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NMR based biomarkers to study age-related changes in the human quadriceps

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ABSTRACT

Age-related sarcopenia is a major health issue. To improve elderly person quality of life, it is important to characterize age-associated structural changes within the skeletal muscle. NMR imaging offers quantitative tools to monitor these changes. We scanned 93 subjects: 33 young adults aged between 19 and 27 years old and 60 older adults between 69 and 80 years old. Their physical activity was assessed using a tri-axial accelerometer and they were classified either as active or sedentary. A standard multi-slice multi-echo (MSME) sequence was run and water T2 maps were extracted using a tri-exponential fit. Fat fraction was quantified using three-point Dixon technique. Each quadriceps muscle was characterized by: water T2 mean value, water T2 heterogeneity and the mean fat fraction.

Statistical analysis (ANOVA) showed that water T2 mean values and its heterogeneity indices as well as fat fraction were significantly higher in the elderly group ($p < 0.05$). Only fat fraction was significantly lower in the active group compared to the sedentary one ($p < 0.05$). Linear regression confirmed the significant impact of age on these NMR parameters whereas physical activity impact was not systematic.

NMR imaging provided a comprehensive assessment of the aging process impact on skeletal muscle composition. Water T2 increase might be related to changes in fiber typology while increased T2 heterogeneities might correlate with some degree of tissue disorganization, like the development of interstitial fibrosis. Fat fraction and water T2 heterogeneity increase was partly slowed down by physical activity. These changes were not gender dependent.

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1. Introduction

Sarcopenia, the loss of muscle mass has become a public health problem worldwide and consequently an important research topic. Understanding age-related structural changes within the muscle is a prerequisite to develop a therapeutic scheme or slow down sarcopenia progression. Several studies have focused on muscle mass and strength evaluation (Frontera et al., 1991, 2000; Kallman et al., 1990; Thom et al., 2005) and they have shown that aging resulted in a decline of muscle performance. While these observations reflect the integrated action of several structural changes they do not explain the mechanisms of muscle wasting and weakness. Nevertheless, this was explained partly by histological analysis in which both a loss of fibers and changes in fibers type and size were reported (Frontera et al., 2000; Lexell et al., 1983, 1988).

Contrary to histology which provides a local insight of muscle changes while being invasive, quantitative NMR imaging allows the screening of large segments of the body and is more adapted to longitudinal analysis. In aging studies, NMR imaging was mainly used to assess the changes in volume and in the cross sectional area in lower limbs (Gray et al., 2011; Maden-Wilkinson et al., 2013; Morse et al., 2005; Ozaki et al., 2011) and upper limbs (Klein et al., 2002). MRI was also used to study the changes in the amount of fat with age (Buford et al., 2012; Zoico et al., 2010), although this was achieved with standard T1 imaging instead of using chemical shift based approaches (Glover and Schneider, 1991). To the best of our knowledge, very few studies on human have dealt with in vivo tissue characterization using NMR biomarkers. One study used diffusion tensor imaging to compare water diffusivity in leg muscles and showed that in the tibialis anterior muscle, the fractional anisotropy was higher in muscles of young men (Galbán et al., 2007). In addition to diffusion parameters, it is relevant to monitor T2 relaxation time because it conveys information on muscle physiological status. For instance, water T2 increases in some pathological conditions such as inflammation and hydrostatic oedema. The impact of aging on the increase of T2 relaxation time was shown in Hatakenaka et al. (2001) and Schwenzer et al. (2009) on the calf muscles.

In the present work we studied the effect of aging on the quadriceps femoris muscles with several contributions: first in addition to

assessment of water T2 relaxation time and fatty infiltration fraction we used a new biomarker that reflects the heterogeneity of the muscular tissue. Second we investigated the difference in the aging process between men and women and third we considered the effect of physical activity.

2. Material and methods

2.1. Population description

This study was approved by local ethical committee and informed consent was obtained from each volunteer. We scanned a total of 93 subjects composed of 33 young adults aged between 19 and 27 years old (19 males and 15 females) and 60 older adults with age comprised between 69 and 80 years old (31 males and 32 females).

2.2. Physical activity assessment

Physical activity was assessed using a tri-axial accelerometry-based monitor (GeneActiv. UK). The participants were instructed to maintain their usual habitual physical activity levels for the duration of monitoring. The accelerometer watches were worn on the wrist of the non-dominant arm. Subjects were instructed to wear the accelerometer(s) day and night for at least 7 consecutive days. The norm of the acceleration vector was integrated over the whole recording session and normalized by the recording duration to get a global daily physical activity indicator. The older group was divided in two sub-groups according to their physical activity measure. The physical activity measure in older volunteers was significantly lower than in the younger ones ($p < 0.001$). A threshold value was selected to be lower than 97% of younger adult physical activity measure; the older ones that had a physical activity record lower than this threshold were considered as sedentary; the others as active.

