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Dynamic contrast-enhanced magnetic resonance imaging of the sarcopenic muscle

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Abstract

Background: Studies about capillarity of the aged muscle provided conflicting results and no data are currently available about the magnetic resonance imaging (MRI) *in vivo* characteristics of the microvascular bed in aged rats. We have studied age-related modifications of the skeletal muscle by *in vivo* T2-relaxometry and dynamic contrast-enhanced magnetic resonance imaging (CE-MRI) at high field intensity (4.7 T). The aim of the work was to test the hypothesis that the ageing process involves microvessels in skeletal muscle.

Methods: The study was performed in 4-month-old (n = 6) and 20-month-old (n = 6) rats.

Results: At MRI examination, the relaxation time T2 of the gastrocnemius muscle showed no significant difference between these two groups. The kinetic of contrast penetration in the tissue showed that in 4-month-old rats the enhancement values of the signal intensity at different time-points were significantly higher than those found in senescent rats.

Conclusion: The reported finding suggests that there is a modification of the microcirculatory function in skeletal muscle of aged rats. This work also demonstrates that CE-MRI allows for an *in vivo* quantification of the multiple biological processes involving the skeletal muscle during aging. Therefore, CE-MRI could represent a further tool for the follow up of tissue modification and therapeutic intervention both in patients with sarcopenia and in experimental models of this pathology.

Background

The process of reduction of the muscle mass and strength in the elderly populations, called sarcopenia, is widely considered as a part of normal aging [1,2]. The etiology of sarcopenia is unclear but several important factors have been identified [3]. Neurological, metabolic, hormonal, nutritional, and physical activity-related changes with age are likely to contribute to the loss of muscle mass [4,5].

Conflicting results about capillarity of the aged muscle have been reported in the literature. Studies performed using traditional methods have found little or no age-related

decline in blood flow capacity in the healthy elderly [6,7]. In human biopsy studies, capillarization has been reported to be maintained [8–10] or reduced [11–13] with increasing age. However, studies in aged mice showed a significant higher capillary-to-muscle fiber ratio and capillary density [14].

At the best of our knowledge, no data are available about the MRI-*in vivo* characteristics of the microvascular bed in aged-rats. Some reports suggest that magnetic resonance imaging (MRI)-based techniques can detect age-related changes in skeletal muscle [15,16]. In the present work, we have studied age-related modification of the skeletal muscle by *in vivo* T2-relaxometry and dynamic contrast-enhanced MRI (CE-MRI) at high field intensity (4.7 T). The latter method allows for measuring the kinetics of penetration of a contrast agent in a tissue and for evaluating functional parameters of the microvascular bed [17]. CE-MRI seems to be a promising approach to test the hypothesis that age-related modifications involve microvessels in skeletal muscle.

Material and Methods

For MRI evaluations, a 4-months-old group (n = 6) and a 20-months-old group (n = 6) of male Wistar rats (Harlan-Nossan, Italy) were anaesthetized with an inhaled mixture of O_2 and air containing 1%-2% halothane. Clinical examination performed at the end of the MRI evaluation revealed that hypothermia was not induced by gas anesthesia in rats. All MRI experiments were carried out using a Biospec System (Bruker, Karlsruhe, Germany) equipped with a 4.7 Tesla (T) Oxford Magnet, 33 cm bore and a SMIS (Surrey Medical Imaging System Ltd., UK) gradient insert. A 72 mm. i.d. birdcage coil was used.

After a pilot scout, coronal and axial multislice T2 weighted (T2W) spin-echo (SE) images were acquired with the following parameters: slice thickness = 2 mm, TE = 60 ms, TR = 2000 ms, 128 × 128 matrix, field of view (FOV) = 8 × 8 cm. In coronal images, the best slice to observe hindlimbs was localized. A dynamic acquisition of twenty coronal T1-weighted (T1W) SE images was started with evolution delay of 1 sec. During the time interval between the first and the second acquisition, a bolus of Gd-DTPA (100 μmol/kg) was injected trough the tail vein. In this case, parameters were: slice thickness = 2 mm, TE = 10 ms, TR = 100 ms, 64 × 64 matrix, FOV = 8 × 8 cm corresponding to an acquisition time of 6.4 sec.

T2 parametric maps were obtained for all rats. Such maps were calculated by a coronal T2W multi-echo single slice SE image acquired with the following parameters: TE = 20–120 ms, TR = 1200 ms, echoes number = 6, 128 × 128 matrix, FOV = 8 × 8 cm. On T2W images, a regions-of-interest (ROI) was selected (5 × 5 mm^2) in the central portion of the gastrocnemius muscles. On these ROIs, the mean T2 values were calculated.

