README — Experimental Design and Methods

Study Overview

This study investigated the effects of treadmill-based aerobic training and doxorubicin (DOX) chemotherapy on tumor progression in mice. Male C57BL/6 mice were preconditioned to treadmill running, underwent graded exercise testing and endurance training, and were subsequently inoculated with Lewis lung carcinoma (LL/2) cells. After palpable tumors were established, mice were randomized to groups and treated with DOX. Tumor growth, body weight, and exercise performance were recorded, and tissues were collected at the study endpoint.

Exercise Protocol

Apparatus: Exer 3/6 treadmill (Columbus Instruments, USA).
Acclimatization: 4 consecutive days, 10 min/day at 6 $m \cdot min^{-1}$.
Graded Maximal Exhaustive Test: Starting at 6 $m \cdot min^{-1}$; speed increased by 3 $m \cdot min^{-1}$ every 3 min until exhaustion (Ferreira et al., 2007).
Single blinded observer (RWAS). Parameters: total running distance (m) and maximal running speed ($m \cdot min^{-1}$).
Training Program: Intensity at 60% of maximal speed from graded test (approximates maximal lactate steady state; Ferreira et al., 2007).

- Acute exercise: one 90-min bout at 60% peak workload; samples collected immediately, 3 h, 6 h, and 24 h post-exercise.
- Endurance training: 6 weeks, 5 days/week at 60% peak workload; graded test repeated at week 3 to adjust intensity.

Sample harvest for trained cohorts occurred 72 h after the last training session to avoid acute exercise effects. Performance data are labeled as Pre-TR, 3 wks, and Post-TR.

Tumor Model

Cell line: LL/2 (CRL-1642, ATCC) cultured in DMEM (4.5 g/L glucose) + 10% FBS at 37 °C, 5% CO_2.
Inoculation: 1×10^6 LL/2 cells injected subcutaneously into the flank; group size n = 8–13 mice per group.
Enrollment/randomization: Upon development of a palpable solitary mass (≈day 5 post-inoculation), mice were randomized into study groups.

Doxorubicin Regimen

Initiation: at palpable tumor detection.
Regimen: 4 $mg \cdot kg^{-1}$ DOX, intraperitoneal, every other day for five doses (≈10 days total).
Groups:

1) LL/2 only (tumor-bearing, no DOX, no training)
2) Non-TR + LL/2 + DOX (sedentary with DOX)
3) TR + LL/2 + DOX (trained with DOX)

Monitoring and Outcomes

Body weight: recorded periodically during treatment.
Tumor volume: measured with a Vernier caliper; formula = (length × width × height)/2 (mm^3).

Tumor mass: excised and weighed at ~day 21 post-inoculation.
Endpoint: ~21 days after inoculation; tumor and cardiac tissues collected.

Blinding and Randomization
Performance tests were conducted by a blinded observer (RWAS).
Randomization occurred at the time of palpable tumor detection, prior to DOX treatment.

Notes
- Figures referenced in the file correspond to performance (A–B), body weight change (C), tumor mass at day 21 (D), and tumor volume trajectory (E).
- Reference for treadmill protocols: Ferreira et al., 2007.