Increased prolyl 4-hydroxylase activity in the myocardium of endurance-trained mice[1]

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Summary. Endurance training over 3, 10 or 20 days increased the activity of prolyl 4-hydroxylase (PH) in the left ventricle of mice. No increase was observed in the weight of the left ventricle, in galactosylhydroxylysyl glucosyltransferase activity or in hydroxyproline concentration. The increase in PH suggests that the synthesis of collagen increases during physiological adaptation of the heart to endurance exercise without changes in the ventricle weight or its total collagen content.

Collagen is the main component of the stress-tolerant connective tissue network that is capable of distributing the force generated by myocardial contraction. Collagen is also associated with the myocardial vasculature (Borg and Caulfield[3] and references cited). Enhanced cardiac collagen synthesis has been observed after the onset of cardiac pressure overload, simultaneously with an overall activation of protein synthesis and the development of hypertrophy[4-6].

Prolyl 4-hydroxylase (PH) and galactosylhydroxylysyl glucosyltransferase (GGT) are enzymes catalyzing reactions involved in the post-translational modification of collagen. Their activities generally show a positive correlation with the rate of collagen biosynthesis, the activity changes in PH often being more prominent than those in GGT[7-9]. PH activity and hydroxyproline content increase in cardiac muscle during cardiac hypertrophy induced by elevated aortic pressure in the rat[10,11].

Physical exercise causes an intermittent increase in cardiac volume load, and when regularly repeated it may result in moderate hypertrophy. The cardiac muscle collagen concentration is usually unchanged in exercise hypertrophy[12-14]. Since the reports on the effects of endurance training on the cardiac collagen content are at variance with those on collagen metabolism in cardiac hypertrophy, we set out to study cardiac collagen content and synthesis during endurance training, taking advantage of the known correlation between the rate of collagen synthesis and the activities of the enzymes involved in the post-translational modifications of collagen.

Methods. 16-20-week-old male NMRI mice were exercised on a motordriven treadmill once a day for 1 h at a speed of 25 m min^{-1}. Three exercise programs were used, one group of mice being exercised once a day for 3 days, another group for 10 days and a third for 20 days. The training and sedentary control animals were fed and housed as described earlier[15].

The mice were killed by cervical dislocation the day after their last training session. The heart was rapidly removed and the left ventricle isolated, washed free of blood, immediately frozen in liquid nitrogen and stored at −70 °C for up to 3 months. Upon thawing, the tissue samples were homogenized with an Ultra-Turrax homogenizer in 2 bursts of 5 sec in a cold solution containing 0.2 M NaCl, 0.1% (w/v) Triton X-100, 0.01% (w/v) soy bean trypsin inhibitor, 0.1 M glycine and 0.2 M Tris-HCl buffer, pH adjusted to 7.5 at 4 °C (0.5 ml buffer per 100 mg of sample). The homogenates were centrifuged at 15,000 × g for 30 min at 4 °C, and aliquots of the supernatants were taken for the assay of the enzyme activities and supernatant protein[16]. The pellet was hydrolyzed for 16 h in 2 ml of 6 N HCl at 120 °C for the measurement of hydroxyproline by the method of Kivirikko et al.[17].

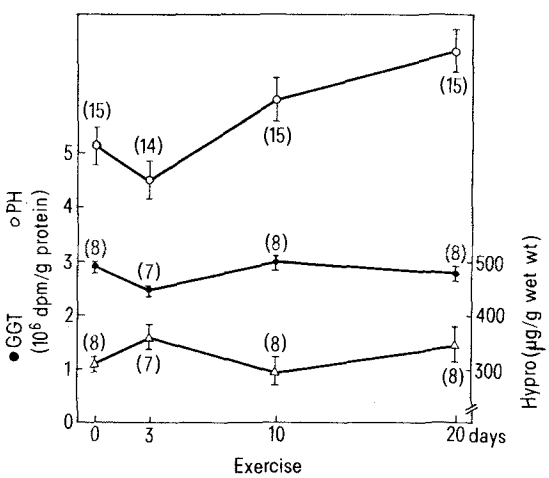
The assay for pH, based on the measurement of labeled hydroxyproline formed from peptide-bound prolyl residues of unhydroxylated, labeled protocollagen substrate, was performed essentially as described by Kivirikko and Myllylä[18] and Juva and Prockop[19]. GGT activity was assayed by determining the amount of radioactive glucosylgalactosylhydroxylysine formed in a gelatinized calf skin collagen substrate as described by Myllylä et al.[20].

