

JEPonline
Journal of Exercise Physiologyonline

Official Journal of The American
Society of Exercise Physiologists (ASEP)

ISSN 1097-9751

An International Electronic Journal
Volume 7 Number 3 June 2004

Clinical Exercise Physiology

EFFECTS OF EXERCISE WITH AND WITHOUT BCG ON THE GROWTH OF
PROSTATE CANCER

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ABSTRACT

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Randy Bryner, Dale Riggs, David Donley, Justin White, Irma Ullrich, Rachel Yeater. JEPonline
2004;7(3):75-83. Numerous animal studies have found that exercise training enhances resistance to
experimentally induced tumor growth although limited data exist on prostate cancer. Virtually no data exist on
the interactive effect of exercise training with immunotherapy. Therefore we examined the effect of moderate
treadmill exercise alone and in combination with Bacillus Calmette Guerin (BCG) on the growth of prostate
cancer in rats. Groups included control-saline (CS: n=13), exercise-saline (ES: n=13), exercise-BCG (EB:
n=12) or control-BCG (CB: n=14). Treadmill exercise consisted of running on a motor-driven treadmill at 20
m/min, 30 min/day, five times/week for eight weeks. Following training, all animals were inoculated with 1×10^6
transplantable rat prostate cancer subcutaneously on the back (day=0). Eight animals from CS and ES were
given a maximal treadmill test pre and post training. Although no significant differences were observed among
groups, total tumor volume tended to be smaller ($p=0.06$) at day 28 in EB vs ES (EB: 2232 ± 1368 vs ES: 3482 ± 1854 ,
 mm^3). The percent change in tumor volume from weeks 4 to 6 tended to be greater ($p=0.07$) in CS vs
CB (CS: 82 ± 12 vs CB: 69 ± 15) and EB vs CB (EB: 83 ± 15 vs CB: 69 ± 15). Exercise training and BCG
immunotherapy did not result in any significant modifications to tumor growth when compared with controls.
However, the combination of exercise training and BCG immunotherapy tended slow the growth of a
transplanted prostate adenocarcinoma during the initial phases of detectable growth. In addition, BCG alone
had a tendency to limit the growth of this tumor during the aggressive phase of growth.

Key Words: Exertion, Immunotherapy, Carcinoma, Tumor, Immune System

INTRODUCTION

Carcinoma of the prostate is the most common cancer and the second most common cause of cancer death
among men in the United States. It is estimated that in 1998 there will be 184,500 new cases of prostate cancer
diagnosed in the United States (15). While the majority of patients will respond to hormonal therapy, average

survival in patients with metastatic disease is less than three years and optimal hormonal therapy increases survival by only seven months (5). Response to chemotherapy is poor and has not been found to prolong survival. Evidence suggests that the development of prostate carcinoma may be related to impaired immune control of malignancy within the gland.

Bacillus Calmette Guerin (BCG) immunotherapy is now the intravesical treatment of choice for bladder cancer (13). Preliminary clinical trials have suggested that BCG may also have significant anti-tumor activity in carcinoma of the prostate (6). Injection of BCG into the adenocarcinoma of the prostate in humans produces granulomata with local destruction of tumor cells resulting in tumor necrosis (18,27). Biopsy of the prostate in patients receiving BCG for carcinoma of the bladder demonstrated granulomatous reactions in 40% of patients (20). Percutaneous BCG application resulted in a significant prolongation of survival in patients with prostate cancer (8). Therefore, intravesical BCG immunotherapy may be effective in the management of carcinoma of the prostate. There are obvious drawbacks to administering live bacteria to patients. Although BCG has been shown to be an effective treatment of and prophylaxis against recurrent stages Ta and T1 tumors and carcinoma in situ of the bladder, and most patients will tolerate this treatment well, adverse side effects can and do occur. BCG therapy may cause systemic side effects varying from mild malaise and fever to, in rare instances, life-threatening or fatal sepsis (14). Therefore, any procedures that could make the host more responsive to smaller doses could be advantageous.

