The effect of regular exercise on development of sarcoma tumor and oxidative damage in mice liver

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Abstract
Regular exercise has the capability of decreasing the incidence and progress of certain cancers. Murine sarcoma, (S-180) cells were transplanted to control (TC), exercise trained (10 week, 1 hour day, 5 times/week) mice, which had the swimming training terminated at the time of transplantation (ETT), and also to a group of mice that continued to exercise during tumor bearing (ETC). Continuous exercise decreased the size of tumor by about 50%. The accumulation of reactive carbonyl groups (RCD), were not significantly different for any group. The oxidative modification of proteins in the liver of the animals decreased in the exercise-trained non-tumor bearing group compared with control or tumor-bearing groups. No significant alteration was detected in the level of mutant p53. The data indicate that regular exercise retards the development of sarcoma solid tumors and it seems unlikely that massive uncompensated oxidative stress takes place in the tumor.

Key words: Exercise, cancer, oxidative stress, DNA damage, reactive carbonyl derivatives.

Introduction
Antioxidant therapies on cancer bearing animals have controversial effects (Gerber, 2000). Depending on the timing or the type of cancer, antioxidants could promote or retard the development of tumors (Gerber, 2000). Regular exercise is known to increase the activity of antioxidant enzymes (Ji and Hollander, 2000), and hence increase protection against the activity of reactive oxygen and nitrogen species (RONS). Similarly to antioxidant administration regular exercise could also promote and/or retard the development of certain cancers, depending on the timing and perhaps on the intensity of the exercise (Thompson et al., 1995). In general, epidemiological data demonstrate that regular exercise decreases the incidence of colon and breast cancers (Daneryd et al., 1995a; 1995b; Hill, 1999; Inger and Eiliv, 1997; Thompson, 1994; Thompson et al., 1995). Shephard and Futcher (Shephard and Futcher, 1997) suggested that the protective mechanism of regular exercise against many types of cancer might be as high as 50%.

In addition to epidemiological data, experimental results indicate that indeed, regular exercise retards the development of certain cancers (Kiningham, 1998; Radak et al., 2001; Thompson, 1994). Several hypotheses have arisen to explain this phenomenon involving the beneficial effects of exercise on the hormonal system (Daneryd et al., 1995b), energy metabolism (Thompson, 1994), and the antioxidant system (Daneryd et al., 1995a). It seems that regular exercise-induced beneficial effects on cancer development and incidence are not due to one particular pathway, but most probably are the result of several mechanisms, which are altered by regular exercise. The purpose of the present investigation was to identify mechanisms by which regular exercise could affect the development (not the incidence) of sarcoma. We hypothesized these to be changes in the RONS generating and antioxidant systems as a result of exercise training, and hence, altered accumulation in reactive carbonyl derivatives (RCD), changes in cellular regulation, including apoptosis, and the accumulation of mutant p53, the wild form of which plays a central role in a number of viral cellular processes.

Methods
Protocol
Thirty-five first-generation hybrid BDF1 (C57B1/6 female and DBA males) adult female mice, weighing 23-25 g, specific pathogen free, were used in the study. The animals were kept in 12 h light/dark rooms and fed a sterilized standard diet (Biofarm) and tap water ad libitum.

Tumor and exercise
Murine sarcoma, (S-180) obtained from the Biological Testing Branch of the National Cancer Institute (USA), were maintained in live BDF1 mice by in vivo inoculation (s.c.) with 5 x 10^6 cells per mouse. Mice were assigned to five groups seven animals per group: control (C); control tumor treated (TC); exercise trained (ETT), with the exercise being terminated at the point of tumor cell transplantation; exercise trained with the animals continuing the exercise (ETC); and continuously exercised non-treated mice (EC). Exercised mice had five, one hour swimming training sessions per week for ten weeks and some group received tumor cells after this period (ETT, ETC). The water temperature was set to 30 C degree. Sarcoma cells were transplanted under the skin of mice and the tumor size was measured every two days using a microcaliper, without cutting the fur. The volume of the tumor was calculated as described by Tomayko and Reynolds (Tomayko and Reynolds, 1989). The ETC continued swimming training until the termination of the study, on the 18th day following transplantation. The animals were
killed and liver and tumor samples were collected and frozen immediately in liquid nitrogen.