2.3. NMR data acquisition

For T2 determination, a standard multi-slice multi-echo (MSME) sequence was acquired with a TR = 3000 ms, nominal flip angles = 90° and 180°, a train of 17 echoes with TEs ranging from 9.5 ms to 161 ms with 9.5 ms echo-spacing. The field of view (FOV) was equal to 224×448 mm^2, with a pixel size of 1.4 mm^2. We acquired 11 slices of 10 mm thickness with a 25 mm inter-space.

The transmit field spatial distribution (B1+) was calculated using the actual flip angle imaging (AFI) method (Yarnykh, 2007). It was performed using two nominal excitation pulses of 60° followed by delays of TR1 and TR2 respectively, with TR2 = 5TR1 and TR1 + TR2 = 100 ms, TE = 2.75 ms, and bandwidth = 550 Hz/voxel. Optimal spoiling of transverse relaxation was ensured by using an improved RF and gradient spoiling scheme as described in Nehrke (2009), assuming an isotropic scalar water diffusion coefficient $D = 0.75$ μm^2/ms. Relevant parameters for spoiling were: diffusion damping = 0.100, and RF spoil phase increment = 129.3°. FOV was equal to $224 \times 448 \times 320$ mm^3, with voxel size of $4 \times 4 \times 10$ mm^3.

Fat quantification was obtained using a standard 3D gradient echo three-point Dixon technique (Glover and Schneider, 1991) with the following parameters: TR = 10 ms, TE1 = 2.75 ms, TE2 = 3.95 ms, TE3 = 5.15 ms and flip angle = 3°. FOV was equal to $224 \times 448 \times 320$ mm^3 with a voxel size of $1 \times 1 \times 5$ mm^3.

2.4. Data processing and NMR biomarker computation

First, all volumes were realigned and Regions of Interest (ROIs) were drawn manually according to anatomical atlases to identify the Vastus Medialis (VM), the Vastus Lateralis (VL) and the Vastus Intermedius (VI). The ROIs delineated the interior of the muscle avoiding fasciae and blood vessels. The Rectus Femoris muscle was not analyzed because it was located in a region where the B1 field was not homogeneous.

2.4.1. Muscle water T2 maps

In each pixel, the water T2 value was computed using the technique proposed in Azzabou et al. (2014). This approach determines the water T2 in fatty infiltrated tissues using a tri-exponential model. Unlike the techniques based on fat saturation, the multi-exponential model accounts for different components such as water and fat. In Fig. 1 there are examples of water T2 maps obtained in both young and older men are presented.

To characterize the muscles of each subject, we were interested in the distribution of water T2 in each muscle. Hence we computed: (i) the mean value of the water T2; (ii) the percentage of pixels with a water T2 higher than 38.8 ms. This threshold was determined according to Azzabou et al. (2014) as the mean value plus two standard deviation of water T2 values of muscles of 10 healthy volunteers; and (iii) the heterogeneity which was defined as the ratio between the standard deviation of water T2 within a muscle and its mean value.

2.4.2. Fat ratio maps

After acquiring three images according to the extended three point Dixon technique (Glover and Schneider, 1991), a signal of water and a signal of fat were computed in each voxel. The fat fraction map was generated by computing the ratio between the fat signal and the sum of water and fat signal. Fig. 2 shows fat ratio maps obtained in both a young and an older men. Each muscle was characterized by the mean value of the fat fraction map.

2.5. Statistical analysis

We used SPSS software (v.19) for statistical analysis. Repeated measure ANOVA was performed with age group and gender as between subjects variables and muscle as within subjects variable. For the older group, we also performed a repeated measure ANOVA with activity level and gender as between subject variables and muscle as within subject ones. The significance level was set to $p < 0.05$. For each muscle, we performed a multi-linear regression with the age and physical activity as parameters controlling the NMR outcome measures. These regressions were performed on each age group separately, because physical activity and age were correlated when considering both elder and younger adults ($R = 0.55$ $p < 0.001$). Besides, in this study the ages of the volunteers were clustered around two values (23 ± 3 years and 74 ± 3 years), which may enforce the effect of the long term evolution of NMR parameter on the regression curve.