Exercise training may prove effective in modulating the host's immune system. Moderate exercise training has been shown to significantly increase serum immunoglobins, monocytes, natural killer (NK) cell number and activity while also decreasing symptoms of upper respiratory disease (21,22). Studies utilizing laboratory rodents have reported that exercise can have a protective effect on the induction of chemically induced mammary tumors (28,29). Chronic exercise retarded percent retention of intravenously injected CIRAS 3 tumor cells in the lungs of treadmill and wheel trained mice compared to that of sedentary groups (17). Moderate exercise training has been shown to reduce the growth of mouse fibrosarcoma L-1 inoculated subcutaneously in BALB/C mice (31). The effects of exercise training, therefore appears to be more global in its ability to stimulate the immune system of the host, much like the effects of BCG immunotherapy.

Very little data exist on the effects of exercise training and the development of prostate cancer. We are aware of no published studies that have compared the interactive effect of exercise training with immunotherapy on this tumor. We hypothesized that subcutaneous injections of BCG would limit the growth of an aggressive transplanted prostate cancer in rats. We also hypothesized that if animals were aerobically trained prior to receiving prostate cancer, they would respond more favorably to BCG by having reduced tumor growth and metastasis.

METHODS

Animals

Fifty-two male Lobund-Wistar rats were randomly assigned to four groups; control-saline (CS: n=13), exercise-saline (ES: n=13), exercise-BCG (EB: n=12), or control-BCG (CB: n=14). Animals were obtained at four to six weeks of age and housed two per cage for a short acclimatization period on a 12 hour reversed light/dark cycle, with an ambient temperature of approximately 21⁰C. After the acclimatization period, all animals were handled daily and ES and EB animals were allowed to walk on the treadmill for short periods of time. This procedure was used to teach the animals to walk on the treadmill without the need for electrical stimulation. Any animal that refused to walk or required excessive stimulation was excluded from the study. Twenty-five animals were trained to walk without the need for any electrical stimulation. Animals were given free access to both food and water throughout the study. This study was approved by the West Virginia University Animal Care and Use Committee.

Exercise Training

Animals in the ES and EB groups exercised five times/week for eight weeks. Exercise consisted of running on a

motor driven treadmill at 20 m/min, 30 min/day. As detailed below, maximal exercise tests were administered at baseline and at the end of week nine. The ES and EB groups exercised an additional two weeks post inoculation.

Maximal Exercise Test

Eight rats from both CS and ES were chosen randomly and given an incremental maximal treadmill exercise test at baseline and at week nine to determine peak oxygen consumption and treadmill time to fatigue (TT). Exercise tests were conducted on a motor driven treadmill entirely enclosed in an environmental chamber. Animals began walking at 10 m/min, zero percent elevation for three minutes at which time the treadmill speed was increased to 15 m/min for three minutes followed by 20 m/min for the remainder of the test. The treadmill elevation was raised 2% every three minutes until the rats refused to walk even with electrical stimulation. Oxygen consumption (VO_2), carbon dioxide production and respiratory exchange ratio (RER) were monitored continuously using an Oxymax metabolic treadmill system (Columbus Instruments International Corporation, Columbus Ohio). The average of the final minute was calculated and recorded to represent maximal values for VO_2 (VO_{2max}) and RER (RERmax).

Tumor Model

The prostate adenocarcinoma (PA III) tumor arose spontaneously from aged Lobund-Wistar rats (23). This tumor model is morphologically and biologically similar to aggressive hormone-independent human adenocarcinoma of the prostate. This tumor model has both a high incidence of tumor occurrence (100%) and metastasis (98%) with a latency period of approximately one month. The PAIII prostate cancer model has been shown to metastasize from the primary tumor site to lung tissue. In the present experiment, the tumor was enzymatically dissociated and then transplanted as a single cell suspension (10^6 cells/recipient) under the skin of the right flank during week nine at least forty-eight hours after maximal exercise tests.

Tumor Measurements

Primary subcutaneous tumor incidence was evaluated by inspection and palpation. Tumor dimensions (length and width) were measured weekly with a Vernier caliper until the time of sacrifice. Tumor volume was calculated using the formula $V = 0.4(L*W^2)$. The same individual with several years of experience with this type of procedure performed all measurements. Because of the high mortality rate, tumor volume measurements were concluded at day 42. Thereafter, the number of animals who survived to the day of sacrifice (day 60 post inoculation) was followed.

