Effects of Jump Training on Procollagen α_1(I) mRNA Expression and Its Relationship With Muscle Collagen Concentration

Christophe Ducomps[1,3], Dominique Larrouy[2], Aline Mairal[2], Jean-Paul Doutreloux[1,3], François Lebas[3], and Pascale Mauriège[1]

Catalogue Data
Ducomps, C.; Larrouy, D.; Mairal, A.; Doutreloux, J-P.; Lebas, F.; and Mauriege, P. (2004). Effects of jump training on procollagen α_1(I) mRNA expression and its relationship with muscle collagen concentration. **Can. J. Appl. Physiol.** 29(2): 157-171. © 2004 Canadian Society for Exercise Physiology.

Key words: *collagen synthesis, high intensity exercise, muscle type, age*
Mots-clés: *synthèse du collagène, exercice de forte intensité, myotype, âge*

Abstract/Résumé

The aim of this study was to examine the effects of a prolonged high-intensity exercise, jumping, on procollagen α_1(I) mRNA level and collagen concentration in different muscles of trained (T) and control (C) rabbits. Procollagen α_1(I) mRNA expression was much higher (2.8 to 23.5 times) in semimembranosus proprius (SMP), a slow-twitch oxidative muscle, than in extensor digitorum longus (EDL), rectus femoris (RF), and psoas major (Psoas) muscles, both fast-twitch mixed and glycolytic, whatever group was considered ($p < 0.001$). Procollagen α_1(I) mRNA level also decreased significantly between 50 and 140 days in all muscles ($0.001 < p < 0.01$). However, mRNA levels were 16 to 97% greater at 140 days in all muscles of T animals compared to C ones ($0.01 < p < 0.05$). Collagen concentrations of EDL and RF muscles were also higher (14 to 19%) in T than in C rabbits at 90 and 140 days ($0.001 < p < 0.05$). In the whole sample, collagen concentration was negatively associated with the procollagen α_1(I) mRNA level in EDL and RF muscles ($-0.49 < r < -0.44$, $p < 0.05$), while being positively related to mRNA expression in SMP and Psoas muscles (0.65

[1]UFR STAPS, Université Paul Sabatier, 118 Rte. de Narbonne, 31062 Toulouse Cedex 4, France; [2]INSERM U 586, Institut Louis Bugnard, CHU Rangueil, 31 Ave. Jean Pouilhès, 31043 Toulouse Cedex 5, France; [3]INRA Station de Recherche Cunicole, Chemin Borde-Rouge, 31326 Auzeville, France.

< r < 0.85, p < 0.01). It is concluded that jump training clearly restricts the decrease of procollagen (I) mRNA level and probably affects collagen synthesis level. In trained rabbit muscles, the maintenance of a better synthesis level could partly explain the higher collagen concentrations found in EDL and RF at 140 days. Nevertheless, the collagen degradation process seems to play the main role in the increase of total collagen concentration with age in EDL and RF muscles.

Le but de cette étude est d'analyser les effets d'un exercice prolongé de forte intensité, sauter, sur la concentration de l'α_1(I) ARNm procollagène et de collagène dans divers muscles de lapins entraînés (T) et témoins (C). L'expression de l'α_1(I) ARNm procollagène est beaucoup plus importante (2,8 à 23,5 fois, p < 0,001) dans le semimembranosus proprius (SMP), un muscle constitué de fibres lentes et oxydatives, que dans l'extensor digitorum longus (EDL), le rectus femoris (RF), et le psoas major (Psoas) constitués de fibres rapides mixtes et glycolytiques. La concentration de l'α_1(I) ARNm procollagène de tous les muscles diminue significativement (0,001 < p < 0,01) entre le 50^e et le 140^e jour. Comparativement aux muscles des animaux témoins, la concentration de l'ARNm est, au 140^e jour, de 16 à 97% plus grande (0,01 < p < 0,05) dans tous les muscles des animaux entraînés. Au 50^e et au 140^e jour, la concentration de collagène dans les muscles EDL et RF est également plus forte (de 14 à 19%) chez les animaux entraînés que chez les témoins (0,001 < p < 0,05). Chez tous les animaux, la concentration de collagène est corrélée négativement au niveau de l'α_1(I) ARNm procollagène dans les muscles EDL et RF (–0,49 < r <–0,44, p < 0,05), mais corrélée positivement à l'expression de l'ARNm dans les muscles SMP et Psoas (0,65 < r < 0,85, p < 0,01). En conclusion, l'entraînement à sauter réduit nettement la baisse de l'α_1(I) ARNm procollagène et modifie probablement le degré de synthèse du collagène. Chez les lapins entraînés, le maintien d'une meilleure synthèse pourrait expliquer en partie les plus fortes concentrations de collagène observées dans les muscles EDL et RF après 140 jours. Quoi qu'il en soit, le processus de dégradation du collagène semble être le principal facteur de l'augmentation de la concentration totale de collagène au fil du temps dans les muscles EDL et RF.

Introduction

There are at least 19 well-identified vertebrate collagen types (Velleman, 1999), but each displays a specific assembly and a typical structural function (Myers et al., 1993). The fibrillar collagens, namely types I, II, III, V, and XI, have the same overall molecular structure, but only types I, III, and V are present in skeletal muscles with type IV collagen. The prevalent forms I and III characterized by the assemblage of triple α helixes are often co-expressed in muscular tissue (Velleman, 1999). Type I collagen is the predominant form, composed of two chains α_1(I) and one α_2(I). Muscular collagen maintains muscle integrity and allows the transmission of forces; therefore the state (native or cross-linked) as well as variations in collagen concentration can act on the mechanical behavior of the muscle (McCormick, 1994). The hydroxyproline content, which is almost constant in type I collagen, can be used to measure the collagen concentration. However, collagen concentration is known to vary according to growth, aging, muscle type, and physical exercise (Alnaqeeb et al., 1984; Kovanen, 1989; Kovanen et al., 1980; Listrat et al., 1998; McCormick, 1994). Indeed, slow-twitch muscles display a higher collagen concentration than fast-twitch glycolytic ones (Kovanen et al., 1984).

