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Load-sensitive adhesion factor expression in the elderly with skiing: relation to fiber type and muscle strength

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We hypothesized that 12 weeks of downhill skiing mitigates the functional deficits of knee extensor muscles in elderly subjects due to the specific recruitment of fast motor units during forceful turns on the slope. Downhill skiing led to a 1.4-fold increase in the mean cross-sectional area of slow ($P = 0.04$)- and fast ($P = 0.08$)-type muscle fibers. Fold changes in the expression of the structural component of focal adhesions, gamma-vinculin, were correlated with alterations in concentric force ($r = 0.64$). Hypertrophy of fast fibers was more pronounced in women than in men (1.7 vs 1.1). Gender-specific structural–functional adjustments of knee extensor muscles and attached patellar tendon were reflected by altered expression of pro- vs de-adhesive proteins and a number of correlations. The de-adhesive protein tenascin-C was selectively increased in women compared with men (1.7 vs 1.1) while the content of the adhesive collagen XII was specifically reduced in women. The pro-adhesive focal adhesion kinase showed a specific increase in men compared with women (1.9 vs 1.1). Our findings indicate that quantitatively matched adaptations in slow and fast motor units of extensor muscle underlie the preventive effect of skiing against sarcopenia and support that hypertrophy and reinforcement of fiber adhesion operate in the improvement of muscle strength.

Deconditioning of muscle strength is a debilitating consequence of reduced load-bearing activity and biological decline with an increase in age (reviewed in Fluck et al., 2003; Yu et al., 2007). This reduction in the capacity to develop force in the elderly is thought to originate from a combined decrease in the mean cross-section (atrophy) of muscle fibers with a preferential loss of fast contractile muscle fibers with aging (termed sarcopenia; (Vandervoort, 2002; Narici & Maganaris, 2006). Recent studies suggest that the aforementioned aspects of sarcopenia can be slowed by exercise stimuli that augment the recruitment of fast motor units under concomitant muscle loading (Jones et al., 2009; Aagaard et al., 2010).

Downhill skiing constitutes a possible physical intervention that can specifically address the strength deficit in anti-gravitational muscle of the healthy elderly. This is explained by the preferential activation of fast-type motor units during forceful, lengthening-type (i.e. eccentric) contractions of knee extensor groups with downhill turns on skis (Berg et al., 1995; Byrne & Eston, 2002). This view is supported by the correspondingly larger cross-section of fast- vs slow-type fibers in ski athletes (Andersen & Montgomery, 1988). Because of the unusually slow nature of the breaking movements with descent on the slope (Berg et al., 1995), it remains to be tested whether the consequent muscular adaptations are selective for the fast fiber pool. Because elderly subjects, women in particular, show a reduced anabolic response to exercise (Bamman et al., 2003; Kumar et al., 2009) and a diminished capacity to repair damaged fibers with muscle overload (Brooks & Faulkner, 1994; Fulle et al., 2005), it is possible that elderly subjects may not demonstrate significant fiber growth in response to an eccentric type of exercise like alpine skiing.

Sarcolemmal focal adhesion complexes (costameres) are important for the structural integrity of muscle by connecting muscle fibers via the surrounding extracellular matrix to adjacent muscle fibers (Grounds et al., 2005). Consequently, costameres are believed to modify lateral force transmission via

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reinforcement of the muscle fiber composite (Huijing, 1999; Bloch & Gonzalez-Serratos, 2003). Culture studies indicate that costamere assembly is also important for muscle growth by controlling sarcomerogenesis via a mechanism that involves focal adhesion kinase (FAK; Quach & Rando, 2006). This is supported by observations showing that enlargements of muscle size with increased loading involve expression changes in costameric proteins FAK, gamma-vinculin and meta-vinculin (Chopard et al., 2005; Durieux et al., 2009; Flueck et al., 2010) and are driven by a FAK-dependent mechanism in skeletal muscle (Durieux et al., 2009). Thereby, adhesive and de-adhesive mechanisms appear to interplay in controlling the attachment of muscle fibers to the surrounding extracellular matrix (Gullberg et al., 1998; Fluck et al., 2008). For instance, expression of the extracellular regulator of focal adhesion disassembly, tenascin-C (Fluck et al., 2002; Midwood & Schwarzbauer, 2002), is strongly up-regulated in the interstitium of muscle that is subjected to eccentric loading paradigms (Crameri et al., 2004; Fluck et al., 2008). In mice, the up-regulation of tenascin-C with muscle overload is important for the maintenance of the mean cross-sectional area (MCSA) of fast-type muscle fibers through the activation of myogenesis in overload-damaged muscle fibers (Fluck et al., 2008). The partial detachment of muscle fibers from the strained extracellular substrate in overloaded muscle is permissive for repair mechanism (Fluck et al., 2008) and is integrated with adhesive processes in the interstitium such as the increased expression of the cross-linker of collagen fibrils, collagen XII (Fluck et al., 2000). The relationship of load-dependent expression of pro- and de-adhesive regulators of focal adhesions (Fluck et al., 2002; Chopard et al., 2005; Flueck et al., 2010) with the growth and reinforcement of muscle fibers in human muscle remains to be explored.

