-term training ($N=13$)	p value
Age (years)	79 (5)	74 (5)	0.004
Barthel Index	94 (6)	100 (0)	0.007
Total number of diagnoses per subject	1.4 (1.3)	0.3 (0.5)	0.061
Total number of medications per subject	5.0 (1.9)	0.6 (0.7)	<0.001
Mean training practice time (years)	—	49 (8)	—
Mean training sessions (sessions/week)	—	4 (1)	—
Mean training load (minutes/session)	—	83 (30)	—

All data are presented as means (SD)

group. The functional independence grade of the SE group was elevated ($BI=94\pm6$); all participants of the TE group showed total independence ($BI=100$). Only the TE group was involved in long-term exercise, with a mean of four training sessions per week (range 3–7 sessions per week); each training session lasted 83 min (range 60–120 min per session) for the previous 49 years (range 40–60 years). After adjusting for age and functional status, the number of medications taken per subject was lower in the TE group. Long-term training was also associated with lower numbers of total medical diagnoses per subject than the SE group, with statistical differences nearly significant. Analysis of the data showed that there were no significant differences between groups based on cognitive impairment, dementia, osteoporosis, depression, heart failure, ischemic heart disease, rheumatoid arthritis, cancer, liver disease, hyperthyroidism, dyslipidemia, and diabetes. In addition, TE group presented a lower incidence of hypertension ($p=0.039$) and chronic obstructive pulmonary disease ($p=0.030$) than SE group.

Cellular inflammatory biomarkers

As shown in Table 2, WBC levels were significantly lower in the trained subjects than in the sedentary ones. A significant decline in blood levels of NEU associated with physical activity was also observed when we compared the TE group to the SE group. No statistical difference was observed between the sedentary versus active elderly groups in the MONO or LINF levels.

Serological inflammatory and endocrine biomarkers

As shown in Table 3, long-term training was associated with a decline in the cytokines IL-6, IL-1ra, IL-10, and sTNF-RI, and higher values of the

chemokine MCP-1. There was no statistical difference in TNF- α , IL-1 β , sIL-6R, or sgp130 levels between the SE and TE groups. Regarding endocrine biomarkers, we observed an increase in IGF-1 plasma levels associated with long-term training. Likewise, DHEA levels were significantly higher in the TE group compared with the SE group. Although there were high differences in the means of GH concentrations between groups, no statistical difference was observed.

Because chronic inflammation and endocrine decline are associated with morbid conditions, additional analysis was conducted to determine whether the incidence of chronic diseases alters the relationship of biological markers and long-term training. When we applied this statistical analysis, all inflammatory and endocrine parameters studied remained statistically significant (data not shown).

Discussion

In the current investigation, we compared two groups of men older than 65 years who maintained a degree of independence. One group consisted of sedentary subjects without a history of regular physical activity and the other group designed to investigate the effects of long-term exercise on the elderly and consisted of subjects who had a history of long-term training and who initiated training during adulthood. The main finding of the current investigation is that long-term training from adulthood to old age induced a healthy profile of emergent geriatric biomarkers in elderly men. Age and BI showed statistically significant differences between groups. Because levels of inflammatory and endocrine biomarkers are intimately associated with age and functional status, we adjusted all biomarkers by these parameters. After eliminating

Table 2 Cellular inflammatory biomarkers in sedentary and long-term trained elderly men

	Sedentary ($N=13$)	Long-term training ($N=13$)	p value
WBC ($\times 10^3/\mu\text{l}$)	8.4 (1.9)	5.8 (0.9)	0.014
NEU ($\times 10^3/\mu\text{l}$)	5.0 (1.3)	3.2 (0.74)	0.020
MONO ($\times 10^3/\mu\text{l}$)	0.6 (0.2)	0.6 (0.1)	0.464
LINF ($\times 10^3/\mu\text{l}$)	2.2 (0.7)	1.8 (0.4)	0.104

WBC white blood cell count, NEU neutrophils cell count, LINF lymphocytes cell count, MONO monocytes cell count

All data are presented as means (SD)