The kinetic of contrast agent penetration in the leg was studied in serial images by calculating, on a pixel-by-pixel basis, the parameter ΔSI(t):

$$\Delta SI(t) = 100\{[SI(t) - SIpre] / SIpre\}$$

At different time points the average ΔSI(t) values were evaluated on the selected ROIs; values were expressed as mean ± SEM.

The volume of muscle and adipose tissue was measured as follows: five transversal contiguous slices were selected within the leg region starting at a distance of 10 mm from the ankle; an operator defined ROI was manually traced on muscle or adipose tissue and the corresponding volume was calculated. These values were then averaged over the five slices for each rat and for left and right leg.

Data analysis

An unpaired t test was used to determine the significance of the difference between the signal intensities in the two groups of rats. The correlation between data from the right and left leg of each rat has been also calculated. A t test was also performed to determine the significance of the difference of hindlimb volume between young and old rats.

Results

At clinical examination, aged (twenty-month-old) rats showed an evident reduction of skeletal muscle tissue. Fig. 1 shows coronal and axial slices acquired for three rats belonging to the young and old group. This figure clearly shows that old rats showed less muscle tissue and more adipose tissue. This finding is confirmed by quantitative data shown in Table 1 and 2 where the average volume (expressed in cm^3) of the muscle and adipose tissue tissue measured in the hindlimbs are reported. No significant difference between left and right leg is observed for both young and old rats. On the contrary, the difference between the same leg of the young and old group is highly significant ($P < 0.05$) for both muscle and adipose tissue.

No significant difference of the relaxation time T2 of the muscle was found between these two groups (four-month-old rats: 34.6 ± 1.6 ms in the left muscle and 33.9 ± 1.1 ms in the right one; twenty-month-old rats: 33 ± 0.5 ms in the left and 33.2 ± 1 ms in the right muscle).

In Fig. 2 we show the time dependence of the SI enhancement in the two groups of rats. The kinetics of contrast penetration in the muscle tissue showed a different pattern. In 4-month-old ones, the analysis of the enhancement at different time-points showed significantly higher

