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**13. ABSTRACT (Maximum 200 Words)**

Physical training-related bone injuries represent an important concern in young military recruits. Diet inquiries showed changes in calcium and phosphorus intakes. This study is aimed at investigating the effects of physical activity on bone tissue in the context of high-phosphorus and low-calcium intakes. Sedentary (SED) and trained (TR) Dark Agouti rats were raised on four different diets: normal, high phosphorus-normal calcium, normal phosphorus-low calcium and high phosphorus-low calcium. TR animal were submitted to a 6-week voluntary exercise. The experimental phase was achieved without problem. All TR rats ran spontaneously distances corresponding to a high range of performance (14.4±1.5 km and 40.5±2.5 km for the first and the last week respectively). Analysis of weekly physical activity and running speed showed no significant difference considering the diet. Trained animals (TR) had a 30-40% higher food intake than corresponding sedentary (SED) animals. A slight difference in body growth appeared on the 28$^{th}$ day of the experiment with lower body weight in TR than in SED animals, independently of the dietary group. Bones and blood were sampled at the end of the experiment. The right tibias are aimed to be processed for histomorphometric analysis and the left tibias for analysis of bone mineral content.

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## INTRODUCTION

The present study consists in a biomedical research aimed at improving bone health under physical training. Physical training-related bone injuries represent an important concern in young and middle-aged male and female subjects. Moreover, they need to be taken into account in military recruits, considering their frequency in this particular population. Diet inquiries conducted in the past few decades showed that high phosphorus and moderate-low calcium intake is one characteristic of teens and young adults in U.S. (Calvo 1993, Calvo and Park 1996) and European countries (Le Francois et al 1997). These authors indicated that daily intakes of phosphorus reached levels as high as 50-100% above RDA. Most concerns about high phosphorus low calcium intakes in young adults relate to diet-related bone loss. Studies in rodents showed that rats fed diets containing excess phosphorus had a significantly reduced bone mass. This result has been related to a state of secondary hyperparathyroidism and an increased resorption activity in bone tissue. In humans, a strong correlation between soft drink consumption and bone fractures was found in high-school girls. However, no data are available regarding the effects of altered calcium and/or phosphorus intakes during physical training. We thus proposed to investigate the effects of physical exercise on skeletal tissue in the context of low-calcium high-phosphorus intakes in the rat.

## ADVANCEMENT OF WORK

### *Animals and Treatment*

The present part of this work began in October 2002. It represents the first step of the experiment : animal training under Ca and/or P controlled intakes, and further biological sampling.

Forty-five 5-week old male Dark Agouti rats (100g bw) have been purchased from Janvier (Le Genest St Isle, France) and randomly assigned to nine groups of 5 animals. Four groups of rats were submitted to physical training (TR) for a 6-week period, and five groups were considered as sedentary controls (SED). Sedentary animals were singly housed in standard plastic cages (32x20x15cm). Trained animals were individually housed in specifically designed cages (40x20x65cm) (BioPharm, Mesland, France), with free access to a stainless steel wheel (26.5cm diameter), in which rats were reported to run distances of more than five kilometers per day (Holy and Zérath, 2000). A specifically designed device of the wheels made possible recording the amount of wheel rotations through a connection to a PC computer which converted wheel rotation informations in running speed and distance data. Animals were maintained in a 12:12h light-dark cycle with the light period starting at 8:00a.m. Temperature was set at 21±1°C and humidity maintained between 45 and 65%.

Three groups of rats (one TR and two SED) were fed a standard commercial rat chow containing 0.7% calcium and 0.6% phosphorus (Ca/P = 1.2). One SED group was sacrificed on the first day of running and was considered as the basic control group, aimed at assessing the effects of growing.

Two groups (one SED and one TR) were fed a diet enriched in phosphorus, with 0.7% calcium and 1.2% phosphorus (Ca/P = 0.6). Diet enriched in phosphorus was formulated by adding dehydrated sodium dihydrogenophosphate.

Two groups (one SED and one TR) were fed a diet low in calcium, containing 0.35% calcium and 0.6% phosphorus (Ca/P = 0.6).

