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RESEARCH ARTICLE

Circulating levels of adipokines and IGF-1 are associated with skeletal muscle strength of young and old healthy subjects

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Abstract It is known that adipose tissue mass increases with age, and that a number of hormones, collectively called adipokines, are produced by adipose tissue. For most of them it is not known whether their plasmatic levels change with age. Moreover, it is known that adipose tissue infiltration in skeletal muscle is related to sarcopenia and loss of muscle strength. In this study we investigated the age-related changes of representative adipokines and insulin-like growth factor (IGF)-1 and their effect on muscle strength. We studied the association between circulating levels of adiponectin, leptin, resistin and IGF-1 and muscle strength. This cross-sectional study included 412 subjects of different age (152 subjects aged 18–30 years and 260 subjects aged 69–81 years) recruited within the framework of the European research network project "Myoage". The levels of adiponectin (both in male and female subjects) and leptin (only in males) were significantly higher in old subjects compared to young, while those of IGF-1 were lower in old subjects. In old subjects adiponectin, resistin and the resistin/IGF-1 ratio (but not IGF-1 alone) were inversely associated with quadriceps torque, while only adiponectin was inversely associated with handgrip strength

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independently from percentage of fat mass, height, age, gender and geographical origin. The ratio of leptin to adiponectin was directly associated with handgrip strength in both young and old subjects. These results suggest that in humans the age-associated loss of strength is associated with the levels of representative adipokines and IGF-1.

Keywords Muscle aging · Adipokines · IGF-1 · Muscle strength · Sarcopenia · Loss of strength

Introduction

The decrease in muscle mass, strength and power that occurs with aging, defined as sarcopenia, leads to a progressive loss of mobility and dependence, with social and economic consequences for the society (Mitchell et al. 2012; Janssen et al. 2004). Several factors intervene in the etiology of muscle atrophy and weakness such as denervation, chronic inflammation, hormonal and nutritional changes, and modification in life-style (Morley 2012). It is known that adipose tissue mass increases with age, and recently it has been observed that infiltration of muscle with fat tissue is strongly associated with the decrease of muscle strength (Koster et al. 2011; Marcus et al. 2012). White adipose tissue (WAT) is considered nowadays as an endocrine organ able to produce and secrete several hormones known as adipokines. Among these, adiponectin, leptin and resistin emerged as important modulators of glucose and lipid metabolism, and inflammation (Harwood 2012; Fantuzzi 2005) and have also effects on muscle tissue (Dyck 2009). In particular, adiponectin and leptin can increase insulin sensitivity by inducing the uptake of glucose and fatty acid oxidation in skeletal muscle increasing AMPK activity (Hajer et al. 2008; Long and Zierath 2006). Adiponectin is described mainly as an anti-inflammatory agent while leptin is considered a pro-inflammatory one (Fantuzzi 2005, 2009). Obese subjects are characterized by high levels of leptin (Fried et al. 2000) and by skeletal muscle leptin resistance (Steinberg et al. 2002), moreover leptin might be an independent risk factor for coronary heart disease (Wallace et al. 2001).

Resistin is a protein that is expressed and secreted from adipocytes, is present in the circulation and is likely to play a role in modulating metabolism and energy balance. Resistin has been proposed to be a potential link between obesity and insulin resistance (Steppan et al. 2001). The physiological role of resistin in altering muscle lipid metabolism is not clear, but it has been shown to impair insulin signaling and consequently, insulin stimulated glucose uptake in muscle (Jørgensen et al. 2009). Insulin-like growth factor (IGF)-1, the major mediator of growth hormone (GH) action, is a potent anabolic hormone mediating muscle growth and regeneration. The age-dependent decrease of IGF-1 may be associated with age-related changes in body composition (Giovannini et al. 2008). It has been previously demonstrated that IGF-1 is an independent predictor of muscle function in subjects displaying low levels of interleukin-6 and that polymorphic variants of IGF-1 receptor affect IGF-1 levels (Barbieri et al. 2003; Bonafè et al. 2003). Recently Vitale et al. (2012) showed that centenarians' offspring had lower IGF-1 levels and bioactivity, and that bioactivity was inversely related to insulin sensitivity supporting a role in the human aging process.

The aim of the present study was to investigate the age-associated changes in the circulating levels of the representative adipokines adiponectin, leptin and resistin, next to IGF-1 and their possible correlation with muscle strength in healthy subjects without mobility limitations.

Materials and methods

Study design and participants

The MYOAGE study is a cross-sectional European research network study of young (aged 18–30 years) and relatively healthy old subjects (aged 69–81 years).