Concerning physical exercise, endurance training has been shown to increase collagen concentration, prolyl-4-hydroxylase (PH) activity (PH catalysing the 4-hydroxylation of prolyl residue), and also to affect collagen type-I mRNA level (Kovanen, 1989; Perhonen et al., 1996; Takala et al., 1986). These variations in both enzyme activity and mRNA level could be used as indirect indices of collagen synthesis level (Goldspink et al., 1994), although collagen concentration may be affected by post-translational changes such as the formation of covalent cross-links (Khün, 1987). However, relationships have already been observed between mRNA level and collagen V protein concentration in granulation experiments in rat (Inkinen et al., 1998; 1999).

Thus it would be relevant to investigate the expression of procollagen α_1(I) mRNA and collagen concentration in response to another type of exercise: jump training. The effects of such a high intensity exercise on muscular collagen could have implications in the field of human health or sports performance. For this study, four muscles were chosen for their different contractile and metabolic fiber types: semimembranosus proprius (SMP), a slow oxidative muscle; extensor digitorum longus (EDL) and rectus femoris (RF), two fast-twitch mixed muscles; and psoas major (Psoas), a fast glycolytic muscle (Alasnier et al., 1996; Gondret et al., 1996; Hämäläinen and Pette, 1993).

The objective of this study was to examine the effects of prolonged jump-training on procollagen α_1(I) mRNA level, and thereby on collagen I synthesis, in different muscle types of rabbit. This animal was chosen because it displays a natural aptitude to jump. To verify whether variations in collagen concentration were related to variations in procollagen α_1(I) mRNA level, the construction of a specific cDNA probe was required. The relationships between mRNA level and collagen concentration were further analyzed in the different muscles of control and trained animals considered together.

Material and Methods

ANIMALS

Sixty hybrid male rabbits (White New Zealand Rabbits 1077) were purchased from the Institut National de la Recherche Agronomique in Toulouse, France. Each animal was initially subjected to a general physical examination by a qualified technician, and any rabbit deemed abnormal was excluded from the study. During the study period, rabbits were housed in a climate-controlled room at an ambient temperature of 20 ± 2 °C, under a regimen of 12 hrs of light/day (lights on at 7 a.m.). Rabbits were cared for and handled in accordance with the principles and guidelines of the Canadian Council on Animal Care. Food (Rablo Formax, Alisud, containing 60% carbohydrate, 17% protein, 3% fat, and 14% cellulose) and water were provided ad libitum throughout the study.

TRAINING PROTOCOL

Sixty weaned male rabbits (31 days old) were randomly assigned to two groups of 30 animals each: a sedentary control group and an exercise-trained group. Control rabbits (C) were housed individually in standard cages which limited their possi-

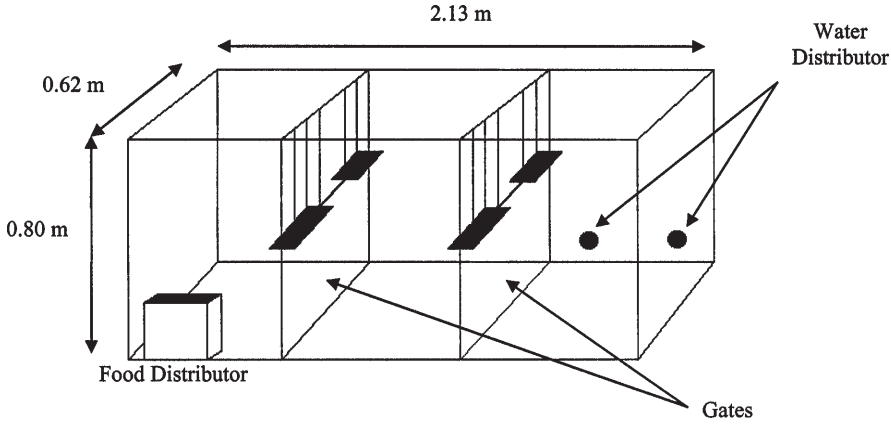

Figure 1. Perspective view of a large cage equipped with two gates.

bilities of movements, thus permitting only a sedentary lifestyle, while trained animals (T) were three per large cage measuring 2.13 m long, 0.62 m wide, and 0.80 m high. These large cages were equipped with two gates which divided each cage in three equal volumes, as shown in Figure 1. The gates were equipped with an obstacle adjustable in height. Trained rabbits had to jump over these obstacles to have access to food and water which were located at each side of the cage. The height of obstacles was determined according to the animals' size and age, as shown in Table 1.

Continuous video monitoring was carried out with color sensitive cameras. These cameras filmed the cages laterally in order to count the number of jumps made by trained rabbits and to check their activity. Video films were recorded over a 24-hr period, one day before sacrifice which occurred at 50, 90, and 140 days. Ten control and 10 trained animals were sacrificed at these different ages. At 140 days of age, rabbits have reached sexual maturity and are fully grown (Gondret et al., 1996). Animals with injuries (hematomas, muscular lesions, etc.) had already been excluded from the study.

mRNA COLLAGEN ASSAYS

EDL, RF, SMP, and Psoas muscles separated from their tendons were frozen, then powdered using a crusher cooled in liquid nitrogen, and finally lyophilized. Hydroxyproline concentration was assayed by a colorimetric method derived from Woessner (1961). Soluble collagen was assessed after solubilization of the same samples in Ringer solution (2.125g NaCl; 62.5mg KCl; 0.1g $CaCl_2(H_2O)_2$, 50mg $NaHCO_3$; H_2O 1,000 ml) at 77 °C for 1 hour under a constant gentle shaking (60 cycles/min), and centrifugation at 4,000g, 30 min, 4 °C, twice in order to recover supernatant. Then hydroxyproline concentration was measured in the supernatant, using the method of Woessner (1961). Cross-linked collagen was estimated by

Table 1 Adjustment of Height of Obstacles According to Animals' Age

Animals' age	Height of obstacles
30 to 50 days	0.25 m
51 to 90 days	0.30 m
91 to 110 days	0.35 m
111 to 140 days	0.50 m

subtracting soluble collagen concentration from total collagen concentration (Kovanen and Suominen, 1989). Soluble collagen concentration could not be determined in EDL and SMP muscles at 50 days of age, as we could not obtain sufficient amounts of lyophilized tissue to perform such experiments.

