STUDIES IN SPORT, PHYSICAL EDUCATION AND HEALTH 9

ANJA KIISKINEN

# ADAPTATION OF CONNECTIVE TISSUES TO PHYSICAL TRAINING IN YOUNG MICE

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ACADEMIC DISSERTATION TO BE PUBLICLY DISCUSSED, BY PERMISSION OF THE FACULTY OF PHYSICAL AND HEALTH EDUCATION OF THE UNIVERSITY OF JYVÄSKYLÄ, AUDITORIUM L-302, ON SEPTEMBER 30, 1976, AT 14 O'CLOCK

UNIVERSITY OF JYVÄSKYLÄ, JYVÄSKYLÄ 1976

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CONTENTS


PREFACE .................................................. 7

1. INTRODUCTION ........................................... 9

2. REVIEW OF THE LITERATURE ............................... 10

2.1. Long bones ........................................... 10

2.1.1. Physical effects of inactivity and denervation ..... 10

2.1.2. Chemical effects of inactivity ..................... 11

2.1.3. Physical and chemical effects of pressure .. 12

2.1.4. Physical effects of exercise ............... 13

2.1.5. Chemical effects of exercise ............... 16

2.2. Tendons, ligaments and skin ........................ 17

2.2.1. Physical effects of inactivity and exercise 17

2.2.2. Chemical effects of inactivity and exercise 19

2.3. Skeletal and heart muscle .......................... 21

2.3.1. Physical and chemical effects of exercise .. 21

3. OUTLINES OF THE PRESENT STUDY ......................... 22

4. RESEARCH METHODS ...................................... 23

5. RESULTS ............................................... 25

5.1. Physical properties ................................. 25

5.2. Chemical properties ................................. 27

6. DISCUSSION ............................................ 28

TIIVISTELMÄ .............................................. 32

REFERENCES ............................................... 34

# PREFACE

This thesis is based on the following original communications referred to as I-VI in the text:

I A. Kiiskinen. Physical training and connective tissues in young mice - Physical properties of Achilles tendons and long bones. University of Jyväskylä, Department of Public Health Publications No 28, 1976[1)].

II A. Kiiskinen and E. Heikkinen. Physical training and connective tissues in young mice. Part 1: Biochemistry of long bones. University of Jyväskylä, Department of Public Health Publications No 29, 1976[1)].

III A. Kiiskinen and E. Heikkinen. Physical training and connective tissues in young mice. Part 2: Biochemistry of Achilles tendons. University of Jyväskylä, Department of Public Health Publications No 29, 1976[1)].

IV A. Kiiskinen and E. Heikkinen. Physical training and connective tissues in young mice - Biochemistry of skin. British J. Dermatol. (in press).

V A. Kiiskinen and E. Heikkinen. Physical training and connective tissues in young mice - Heart. Europ. J. appl. Physiol. 35, 167-171 (1976).

VI A. Kiiskinen and H. Suominen. Blood circulation of long bones in trained growing rats and mice. Europ. J. appl. Physiol. 34, 303-309 (1975).

The present work aims at obtaining a clearer picture of some chemical and physical changes induced by physical training in connective tissues. This publication reviews the literature and summarizes the methods and results of 6 earlier communications. Attention is mainly focused on chemical changes in both cells and matrix. Training-induced responses are compared in

[1)] This report will also be published in an international journal.

structurally different types of tissues: long bones, tendons, skin, and heart. Finally, mechanisms adapting connective tissues to physical training are considered.

This work was carried out at the Department of Public Health, University of Jyväskylä. I am very much indebted to Professor Eino Heikkinen, M.D., for suggesting to me the subject of this investigation, and for his valuable guidance during the work. I am also very much indebted to the Head of the Department, Professor Jeddi Hasan for his kindness in placing the facilities of the Department at my disposal.

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Anja Kiiskinen

# 1. INTRODUCTION

The potential for the growth of organs and tissues is determined by hereditary factors. Environmental factors, however, regulate to what extent the growth potential is utilized. Physical exercise subjects the locomotor system to tensile and compressive forces both statically and dynamically and causes circulatory, respiratory, and metabolic reactions in the body. Yet, little is known about the long-term effects of physical training on the growth and development of connective tissues. Present knowledge is based on studies of adults, athletes, occupational groups, and animals. Some information has been derived from research on the effects of immobilization, bed rest, compressive and tensile stress, and limb denervation. Most of the published studies concentrate on physical and morphological parameters. Only few also include chemical aspects. Published results show unsatisfactory variation (see reviews by Steinhaus 1933; Rarick 1960; Malina 1969; Larson 1973; Viidik 1973; Booth and Gould 1975). Reasons for this can probably be found in differences in methods, animals, and even in the presentation of data.