To gain an understanding of the mechanism underlying the suggested benefit of ski training in the elderly, we aimed at addressing changes in the MCSA of slow and fast contractile muscle fibers in the major knee extensor muscle, *m. vastus lateralis*, in relation to muscle abundance of mechano-sensitive adhesion proteins and improved function of the implicated muscle–tendon complex. Our hypothesis was that a 12-week intervention of downhill skiing would produce hypertrophy of muscle fibers, which would be larger in fast- than slow-type muscle fibers and be related to expression changes of costameric proteins. Because of the reported blunted anabolic response in women compared with men (Kumar et al., 2009), we speculated that the hypertrophic response to downhill skiing would be amplified in males compared with females.

Material and methods

Study design

This study reports on muscle parameters of 20 elderly Austrian subjects (11 males; nine females) who completed the 12-week downhill skiing protocol of the Salzburg Alpine Skiing for Elderly Study (SASES) described in the accompanying paper (see Müller et al., 2011a). Subjects were recruited from the local population based on the criteria of previous experience with skiing. Anthropometry and muscle strength measures, i.e. "maximal isokinetic strength" using a leg press with both legs and for each single leg and "maximal isometric strength" (moment) during both leg extension and flexion of the knee at 90° were performed before and after the 12 weeks of skiing. A muscle biopsy was collected at baseline and after training with a Weil-Blakely conchotome (Gebrueder Zepf Surgical Instruments, Dürbheim, Germany) from *m. vastus lateralis* of the left leg, snap frozen during rapid shaking in liquid nitrogen and stored at −80 °C for the analysis of muscle fiber types and expression of adhesion proteins. Muscle samples from two of the originally 22 subjects who completed the study were not assessed by biochemical and cellular means due to unsatisfactory yield of protein isolation and sample integrity, respectively. All study protocols were approved by the Ethics Committee of the University of Salzburg in Austria and a written informed consent was obtained from all participants before any testing.

Protein expression

The content of adhesion proteins per skeletal alpha actin was basically assessed as described previously (Giraud et al., 2005; Fluck et al., 2008). Twenty-five-micrometer cryosections from the frozen biopsy material corresponding to an estimated volume of $10\,mm^{-3}$ were collected in a frozen 2 mL tube (Eppendorf, Cambridge, UK). Cryosections were homogenized in 200 µL of an ice-cold modified radio immuno precipitation buffer [50 mM 2-Amino-2-(hydroxymethyl)-1,3-propanediol-hydrochloride (Tris-HCl) pH 7.5, 150 mM NaCl, 1 mM ethylenediaminetetraacetic acid, 1% Nonident P-40, 0.25% sodium deoxycholate at 90%, 1 mM sodium orthovanadate] containing protease inhibitors (1 µg/mL leupeptin, 2 µg/mL pepstatin, 1 µg/mL aprotinin, 0.1 mM phenylmethylsulfonyl fluoride; Sigma, Buchs, Switzerland) using a rotor stator mixer (Ultraturrax, IKA Werke GmbH & Co. KG, Staufen, Germany). The protein concentration in the resulting homogenate was estimated using the bicinchoninic acid protein assay (Pierce, Thermo Fisher Scientific, Cramlington, UK) against bovine serum albumin (BSA). Total homogenate was solubilized at 1 mg/mL in Laemmli buffer (50 mM Tris-HCL, pH 6.8, 10% glycerol, 2% SDS, 2% mercaptoethanol, 0.1% bromophenol blue), denatured by heating for 5 min at 95 °C and spun (1 min at 10 000 g). Equal amounts of denatured total muscle protein per sample lane were resolved via 7.5% sodium dodecyl sulfate-poly acrylamide gel electrophoresis using a Mini-Protean III system (Bio-Rad Laboratories Ltd, Hemel Hempstead, UK) and blotted on a nitrocellulose membrane.

The membrane was stained with Ponceau S to inspect for the quality of protein transfer and the signal of the alpha actin band at 42 kDa was determined (Gel-Doc system, Bio-Rad Laboratories Ltd). Subsequently, membranes were subjected to immunodetection for different adhesion proteins [pro-adhesive: FAK, gamma-vinculin, meta-vinculin and collagen XII; de-adhesive: tenascin-C and FAK-related non-kinase (FRNK)]. In brief, the membrane was blocked within 2.5% semi-dried milk/1% BSA in Tween-Tris-buffered saline (1% Tween-20, 10 mM Tris pH 7.4, 0.9% NaCl), incubated with

Table 1. List of detected adhesion proteins and their antibodies being used

Protein	Molecular weight (kDa)	Antibody reference
Tenascin-c	201, 231, 241	Fluck et al. (2000)
Collagen XII	206	Fluck et al. (2000)
Focal adhesion kinase (FAK)	125	Fluck et al. (1999)
FAK-related non-kinase (FRNK)	42	Fluck et al. (1999)
Gamma-vinculin	130	Glukhova et al. (1990)
Meta-vinculin	150	Glukhova et al. (1990)

first antibody against adhesion proteins and secondary antibody with washing steps in between (see Table 1). Signal detection was carried out with enhanced chemoluminescence using a Geldoc system (Bio-Rad Laboratories Ltd).

