Effect of a High-Intensity Exercise Training on the Metabolism and Function of Macrophages and Lymphocytes of Walker 256 Tumor–Bearing Rats

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Epidemiologic studies suggest that moderately intense training promotes augmented immune function, whereas strenuous exercise can cause immunosupression. Because the combat of cancer requires high immune function, high-intensity exercise could negatively affect the host organism; however, despite the epidemiologic data, there is a lack of experimental evidence to show that high-intensity training is harmful to the immune system. Therefore, we tested the influence of high-intensity treadmill training (10 weeks, 5 days/week, 30 mins/day, 85% VO_2max) on immune system function and tumor development in Walker 256 tumor–bearing Wistar rats. The metabolism of glucose and glutamine in lymphocytes and macrophages was assessed, in addition to some functional parameters such as hydrogen peroxide production, phagocytosis, and lymphocyte proliferative responses. The metabolism of Walker 256 cells was also investigated. Results demonstrated that high-intensity training increased the life span of tumor-bearing rats, promoted a reduction in tumor mass, and prevented indicators of cachexia. Several changes, such as a reduction in body weight and food intake and activation of glutamine metabolism in macrophages and lymphocytes induced by the presence of Walker 256 tumor, were prevented by high intensity training. The reduction in tumor growth was associated with an impairment of tumor cell glucose and glutamine metabolism. These data suggest that high-intensity exercise training may be a viable strategy against tumors. Exp Biol Med 232:1289–1299, 2007

Key words: exercise; glucose; glutamine; cancer; immune function

Introduction

Several studies have shown that exercise may improve the physical fitness and quality of life of cancer patients (1, 2) and also suggest that regular exercise may have a helpful effect in reducing all-site cancer rates (3, 4). Quist *et al.* (5) reported that, in general, studies on physical activity and cancer have inadequate control for intensity and volume, making the acquisition of knowledge about prescription difficult because the responses of the organism are dependent upon exercise loading (6). Epidemiologic studies suggest that moderate exercise loading reduces the number of infections, whereas heavy exercise loading increases infectious episodes (7, 8), probably by blunting the immune function (9). However, a recent study demonstrated that high-intensity exercise (resistance and aerobic training) presented positive effects in cancer patients (5). Additionally, it is important to note that in contrast to the great number of epidemiologic studies, there are limited experimental data to show that intense exercise blunts immune function and that strenuous (not moderate) exercise is causally linked to exercise-induced changes in immune functions (10).

The effects of exercise on cancer could be partially explained by modulation of the immune system (11, 12), which is mediated by a complex interplay among hormones,

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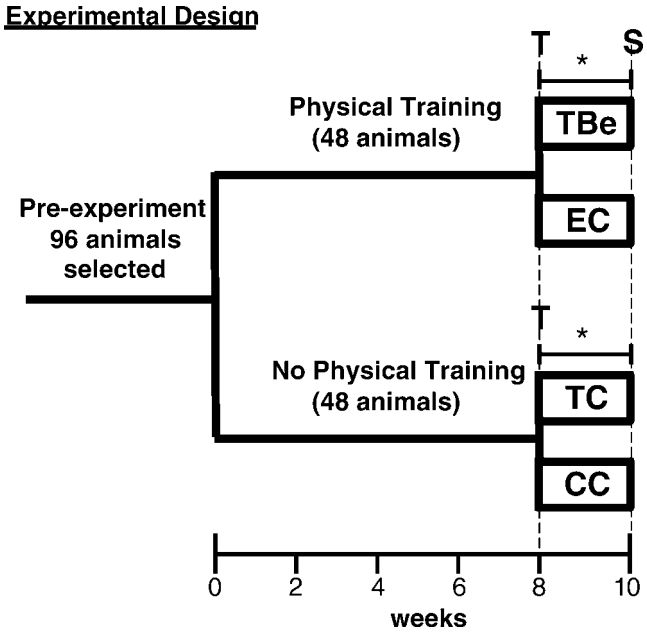

Figure 1. Experimental design. TBe, tumoral implant and exercise; EC, exercise control; TC, tumor control; CC, double control; T, tumor inoculation (only for TBe and TC groups); S, sacrifice of animals. *, period required for tumor development.

cytokines, and neural and hematological factors (13). Despite such hypotheses, most studies on exercise and cancer have separately analyzed the effect of exercise loading upon immune and neuroendocrine responses (7, 14, 15). Therefore, it is necessary to associate endocrine changes, promoted by exercise, with those of metabolism and function of cells of immune system, such as lymphocytes and macrophages that play a central role in the response against tumorigenesis (16–18). These cells utilize glucose and glutamine at high rates in a manner strictly related to function (19–21).

The Walker 256 tumor is a carcinosarcoma that spontaneously appeared in a pregnant rat in 1928 (22). Since then it has been used in several studies because it mimics many of the alterations induced by human tumors, is easily transplanted, is specific to the rat species, and grows rapidly (mean survival time of only 15 ± 1 days; Refs. 22–24). In this investigation, we studied the effect of high-intensity training upon glucose and glutamine metabolism in Walker 256 tumor–bearing Wistar rats, as well as the metabolism of these substrates in the immune system (lymphocytes and macrophages) and in cells isolated from the tumor. Our hypothesis was that high-intensity exercise without a high-volume (frequency and duration) program could prevent metabolic changes induced in immune cells and impair tumor development.

Materials and Methods

Animals and Reagents. Male adult Wistar rats from 150–200 g (8 weeks old) from the animal Breeding Unit, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo Brazil, were housed in a temperature-controlled room at 23°C under an inverted photoperiod regimen of 12:12-hr light:dark cycle (lights on at 1800 hrs) with water and commercial food *ad libitum*. The animals were maintained in accordance with the guidelines of the Brazilian Association for Laboratory Animal Science, and all experimental procedures were approved by the Ethical Committee on Animal Experimentation of the Institute of Biomedical Sciences, University of Sao Paulo. All reagents were purchased from Sigma (St. Louis, MO), except glucose and glutamine, which were purchased from Merck (Darmstadt, Germany).