Table 3 Serological inflammatory and endocrine biomarkers in sedentary and long-term trained elderly men

	Sedentary (N=13)	Long-term training (N=13)	p value
TNF- α (pg/ml)	13.5 (2.0)	12.8 (1.8)	0.322
sTNF-RI (ng/ml)	3.3 (1.3)	1.9 (0.4)	0.001
IL-1 β (pg/ml)	2.4 (1.5)	2.6 (1.3)	0.120
IL-1ra (pg/ml)	139.4 (39.3)	70.9 (20.7)	<0.001
IL-10 (pg/ml)	7.8 (1.3)	5.8 (0.9)	0.013
IL-6 (pg/ml)	11.0 (7.3)	2.6 (1.7)	0.002
sIL-6R (pg/ml)	141.3 (42.1)	125.6 (24.8)	0.891
sgp130 (ng/ml)	220.4 (8.9)	222.3 (53.7)	0.944
MCP-1 (pg/ml)	125.9 (42.5)	201.7 (68.7)	0.011
DHEA (ng/ml)	4.8 (1.5)	7.0 (1.6)	0.023
IGF-1 (ng/ml)	28.4 (7.4)	53.3 (20.4)	0.020
GH (pg/ml)	229.1 (126.6)	813.3 (838.4)	0.259

TNF- α tumor necrosis factor- α , sTNF-RI soluble TNF receptor-I, IL-1 β interleukin-1 β , IL-1ra interleukin-1 receptor antagonist, IL-10 interleukin-10, IL-6 interleukin-6, sIL-6R soluble interleukin-6 receptor, sgp130 soluble gp130, MCP-1 monocyte chemoattractant protein-1, DHEA dehydroepiandrosterone, IGF-1 insulin-like growth factor, GH growth hormone

All data are presented as means (SD)

the possible interference of the difference in age and BI between groups on the studied biological markers, the participants with the history of long-term physical activity displayed lower concentrations of the following inflammatory biomarkers: WBC (percentage of change 31%), NEU (36%), IL-6 (76%), IL-1ra (49%), sTNF-RI (42%), and IL-10 (26%). We noted an increase in the concentration of the anabolic hormones DHEA (46%) and IGF-1 (88%) in the TE group. Long-term exercise was also associated with an increase in MCP-1 level (60%). Furthermore, long-term exercise was associated with reduced medication intake per subject and a decreased number of diseases per subject with statistical differences nearly significant, both conditions associated with a better quality of life. Taken together, our results suggested that long-term training is associated with a healthy profile of emergent geriatric biomarkers and could improve the inflammatory–endocrine imbalance associated with disease, frailty, functional decline, and mortality in elderly men.

Inflammatory responses to specific insults involve a cascade of well-defined and distinct cellular and molecular events. Because cytokines, chemokines, and immune cells work in networks, the effects of individual mediators on physiological functions depend on their place within the network (Hotamisligil 1999). Cross-sectional single inflammatory mediator measurements

likely do not reflect the true complexity of relevant inflammatory processes in vivo (Leng et al. 2008). The analysis of multiple inflammatory biomarkers could provide a reliable general view of clinical status (Hsu et al. 2009). Therefore, we have investigated the impact of long-term exercise in several cellular and serological inflammatory biomarkers.

Leukocytes are important cellular components of the inflammatory system. Increased WBC is a well-recognized cellular marker of systemic inflammation, primarily due to acute infection. Studies have demonstrated that high total WBC is associated with a greater risk for cardiovascular disease and mortality (De Martinis et al. 2006). However, relatively little is known about the alterations in total leukocyte population and leukocyte subpopulation levels in long-term exercising elderly subjects. Excluding potential acute infection, the current data showed that long-term training is associated with a decline in WBC in elderly men. We also reported a decrease in NEU levels, but not in MONO or LINF levels, in the TE group. These results are in line with previous investigations reporting lower levels of inflammatory cellular biomarkers in physically active elderly populations (Wannamethee et al. 2002; Elosua et al. 2005). Our finding indicated that long-term training seems to be associated with a reduction in the total number of leukocytes. Moreover, because we only

observed a significant decline in NEU, these results revealed differential responses of the distinct leukocyte subpopulations to long-term training.