3. Results

3.1. Multi-linear regression

In Table 1 we report the results obtained for the different NMR parameters in both groups. Regarding young subjects, they indicated that a simple mean model was better than the multi-linear model. They also showed that age and physical activity did not have an impact on the water T2 value as well as its coefficient of variation, nor on the fat content within the muscle.

The results for the older subjects showed that in most of the cases, the multi-linear fit worked better in estimating the NMR parameters using age and physical activity than the predictor that only computes the mean value. For instance, the water T2 values had a significant relationship with age with a positive slope for the three muscles, whereas the influence of physical activity on water T2 was negligible. The age significantly increased the ratio of pixels with high water T2 in the VM and VI muscles, whereas no significant dependence to physical activity was shown. The impact of age on the coefficient of variation was observed only for the VI muscle, with a positive slope whereas negative slope with physical activity was found in the case of VI and VM muscles.
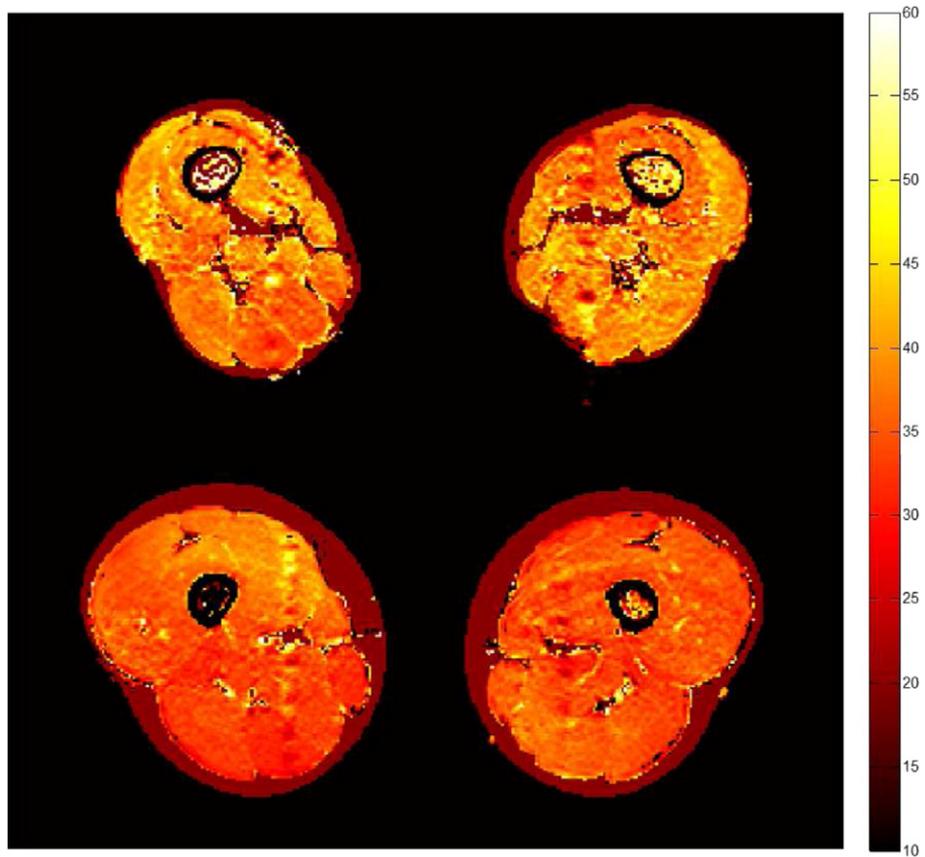
Fig. 1. Water T2 maps (ms) in an axial slice of the thigh of an older volunteer (upper line) and a young volunteer (bottom line).
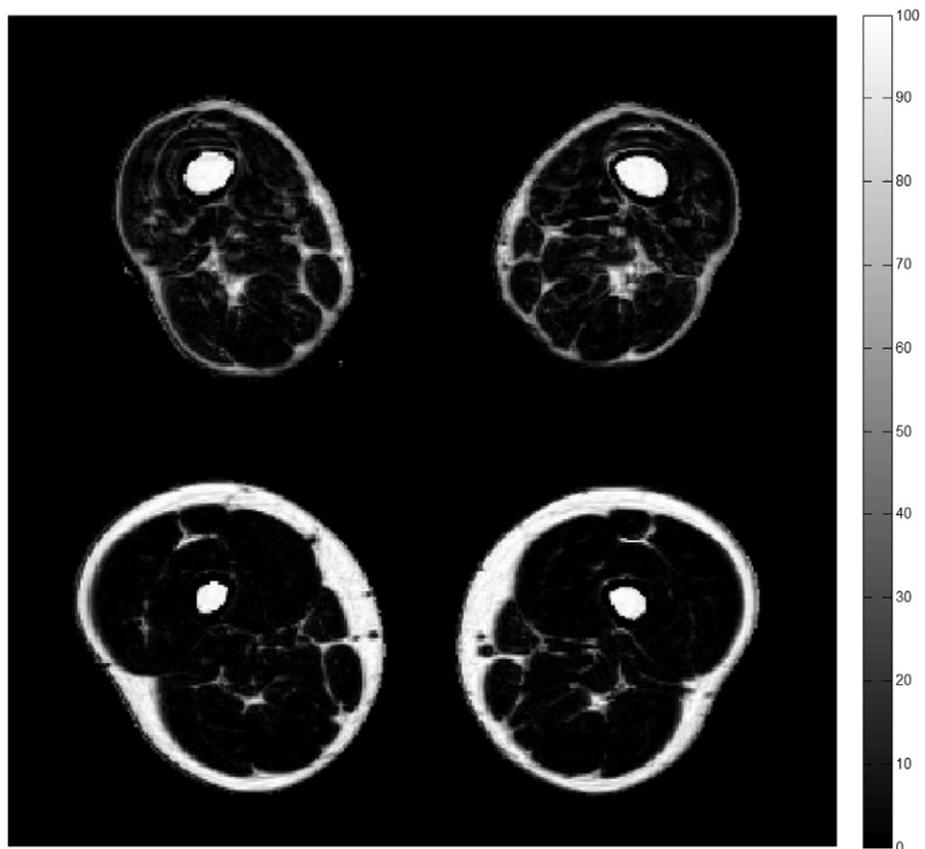

