Influence of exercise training on tissue chromium concentrations in the rat[1-3]

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ABSTRACT This study reports on the effects of exercise training on the chromium concentrations in the heart, liver, kidney, and gastrocnemius muscle of normal Sprague-Dawley rats. A pair-fed (to the trained rats' intake) and a preexperimental group were also studied in order to control food intake and to ascertain any age-related influence on tissue chromium levels, respectively. Four groups of animals were examined: exercise-trained, pair-fed, preexperimental, and sedentary control. Chromium determination was performed by flameless atomic absorption spectrophotometry. The results from this study show that exercise training increases while pair-feeding and normal aging both decrease chromium levels in tissues. It is suggested that the male Sprague-Dawley rat adapts to exercise training by enhancing tissue levels of chromium or by simply maintaining the high levels of the element found at a younger age. *Am J Clin Nutr* 1984;39:402-409.

KEY WORDS Age, atomic absorption spectrophotometry, glucose tolerance factor, pair-feeding, treadmill running

Introduction

Trivalent chromium is an essential trace element for the mammal which must be provided by the diet (1, 2). It is also known that dietary chromium is mostly in an inactive form which must be converted into an active form, known as the glucose tolerance factor, before exerting any physiological role which is thought to be as a cofactor for insulin action (2-5).

A chromium deficiency state in the mammal has been associated with an insufficient dietary intake of chromium, a weak gastrointestinal absorption, an excessive urinary excretion, aging, or diabetes mellitus (1, 2, 4, 6-10). It has been characterized by a reduced insulin sensitivity of peripheral tissues and an impaired glucose tolerance profile (2, 9, 11). In contrast, exercise training is known to bring about a significant reduction of insulin requirements that is associated with an unchanged or even improved glucose tolerance (12, 13). The hypothesis that tissue chromium levels are implicated in the enhancement of insulin sensitivity of peripheral tissues during exercise training has not been verified. However, it was recently reported that one bout of strenuous exercise significantly increased the urinary output of chromium in man (14), thus implying the possibility that repeated exposure to exercise could lead to a depletion of chromium body stores. This proposition is partly supported by the results of a study which showed that exercise training aggravates the chromium deficiency state of chromium-deficient rats (15). However, tissue chromium levels were not assessed in that particular study.

The present study was thus undertaken in normal rats to determine the influence of

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exercise training on the chromium concentrations of insulin-sensitive tissues such as the liver, heart, gastrocnemius muscle, as well as in the kidney where absorbed chromium is ultimately excreted in the urine or reabsorbed into the blood for use with insulin or for tissue storage (16, 17). In addition, a pair-fed group (to the trained rats' intake) was used to control food intake. Finally, a preexperimental group was also studied in order to determine any age-related influence on tissue chromium.

Materials and methods

Animals and experimental design

Seven-week-old male Sprague-Dawley rats weighing about 195 to 225 g each (Charles River, St-Constant, Québec, Canada), were randomly divided in four groups of animals: exercise-trained, pair-fed, preexperimental, and sedentary control. All experiments were conducted in conformance with the Guiding Principles in the Care and Use of Animals of the American Physiological Society.

Experimental procedures

In order to avoid chromium contamination, all animals were housed in individual plastic cages with a wire-mesh floor which enabled urine and feces to drop out of the cage (18). Moreover, all food dishes were in clay and distilled water was provided ad libitum in glass dispensers. After a 4-day period of familiarization with the environment, the preexperimental rats were killed following these procedures: fasted for 12 h, slightly anaesthetized with diethyl ether, and decapitated.

The animals in the exercise group were then progressively trained on a running treadmill (Quinton Rodent Treadmill, Seattle, WA) and followed a mild exercise program: 26.7 m/min, 60 min, 8° incline, six sessions/wk, for 12 wk (19). Control and trained rats ate ad libitum (Agway's Charles River Rat, Mouse, and Hamster Formula, Syracuse, NY), while each pair-fed rat received daily for 12 wk a quantity of food equal to the intake on the previous day of its trained counterpart (20). Pair-fed animals were used for the purpose of obtaining a sedentary group with a dietary intake of chromium similar to that of the trained rats. All control and pair-fed rats walked for 5 min on the treadmill at 13.3 m/min, 0° incline, six sessions/wk, for 12 wk, a procedure wherein all animals were handled daily and also exposed to the stresses inherent to forced treadmill running.