Concerning serological biomarkers, we reported a significant decrease in the concentrations of the cytokines IL-6, IL-1ra, IL-10, and sTNF-RI in the TE group compared to the SE group. Our data are consistent with emerging evidence that physical activity results in a reduced inflammatory state in the elderly population (Bruunsgaard 2005; Elosua et al. 2005). These data have important clinical implications. Persistent elevation of systemic inflammation biomarkers, even within the clinically normal range, is prospective risk factor for adverse health conditions in elderly subjects (Nicklas and Brinkley 2009). Likewise, Stowe and colleagues (Stowe et al. 2010) proposed that successful aging is intimately associated with lower levels of cytokines, such as IL-1ra. Interestingly, we have also demonstrated that the concentration of the chemokine MCP-1 was increased in the TE group compared to the SE group. These data may be controversial. MCP-1 has been identified as both a traditional risk factor for atherosclerosis as well as a risk factor for death and myocardial infarction (de Lemos et al. 2003). It has been reported that significant reductions in MCP-1 after a 12-week training program were observed in exercised individuals (Troseid et al. 2004). However, because exercise plays a role in physiological repair and/or compensatory mechanisms to promote angiogenesis and vascular regeneration (Wardyn et al. 2008), and MCP-1 has been recognized as an angiogenic chemokine (Niu et al. 2008), it could be conceivable that long-term training could cause an increase in MCP-1. It is important to note that investigations based on long-term exercised subjects are scarce; therefore, in light of the present results, these data deserve a more detailed investigation.

In the current investigation, we have shown similar values of TNF- α and IL-1 β in the SE and TE groups. These results are surprising because elevated levels of TNF- α and IL-1 β have been associated with age-related decline (Bruunsgaard et al. 1999, 2003; Chung et al. 2009). It would be interesting to speculate about local anti-inflammatory effects of training that may not be evident in the systemic circulation but that may be relevant to clinical outcomes (Beavers et al. 2010b). Indeed, Gielen and colleagues (Gielen et al. 2003) reported that 6 months of exercise training did

not reduce TNF- α or IL-1 β serum levels in male patients with congestive heart failure but did reduce gene expression of TNF- α and IL-1 β in skeletal muscle. Furthermore, Pedersen (2009) reported that the cytokine cascade induced by exercise is independent of TNF- α and IL-1 β . The fact that TNF- α and IL-1 β did not change with long-term training could be in line with the hypothesis proposed by this author.

Soluble receptors for several cytokines, such as sTNF-Rs, are believed to modulate the cytokine response by binding the ligand, thereby reducing its bioavailability or, such as IL-1ra, competing with IL-1 β for receptor interaction. In the case of IL-6, the situation is more complex. IL-6 receptor (IL-6R) is only present on a comparatively small number of cells: hepatocytes, monocytes, neutrophils, and some T- and B-cells (Kallen 2002). For IL-6 to signal in tissues where membrane IL-6R expression is low or absent, a soluble receptor form, sIL-6R, exists (Memoli et al. 2005). In the circulation, IL-6 can combine with sIL-6R to form an IL-6/sIL-6R complex, which increases the half-life of IL-6 and allows signaling to occur in tissues devoid of membrane IL-6R, a process termed “trans-signaling” (Kallen 2002). This complex can be rendered inactive by a soluble form of the gp130 receptor (Giuliani et al. 2001). Although there is an abundance of research characterizing the response of IL-6 to exercise (Colbert et al. 2004; Jankord and Jemiolo 2004; Bruunsgaard 2005; Goldhammer et al. 2005; Pedersen and Febbraio 2008), few studies have investigated the influence of physical activity on sIL-6R and sgp130 levels (You et al. 2004; Gray et al. 2009; Beavers et al. 2010b). This is somewhat surprising because these regulatory cytokines have been shown to be crucial in determining the biological activity of IL-6 and in the pathology of many chronic inflammatory diseases, such as cancer, osteoporosis, sarcopenic obesity, and cardiovascular disease (Kallen 2002; Schrager et al. 2007). In the current study, we show similar sIL-6R and sgp130 levels in the SE and TE groups. However, plasma IL-6 concentration decreased with long-term training. The present data could indicate that long-term exercise modulated the trans-signaling phenomenon altering IL-6 blood concentration, but not sIL-6R or sgp130 levels. Indeed, sIL-6R and sgp130 have been reported to fluctuate much less than IL-6 (Maggio et al. 2006). Therefore, as Gray and colleagues (Gray et al. 2009) proposed, IL-6