Fig. 2. Fat ratio maps (%) in an axial slice of the thigh of an older volunteer (upper line) and a young volunteer (bottom line).

Table 1
Multi-linear regression analysis for the younger and adults group with age and physical activity measure as the independent variables and the NMR parameters as the dependent variables. The significance level of each coefficient are * for p < 0.05 and ** for p < 0.01.

	Young subjects			Older subjects		
	VM	VL	VI	VM	VL	VI
T2 (ms)						
Intercept	38.91	36.77	39.20	28.20	27.52	26.78
Age	−0.11	−0.06	−0.11	**0.12***	**0.11****	**0.13****
Phy activity	−2.8E-07	−3E-07	−5.3E-07	2.3E-07	7.3E-08	1.4E-07
R²	0.09	0.03	0.13	**0.10***	**0.13***	**0.12***
Ratio high T2 (%)						
Intercept	17.78	15.02	22.80	−74.42	−53.76	−70.60
Age	−0.34	−0.30	−0.46	1.28	**0.79***	**1.11****
Phy activity	−1.0E-06	−1.9E-06	−2.6E-06	4.2E-07	1.3E-06	1.3E-06
R²	0.03	0.05	0.08	0.08	**0.11***	**0.12***
T2 coef of variation (%)						
Intercept	1.41	7.27	5.81	4.54	3.22	−6.10
Age	0.12	−0.06	0.01	0.07	0.03	**0.21****
Phy activity	6.3E-07	−1.5E-07	−1.2E-07	**−8.9E-07***	3.8E-08	**−8.3E-07***
R²	0.16	0.06	0.01	**0.10***	0.01	**0.17***
Fat ratio (%)						
Intercept	1.53	2.00	2.41	−3.08	−0.43	−5.03
Age	−0.01	−0.02	−0.03	**0.09****	0.06	**0.13****
Phy activity	7.3E-08	0.00	5.1E-08	−2.4E-07	−3.6E-07	**−3.8E-07***
R²	0.02	0.03	0.04	**0.16***	**0.10***	**0.23***

Regarding the fat ratio, it also increased with age (except for the VL) and decreased with physical activity but this was significant only in the VI muscle.

3.2. Age & gender related differences

Water T2 was different between the three muscles that we studied. It was also significantly higher in older volunteers when compared to the younger ones. There was a significant difference in water T2 between men and women muscles but this difference was muscle dependent. The highest and most significant was observed in the VL muscle. These results are shown in Fig. 3 and the descriptive statistics of the different NMR parameters are reported in Table 2.