For the purpose of comparing the protein content between samples, a specific loading design was used: 10 μg of total protein in Laemmli buffer from Pre- and Post-training pairs were loaded in adjacent lanes. Four pairs were loaded per gel. For each protein, the relative content was calculated by first subtracting the background from an empty sample lane and then standardizing the resulting difference for each sample to the intensity of the alpha actin band of the respective sample/lane. Standardized protein signals from each immunodetection on a blotted membrane were then normalized to the mean of the protein signals in the Pre-samples on each blot and pooled between immundetection experiments to reveal for each sample and protein the relative protein content with respect to the baseline (i.e. Pre-ski levels).

Muscle fiber types

Twelve-micrometers cryosections were prepared from *m. vastus lateralis* biopsies and subjected to staining for type I or type II myosin heavy chain and nuclei essentially as described by Fluck et al. (2008). For the purpose of accurately assessing the MCSA, cryosections were inspected for optimal perpendicular cutting angle by visualizing the fiber profiles under a microscope. The criteria for accepting a specific cutting angle were that the majority of the fiber profiles showed a ratio between the smallest and the largest diameter of 0.66 and above and that no further reduction of this ratio was apparent despite tilting the biopsy before sectioning. Fiber profiles of 15 Pre/Post-skiing biopsy pairs were of sufficient quality for a quantitative analysis based on the criteria of optimal cutting angle and freeze fractures.

Type I and type II myosin heavy chain stained sections were recorded digitally at 10 × magnification on an Axioskop 2 microscope (Carl Zeiss Ltd, Welwyn Garden City, UK) that was operated with AxioVision software (Carl Zeiss Ltd). The comparison indicated that the majority of fibers were pure fibers. Subsequently, one field from type II myosin heavy chain stained sections was assessed for cross-sectional area of stained (fast type) and unstained (slow type) fibers against a scale by manually recording the fiber periphery of each assessed fiber within the ARDOM software (Degens et al., 2002). These numbers were used to calculate the percentage and MCSA of slow- and fast-type muscle fibers and the area content of slow-type fibers. The latter parameter was calculated using the formula: [area content of slow-type fibers = percentage of slow-type fibers × mean cross-sectional area of slow-type fibers/100%]. On average, 56 slow- and 49 fast-type muscle fibers were counted per muscle cross-section.

Statistics

The effects of "training" and the interaction effect of "training" × "gender" were evaluated for each parameter separately based on a repeated analysis of variance (ANOVA) using Statistica 9.0 (Statsoft, Tulsa, Oklahoma, USA). *Post hoc* effects were localized with a Fisher test. Gender effects before exercise were evaluated with an unpaired *T*-test. In cases where a one-sided hypothesis could be formulated based on published evidence (i.e. tenascin-C, collagen XII, FAK, fiber cross-sectional area; Fluck et al., 2002, 2008; Crameri et al., 2004; Jones et al., 2009), a one-sided *post hoc* test was applied. *P*-values <0.05 were considered significant and $0.05 \leq P < 0.10$ were considered as a tendency.

Linear relationships between parameters were assessed using Pearson's correlations (Statistica 9.0). Correlations with $P<0.05$ and $r>0.60$ were deemed biologically significant. In order to localize linear relationships, the correlation coefficients of significant correlations were displayed in color coding from gray (negative) to black (positive) in a correlation matrix with Treeview as described (Fluck et al., 2005). In order to warrant the best visual resolution, image contrast was set to 0.9 and correlations with *r*-values below 0.60 or *P*-values >0.05 were masked as white for display in Treeview (software publicly available through http://rana.lbl.gov/EisenSoftware.htm). The resulting figure was exported into CorelDRAW X3 vs 13 (Corel Coorporation, Fremont, California, USA) to allow for final assembly in Microsoft Powerpoint 2002 (Microsoft, Kildare, Ireland).

Results

Baseline data

Anthropometry, parameters of extensor muscle function and the MCSA of fiber types in extensor *m. vastus lateralis* of the subjects before downhill ski training are provided in Table 2.

Effect of downhill skiing on muscle fibers and adhesion factors

The 12 weeks of downhill skiing increased the MCSA of slow-type fibers in *m. vastus lateralis* by 1.4-fold (Fig. 1). MCSA of fast-type fibers showed a tendency ($P = 0.08$) toward a 1.4-fold increase after skiing. Concomitantly, maximal isokinetic strength of extensor muscles and patellar tendon stiffness were increased by 15%, each, in the subjects whose biopsy samples were subjected to cell-molecular analysis.

From the assessed adhesion proteins, the levels of the extracellular protein tenascin-C and its downstream effecter, FAK, were increased 1.6-fold and 1.7-fold, respectively (Fig. 2).

Relationships between myocellular and functional parameters at baseline

The main observation was that positive, linear relationships with $r>0.6$ and $P<0.05$ existed between MCSA of slow- and fast-type fibers and maximal isokinetic strength in both legs [Fig. 3 (a and b)].