could likely be the limiting factor in the trans-signaling process. Considering the paucity of literature on the subject, our findings provide additional information about IL-6, sIL-6R, and sgp130 levels in long-term exercising elderly subjects and about the role of IL-6 in the regulation of inflammatory processes.

Because low-grade inflammation and endocrine decline are intimately associated, we analyzed the endocrine mediators IGF-1, DHEA, and GH in both study groups. The GH levels of the groups were not statistically different, a finding that likely resulted from the small sample size. However, we observed elevated IGF-1 and DHEA concentrations in the TE group compared to the SE group. Our data indicated that long-term exercise is associated with higher levels of IGF-1 and DHEA. These results are in agreement with several studies that have shown increases in hormone concentrations and anabolic cytokines, such as interleukin-15, in more active elderly subjects (Tissandier et al. 2001; Nielsen et al. 2007; Aldred et al. 2009). This situation could be unclear because the role of IGF-1 and IGF receptor in association with longevity and cancer survival remains controversial (Suh et al. 2008). Nevertheless, the data indicated that long-term exercise could be a serious strategy to increase the levels of anabolic mediators in the elderly population. These results have important clinical consequences because the decline in anabolic hormones increases the risk of adverse outcomes in elderly subjects (Joseph et al. 2005; Maggio et al. 2005, 2007, 2010).

Due to the ubiquitous nature of low-grade inflammation and endocrine decline as an underlying risk factor for adverse outcomes in the elderly population, long-term training could be effective in treating multiple adverse health conditions. Indeed, our results point to the benefit of prolonged exercise from adulthood to old age. We have shown a significant decrease in the number of diseases, with statistical differences nearly significant, and medications taken in elderly men engaging in long-term regular exercise compared to sedentary ones. Furthermore, although subclinical inflammation and endocrine decline are thought to be important contributors to age-related disease, there are no known definitive treatments for reducing these conditions in the elderly. The use of anti-inflammatory medications or substitutive hormonal

therapies may induce a beneficial response, but the side effects of these medications are likely to reduce their clinical application for the ongoing treatment of inflammatory–endocrine imbalance (Grundy et al. 2005; Nicklas and Brinkley 2009; Clayton et al. 2010). Long-term regular exercise seems to be an important alternative to long-term pharmacological treatments.

Several limitations of this study must be considered. The primary limitation of this study is the small sample size due to the problems of finding elderly subjects with the training characteristics presented in this study. A population sample >13 participants would have been desirable. Second, we sample blood from sedentary participants who were institutionalized; this may have biased the study results because it is likely that these subjects would have higher disease levels (Bonanini et al. 2008). To minimize this situation, we studied well-functioning sedentary subjects ($BI=94\pm6$). Finally, the questionnaire to determine the characteristics of training is based on participant recall; therefore, we tried to make it as simple as possible. It is difficult to establish the training history after a mean training period of 49 ± 8 years. Our intention was analyzed an overview of long-term training without going in specific details. The questionnaire required that the participant provide data of past and current physical activities. Furthermore, we only characterized the modalities of endurance or resistance exercise, which are the main kinds of exercise. Because the training-specific adaptations are related to the nature (aerobic-based, resistance-based, balance-based training), intensity, and duration of the exercise training (Chodzko-Zajko et al. 2009), current report is not able to propose a reliable long-term training program to counteract age-related decline. Cautious interpretation of these results is warranted.