The procedures for killing the exercise-trained, pair-fed, and control rats were identical to those for the preexperimental rats. In addition, the animals were rested for 44 h and killed in groups of three rats (one of each group) with a maximum of 36 h between the first and last death. The heart, liver (right upper lobe), kidneys, and gastrocnemius muscles were rapidly removed, weighed, frozen in liquid nitrogen, and stored at −80°C for later analyses. The right gastrocnemius and kidney were not excised from the preexperimental rats; also, their left kidney was not weighed.

Determination of chromium

Tissue chromium concentrations were determined by atomic absorption spectrophotometry with electrothermal volatilization (Perkin-Elmer 370A and HGA Ramp). Samples were oxidized in an oxygen plasma low temperature asher (LTA 401). Ashes from the oxidized samples were diluted (1% HNO_3) and mechanically injected into the graphite tube where the electrothermal volatilization took place in three steps exactly as reported in Table 1 (21). Materials were of the highest commercial quality and acid purity was Suprapur throughout the analysis (Merck, West Germany). Standard procedures were taken to eliminate as much as possible any contamination with chromium. Coefficients of variation of 10 aliquots of the same sample, and of five aliquots of different samples were 7.3 and 11.7%, respectively. The accuracy of the methodology was verified with a certified standard reference material, the NBS brewer's yeast (NBS 1569). Results were within 96% (or a mean of 2.04 μg/g) of the certified chromium content (2.12 μg/g). Control values of tissue chromium obtained in this study are in agreement with most (22–25), but not all (26), previously reported data. These latter differences could be due to variations in the chromium content of the diet and its bioavailability, and to different procedures (27).

Statistical analyses

One-way analyses of variance were used with Newman-Keuls post hoc tests for all cases where the exercise-trained, pair-fed, and control groups were compared (28). A two-way analysis of variance with repeated measures was also used to compare food intake profiles throughout time between the exercise-trained and control groups (28). Raw data were transformed in $\log_{10}$ in order to establish homogeneity of variances, when there were significant differences of variances (29). Independ-

TABLE 1
Chromium determination*

Analytical conditions	
Wavelength	357.9 nm
Background correction:	no
Measure mode:	Highest absorbance peak
Sample volume:	25 μl
Dessication:	56 s (40 s) at 150°C
Carbonization:	12 s (9 s) at 1250°C
Atomization:	8 s at 2650°C
Purge:	Argon
Graphite tube:	Pyrolized
Calibration:	Standard curve; $CrCl_3$, aqueous solution

*Chromium determination was performed by atomic absorption spectrophotometry with electrothermal volatilization in three steps: dessication, carbonization, and atomization of the sample. The number of seconds indicated in parentheses at each of these steps represents the time required to reach the desired temperature.

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Results

The number of animals in the exercise-trained, pair-fed, preexperimental, and control groups was nine, nine, 10, and 10, respectively, and was the same throughout the study. The results reported in **Figure 1** indicate that the body weight and total weight gain of the exercise-trained rats were significantly lower than those of the pair-fed, as well as the control rats, at the end of the training program ($p < 0.01$). The body weight gain for the exercise-trained, pair-fed, and control rats was, respectively: 159.2 ± 4.5, 246.0 ± 7.3, and 271.4 ± 8.3 g. Pair-feeding also significantly decreased the total weight gain compared to controls ($p < 0.05$), although the final body weight was not significantly affected by this treatment. The body weight of the preexperimental rats was 203.5 ± 3.2 g, as they were killed a few days before the beginning of the experiment.