Maximum Oxygen Consumption (VO_2max). One week before beginning the training period, the VO_2max of the animals was determined as described by Brooks and White (25). These data were used to determine the speed of the treadmill (ESD-01; FUNBEC, Sao Paulo, Brazil) corresponding to 85% VO_2max. This parameter was re-evaluated every 15 days to allow the correction of exercise intensity, which was maintained at 85% VO_2max. The animals ran at speeds of 0.4, 0.6, 0.8, 0.9, 1.08, 1.2, 1.5, 1.6, 1.7, and 1.8 km/hr for Weeks 1–10 of training, respectively.

Training Protocol. The animals were submitted to exercise for 10 weeks at 85% VO_2max on a level treadmill, 5 days/week and 30 mins/day, beginning at 0900 hrs. The animals were not submitted to any kind of reinforcement.

Experimental Groups. At the beginning of the study, 96 animals were selected with the same age and weight; rats were randomly divided into two groups of 48 animals. One of these groups initiated the training program and the second group remained without training. After 8 weeks of high-intensity training, exercised animals were divided in two groups (24 animals each), exercise control (EC) and tumor implant and exercise (TBe); the animals from the second group were also divided into two groups (24 animals each), double control (CC) and tumor control (TC). Animals from TC and TBe groups were inoculated subcutaneously in the right flank with 1 ml of sterile suspension of 2×10^7 Walter 256 tumor cells (Fig. 1). This number of cells ensures the development of a tumor mass equivalent to 15% of total body mass after 15 days (26). Animals from the CC and EC groups were injected with 1 ml of phosphate-buffered saline (PBS). After tumor implantation, TBe returned to high-intensity exercise program for 15 additional days. EC animals did not receive tumor, but underwent the same exercise treatment as TBe. All outcomes were conducted on the 15th day after tumor cell inoculation, except when the animal was used to measure the change in life span (this was obtained from TBe group). The TC group, formed by sedentary rats, was not submitted to exercise at any time, but the animals were inoculated with tumor cells on the same day as TBe.

Tumor Cell Isolation. Walker 256 tumor–bearing rats were killed by decapitation, and the tumoral tissue was removed and fragmented in PBS (0.14 *M* NaCl, 4.7 m*M*

KCl, 10 m*M* $MgSO_4$, 1.2 m*M* KH_2PO_4, 4 m*M* Na_2HPO_4, 1.3 m*M* NaH_2PO_4, 1.3 m*M* $CaCl_2$; pH 7.2). The material was then centrifuged at 150 *g* for 10 mins at 4°C. The pellet was resuspended in PBS. The viable cells were used for tumor implantation in the TC and TBe groups. For metabolic measurements, tumor cells were obtained from TC and TBe animals.

Body Mass and Food Intake. The food intake and the weight of animals were assessed daily in the early morning before and after tumor inoculation.

Cell Separation. PBS was injected (6 ml) intraperitoneally and, after 30 secs, peritoneal macrophages were collected. Cell viability was confirmed by Trypan blue exclusion (>95%). At least 92% of the peritoneal exudate cells were macrophages, as determined by differential counting. The lymphocytes were obtained from the mesenteric lymph nodes, which were pressed against a steel mesh as described by Ardawi and Newsholme (27). The cell suspension was filtered (Whatman plc, Middlesex, UK) and centrifuged at 150 *g* for 15 mins at 4°C. The pellet was resuspended in extraction buffer that was specific for each enzyme or in culture medium (GIBCO RPMI 1640; Invitrogen, Carlsbad, CA). The total contamination with macrophages was lower than 1%.

Lymphocyte Proliferation. Mesenteric lymphocytes were cultivated in 96-well plates (1×10^5 cells per well; Corning, One Riverfront Plaza, NY) under sterile conditions in GIBCO RPMI 1640 medium for 48 hrs at 37°C in an artificially humidified atmosphere of 5% CO_2 in a microprocessor incubator (LAB LINE, Boston, MA). Cells were also cultivated in the presence of concanavalin A (ConA; 5 μg/ml) or lipopolysaccharide (LPS; 10 mg/ml). After 48 hrs in culture, more than 98% of the lymphocytes were still viable, as measured by Trypan blue exclusion. The cells were labeled with 7400 Bq ^{14}C-thymidine (Amersham-GE Healthcare, Uppsala, Sweden) diluted in sterile PBS yielding a final concentration of 1 μg/ml. The cells were maintained under these conditions for an additional 15 hrs and automatically harvested using a multiple-cell harvester and filter paper (Skatron Combi, Sulfolk, UK). The paper discs containing the labeled cells were counted in 5 ml Bray's scintillation cocktail (Sigma, St. Louis, MO) in a Beckman-LS 500 liquid scintillator (Beckman Instruments, Fullerton, CA).

Metabolites. Glucose consumption was determined as previously described by Trinder (28). Lactate production was determined as previously described by Engle and Jones (29). Glutamine consumption was determined using the method described by Windmueller and Spaeth (30). Glutamate and aspartate production were determined as previously described by Bernt and Bergmeyer (31) and Bergmeyer *et al*. (32), respectively. Macrophages and lymphocytes of individual rats were incubated (1×10^6 per flask) at 37°C in the Krebs Ringer medium with 2% fat-free bovine serum albumin (BSA) in the presence of glucose (5 m*M*) and glutamine (2 m*M*). After 1 hr, the supernatant was collected and stored at −70°C for the measurement of metabolites. For the assay of glucose and glutamine consumption, cells were disrupted with 200 μl 25% (w/v) trichloroacetic acid, and the sample was neutralized with 100 μl of 0.5 *M* Tris containing 2.0 *M* KOH for the measurement of metabolites.

Glucose and Glutamine Oxidation. The $^{14}CO_2$ produced from ^{14}C-glucose and ^{14}C-glutamine (Amersham-GE Healthcare) oxidation was determined as described by Curi *et al*. (33). Macrophages and lymphocytes were incubated for 1 hr in the presence of one of the radiolabeled substrates in a sealed Erlenmeyer flask (25 ml) with one compartment for cell incubation and a second one for CO_2 collection, as previously described by Kowalchuck *et al*. (34).

