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Original Article

Effects of Physical Exercise on Food Intake and Body Weight: Experimental Study in Tumor-Bearing Rats

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Abstract

Cancer cachexia is frequently observed in tumor-bearing patients or animals with diabetes-like syndrome. Physical exercise has been shown to reduce insulin requirements in diabetics. **Objectives**: To evaluate the effects of daily physical exercise on food intake and body weight in an experimental tumor-bearing rat model (Walker 256 carcinosarcoma). **Methods**: Wistar adult rats were submitted to a similar daily manipulation protocol except for physical exercise (running pad continuously for 60 minutes/day), and randomly distributed as to the presence of tumor or exercise into eight groups: Group 1: (Saline day 1 + No exercise for 50 days), Group 2: (Tumor day 1 + No exercise for 50 days), Group 3: (Saline day 1 + Exercise for 50 days), Group 4: (Tumor day 1 + Exercise for 50 days), Group 5: (Tumor day 1 + Exercise for 25 days + No exercise for following 25 days), Group 6: (Tumor day 1 + No exercise for 25 days + Exercise for following 25 days), Group 7: (Exercise for 25 days + Tumor day 25 + Exercise for following 25 days), Group 8: (Exercise for 25 days + Saline day 25 + Exercise for following 25 days). **Results**: Body weight was significantly lower in Exercise (mean coefficient: 1.29 ± 0.17), than in No exercise (mean coefficient: 2.2 ± 0.16) - $p<0.001$. In the tumor-bearing rats, exercise did not affect significantly daily food intake - $p=0.415$. **Conclusions**: Daily physical exercise significantly affected body weight in this animal model, but did not have impact on food intake.

Keywords: Carcinoma 256, Walker; Exercise; Cachexia; Neoplams

Introduction

Cancer cachexia is the leading condition associated with tumor growth in patients, with all the undesirable consequences on the host. Progressive decrease of body weight, proteolysis, lipolysis,[1] and overall catabolic balance significantly affect the immune system, as well as all basic organ functions. An estimated 70% of cancer patients will die in consequence of cachexia, rather than from the direct effects of metastatic or primary tumor localization.[1] Several alternatives were evaluated over the last decades trying to halt or reverse catabolic metabolism in cancer patients, as well as in experimental tumor-bearing animals. Nutritional, hormonal, and anti-cytokine antibody manipulation has been extensively studied, with no clear benefits reported in the recent medical literature. Insulin and anti-interleukin 6 antibodies have been shown to reverse some of the metabolic alterations induced

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by tumor in animal models but not in patients.[2] One of the basic metabolic alterations seen in cancer cachexia is peripheral resistance to insulin, producing a diabetes-like situation. Previous studies in diabetic patients showed that regular physical exercise significantly decreased the amount of insulin needed to maintain euglycemia in those patients.[3] This effect is seemingly due to better glucose utilization by body tissues, despite low insulin levels. The present study evaluates the potential effects of daily physical exercise on food intake, and body weight, in an experimental rat model.

Material and Methods

Wistar adult rats (250g - 300g) were used for this study. All animals were kept in individual metabolic cages, and offered food and water *ad libidum*. The rats were submitted to a similar daily manipulation protocol except for physical exercise.

Following a conditioning period of four days in the individual cages, the rats were randomly distributed to eight groups, with randomization including more than 10 rats every time, in order to insure homogeneity in the distribution of animals receiving the same tumor cells, prepared in the same technique, and injected under the same conditions, among the following different groups:

- Group 1
  Saline on day 1 + No exercise for 50 days
- Group 2
  Tumor on day 1 + No exercise for 50 days
- Group 3
  Saline on day 1 + Exercise for 50 days
- Group 4
  Tumor on day 1 + Exercise for 50 days
- Group 5
  Tumor on day 1 + Exercise for 25 days + No exercise for following 25 days
- Group 6
  Tumor on day 1 + No exercise for 25 days + Exercise for following 25 days
- Group 7
  Exercise for 25 days + Tumor on day 25 + Exercise for following 25 days
- Group 8
  Exercise for 25 days + Saline on day 25 + Exercise for following 25 days

Day 1 corresponds to the first day in the study (day of randomization). The total duration of the experiment was 50 days, divided into two periods of 25 days. Tumor cells or placebo saline injections were administered on the days specified for each group.

Tumor Cells and Implant Technique

We used Walker 256 carcinosarcoma tumor cells stored at the Department of Surgery of the University of São Paulo. Cells were injected into the abdominal cavity of Wistar rats in order to multiply the number of cells, and prepare for injection into experimental animals. The rats were anesthetized seven days following that procedure, the ascitic peritoneal fluid was then aspirated by a puncture with sterile technique, and the fluid was immediately kept on ice. The fluid was then diluted in saline (1:200), and a sample (40 mcL) of that solution was separated in an Eppendorf tube with 20 mcL of Trypan Blue. After homogenizing the solution, it was analyzed in a Neubauer chamber, in order to count viable tumor cells. Following the counting procedure, a solution of 100,000 cells/ml saline was prepared. Tumor cells were injected in one ml volume in the left flank of the rats randomized to receive tumor. Rats randomized to receive only saline were injected in the same area with one ml of saline (0.9% NaCl solution).