# 2. REVIEW OF THE LITERATURE

## 2.1. Long bones

### 2.1.1. Physical effects of inactivity and denervation

Various publications describe inactivity as leading to a loss in both the mass and size of bone i.e. bone atrophy (e.g. Whedon 1960; Birge and Whedon 1968; Hattner and McMillan 1968; Mattsson 1972). Steinhaus (1933) summarized the results of early studies done in Germany with the statement that immobilization of limbs in growing animals (dogs and rabbits) increases the length and water content of long bones and accelerates the growth in the epiphyseal zones, but decreases the bone mineral content. On the other hand Allison and Brooks (1921) observed that immobilization retarded the bone growth in inactive limbs in growing dogs; the bones remained shorter and slimmer in the shafts while the epiphyses broadened relatively. In adult dogs they described bone atrophy as a decrease in cortical thickness together with a higher shaft porosity but the typical shape of the bones was maintained. Engström and Amprino (1950) found no differences in the fine structure of bone and the rate of bone formation in the immobilized legs of dogs compared to the active legs. Among others Laros et al. (1971) demonstrated that simple caging for six weeks or more produced subperiosteal bone resorption at the ligament-bone attachment in inactive dogs which healed over a period of six weeks or more as fibrous tissue replaced resorbed bone and then became mineralized although caging was continued.

Disuse has been found to result in decreased breaking strength (Allison and Brooks 1921; Semb 1966; Haike et al. 1967), smaller bending moments (Gillespie 1954), and decreased

(Haike et al. 1967) or unchanged (Gillespie 1954) elasticity in the atrophied bones. Mattsson (1972) demonstrated that for rats a 48-week remobilization period after 16 weeks' immobilization evoked practically no reversibility of bone atrophy, which was also observed by Haike et al. (1967).

Cortex weight and diameter have been found to remain below average in the paralyzed hind legs of rats (Gillespie 1954). Paralysis has been found to reduce both size and density of bones in adolescents, but only density in adults (Goisman and Compere 1938). Tower (1939) reported that the intact nerves alone have no effect on bone growth in the absence of muscular activity. Tachovska et al. (1963) and Doskocil et al. (1965) concluded that paralysis reduces the longitudinal growth of limbs in direct relation to the inactivity of muscles. The specific gravity of bone from denervated (Gedalia et al. 1966) and immobilized (Mattsson 1972) limbs has been observed to be lower than in contralateral control limbs. Bone atrophy after inactivation of motor nerves has been considered to be either due to the unbalanced apposition (Strandh et al. 1964) and/or resorption (Kharmosh and Saville 1965) of calcium.

### 2.1.2. Chemical effects of inactivity

Several investigations have shown that within 5 to 6 weeks bed rest causes increased calcium and nitrogen excretion into urine (e.g. Abramson 1948a; Deitrick et al. 1948; Issekutz et al. 1966) as well as hydroxyproline excretion (Donaldson et al. 1970) in healthy men. Equal proportions of bone matrix and bone mineral are lost during bone atrophy and thus the composition of remaining bone is not altered (Allison and Brooks 1921; Klein et al. 1968). This statement is supported by Mattsson (1972) who reported no significant differences in calcium, hydroxyproline, glycosaminoglycan, and organic components when expressed as a percentage of the total dry weight of cortical bone from the femur and tibia of both control and immobilized limbs in rats.

During prolonged bed rest on a Sanders slowly oscillating bed which rocks from the horizontal to a $20^{o}$ footdown position and back every 2 minutes has been found to reduce urinary calcium excretion by 50% in young healthy men who were immobilized from their umbilicus down in plaster casts as compared to the same group of men immobilized in the same manner, but without the Sanders bed (Whedon et al. 1949; Birge and Whedon 1968). This effect could not be produced in patients with acute anterior poliomyelitis (Whedon and Shoor 1957). The authors concluded that the contractions of the antigravity muscles in the normal men immobilized on the Sanders bed was responsible for the changes. On the other hand bicycle ergometer work in a supine position at a load of 600 kpm/min for 1 to 4 h a day could not prevent increased urinary calcium excretion in healthy young men after 6 weeks' bed rest; quiet standing for 3 h daily in addition to supine bed rest restored normal calcium excretion. They obtained similar results when pressure equal to body weight was applied against the longitudinal axes of bones in supine position (Issekutz et al. 1966). This supports the assumption that longitudinal pressure as maintained by the pressure of gravity during normal ambulatory existence is at least equally important for maintaining a balanced chemistry of bones as is physical activity.

### 2.1.3. Physical and chemical effects of pressure

According to Wolf's law bone formation is higher in places of heavier stress. Jansen (1920) and Rodahl et al. (1966) have stated that pressure at least equal to gravity against the longitudinal axes of bones is necessary for the normal growth of bones. Evans (1957), however, demonstrated that tensile forces also stimulate the growth of bones, but Gooding and Neuhauser (1965) found an increase in the longitudinal growth of bones in the absence of gravity. Retarded maturation and lower bone density were observed in connection with below normal weight bearing ability (Makin 1965; Shopfner and Coin 1966). A constant pressure has been found to impair the growth of long bones and, according to estimates by Blount and Zeier (1952), for complete stopping of the growth in adolescents a weight of at least 400 kilos is needed. Thus, it is not known what amount of physical stress is optimal for the growth and functioning of bones.

Abramson (1948b) noted that the ambulatory position prevented the development of osteoporosis in the pelvis and legs of weight-bearing paraplegics whereas paraplegics who were non-ambulatory showed bone atrophy. In addition Saville and Nilsson (1966) observed that women with symptomatic osteoporosis were lighter than controls matched for age as well as women with fractures of the neck of the femur (Alffram 1964).