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A detailed description of the study design and physiological evaluation has been provided elsewhere in this Journal Edition (McPhee et al. 2013). Participants were recruited by focused advertisement in newspapers, the third generation university, association of emeriti and universities, which allowed us to select cognitively active subjects. In total, 461 participants were included consisting of 110 recruited in Leiden, the Netherlands, 105 in Jyväskylä, Finland, 100 in Tartu, Estonia, 62 in Paris, France, and 84 in Manchester, United Kingdom. In this study, 412 participants were included, with available data for the analysis of adipokines and IGF-1.

Exclusion criteria were used to ensure the selection of healthy participants and minimize the confounding effect of comorbidity on sarcopenia. In short, exclusion criteria were: dependent living situation, unable to walk a distance of 250 m, presence of morbidity (neurologic disorders, metabolic diseases, rheumatic diseases, recent malignancy, heart failure, severe chronic obstructive pulmonary disease (COPD), haemocoagulative syndromes), use of medication (immunosuppressive drugs, insulin), immobilization for 1 week during last 3 months, and orthopedic surgery during the last 2 years or still causing pain or functional limitation.

Measurements were performed according to unified standard operating procedures during visits to the local study centers. The local medical ethical committees of the respective institutions approved the study. Written informed consent was obtained from all subjects.

Subjects characteristics

Standing height and weight was measured for each participant. Information about lifestyle factors such as smoking, alcohol use, living status, and education were self-reported using a questionnaire. Smoking was defined as current smoking. Excessive alcohol use was defined as more than 21 units per week for men, or more than 14 units per week for women. Diseases were registered and categorized into cardiovascular disease (including cardiovascular events, arterial surgery, and hypertension), non insulin dependent diabetes mellitus, mild COPD, thyroid disease, and osteoarthritis. The sum score of diseases including these diseases was calculated. The use of medication was registered and a sum score of all oral and inhaled medication was calculated.

Muscle strength

Isometric knee extension torque was measured with a knee extension dynamometer (Netherlands: Forcelink B. V., Culemborg, the Netherlands; Finland: custom made; Estonia: custom made; France: Biodex system 3 Pro isokinetic dynamometer, Biodex Medical Systems, Shirley, New York, USA; UK: custom made). Common torque calibrations were obtained across the testing laboratories to ensure that the same torque reading in response to standard loads could be obtained. The participants were seated in an upright position, with straps to fix the hips to the chair and the ankle to a force or torque transducer. The knee angle was set at 90°. Lever arm length was recorded as the distance between the knee axis of rotation and the point of force application against the transducer. After three warm up trials at 50 and 90 % of self-perceived maximal strength, three trials were conducted to measure maximal voluntary contraction (MVC) force of the knee extension muscles. For each attempt, maximal force or torque was recorded by the transducer and saved on the computer. Each trial was separated by 1 min of rest. Knee extension torque was obtained either directly or by multiplying recorded peak force with the lever arm length. The trial with the highest torque output was taken for analyses (Lauretani et al. 2003).

Handgrip strength was measured using the Jamar dynamometer handle (Sammons Preston Inc, Bolingbrook, IL). The width of the dynamometer was adjusted for each participant separately for optimal fit. Participants were instructed to stand upright and with the dynamometer besides but not against their body. For both hands the strength was measured three times and recorded in kilograms. The best of all attempts was used for further analysis (Taekema et al. 2010).

Dual-energy X-ray absorptiometry

A whole body scan to detect fat body mass was performed using Dual-energy X-ray absorptiometry (DXA) (Netherlands: Hologic QDR 4500, version 12.4, Hologic Inc., Bedford, USA; Finland: Lunar Prodigy, version EnCore 9.30; Estonia: Lunar Prodigy Advanced, version EnCore 10.51.006; France: Lunar Prodigy, version EnCore 12.30; United Kingdom: Lunar Prodigy Advance, version EnCore 10.50.086).

During the measurements a light cotton shirt was worn by the participants, reducing measurement errors due to clothing absorption. A trained technician performed the dual-energy X-ray absorptiometry. Fat mass percentage was calculated as fat mass divided by body mass in percentage.

Laboratory measurements

Overnight fasting blood samples were obtained in the morning. Plasma and serum samples were obtained following a centrifugation for 15 min at 2,750×*g* at 4 °C. Aliquots of both plasma and serum were rapidly frozen and stored at −80 °C until determination.