Enzymes. The activities of glucose-6-phosphate dehydrogenase (G6PDH), hexokinase (HK), and glutaminase (GLUTase), enzymes that catalyse, respectively, the first reaction of pentose phosphate and glycolytic and glutaminolytic pathways, were measured as previously described by Bergmeyer *et al*. (32), Crabtree and Newsholme (35), and Curthoys and Lowry (36), respectively. Citrate synthase (CS), an important enzyme from Krebs cycle, was measured as described by Alp *et al*. (37). The extraction media for enzymes were: 25 m*M* Tris-HCl buffer containing 1 m*M* EDTA and 30 m*M* β-mercaptoethanol (for HK; pH 7.4), 50 m*M* Tris-HCl containing 1 m*M* EDTA (for GLUTase; pH 8.6), 50 m*M* Tris-HCl containing 1 m*M* EDTA (for CS; pH 7.4), and 50 m*M* Tris-HCl containing 1 m*M* EDTA (for G6PDH; pH 8.0). For all enzyme assays, Triton X-100 was added to the medium to a final concentration of 0.05% (v/v). For HK activity, the following incubation medium was used (pH 7.5): 75 m*M* Tris-HCl containing 7.5 m*M* $MgCl_2$, 0.8 m*M* EDTA, 1.5 m*M* KCl, 4.0 m*M* β-mercaptoethanol, 0.4 m*M* creatine phosphate, 1.2 m*M* (final concentration) creatine kinase, 0.1 m*M* (final concentration) glucose 6-phosphate dehydrogenase, and 0.4 m*M* NAPD. The assay buffer for CS activity (pH 8.1) consisted of 100 m*M* Tris-HCl, 0.2 m*M* 5,5′-dithio-bis-2-nitrobenzoic acid, 15 m*M* acetyl-coenzyme A, and 0.5 m*M* oxaloacetate. The buffer for G6PDH (pH 7.6) consisted of 86 m*M* Tris-HCl containing 6.9 m*M* $MgCl_2$, 0.4 m*M* $NADP^+$, 1.2 m*M* glucose-6-phosphate, and 0.5% Triton X-100. The assay medium for GLUTase (pH 8.6) consisted of 50 m*M* potassium phosphate buffer containing 0.2 m*M* EDTA and 20 m*M* glutamine. In all cases, the final assay volume was 1.0 ml. CS activity was determined by the absorbance at 412 nm and the other enzymes at 340 nm. All spectrophotometric measurements were performed in a Hitachi U-2001 spectrophotometer (Hitachi, Tokyo, Japan) at 25°C.

H_2O_2 Release and Phagocytosis. The release of H_2O_2 was measured using a modification of the method described by Pick and Mizel (38). The cells were incubated in siliconized flasks (25 ml) in 1 ml PBS in the presence of glucose (5 m*M*) under an atmosphere of 5% CO_2/95% air at 37°C. After 1-hr incubation, 100 μl of a mixture of phenol red (200 μl/ml) and horseradish peroxidase (19 U/ml, final concentration) were added. After 10 mins, the reaction was

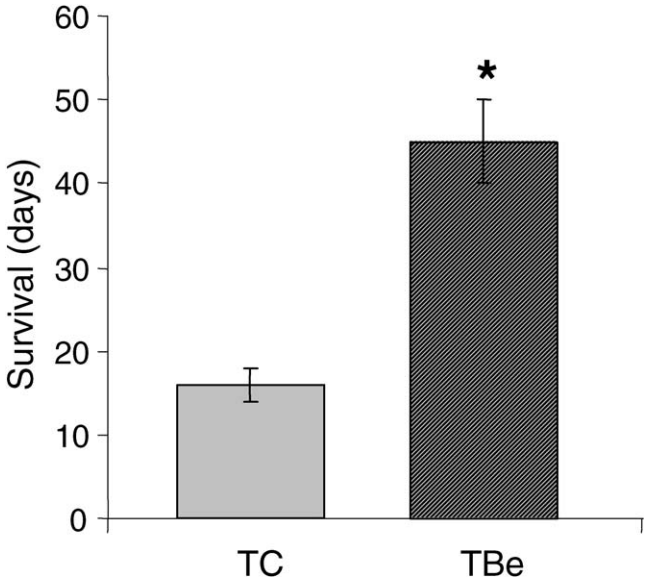

Figure 2. Life span of tumor-bearing rats. The values are expressed as days of survival, counted from the day of tumor cell inoculation. The results are presented as means ± SEM of 24 animals for each group. TC, tumor control; TBe, tumor implant and exercise. *, $P < 0.05$ compared to TC group.

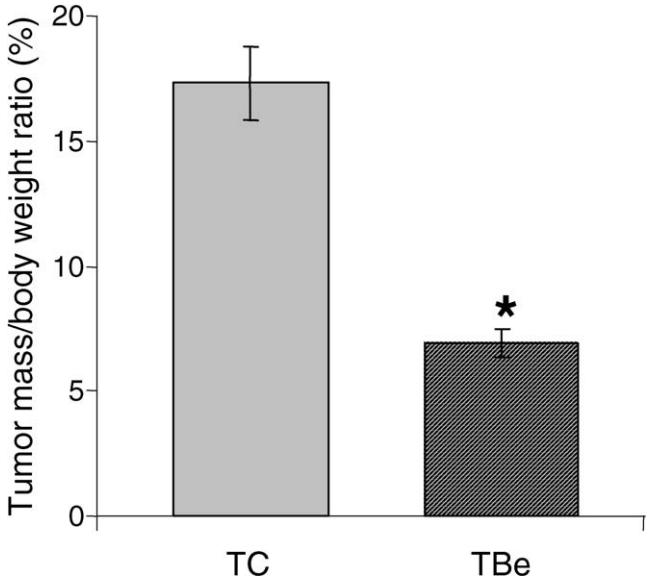

Figure 3. Tumor mass and body weight ratio. The results are presented as means ± SEM of 24 animals for each group. TC, tumor control; TBe, tumor implant and exercise. *, $P < 0.05$ compared to TC group.

stopped with 100 µl 1 *M* NaOH and the absorbance measured at 620 nm. The rate of H_2O_2 release was linear over a period of 1 hr, and there was no significant difference in the H_2O_2 release if the cells were incubated in the presence or in the absence of bicarbonate-containing medium. The rate of phagocytosis was measured in macrophages incubated in siliconized flasks (25 ml) in 1 ml PBS in the presence of glucose (5 m*M*), glutamine (2 m*M*), and zymosan particles under an atmosphere of 5% CO_2/95% air at 37°C. After 40 mins, an aliquot was obtained, and after dilution (10:1) in PBS (pH 7.2), the aliquot was evaluated with light microscopy, and the rate of phagocytosis was determined.