The results on the tissue weights are shown in **Table 2** and were found similar in all groups of animals, except for the heart and left gastrocnemius muscle weight of the trained rats which were significantly higher than those of pair-fed and control rats. In addition, preexperimental rats had a significantly higher heart muscle weight (385.5 ± 12.7 mg/100 g body weight; $p < 0.01$) and liver weight (1,095.6 ± 47.0 mg/100 g body weight; $p < 0.05$) compared to controls; their left gastrocnemius muscle weight (508.2 ± 10.9 mg/100 g body weight) was however not different than controls.

Weekly and total food intakes of the trained rats were not significantly different than those of controls (**Fig 2**). The total food intake of the trained and control rats was 2.036 ± 0.132 and 1.961 ± 0.061 kg, respectively. An interesting pattern of food intake

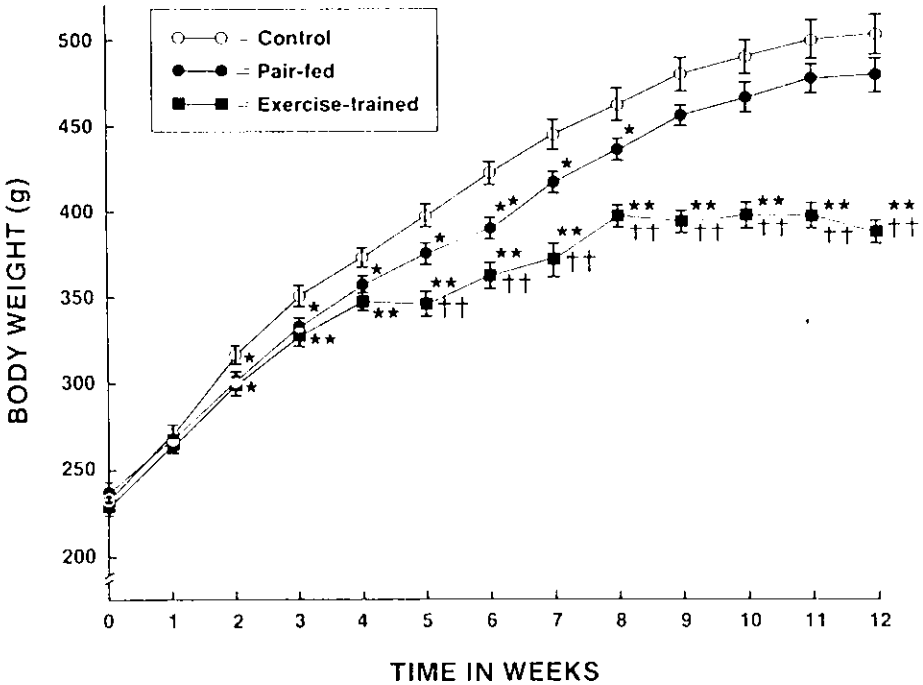

FIG 1. Body weight changes throughout the experimental period in exercise-trained, pair-fed, and control male Sprague-Dawley rats. Rats in the exercise group were progressively trained on a running treadmill over a period of 12 wk following a mild running program: 26.7 m/min, 8° incline, 60 min/day, 6 sessions/wk. Each pair-fed rat received daily an amount of food equal to the intake on the previous day of its trained counterpart, while trained and control rats ate ad libitum. Significant differences from the control and pair-fed groups are respectively indicated by the following symbols: *, † ($p < 0.05$). Two identical symbols refer to: $p < 0.01$.

TABLE 2
Effects of exercise-training and pair-feeding on ratio of tissue wt to body wt*

Groups	Heart	Liver	Left kidney	Right kidney	Left gastrocnemius	Right gastrocnemius
			mg tissue/100 g body wt			
Control (10)	284.1 ±10.8	975.4 ±30.4	294.3 ±2.5	295.1 ±7.9	520.2 ±9.0	525.6 ±12.6
Pair-fed (9)	266.9 ±9.1	919.9 ±36.3	294.9 ±13.2	297.6 ±11.4	518.0 ±10.7	517.3 ±11.0
Exercise-trained (9)	316.2* ±9.0††	944.9 ±25.1	316.6 ±9.5	327.8 ±10.8	563.9** ±10.9††	548.9 ±11.8