Determination Of Tumor Metastasis

At day 60, all animals were sacrificed and the lung tissue was collected and fixed in Bouin's fluid which stained metastasis bright yellow. The lungs were then rinsed with 70% alcohol and tumor metastasis counted by visual inspection.

Determination of Adrenal Gland Weights

One adrenal gland was removed from a subset of animals in each group and served as a measure of induced stress as indicated by adrenal hypertrophy. The adrenal glands were removed, trimmed of excess fat and connective tissue, blotted dry and wet weighed.

Bacillus Calmette-Guerin (BCG)

ImmunCyst BCG was supplied as a lyophilized preparation by Connaught Laboratories Limited (Ontario, Canada) and was re-suspended to 1×10^6 cfu concentration using phosphate buffered saline. This concentration was selected because it represents the lowest dose believed to be effective.

Statistical Analyses

Differences between groups in tumor volume across all measurements were determined using a two-way ANOVA with replications. Differences between the subset of rats from ES and EB for VO_{2max} , TT, and RERmax measured at baseline and week 8 were determined using a two-way ANOVA with replications. Fisher exact analysis was used to determine differences in animal survival. Throughout this paper, results are presented as means \pm SD. A probability level of 0.05 was selected as the criterion for statistical significance. Because this study is the first to examine the combination of exercise training and immunotherapy and because of its potential importance to the field, results that obtain a p value between 0.05-0.1 will be defined as a trend and will be discussed accordingly.

RESULTS

Tumor volume measures can be found in Figure 1. The first day that tumors could be palpated with confidence in either group was day 21 post-inoculation. There was no significant group by day interaction for tumor volume. There was also no significance difference in tumor volume among groups at any measured time point. As shown in figure 1, the pattern of tumor growth appears to be similar for the CS, ES and EB groups. However, tumor growth tended to be suppressed in the CB group. At day 28, total tumor volume tended to be smaller ($p=0.06$) in EB vs. ES (EB: 2232.02 ± 1368.2 vs. ES: 2399.02 ± 841.6). Figure 2 shows that the mean tumor volume across all measurements. Again it appears that the mean tumor volume was lowest in the CB group, although this did not reach statistical significance ($p=0.1$) due to the large standard deviation among groups. The percent change in tumor volume from day 28 to 42 was as follows: CS: 81.6 ± 12.1 , ES: 79.9 ± 14.6 , EB: 83.1 ± 15.4 , and CB: 69.4 ± 15.0 . The percent change tended to be smaller ($p=0.07$) in CB vs CS and CB vs EB.

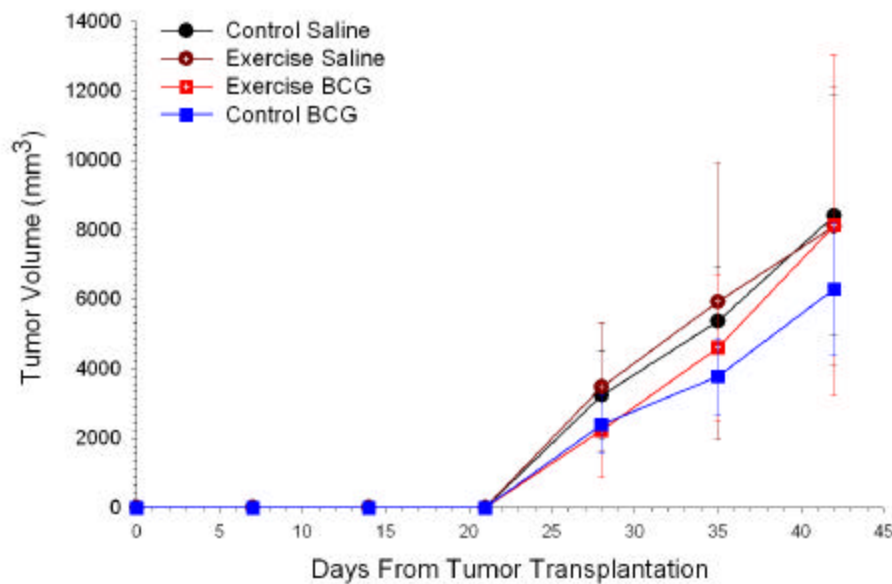


Figure 1. Change in tumor volume (mm^3) from the day of tumor transplantation. Tumor growth was measured weekly from the time of inoculation (day 0) to the last day of tumor measurement (day 42). Palpable tumors were first recorded on day 21 post inoculation. The first actual measurement of tumor growth was recorded on day 28 post-inoculation.

