Journal of Dermatological Science (2005) xxx, xxx–xxx

Journal of Dermatological SCIENCE

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LETTER TO THE EDITOR

KEYWORDS
Growth/differentiation factors;
Bone morphogenesis proteins;
GDF-5;
Brachypodism;
Skin

GDF-5 deficiency alters stress–relaxation properties in mouse skin

To the Editor:

Growth/differentiation factors (GDFs) are a subset of the bone morphogenetic proteins (BMPs), a class of proteins known to have diverse effects on musculoskeletal connective tissue. GDF-5 (*a.k.a.* BMP-14) is the most closely studied GDF to date. A GDF-5 deficient animal model (the *brachypodism* mouse) exists [1], and studies of this mouse demonstrate altered biomechanical, ultrastructural, and compositional properties in numerous tissues rich in type I collagen [2–4]. Achilles' tendons from mutant mice contained less collagen per DNA and were more compliant and weaker than control tendons in quasi-static tests. In tail tendons, mutants did not differ from controls in glycosaminoglycan or collagen quantity, but demonstrated an increase in irregularly shaped fibrils [3]. Furthermore, although GDF-5 deficient tail tendons demonstrated no differences in quasi-static testing, they exhibited 11% slower relaxation and a 4% smaller extent of relaxation during time-dependant stress–relaxation tests. Finally, GDF-5 deficient femora exhibited 31% lower maximum torque to failure and were 57% more compliant than controls [4], due at least in part to inferior material properties (31% smaller shear modulus).

In total, although no consistent pattern of changes between weight-bearing (e.g. long bone, Achilles tendon) and non-weight-bearing (e.g. tail tendon) tissues has been demonstrated, studies suggest that a primary effect of GDF-5 deficiency may be on the biomechanical behavior of type I collagen-rich tissue. We were interested in how GDF-5 deficiency affects the biomechanical behavior of other non-weight-bearing tissues containing large proportions of type I collagen, and hypothesized that skin from mice deficient in GDF-5 would also exhibit altered biomechanical properties.

Skin from 12 GDF-5 deficient (−/−) mice was compared with skin from 12 age-matched heterozygous (+/−) littermates. Six mice from each group were used for quasi-static tests, and six for viscoelastic tests. Dorsal samples oriented symmetrically across the midline were harvested, and using a custom punch-out tool, a standardized dumbbell-shaped specimen (also longitudinally centered) was cut. Specimen thickness and width was recorded, and for quasi-static tests, samples were tested to failure in tension at a strain rate of 10% per second (Fig. 1A). Load versus extension data were recorded and stress versus strain curves constructed. Maximum stress to failure, strain at maximum stress, modulus of elasticity (slope of the linear region of the stress versus strain curve), and strain energy density (area under the curve) were compared between groups. For time-dependant analyses (Fig. 1B), specimens were maintained for 90 min at 38% strain (this value fell safely on the linear region of the quasi-static curve for all previous specimens tested). Load versus time data were recorded, stress versus time curves calculated and peak stress, equilibrium stress, and extent of relaxation [(1-end stress/peak stress) × 100] determined. The relaxation time constant was obtained by fitting a power curve [stress = $a \times \text{time}^{-b}$] to each specimen, with b = relaxation constant.

Qualitative analysis of collagen fibril size, shape, and density was performed on additional samples from one GDF-5 (−/−) and one GDF-5 (+/−) mouse. After preparation, 70 nm sections were cut, stained, and photographed at 40,000×. For each photo, mean fibril diameter, fibril numerical density (number of fibrils per area), and fibril area fraction (percentage of photo occupied by fibrils) were determined. A polymorphism factor (PF) was calculated for each fibril by dividing perimeter by the circumference of the largest circle residing entirely inside that fibril.

0923-1811/$30.00
doi:10.1016/j.jdermsci.2005.07.002

Table 1A Biomechanical and ultrastructural results (mean ± S.D.) of GDF-5 deficient skin

	GDF-5 (+/−) (N = 6)	GDF-5 (−/−) (N = 6)
Quasi-static tests		
Maximum stress (MPa)	3.28 ± 0.27	2.96 ± 0.52
Strain at maximum stress (%)	58.7 ± 10.8	57.7 ± 4.7
Modulus of elasticity (MPa)	10.04 ± 2.16	8.65 ± 2.07
Strain energy density (MPa)	0.728 ± 0.082	0.713 ± 0.134
Stress–relaxation tests		
Maximum (peak) stress (MPa)	1.070 ± 0.226	0.717 ± 0.287†
Equilibrium (end) stress (MPa)	0.242 ± 0.049	0.186 ± 0.044
Extent of relaxation (%)	77.13 ± 2.42	73.51 ± 3.03†
Relaxation time constant	0.156 ± 0.013	0.141 ± 0.010‡
Ultrastructural analysis[a]		
Mean fibril diameter (nm)	88 ± 6	91 ± 7
Fibril numerical density (μm^2)	61 ± 11	52 ± 9
Fibril area fraction (%)	62 ± 4	54 ± 3
Polymorphism factor (PF)	1.13 ± 0.02	1.11 ± 0.02

[a] N = 1 for each group.
† $p < 0.05$.
‡ $p \approx 0.055$.

Fibrils with a PF of 1.0–1.1 were considered circular; PF values greater than 1.1 were considered polymorphic. Parameters from all photos for each mouse were averaged and qualitatively compared.