L-[U-^{14}C]proline (> 225 Ci/ml) was purchased from Amersham International (Amersham, Bucks, U.K.) and uridine diphosphate D-[U-^{14}C]glucose (229 Ci/mole) from New England Nuclear Co. (Boston, Mass., USA). Nonradioactive UDP-glucose was from Sigma Chemical Co. (St. Louis, Mo., USA). ^{14}C-Proline-labeled, unhydroxylated protocollagen substrate was prepared in freshly isolated chick-embryo tendon cells[21]. Gelatinized calf skin collagen was prepared as described elsewhere[20] and heat-denatured immediately before use[22].

Results and discussion. The training program did not increase the left ventricular weight, which was 124.4 ± 2.7 mg (± SE) in the control group. The left ventricular PH-activity was measured in 2 independent training series (n = 59), whereas the hearts of a single series (n = 31) were used for the GGT and hydroxyproline assays. The left ventricular PH activity increased during training in both series ($p < 0.01$ by analysis of variance). No changes were observed in GGT activity or hydroxyproline concentration during the training period (fig.).

The increase in left ventricular PH activity suggests that an intermittent moderate increase in volume load of the heart may cause an increase in collagen biosynthesis even when the cardiac work load does not produce hypertrophy. The PH activity in skeletal muscle is also increased by training[23].

The fact that GGT activity remained unaltered is in accordance with previous observations of a stronger re-



Effect of treadmill exercise on the activities of prolyl 4-hydroxylase (PH) and galactosylhydroxylysyl glucosyltranferase (GGT) and the concentration of hydroxyproline (Hypro) in the left ventricle of the mouse heart. The values are means ± SEM (vertical bars). Number of experiments in parentheses.

sponse in PH than GGT activity during increased collagen biosynthesis[7-9]. The hydroxyproline concentration and total hydroxyproline content of the cardiac muscle were also unchanged in the present study. The mean cardiac collagen concentration usually increases only during extensive myocardial hypertrophy, e.g.[6,10,24]. It should be noted that the hydroxyproline content reflects long-term events of collagen synthesis and degradation. Moreover, the turnover in cardiac collagen is slow, 0.56% per day[6]. It is also possible that alterations in cardiac muscle collagen concentration may be nonuniformly distributed transmurally during hypertrophy[24,25]. Thus, the relatively small increase in the rate of collagen synthesis need not result in a measurable increase in total collagen content in a non-hypertrophic adaptation model such as the present one.

It has been suggested that myocardial collagen may be composed of collagen of types I, III, IV and V at least[26], and the possible differential effects of exercise on the turnover or net synthesis of different collagen types remain to be established. The present results indicate that, although not reflected in the tissue collagen content during the present observation time, collagen synthesis is involved in non-hypertrophic physiological adaptation of the heart to volume overload induced by physical exercise.

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Occurrence of a pheromone-producing gland in female tobacco beetles

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Summary. A pheromone-producing gland was discovered in the second abdominal segment of virgin female tobacco beetles, *Lasioderma serricorne* (Fabricius). The gland duct extends to an orifice below the genital pore and is supported by a rigid invagination of the integument. Hexane extracts of intact pheromone glands were found attractive to male tobacco beetles and also induced high receptor potentials in the olfactory sensilla of the antennae of male *L. serricorne*. A surface extract of virgin females proved to be significantly more attractive than an extract of pheromone glands.

The sex pheromone emitted by female tobacco beetles (*Lasioderma serricorne* F.) has been investigated mostly from a chemical viewpoint[1-8], whereas the biological aspects of this subject have received less attention[9-13]. Since studies on the site of pheromone production have been neglected so far, a detailed investigation of the exocrine gland producing the sex attractant was undertaken.

The tobacco beetles used were reared under constant climatic and nutritional conditions[10]; unmated females and males were frozen at the age of 1-2 weeks after pupal-imaginal ecdysis, decapitated and subjected to fixation and embedding, subsequently cut into thin sections (average thickness 10 µm) and stained by Ehrlich's hematoxylin[14].

The apodeme (integumental invagination) was removed together with the adjoining gland (fig. 1a) from the abdomen of virgin females of *L. serricorne* (1-2 weeks of age) which had been frozen previously (−20 °C for 24 h). The 5th abdominal segment was gently compressed, ovipositor and ovaries removed, whereupon the apodeme and adjoining gland were pulled out with pointed forceps and cleaned of adhering tissues. Batches of 200 extirpated glands, either homogenized or intact, were immersed in 2-ml portions of n-hexane for 48 h (20 ± 1 °C) and the resulting extracts diluted to provide gland equivalents ranging from 10^{-4} to 10^{0}. The gland and surface extracts of virgin females and males were used for studies on the olfactory responses[15]