Plasma adiponectin, leptin, and resistin, and serum IGF-1 were determined using a commercial ELISA kit (Quantikine® R&D Systems, Minneapolis, USA) according to the manufacturer's instructions. In all the samples, adiponectin, leptin, resistin, IGF-1 and insulin were measured in duplicate and final data were obtained in a blind set up by the operators. Leptin/adiponectin ratio was calculated by dividing the value of the concentration of leptin with that of adiponectin (in ng/ml) for each subject. Value of this ratio was multiplied for 1,000 (Ostan et al. 2012). Resistin/IGF-1 was calculated in accordance to previous study (Ostan et al. 2012).

Statistical analyses

Continuous variables with Gaussian distribution are presented as mean (standard deviation) and those with non-Gaussian distribution as median (interquartile range (IQR)). The differences between male and female subjects (stratified for age) as well as the differences between young and old subjects (for the whole group and stratified for gender) were tested by independent sample *t* test and Mann–Whitney *U* test for normally and not-normally distributed data. χ^2 test was used to analyze the distribution of categorical variables.

Results from the different countries were first analyzed separately, and were pooled if the effect sizes were comparable. In pooled analyses, quadriceps torque, handgrip strength, and fat percentage were standardized into country specific *z* scores, to minimize possible effects due to differences in equipment. Not normally distributed data were log transformed and the log transformed values were used in the regression model.

Linear regression analyses were used to identify associations of adipokines and IGF-1 with muscle strength, including adjustments for age, sex, fat percentage, height and geographical origin (Country). Correlation between circulating levels of adiponectin, leptin, resistin and *z* scores of fat percentage was calculated by Pearson's or Spearman's correlation where appropriate.

SPSS 17.0 for Windows was used for all analyses. *P* values <0.05 were considered statistically significant.

Results

The study was conducted in a total of 412 European subjects: 152 young subjects (74 males and 78 females, mean age ± SD: 23.3 ± 4 years) and 260 old subjects (128 males and 132 females, mean age ± SD: 74 ± 5.5 years). In Table 1 the baseline characteristics of the study population are reported. Old subjects (both males and females) had a significantly higher body mass index and fat percentage and a significantly reduced handgrip strength and quadriceps torque compared to young subjects. Plasma levels of adiponectin were higher in old subjects, both in males and in females while plasma levels of leptin were higher in old males when compared to young. IGF-1 was lower in old subjects as compared to young (both males and females). Plasma levels of adiponectin and leptin and total fat mass (kg and %) were significantly higher in female compared to male subjects, while handgrip and quadriceps strength were lower in women with respect to men, as expected (Table 1).

Resistin did not change significantly between young and old. According to previous studies, the ratio between leptin and adiponectin as well as resistin and IGF-1 are associated with metabolic syndrome in old people (Ostan et al. 2012), therefore we analyzed these ratios in our population. Leptin/adiponectin ratio was not different between young and old subjects, while resistin/IGF-1 ratio was significantly higher in old subjects (both in male and in female subjects), due to a lower level of IGF-1 in old subjects (Table 1). Adipokines were correlated with the percentage of fat mass and in particular, leptin was strongly associated with fat percentage in all age and gender groups