Hormones and Cytokines. The plasmatic levels of insulin, growth hormone (GH), testosterone, and corticosterone were measured using commercially available kits for radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA). The counting was performed in a Beckman L6000 (Beckman Instruments, Fullerton, CA). The plasmatic concentrations of interleukin-1 (IL-1), interleukin-2 (IL-2), and tumor necrosis factor-α (TNF-α) were measured using commercially available ELISA kits (Amersham-GE Healthcare).

Fat Content Evaluation. Samples from various tissues were extracted three times with ethanol and petroleum ether. After evaporation of the ether, fat content was determined as described by Stansbie *et al*. (39).

Protein Measurement. The protein content of samples was measured by the method of Bradford (40). BSA was used as standard.

Statistical Analysis. Analysis was performed using GraphPad-Prism. When differences among the groups were detected by two-way factorial ANOVA, the Tukey test was used. The level of significance of $P < 0.05$ was chosen for all statistical comparisons. Data are presented as means ± SEM.

Results

Life Span. Training increased the lifespan of the TBe group by 2.8-fold (16 ± 2 vs. 45 ± 5 days; Fig. 2) in comparison to values observed for animals without exercise (TC group).

Tumor Mass. Figure 3 shows that tumor mass in TC rats corresponds to 17.33% of animal body mass (38.8 ± 3.2/224 ± 2.0 = 0.1733). On the other hand, the tumor mass in TBe rats corresponded to 6.96% of animal body mass (22.2 ± 1.8/319 ± 4.0 = 0.0696).

Cachexia-Related Parameters. TC rats presented a 29.33% reduction in body weight in comparison to CC rats (Fig. 4). EC and TBe rats presented a body weight similar to that observed in CC rats (Fig. 4). Data regarding food intake and adiposity are presented in Table 1. Animals from the TC group ingested 33.6% less food in comparison to rats from the CC group. High-intensity–trained tumor-bearing rats partially prevented this reduction because TBe rats consumed 22% more food than TC animals. Exercise training in non–tumor-bearing rats also reduced food intake by 14% in relation to CC animals. In accordance with the minor food intake, Walker 256 tumor–bearing rats presented only 53.27% of CC total body fat. Exercise training in tumor-bearing rats prevented the reduction in total body fat promoted by the tumor. TBe animals presented an increase of 2.14-fold in epididymal fat in comparison to animals from the TC group.

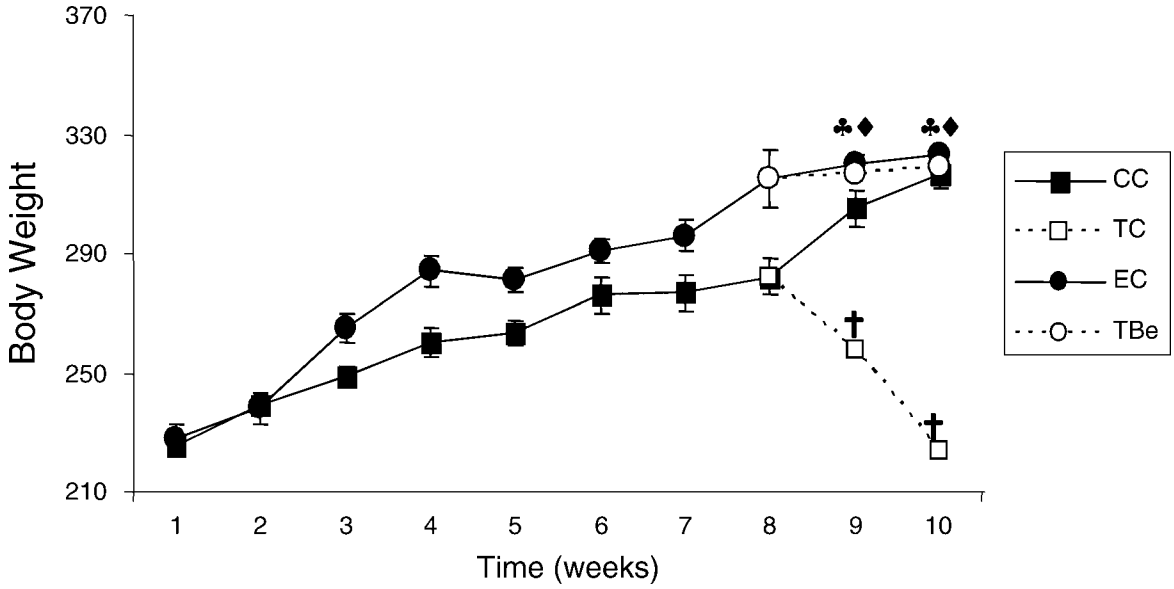

Figure 4. Body weight of animals in the experimental period. The values are expressed as grams and presented as mean ± SEM of 24 animals for each group. CC, double control; TC, tumor control; EC, exercise control; TBe, tumor implant and exercise. †, $P < 0.05$ compared to CC group. ♦, $P < 0.05$ compared to TC group. ♣, $P < 0.05$ compared to EC group.