Despite these limitations, the results of this study have important clinical and conceptual implications. We show that long-term exercise from adulthood to old age appears to be an important non-pharmacological intervention that clearly induces a health profile of disease-, frailty-, and mortality-relevant emergent biomarkers. Moreover, long-term training seems to improve the inflammatory–endocrine imbalance in elderly men. Future investigations should study a large long-term exercised sample to corroborate present results.

Acknowledgments Part of this investigation has been awarded with the second place in XIII National Sport Medicine Research Award 2010 from Spain. The authors thank the Residencia Santa Teresa personnel for their excellent work. We also want to thank the Hospital Monte Naranco. We are part of the INPROTEOLYS group and national net RETICEF for Aging Studies. This work was partly supported by the following grants: FISS-06-RD06/0013/0011 from the Instituto Carlos III, INIA-070RTA2007-00087-C02-02 from the Ministerio de Agricultura and FEDER funds, and FICYT IB09-134 from the Gobierno del Principado de Asturias. David de Gonzalo Calvo is a FICYT pre-doctoral fellow from the Gobierno del Principado de Asturias. Marina García-Macia is a FISS pre-doctoral fellow from Instituto de Salud Carlos III.

References

- Aldred S, Rohalu M, Edwards K, Burns V (2009) Altered DHEA and DHEAS response to exercise in healthy older adults. *J Aging Phys Act* 17(1):77–88
- Bar-Shai M, Carmeli E, Ljubuncic P, Reznick AZ (2008) Exercise and immobilization in aging animals: the involvement of oxidative stress and NF-kappaB activation. *Free Radic Biol Med* 44(2):202–214
- Beavers KM, Brinkley TE, Nicklas BJ (2010a) Effect of exercise training on chronic inflammation. *Clin Chim Acta* 411(11–12):785–793
- Beavers KM, Hsu FC, Isom S, Kritchevsky SB, Church T, Goodpaster B, Pahor M, Nicklas BJ (2010b) Long-term physical activity and inflammatory biomarkers in older adults. *Med Sci Sports Exerc* 42(12):2189–2196
- Bonanini M, Veronesi L, Colucci ME, Guidotti R, Tanzi ML (2008) Oral health conditions and systemic diseases prevalence in long term institutionalized elderly. A cross sectional study in Parma province. *Ig Sanità Pubbl* 64 (2):149–161
- Brunsgaard H (2005) Physical activity and modulation of systemic low-level inflammation. *J Leukoc Biol* 78 (4):819–835
- Brunsgaard H, Andersen-Ranberg K, Jeune B, Pedersen AN, Skinhoj P, Pedersen BK (1999) A high plasma concentration of TNF-alpha is associated with dementia in centenarians. *J Gerontol A Biol Sci Med Sci* 54(7):M357–M364
- Brunsgaard H, Ladelund S, Pedersen AN, Schroll M, Jorgensen T, Pedersen BK (2003) Predicting death from tumour necrosis factor-alpha and interleukin-6 in 80-year-old people. *Clin Exp Immunol* 132(1):24–31
- Byberg L, Melhus H, Gedeberg R, Sundstrom J, Ahlbom A, Zethelius B, Berglund LG, Wolk A, Michaelsson K (2009) Total mortality after changes in leisure time physical activity in 50 year old men: 35 year follow-up of population based cohort. *Br J Sports Med* 43(7):482
- Cappola AR, Xue QL, Ferrucci L, Guralnik JM, Volpato S, Fried LP (2003) Insulin-like growth factor I and interleukin-6 contribute synergistically to disability and mortality in older women. *J Clin Endocrinol Metab* 88 (5):2019–2025