Table 1 Characteristics of the study population

	Total, $N = 412$			Men, $N = 202$			Women, $N = 210$		
	Young, $N = 152$	Old, $N = 260$	P value	Young, $N = 74$	Old, $N = 128$	P value	Young, $N = 78$	Old, $N = 132$	P value
Age, years	23.25 (3.95)	73.95 (5.50)	**<0.001**	23.76 (2.75)	74.11 (5.50)	**<0.001**	22.64 (3.53)	73.54 (5.59)	**<0.001**
Height, m[a]	1.74 (0.09)	1.67 (0.09)	**<0.001**	1.81 (0.06)	1.74 (0.06)	**<0.001**	1.68 (0.06)[#]	1.61 (0.07)[#]	**<0.001**
Current Smoking, *n* (%)	18 (11.8)	12 (4.6)	**0.006**	9 (12.2)	8 (6.2)	0.150	9 (11.5)	4 (3.0)	**0.013**
Alcohol use, *n* (%)[b]	31 (20.4)	29 (11.2)	**0.010**	19 (25.7)	19 (14.8)	0.058	12 (15.4)	10 (7.6)	0.074
Number of diseases	0 (0–0)	1 (0–1)	**0.001**	0 (0–0)	1 (0–1)	**<0.001**	0 (0–0)	1 (0–1)	**<0.001**
Number of medication	0 (0–1)	1 (0–3)	**0.001**	0 (0–0)	1 (0–3)	**<0.001**	0 (0–1)	1 (0–3)	**<0.001**
Weight, kg[a]	69.47 (11.81)	71.89 (12.56)	0.054	75.91 (10.71)	78.70 (11.31)	0.087	63.36 (9.34)[#]	65.33 (14.28)[#]	0.156
BMI, kg/m^{2a}	22.88 (2.94)	25.55 (3.31)	**<0.001**	23.24 (2.80)	25.89 (3.04)	**<0.001**	22.53 (3.04)	25.21 (3.53)	**<0.001**
Handgrip, kg	40.00 (19.20)	32.00 (13.50)	**<0.001**	52.00 (11.70)	39.45 (10.38)	**<0.001**	33.25 (7.50)[#]	26.20 (7.10)[#]	**<0.001**
Qudriceps torque best effort, N/m	189.70 (84.75)	118.50 (59.50)	**<0.001**	230.50 (84.66)	148.89 (47.00)	**<0.001**	151.75 (48.83)[#]	94.00 (31.24)[#]	**<0.001**
Total fat mass, kg[a]	16.25 (6.78)	21.61 (7.38)	**<0.001**	13.22 (5.67)	19.52 (7.39)	**<0.001**	19.12 (6.51)[#]	22.88 (7.16)[#]	**<0.001**
Total fat mass, %[a]	23.50 (8.94)	30.00 (8.00)	**<0.001**	16.95 (6.00)	25.35 (6.41)	**<0.001**	29.72 (6.50)[#]	34.44 (6.75)[#]	**<0.001**
Adiponectin, μg/ml	8.35 (6.86)	12.37 (11.32)	**<0.001**	6.06 (6.37)	9.25 (6.98)	**<0.001**	10.31 (6.49)[#]	16.95 (11.38)[#]	**<0.001**
Leptin, ng/ml[a]	10.22 (11.24)	12.51 (12.66)	0.068	3.79 (4.40)	6.92 (6.25)	**<0.001**	16.16 (12.34)[#]	17.63 (14.75)[#]	0.460
Leptin/Adiponectin ratio[ac]	1.42 (1.87)	1.61 (4.90)	0.658	0.74 (1.10)	1.50 (5.44)	0.271	2.01 (2.22)[#]	1.71 (4.37)	0.563
Resistin, ng/ml[a]	6.95 (2.54)	7.07 (3.00)	0.692	6.83 (1.92)	7.07 (3.00)	0.539	7.07 (3.01)	7.07 (3.04)	0.997
IGF-1, ng/ml	156.15 (87.95)	83.15 (45.20)	**<0.001**	154.90 (82.09)	82.00 (51.48)	**<0.001**	159.40 (85.30)	83.30 (41.88)	**<0.001**
Resistin/IGF-1 ratio	0.04 (0.03)	0.08 (0.05)	**<0.001**	0.06 (0.05)	0.09 (0.05)	**<0.001**	0.04 (0.03)	0.08 (0.05)	**<0.001**

All data are expressed as median and interquartile range (IQR) in parenthesis. P values are derived from Mann–Whitney U test or independent sample t test for not normally and normally distributed variables and from Chi square test for categorical variables

Significant values are highlighted in bold

n Number, % percentage, *N* Newton

[#] $P < 0.001$ between men and women

[a] Data are expressed as mean and standard deviation (SD) in parenthesis

[b] Excessive alcohol used defined as for males > 21 units/week and females > 14 units/week

[c] Values were multiplied for 1,000

(Fig. 1). Adiponectin but not resistin was inversely correlated with the percentage of fat mass in old female subjects (Fig. 1).

The association of the above mentioned circulating mediators with quadriceps and handgrip strength is reported in Table 2 and 3 respectively. In young