Numerous investigations have revealed that weightlessness during space flights raises the calcium release from bones (e.g. Mack et al. 1967; Hattner and McMillan 1968; Berry 1969; Mack 1971) while increased weight-bearing has the reverse effect on the bone calcium metabolism (Abramson and Delagi 1961; Ragan and Briscoe 1964; Walser 1969). Rosenfeld et al. (1973), however, found a decrease in the total calcium content of tibio-fibular bone and retarded body weight development in growing rats subjected to long-term acceleration[1).

### 2.1.4. Physical effects of exercise

The effects of physical exercise on the growth and development of bones have mostly been studied in animals although certain

1) The body weight development decreased 14.8% ($p < 0.005$) and the calcium content of tibio-fibula was 12.7% ($p < 0.005$) lower than that of control rats. No weights of bones were given and therefore it is not known whether the concentration of calcium also changed in these bones. Probably the decrease in calcium content was due to lower bone weights of lighter rats in the acceleration group.

conclusions have been drawn from observations of humans. Whether physical exercise enhances or retards the longitudinal growth of long bones has been a subject of debate for decades (c.f. Steinhaus 1933; Rarick 1960; Malina 1969). Prolonged physical training has been reported both to increase the length of long bones in rats and dogs (Steinhaus et al. 1932) and to decrease the length and weight of long bones in trained rats (Lamb et al. 1969; Tipton et al. 1972). Saville and Whyte (1969) found an increase in long bone volume and weight, but no change in bone density and breaking load (compression) in growing rats after low intensity running exercise. In these animals muscle and bone hypertrophy developed accordingly. King and Pengelly (1973) also found no change in bone density following less strenuous training, but after high intensity training for 10 weeks a marked increase in the cortical density of rat bones was found. Isometric exercise further resulted in an increase in the density and breaking load of long bones in the hind limbs of bipedal rats (Saville and Smith 1966).

Exercise has been observed to affect the physical characteristics of human bones as well. Negro women who had engaged in heavy labour from early childhood were taller and heavier and their breast dimensions, and knee and hip width were larger than those of women doing light work (Adams 1938). Van Dusen (1939) found in comparing the size of the right and left hands and arms of 1 to 4 year old children and of 5 to 8 year old children that the older age group tended to have longer right arms, forearms, and hands, and wider palms, while these characteristics were less marked in the younger age group. Similar discrepances between the right and left sides of the body, but greater in magnitude, were observed in college-age adolescents. Buskirk et al. (1956) demonstrated with anthropometric measurements that the hands and forearms of tennis players showed greater bone and muscle development in the dominant than in the non-dominant member. Ingelmark (1947) also reported that the dominating leg of to 20 years old children and adolescents was longer than the non-dominating one. In 13 - 14 years old baseball players, an accelerated epiphyseal growth of the humerus was noticed in the throwing arm, which was also wider, demineralized, and showed apparent fragmentation without evidence of avascular bone necrosis (Adams 1966). In his review of the literature Rarick (1960) concluded that heavy exercise appears to affect favorably the growth in weight and diameter of bone tissue, particulary in the non-weight-bearing segments of the body. Kato and Ishiko (1966), however, found that 116 children out of 4000 doing heavy physical work were on the average shorter than children doing lighter work. They reasoned that the epiphyseal zones of the tibio-fibula and the humerus of these bones were closed a few years before normal.

Changes in the diameters of long bones, in their internal structure, and to a lesser extent in their length were also reported in the investigation of athletes by Ivanitsky (1962). According to Larson's (1973) quotation diameters of the femurs of long-term soccer players were frequently larger than those of non-athletes; an enlargement in the bone marrow cavity of the tibia was observed in runners who had been active for more than 5 years; the pelvises of young girls who started gymnastics before the age of 14 were found to be smaller than those of girls who had taken up sports after reaching that age. In addition changes in bone density were observed in competition runners active more than 25 years (Dalén and Olsson 1974) and in young athletes: the bones of weight lifters were the densest followed by those of runners, while the bones of swimmers were the least dense (Nilsson and Westlin 1971).

The effects of physical exercise on the height of children are contradictory. Endurance training for 6 to 26 months was reported to increase the height of 11-year-old boys (Ekblom 1969). Similar results were obtained in the earlier study by Schwartz et al. (1928). No training-induced height changes in 11 to 15 years old children, however, were observed by Parizkova (1968). Bugyi and Kausz (1970) noted an accelerated maturation of bone age in relation to chronological age in adolescent swimmers.

## 2.1.5. Chemical effects of exercise

In the field of studies concerning the effects of physical exercise on the chemical composition of bones the interest has mostly been focused on calcium. Published results are, however, somewhat contradictory. Ingelmark (1957) found that calcium concentration in the bones of well-trained animals can reach 65% of fat-free dry weight, while inactivity can bring it down to 40%. A running exercise was reported to have increased the calcium concentration in the long bones of 2-month-old rats after 7 weeks high intensity training (3 h/day), but not after prolonged 6 months' low intensity (1 h/day) training, although the calcium-hydroxyproline ratio decreased (Chvapil et al. 1973)[1). Rosenfeld et al. (1973) reported that climbing exercise decreased the calcium content of the long bones and the gain of body weight in growing rats[2)]. King and Pengelly (1973) claimed that, after exhaustive swimming, metabolic changes similar to osteoporosis were found in rat bones. Some authors have suggested that bone calcium loss in old people can be prevented by physical activity. Physical exercise combined with physical therapy for 8 months proved to be superior to conventional physical exercise in increasing bone mineralization in 55 to 94 years old persons (Smith and Bacock 1973).