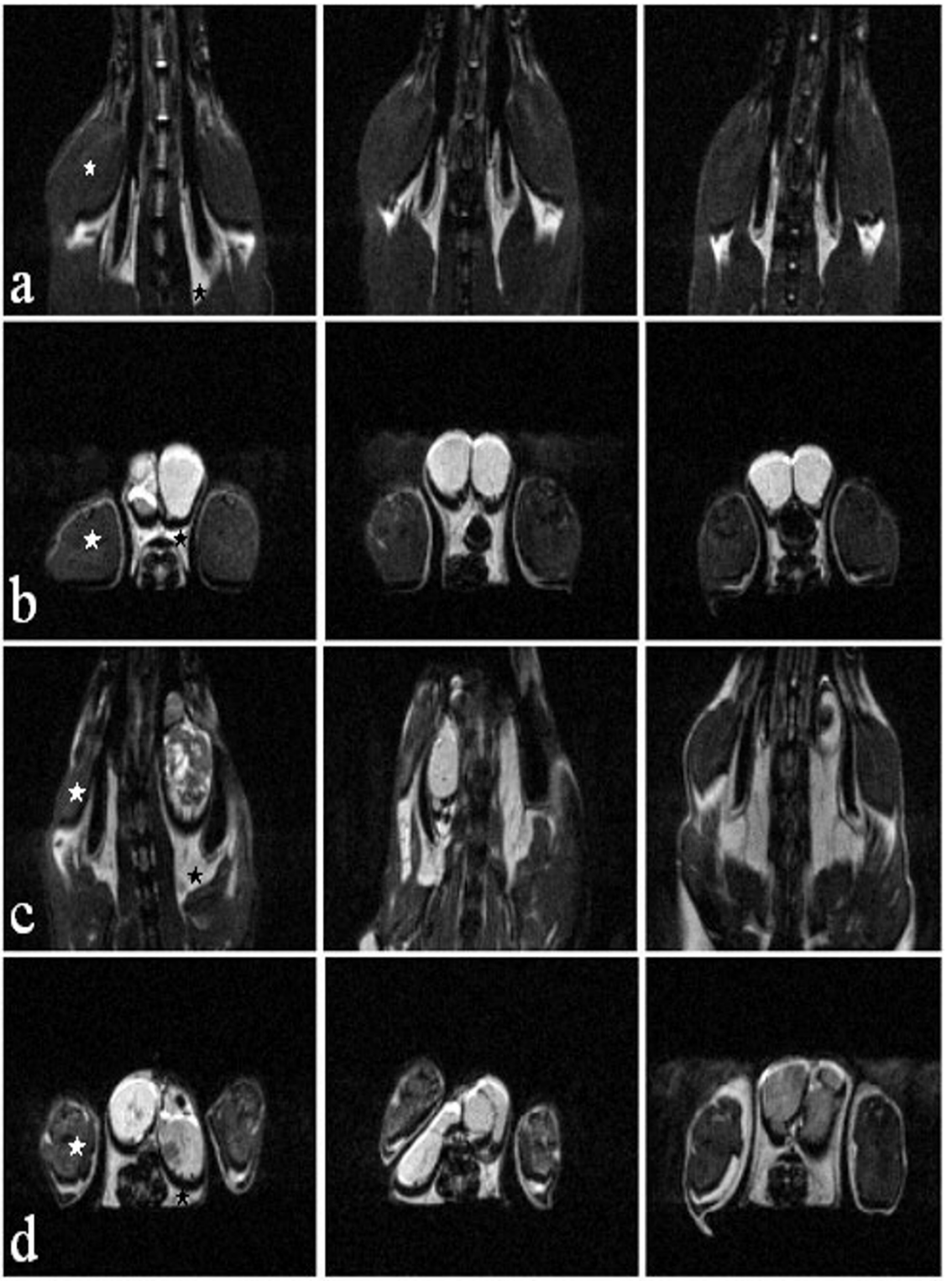

Figure 1
Coronal and axial images (TR/TE = 1200/20) of hindlimbs of three young (a and b) and three old (c and d) rats. FOV = 8 × 8 cm and 128 × 128 matrix. Adipose tissue (black stars) and skeletal muscle (white stars) are shown.

Table 1: Average volume (cm^3) of the muscle of the hindlimb.

	4-MONTH-OLD		20-MONTH-OLD	
	LEFT	**RIGHT**	**LEFT**	**RIGHT**
1	27,6	30,5	19,7	21,4
2	28,9	30,1	15,5	16,0
3	28,2	34,5	22,3	20,5
4	32,8	32,5	21,3	20,0
5	32,1	34,6	23,0	23,0
6	29,1	30,7	21,1	22,1
MEAN	**29,7**	**32,1**	**20,4**	**20,5**
SD	**1,9**	**1,8**	**2,4**	**2,2**

Table 2: Average volume (cm^3) of the adipose tissue of the hindlimb.

	4-MONTH-OLD		20-MONTH-OLD	
	LEFT	**RIGHT**	**LEFT**	**RIGHT**
1	6,0	5,0	12,0	13,0
2	5,0	6,0	13,0	12,0
3	5,0	4,0	10,0	11,0
4	7,0	6,0	8,0	11,0
5	5,0	5,0	12,0	11,0
6	4,0	5,0	18,0	17,0
MEAN	**5,3**	**5,1**	**12,1**	**12,5**
SD	**0,9**	**0,6**	**3,0**	**2,1**

values than those found in senescent rats. In aged rats, the analysis of the enhancement curves shows a clear alteration of the first phase, which mainly expresses a retard of contrast medium arrival in the tissue. The maximal difference between young and senescent rats occurred at about 98 sec after injection of the contrast agent (scan 14). In Table I we have reported the enhancement of the signal intensity measured at the point of maximal difference (scan 14). Data obtained from the legs of each rat showed high correlation in young rats ($r^2 = 0.98$) and a worse correlation ($r^2 = 0.59$) in senescent rats. Unpaired t-test performed on these data confirms a significant difference ($P < 0.05$) between old and young rats on both legs.

Table 3: Dynamic percentage of enhancement at about 2 minutes after contrast injection.

	4-MONTH-OLD		20-MONTH OLD	
	LEFT	**RIGHT**	**LEFT**	**RIGHT**
1	82,4	80,9	21,9	28,9
2	42,4	41,1	48,1	38,3
3	50,4	49,7	45,8	50
4	50,9	46,6	24,4	32,5
5	49,5	48,8	28	25,7
6	63,4	67,6	45,8	29,1
MEAN	**56,5**	**55,8**	**35,7**	**34,1**
SD	**14,4**	**15,2**	**12,1**	**8,9**
	CORRELATION LEFT-RIGHT			
	0,98		**0,59**	

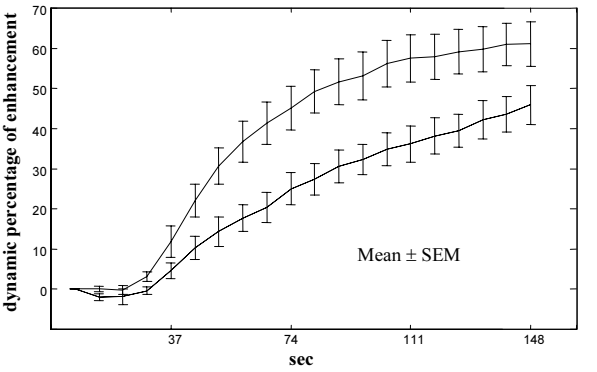

Figure 2
Dynamic percentage of enhancement $\Delta S(t) = 100\{[SI(t)-SIpre]/Sipre\}$ on the ROIs selected on the gastrocnemius of the 4-moth-old (solid line) and on the 20-moth-old (dotted line). Values and error-bars represent mean ± SEM of both legs. Each dynamic scan was 7.4 sec long. The maximal difference between the two groups was around the 14^{th} scan (at about 2 minutes after contrast injection).