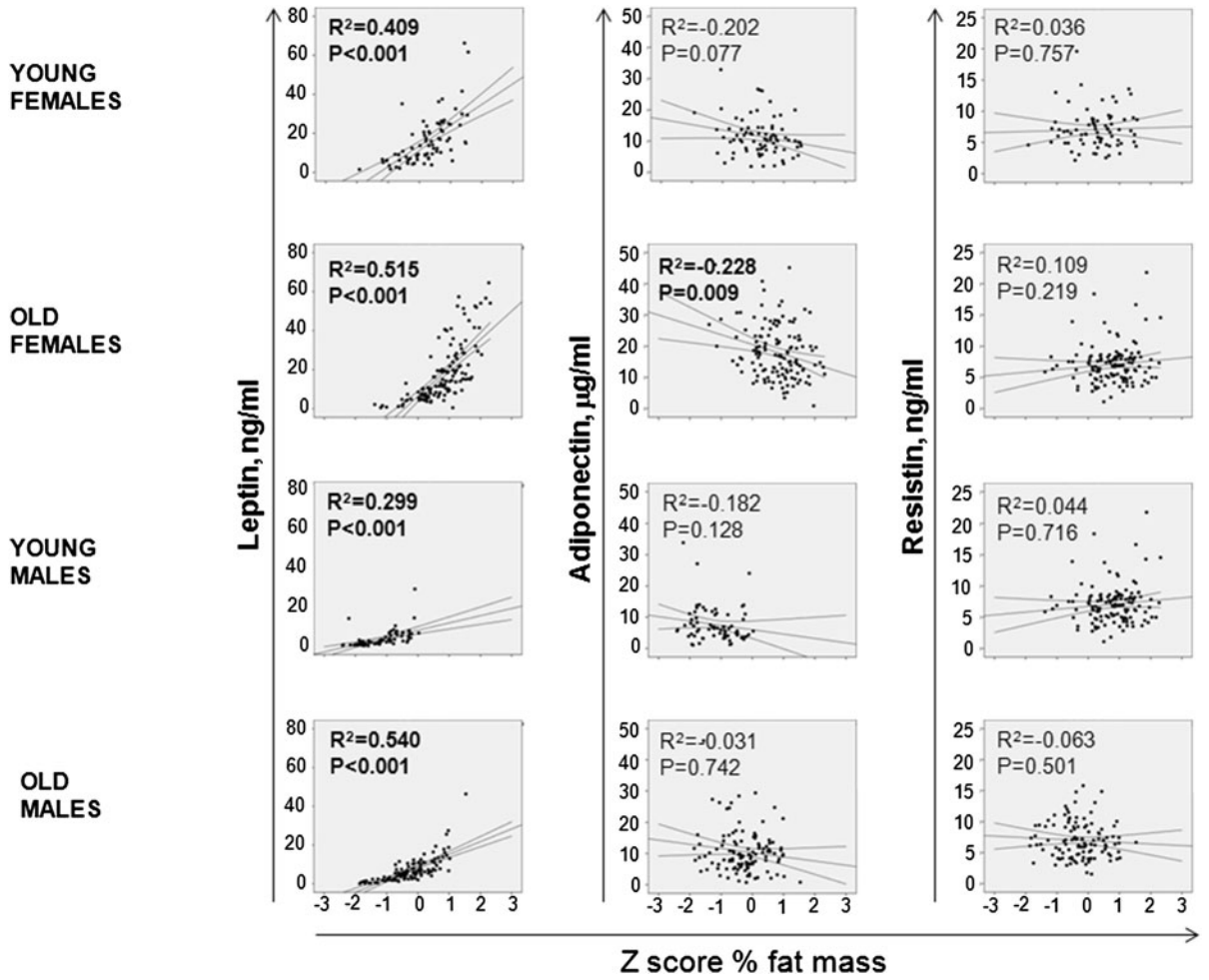

Fig. 1 Correlation analysis of adiponectin, leptin and resistin with z scores % fat mass in all subjects according to age and gender. R^2 was derived from Pearson or Spearman correlation

subjects none of the mediators were significantly associated with quadriceps torque except for the leptin/adiponectin ratio that was positively and significantly correlated. In old subjects we observed a significant inverse association of adiponectin, resistin, resistin/IGF-1 ratio with quadriceps torque (Table 2). Since adiponectin and leptin are known to display a gender dimorphism, we performed the same association analyses considering male and female subjects separately. In young subjects, none of the mediators were associated with quadriceps torque in men, while in women only the leptin/adiponectin ratio was positively associated (Table 2). In old males, there was a positive association between IGF-1 and quadriceps torque and a negative association of resistin/IGF-1 ratio and adiponectin with quadriceps torque. In old females only adiponectin was negatively associated with quadriceps force (Table 2).

Considering the association of the same circulating mediators with handgrip strength we noticed that the leptin/adiponectin ratio was significantly and positively associated with handgrip in both age groups while adiponectin showed a negative association with handgrip strength only in old subjects (Table 3). When considering genders, in young subjects a positive association was found for the leptin/adiponectin ratio and muscle strength in females, while in old subjects it was found for males (Table 3). Moreover, adiponectin

Table 2 Regression analyses showing the association of leptin, adiponectin, resistin, IGF-1, leptin/adiponectin and resistin/IGF-1 ratios with quadriceps torque in all young and old subjects and according to gender