Table 2. Structural and functional parameters of the subjects at baseline

	All	Male	Female	*P*-value
Age (years)	67.0 ± 0.5 (20)	67.0 ± 0.7 (11)	67.0 ± 0.6 (9)	0.28
BMI (kg/m^2)	26.9 ± 0.7 (20)	27.9 ± 0.8 (11)	25.4 ± 1.2 (9)	0.13
Maximal isokinetic strength (N) during knee extension				
Both legs	1911.3 ± 159.9 (18)	2855.6 ± 168.8 (9)	1582.9 ± 97.4 (9)	<0.001
Right leg	1185.8 ± 69.8 (19)	1426.7 ± 70.4 (10)	1007.6 ± 57.2 (9)	<0.001
Left leg	1194.1 ± 86.0 (18)	1471.3 ± 100.4 (10)	930.5 ± 56.3 (8)	<0.001
Maximal isometric strength (N m) at 90°				
Knee extension	140.9 ± 10.1 (20)	187.5 ± 7.8 (11)	99.5 ± 7.1 (9)	<0.001
Knee flexion	68.7 ± 5.0 (19)	89.5 ± 5.0 (10)	51.5 ± 3.7 (9)	<0.001
Patellar tendon stiffness (N/mm)	2325.2 ± 132.3 (19)	2567.8 ± 202.6 (10)	1994.0 ± 138.5 (9)	0.04
Fiber cross-section (μm^2)				
Slow type	5500.0 ± 390.5 (17)	5770.0 ± 508.3 (11)	5192.9 ± 419.4 (6)	0.08
Fast type	4899.8 ± 502.5 (17)	5160.0 ± 552.5 (11)	3265.0 ± 382.2 (6)	0.003
Slow fiber%	53.3 ± 4.0 (17)	54.9 ± 5.3 (11)	50.3 ± 6.1 (6)	0.60

Values represent median ± standard error. Numbers in brackets reflect the number of subjects. *P*-values refer to the statistical significance of differences between genders.

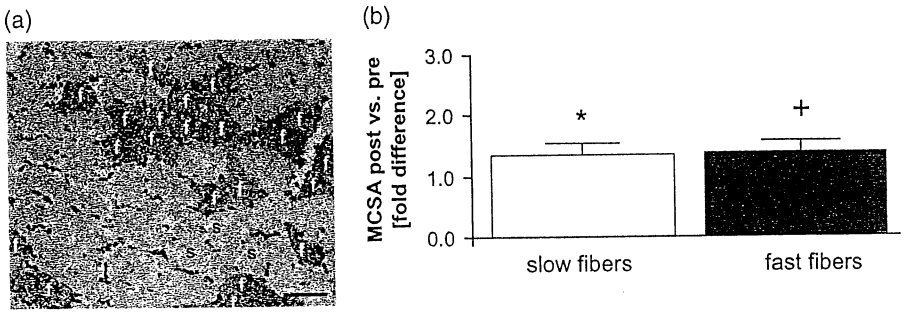

Fig. 1. Overview of the changes in the mean cross-sectional area (MCSA) in slow- and fast-type muscle fibers with 3 months of downhill skiing. (a) Black and white image showing fiber type detection in a microscopic field of an immunohistochemically stained cryosection from *m. vastus lateralis* of a male subject before ski training. All visible fast-type fibers (denoted as "f") and three slow-type fibers (s) are indicated. Bar denotes 100 μm. (b) Bar graphs indicate the mean fold change and standard error (SE) with 3 months of downhill skiing for MCSA of slow and fast fiber types in the assessed subjects ($n = 15$). Asterisk and cross denote significant ($P<0.05$) and tendinous ($0.05 \leq P<0.10$) effect of training based on a paired, one-tailed *t*-test.

MCSA of fast-type fibers was correlated with maximal isometric strength at 90° knee extension [Fig. 3 (a and b)]. Equally, a significant positive correlation existed between collagen XII content and fast fiber percentage [$r = 0.75$ and $P<0.05$; Fig. 3(b)].

Correspondences between myocellular and functional changes with skiing

Considering the training effect, it appears that the fold changes in the MCSA of slow and fast fibers were positively correlated [*r*-value of 0.91; Fig. 3(b)]. Fold changes in tenascin-C expression correlated positively with the fold changes in the area content of slow-type fibers ($r = 0.79$, $P = 0.001$). At a lower significant level, the fold changes in MCSA of slow-type fibers were correlated with the fold changes in maximal isometric strength at 90° knee extension ($r = 0.55$, $P = 0.047$). The fold changes in the content of the costamere component gamma-vinculin and maximal isometric strength of knee extensors were correlated ($r = 0.64$, $P = 0.01$). The positive relationships between MCSA of fast- and slow-type fibers and isokinetic maximal strength were main-