Construction of a Rabbit Procollagen α_1(I) mRNA-Specific cDNA Probe. A 780-bp type I collagen cDNA fragment was obtained by RT-PCR on rabbit EDL total RNA using 5'-GTGACAAGGGTGAGACAGGC-3' as forward primer and 5'-AGGCGCAGGAAGGTCAGCTG-3' as reverse primer. The cDNA was then cloned in PGEMT-Easy plasmid (Promega) and sequenced with an automatic DNA sequencer (ABIPRISM 310). Rabbit procollagen α_1(I) mRNA was 80% homologous to human procollagen α_1(I) mRNA.

Northern Blot Analysis. Total RNA from 200 mg of EDL, RF, SMP, and Psoas muscles were extracted with the STAT-60 isolation reagent (AMS Biotechnology, Abingdon Oxon, UK), electrophoresed in a 1% (w/v) agarose / 2.2 M formaldehyde gel, transferred to nylon membrane (Hybond N, Amersham, Saclay, France), and cross-linked with UV. Blots were hybridized for 16 hrs at 65 °C in 0.5 M Na_2HPO_4 / 1 mM EDTA / 7% (w/ v) sodium dodecyl sulfate (SDS) / 1% (w/v) bovine serum albumin (BSA) with the 780-bp rabbit collagen ^{32}P-labeled cDNA probe. Blots were then washed at a final stringency of 15 mM NaCl / 1.5 mM citric acid / 0.1% SDS at 65 °C and subjected to digital phosphore imager (Molecular Dynamics, Saclay, France). Homogeneity of RNA loading was assessed by hybridization with a 1300-bp rat glyceraldehyde phosphate dehydrogenase (GAPDH) cDNA probe.

STATISTICAL ANALYSES

Values presented in the figures are means ± standard deviation. Data were analyzed using a two-way (age, training) analysis of variance with post hoc tests (Student *t*-test). A Mann-Whitney U-test was used to test the difference between groups concerning mRNA data. Correlations between two variables were determined using Pearson correlation coefficient. Statistical analyses were conducted with the software SPSS® (1999). In all cases, statistical significance was set at $p < 0.05$.

Results

The anthropometric data revealed no significant differences between the muscle weights and body weights of the T and C animals at their three stages of age. The mean body weights were 1722 ± 150g for T and 1779 ± 141g for C rabbits at 50 days; 3073 ± 222g for T and 3082 ± 239g for C rabbits at 90 days; and 4013 ± 344g for T and 4074 ± 169g for C rabbits at 140 days. During the study one trained rabbit died and three presented muscular, osseous, and joint lesions on their rear limb. Some rabbits presented muscular hematomas, probably due to torn muscles. Videos revealed an average of 90 ± 9, 180 ± 13, and 60 ± 5 jumps daily per rabbit at 50, 90, and 140 days of age, respectively.

EXPRESSION OF PROCOLLAGEN $\alpha_1(1)$ mRNA

The northern hybridization with the specific cDNA probe revealed the presence of one procollagen a_1(I) mRNA. In our gel system, this mRNA is located near 28S rRNA at approximately 5.0 kb (Figure 2).

At 50 days, procollagen α_1(I) mRNA expression was higher in SMP than in EDL, RF, and especially the Psoas muscles, whatever group was being considered (Figure 3). At the other stages of age, the mRNA level was 2.8 to 23.5 times higher in SMP than in the three other muscles ($p < 0.001$). Procollagen α_1(I) mRNA level decreased significantly between 50 and 140 days in all muscles ($0.001 < p < 0.01$) (Figure 3). From 50 days, procollagen mRNA level was higher in EDL muscles of T rabbits than in those of C animals ($p < 0.01$). From 90 days, there was a similar pattern of response for RF and SMP muscles (*p* values ranging from 0.01 to 0.05).

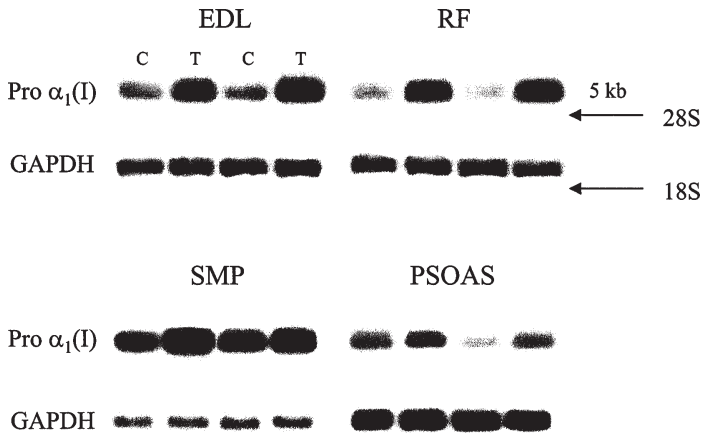

Figure 2. Northern blot analysis of procollagen α_1(I) mRNA in EDL, RF, SMP, and Psoas muscles from control (C) and trained (T) animals at 140 days. Hybridization at 1,300 bp rat GAPDH cDNA probe was performed to verify the homogeneity of RNA loading. Arrows mark the migration of rRNA (18S and 28S) and each blot represents a sample. Pro α_1(I): Procollagen α_1(I); GAPDH: glyceraldehyde phosphate dehydrogenase.