	Young					
	All, N = 152		Males, N = 74		Females, N = 78	
	β coefficient (95 % CI)	*P* value	β coefficient (95 % CI)	*P* value	β coefficient (95 % CI)	*P* value
Leptin, ng/ml	0.01 (−0.00 to 0.03)	0.078	0.04 (−0.02 to 0.10)	0.162	0.00 (−0.01 to 0.01)	0.880
Adiponectin, μg/ml*	−0.25 (−0.67 to 0.16)	0.224	−0.29 (−0.98 to 0.40)	0.405	−0.35 (−0.79 to 0.09)	0.116
Leptin/Adiponectin ratio	**89.60 (20.71 to 158.48)**	**0.011**	176.56 (−25.65 to 378.77)	0.086	**57.21 (3.02 to 111.41)**	**0.039**
Resistin, ng/ml	0.02 (−0.02 to 0.07)	0.316	0.07 (−0.04 to 0.14)	0.515	0.01 (−0.03 to 0.05)	0.690
IGF-1, ng/ml*	−0.05 (−0.62 to 0.52)	0.862	−0.54 (−1.43 to 0.35)	0.229	0.34 (−0.33 to 1.02)	0.316
Resistin/IGF-1 ratio*	0.16 (−0.33 to 0.65)	0.511	0.47 (−0.39 to 0.27)	0.284	−0.05 (−0.55 to 0.45)	0.845
	Old					
	All, *N* = 260		Males, *N* = 128		Females, *N* = 132	
	β coefficient (95 % CI)	*P* value	β coefficient (95 % CI)	*P* value	β coefficient (95 % CI)	*P* value
Leptin, ng/ml	−0.00 (−0.01 to 0.00)	0.328	−0.00 (−0.03 to 0.02)	0.758	−0.00 (−0.01 to 0.00)	0.273
Adiponectin, μg/ml*	**−0.37 (−0.59 to −0.15)**	**0.001**	**−0.38 (−0.72 to −0.04)**	**0.030**	**−0.38 (−0.66 to −0.11)**	**0.006**
Leptin/Adiponectin ratio	7.15 (−5.61 to 19.91)	0.271	2.86 (−18.17 to 23.89)	0.788	7.71 (−7.362 to 23.04)	0.321
Resistin, ng/ml	**−0.02 (−0.05 to −0.00)**	**0.019**	−0.037 (−0.08 to 0.00)	0.058	−0.01 (−0.03 to 0.01)	0.256
IGF-1, ng/ml*	0.31 (−0.04 to 0.65)	0.083	**0.67 (0.07 to 1.26)**	**0.029**	0.02 (−0.37 to 0.41)	0.912
Resistin/IGF-1 ratio*	**−0.38 (−0.63 to −0.13)**	**0.003**	**−0.73 (−1.17 to −0.28)**	**0.002**	−0.07 (−0.33 to 0.20)	0.618

Data are presented as β coefficient (95 % CI). Adjusted for age, gender, *z* score of fat mass percentage height and country

Significant associations are highlighted in bold

* Log-transformed data

Table 3 Regression analyses showing the association of leptin, adiponectin, resistin, IGF-1, leptin/adiponectin and resistin/IGF-1 ratios with handgrip strength in all young and old subjects and according to gender

	Young					
	All, $N = 152$		Males, $N = 74$		Females, $N = 78$	
	β coefficient (95 % CI)	*P* value	β coefficient (95 % CI)	*P* value	β coefficient (95 % CI)	*P* value
Leptin, ng/ml	0.11 (−0.00 to 0.03)	0.115	0.01 (−0.50 to 0.07)	0.772	0.01 (−0.00 to 0.02)	0.169
Adiponectin, μg/ml*	−0.31 (−0.71 to 0.08)	0.119	−0.32 (−1.00 to 0.36)	0.351	−0.31 (−0.72 to 0.10)	0.133
Leptin/Adiponectin ratio	**75.31 (8.73 to 141.90)**	**0.027**	138.69 (−64.16 to 341.55)	0.177	**57.51 (7.21 to 107.81)**	**0.026**
Resistin, ng/ml	0.00 (−0.04 to 0.05)	0.840	−0.01 (−0.12 to 0.09)	0.791	0.00 (−0.03 to 0.04)	0.811
IGF-1, ng/ml*	−0.31 (−0.85 to 0.23)	0.262	−0.36 (−1.25 to 0.53)	0.417	−0.49 (−0.17 to 0.10)	0.112
Resistin/IGF-1 ratio*	0.14 (−0.33 to 0.60)	0.569	0.10 (−0.77 to 0.97)	0.819	0.23 (−0.26 to 0.67)	0.315
	Old					
	All, $N = 260$		Males, $N = 128$		Females, $N = 132$	
	β coefficient (95 % CI)	*P* value	β coefficient (95 % CI)	*P* value	β coefficient (95 % CI)	*P* value
Leptin, ng/ml	0.01 (−0.00 to 0.02)	0.069	0.02 (−0.01 to 0.05)	0.242	0.00 (−0.01 to 0.01)	0.391
Adiponectin, μg/ml*	**−0.32 (−0.57 to −0.07)**	**0.012**	−0.35 (−0.74 to 0.03)	0.069	−0.28 (−0.59 to 0.04)	0.082
Leptin/Adiponectin ratio	**25.50 (11.46–39.55)**	**<0.000**	**34.22 (11.56–56.88)**	**0.003**	10.65 (−6.55 to 27.85)	0.223
Resistin, ng/ml	−0.02 (−0.04 to 0.01)	0.133	−0.01 (−0.06 to 0.03)	0.568	−0.02 (−0.05 to 0.00)	0.059
IGF-1, ng/ml*	0.14 (−0.25 to 0.53)	0.488	0.14 (−0.55 to 0.83)	0.687	0.14 (−0.29 to 0.58)	0.517
Resistin/IGF-1 ratio*	−0.26 (−0.54 to 0.03)	0.080	−0.25 (−0.77 to 0.27)	0.342	−0.25 (−0.55 to 0.04)	0.093