Metabolic studies have shown that exercise maintains a higher turn-over in the collagen and mineral components of

1)The bone calcium concentration of rats trained for 6 days were not included in the report but had to be re-calculated on the basis of given data. According to my calculations there was a significant decrease in the calcium concentration of these bones after training.

2)The body weight development of exercised rats was retarded by 15.7%, the calcium content of the tibio-fibula and humerus were 10.2% and 15.4% lower respectively. Since the bone weights were not given the calcium concentration of the bones remains unknown. Probably the reported decrease in the total calcium content was due to lower bone weights paralleling body weight differences.

bones. Even after a short period of exercise (11 days), pigs retained more $^{85}Sr$ and hydroxyproline as compared to their foregoing period of non-exercise; re-adaptation to normal did not occur within 10 days (Anderson et al. 1971). Exercise over 1 month accelerated the metabolism of proteins and collagen in the long bones of relatively old mice (Heikkinen and Vuori 1972). They did not find changes in nitrogen and hydroxyproline concentrations, although the hydroxyproline-nitrogen ratio as well as the hexosamine concentration increased. Chvapil et al. (1973) reported that hydroxyproline concentration of the femur increased in adult rats, but not in young rats when trained for 7 weeks. In young rats, however, such a change was produced when the training was continued for 6 months.

Enzymes functioning in the energy metabolism respond to exercise by promoting the activities of the isocitrate dehydrogenase (NAD), malate dehydrogenase, NADH-oxidase, hexokinase, pyruvate kinase, glycose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in the long bones of young trained mice (Heikkinen et al. 1975). Heikkinen et al. (1974) have reported the enhanced callus formation in the bones of mice which had been trained before experimental fracturing. The effects of exercise on the cells, ground substance and blood circulation of bones remain to be studied.

## 2.2. Tendons, ligaments and skin

### 2.2.1. Physical effects of inactivity and exercise

During recent years a number of observations have been made regarding the effects of inactivity and physical activity on ligaments and tendons. Immobilization has been observed to decrease the thickness of fiber bundles in rats and dogs

(Tipton et al. 1970, 1971) and activity either to increase (Ingelmark 1948) or not to influence the size of tendons in rabbits (Viidik 1967a). Ingelmark (1945, 1948) observed that long-term exercise increased the number of cells and the amount of ground substance in the tendons of growing rabbits, the increase in ground substance being proportionally greater than that of cells, while comparable treatment of adult animals led only to the hypertrophy of muscles. On the other hand Tipton et al. (1970) were able to demonstrate that training increased the size of the collagen fiber bundle in mongrel dogs older than one year. The studies of Viidik (1967a) showed that there was no change of weight and water content in the tendons of adult rabbits after prolonged exercise. According to the microscopic and electron-microscopic studies of Laros et al. (1971) there were no ultrastructural alterations in the ligaments of dogs which could have been related to activity level, although after immobilization the insertion point of ligament to cortex of the tibia revealed marked bone resorption. Adams (1966) also found no microscopic changes in the structural appearance of cells, bundles of fibres, and in the individual fibres of ligaments in trained female rats, though the strongest ligaments of the trained animals appeared more opaque and shiny.

Several investigations show that there is a direct relation between the increased breaking load[1)] of bone-ligament-bone and/or tendon-bone preparates after above average physical activity (Adams 1966; Tipton et al. 1967; Viidik 1968). Only two reports failed to show any alteration in knee ligament separation forces (Booth and Tipton 1969; Rasch et al. 1960). The breaking stress of the knee ligaments of trained female rats did not increase by training over 9 weeks (Booth and Tipton

[1)] Breaking load denotes the force (in e.g. newton or lbf) required to rupture a specimen. Separation force and breaking resistance are synonymously used when describing composite specimens (e.g. bone-ligament-bone). On the other hand breaking stress (or ultimate tensile or compressive stress) is breaking load per cross-sectional area (e.g. in newton/$mm^2$).

1969). On the other hand restricted physical activity weakens the strength of these junctions (e.g. Zuckerman and Stull 1973) owever, detraining for 8 weeks did not reduce ligamental rupture-force to the level acquired without training. Similar results were obtained in an earlier study by Tipton et al. (1967).

The ligaments from immobilized limbs of rats and dogs (Tipton et al. 1967, 1970, 1971) show greater extensibility per unit of load. Viidik (1967a) demonstrated an increase in the maximal breaking load and maximal linear load of the isolated tendons of trained rabbits[1]. He also found that the elasticity and elongation of the trained tendons increased (Viidik 1969) and thermal contraction decreased (Viidik, personal communication;1973). There were, however, some differences in these results between different ligaments and tendons. The observation suggests that trained tendons are molecularly less stable, which also was confirmed by Byrd (1973). On the other hand Peacock (1963) found the thermal shrinkage of fibres from ligaments in immobilized limbs to be less.