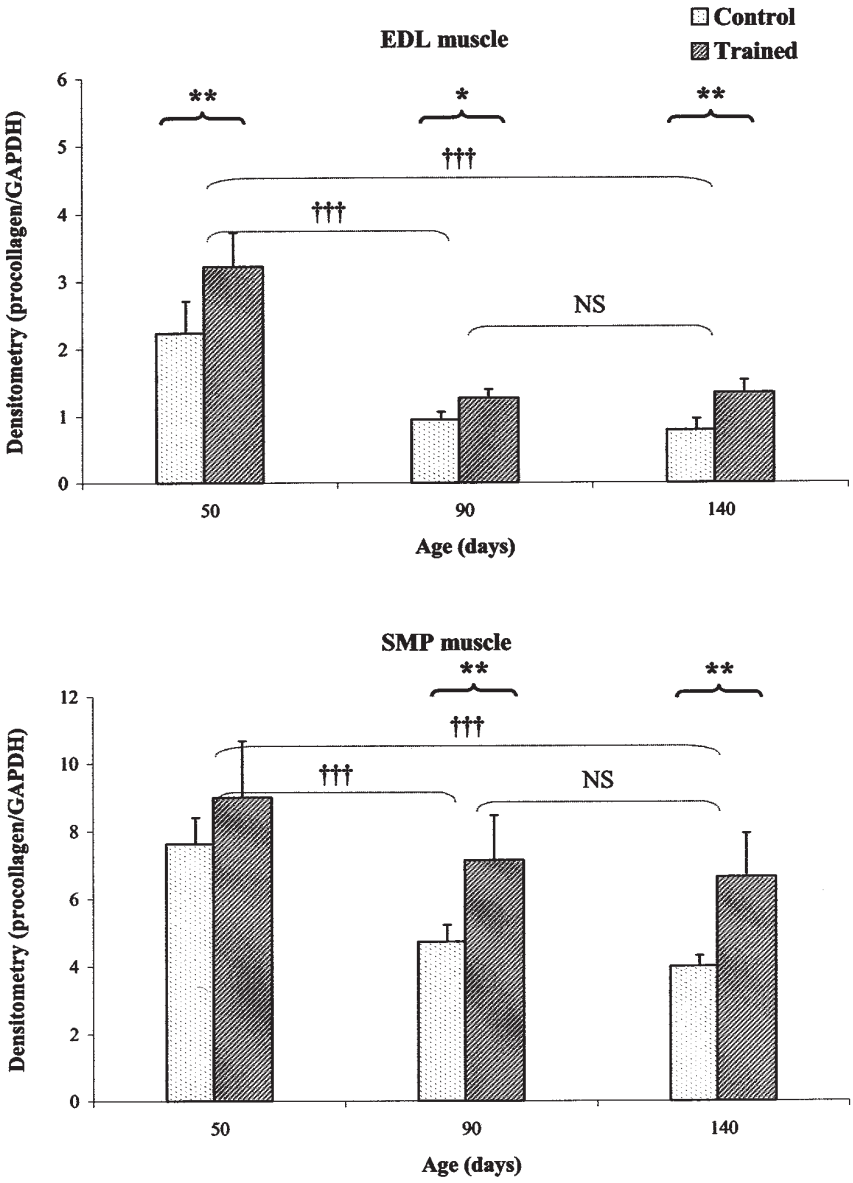

Figure 3. Densitometric analysis of procollagen α_1(I) mRNA expression normalized for GAPDH in EDL (top) and SMP (bottom) muscles, from control and trained animals at 50, 90, and 140 days. Values are means ± *SD* of 5 muscles. Significant difference between groups of similar age, at $^{*}p < 0.05$ and $^{**}p < 0.01$. Significant increase with age in T and C group at $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, and $^{\dagger\dagger\dagger}p < 0.001$. NS = nonsignificant. (*continued*)