Regarding the ratio of pixels with high water T2, the statistical analysis revealed that there was a difference between muscles and that it was larger for older subjects (Fig. 4). The difference between older and younger adults was muscle dependent. No significant differences between men and women were observed (Fig. 4).

The water T2 coefficient of variation increased with age (Fig. 5). The VL muscle had lower water T2 coefficient of variation in comparison with the VM and the VI and it was the least affected muscle with age. Fat ratio was higher in the muscles of older volunteers compared to the younger volunteers with no significant differences between women and men (Fig. 6). VM has lower fat ratio than VL and VI.

3.3. Physical activity & gender related differences

The mean water T2, the ratio of pixels with elevated T2 as well as the coefficient of variation, were not significantly different between active and sedentary groups. However, the fat ratio was significantly higher in the sedentary group. The results are shown in Fig. 7 and the descriptive statistics are given in Table 3.

4. Discussion

In this work we studied the effect of aging on the quadriceps femoris muscles using NMR biomarkers reflecting the fat content and the water T2 relaxation properties.

We showed in this paper that the mean fat ratio within the quadriceps muscles was not only higher for older adults (80% of increase in average) but is still increasing with age in this group. A few earlier studies showed already an increase in fat content in older adult muscles using T1 weighted images where inter-muscular fat (IMAT) was detected by classification algorithms (Buford et al., 2012; Zoico et al., 2010). Such an approach may suffer from inhomogeneity problems and partial volume effects. In this study we were interested in the intramuscular fat and we consequently used a specific imaging sequence dedicated to fat quantification based on the chemical shift between fat and water (Ma, 2008). Dixon based technique are not prone to B1 inhomogeneity problems and they estimate a percentage of fat in each voxel. The results we obtained brought additional information about fat progression inside the muscle. Similar findings were reported in a study on the calf muscles where the fat percentage was evaluated using a spectrally fat selective sequence to assess the intramuscular fat (Schwenzer et al., 2009). The increase in fat ratio reported in Schwenzer et al. (2009) was more than 100% on average, which was higher than the value we found.
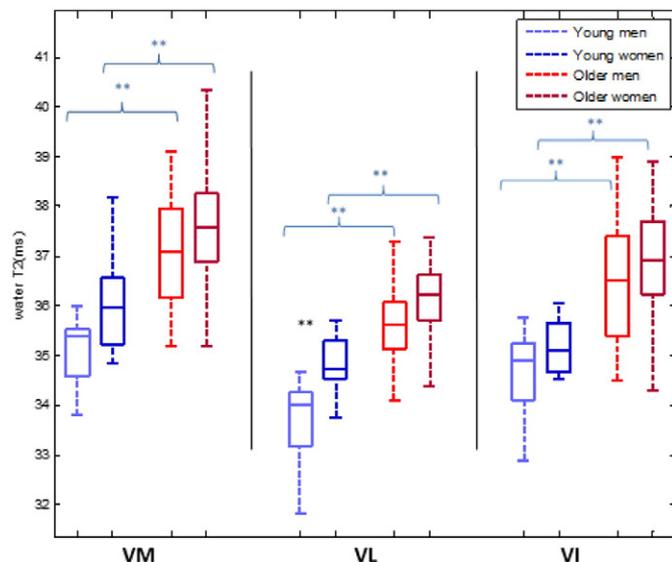

Fig. 3. Boxplot of water T2 mean value for each muscle. Water T2 was higher in older adults and in women compared to men's muscle (** for p < 0.01 and * for p < 0.05).

Table 2
Mean value and standard deviation of the different NMR parameters. * and ** correspond respectively to the significance level p < 0.05 and p < 0.01 of the comparison between young and older groups.

	Gender	Age group	VM		VL		VI	
			Mean	std	Mean	std	Mean	std
Water T2 (ms)	Men	Old	37.19**	1.34	35.64**	0.99	36.47**	1.22
		Young	35.05	0.75	33.73	0.77	34.60	0.83
	Women	Old	37.58**	1.17	36.24**	0.91	36.85**	1.14
		Young	35.99	0.89	35.09	0.97	35.36	0.91
Ratio of pixels with elevated water T2 (%)	Men	Old	20.55**	15.14	6.8*	8.13	13.36**	10.28
		Young	3.50	1.88	0.49	0.60	2.40	1.89
	Women	Old	22.71*	14.57	9.13*	8.07	16.74**	10.28
		Young	8.82	7.42	3.71	6.95	4.96	8.09
Water T2 coefficient of variation (%)	Men	Old	7.8*	2.12	6.00*	1.04	8.02*	2.50
		Young	6.25	1.00	5.09	0.64	5.88	1.02
	Women	Old	6.84	1.77	5.44	0.69	7.11*	1.48
		Young	6.44	1.03	5.53	0.72	5.52	0.68
Fat ratio (%)	Men	Old	3.00**	0.90	3.41	0.96	3.48*	1.02
		Young	1.66	0.33	1.88	0.61	1.87	0.67
	Women	Old	2.81**	0.72	3.48*	1.02	3.51**	1.01
		Young	1.64	0.39	1.87	0.38	1.83	0.31