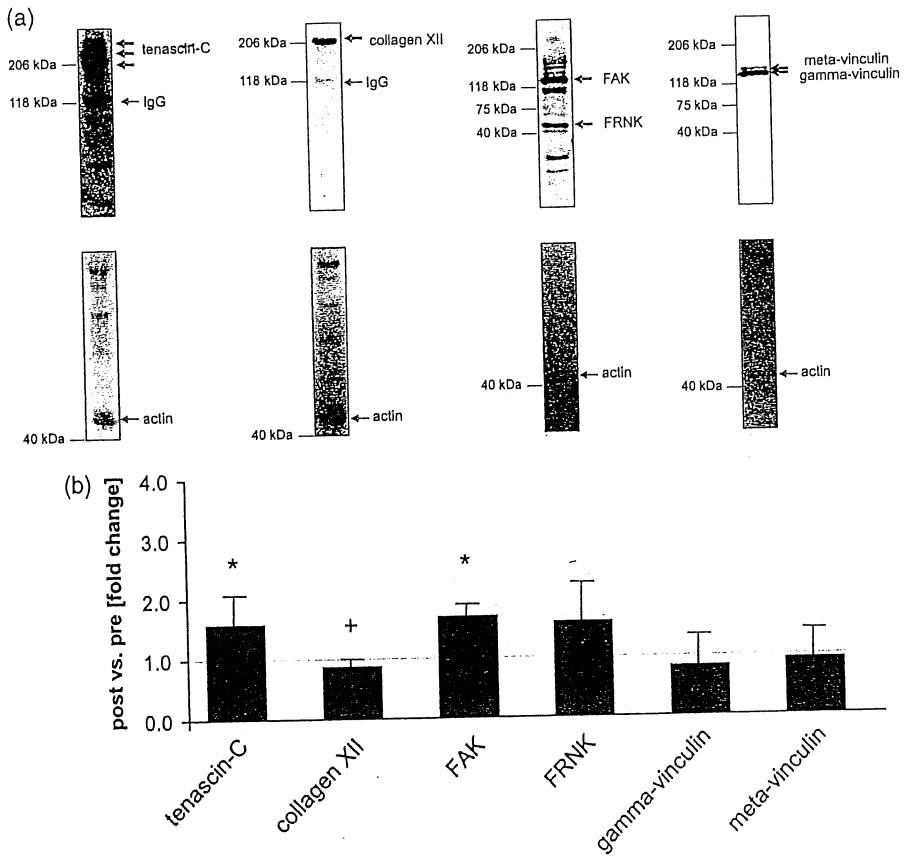

Fig. 2. Overview of ski training-induced changes in adhesion proteins. (a) Examples of detected proteins in immunoblots Pre- and Post-training. (b) Bar graphs showing the median fold change and standard error in respective protein expression with skiing. Asterisk and cross denote a significant effect ($P<0.05$) and trend for an effect ($0.05 \leq P<0.10$) of ski training. The extended horizontal line indicates the ratio of 1.

tained in ski-trained muscle (r-values of 0.70 and 0.59, respectively).

Gender effects

ANOVA identified a main effect of gender for MCSA of fast-type muscle fibers and the content of the endogenous FAK inhibitor, FRNK at baseline. Fast fiber MCSA was 48% lower in females than in males. The FRNK content was 95% higher compared with males.

A tendency toward a "gender" × "ski training" interaction was evident for changes in collagen XII ($P=0.06$) due to a selective down-regulation in females in ski-trained muscle [Fig. 4(a)]. Gender-specific regulation with ski training was also noted for other factors, without the gender × training effect reaching significance. This was for MCSA of fast-type fibers, which was significantly 1.7-fold increased in females but not altered in males [$P=0.29$, one-way ANOVA; Fig. 4(e)]. The extracellular protein tenascin-C was 1.7-fold increased in the female population, whereas the change in males was borderline significant [1.1-fold, $P=0.06$; Fig. 4(c)]. Conversely, the FAK levels were 1.9-fold elevated in males [Fig. 4(b)] but unaltered in females (1.1-fold, $P=0.22$).

Gender-specific relationships

A number of linear relationships were apparent between adhesion proteins and muscle variables that differed between genders [Fig. 3 (c and d)]. At baseline, this was for correlations for the focal adhesion regulators, FAK and tenascin-C in males and females, respectively, with the composition and MCSA of fiber types [Fig. 3 (c and d)]. In males, there was also a negative linear relationship between the FAK content and the percentage of slow-type fibers [$r=-0.72$, $P=0.03$; Fig. 3(c)].

Gender-specific correlations in fold changes with skiing led to the negative correlation between FAK content and the percentage of fast-type fibers ($r=-0.97$, $P=0.006$) and between meta-vinculin and maximal isometric strength of knee extension at 90° in females [$r=-0.74$, $P=0.04$; Fig. 4(d)]. In males, positive correlations were seen between fold changes in FAK and collagen XII ($r=0.60$), changes in gamma-vinculin and maximal isometric strength