### 2.2.2. Chemical effects of inactivity and exercise

Research has paid more attention to physical than to chemical changes in soft connective tissue exposed to an above average physical exercise. The collagen component of the tendons and ligaments has been the preferred topic since this probably has been considered as the only force-resisting component of soft

[1] This statement was made on the basis of results which were achieved after relating the absolute maximal load to the maximal load per fresh weight/length unit of the sample. This data presentation is comparable to the data expression per cross section area according to the authors' studies (Viidik 1967b). If the same results had been related to the hydroxyproline concentration of the sample/length unit (which is the data expression of the author in later reports), a slight decrease in the maximal breaking and maximal linear load would have been observed.

connective tissues against loading. The effects of exercise on the hydroxyproline concentration of tendons or ligaments, however, are contradictory (Viidik 1967a; Tipton et al. 1970; Chvapil et al. 1973). Physical activity has been reported either to increase (Tipton et al. 1970) or not to affect (Tipton et al. 1975; Viidik 1967a) hydroxyproline concentrations of tendons or ligaments. Heikkinen and Vuori (1972) found an accelerated metabolic turn-over of collagen and other proteins after physical training and a decreased turn-over of these substances after physical inactivity. Immobilization, however, was not found to change the hydroxyproline concentration in ligaments (Tipton et al. 1971) or tendons (Peacock 1963). After immobilization the glycosaminoglycan concentration in ligaments has been observed either to remain unchanged (Tipton et al. 1970) or to decrease (Akeson 1961).

The concentrations of adenosine triphosphatase, alkaline and acid phosphatase, and of leucinoaminopeptidase were found to be up to 3 times above normal values in the fibrocytes of calcaneus tendons in rats after training (Langhoff and Münzenmaier 1973). The activities of energy metabolism enzymes especially isocitrate dehydrogenase (NAD), malate dehydrogenase and NADH-oxidase were also higher in the tendons of trained mice as well as lactate dehydrogenase as compared to controls (Heikkinen et al. 1975).

A slight retardation in the metabolism of collagen was observed in the skin of inactive mice. The concentrations of hexosamines and nitrogen were highest in the skin of exercised mice (Heikkinen and Vuori 1972).

## 2.3. Skeletal and heart muscle

### 2.3.1. Physical and chemical effects of exercise

It is well established that physical exercise causes enlargement of the heart and skeletal muscles. During recent years various reports on heart hypertrophy have been published (e.g. Meerson 1969; Fanburg 1970). Results from experimental cardiac hypertrophy demonstrate that collagen concentration depends on the type of stimulus (Bartosova et al. 1969) and the age of animals (Chvapil et al. 1973). Chvapil et al. (1973) found that in cardiac hypertrophy induced by physical exercise both total collagen content and concentration increased in the heart muscle of young rats whereas in adult rats similar changes were not observed. Tomanek et al. (1972), however, found no alteration in the hydroxyproline concentration of heart tissue after physical training in either young or old rats. A slightly accelerated protein turn-over was caused by exercise and a slight retardation by inactivation in the heart of relatively old mice (Heikkinen and Vuori 1972). The activation of protocollagen proline hydroxylase was found to precede the increase in the collagen content of hypertrophied heart and skeletal muscles in rats (Lindly et al. 1972; Turto et al. 1974), as well as in trained mice (Suominen and Heikkinen 1975a) and habitually trained endurance runners (Suominen and Heikkinen 1975b).

## 3. OUTLINES OF THE PRESENT STUDY

As the literature review revealed there are many investigations somehow related to the subjects of the present study. However, many basic questions are still unsolved and await further elucidation. In these experiments the main attention was focused on the chemical parameters, although some physical parameters were also studied in young mice trained at two training intensities either during growth only or until after reaching maturity. This paper summarizes the results of original communications (I-VI) and evaluates the results from the point of view of the following problems:

1) Whether physical training enhances or retards the growth of animals and their connective tissues, and whether this is related to training intensity;
2) Whether the growth responses are affected by the state of maturation (the weight of animals) at the commencement of training;
3) Whether physical training results in the strengthening of isolated femoral bones and patella tendons;
4) Whether physical training enhances the vascularity of long bones;
5) Whether chemical responses evoked by training in cells and matrix are tissue-specific or parallel in different types of connective tissues; and
6) Whether there are differences in the time of onset of chemical responses in different types of connective tissues.

# 4. RESEARCH METHODS

Effects of physical activity on the growth of Achilles tendons, long bones, skin and heart were studied in Wistar male mice of NMRI-strain. The mice to be trained and their controls were about 2 weeks old (14 ± 2 days) at the beginning of the training, which took place on a $5^{o}$ inclined treadmill 5 days a week. The duration of daily exercise was increased progressively over 3 weeks. The final daily exercise bouts were 50 and 80 minutes for moderate programs and 180 minutes for the intensive program at a speed of 30 cm/s. The training lasted for 3-22 weeks in the moderate and 3-12 weeks in the intensive training program. At the end of each experiment the Achilles tendons, the long bones of hind and fore limbs, the heart, the skin and a blood sample were taken for analysis. The parameters investigated together with references for analytical methods are listed in Table 1. More detailed information on the handling of samples and analytical and statistical methods are given in each original communication (I-VI).