This might be related to the fact that we did not study the same muscles, but it is more likely related to the difference of fat percentage definition. In Schwenzer et al. (2009), they used a fat signal image and evaluated the ratio of mean signal intensity inside the muscle and inside the bone marrow (considered as a reference with 100% of fat). Overall, and in spite of the sensitivity to B1 and B0 field inhomogeneity of the technique used in Schwenzer et al. (2009), the difference in fat content between old and young was high enough to be detected regardless of the robustness of the techniques. This was also confirmed by the correlation values between age and fat ratio when both groups were pooled. They found in the calf muscle an average correlation equal to 0.67 ± 0.13 whereas in our study it was 0.68 ± 0.04 for the quadriceps muscle.

We found that the mean intramuscular fat ratio was similar between men and women which was also the case for the calf muscles (Schwenzer et al., 2009). This conclusion was confirmed in younger healthy volunteers, where a comparison of extra- and intra-myocellular (IMCL) lipid content was performed in the calf and thigh muscles using high-spatial-resolution magnetic resonance spectroscopic imaging (MRSI) and which did not reveal any significant difference between men and women (Ortiz-Nieto et al., 2010).
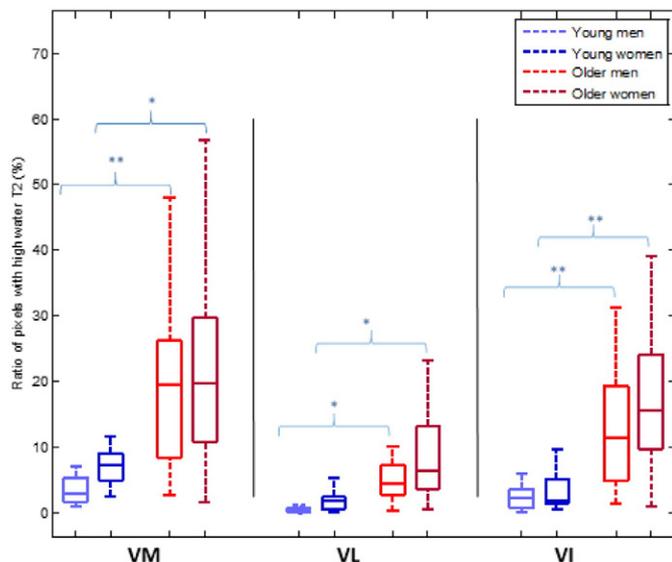
In our work we observed in the older adult group, a significant positive slope between the age and the mean fat ratio, which indicated that the process of fat replacement was still active after the age of 70 years. These results corroborate the observations made in Castillo et al. (2003) on the prevalence in elderly population of sarcopenia that was defined as 2.0 SDs or more below the fat-free mass mean of a young reference group. In Castillo et al. (2003) it was shown that the proportion of subjects with sarcopenia, was increasing with age starting around the age of 65.

Water T2 changes were important to monitor because they convey relevant information about the structural changes within the muscle. This study showed that T2 increased with age and this was also the case of the ratio of pixels with elevated water T2. Increase of T2 was also reported in Hatakenaka et al. (2001) and Schwenzer et al. (2009) for calf muscles and in Esposito et al. (2013) in the tibialis anterior and gastrocnemius muscles in young and old mice. One explanation could be the change in muscle fiber structure with age. For instance, some studies (Larsson et al., 1978; Lexell et al., 1983; Nilwik et al., 2013) showed that the proportion of type II fibers decreased as a consequence of a significant loss of satellite cells associated with this type of fibers (Verdijk et al., 2007). On the other hand, in vivo (Bonny et al.,