**Assays**
Reagents used in this study were obtained from Sigma (St. Louis, MO, USA) otherwise is stated. The mutant p53 protein ELISA was purchased from Calbiochem and measured according to the supplier (cat. no: QIA 03). The oxidative modification of amino acid residues was measured by the accumulation of reactive carbonyl derivatives (RCD) as described previously (Radak et al., 1997). In brief, proteins precipitated with trichloroacetic acid were suspended and incubated in a solution containing 10 mM 2,4-dinitrophenylhydrazones (DNPH) and 2 N HCl for 1 hour at 15 C. The resulting protein hydrazones were pelleted in a centrifuge at 11000 x g for 5 minutes. The pellets were washed three times with ethanol-ethyl acetate (1:1) and then once with ice-cold acetone. The final precipitates were dissolved in 1 ml buffer containing 8 M urea and 5% 2-mercaptoethanol. The protein content was re-measured following the RCD spectrophotometric measurement and in some cases the same samples were further used for Western blots. Duplicate polyacrylamide gel electrophoresis of derivatized proteins was carried out in 12% polyacrylamide gels containing 0.1% sodium dodecyl sulfate. After electrophoresis, the proteins were transferred to nitrocellulose membranes. The membranes were soaked in phosphate-buffered saline containing 3% skim milk, 0.05% Tween, and 0.05% sodium azide and then treated with anti-DNPH antibody. After washing in buffer without antibodies, the membranes were treated with ³²²I-Protein A. Finally, the radioactive signals were quantified by BAS 2000 Bioimaging Analyzer (Fuji Film. Co., Tokyo). The Western blot data of five animals from each group were quantified by densitometer and expressed in arbitrary units.

**Statistical analysis**
Data were normally distributed and expressed as means SD. Analysis of variance (ANOVA) was used to compare groups. Post hoc analysis was performed using the Tukey test. A p < 0.05 was considered statistically significant.

**Results**
Tumor size was significantly smaller in ETC animals compared to TC on the 18th day following transplantation (Figure 1). The level RCD accumulation was not significantly different between the groups (Figure 2). The oxidative modification of proteins, assessed also by reactive carbonyl groups, decreased in the liver of E, compared with C, indicating a beneficial adaptive response to exercise training (Figure 3). No significant alteration was detected in the protein level of mutant p53 (Figure 4).

![Figure 1](image1.png)
**Figure 1.** The tumor volume of continuously exercised (ETC) animals was significantly smaller on the 18th day following transplantation compared with control tumor-bearing (TC) animals. The tumor size of the animal which terminated the exercise at the time of tumor transplantation (ETT) was slightly, but not significantly smaller than TC. Values are means ± SD of 7 animals. * p < 0.05 versus TC.

![Figure 2](image2.png)
**Figure 2.** Accumulation of reactive carbonyl derivatives (RCD) in the tumors of mice was determined by spectrophotometer and immunoblot methods. The graph shows no significant alteration in RCD content in tumors of TC, ETT, and ETC mice (similar data were obtained spectrophotometrically). The immunoblot data reveals protein specific increases in RCD levels. Values are means ± SD of 6 animals.

![Figure 3](image3.png)
**Figure 3.** Exercise training resulted in decreased accumulation of RCD levels in the liver of mice compared with control non-tumor bearing and tumor-bearing animals (data obtained spectrophotometrically). Values are means ± SD of 7 animals. * p < 0.05 versus tumor-bearing animals (TC, ETT, ETC) and # p < 0.05 versus C.

**Discussion**
Our hypothesis that regular exercise-induced adaptation might be an important means by which exercise decreases the development of tumors proved to be correct and in accordance with data obtained in other studies on cancer and exercise (Daneryd et al., 1995a; 1995b; Hill, 1999; Inger and Eiliv, 1997; Thompson, 1994; Thompson et al., 1995). Interestingly, pre-training also had some beneficial effects on tumor development, however the difference did not reach the statistical level. However, the decrease in
tumor size was more apparent in those animals that exercised during cancer development. The underlying mechanism is not known, but it could be due to altered angiogenesis in the tumors (Lane and Benchimol, 1990) and/or different energy utilization, or to an altered immune system (Fairey et al., 2002; Kritchevsky, 2001).

The lack of accumulation of RCD also suggests that solid sarcoma tumors do not result in increased accumulation of oxidatively modified proteins. On the other hand, it appears that regular exercise training decreases the accumulation of RCD in the liver of non-tumor-bearing animals. Exercise increases the formation of free radical species, which might lead to increased insult on macromolecules (Davies et al., 1982). However, regular exercise provides an excellent possibility to respond to the repeated oxidative challenge by the up-regulation of antioxidant and repair mechanisms (Ji, 1999; Radak et al., 1999). A beneficial adaptation could result in a lower level of oxidative damage as noted in the present study. We suggest that the lower level of RCD could be partly due to the increased degradation of RCD by the proteasome complex and that this is an important part of exercise-induced oxidative adaptation (Radak and Goto, 2000). Moreover, proteasome inhibitors are widely used in cancer therapy, which even can suggest that accumulation of oxidized proteins during cancer could curb the progress of diseases (Wiedmann and Caca, 2005; Wiedmann and Mossner, 2010).

In conclusion, regular exercise significantly decreases the development of solid sarcoma tumors. Exercise during tumor-bearing has more prominent effects: the livers of tumor-bearing mice do not suffer significant oxidative damage; and cellular regulation is not disturbed judging from the accumulation of mutant p53. Regular exercise-induced adaptation decreases the accumulation of oxidatively modified proteins in the liver, which could beneficially affect the function of this organ.

**References**


Key points

- Regular exercise has a capability to reduce the incidence and progress of certain cancers.
- Free radicals could act as promoters and suppressors of cancers.
- Exercise can suppress the development of Sarcoma, but the underlying mechanisms are not known.