Plasmatic Parameters. The results regarding corticosterone, testosterone, and glutamine plasmatic levels are presented in Table 2. The Walker 256 tumor promoted increased plasmatic levels of corticosterone in comparison to control animals. Exercise training in non–tumor-bearing animals also promoted an increase of 21.5-fold in plasmatic levels of corticosterone in comparison to the control group. Non–tumor-bearing animals, submitted to high-intensity training, presented higher levels of testosterone in relation to animals from TC and TBe groups. TC promoted a marked increase (1.63-fold) in plasmatic glutamine concentration in comparison to values observed in CC rats. EC promoted a 13% augment in glutamine plasmatic levels in comparison to CC rats. TBe rats presented a reduction of 26.66% in glutamine levels in comparison to TC rats.

Walker 256 Tumor Cells. Table 3 shows the results for glucose and glutamine metabolism. With the exception of glucose consumption, the high-intensity exercise training changed the pattern of glucose and glutamine metabolisms presented by Walker 256 tumor cells isolated from animals of the TC group. For glucose metabolism, the exercise reduced lactate production by 72% and glucose decarboxylation by 94.32%. For glutamine metabolism, exercise reduced glutamine decarboxylation by 98.24% and increased all other parameters evaluated.

Macrophages. Due to the importance of macrophage tumoricidal activity, we evaluated phagocytosis and H_2O_2

Table 1. Food Intake, Total Body Fat Content, and Fat Content in Specific Sites from Sedentary (CC, TC) and Trained (EC, TBe) Rats[a]

	CC	TC	EC	TBe
Food intake[b]	25 ± 0.4	16.6 ± 0.5[e]	21.5 ± 0.3[e,f]	20.3 ± 0.4[e,f]
Total body fat[c]	65.7 ± 6.4	35 ± 1.4[e]	51.1 ± 7.3	66.4 ± 12.8
Liver fat weight[d]	25.8 ± 3.3	32 ± 1.7	28.2 ± 3.7	32.5 ± 0.8
Gastrocnemius fat weight	12.4 ± 1.1	8.3 ± 0.3	11.8 ± 1.2	13.2 ± 1.8[f]
Heart fat weight	29.3 ± 3.4	23.8 ± 4.4	34.8 ± 0.5	28.6 ± 4
Retroperitoneal fat weight	818.2 ± 18.2	381.4 ± 25.1	657.1 ± 64.7	659.5 ± 47.8
Epididymal adipose weight	834.8 ± 11.4	511.5 ± 18.7	832.5 ± 21.8	1095.7 ± 219.8[f]
BAT fat content	495 ± 59.5	28.6 ± 9.8[e]	138.2 ± 89[e]	420 ± 20[f,g]

[a] Values are presented as mean ± SEM of 24 animals for each group. CC, double control; TC, tumor control; EC, exercise control; TBe, tumor implant and exercise; BAT, brown adipose tissue.
[b] Values are expressed as grams per day.
[c] Values are expressed as mg per grams of carcass.
[d] Fat content for other parameters expressed as mg per grams of tissue.
[e] $P < 0.05$ compared to CC group.
[f] $P < 0.05$ compared to TC group.
[g] $P < 0.05$ compared to EC group.

Table 2. Levels of Hormones, Cytokines, and Glutamine from Sedentary (CC, TC) and Trained (EC, TBe) Rats[a]

Parameter	CC	TC	EC	TBe
Hormone				
Insulin (μU/ml)	4.4 ± 0.30	3.9 ± 0.20	5.2 ± 0.35	2.9 ± 0.25
GH (ng/ml)	1.5 ± 0.20	1.3 ± 0.30	2.3 ± 0.40[c]	1.0 ± 0.06[d]
Testosterone (pg/ml)	6.0 ± 0.10	7.9 ± 3.50	94.4 ± 3.30[b,c]	1.1 ± 0.20[d]
Corticosterone (μg/dl)	0.02 ± 0.01	0.15 ± 0.05[b]	0.43 ± 0.08[b,c]	0.24 ± 0.03[b]
Cytokine				
IL-1 (pg/ml)	1.2 ± 0.91	15.4 ± 2.1[b]	1.7 ± 0.50[c]	28.0 ± 2.5[b,c,d]
IL-2 (pg/ml)	13.7 ± 0.04	102.1 ± 39.4[b]	11.5 ± 0.08[c]	115.1 ± 53.6[b]
TNF-α (pg/ml)	53.1 ± 0.02	456.7 ± 18.66[b]	53.1 ± 0.02	673.0 ± 28.05[b,c]
Aminoacid				
Glutamine (nmol/ml)	1299 ± 104	2119 ± 100[b]	1476 ± 176[b,c]	1554 ± 15[c]

[a] The values are presented as mean ± SEM of 24 animals for each group. CC, double control; TC, tumor control; EC, exercise control; TBe, tumor implant and exercise; IL-1, interleukin-1; IL-2, interleukin-2; TNF-α, tumor necrosis factor-α.
[b] $P < 0.05$ compared to CC group.
[c] $P < 0.05$ compared to TC group.
[d] $P < 0.05$ compared to EC group.

production of macrophages from rats of the TC group. The Walker 256 tumor produced a potent stimulus for H_2O_2 production in comparison to the CC animals (Table 4). Glucose consumption increased by 3.15-fold (Table 3) and the maximal activity of G6PDH increased by 1.58-fold (Table 5) in macrophages from TC rats in comparison to cells from CC animals. Despite these alterations, phagocytosis did not change in macrophages from rats of the TC group (Table 4). The increase in the lactate (5.39-fold; Table 3) and CO_2 (1.36-fold; Table 3) production and in CS activity (1.80-fold; Table 5) demonstrated that glucose was also used at higher rates for energy production in comparison to cells from CC animals. The activation of macrophages by Walker 256 tumor was confirmed by

Table 3. Biochemical Parameters Related to Glucose and Glutamine Metabolism in Immune Cells and Walker 256 Tumor Cells from Sedentary (CC, TC) and Trained (EC, TBe) Rats[a]