Table 1. Parameters measured in different tissues with references to the original communications (I-VI) and the methods used.

| Parameters | Tissues | | | | Methods in references |
|---|---|---|---|---|---|
| | Long bone | Ten-don | Skin | Heart | |
| Physical parameters: | | | | | |
| - dry weight | I | I | IV | V | I, IV and V |
| - volume | I | . | . | . | I |
| - density | I | . | . | . | I |
| - length | I | . | . | . | I |
| - breaking load | I | I | . | . | I |
| - vascularity | VI | . | . | . | Brookes (1965) |
| Chemical parameters: | | | | | |
| - nitrogen | II | III | IV | V | Minari & Zilversmith (1963) |
| - hydroxyproline | II | III | IV | V | Woessner (1961) |
| - hexosamines | II | III | IV | V | Blix (1948); Boas (1953) |
| - uronic acids | II | III | IV | . | Bitter & Muir (1962) |
| - calcium | II | . | . | . | Pybus et al. (1970) |
| - DNA | II | III | IV | . | Burton (1956) |
| - RNA-ribose | II | III | IV | . | Ceriotti (1955) |

# 5. RESULTS

A schematic presentation of training-induced effects on the growth of mice and their connective tissues are shown in Table 2 which summarizes the results from original communications I-VI.

## 5.1. Physical properties

The results suggest that physical training during growth can either accelerate or retard the growth and development of mice depending on the intensity of training and the age of the animals: moderate training over 5 and 7 weeks' duration evoked a transient increase in the weights of long bones, Achilles tendons and to a lesser degree of the heart (I: Tables 2 and 3, Fig. 5). Intensive training of equal duration, however, retarded the gain in body and tissue weights (excluding the skin) and reduced the femoral length (I: Tables 4 and 5, Fig. 5). The younger (lighter) the mice were at the beginning of training the more extensive these changes were. The differences between moderate and intensive programs for the younger mice, were significant as to dry weight, density and length of long bones, (I: Table 6). Regardless of the above-mentioned changes these experiments failed to demonstrate any clear functional advantages in the total breaking load of femoral bones which increased only after one moderate training program but did not change in patella tendons (I: Tables 6, 7 and 8). An indication of an enhanced capacity for blood circulation in femoral bones was found where the results were related to the volume of these bones (VI: Table 4).

Table 2. Effects of moderate and intensive training on the growth of mice and their connective tissues during growth and after reaching maturity.

**5-9 WEEKS' OLD MICE**
**trained for 3-7 weeks**

| INTENSIVE TRAINING (180 min/day) | MODERATE TRAINING (80 min/day) |
|---|---|
| **Physical effects** | |
| - reduced gain in dry weight in long bones, Achilles tendons, and heart as well as in body weight | - enhanced gain in dry weight in long bones, Achilles tendons and to a lesser degree in heart; no change in body weight |
| - no difference in total breaking load in femur or patella tendon | - slight increase in total breaking load in femur, no difference in patella tendon |
| - decrease in bone volume and length, increase in density and slight increase in vascularity | - slight increase in bone volume, length, density and vascularity |
| **Chemical effects[1)]** | |
| - increase in bone and tendon nitrogen, decrease or no change in hydroxyproline; increase in skin nitrogen, hydroxyproline and hexosamines | - no change in bone or tendon nitrogen and hydroxyproline; increase in skin nitrogen, hydroxyproline and hexosamines |
| - no change or decrease in skin and bone DNA; increase in tendon DNA | - no change or decrease in skin and bone DNA; increase in tendon DNA |
| - no change in heart | - no change in heart |

**14-24 WEEKS' OLD MICE**
**trained for 12-22 weeks**

(NO MAJOR DIFFERENCES BETWEEN THE TWO TRAINING PROGRAMS)

**Physical effects**

- permanent decrease in femoral length
- no difference in dry weight in long bones and Achilles tendons, increase in heart dry weight

**Chemical effects[1)]**

- increase in glycosaminoglycans (hexosamines and uronic acids) and nitrogen in long bones, Achilles tendons and skin
- increase in hexosamine-hydroxyproline ratio and decrease in hydroxyproline-nitrogen ratio in long bones, Achilles tendons and skin
- no change in heart

1) in concentration units

## 5.2. Chemical properties

The chemical responses were rather similar with respect to both training intensities although responses to intensive training tended to be of greater magnitude and appear earlier. The chemical analyses did not reveal any significant differences in the bone calcium concentration (II: Tables 3 and 4) between the trained and control mice which could have been related to the observed increase in bone density (I: Table 6). After short training programs the concentrations of hexosamines, hydroxyproline and nitrogen increased significantly in the skin (IV: Tables 3 and 4), but not in the long bones, Achilles tendons and heart of growing mice (II, III, V: Tables 3 and 4). The only exception to this was the increase in bone nitrogen after only 3 weeks' intensive training (II: Table 4). After prolonged training the hexosamine and nitrogen concentrations began to increase in the long bones (II: Tables 3 and 4) and Achilles tendons (III: Tables 3 and 4) of the matured mice. The nitrogen and hydroxyproline concentrations were maintained at an above average level also in the skin (IV: Tables 3 and 4). These changes resulted in a decrease in the hydroxyproline-nitrogen ratio and an increase in the hexosamine-hydroxyproline ratio in the long bones, Achilles tendons and skin of trained animals compared to the controls (II and III: Fig. 1; and IV: Tables 3 and 4). The changes in the uronic acid concentrations of long bones and Achilles tendons were parallel to those in hexosamines, although not quite so systematic (II and III: Tables 3 and 4). A slight decrease in the mean DNA concentrations was found after most training programs in the long bones (II: Tables 3 and 4), and in the skin (IV: Tables 3 and 4), whereas a marked increase was observed in the Achilles tendons (III: Tables 3 and 4). No systematic changes were observed in the RNA-ribose concentrations in any tissue (II, III and IV: Tables 3 and 4).