Two groups (one SED and one TR) were fed a diet enriched in phosphorus and low in calcium, with 0.35% calcium and 1.2% phosphorus (Ca/P = 0.3). Diet enriched in phosphorus was formulated by adding dehydrated sodium dihydrogenophosphate.

Standard and enriched diets were purchased from UAR (Villemoisson, France).

Animals were fed *ad libitum* and had free access to water. The amount of food ingested daily was recorded and animals were weighed daily except weekends.

In order to label the sites of mineralization, two doses of calcein (10 mg/kg bw) were injected intra-peritonealy respectively 6 and 2 days before the end of the experiment. On the first day of running for basic control animals, and at the end of the training period for the eight remaining groups, animals were anaesthetized, blood was collected, and the tibias were removed. The right tibias are aimed to be processed for histomorphometric analysis and the left tibias for analysis of bone mineral content.

Results are expressed as mean ± SEM. Data were analyzed by a two-factor analysis of variance (ANOVA) using a statistical software Statview (SAS Institute Inc, Cary, NC, USA). A minimal level of $p<0.05$ was considered significant.

*Results of the training period*

1 – Physical activity

Animals ran spontaneously in the above presented conditions, as previously described (Holy and Zérath 2000).

During the first week, standard diet rats ran 14.4 ± 1.5 km. Physical activity increased with time and distances reached 30.3 ± 5.0, 31.9 ± 4.8, 40.9 ± 4.1, 33.2 ± 4.1 and 40.5 ± 2.5 km during the weeks 2, 3, 4, 5 and 6, respectively. As expected, it was observed that animals ran exclusively during the light off period. The time spent each day by the animals in the running wheels slightly increased with time from 152 ± 11 min to 338 ± 18.8 min at the end of the experiment. A plateau was observed after 28 days of training. Speed training increased from 8.3 ± 0.4 m/min at the beginning to 20.6 ± 0.6 m/min at the end of the 6-week training.

Fig 1 and 2 show the distance run and the maximal speed reached by each group of rats per week. We didn't observe any died related significant for any physical activity parameter.