* Animal tissue weight expressed as mg of tissue/100 mg of body weight for the heart, liver (right upper lobe), left and right kidneys, as well as left and right gastrocnemius muscles. Symbols for statistical differences are as in Figure 1.

is nevertheless shown in Figure 2: exercise-trained rats ate an average of 5.8% less food than controls during the first 6 wk, but 12.5% more food in the last 5 wk. These data were further analyzed by a two-way analysis of variance with repeated measures of food intake. The results showed that food intake increased throughout time whatever the treatment condition ($p < 0.01$). The results also showed that the effect of exercise training was not the same as a function of time ($p < 0.01$), even though the main effect of exercise training was without influence on the food intake profile compared to controls ($p > 0.68$). The 12th wk of the experiment was incomplete due to the period during which the rats were killed and was therefore not shown in Figure 2. Food intake was still measured for 4 days, and the food intake of the trained (122.7 ± 9.9 g) and control rats (102.8 ± 4.2 g) remained not significantly different. Since each pair-fed rat was nourished daily according to its respective trained counterpart, food intake was thus similar in all posttest groups. However, it should be pointed out that near the end of the experiment, some pair-fed rats did not eat all the food that was offered. This usually small residual amount of food was not taken into account since the main goal of the treatment was for them not to eat more than trained animals.

The results of the tissue chromium concentrations are reported in **Figure 3**. Exercise training produced a significant enhancement of the chromium levels in the heart ($p < 0.05$) as well as in both kidneys ($p < 0.01$) compared to pair-fed animals. These results represent increases of 141.2, 371.3, and 188.8%, respectively, over pair-fed rats' values. When compared to controls, the trained rats showed significantly higher chromium levels in both kidneys only, which were 179.2 and 99.3% higher than controls ($p < 0.01$). In contrast, pair-feeding significantly decreased by 41% the chromium concentrations in one kidney compared to controls ($p < 0.05$). Similarly, a significant age-related influence on the chromium concentrations is seen in one kidney of the preexperimental rats compared to controls ($p < 0.001$), and correspond to a reduction of 180.6%. Finally, the chromium concentrations in the liver and gastrocnemius muscle were unaffected by the various treatments.

Discussion

Exercise training

The reductions in body weight and total weight gain, as well as the increase in the ratio of some tissue weights per unit of body weight, have been previously reported by many authors (30, 31) after a period of exercise training in male rats, and are confirmed in this study. These changes are explained by an increased energy expenditure over energy intake compared to pair-fed and control rats, thereby reducing adiposity in exercise-trained animals (30, 31).

Results from this study demonstrated that tissue chromium levels were markedly increased in animals trained on a running treadmill for 12 wk (Fig 3). Remarkably, this