- Chodzko-Zajko WJ, Proctor DN, Fiatarone Singh MA, Minson CT, Nigg CR, Salem GJ, Skinner JS (2009) American college of sports medicine position stand. Exercise and physical activity for older adults. *Med Sci Sports Exerc* 41 (7):1510–1530
- Chung HY, Cesari M, Anton S, Marzetti E, Giovannini S, Seo AY, Carter C, Yu BP, Leeuwenburgh C (2009) Molecular inflammation: underpinnings of aging and age-related diseases. *Ageing Res Rev* 8(1):18–30
- Clayton PE, Banerjee I, Murray PG, Renehan AG (2010) Growth hormone, the insulin-like growth factor axis, insulin and cancer risk. *Nat Rev Endocrinol*
- Colbert LH, Visser M, Simonsick EM, Tracy RP, Newman AB, Kritchevsky SB, Pahor M, Taaffe DR, Brach J, Rubin S, Harris TB (2004) Physical activity, exercise, and inflammatory markers in older adults: findings from the health, aging and body composition study. *J Am Geriatr Soc* 52 (7):1098–1104
- Cotman CW, Berchtold NC, Christie LA (2007) Exercise builds brain health: key roles of growth factor cascades and inflammation. *Trends Neurosci* 30(9):464–472
- de Gonzalo-Calvo D, Neitzert K, Fernandez M, Vega-Naredo I, Caballero B, Garcia-Macia M, Suarez FM, Rodriguez-Colunga MJ, Solano JJ, Coto-Montes A (2010) Differential inflammatory responses in aging and disease: TNF-alpha and IL-6 as possible biomarkers. *Free Radic Biol Med* 49(5):733–737
- de Gonzalo-Calvo D, Neitzert K, Fernandez M, Vega-Naredo I, Caballero B, Garcia-Macia M, Suarez FM, Rodriguez-Colunga MJ, Solano JJ, Coto-Montes A (2011) Defective adaption of erythrocytes during acute hypoxia injury in an elderly population. *J Gerontol A Biol Sci Med Sci* 66 (4):376–384
- de Lemos JA, Morrow DA, Sabatine MS, Murphy SA, Gibson CM, Antman EM, McCabe CH, Cannon CP, Braunwald E (2003) Association between plasma levels of monocyte chemo-attractant protein-1 and long-term clinical outcomes in patients with acute coronary syndromes. *Circulation* 107(5):690–695
- De Martinis M, Franceschi C, Monti D, Ginaldi L (2006) Inflammation markers predicting frailty and mortality in the elderly. *Exp Mol Pathol* 80(3):219–227
- Elosua R, Bartali B, Ordovas JM, Corsi AM, Lauretani F, Ferrucci L (2005) Association between physical activity, physical performance, and inflammatory biomarkers in an elderly population: the InCHIANTI study. *J Gerontol A Biol Sci Med Sci* 60(6):760–767
- Frost RA, Lang CH (2003) Regulation of insulin-like growth factor-I in skeletal muscle and muscle cells. *Minerva Endocrinol* 28(1):53–73
- Gielen S, Adams V, Mobius-Winkler S, Linke A, Erbs S, Yu J, Kempf W, Schubert A, Schuler G, Hambrecht R (2003) Anti-inflammatory effects of exercise training in the skeletal muscle of patients with chronic heart failure. *J Am Coll Cardiol* 42(5):861–868
- Giuliani N, Sansoni P, Girasole G, Vescovini R, Passeri G, Passeri M, Pedrazzoni M (2001) Serum interleukin-6, soluble interleukin-6 receptor and soluble gp130 exhibit different patterns of age- and menopause-related changes. *Exp Gerontol* 36(3):547–557
- Goldhammer E, Tanchilevitch A, Maor I, Beniamini Y, Rosenschein U, Sagiv M (2005) Exercise training modulates cytokines activity in coronary heart disease patients. *Int J Cardiol* 100 (1):93–99