## 6. DISCUSSION

The discussion is limited to a synoptical evaluation of the main results obtained in the experiments (I-VI) as they relate to the outlines of the present study on page 22, and to considering mechanisms adapting connective tissues to physical training. Individual parameters with references to the literature have been scrutinized in each original communication. Another reason for limiting discussion is that in the literature there is no comparable investigation describing various connective tissues in the same experimental animals simultaneously a valid comparison is not possible.

The results of the experiments were in general fairly systematic in all the tissues studied,clarifying some old fundamental subjects of debate and revealing some undetected chemical phenomena.

Age of animals and intensity of training. During growth physical training may lead either to accelerated (Rarick 1960; Bugyi and Kausz 1970; Chvapil et al. 1973) or retarded growth of bones (Lamb et al. 1969; Tipton et al. 1972) depending on the training intensity applied and/or the age of mice at the beginning of training. According to the results obtained in the present studies the decreases in bone volume, length, dry weight and the increase in the bone density as well as decreases in body and other tissue weights were most evident in the youngest mice on the most intensive training program. After short training periods the changes were, however, almost completely limited to quantitative physical properties and were not accompanied by concomitant qualitative chemical changes in the Achilles tendons, long bones or heart. The results agree with those of Saville and Whyte (1969) as to bone calcium, but not as to the bone volume. On the other hand the concentrations of hydroxyproline, nitrogen and hexosamines increased in the skin already after short training periods. The results suggest that the intensity of training determines to what extent genetic growth potential is realized providing the animals are of equal age (weight), or in other words the age (weight) of animals at the beginning of training determines the magnitude and onset of responses to the same training intensity.

Maturation and aging. After cessation of growth the only lasting training-induced change was the decreased length of femoral bones in the trained mice compared to the controls, whereas no differences in the tissue weights of Achilles tendons or long bones were found between the groups. A decrease in bone length has also been observed both in humans (Kato and Ishiko 1966) and in animals (Lamb et al. 1969; Tipton et al. 1972) after strenuous physical activity. These observations show that not only the diameter of bones is influenced by excercise but also the length which Howell (1917) believed to be determined by inherent factors only. Nitrogen, hydroxyproline and glycosaminoglycan reacted similarly to both training intensities in the Achilles tendons and long bones, although the changes tended to appear later in the long bones. The overproportional increase in glycosaminoglycan components, especially that of hexosamines, in relation to hydroxyproline has been regarded as an indication of tissue age (e.g. Sobel and Marmoston 1956) thus suggesting that prolonged training may retard the rate of tissue aging in long bones, Achilles tendons and skin. The statement is also supported by the observations of Akeson et al. (1968) demonstrating a decrease in tendon glycosaminoglycan concentrations after immobilization, and by thermal skrinkage measurements showing molecularly less stable collagen in the trained animals (Viidik personal communication; Byrd 1973). Although the responses to the two training intensities after prolonged (12 weeks) training were about similar one has to note that the higher mortality in the group of intensively trained mice may have caused a selective bias in favour of the intensive program.

The connective tissue components of heart muscle did not respond to training by any chemical alteration in collagen concentration neither in normal sized nor hypertrophied heart, which agrees with Tomanek et al.(1972), but disagrees with Bartosova et al. (1969) and Chvapil et al. (1973). The results indicate that the quantitative changes of heart muscle are obviously appropriate in meeting functional requirements under various states of loading.

Adaptation mechanisms to training. Physical training-induced chemical alterations in the skin demonstrated that the effects of exercise are not limited to the locomotor system only, which suggests that hormonal control mechanisms also play a role in adapting connective tissues to training. Administering interstitial cell-stimulating hormone, testosterone and thyrotropin contributed to the higher breaking load values of bone-ligament-bone preparates in trained rats (Tipton et al. 1971). Booth and Tipton (1969) and Tipton et al. (1974) have discovered sex-linked differences in the breaking load values between female and male rats of similar age after comparable training programs, which suggests that testosterone may influence responces as well.

Hormones also seem to take part in regulating calcium metabolism under physical training as demonstrated by simultaneous experiments with calciferol and physical training (Rosenfeld et al. 1973). They found that administering calciferol in physiological doses affected the calcium accumulation in long bones, heart and kidney and normalized body weight development in the trained rats.

Physical training may also increase the volume of blood circulation in long bones, which exposes more surface area for metabolism. In addition to this, according to the assumption introduced by Trueta (1964), muscular contraction impeds the venous outflow of blood and increases the blood pressure, which opens inactive vascular channels and forces oxygenated blood to areas of the intra-osseus spaces which otherwise would remain practically anoxic. The same is probably true concerning other tissues as well. These changes could lead to an increased metabolic rate of organic and mineral substances, as has been demonstrated by Anderson et al. (1971) in swine and by Heikkinen and Vuori (1972) in various connective tissues of mice.

The results of the present study, however, show that enhanced metabolism does not necessarily lead to higher concentrations of these substances, and that the increased density of long bones was not explained by an increased calcium concentration in these bones. Probably such factors as proportional changes in the size of compact and porous bone and/or bone medullary channel are responsible for the increased bone density observed by measurements based on the Archimedes principle, and not proportional changes in different chemical components.