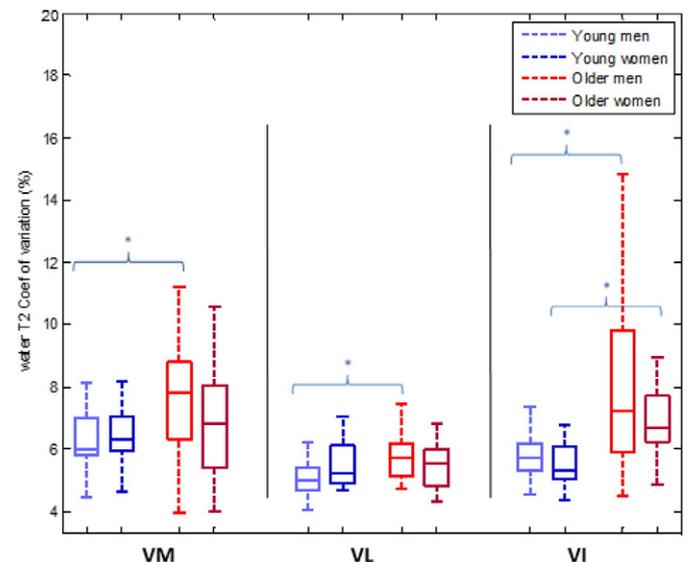**Fig. 4.** Boxplot of pixel ratio with elevated water T2 for each muscle. It was higher in older adults (** for p < 0.01 and * for p < 0.05).

Fig. 5. Boxplot of water T2 coefficient of variation in each muscle. It was higher in older adults (** for p < 0.01 and * for p < 0.05).
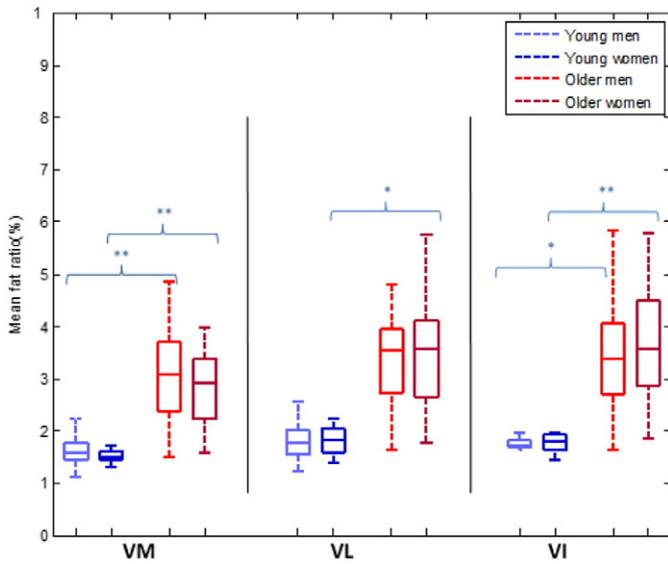
Fig. 6. Boxplot of mean value of the fat ratio in each muscle. It was higher in older adults (** for p < 0.01 and * for p < 0.05).

Table 3
Mean and standard deviation of the different NMR parameters in older group.

		VM		VL		VI	
		Mean	std	Mean	std	Mean	std
Water T2 (ms)	Active	37.43	1.41	35.97	1.17	36.74	1.32
	Sedentary	37.34	1.14	35.91	0.82	36.59	1.08
Ratio of pixels with elevated water T2 (%)	Active	20.74	16.60	8.93	10.34	15.97	11.97
	Sedentary	22.41	13.19	7.15	5.53	14.26	8.78
Water T2 coefficient of variation (%)	Active	6.79	2.16	5.75	1.01	7.36	1.77
	Sedentary	7.77	1.75	5.68	0.85	7.74	2.34
Fat ratio (%)	Active	2.63*	0.87	3.08*	1.09	3.08*	1.04
	Sedentary	3.15	0.69	3.76	0.77	3.86	0.83

* Correspond to the significance level p < 0.05 of the comparison between active and sedentary groups.

demonstrated by the significant positive slope between age and T2 parameters in linear regression results for some muscles. Mean water T2 values within the VL were significantly different between men and women. To the best of our knowledge, such differences were not reported before. However, in Simoneau and Bouchard (1989) it was shown that the mean proportion of type I fibers in the VL muscle was lower in men then in women which corroborates our T2 results. It is also important to note that in spite of the difference between men and women, the changes in water T2 mean values were not gender dependent.