The number of animals surviving to day 60 can be found in Table 1. The overall time to survival was significantly enhanced ($p=0.05$) in the CB vs the ES. No other differences were observed among groups. Because of the high mortality rate in the ES group, statistical analysis for lung metastasis was only performed on CS, EB, and CB. There were no differences in the number of lung metastatic lesions at day 60 among CS, EB and CB. The mean (\pm SD) adrenal weight taken at sacrifice from of each group was as follows: CS: 0.0229 ± 0.0047 g, ES: 0.0232 ± 0.0031 g, EB: 0.0198 ± 0.001 g, and CB: 0.0218 ± 0.0024 . Analysis across all groups revealed no statistically significant difference for the mean adrenal weights.

Table 1. Number of animals surviving to Day 60.

Group	N	# Alive	% Alive
Control-Saline	13	6	46.1
Exercise-Saline	13	5	38.5
Exercise-BCG	12	8	66.6
Control-BCG	14	11	78.8*

The data for VO_2 max, TT, RERmax and body weight measured in eight animals in CS and ES at baseline and at week nine can be found in Table 2. There was a significant ($p<0.01$) group by test interaction for RERmax. As observed in Table 2, both groups experienced an increase in RERmax during the week nine maximum treadmill test compared to baseline. However, animals from ES experienced a greater increase compared with CS. No significant interactions were observed for the VO_2 max, TT or body weight. However, the main effect for the TT tended to be different ($p=0.09$) between groups.

Table 2. Physiological variables measured in eight animals in CS and ES at baseline and at week nine.

VARIABLE	Control Saline	Exercise Saline
Weight (g)		
Baseline	185.7 ± 24.9	155.2 ± 19.2
Week 9	349.9 ± 4.3	313.4 ± 25.2
VO_2max (ml/kg/min)		
Baseline	68.5 ± 4.3	73.4 ± 4.0
Week 9	71.4 ± 5.8	76.8 ± 6.2
TT (s)		
Baseline	783.0 ± 228.6	835.2 ± 339.6
Week 9	1149.5 ± 202.4	1406.0 ± 54.1
RER		
Baseline	0.911 ± 0.051	0.861 ± 0.087
Week 9	0.966 ± 0.143	1.059 ± 0.126

CS=Control saline group (N=8); ES=Exercise saline group (n=8)

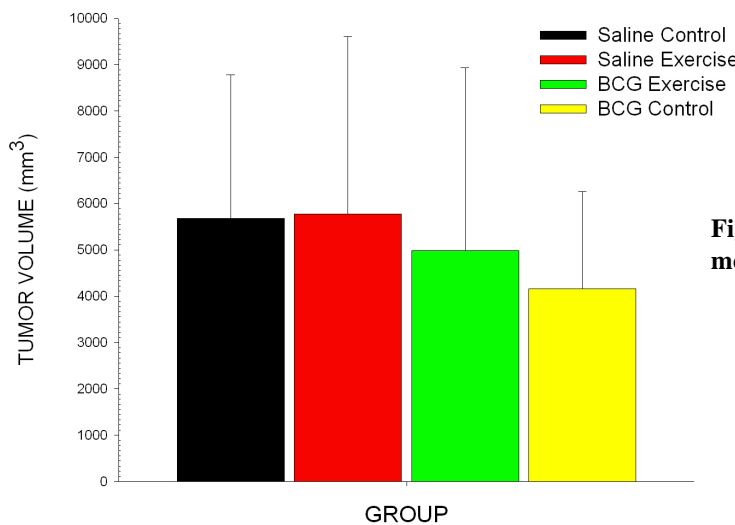


Figure 2. Total tumor volume (mm³) across all measurements.