Results are provided in Table 1A. GDF-5 deficient skin was 9.8% thinner than control skin (0.479 ± 0.050 mm versus 0.531 ± 0.052 mm; p = 0.021). Maximum stress to failure was 10% lower in mutants, but this difference was not significant (p = 0.20). In stress–relaxation conditions, maximum stress was 33% less in GDF-5 deficient skin (p = 0.04), whereas differences in equilibrium stress did not reach significance (−23% in mutants; p = 0.12). Mutant skin also exhibited a 5% lower extent of relaxation (p = 0.046). Finally, the relaxation time constant was 10% lower in the GDF-5 deficient skin. No striking differences were noted in mean fibril diameter or polymorphism (Fig. 1C), though numerical density and area fraction were lower in mutants (−15 and −13%, respectively).

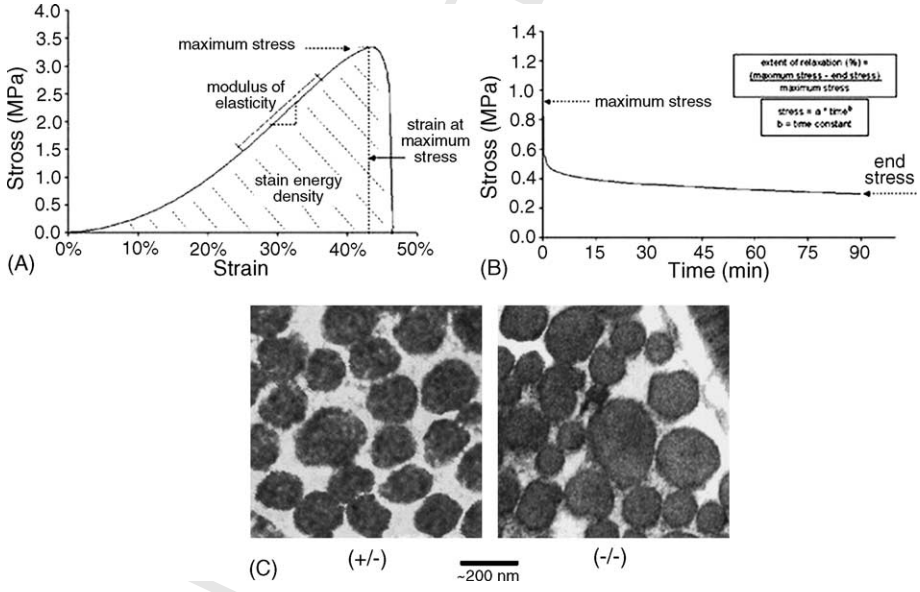

Fig. 1 (A) Representative quasi-static mechanical test from one sample; (B) representative stress–relaxation mechanical test from one sample; (C) representative transmission electron microscopy photos of collagen fibrils from control (left) and GDF-5 deficient (right) skin samples.

Table 1B Comparison of changes in GDF-5 deficient skin and tail tendon [relative to GDF-5 (+/−) controls][3]

	GDF-5 (−/−) skin	GDF-5 (−/−) tail tendon
Extent of relaxation (%)	−5†	−4†
Relaxation time constant (%)	10‡	−11†

† $p < 0.05$.
‡ $p \approx 0.055$.

This data demonstrates that GDF-5 deficiency in mouse skin results in alterations in viscoelastic behavior without alterations in quasi-static behavior. These results are strikingly similar to data previously reported from GDF-5 deficient tail tendons (Table 1B) [3], suggsesting that effects of GDF-5 deficiency are manifest in many type I collagen-rich tissues, and that the specific alterations in multiple non-weight-bearing anatomic locations are similar. The underlying cause of these changes is not clear. In the study of tail tendons, mutants contained an increase in irregularly shaped fibrils [3]; although not statistically significant, it was suggested that the differences might contribute to the viscoelastic alterations. Our study did not detect increased mutant polymorphism, and in fact, both GDF-5 (−/−) and (+/−) skin exhibited a significant percentage of "polymorphic" fibrils. We did find fibril density and area fraction to be qualitatively lower in mutants, however. It is accepted that collagen content and fibril ultrastructure affect the mechanical behavior of skin and other collagenous tissues [5–8], but specific effects of fibril shape, size, and density are not well described. Although conclusions cannot be made from our analysis of one animal per group, it is possible that such changes might alter matrix–fibril and fibril–fibril interactions, thus causing slower relaxation. We have not yet, however, documented a consistent pattern of alterations in fibril morphology across multiple GDF-5 deficient tissues.

Fibril orientation is also an important influence on biomechancial behavior. Numerous studies demonstrate that the mechanical properties of skin are, in large part, dependant on the direction of testing [9,10]. We took rigorous measures to control such effects, but this does not necessarily control for intrinsic differences in fibril arrangement, however. It is possible that one effect of GDF-5 deficiency is disordered microscopic fibril alignment or derangement of the fibril straightening process. Finally, we found a small but significant difference in skin thickness, with mutant skin approximately 4% thinner, suggesting that there may be gross differences in collagen or matrix quantity between groups. Although no differences in glycosaminoglycan content or collagen content were found in tail tendons [3], Achilles' tendon from GDF-5 deficient mice contains 40% less total collagen [2]. This discrepancy could be due to differences in mechanical loading environment – as tail is not a load-bearing structure in mice – or due to site-specific actions of GDF-5. The next steps will include similar comparison of cell numbers, GAG content, collagen, and matrix protein quantities in skin samples. Regardless, our results demonstrate that the biomechanical behavior and ultrastructure of mouse skin are affected by GDF-5 deficiency. Considered with earlier studies, our findings suggest that GDF-5 influences multiple tissues composed primarily of type I collagen, with consistent biomechanical effects on non-weight-bearing tissues such as tail tendon and skin.

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28 April 2005