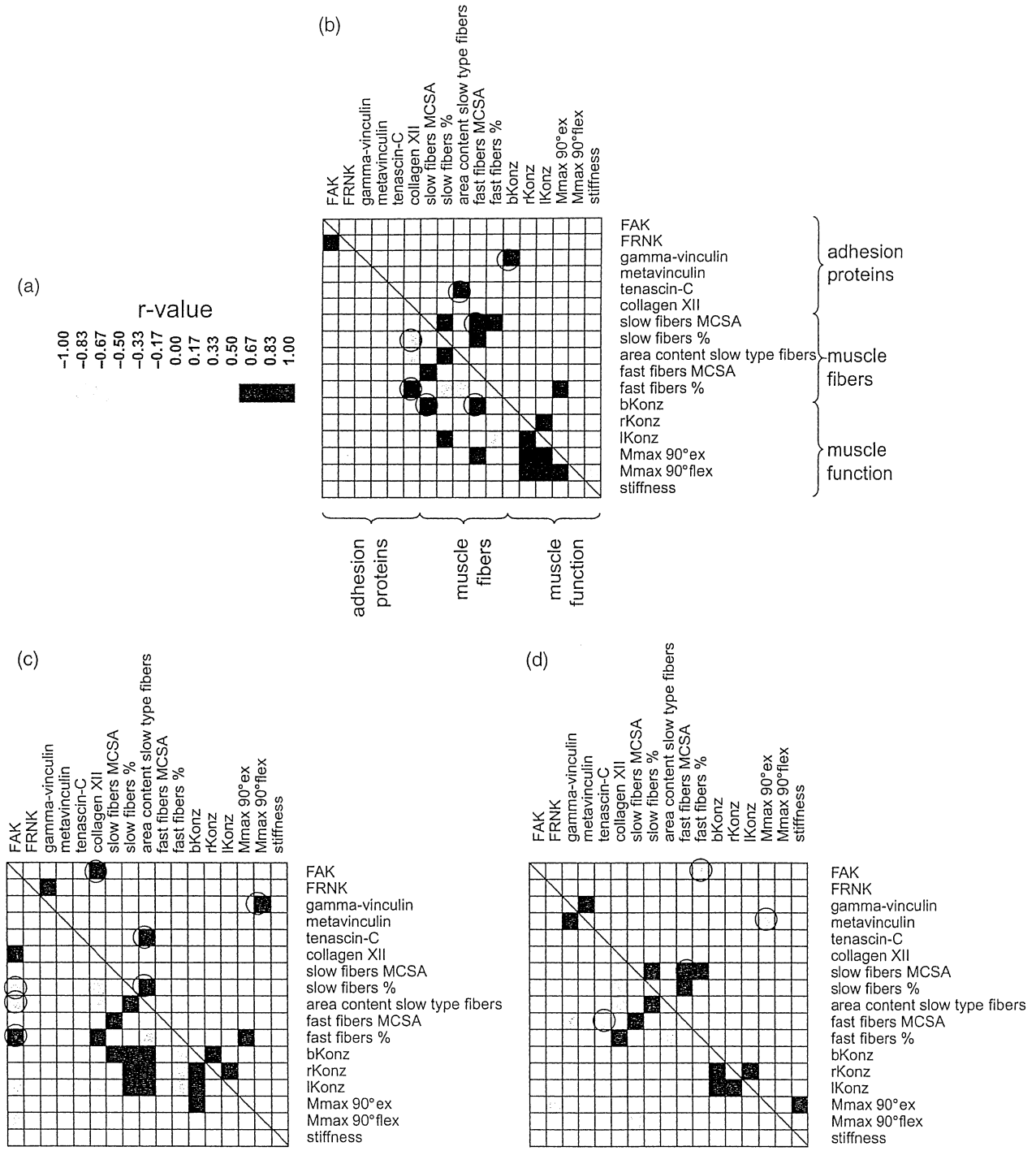

Fig. 3. Matrices visualizing Pearson's correlations between measured muscle parameters at baseline and the fold change with downhill skiing. (a) Black and white scale of the correlation matrix. Matrices over the entire study population (b) and separated for gender; males (c) and females (d). *R*-values are given in black and white coding as squares, with black and gray indicating positive and negative *r*-values, respectively. Relationships with $r < 0.65$ are given in black. Gray pixels along the diagonal reflect "line of identity." Squares below the "line of identity" refer to correlations at baseline; those above the line reflect correlations between fold changes with skiing. Squares reflecting correlations that are of particular interest are circles. bKonz, maximal isokinetic strength of both legs during knee extension; rKonz, maximal isokinetic strength (concentric force) of the right leg during knee extension; lKonz, maximal isokinetic strength of the left leg during knee extension; Mmax 90°ex, maximal isometric strength (moment) at 90° during knee extension; Mmax 90°flex, maximal isometric strength (moment) at 90° during knee flexion; stiffness; stiffness of the patellar tendon.

of knee flexion at 90° ($r = 0.78$), and between changes in tenascin-C content and area content of slow-type fibers ($r = 0.85$). After skiing, a positive relationship was found between the FAK content and the slow fiber type percentage in the *m. vastus lateralis* of male subjects ($r = 0.71$, $P = 0.08$).

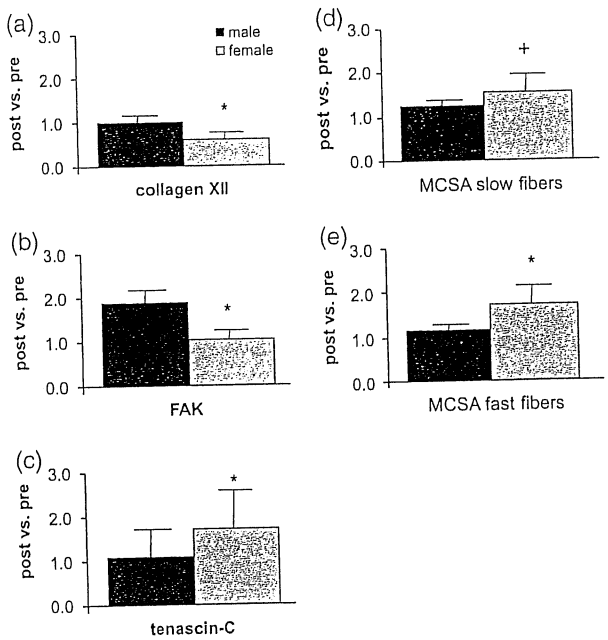

Fig. 4. Bar graphs visualizing the gender effect on muscle variables. (a–c) Median and standard error of fold changes in the content of collagen XII and focal adhesion kinase (FAK) and tenascin-C protein in the male and female subset with skiing. (d, e) Fold changes in the mean cross-sectional area (MCSA) of slow- and fast-type fibers with skiing. Asterisk and cross denote significant effect ($P<0.05$) and trend for an effect ($0.05 \leq P<0.10$) of ski training.