Daily measurement of tumor burden

The tumor was measured daily starting from the first day following injection. Perpendicular diameters of the palpable nodule were measured by using a skinfold caliper, and the volume was calculated. In a separate previous study done with the same tumor, we determined the correlation between tumor volume and its actual weight by a linear regression equation. Tumor volume tumor was estimated to be an ovoid with the equation: volume = $4/3\ pa^2 \times b$. Carcass weight of each animal was calculated daily by subtracting tumor weight from rat total body weight on the same day.

Food intake and animal weight were determined daily, at the same time of the day (morning, before any physical activity).

Exercise

The rats randomized to exercise were placed on a running pad, with electrical stimulus, continuously for 60 minutes/day, during the pre-established period. Rats that did not adapt to the running pad, and did not run for full period during the first three days of the experiment, were excluded.

Statistical analyses

Tumor volume, total body weight, and carcass body weight curves were analyzed by determining through regression analysis the coefficient of tangency of each curve for the first 25 days, and then for the final 25 days of the experiment. Curves at these periods of observation had linear behavior, which enabled us to calculate the coefficient of tangency by linear regression ($r>0.85$) for all curves. Coefficients were determined individually for each individual rat, and then compared by variance analysis between groups. Coefficients were grouped for each variable analyzed: presence or absence of a variable (Tumor X No tumor, Exercise X No exercise), or the combination of variables (Tumor-bearing with exercise X Tumor-bearing with no exercise). Variance analysis (ANOVA) or Student's t-test were used to compare groups coefficients, or groups subsets defined within the experimental population.

Data are presented as mean ± standard error of the mean (SEM). Differences were considered significant if $p<0.05$.

Results

Figure 1 shows mean curves for body carcass weight during the experiment for all rats. Mean coefficients for the curves in each group variable regarding total body weight and carcass weight were determined and analyzed:

First 25 Day Period

a) Total body weight increased significantly more in No Exercise (mean coefficient :2.2 ± 0.16), than in Exercise (mean coefficient: 1.27 ± 0.16), with $p<0.001$. Tumor-bearing rats had significantly lower increase in body weight (mean coefficient: 1.69 ± 0.12) than non-tumor bearing rats (mean coefficient: 2.12 ± 0.1), $p=0.009$. When we analyzed only animals injected with tumor, body weight was significantly lower in Exercise (mean coefficient: 1.29 ± 0.17), than in No exercise (mean coefficient: 2.2 ± 0.16), with $p<0.001$.

b) Carcass body weight (Total body weight - tumor weight) increased at a faster rate in No exercise (mean coefficient: 0.92 ± 0.22) than in Exercise (mean coefficient: -0.32 ± 0.31)

c) Daily food intake was significantly higher in rats with No tumor (mean intake/day: 27.9g ± 0.72), compared to rats with Tumor (mean intake/day: 25.0g ± 0.71), with $p=0.011$. If we include only tumor-bearing rats, exercise did not affect significantly daily food intake: Exercise tumor bearing (mean intake/day: 24.3g ± 0.83); No exercise tumor bearing (25.3g ± 0.90), with $p=0.415$.

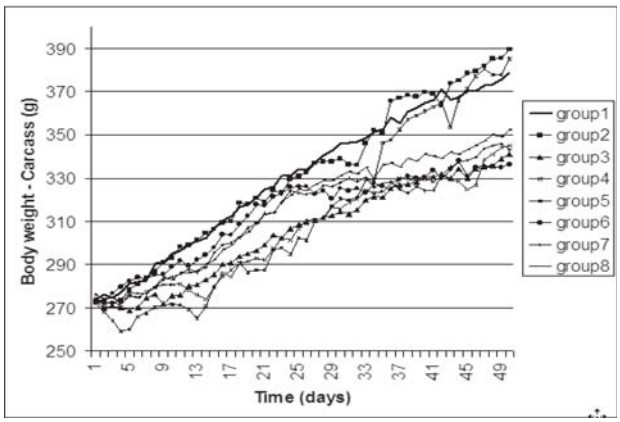

Figure 1 - Carcass body weight during the experiment (mean/group)

Second 25 Day Period

a) Carcass body weight was similar in the four groups during the last 25 day period (Group 2: mean coefficient - 0.44 ± 1.32; Group 4: mean coefficient 0.23 ± 0.67; Group 5: mean coefficient 0.36 ± 0.79; Group 6: mean coefficient - 2.3 ± 0.98 - $p=0.148$). When we compared carcass weight between e rats that exercised and rats in rest, there was no significant difference (Exercise: mean coefficient -0.01 ± 0.75; No exercise: mean coefficient -1.08 ± 0.64 - $p=0.275$). When we looked at rats that were in rest during the last 25 days of

the experiment, we did not observe any significant influence of exercise in the first 25 day period (p=0.596) On the other hand, rats that had exercised during the first 25 days and continued to exercise in the last period gained carcass weight, while rats in rest during the first 25 days and exercising in the last 25 days lost carcass weight (p=0.043).

b) Food intake was significantly higher in non-tumor bearing rats (mean intake/day: 30.4g ± 0.65) than in tumor-bearing rats (mean intake/day: 25.3g ± 0.67), with p<0.001. When we included only tumor-bearing rats, exercise significantly increased food intake (mean intake/day: 26.5g ± 1.06) compared to No exercise tumor-bearing animals (mean intake/day: 22.0g ± 1.22), p=0.008.