The coefficient of variation of water T2 is a parameter that reflects the tissue heterogeneities. Similar indices were used in Thibaud et al. (2012) to monitor the changes in Golden Retriever dogs with muscular dystrophy. In the current study, we considered a parameter that is more robust to both image inhomogeneities and fatty infiltration. Our results revealed that water T2 variability increased with age. The absence of
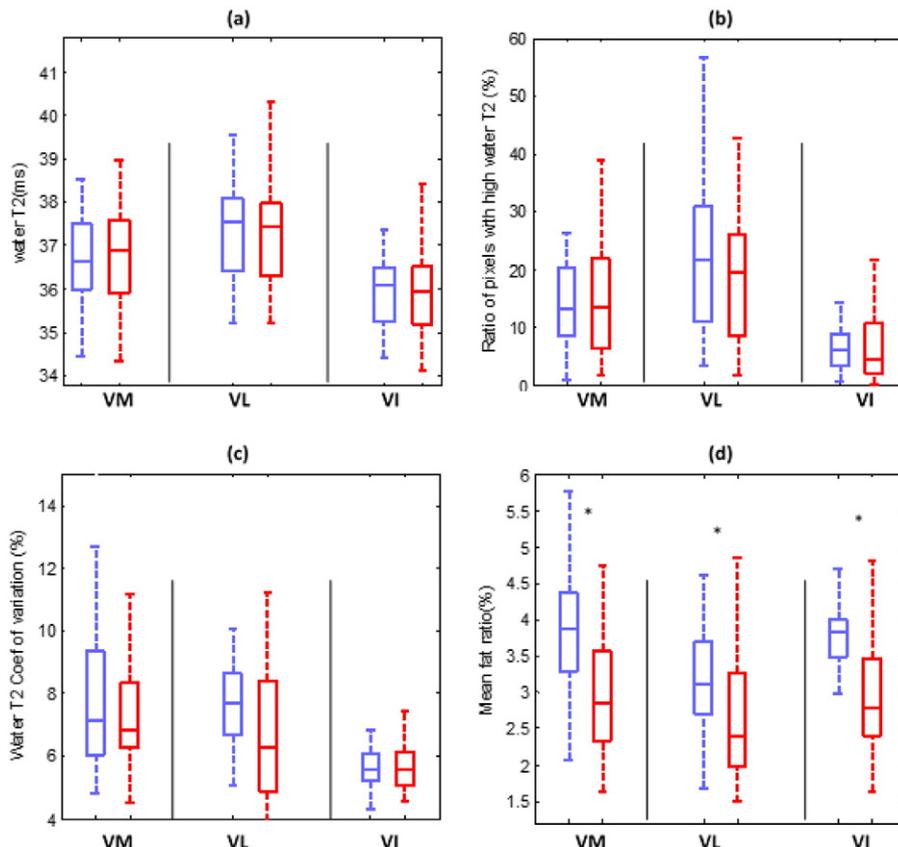

Fig. 7. Boxplot of the different NMR parameters computed in older group muscles: (a) Water T2 mean value and (b) ratio of pixel with high water T2 (%). (c) Coefficient of variation of water T2. (d) Mean fat ratio. The blue and the red box are respectively for active and sedentary volunteers. (* for p < 0.05).

correlation between the heterogeneity and the amount of fatty infiltration as well as the water T2 mean value, means that it reflects a mechanism of tissue disorganization other than fat infiltration, inflammation or fiber typology. Elevated heterogeneity indices on T2 weighted image of hamsters with cardio-myopathy and dogs with muscular dystrophy were reported (Parzy et al., 2007; Thibaud et al., 2012). So, it would make sense to attribute the increase of water T2 heterogeneities to the development of interstitial fibrosis.

Considering the impact of physical activity, the linear regression between accelerometry values and NMR biomarkers, showed a significant negative relation only for the fat ratio for VI and water T2 coefficient of variation for VI and VM. In general, fat ratio was significantly lower in the case of physically active group. These findings were concordant with the claim that physical activity is beneficial in sarcopenia prevention as reported in Castillo et al. (2003) and Degens and Korhonen (2012). We expected a similar trend on the impact of physical activity on water T2, but no significant changes were observed. This discrepancy could be related to the variability of the activity of the different subjects that were not probably intensive enough to make changes in water T2.

In summary, we presented several NMR based biomarkers to monitor the effect of aging on the quadriceps tissue and to study the impact of physical activity and gender difference. We showed that tissue properties significantly changed with age, but still a correlation with histology or more advanced sequence like Ultra-short Time-to-Echo sequences are necessary to confirm the link between the T2 heterogeneity and the source of tissue relative disorganization. This study did not reveal any gender difference in the aging process and it demonstrated that physical activity had an impact on the T2 heterogeneity and fat ratio within the VI and VM muscles.

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