Discussion

Skiing places a particularly high mechanical demand on activated muscle groups during downhill turns (Berg et al., 1995). Based on observations in skilled skiers, it is thought that downhill ski training is associated with a specifically improved force in extensor muscles at low angular velocities (Tesch, 1995; Turnbull et al., 2009). It is not understood to what extent myocellular adjustments contribute to these strength gains and what might occur in untrained subjects. We addressed this question in the extensor *m. vastus lateralis* in an elderly population of men and women because this muscle is known for its strength deficits with aging (Tracy & Enoka, 2002; Yu et al., 2007; Turnbull et al., 2009). Our results show for the first time that downhill skiing promotes hypertrophy of fast- and slow-type muscle fibers in connection with adjustments in the expression of adhesive proteins and gender-specific improvements in extensor muscle function.

A number of limitations of our study are worth noting. The first constraint relates to the small number of subjects for whom biopsy material of sufficient quality for the measure of muscle fiber structure Pre- and Post-training (i.e. 15 out 28) was available. The number of correlations between molecular, cellular and functional variables of the path that sets muscle strength that met the selective criteria of $P>0.60$ and $r<0.05$ supports that our approach developed an acceptable biological power despite numerical limitations in the sample number. The notion of the good resolution of our correlative approach is supported by the observation showing that correlations between cell-molecular variables and "maximal isokinetic strength" at baseline were more extensive for the leg where the biopsy was collected. For instance, additional relationships are apparent between slow and fast fiber percentage (i.e. "slow fiber%," "fast fiber%") as assessed in biopsies from the left leg vs the "maximal isokinetic strength" of single legs [Fig. 3(b)]. Significant correlations are seen between fiber percentages and "maximal isokinetic strength" of the left leg (lKonz) but not the right leg (rKonz). Equally, exclusive linear relationships are evident between "maximal isokinetic strength" of the left, but not the right leg, and "FAK" and "collagen XII" in biopsies from male participants [Fig. 3(c)]. A second limitation relates to the fact that the skiing "intensity" of participants was adjusted to the skill level before the intervention. Thereby, the lower ability of female participants to develop force likely carried over on the imposed training stimulus and its effect. This view is supported by the observation that hypertrophy of slow and fast fibers was negatively correlated with MCSA of the respective fiber type at baseline, i.e. r values of -0.55 and -0.58 ($P<0.04$). This compares to an increase in MCSA of slow fibers and a tendency toward an increase in fast fibers with ski training [Fig. 1(b)]. This relationship indicates that the variability in fiber growth with recreational skiing largely reflects the functional deficit of the population at baseline. Lastly, due to the inclusion criteria, our findings on muscle adjustments with downhill skiing may only reflect the situation in the elderly population.

Morphometric analysis of the biopsy samples identified that maximal concentric force (i.e. maximal isokinetic strength) of ski-trained muscles was overall correlated with MCSA of fast-type fibers ($r = 0.70$, $P = 0.003$) and at a lower r-level with MCSA of slow-type muscle fibers ($r = 0.59$, $P = 0.016$). This correspondence represents the role of muscle fibers in force production (Reggiani et al., 2000). Alike, the content of the costamere components, gamma-vinculin and meta-vinculin, were correlated with changes in maximal isokinetic strength and maximal isometric strength of knee extension [Fig. 4 (b and d)]. This indicates that the variability in improvement of muscle strength in the elderly with downhill skiing can be explained to a considerable degree by the reinforcement of the contact zone between muscle fibers. This suggests a role of both fiber hypertrophy and modified fiber adhesion in strength gains due to skiing. This also highlights the important, but compared with neurological factors often

underestimated, role of mechanical factors in the prevention of sarcopenia (Vandervoort, 2002; Aagaard et al., 2010).

In contrast to our hypothesis, we did not find evidence for a significantly different hypertrophy of fast compared with slow-type fibers independent on whether data were analyzed separately for gender or combined for the entire population. In fact, the changes in MCSA of slow- and fast-type muscle fibers were highly correlated ($r = 0.94$, $P < 0.001$). This similarity in muscle hypertrophy between fiber types with ski training in a population with previous skiing experience suggests that the training scheme activated both slow- and fast-type motor units. Possibly this relates to the generally slow nature of eccentric movements with downhill skiing (Berg et al., 1995).