Discussion

Magnetic resonance imaging (MRI) has the ability to discriminate between various soft tissues in vivo [18]. Both cross-sectional and longitudinal studies on human have shown age-related changes in body composition: with advancing age, elderly men and women tend to became more obese, their amount of visceral fats tends to increase, and their skeletal muscle mass declines [19–21].

Our quantitative data show that MRI can detect body composition change with age, in particular hindlimb composition change, with increase in adipose tissue and decline in skeletal muscle, also in small laboratory species. Increase in adipose tissue is probably due to a process of differentiation of myoblasts into adipocyte [22]. Previous study shows that myoblasts isolated from mouse hindlimb skeletal muscle demonstrate increased adipogenic potential as a function of age. This change suggests that a default program may be activated in mesenchimal cells with increasing age resulting in a more adipogenic-like phenotype. However, whether this change in differentiation potential contributes to the increased adiposity in muscle with age remains to be determined [23].

Furthermore, muscle modifications we found are quantitative as well as qualitative.

Data of CE-MRI show that the enhancement of the signal intensity in muscle is significantly higher in young rats that in old rats. This finding has never been described in the literature and demonstrates that a modification of the vasculo-stromal component of the muscle occurs in elderly. The tissue determinants of such modification are not clear because several parameters may change MRI-signal enhancement: the blood volume, the blood flow, the permeability of the microcirculatory bed, the axial area or number of microvessels and the extracellular volume. In the sarcopenic muscle, all this parameters are probably altered. Data about age-related changes in vascular permeability of the rat skeletal muscle have not been reported yet; however data obtained on humans demonstrated high amount of water in the skeletal muscle of elderly population [16]. About blood volume, a meta-analysis of literature suggests that a certain degree of reduction in capillarization can exist in elderly also if data are discordant. Reduced capillary density and reduced blood flow have been reported in some studies [7,24]. Alteration in heart rate and cardiac output with age such as the decrease of basal metabolic rate (BMR) acquired with aging could be factors affecting the hemodynamic pattern, while an age-related increased collagen concentration could modify the MRI characteristics of the muscle. It has been found that the anaesthesia could also induce a lower blood flow in many tissues of the elderly rat compared to the adult, but data for the skeletal muscle are not in trend with the other organs [25].

The above reported considerations show that tissue modification of the sarcopenic muscle is very complex and, taken together, they seem to suggest that the age-related impairment of the signal enhancement is a multifactorial event. However, in aged rats, the analysis of the enhancement curves shows a clear alteration of the first phase, which mainly expresses a retard of contrast arrival in the tissue. Therefore, *in vivo* data, also if not conclusive, seem to suggest a reduction in blood flow in the sarcopenic muscle, whose possible origin from macroangiopathy should be excluded since the dynamics are quite different from those we have recently described in experimental arterial occlusion [17]. Instead, the pattern that we have found in aged animals is quite similar to that described in an experimental model of myocardium ischemia [26], where neo-angiogenesis by VEGF gene transfer significantly increased functional performances of the organ and magnetic resonance mapping demonstrated benefits of VEGF-induced myocardial angiogenesis.

Data obtained from the two different legs of each animal showed a greater homogeneity in young with respect to senescent rats. This finding seems to be in accordance with the existence of a microangiopathy, which may differently involve the left and right hindlimb.

Conclusion

Our functional MRI-findings suggest the existence of an alteration of the microcirculatory function in skeletal muscle of aged animals. Further studies, using MRI on longitudinal observation, will allow to show if the microcirculatory alteration we described are foregoing or subsequent to the process of differentiation of myoblasts into adipocyte.

Moreover, further studies will be necessary to evaluate the tissue determinants of CE-MRI modification that we have detected in the muscle of aged animals. It could be hypothesised that such a modification could be the expression of multiple biological processes involving the sarcopenic skeletal muscle. This work clearly demonstrates that these events are quantifiable *in vivo* and that CE-MRI could represent a further tool for following up tissue modification and therapeutic intervention both in patients with sarcopenia and in experimental models of this pathology.

Author's contribution

Author1: Conceived of the study, carried out the experiments and drafted the manuscript.

Author 2: Participated in analysis of data and manuscript writing.

Author 3: Veterinary advice.

Author 4: Conceived of the study, set up MRI sequences.

Author 5: Participated in analysis of data and manuscript writing.

Author 6: Laboratory sponsor who participated in experiment design. Wrote the final version of the manuscript.

Author 7: Laboratory sponsor who conceived and coordinated the overall study.

Competing interests

None declared.

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