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LETTER TO THE EDITOR

Age-related structural alterations of skeletal muscles and associated capillaries

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Abstract

Aging is associated with a progressive decline in muscle mass, strength, and quality. We have previously demonstrated the important role of the blood vasculature system in ultraviolet (UV) light-induced changes in skin and its molecular mechanisms. Whereas recent findings revealed structural alterations of the cutaneous vasculature in aged and photoaged human skin, structural changes of blood vessels in skeletal muscles with age have remained unclear. Although, facial skeletal muscles could be involved in skin-aging, here, we show—for the first time—that, in the lateral great muscle, the cross-sectional muscle fiber area and vessels size were decreased in older skin compared with that in younger skin. In the orbicularis oculi muscle, no significant interaction between age and the muscle fiber area was observed. However, a significantly decreased ratio of muscle area was indicated in older skin compared with that in younger skin. Interestingly, the pericyte-covered vessels ratio was decreased in older skin. Therefore, we found that the skeletal muscle capillary destabilizes with age. In summary, we revealed that the lateral great muscle and the orbicularis oculi muscle fibers become thinner with age due to the destabilization of skeletal muscle capillaries. Therapeutic targeting of muscle capillaries might affect the decline of skeletal muscles with age and could potentially regulate muscle/skin-aging.

Keywords Aging · Capillary · Lateral great muscle · Orbicularis oculi muscle

Letter to the editor

Aging is associated with a progressive decline in muscle mass, strength, and quality. This is generally referred to as the "sarcopenia of aging." These age-related changes have been reported even among healthy and physically active subjects, with the estimated rate of muscle loss ranging from 1 to 2% per year when the subjects exceed 50 years of age [1].

This muscular decline is also seen in facial muscles. It is widely agreed that the aging process results in the gradual loss of elastic fibers and skin sagging, giving it a loose appearance. It is also argued that muscle ptosis may be attributable to the aging appearance of the upper eyelid [2]. Although the eyelids are probably the first parts to be affected on the face, the research conducted in this field is still slight.

In this study, it was found that ptosis is correlated with dense networks of capillaries. It is known that capillaries help the exchange of oxygen, blood-borne energy, metabolites, and heat between the blood and muscle tissue [3]. Therefore, we supposed that aging-induced muscle weakness may be involved in the changes to the capillaries. Thus, this study focused on investigating how aging affects the muscles, such as lateral great muscle and the orbicularis oculi muscle, as well as their capillaries. Completed materials and methods are in the supplemental data with consensus guidelines [4].

To clarify age-related changes in the skeletal muscles and capillary networks, we examined the size of individual skeletal muscle fibers, as well as the capillary luminal size and the structure, assessed as the ratio of capillary coverage by pericytes, in young and old healthy subjects (from 19 to 62 years old).

All morphometric parameters for capillary and muscle fibers were determined in the cross sections of the fixed

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lateral great muscles and the orbicularis oculi muscles. The fiber size, capillary size, and capillary coverage were larger in young lateral great muscles.

Micrographs of transverse sections of lateral great muscles in young and old muscles are shown Fig. 1a. Compared with the young samples (3719.6 ± 1141.3 μm^2), the average of cross-sectional area of individual muscle fibers decreased in the old samples (2354.2 ± 1253.5 μm^2) ($p < 0.05$). Capillary morphometric data are presented in Fig. 1b. A significantly ($p < 0.01$) decreased average of vessel size was indicated in the old group compared with the young group (young 26.0 ± 11.6 μm^2; old