When the response of fast-type muscle fibers to skiing was analyzed separately, it revealed that the changes in fast muscle fiber MCSA showed gender dependence, resulting in a significant 1.3-fold increase in females ($P = 0.047$) vs a non-significant 1.1-fold increase in males ($P = 0.310$). This observation for larger fiber hypertrophy during load-bearing ski training in female compared with male participants is in contrast with the literature. It has been reported that fast (and slow) fiber types show a lower degree of hypertrophy with conventional resistance training in elderly women than elderly men (Bamman et al., 2003). Our finding on the effects of downhill skiing thus rejects the assumption of a generally reduced hypertrophic response in women compared with men with load-bearing types of exercise. This finding indicates that the postulated anabolic resistance in untrained female subjects is overridden by repeated exercise (Kumar et al., 2009). In this regard, the elevated content of the negative regulator of the FAK-dependent protein synthetic pathway, FRNK, in females before training is of interest (Qin & Liu, 2006; Durieux et al., 2009; Kumar et al., 2009). It suggests FRNK as a new candidate signaling factor that underlies basal anabolic resistance to load-bearing exercise in healthy, elderly subjects.

The notion of gender-specific muscular reactions to downhill skiing was corroborated by the altered content of adhesion factors FAK, collagen XII and tenascin-C in *m. vastus lateralis* (Fig. 4). The selective increase in the content of FAK [Fig. 4(b)] with skiing relates to our previous finding in rodents that demonstrate increases of subsarcolemmal FAK content in load-bearing muscle fibers (Fluck et al., 2002). Interestingly, the changes in the FAK content in male participants were correlated with the fold changes in the collagen type I fibril-associated collagen XII (Fluck et al., 2000). Coincidentally, the male participants demonstrated an inversion of the linear relationship between FAK content and slow fiber type percentage at baseline with skiing (i.e. $r = -0.72$ vs $r = 0.71$). These relationships support the notion of a "feed forward" control over fiber adhesion in human muscle by muscle activity/loading as pointed out previously in rats (Fluck et al., 2002).

The skiing-induced association of changes in the content of the structural components of costameres, i.e. gamma-vinculin and meta-vinculin, with parameters of muscle strength is of fundamental interest in light of the postulated role of costameres in force transmission (Huijing, 1999; Bloch & Gonzalez-Serratos, 2003). Concerning the extensor function of *m. vastus lateralis*, correlations were revealed between the costamere marker, gamma-vinculin, with gains in maximal isokinetic strength of extensor muscle after skiing [$r = 0.64$, $P = 0.01$; Fig. 3(b)], and between changes in meta-vinculin content with changes in maximal isometric strength of knee extension at 90° [$r = -0.74$, $P = 0.04$; Fig. 3(d)]. These linear relationships provide the first support indicating that changes in biomechanical muscle functioning in humans occur in parallel with changes in costamere composition.

The notion of a relationship between fiber adhesion and altered muscle strength is supported by the elevated content of the de-adhesive protein tenascin-C in females with ski training. Tenascin-C content is important for fiber hypertrophy in overloaded muscle of rodents (Fluck et al., 2008) and its up-regulation relates to gains in muscle mass with overload (Fluck et al., 2005). To our understanding, the identified parallelism between elevated tenascin-C content, muscle hypertrophy and enhanced concentric force of knee extension in female participants provides the first support for a role of tenascin-C in control of muscle anatomy and function in humans.

The former observations relate to the important control of tenascin-C expression in musculo-skeletal tissues (i.e. muscle, tendon, bone) by mechanical loading in a number of vertebrate species (Fluck et al., 2000, 2003; Chen et al., 2003; Crameri et al., 2004). In this regard, the tendency toward a positive correlation (i.e. $P = 0.10$, $r = 0.62$) between tenascin-C content in extensor muscle and the 1.14-fold improved maximal isometric strength of knee flexion in females is of interest. By contrast, this linear relationship was negative in males ($r = -0.64$, $P = 0.05$) whose maximal isometric strength of knee flexion was not altered ($P = 0.24$). This gender-specific inversion relates to the interaction effect of changes and training on patellar tendon stiffness with skiing ($P = 0.04$), where males showed significantly larger increases (Seynnes et al., 2011). Coincidentally, we found that the elevated tenascin-C expression in ski-trained female muscle correlated to stiffness of the attached patellar tendon as well ($r = 0.67$, $P = 0.07$). These relationships are puzzling,

given that the *m. vastus lateralis* is frequently subjected to eccentric loading with knee flexion during downhill turns with the skis on the slope (Berg et al., 1995). Our findings now suggest a connection between maxima of the possible flexor moments in a lab test with the content of a protein (tenascin-C) known to be controlled by eccentric loading. This correspondence supports the idea that the regulation of tenascin-C protein expression and fiber hypertrophy in *m. vastus lateralis* with ski-training directly relates to eccentric loading of the implicated muscle–tendon complex with a maximal effort during downhill skiing.

Perspectives

The correspondence identified between alterations in size and adhesion of muscle fibers and extensor muscle function and attached tendon has relevance for rehabilitation and exercise physiology. It points out that skiing is a suitable countermeasure against sarcopenia in a healthy elderly population. The gender effect identified warrants further exploration as it suggests that the therapeutic effect of downhill skiing is pronounced in subjects with a deficit in muscle functionality before such an intervention.

Key words: muscle, human, eccentric, extracellular matrix, focal adhesion, costamere, tenascin-C, focal adhesion kinase.

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