Data are presented as β coefficient (95 % CI). Adjusted for age, gender, *z* score of fat mass percentage height and country

Significant associations are highlighted in bold

* Log-transformed data

showed a trend towards an inverse association with handgrip strength in both male and female old subjects; while resistin showed a borderline significant inverse association with handgrip strength only in female old subjects (Table 3).

Discussion

In this study we found out that the plasma levels of representative adipokines change with age and are associated with strength of the quadriceps and handgrip muscles. In particular, adiponectin is higher in old subjects, and leptin is higher in old males compared to young males; the levels of total adiponectin, resistin and the ratio between resistin/IGF1 are inversely associated with quadriceps strength, while levels of adiponectin are inversely correlated with handgrip strength in old subjects. Moreover, for the first time a positive association of the leptin/adiponectin ratio with muscle strength (both quadriceps torque and handgrip strength) was observed in young and old subjects.

Little is known about the precise role of circulating levels of adipose-tissue-derived hormones on muscle function. Adiponectin has been studied for its possible association with cardiovascular diseases (CVD) but contrasting data are present in the literature, since higher levels of adiponectin have been described as both risk and protective factor for CVD (Ai et al. 2011; Persson et al. 2012). Cross-sectional studies have shown that adiponectin is positively associated with insulin sensitivity measured by hyperinsulinemic clamp (Tschritter et al. 2003), and prospective studies have demonstrated that hypoadiponectinemia is associated with an increase in insulin resistance (Yamamoto et al. 2004), higher risk of developing diabetes (Duncan et al. 2004) and metabolic syndrome (Ostan et al. 2012). Little is known regarding the effects of adiponectin on muscle strength. In general it appears that adiponectin is associated with positive effects on muscle metabolism (Dyck 2009), and low levels of circulating adiponectin are associated with larger muscle fibers typical of obese subjects (Pisto et al. 2012) and that high molecular weight adiponectin is inversely associated with intramyocellular fat accumulation (Bredella et al. 2011). In our study, in old subjects adiponectin appears to be inversely associated with muscle strength (both quadriceps torque and handgrip strength) while leptin resulted not to be associated with muscle strength. Consistently, the ratio between leptin and adiponectin resulted to be positively associated with quadriceps torque (for the young group) and handgrip strength (for both age groups). Our results are in agreement with previous studies showing that circulating levels of adiponectin are inversely associated with handgrip in old people (Kizer et al. 2010) and with muscular fitness in adolescent subjects (Martinez-Gomez et al. 2012). The mechanisms by which adiponectin impact on muscle strength are at present unclear. It has been reported that adiponectin up-regulates the IRS-1/Akt signaling pathway with protective effects on muscle protein degradation (Zhou et al. 2007). Therefore, the increased amount of adiponectin found in old subjects and its inverse correlation with muscle strength appear to be paradoxical. Three possible mechanisms to solve this paradox can be hypothesized: first of all the increased adiponectin levels can be a simple mirror of an ongoing adiponectin resistance as already proposed (Kizer et al. 2010). Second, we can hypothesize that the inverse correlation with muscle strength may be mediated by adiponectin effects on adipose tissue rather than muscle fibers. Indeed, it is reported that adiponectin promotes adipogenesis (Fu et al. 2005), and, as mentioned above, the deposition of adipose tissue infiltrating muscle fibers is a leading cause of loss of muscle strength (Marcus et al. 2012). Third, it is also possible that the activation of the adiponectin downstream pathways is blunted in old subjects as observed for insulin pathway (Wilkes et al. 2009). Recent data also suggest the importance of the different molecular weight isoforms of circulating adiponectin in modulating and mediating metabolic changes in muscle and liver (Vu et al. 2007). It is possible that in the case of muscle tissue the different isoforms can play a specific role. Future studies are needed to test this hypothesis.