- Gray SR, Clifford M, Lancaster R, Leggate M, Davies M, Nimmo MA (2009) The response of circulating levels of the interleukin-6/interleukin-6 receptor complex to exercise in young men. *Cytokine* 47(2):98–102
- Grundy SM, Cleeman JJ, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC Jr, Spertus JA, Costa F (2005) Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation* 112(17):2735–2752
- Hotamisligil GS (1999) The role of TNF α and TNF receptors in obesity and insulin resistance. *J Intern Med* 245(6):621–625
- Hsu FC, Kritchevsky SB, Liu Y, Kanaya A, Newman AB, Perry SE, Visser M, Pahor M, Harris TB, Nicklas BJ (2009) Association between inflammatory components and physical function in the health, aging, and body composition study: a principal component analysis approach. *J Gerontol A Biol Sci Med Sci* 64(5):581–589
- Jankord R, Jemiole B (2004) Influence of physical activity on serum IL-6 and IL-10 levels in healthy older men. *Med Sci Sports Exerc* 36(6):960–964
- Ji LL, Dickman JR, Kang C, Koenig R (2010) Exercise-induced hormesis may help healthy aging. *Dose Response* 8(1):73–79
- Joseph C, Kenny AM, Taxel P, Lorenzo JA, Duque G, Kuchel GA (2005) Role of endocrine-immune dysregulation in osteoporosis, sarcopenia, frailty and fracture risk. *Mol Aspects Med* 26(3):181–201
- Juge-Aubry CE, Somme E, Giusti V, Pernin A, Chicheportiche R, Verdumo C, Rohner-Jeanrenaud F, Burger D, Dayer JM, Meier CA (2003) Adipose tissue is a major source of interleukin-1 receptor antagonist: upregulation in obesity and inflammation. *Diabetes* 52(5):1104–1110
- Kallen KJ (2002) The role of transsignalling via the agonistic soluble IL-6 receptor in human diseases. *Biochim Biophys Acta* 1592(3):323–343
- Karolkiewicz J, Michalak E, Pospieszna B, Deskur-Smielecka E, Nowak A, Pilaczynska-Szczesniak L (2009) Response of oxidative stress markers and antioxidant parameters to an 8-week aerobic physical activity program in healthy, postmenopausal women. *Arch Gerontol Geriatr* 49(1):e67–e71
- Leng SX, McElhaney JE, Walston JD, Xie D, Fedarko NS, Kuchel GA (2008) ELISA and multiplex technologies for cytokine measurement in inflammation and aging research. *J Gerontol A Biol Sci Med Sci* 63(8):879–884
- Maggio M, Cappola AR, Ceda GP, Basaria S, Chia CW, Valenti G, Ferrucci L (2005) The hormonal pathway to frailty in older men. *J Endocrinol Invest* 28(11 Suppl Proceedings):15–19
- Maggio M, Guralnik JM, Longo DL, Ferrucci L (2006) Interleukin-6 in aging and chronic disease: a magnificent pathway. *J Gerontol A Biol Sci Med Sci* 61(6):575–584
- Maggio M, Lauretani F, Ceda GP, Bandinelli S, Ling SM, Metter EJ, Artoni A, Carassale L, Cazzato A, Ceresini G, Guralnik JM, Basaria S, Valenti G, Ferrucci L (2007) Relationship between low levels of anabolic hormones and 6-year mortality in older men: the aging in the Chianti area (InCHIANTI) study. *Arch Intern Med* 167(20):2249–2254
- Maggio M, Cattabiani C, Lauretani F, Ferrucci L, Luci M, Valenti G, Ceda G (2010) The concept of multiple hormonal dysregulation. *Acta Biomed* 81(Suppl 1):19–29
- Mahoney FI, Barthel DW (1965) Functional Evaluation: the Barthel Index. *Md State Med J* 14:61–65
- Memoli B, Grandaliano G, Soccio M, Postiglione L, Guida B, Bisesti V, Esposito P, Procino A, Marrone D, Michael A, Andreucci M, Schena FP, Pertosa G (2005) In vivo modulation of soluble "antagonistic" IL-6 receptor synthesis and release in ESRD. *J Am Soc Nephrol* 16(4):1099–1107
- Michishita R, Shono N, Inoue T, Tsuruta T, Node K (2010) Effect of exercise therapy on monocyte and neutrophil counts in overweight women. *Am J Med Sci* 339(2):152–156
- Nicklas BJ, Brinkley TE (2009) Exercise training as a treatment for chronic inflammation in the elderly. *Exerc Sport Sci Rev* 37(4):165–170
- Nielsen AR, Mounier R, Plomgaard P, Mortensen OH, Penkowa M, Speersneider T, Pilegaard H, Pedersen BK (2007) Expression of interleukin-15 in human skeletal muscle effect of exercise and muscle fibre type composition. *J Physiol* 584(Pt 1):305–312
- Niu J, Azfer A, Zhelyabovska O, Fatma S, Kolattukudy PE (2008) Monocyte chemotactic protein (MCP)-1 promotes angiogenesis via a novel transcription factor, MCP-1-induced protein (MCPIP). *J Biol Chem* 283(21):14542–14551
- O'Connor JC, McCusker RH, Strle K, Johnson RW, Dantzer R, Kelley KW (2008) Regulation of IGF-I function by proinflammatory cytokines: at the interface of immunology and endocrinology. *Cell Immunol* 252(1–2):91–110
- Pedersen BK (2009) The diseases of physical inactivity—and the role of myokines in muscle—fat cross talk. *J Physiol* 587(Pt 23):5559–5568
- Pedersen BK, Febbraio MA (2008) Muscle as an endocrine organ: focus on muscle-derived interleukin-6. *Physiol Rev* 88(4):1379–1406
- Petersen AM, Pedersen BK (2005) The anti-inflammatory effect of exercise. *J Appl Physiol* 98(4):1154–1162
- Polak J, Klimcakova E, Moro C, Viguier N, Berlan M, Hejnova J, Richteroval B, Kraus I, Langin D, Stich V (2006) Effect of aerobic training on plasma levels and subcutaneous abdominal adipose tissue gene expression of adiponectin, leptin, interleukin 6, and tumor necrosis factor alpha in obese women. *Metabolism* 55(10):1375–1381
- Schrager MA, Metter EJ, Simonsick E, Ble A, Bandinelli S, Lauretani F, Ferrucci L (2007) Sarcopenic obesity and inflammation in the InCHIANTI study. *J Appl Physiol* 102(3):919–925
- Stowe RP, Peek MK, Cutchin MP, Goodwin JS (2010) Plasma cytokine levels in a population-based study: relation to age and ethnicity. *J Gerontol A Biol Sci Med Sci* 65(4):429–433
- Suh Y, Atzmon G, Cho MO, Hwang D, Liu B, Leahy DJ, Barzilai N, Cohen P (2008) Functionally significant insulin-like growth factor I receptor mutations in centenarians. *Proc Natl Acad Sci USA* 105(9):3438–3442
- Tiainen K, Hurme M, Hervonen A, Luukkaala T, Jylha M (2010) Inflammatory markers and physical performance