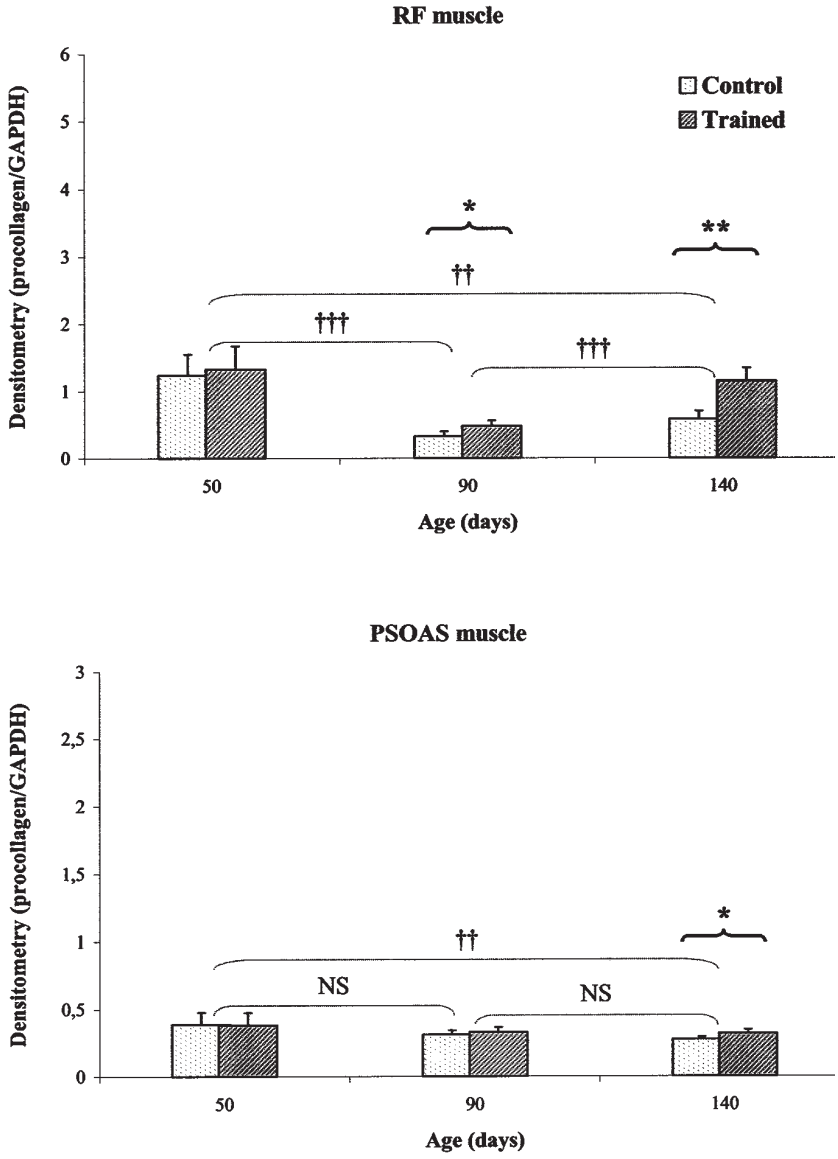

Figure 3 (*Cont.*). Densitometric analysis of procollagen α_1(I) mRNA expression normalized for GAPDH in RF (top) and Psoas (bottom) muscles, from control and trained animals at 50, 90, and 140 days. Values are means ± *SD* of 5 muscles. Significant difference between groups of similar age, at $^{*}p < 0.05$ and $^{**}p < 0.01$. Significant increase with age in T and C group at $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, and $^{\dagger\dagger\dagger}p < 0.001$. NS = nonsignificant.

Table 2 Collagen Concentration (*M* ± *SD*) in Different Muscles of Trained and Control Rabbits at 50, 90, and 140 Days

Muscles	Fraction	Collagen concentration (mg / g dry weight)					
		50T	50C	90T	90C	140T	140C
EDL	Total	88.1	84.1	102.2*	89.7	113.8**	98
		±8.9	±8.5	±10.4	±9.9	±7.6	±6.8
	Soluble	–	–	31.1	33.7	18.6	19.3
				±1.9	±2.9	±1.3	±2.1
RF	Total	62.8	62.7	72.1**	63.4	83.9***	70.5
		±5.1	±5.7	±7.1	±5.1	±8.7	±8.2
	Soluble	19.6	19.9	15.4	15.3	14.4	14.4
		±1.4	±1.1	±1.6	±1.4	±1.4	±1.7
SMP	Total	81.2	83.9	73.8	72.3	68.7	64.7
		±6.8	±7.8	±8.4	±3.6	±8.5	±6.2
	Soluble	–	–	7.7*	9.5	3.5	3.9
				±0.6	±0.8	±0.3	±0.2
Psoas	Total	18.4	18.2	12.2	10.9	8.4	7.5
		±1.3	±1.3	±1.2	±1.5	±0.6	±0.7
	Soluble	2.4	2.6	1.3	1.1	1	0.9
		±0.2	±0.2	±0.2	±0.1	±0.1	±0.05

Note: EDL = extensor digitorum longus; RF = rectus femoris; SMP = semimembranosus proprius; Psoas = psoas major. Significant difference between trained (T) and control (C) rabbits of similar age, at * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

At 140 days, procollagen mRNA level was 16 to 97% higher in T compared to C muscles ($0.01 < p < 0.05$) (Figure 3). Considering the mRNA level, a significant interaction effect between age and training was observed for all muscles ($p < 0.001$).

Collagen Assays. Collagen concentration in EDL and RF muscles increased ($p < 0.001$) between 50 and 140 days of age, although it decreased in SMP and Psoas muscles ($p < 0.001$) in both groups (Table 2). Soluble collagen concentration in RF and Psoas muscles decreased significantly between 50 and 140 days of age in both T and C rabbits ($p < 0.001$). A similar pattern of response was observed between 90 and 140 days for EDL and SMP muscles ($p < 0.001$). EDL and RF muscle collagen concentrations were also 13.7 to 19.1% higher at 90 and 140 days in the T than in the C group ($0.001 < p < 0.05$). However, SMP and Psoas muscle collagen concentrations were similar in both groups. Finally, soluble collagen concentration did not vary between groups, whatever the muscle or the animal's age (Table 2).

To understand further the relationships between procollagen α_1(I) mRNA level and collagen concentration, we performed correlation analyses between these two variables at various stages of age and for each muscle.

CORRELATIONAL ANALYSES

Positive relationships were observed between collagen concentration of rabbit muscles, considered together, and collagen-I mRNA level, in both T and C animals at 50, 90, and 140 days ($0.39 < r < 0.82$, p values ranging from 0.001 to 0.05, not shown). Correlation coefficients decreased with advancing age, the values being 0.82, 0.74, and 0.39 at 50, 90, and 140 days, respectively. A negative association was found between collagen concentration and procollagen-I mRNA level in EDL and RF muscles ($-0.49 < r < -0.44$, $p < 0.05$; Figure 4). In contrast, a strong and positive correlation was noted between collagen concentrations of SMP and Psoas muscles and procollagen-I mRNA level ($0.65 < r < 0.85$, $p < 0.01$; Figure 4).

Discussion

This study was conducted to verify the effects of a high intensity exercise (jump training) on collagen I gene expression, by determining procollagen α_1(I) mRNA level, as this collagen type is the prevalent form in conjunctive skeletal muscle compared to type III collagen (Friess, 1998). For this purpose, a specific cDNA probe of rabbit procollagen α_1(I) mRNA was synthesized. In this experiment young rabbits were preferred to adult males for several reasons. Young animals display a better ability to adapt to the particular living conditions compared to adults. Furthermore, male adult rabbits are aggressive toward each other and thus it is impossible to keep several of them in the same cage because of the risk of mortality.

Concerning the mode of training chosen in our study, it has rarely been used and corresponds to a high intensity exercise (Pousson et al., 1991; Watt et al., 1982). Indeed, rabbits were not endurance-trained on a treadmill several hours per day, but they were stimulated to jump by the presence of food according to their own rhythm of activity during the light or dark phases. It is also interesting to note that this training mode is more respectful of the animal's biorhythm, as rabbits do not perform jumps under constraint. Nevertheless, the presence of muscular lesions and the great frequency of jumps carried out by trained animals, compared notably to Pousson et al. (1991), highlight the intensity of the stimulation as characteristic of a highly intensive exercise.