Resistin is expressed by monocytes and macrophages infiltrating the adipose tissue, and it has been correlated with coronary heart disease (CHD) risk, renal dysfunction and heart failure and can be considered a molecule with potent proinflammatory properties (Butler et al. 2009; Qi et al. 2008). A decline in the baseline and immediately after resistance training levels of resistin has been shown compared with pre-training in a cohort of elderly postmenopausal women indicating that periodized resistance

training can be an important intervention to reduce systemic inflammation (Prestes et al. 2009). In patients with stable CHD, elevated serum resistin levels were associated with poor exercise capacity calculated as the total number of metabolic equivalents achieved at peak exercise (Zhang et al. 2010). In vitro and animal studies suggested that in skeletal muscle resistin impairs the insulin-stimulated glucose uptake through impaired phosphorylation of IRS-1, IRS-2, AKT and AMPK leading to whole body insulin resistance. Since AMPK is a fundamental regulator of fatty acid (FA) metabolism in muscle, resistin may reduce FA oxidation leading to increased storage of cytosolic lipids (Moon et al. 2003; Pravenec et al. 2003; Satoh et al. 2004). Furthermore, resistin inhibits myogenic differentiation of myoblasts (Sheng et al. 2013). No data are available regarding the role of resistin in muscle metabolism and strength. We found that resistin is inversely associated with quadriceps strength in old subjects and especially in males. Since resistin is known to have pro inflammatory properties and impairs glucose and lipid metabolism, as discussed, it is possible that it exerts negative effects on muscle strength by modulating energy metabolism pathways at skeletal muscle level.

A recent study showed a positive association of IGF-1 with handgrip in very old women that remained even after adjusting for several confounders (Taekema et al. 2011). In our population of older men IGF-1 showed a positive association with quadriceps torque but not with handgrip strength. The discrepancy between these results and our own may be accounted for by the age of the subjects and by the different adjustments performed.

Recently, it has been shown that the prevalence of metabolic syndrome increases dramatically with the increase of resistin/IGF-1 ratio in subjects with no history of familial longevity (Ostan et al. 2012). In fact, it has been observed that IGF-1 down-regulates resistin gene expression via IGF-1R-dependent and MEK1-, p38 MAPK-, and phosphoinositide 3-kinase-independent pathways (Chen et al. 2005). The lower levels of IGF-1 observed in old subjects may lead to an over-expression of resistin by adipocytes affecting muscle glucose uptake and contributing to insulin resistance. Interestingly, in the present study the resistin/IGF-1 ratio was inversely correlated with quadriceps strength in old men but not in old women. This gender difference could be explained by the different localization and composition of adipose tissue between males and females. Indeed, males accumulate more abdominal fat that could have higher pro-inflammatory effects with respect to other fat depots, due to an increase of macrophages infiltration (Alvehus et al. 2010; Harman-Boehm et al. 2007).

As a whole, these data indicate that adiponectin, leptin (in males) and the resistin/IGF-1 ratio are higher in old subjects, while IGF-1 is lower. Moreover, adiponectin, resistin, resistin/IGF-1 ratio and IGF-1 are associated with the muscle strength in relatively healthy old subjects. The main strength of this study is that it is conducted on a representative sample of the European population recruited in 5 different countries, where the enrolled subjects were carefully selected for their health status and well characterized. This allowed us to correct the data for a number of confounding variables in particular for the fat percentage. Nevertheless, it is to note that this is a cross-sectional study, which is an obvious limitation.

Conclusions

Our data reinforce the hypothesis that age-associated sarcopenia and loss of strength are more than just a muscular deficit and should be considered a multifactorial condition in which also alterations in adipokines and IGF-1 play a role. Of note, each of the different mechanisms proposed so far to account for sarcopenia (i.e. oxidative stress, alteration in growth factors activity, increased levels of inflammatory cytokines, altered circulating levels of adiponectin, leptin, resistin) is able to induce signs of muscle aging; however none of them is able to entirely recapitulate the severity of sarcopenia. In this complex scenario, the age-related changes in the relative amount of these soluble mediators at systemic level may lead or reflect an inappropriate or altered activation of important signaling pathways affecting differently the metabolism/physiology of muscle and adipose tissues, as well as of the immune cells that infiltrate adipose tissue itself (for example pro- and anti-inflammatory macrophages).

The way in which the different age-related changes in signaling pathways synergize to trigger sarcopenia remains to be fully understood and further studies are needed to clarify the molecular mechanisms behind it.

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