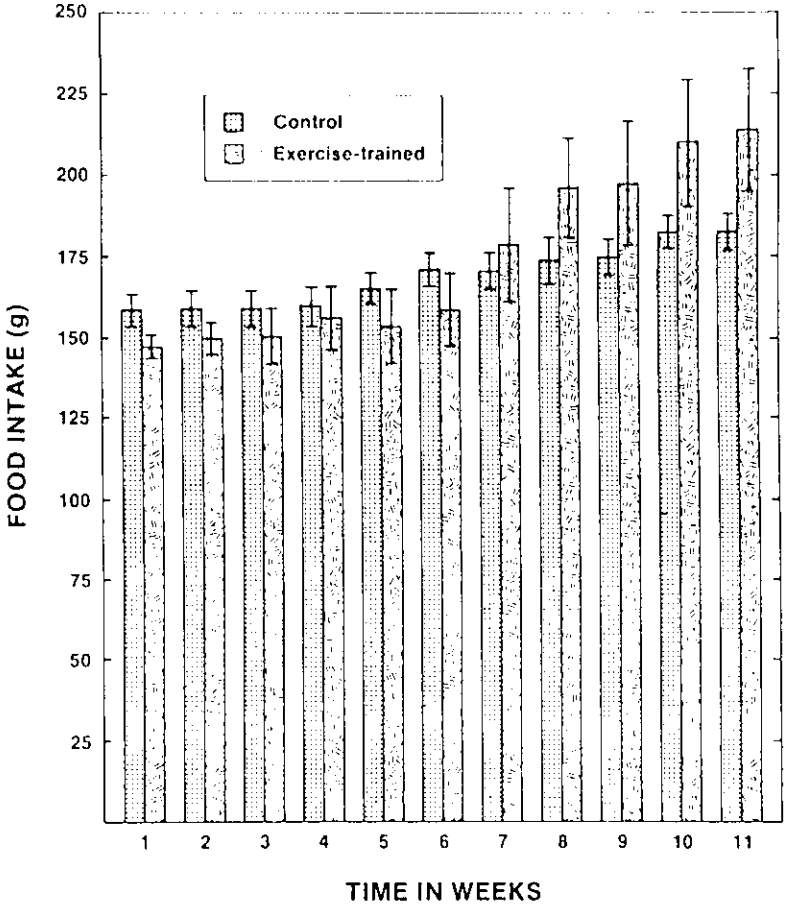

FIG 2. Food intake variations throughout the experimental period in control and trained rats. Pair-fed rats (not shown) were given the same amount of food as the trained rats consumed each day. The last week of the experimental period is incomplete due to the period when the rats were killed. Results are not significantly different throughout. Total food intakes are reported in "Results" and were similar in both groups.

enhancement in chromium concentrations occurred regardless of the adverse influence of food intake (shown by pair-feeding) and normal aging which both lowered chromium levels. Indeed, a significant increase in chromium concentrations was observed in the kidneys of the trained animals compared to control and pair-fed rats, as well as in the heart muscle in comparison to pair-fed animals. These higher chromium levels in trained rats are interpreted as an elevation or preservation of chromium body stores. This phenomenon could result from an improved gastrointestinal absorption of food chromium, although there is no available evidence to support this interpretation. Another explanation of these results would be a reduced urinary excretion of chromium. Indeed, this study expands the work of Anderson et al (14), who reported an exercise-induced loss of urinary chromium, and suggests that exercise training provides a mechanism of adaptation which increases tissue levels of chromium or at least maintains the high levels found at a younger age. Therefore, the present results do not support the work hypothesis that repeated exposure to exercise leads to a depletion of chromium body stores, possibly through the urines.

Although it could not be ascertained, changes could have also occurred in the ratio of biologically active chromium (GTF) to inactive chromium in insulin-sensitive tissues such as the skeletal muscles and liver.