Parameter	CC	TC	EC	TBe
Lymphocytes				
Glucose	57.4 ± 6	42.3 ± 3[b]	43.2 ± 7.4	15.9 ± 7[b,c,d]
Lactate	96.9 ± 7.4	98.3 ± 5.3	38.9 ± 0.2[b,c]	5.5 ± 0.3[b,c,d]
Glucose decarboxylation	12.6 ± 0.7	3.4 ± 3.1[b]	11.4 ± 0.7[c]	6.4 ± 2.1[b,d]
Glutamine	43 ± 0.3	123 ± 0.6 [b]	20.5 ± 1.7[b,c]	9.5 ± 1.5[b,c,d]
Glutamate	13.5 ± 1.7	35.8 ± 3.5[b]	11.3 ± 2[c]	4.2 ± 2.4[b,c]
Aspartate	13.6 ± 1.3	15.7 ± 1.4	6.1 ± 0.7[b,c]	3.5 ± 0.7[b,c]
Glutamine decarboxylation	16.8 ± 0.9	29.7 ± 1.4[b]	16.2 ± 0.8[c]	2.5 ± 0.01[b,c]
Macrophages				
Glucose	80.6 ± 3.1	254.2 ± 13.3[b]	160.6 ± 5.2[b,c]	118 ± 3.4[b,d]
Lactate	71.6 ± 1.1	386.3 ± 24.5[b]	71.1 ± 2.1[c]	24.1 ± 1.4[b,c,d]
Glucose decarboxylation	45.1 ± 2.7	61.4 ± 1.8[b]	60.2 ± 1.3[b]	31.6 ± 3.3[b,c,d]
Glutamine	28 ± 2.3	145 ± 3.5[b]	25.9 ± 6.6[c]	6.6 ± 0.9[b,c,d]
Glutamate	14.4 ± 4.1	97.7 ± 1.8[b]	12.1 ± 0.6[c]	4.8 ± 1.7[b,c]
Aspartate	14.2 ± 0.6	33.9 ± 0.3[b]	3.7 ± 0.6[b,c]	1.98 ± 0.3[b,c]
Glutamine decarboxylation	16.2 ± 0.7	24.7 ± 1.9[b]	25.3 ± 2.1[b]	9.04 ± 1.5[d]
Tumor cells				
Glucose	—	75.6 ± 2.1	—	72.2 ± 4.7
Lactate	—	50 ± 3.1	—	14.1 ± 3.2[c]
Glucose decarboxylation	—	28.2 ± 0.3	—	1.6 ± 0.05[c]
Glutamine	—	112.1 ± 3.5	—	134.1 ± 4[c]
Glutamate	—	55.2 ± 7.4	—	105.8 ± 1.3[c]
Aspartate	—	21.3 ± 1.2	—	38.1 ± 0.8[c]
Glutamine decarboxylation	—	30.2 ± 0.3	—	0.53 ± 0.02[c]

[a] The values are expressed as nanomoles per hour per milligram of protein and are presented as mean ± SEM of 24 animals for each group. CC, double control; TC, tumor control; EC, exercise control. TBe, tumor implant and exercise.
[b] $P < 0.05$ compared to CC group.
[c] $P < 0.05$ compared to TC group.
[d] $P < 0.05$ compared to EC group.

Table 4. Hydrogen Peroxide Production and Rate of Phagocytosis in Macrophages from Sedentary (CC, TC) and Trained (EC, TBe) Rats[a]

	Parameter	
Group	Phagocytosis (%)	Hydrogen peroxide[b]
CC	23.4 ± 0.4	12.4 ± 0.7
TC	17.6 ± 4.6	52.3 ± 1.4[c]
EC	56.0 ± 1[c,d]	14.6 ± 1.3[d]
TBe	15.0 ± 0.1[e]	8.4 ± 0.2[c,d,e]

[a] Values are presented as mean ± SEM of 24 animals for each group. CC, double control; TC, tumor control; EC, exercise control; TBe, tumor implant and exercise.
[b] Hydrogen peroxide values are expressed as nanomoles per hour per milligram of protein.
[c] $P < 0.05$ compared to CC group.
[d] $P < 0.05$ compared to TC group.
[e] $P < 0.05$ compared to EC group.

greater glutamine consumption (5.17-fold; Table 3), glutamate (6.78-fold; Table 3), aspartate (2.38-fold; Table 3) and CO_2 (1.52-fold; Table 3) production, and the increase in maximal activity of glutaminase (1.31-fold; Table 5) in TC macrophages in comparison to CC cells. IL-1 plasmatic levels increased by 12.8-fold in TC rats in comparison to CC rats (Table 2), and TNF-α increased by 8.6-fold in animals from the TC group in comparison to animals from the CC group (Table 2). With the exception of phagocytosis, which did not change in TC rats, all changes induced in macrophage metabolism (consumption and production, Table 3; enzymatic activity, Table 5; in all cases TBe macrophages in comparison to TC macrophages) and function (hydrogen peroxide; Table 4) were prevented by training. The converse was observed, however, for IL-1 and TNF-α plasmatic levels, which increased by 1.81- and 1.47-fold, respectively, in TBe animals in comparison to TC rats (Table 2). The reversal of changes induced by Walker 256 in the macrophages from of the TBe rats, in most cases, were small in comparison to values observed for macrophages from rats of the CC group (except for glucose consumption and G6PDH activity). High-intensity training increased phagocytosis by 2.39-fold in comparison to cells from CC animals (Table 4) and the oxidative metabolism of glucose (1.99 and 1.33-fold, respectively, for glucose consumption and CO_2 production; Table 3). The increase in the oxidative metabolism of glucose was accompanied by an increase in the maximal activity of citrate synthase (1.1-fold; Table 5).