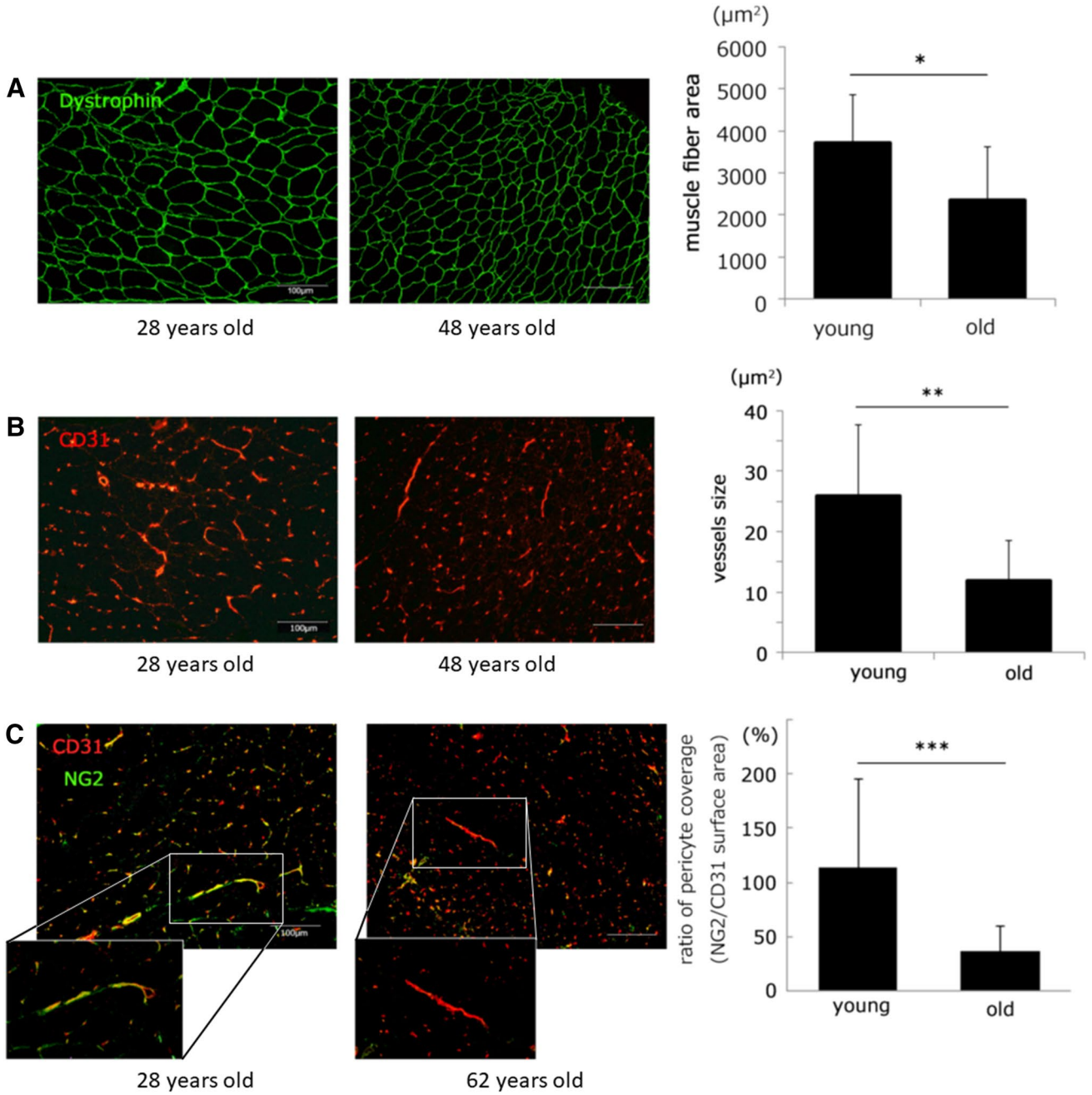

Fig. 1 Structural changes of lateral great muscle with age. **a** Immunofluorescence staining for dystrophin (green) revealed the atrophy of the muscle fiber. **b** Quantitative analysis of the muscle fiber cross-sectional area (μm^2). **c** Immunofluorescence staining for CD31 (red) revealed the capillary thinning with age. **d** Quantitative analysis of the vessels size (μm^2). **e** Analysis pericyte coverage of blood vessels. Representative photomicrographs (E) and quantitative analysis (F) of the ratio of NG2/CD31 surface area in lateral great muscle. Sections were stained green with an anti-NG2 antibody and red with an anti-CD31 antibody. Data are means ± SD. Scale bar: 100 μm. $^{*}p < 0.05$ $^{**}p < 0.01$

$11.9 \pm 6.6\ \mu m^2$). The ratio of NG2/CD31 surface areas was $114.3 \pm 81.1\%$ in the young muscles and $36.8 \pm 22.9\%$ in the old ones (mean ± SD). There was a statistical ($p < 0.01$) difference between the two groups (Fig. 1c).