DISCUSSION

The primary finding of the present study was that exercise training along or in combination with BCG immunotherapy did not produce a statistically significant effect on the volume of a transplanted prostate tumor as compared to controls. However, the combination of treadmill running and BCG immunotherapy tended to produce a smaller ($p=0.06$) prostate tumor volume relative to treadmill running alone during the early phase of tumor detection and growth. In addition, only the treatment with BCG alone tended to limit the growth of this tumor during the later aggressive phase expressed as the percent change from days 28 to 42. Also, the number of animals surviving until day 60 after tumor transplantation was significantly greater in the CB versus ES group. No treatment had any effect on the spread of the prostate tumor to the lung as measured by the number of lung metastases.

Epidemiological studies have indicated a potential relationship between physical activity and the incident rate of

a number of site-specific cancers. To the authors' knowledge, this is the first study that examined the effects of treadmill exercise combined with immunotherapy in rodents on the growth of an experimentally transplanted prostate adenocarcinoma and its metastases. Animal studies have used numerous tumor models to determine the effectiveness of exercise training on cancer induction, development and growth and have found varying results. Jadeski and Hoffman-Goetz, (11) reported that mice which exercised 20 m/min, 30 min/day, five times/ week for nine weeks had a significantly lower retention of radiolabelled H-ras-transformed fibroblasts (CIRAS 1) as compared to sedentary control. Chronic exercise retarded retention of intravenously injected CIRAS 3 tumor cells in the lungs of treadmill-trained mice below that of the sedentary groups (17). Thompson et al., (28) reported that high intensity treadmill running five days/week was associated with a protective effect for rats that developed chemically induced mammary tumors. Only the high intensity training (70% maximal treadmill intensity) whether 20 or 40 min/day and not the low intensity (35%) afforded the animals protection. Time of tumor appearance was significantly sooner in mice that ran three days prior to and 14 days post inoculation of 2.5×10^5 mammary adenocarcinoma cells compared with a group of sedentary controls (32). The contradictory results found in the literature may be explained, in part, by the use of different experimental designs. In the present study, treadmill exercise training was not associated with a greater improvement in the maximum time to fatigue or VO_2 max compared with sedentary animals. However, there was a slight tendency for an overall group effect with regards to the maximum treadmill time to fatigue with the ES group running longer. The low power of the performed statistical test (0.281) leads to the assumption that a statistical difference may have been observed had more than eight animals per group been tested.

BCG is an attenuated strain of the bovine tuberculous bacterium and consists of living bacilli, dead microorganisms and subcellular debris (14). When instilled in the bladder to treat carcinoma, BCG attaches to the bladder wall and causes granulomatous inflammatory reactions and mononuclear cell infiltration predominantly in the subepithelial layer (12). BCG immunotherapy is considered a non-specific immune response modifier, which has been shown to be effective in rats against an epithelioma and hepatocellular carcinoma (1) and against prostate adenocarcinoma-III (25), and in human patients against superficial transitional cell carcinoma of the urinary bladder (19). Therefore, its anticancer affect is not limited to a single tumor type or species (26). Because it is considered a non-specific immune response modifier, the purpose of the present study was to test the combined effect of BCG immunotherapy with exercise training, which has also been shown to modify the immune system. Exercise-induced immune modulation of cytokines has been proposed as a mechanism conferring protection against certain cancers (10). Additionally, exercise induced increases in NK cell number and activity (16) may also be able to limit tumor development and metastasis. In the present study, the combination of exercise with BCG tended to be the most effective treatment for the transplanted prostate cancer in the early phase of growth. However, this combined treatment was unable to prevent further growth of the tumor and was not different from controls from days 28 to 42 post-inoculation. Only the BCG treatment alone approached significance ($p=0.07$) in slowing the growth rate of the tumor when compared with the control saline and exercise BCG groups.

Exercise training alone was not effective in slowing the growth rate of the tumor. In fact, the mortality rate at day 60 post-inoculation was the highest in the ES group. The number of animals surviving until day 60 was significantly greater in the CB versus ES group. There may be a number of plausible explanations for why exercise training was ineffective in its ability to slow the growth rate of the transplanted prostate tumor. The type of tumor model used in an experiment can contribute to the potential outcome. The prostate adenocarcinoma tumor used in the present study arose spontaneously and was originally discovered in aged germ free Lobund Wistar rats (23); transplanted tumor cells from this model are normally fast acting and aggressive with a latency period of approximately one month in control animals. Jadeski and Hoffman-Goetz (11), demonstrated in mice that moderate exercise conditioning can reduce tumor retention but only for tumor cells considered mildly aggressive (CIRAS 1). This same study showed no effect of exercise training for a tumor model that was more aggressive (CIRAS 3). The tumor model used in the present study may have been too aggressive to accurately test the long-term benefits of exercise training on prostate cancer prevention. BCG

immunotherapy has been shown to be effective with this tumor when applied directly to the area of cell growth (26). Although treatment with BCG was unable to prevent tumor development in the present study, results did indicate that it was moderately effective in slowing the growth. The dose of BCG used in the present study may have been too low to achieve maximal results.