Results show an important procollagen-I mRNA level in SMP muscle, compared to EDL, RF, and especially Psoas muscles in trained (T) and control (C) animals, from 50 days. Therefore this mRNA expression was higher in a slow-twitch oxidative muscle (SMP) than in fast-twitch mixed and glycolytic muscles (EDL, RF, and Psoas) at each stage of age. These discrepancies in mRNA level seem to be linked to the muscle contractile and metabolic types, which has already been suggested concerning collagen concentration (Alasnier et al., 1996; Kovanen et al., 1984). Thus the muscle collagen concentration, in type I or type II muscles, seems to be determined genetically according to its future adult contractile type.

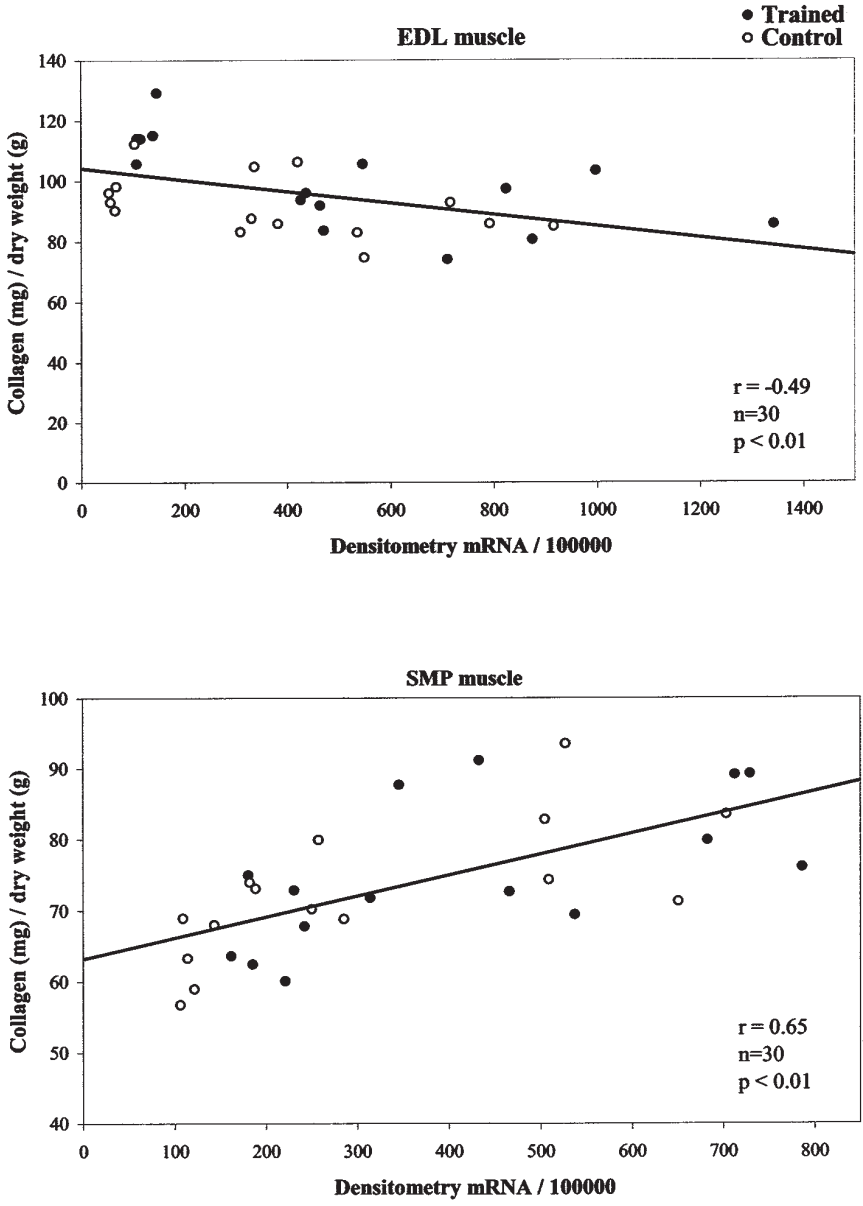

Figure 4. Relationships between densitometric procollagen α_1(I) mRNA expression and collagen concentration of EDL (top) and SMP (bottom) muscles, between 50 and 140 days, in control and trained rabbits. NS = nonsignificant. (*continued*)