Interestingly, in the orbicularis oculi muscles, we found that the fiber size had no change, but the fiber density and the capillary coverage were decreased with age. Micrographs of transverse sections of the orbicularis oculi muscles in the young and old groups are shown in Fig. 2a. No

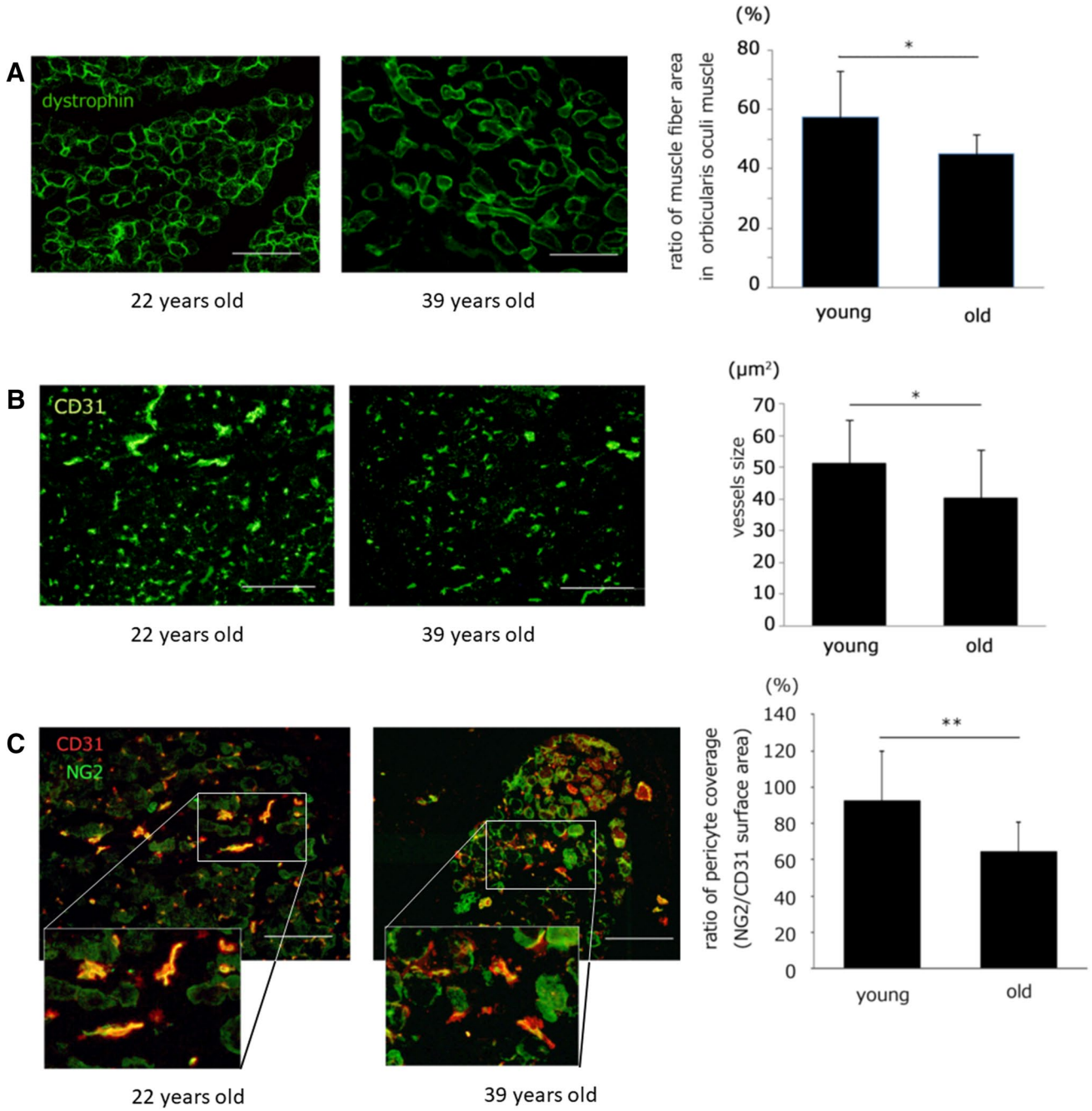

Fig. 2 Structural changes of orbicularis oculi muscle with age. **a** Immunofluorescence staining for dystrophin (green) revealed the atrophy of the muscle fiber. **b** Morphometric photomicrographs and quantitative analysis of the ratio of muscle fiber area in orbicularis muscle (%). **c** Immunofluorescence staining for CD31 (green) revealed the capillary thinning with age. **d** Representative photomicrographs and quantitative analysis of the vessels size (μm^2). Representative photomicrographs (E) and quantitative analysis (F) of the percentage of the ratio of NG2/CD31 surface area in orbicularis oculi muscle. Sections were stained green with an anti-NG2 antibody and red with an anti-CD31 antibody. Data are means ± SD. Scale bar: 100 μm. $p < 0.05$ $^{**}p < 0.01$

significant interaction between age and muscle fiber areas was observed. However, a significantly ($p < 0.05$) decreased ratio of muscle area in the orbicularis oculi muscle was indicated in the old group (45.2 ± 6.2 μm^2) compared with the young one (57.2 ± 15.4 μm^2). Figure 2b shows capillary morphometric data for the orbicularis oculi muscles. Capillary density did not differ between young and old muscles (data not shown). However, the average vessel size showed a significant difference ($p < 0.05$) (young 51.1 ± 13.6 μm^2; old 40.3 ± 15.2 μm^2). In addition, the ratio of pericyte coverage was $90.0 \pm 27.9\%$ in the young group and $64.2 \pm 16.3\%$ in the old group. (Fig. 2c).

Results from this study showed that skeletal muscle capillaries become thinner with age. To the best of our knowledge, this is the first study in which the ultrastructural phenotype of skeletal muscle capillaries has been evaluated in the skeletal muscles of non-diseased, young and old humans. Also, we found that the pericyte coverage of capillaries reduced by age. In other words, the destabilization of capillaries seems to occur with age. We hypothesize that skeletal muscle capillaries become destabilized and cannot deliver sufficient oxygen or metabolites to the muscles. Thus, the muscle fibers become thinner with age.