Lymphocytes. Lymphocytes from TC rats in contrast to cells from CC animals presented a reduced proliferative capacity in all evaluated conditions (57.36%, 51.44%, and 58.31, respectively, for control, ConA, and LPS; Table 6). This reduction was accompanied by a reduction of 32.92% in maximal activity of G6PDH (Table 5). The tumor also promoted a reduction in glucose utilization for energy. Lymphocytes from TC rats consumed 26.3% less glucose (Table 3) and presented a reduction of 73.01% in glucose decarboxylation (Table 3) in comparison to cells from CC rats. The reduction in production of energy from glucose was accompanied by a reduction in the maximal activities of hexokinase (59.77%; Table 5) and CS (42.16%; Table 5). In contrast to glucose metabolism, glutamine metabolism was increased by the Walker 256 tumor. Glutamine consumption was 2.86-fold greater in lymphocytes from TC rats in comparison to cells from CC animals (Table 3). In addition, glutamate production (2.65-fold; Table 3) and glutamine decarboxylation (1.76-fold; Table 3) increased in lymphocytes from TC animals in comparison to cells from CC rats. IL-2 plasmatic levels in animals from the TC group presented an increase of 7.45-fold in comparison to CC animals (Table 2). As described

Table 5. Maximal Activity of Enzymes of Lymphocytes and Macrophages Metabolism in Sedentary (CC, TC) and Trained (EC, TBe) Rats[a]

	Activity			
Enzyme	CC	TC	EC	TBe
Lymphocytes				
G6PDH	81.7 ± 6.4	54.8 ± 1.3[b]	85.3 ± 2.1[c]	53.7 ± 1.4[b,d]
HK	62.4 ± 2.1	25.1 ± 1.1[b]	74.3 ± 2.1[b,c]	35.1 ± 1.2[b,c,d]
CS	294.3 ± 7.2	170.2 ± 4.8[b]	153.03 ± 4.1[b]	81.1 ± 1.4[b,c,d]
Glutaminase	68.4 ± 1	67.2 ± 7	34.1 ± 2.2[b,c]	14.3 ± 4.4[b,c,d]
Macrophages				
G6PDH	14.1 ± 0.7	22.3 ± 1.3[b]	13.9 ± 0.4[c]	11.3 ± 0.7[c]
HK	224.5 ± 13.7	503.2 ± 21.3[b]	210.3 ± 15.2[c]	90.3 ± 5.6[b,c,d]
CS	27.3 ± 0.3	49.3 ± 1.7[b]	30.2 ± 0.7[b,c]	33.2 ± 1.4[b,c]
Glutaminase	128.3 ± 4.5	168.4 ± 7.3[b]	116.5 ± 3.9[c]	77.6 ± 3.7[b,c,d]

[a] The values are expressed as nanomoles per minute per milligram of protein and are presented as mean ± SEM of 24 animals for each group. CC, double control; TC, tumor control; EC, exercise control; TBe, tumor implant and exercise; G6PDH, glucose-6-phosphate dehydrogenase; HK, hexokinase; CS, citrate synthase.
[b] $P < 0.05$ compared to CC group.
[c] $P < 0.05$ compared to TC group.
[d] $P < 0.05$ compared to EC group.

Table 6. Proliferation Index of Lymphocytes Obtained from Sedentary (CC, TC) and Trained (EC, TBe) Rats[a]

Treatment	CC	TC	EC	TBe
Control	1276.4 ± 98.7	544.2 ± 71.3[b]	986.4 ± 51.4[b,c]	816.6 ± 27.2[b,c]
ConA	4003.9 ± 34.9	1944.2 ± 104.5[b]	3175.76 ± 48.5[c]	556 ± 46.3[b,d]
LPS	3467.3 ± 82.3	1445.2 ± 74.1[b]	2689.4 ± 88.2[b,c]	1868 ± 56.8[b,c,d]

[a] The values are expressed as nanomoles per hour per milligram of protein and are presented as mean ± SEM of 24 animals for each group. CC, double control; TC, tumor control; EC, exercise control; TBe, tumor implant and exercise; ConA, concanavalin A; LPS, lipopolysaccharide.
[b] $P < 0.05$ compared to CC group.
[c] $P < 0.05$ compared to TC group.
[d] $P < 0.05$ compared to EC group.

above, Walker 256 tumor inhibited glucose metabolism and stimulated glutamine metabolism. The combination of tumor and exercise not only compromised glucose, but also glutamine metabolism, in lymphocytes obtained in TBe rats. All metabolic parameters evaluated, with the exception of glucose decarboxylation (Table 3) and HK maximal activity (Table 5), were reduced in lymphocytes from TBe animals in comparison to cells from TC rats. However, even in the case of glucose decarboxylation and HK activity, the values observed were low in TBe cells in comparison to CC cells. In relation to the proliferative index, lymphocytes from TBe rats presented greater values under control (without mitogen) and LPS conditions when compared to cells from TC rats (Table 6). T lymphocytes from TBe animals proliferated 71.4% less in comparison to cells from TC animals (Table 6). It is important to note that, in all conditions evaluated, the proliferative index observed in TBe lymphocytes was low in comparison to those observed for CC cells (36.02%, 86.11%, and 46.12%, respectively, for control, ConA, and LPS; Table 6). Lymphocytes B from trained animals without tumor (EC), as well as lymphocytes without mitogen stimulus, presented a reduction in the proliferative index in comparison to cells from the CC group (22.72% and 22.43%, respectively, to control condition [without mitogen] and LPS; Table 6). This reduction was accompanied by the minor aspartate production in EC lymphocytes in comparison to CC cells (55.14%; Table 3) and by the reduction in maximal activity of GLUTase (50.14%; Table 5). In agreement, glutamine consumption was reduced in lymphocytes of exercised animals in comparison to cells from control animals (52.32%; Table 3). Maximal activity from CS was reduced in trained animals (42.16%; Table 5) in comparison to CC rats.

Discussion

Epidemiologic evidence suggests that moderately intense exercise is positive for immune function, whereas highly intense exercise could increase the susceptibility to infections (7, 41). In contrast to these previous reports, the present study demonstrates that high-intensity exercise promotes an increase of 2.8-fold in the life span of tumor-bearing rats and a reduction of 10% in tumor mass. These effects were accompanied by a reduction in the symptoms associated with cachexia, such as excessive body weight reduction and decrease in food intake.