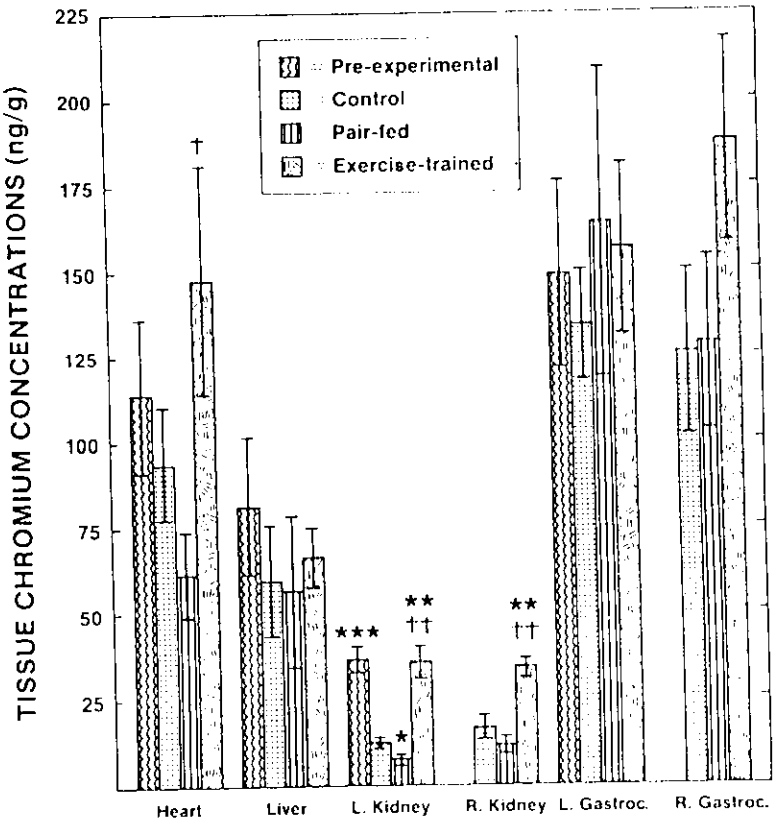

FIG. 3. Tissue chromium concentrations expressed as nanograms of chromium/g of wet tissue for the heart, liver, left and right kidneys as well as left and right gastrocnemius muscles. Symbols for statistical significance of differences are as in Figure 1. In addition, *** represents a highly significant difference from the control group ($p < 0.001$).

Finally, exercise training exerts an intense positive influence on chromium metabolism in tissues, despite the fact that normal aging and the food intake pattern of the trained rats, which was reproduced in the pair-fed rats, both induced a loss of tissue chromium levels.

Pair-feeding

As an experimental treatment, pair-feeding produced a significantly lower total body weight gain compared to controls (Fig 1). Indeed, the pair-fed rats, as did the trained rats to which they were paired, ate less food than controls during wk 1 to 6, and more food in wk 7 to 12, but neither the weekly intakes nor the total food intake were significantly different (Fig 2). However, the ratios of tissue weights to body weight, as well as the final body weight of the pair-fed rats were all comparable to controls, hence indicating the marginal effect of pair-feeding on these variables. Nevertheless, a significant decrease in chromium concentrations was observed in one kidney compared to controls (Fig 3). It appears unlikely that this reduction could be entirely due to the fact that, near the end of the experiment, some pair-fed rats left a small amount of food in their dish. A conceivable explanation might be that a small restriction of dietary chromium at an early age in the rat reduces its levels in tissues. In this line of reasoning, the absence of a recovery of tissue chromium levels in the pair-fed rats remains unexplained in relation to their higher food intake during wk 7 to 12. It is noteworthy that exercise training increased tissue chromium levels on approximately the same dietary

intake of chromium that induced a decrease in pair-fed rats.

Age-related effects

Nineteen-wk-old control rats showed a significant loss of the chromium concentrations in one kidney when compared to 7-wk-old preexperimental animals (Fig 3). Similarly, significant reductions of chromium levels have been reported as a function of age in plasma (32), urine (33), head hair (6), and in tissues of normal men (10, 22), while a similar trend was also observed in female rats older than 2 yr (34). The significant age-related loss of tissue chromium in normal rats reported in this study appears to be independent of the total chromium content of the diet. Indeed, a similar lab food (35) has been shown to contain more than 10 times the amount of chromium necessary to avoid deficiency symptoms (ie, 100 ng of chromium per g of food) (2). Furthermore, the intestinal absorption of chromium has been postulated to decrease with age by some authors (1), but not all (4). Moreover, the absorbency of food chromium might have been reduced by the various refining steps required for the making of the laboratory food (15, 35). Consequently, it is argued that the bioavailability of chromium, not the total chromium content of the food, is inadequate to maintain the initial tissue concentrations of the element.

In conclusion, exercise training in the male Sprague-Dawley rat increases tissue levels of chromium, or at least maintains the high levels of the element found at a younger age. In contrast, pair-feeding (to the trained rats' intake) and normal aging (7 to 19 wk of age) both decrease chromium concentrations in tissues. Further studies on the physiological significance of these variations in tissue chromium levels should be particularly appealing in relation to glucose tolerance as well as insulin sensitivity of peripheral tissues.

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