An additional explanation for the ineffectiveness of exercise training may have been the added stress burden of the forced exercise used in this study. Although animals were trained at a very young age to walk on the treadmill without the need for electrical stimulation, this type of exercise is not natural to these animals. Investigators cite observations that rodents naturally run in multiple short intervals as opposed to the typical 15 to 60 minutes used in most treadmill exercise studies. It has been reported from videotape analysis that mice, given free access to running wheels, will typically run for 20-30 seconds, exit from the wheel, and then return a minute or two later (9). Additionally, it has been shown that rats do not respond the same metabolically to the same exercise intensity. In a previous study conducted in our laboratory the metabolic cost (i.e. oxygen consumption) of running 20 m/min was approximately 73.41 ml/kg/min with a range of 54.67 to 98.80 (SD: 13.13) (3). This would indicate that although the treadmill speed was consistent for all animals, some animals exercised at a much higher intensity than others and that they were different with respect to exercise efficiency. This fact could lead to a greater stress response to exercise in some animals compared to others with a subsequent detrimental effect on the immune system and interference with anticancer activity. Enhanced stress may alter the animals' immune system and subsequent immune mediated response to cancer. A sustained suppression of NK cell numbers has been observed following intensive or prolonged exercise (2). In addition, natural killer cell activity has been reported to be suppressed following exercise of high intensity (75% of maximum VO_2) but not following exercise of moderate (50%) or low (25%) intensity (30). On the other hand, running which is voluntary may not have such an adverse effect. For this reason, protocols which allow rodents free access to running wheels may be more appropriate to study the effects of exercise training on cancer incidence and progression. Recent evidence has shown that voluntary running on running wheels for 19 weeks was associated with a significant reduction in overall tumor volume and a significantly smaller tumor at day 38 post inoculation compared with a sedentary control (4). The tumor used in the study was the same used in the present study indicating that exercise can have an effect on its growth (4). In the present study, a single adrenal was collected and weighed at the time of sacrifice. This procedure was used because adrenal hypertrophy has been associated with extreme stress in rodents (7). No differences were observed in the mean adrenal weights across groups. However, this method is probably too insensitive to detect subtle changes in stress.

A final reason that may explain the ineffectiveness of exercise could be the schedule of training used in the present study. Treadmill exercise training was terminated in the ES and EB group at day 14 post-inoculation. Exercise was stopped at this time because it was feared that it may augment the growth of the tumor which was placed on the flank region. It is possible; however, that this may have limited the effectiveness of the exercise and that had the animals been allowed to train for a longer period of time post inoculation a different outcome may have occurred.

The number of pulmonary metastases was not different among groups. BCG with or without exercise training was unable to decrease the number of observable lung metastatic lesions. The route of BCG administration may be important in its ability to prevent the spread of PA-III cells. Inoculation of BCG was shown not to induce a systemic anticancer effect unless PA-III cells and BCG occupied the same anatomical compartment (26). Only intravenous inoculation of BCG interfered with the passage of PA-III cells to the lungs (26). Although BCG has been shown to be affective in preventing cancerous growths when given directly at the site of the lesion, the subcutaneous route may have limited its effectiveness in preventing the metastatic spread to the lungs (25).

CONCLUSIONS

Exercise training along or in combination with BCG immunotherapy did not have a significant effect on the occurrence or growth rate of a transplanted prostate tumor in rats. The combination of exercise training and BCG immunotherapy tended to slow the growth of a transplanted prostate adenocarcinoma during the initial phases of detectable growth. BCG alone was the only treatment that tended to limit the growth of this tumor during the aggressive phase of growth.

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