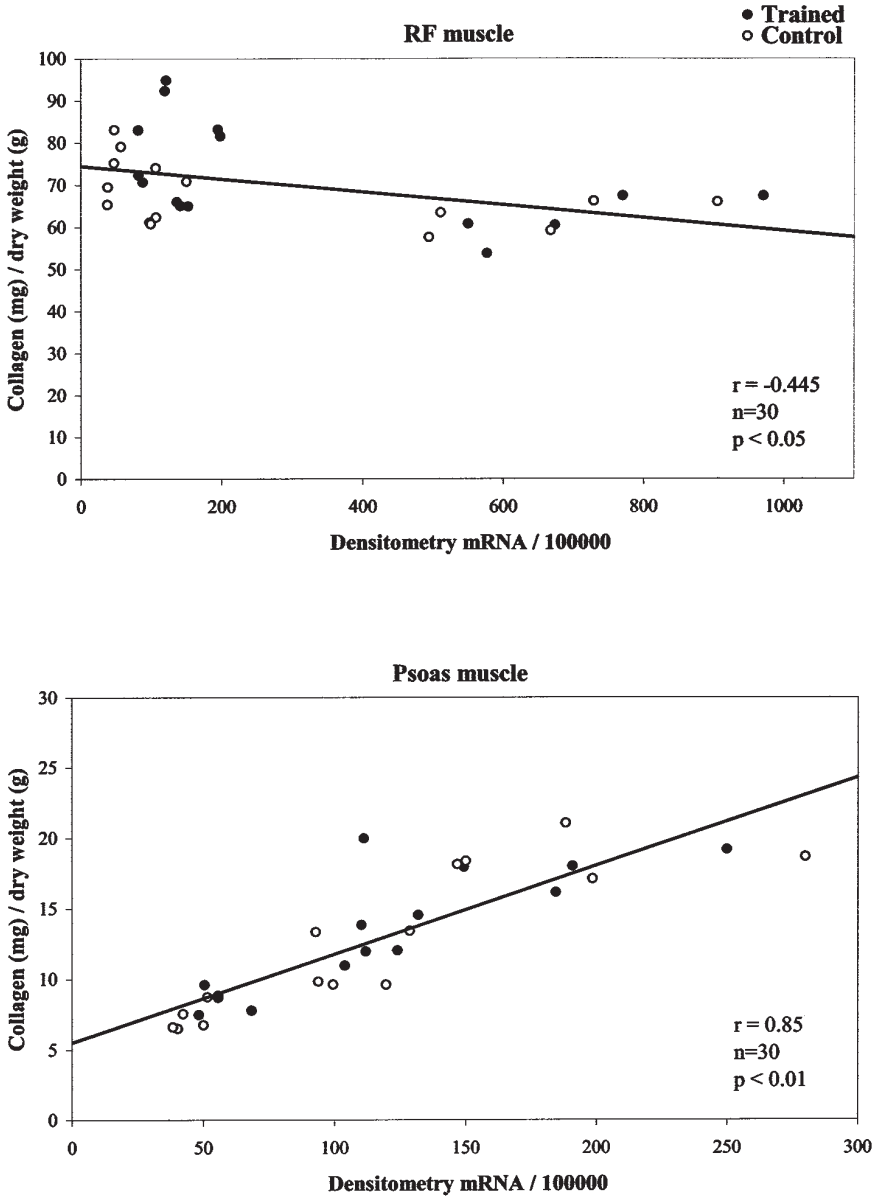

Figure 4 (*Cont.*). Relationships between densitometric procollagen α_1(I) mRNA expression and collagen concentration of RF (top) and Psoas (bottom) muscles, between 50 and 140 days, in control and trained rabbits. NS = nonsignificant.

Our data revealed a significant decrease in the procollagen-I mRNA level with age between 50 and 140 days in all muscles. The decreased procollagen α_1(I) mRNA level could be due to a reduction in collagen synthesis with age, as previously observed in rats (Mays et al., 1991). This finding is surprising, however, in EDL and RF muscles of C rabbits, which display a great decreased procollagen α_1(I) mRNA level vs. an increased collagen concentration. Therefore our results seem to confirm the predominant role of the degradation process (which is influenced by collagen cross-links) in reducing the collagen turnover (Laurent, 1987; Laurent et al., 1985). Thus one should be cautious when trying to establish a relationship between mRNA level and final protein concentration, notably in view of the post-translational transformations (Khün, 1987).

At 140 days, the significant higher procollagen α_1(I) mRNA level in T rabbit muscles and the interaction between age and training seem to confirm an effect of jump training on collagen I gene expression. These data are in agreement with previous studies which reported an increase of procollagen α_1(I) mRNA level after a period of exercise training (Perhonen et al., 1996). As a result, this type of exercise could allow the mRNA level to be maintained higher, and thus might be able to modify the collagen synthesis level. The relationships observed between procollagen α_1(I) mRNA level and muscle collagen concentration seem to confirm the existence of a link between these two variables, regardless of age. However, the decrease in correlation coefficients noted between 50 and 140 days (muscles being considered together), as well as the strong negative relationships observed in the case of pennate EDL and RF muscles, re-emphasize the importance of the cross-link phenomenon in collagen concentration.

Finally, although the decrease in collagen turnover seems to be responsible for the increase in collagen concentration of EDL and RF muscles, the maintenance of a higher collagen synthesis level in EDL and RF muscles of T rabbits could provide a valuable explanation for the presence of a higher collagen concentration in these muscles. The strong relationships observed between mRNA level and collagen concentration in SMP and Psoas muscles point out a reduction in collagen synthesis with age, as previously reported (Listrat et al., 1998; Mays et al., 1991). Another factor involved in the reduction of collagen concentration could be the delaying of the cross-link process, thus contributing to maintain a high turnover. However, SMP and Psoas muscles display an important cross-linked fraction in our study, as their soluble collagen concentration is lower compared to EDL and RF muscles.

This observation might be explained by a greater type III collagen concentration in these two muscles. Indeed, type III collagen is known to affect the test of thermal solubility because of its greater resistance to the rise of temperature (less solubility compared to type I collagen) induced by the presence of numerous pyridinium cross-links (Burson et al., 1986; Maiorano et al., 1995). However, pyridinium cross-links appear late (with ageing), and therefore the presence of a greater level of native collagen (i.e., not cross-linked) which is easily degradable could contribute to the decrease in total collagen concentration.

In conclusion, this study confirms that high intensity exercise such as jumping can act on collagen concentration and procollagen α_1(I) mRNA level after a

15-week training program. Indeed this type of exercise limits the decrease of procollagen α_1(I) mRNA level in muscles of trained rabbits, which probably contributes toward maintaining a greater collagen I synthesis level. This greater collagen synthesis level could explain the higher collagen concentration found in EDL and RF muscles of exercised rabbits at 140 days. However, the decrease in the collagen degradation process, which could be due to the presence of cross-linked collagen, seems to be the predominant factor for the increase in collagen concentration. The results of this study, which highlight the effects of high intensity exercise training on collagen expression, suggest several perspectives for research. Applications are notably possible in humans, and especially for athletes in the domain of injury prevention or performance improvement, thanks to adapted jump training programs that could strengthen conjunctive tissue.