- among nonagenarians. *J Gerontol A Biol Sci Med Sci* 65 (6):658–663
- Tissandier O, Peres G, Fiet J, Piette F (2001) Testosterone, dehydroepiandrosterone, insulin-like growth factor 1, and insulin in sedentary and physically trained aged men. *Eur J Appl Physiol* 85(1–2):177–184
- Topinkova E (2008) Aging, disability and frailty. *Ann Nutr Metab* 52(Suppl 1):6–11
- Troscid M, Lappegard KT, Claudi T, Damas JK, Morkrid L, Brendberg R, Mollnes TE (2004) Exercise reduces plasma levels of the chemokines MCP-1 and IL-8 in subjects with the metabolic syndrome. *Eur Heart J* 25(4):349–355
- Urso ML, Pierce JR, Alemany JA, Harman EA, Nindl BC (2009) Effects of exercise training on the matrix metalloproteinase response to acute exercise. *Eur J Appl Physiol* 106(5):655–663
- Wannamethee SG, Lowe GD, Whincup PH, Rumley A, Walker M, Lennon L (2002) Physical activity and hemostatic and inflammatory variables in elderly men. *Circulation* 105 (15):1785–1790
- Wardyn GG, Rennard SI, Brusnahan SK, McGuire TR, Carlson ML, Smith LM, McGranaghan S, Sharp JG (2008) Effects of exercise on hematological parameters, circulating side population cells, and cytokines. *Exp Hematol* 36(2):216–223
- You T, Berman DM, Ryan AS, Nicklas BJ (2004) Effects of hypocaloric diet and exercise training on inflammation and adipocyte lipolysis in obese postmenopausal women. *J Clin Endocrinol Metab* 89(4):1739–1746