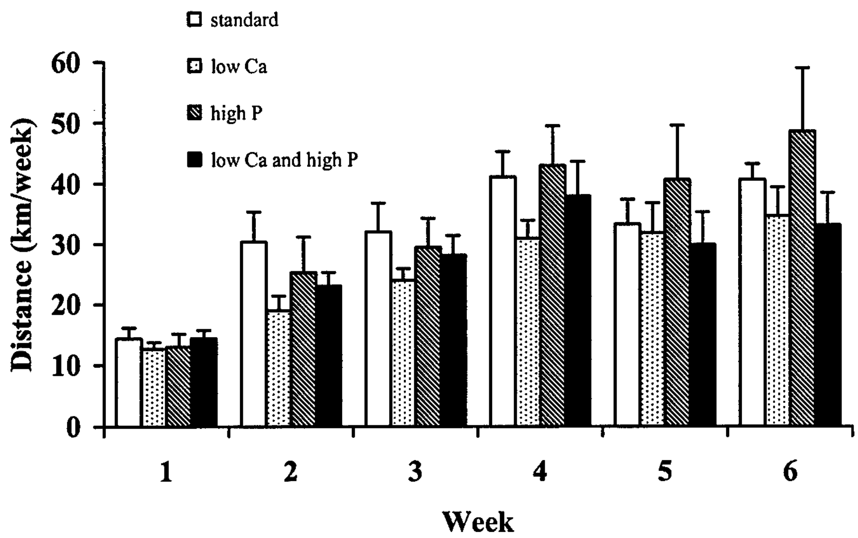


*Fig 1: mean weekly run distance*

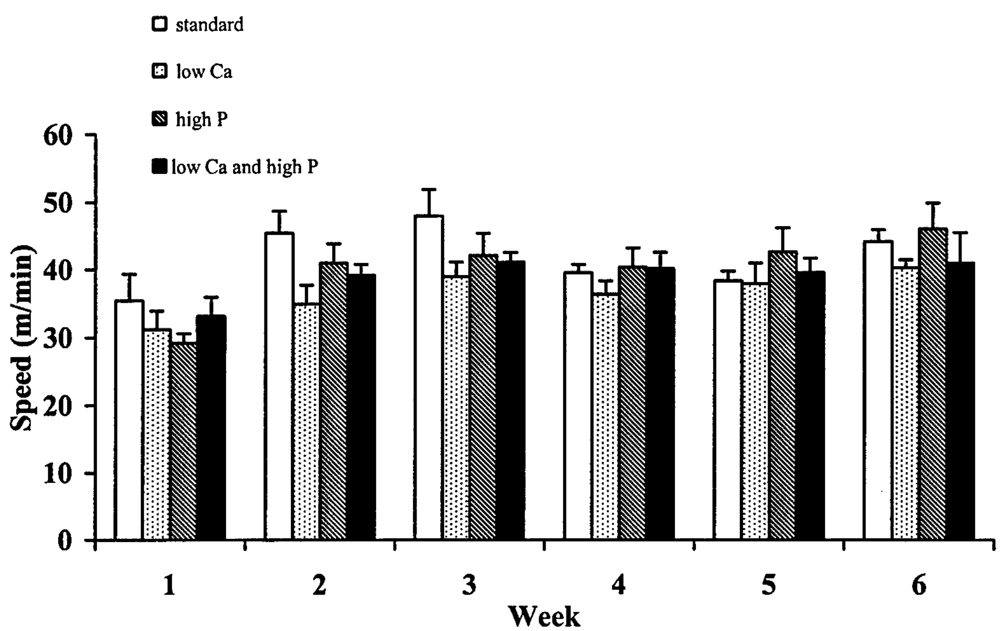


*Fig 2: mean running speed from week 1 to week 6 of the experiment*

2 – food intake

As already described trained animals (TR) had a higher food intake than corresponding sedentary (SED) animals. This trend appeared in the very first days of training. After 14 days, TR ate 30-40 % more than SED rats (fig 3) independently of the type of diet.

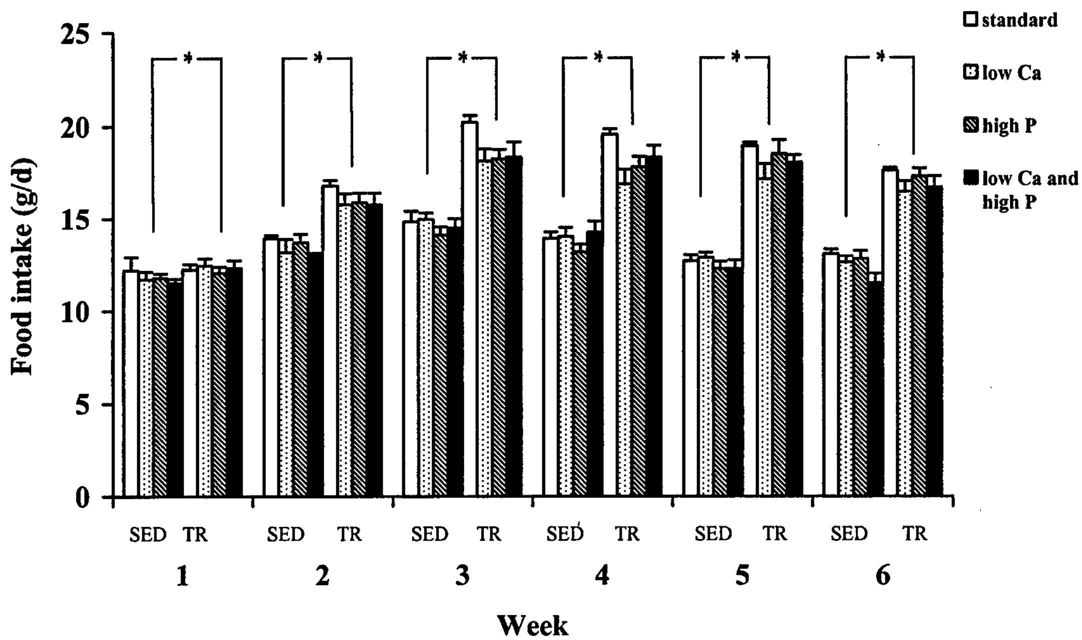


*Fig 3: Food intake*

3 – Growing rate (fig 4)

A slight difference in body growth appeared between SED and TR rats, independently of the dietary group, and became significant 28 days after the beginning of training, with lower body weight in TR than in SED age-matched animals.