Changes in enzymes taking part both in energy metabolism and synthesis in various connective tissue (Lindy et al. 1972; Langhoff and Münzenmaier 1973; Heikkinen et al. 1975) and tissues-specific alterations in DNA concentrations demonstrate that local adaptation mechanisms are triggered in different ways by physical training. These scattered observations on adapting mechanisms operating during physical activity reveal how complex and inadequately understood these mechanisms are.

# TIIVISTELMÄ

Tutkimuksen ensisijaisena tarkoituksena on ollut selvittää liikunnan vaikutusta hiiren sidekudoksen kemiallisiin ominaisuuksiin, joskin myös fysikaalisia ominaisuuksia on tutkittu. Tutkimuksen muuttujat on valittu siten, että fysikaalisilla mittauksilla selvitetään sidekudoksen toiminnallista tilaa ja kemiallisilla muuttujilla rakenteellisia muutoksia, jotka edustavat kaikkia sidekudoksen rakenneosia, soluja, soluväliainetta, säikeitä ja mineraaleja. Koe-eläiminä käytettiin nuoria NMRI-hiiriä, joista osaa harjoitettiin kohtuullisella ja osaa intensiivisellä ohjelmalla. Osalla koe-eläimistä harjoitusta jatkettiin vain kasvun ajan, osalla täysikasvuisuuteen saakka. Kysymyksenasettelussa kiinnitettiin huomiota erityisesti seuraaviin seikkoihin:

1) kiihdyttääkö vai hidastaako liikunta hiirien ja niiden sidekudosten kasvua ja riippuuko tällainen muutos harjoituksen voimakkuudesta (I),
2) ovatko kasvun muutokset riippuvaisia hiiren iästä ja/tai painosta harjoituksen alkaessa (I),
3) vaikuttaako harjoitus reisiluun ja polvijänteen kestävyyteen (I),
4) lisääntyykö luiden vaskulaarisuus harjoituksen vaikutuksesta (VI),
5) ilmenevätkö kemialliset muutokset eri tyyppisissä sidekudoksissa harjoituksen samassa vaiheessa vai eri aikaan (II-V) ja
6) ovatko kemialliset harjoitusmuutokset samansuuntaisia eri tyyppisissä sidekudoksissa (II-V).

Harjoituskauden alussa koe- ja kontrollihiiret olivat noin 2 viikon ikäisiä (14 ± 2 vrk). Harjoitusryhmän hiiriä juoksutettiin 5 päivänä viikossa juoksumatolla nopeudella 30 cm/sek. Harjoituksen intensiteettiä lisättiin asteittain kolmen ensimmäisen viikon aikana lisäämällä päivittäistä juoksuaikaa. Lo-

pulliset päivittäiset harjoitusajat olivat 50 ja 80 min. keskiraskailla ohjelmilla ja 180 min. raskaalla ohjelmalla harjoitteleville ryhmille. Harjoitusjaksojen pituudet vaihtelivat 3-22 viikkoon. Harjoituskauden lopussa eläimet tapettiin ja etu- ja takajalat, sydän, iho ja verinäyte otettiin tutkimuksia varten.

Tutkittuina fysikaalisina suureina olivat eläinten paino, niiden sydämen paino, Achilles-jänteen ja pitkien luiden kuivapainot, reisiluun pituus, pitkien luiden tilavuus, pitkien luiden tiheys, polvijänteen ja reisiluun murtumiskestävyys sekä pitkien luiden vaskulaarisuus. Lisäksi tutkittiin seuraavia kemiallisia muuttujia: kokonaistyppi, hydroksiproliini, heksosamiinit, uronihapot, kalkki, DNA ja RNA-riboosi.

Työn tärkeimmät tulokset ovat seuraavat: liikunta joko kiihdytti tai hidasti kasvua riippuen harjoituksen intensiteetistä sekä/tai hiiren iästä, vaikutti reisiluun pituuteen siten, että ne olivat lyhyemmät harjoitetuilla varttuneilla hiirillä, lisäsi hieman pitkien luiden kiertävää verivolyymia, aiheutti sydämen hypertrofiaa muuttamatta sen sidekudoksen koostumusta sekä lisäsi joissakin tapauksissa reisiluun murtumiskestävyyttä, mutta ei vaikuttanut polvijänteen vetolujuuteen. Todetut kemialliset muutokset olivat yleensä samanlaisia eri tyyppisissä sidekudoksissa; kuitenkin ne todettiin systemaattisimpina ja ensimmäisenä ihossa, sitten Achilles-jänteessä ja pitkissä luissa. Muutoksista tyypillisimpiä olivat heksosamiini/hydroksiproliini-osamäärän kasvu sekä hydroksiproliini/kokonaistyppi-osamäärän aleneminen, jotka todettiin varttuneiden hiirien pitkissä luissa, Achilles-jänteessä sekä ihossa. Lisäksi havaittiin DNA-pitoisuuden lisääntyminen Achilles-jänteessä, mutta raajojen pitkien luiden ja ihon DNA-pitoisuus pyrki alenemaan.

Loppupäätelmänä voidaan todeta, että kohtuullisen liikunnan vaikutukset hiirien kasvuun ja sidekudoksen kasvuun ja fysikaaliseen kokoonpanoon olivat suhteellisen vähäisiä ja kemialliset muutokset olivat vastakkaisia sidekudoksen vanhenemismuutoksille.

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