Discussion

The influence of exercise on tumor-bearing hosts is still controversial. Although physical activity is highly recommended in cancer patients, it is not clear how - if ever - this activity could affect the body and/or the tumor in those patients. Several studies showed that cancer cachexia occurs in most patients, mainly at the end of the course of their disease, resulting in progressive dysfunction of multiple organs, with the subsequent deterioration of vital mechanisms of response to stress and infection.[4,5] One of the basic mechanisms underlying metabolic disturbances seen in patients with cancer is insulin resistance at peripheral tissues, mainly muscles and fat.[5-7] This imbalance causes a catabolic reaction leading to proteolysis, lipolysis, and gluconeogenesesis.[8,9] Alterations of cytokine and hormone activities were identified as paramount for those alterations.[10-12]

Intracellular effectors are shown to be responsible for some of the alterations, probably directly influenced by hormones and/or cytokines. Previous studies have shown that diabetic patients submitted to programs of physical exercise maintain their plasma glucose levels lower, for the same amount of insulin injected, compared to sedentary diabetic patients.[3] It is speculated that exercise could affect cellular permeability to glucose, independently of insulin level. The basic mechanism is still unclear.

The objective of this study was to evaluate the possibility of continuous exercise to affect body weight and food intake in a tumor-bearing animal. Body weight increased in a steady way in the first 25 days of the experiment in all groups. Total body weight increased at a faster rate in resting rats (No exercise), compared to animals submitted to daily exercise. Tumor bearing rats showed similar trends, with exercise negatively influencing the rate of total body weight increase. Carcass body weight, which subtracts tumor weight from total body weight, and therefore correlates with the host real weight host, also increased at a faster rate in rats not subjected to exercise during this period. These results taken separately would suggest a worse progression of cachexia in tumor bearing animals under strict exercising program. One should be cautious when analyzing the present data, as we did not examine in this protocol the compartmental distribution of weight, neither did we assess water content in the animals. It is known that exercise could redistribute weight between fat and muscle compartments, as well as increase the host protein content without affecting overall body weight. Daily food intake was significantly higher in no-tumor animals, compared to tumor bearing rats. In the latter group, exercise did not alter significantly food intake.

The results of this study showed that daily physical exercise significantly affects body weight in this animal model, but did not have impact on food intake. Further studies are planned to evaluate metabolic alterations induced by exercise in tumor-bearing animals, and to identify water and protein tissue distribution. The conceivable impact on the rate of development of protein wasting phenomenon associated with cancer will be clarified.

References

1 Younes RN, Vydelingum NA, Noguchi Y. Lipid kinetic alterations in tumor-bearing rats: Reversal by tumor excision. J Surg Res 1990;48:324-8.

2 Sherry BA, Gelin J, Fong Y. Anticachectin/tumor necrosis factor-alpha antibodies attenuates development of cachexia in tumor models. FASEB J 1989;3:1956-62.

3 Devlin JT. Effects of exercise on insulin sensitivity in humans. Diabetes Care 1992;15:1690-3.
4 Heber D, Chlebowski RT, Ishibashi DE. Abnormalities in glucose and protein metabolism in noncachectic lung cancer patients. Cancer Res 1982;42:4815-20.
5 Lundholm K, Edstrom S, Karlberg I. Glucose turnover, gluconeogenesis from glycerol, and estimation of net glucose cycling in cancer patients. Cancer 1982;50:1142-7.
6 Shaw JHF, Wolfe RR. Glucose and urea kinetics in patients with early and advanced gastrointestinal cancer. Surgery 1986;101:181-8.
7 Jeevanandam M, Lowery SF, Horowitz GD. Cancer cachexia and protein metabolism. Lancet 1984;1:1423-7.
8 Fearon KC, Hansell DT, Preston T. Influence of whole body protein turnover rate on resting energy expenditure on patients with cancer. Cancer Res 1988;48:2590-5.
9 Noguchi Y, Vydelingum NA, Younes RN. Tumor-induced alterations in tissue lipoprotein lipase activity and mRNA levels. Cancer Res 1991;51:863-9.
10 Oliff A, Defeo-Jones D, Boyer M. Tumors secreting human TNF/cachectin induce cachexia in mice. Cell 1987;50:555-63.
11 Darling G, Fraker DL, Jensen JC. Cachectic effects of recombinant human tumor necrosis factor in rats. Cancer Res 1990;50:4008-13.
12 Knox LS, Crosby LO, Feurer ID. Energy expenditure in malnourished cancer patients. Ann Surg 1983;197:152-6.