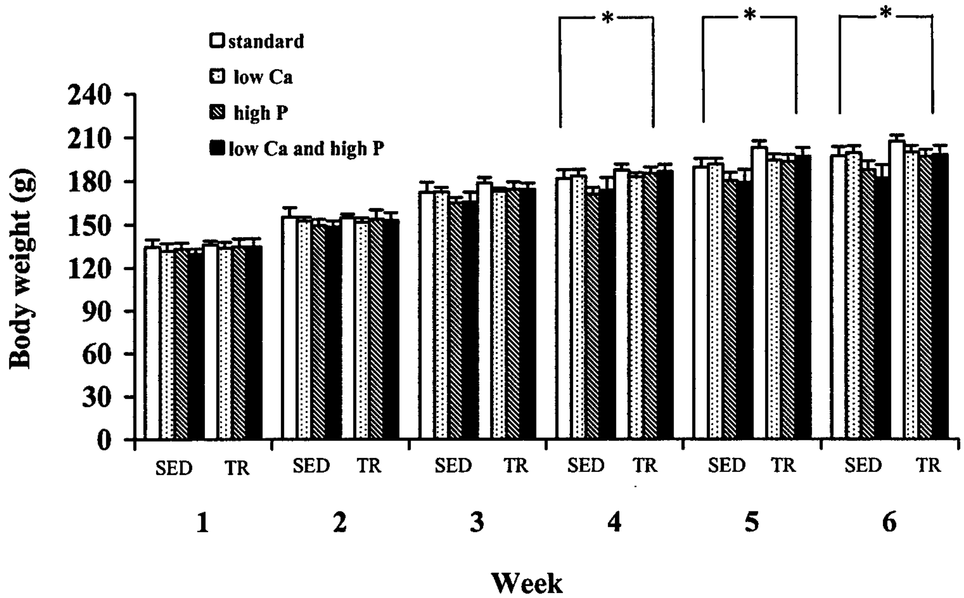


*Fig 4: Body weight*

*Discussion*

The present report brings data relative to physical activity in spontaneously running young male Dark Agouti rats. In contrast of most studies conducted in rats, we employed voluntary wheel exercise for the advantages of this model : absence of conditioning, accordance with the nocturnal activity of the rat and opportunity to eat or drink during the activity period. Dark Agouti has been chosen for his well-known spontaneous activity performance (Oudot et al 1996). In spite of inter-individual variation of daily performance, no animal refused to run and the average distance covered by each animal (20 rats) largely exceeded the distance of training usually imposed in treadmill conditions (6-7 km/week) corresponding to a high range of performance. Dietary interventions did not affect the physical activity in our conditions. Therefore, the effects of dietary can be now investigated in bone parameters in the same training conditions.

## KEY RESEARCH ACCOMPLISHMENTS

| | |
|---|---|
| Experimental phase with animals : | Accomplished |
| Training data collection : | Accomplished |
| Sample collection : | Accomplished |
| Analysis of training data : | Accomplished |
| Analysis of general parameters : | Accomplished |
| Histomorphometric analysis : | To be performed * |
| Mineral content : | To be performed * |
| Biochemical analysis : | To be performed * |
| | * see conclusion |

## REPORTABLE OUTCOMES

At the end of the experiment.

## CONCLUSIONS

Voluntary training in male Dark Agouti rats has been performed without problem and with a high level of activity during the 6 weeks of training. Sampling of blood and bones have been done and the experimental phase with animals is thus completed. Results of physical activity and diet intake are available. They are in accordance with our previous data. Bone analyses remain to be performed.

Please, note that, for administrative problems of money transfer, we do not have recovered funds that were necessary to accomplish this work. This first part, that has been reported here, has been done using our own lab funding. We hope that this problem will be solved and are waiting for the funding in order to complete on this experiment.

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**APPENDICES**

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