The orbicularis oculi are muscles in the face that close the eyelids. It is agreed that the aging process results in the gradual loss of elastic fibers and skin sagging, giving it a loose appearance. It is argued that the eyelids are perhaps the first structures in the face to be affected by aging [2]. In this study, changes in the orbicularis oculi muscle fiber resulting from aging were examined. No statistical difference was found in the mean muscle fiber cross-sectional areas between young and old. However, the ratio of muscle fibers in the muscle tissue was reduced. In contrast to the lateral great muscle, the orbicularis oculi muscle fibers are not thick, and the distribution is not uniform. Consequently, in aged orbicularis oculi muscles, the increase in gaps between muscle fibers leads to the states such as edema.

Edema are known to be closely related to the capillaries. In our previous study, it was revealed that vascular permeability leads to edema formation [5]. Recently, it was also established that angiopoietin-1 (Ang1) promotes lymphatic vessel formation and function by activating the receptor tyrosine kinase Tie2 on lymphatic endothelial cells (LECs). The activation of Ang1/Tie2 signaling attenuates inflammation by promoting lymphatic integrity as well as inhibiting blood vascular hyperpermeability in inflamed tissue. Tie2 activation is thought to be involved in promoting lymphatic function in vitro and improving edema in lower limbs. Then, we hypothesize that Ang1/Tie2 signaling has the potential to suppression skeletal muscle capillary destabilization in the aging capillaries.

In summary, we revealed that, due to the destabilization of skeletal muscle capillaries, the lateral great muscle and the orbicularis oculi muscle fibers become thinner with age. Therapeutic targeting of muscle capillaries might affect the decline of skeletal muscles with age and could potentially regulate muscle aging.

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Compliance with ethical standards

Conflict of interests All of the authors are employees of Shiseido Co., Ltd.

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