According to Costa Rosa (42), two main research approaches have emerged in trying to establish a link between exercise and the immune system: a metabolic approach involving glutamine metabolism and a second, neuroendocrine approach, which considers changes in the neuroendocrine milieu as a mechanism for immunomodulation. Therefore, in response to these considerations (43, 44), in the present study we sought to integrate immunologic, endocrine, and metabolic parameters to obtain a better understanding of the ability of exercise to modulate immune function.

The excessive production of proinflammatory cytokines such as IL-1, IL-2, interferon γ, and TNF-α by the immune system is probably the most common cause of cachexia (45). Hormones such as glucocorticoids, stimulated by cytokines, could also be involved in this phenomenon (46). Lymphocytes and macrophages present high rates of glucose and glutamine consumption in a manner strictly related to their function. Whereas glucose is utilized mainly to synthesize macromolecules, glutamine is important for energy production and synthesis of purines and pyrimidines (19, 20, 47). Thus, the increase in cytokines was accompanied by an increase in the metabolism of glutamine in macrophages and lymphocytes and in glucose metabolism in macrophages, signifying that the Walker 256 tumor was a powerful stimulus for these cells. The increase in life span and reduction in tumor mass was also associated with the prevention of the changes induced by the tumor in the metabolism of macrophages and lymphocytes. It is noteworthy that even though high-intensity exercise training was able to prevent several changes induced by the tumor, some alterations were not avoided. For example, levels of cytokines remained high in TBe animals, but the signs associated with cachexia were absent, suggesting that cytokines present different effects in sedentary and trained animals.

Another change promoted by the tumor, and prevented by exercise training, was the increase in plasma glutamine levels. Increased concentrations of glutamine are important for tumor growth (48). Thus, the reduction in glutamine levels observed in the plasma could be involved with the minor tumor development in TBe rats. However, in

accordance with the glutamine hypothesis (49), a reduction in plasmatic glutamine levels (due to intense exercise) could decrease the capacity of the lymphoid system. This is in agreement with studies that have maintained plasmatic glutamine levels by means of branched-chain amino acids or carbohydrate supplementation and observed a reversal in immune function impairment after exercise (50–52). In this sense, the reduction in glutamine levels observed in TBe animals could compromise immune function, which is in contrast with the results for life span and tumor growth. It is possible, however, that glutamine levels and immune function did not present a cause-effect relationship, because in other studies in which glutamine concentration was maintained by administration of this amino acid impairment of some immune parameters was observed (53–56).

Because metabolic alterations observed in the presence of cachexia appear to be mediated by humoral factors produced by the host organism as well as by the tumor (57), it is tempting to speculate that exercise also compromised metabolism of Walker 256 tumor cells. The evolution of this tumor follows the organic changes caused by the tumor; among these changes are the impairment of the metabolism and function of lymphocytes and macrophages (24, 58). Fernandes *et al*. (21) demonstrated that Walker 256 tumor cells, in comparison to lymphocytes, produced more lactate and CO_2 from glucose and more glutamate and CO_2 from glutamine. Therefore, the metabolic changes induced by high-intensity training could also explain the minor tumor growth, because tumor cells from TBe animals presented a different pattern of glucose and glutamine metabolism in comparison to tumor cells of animals from the TC group.

Some studies suggest that cachexia affects biorhythm regulation (59, 60), though exercise can shift circadian rhythms if performed at the right time of the day. A significant finding was the demonstration that an increase in life span and a reduction in tumor growth were observed in Walker 256 tumor–bearing rats submitted to moderate-intensity training at the same time each day (61). On the other hand, no effects were observed in these parameters if exercise sections were performed randomly through the day (42). As such, in this scenario, and considering the data about life span and tumor growth, the reduction in glucose and glutamine metabolism observed in lymphocytes and macrophages of TBe rats was not truly immunosuppressant. In support of this, changes observed in the parameters of EC animals were not associated with negative outcomes, such as great changes in immune cell function (phagocytosis, H_2O_2 production, and proliferation index) or reduction in body weight or total body fat content.

The apparent contradiction of our results in relation to epidemiologic evidence could be explained by the fact that the efficiency of physical training essentially depends on intensity, volume, and periodization of training stimuli (62). The combination of these training parameters with individual tolerance to stress as well as external facts such as diseases and social problems, determine whether the training load will be excessive or not (63). Another important fact is that, during competition, athletes normally overcome their own limits by characterizing an uncommon overload even for a healthy individual. Although our exercise training program was of high intensity, its volume was low (30 mins/day). This is in agreement with the results obtained in other models of high-intensity exercise, such as resistance training, which was shown not to compromise immune function (64, 65). In addition, Quist *et al*. (5) recently demonstrated that 6 weeks of high-intensity resistance training (30 mins, 2–3 times per week at 85%–100% of 1RM) and high-intensity aerobic training (10 mins on stationary bikes at 85%–95% of maximal heart rate; this was the first study to use such intensity) increase maximal strength, aerobic fitness, and body weight in cancer patients undergoing chemotherapy. These results were observed in patients with different diagnoses and different stages of disease, even in the most advanced cases. The authors did not report negative intercurrences in consequence to intensive training.

It is important to note that at the time of tumor inoculation, the TBe group had already been training for 8 weeks. This was due to the impossibility to adapt animals to exercise training before the 15th day (the time until death caused by the tumor). As such, we are not able to distinguish whether our results are due to training during the pre- or postinoculation (or both) period. Although these results reflect the effect of high-intensity exercise, it is not possible to establish whether they are related to prevention (if a previously trained organism is more resistant to tumor) or cure (once the tumor is present, the training may eliminate it). Future studies utilizing other experimental designs may be used to solve this problem. Because our data demonstrated that high-intensity exercise could be used against tumors, studies concerning combinations of intensity (low vs. moderate vs. high) and volume (low vs. moderate vs. high) are necessary to establish appropriate exercise programs for cancer treatment.

In conclusion, high-intensity training prevents most of the changes promoted in the metabolism and function of macrophages and lymphocytes of tumor-bearing rats, increasing their life span and reducing tumor size.

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