Acknowledgments

We wish to express our gratitude to the technicians, engineers, and researchers of INSERM Unit 586 for their collaboration and advice in the elaboration of the cDNA probe.

References

Alasnier, C., Rémignon, H., and Gandemer, G. (1996). Lipid characteristics associated with oxidative and glycolytic fibres in rabbit muscles. **Meat Sci.** 43: 213-224.

Alnaqeeb, M.A., Al Zaid, N.S., and Goldspink, G. (1984). Connective tissue changes and physical properties of developing and ageing skeletal muscle. **J. Anat.** 139: 677-689.

Burson, D.E., Hunt, M.C., Unruh, J.A., and Bikeman, M.E. (1986). Proportion of types I and III collagen in *Longissimus* collagen from bulls and steers. **J. Anim. Sci.** 63: 453-456.

Friess, W. (1998). Collagen-biomaterial for drug delivery. **Pharmac. & Biopharmac.** 45: 113-136.

Goldspink, G., Fernandes, K., Williams, P.E., and Wells, D.J. (1994). Age-related changes in collagen gene expression in the muscles of mdx dystrophic and normal mice. **Neuro. Disor.** 4: 183-191.

Gondret, F., Lefaucheur, L., D'Albis, A., and Bonneau, M. (1996). Myosin isoform transitions in four rabbit muscles during postnatal growth. **J. Muscle Res. Cell. Motility** 17: 657-667.

Hämäläinen, N., and Pette, D. (1993). The histochemical profiles of fast fiber types IIB, IID and IIA in skeletal muscles of mouse, rat and rabbit. **J. Histochem. Cytochem.** 41: 733-743.

Inkinen, K., Turakainen, H., Wolff, H., and Ahonen, J. (1999). Cloning of cDNA for rat Proα1(V) collagen mRNA. Expression patterns of type I, type III and type V collagen genes in experimental granulation tissue. **Connect. Tissue Res.** 40: 209-220.

Inkinen, K., Wolff, H., Von Boguslawski, K., and Ahonen, J. (1998). Type V collagen in experimental granulation tissue. **Connect. Tissue Res.** 39: 281-294.

Khün, K. (1987). Structure and function of collagen types. In: R.P. Mecham (Ed.), **Biology of Extracellular Matrix**, pp. 88-102. London: Academic Press.

Kovanen, V. (1989). Effects of ageing and physical training on rat skeletal muscle. **Acta Physiol. Scand.** 135(Suppl. 577): 1-56.

Kovanen, V., and Suominen, H. (1989). Age and training-related changes in the collagen metabolism of rat skeletal muscle. **Eur. J. Appl. Physiol.** 58: 765-771.

Kovanen, V., Suominen, H., and Heikkinen, E. (1980). Connective tissue of "fast" and "slow" skeletal muscle in rats – Effects of endurance training. **Acta Physiol. Scand.** 108: 173-180.

Kovanen, V., Suominen, H., and Heikkinen, E. (1984). Collagen in slow twitch and fast twitch muscle fibres in different types of rat skeletal muscle. **Eur. J. Appl. Physiol.** 52: 235-242.

Laurent, G.J. (1987). Dynamic state of collagen: Pathways of collagen degradation in vivo and their possible role in regulation of collagen mass. **Am. J. Physiol.** 252: Cl-C9.

Laurent, G.J., McAnulty, R.J., and Gibson, J. (1985). Changes in collagen synthesis and degradation during skeletal muscle growth. **Am. J. Physiol.** 249: C352-C355.

Listrat, A., Picard, B., and Geay, Y. (1998). Age-related changes and location of type I, III and IV collagens during skeletal muscle development of double-muscled and normal bovine foetuses. **J. Muscle Res. Cell. Motility** 19: 1-14.

Maiorano, G., McCormick, R.J., Field, R.A., and Snowder, G.D. (1995). Collagen characteristics of skin, fell and epimysium from rams, wethers and zeranol-implanted ram lambs. **J. Anim. Sci.** 73: 393-398.

Mays, P.K., McAnulty, R.J., Campa, J.S., and Laurent, G.J. (1991). Age-related changes in collagen synthesis and degradation in rat tissues. **Biochem. J.** 276: 307-313.

Mc Cormick, R.J. (1994). The flexibility of the collagen compartment of muscle. **Meat Sci.** 36: 79-91.

Myers, J.C., Sun, M.J., D'Ippolito, J.A., Jabs, E.W., Neilson, E.G., and Dion, A.S. (1993). Human cDNA clones transcribed from an unusually high-molecular-weight RNA encode a new collagen chain. **Gene** 123: 211-217.

Perhonen, M., Han, X., Wang, W., Karpakka, J., and Takala, T.E.S. (1996). Skeletal muscle collagen type I and II mRNA, prolyl-4-hydroxylase, and collagen in hypobaric trained rats. **J. Appl. Physiol.** 80: 2226-2233.

Pousson, M., Pérot, C., and Goubel, F. (1991). Stiffness changes and fibre type transitions in rat soleus muscle produced by jumping training. **Pflügers Arch.** 419: 127-130.

SPSS® (1999). **SPSS User Guide**. Chicago: SPSS.

Takala, T.E.S., Vuori, J., Anttinen, H., Väänänen, K., and Myllylä, R. (1986). Prolonged exercise causes an increase in the activity of galactosylhydroxylysyl glucosyltranferase and the concentration in type III procollagen aminopropeptide in human serum. **Pflügers Arch.** 407: 500-503.

Velleman, S.G. (1999). The role of the extracellular matrix in skeletal muscle development. **Poultry Sci.** 78: 778-784.

Watt, P.W., Kelly, F.J., Goldspink, D.F., and Goldspink, G. (1982). Exercise-induced morphological and biochemical changes in skeletal muscles of the rat. **J. Appl. Physiol.** 53: 1144-1151.

Woessner, J.F. (1961). The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. **Arch. Biochem. Biophys.** 93: 440-447.

Received January 8, 2003; accepted in final form October 22, 2003.