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Physical exercise effects on metastasis: a systematic review and meta-analysis in animal cancer models

Cecilia Rincón-Castanedo[1] · Javier S. Morales[1] · Asunción Martín-Ruiz[1] · Pedro L. Valenzuela[2] · Manuel Ramírez[3] · Alejandro Santos-Lozano[4,5] · Alejandro Lucia[1,5] · Carmen Fiuza-Luces[5]

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Abstract

Physical exercise is considered a well-tolerated adjuvant therapy to mitigate cancer-related side effects, but its impact on metastasis is unclear. The present systematic review and meta-analysis aimed to summarize the evidence on the effects of exercise on metastasis in animal cancer models. A systematic search was conducted to identify controlled studies in animals analyzing the impact of exercise interventions on any marker of metastasis incidence or severity. The pooled mean differences (PMD) were calculated for those endpoints for which a minimum of three studies used the same assessment method. We also calculated the pooled odds ratio (OR) of metastases. Twenty-six articles were included in the systematic review, of which 12 could be meta-analyzed. Exercise training in murine cancer models did not significantly modify the number of metastatic foci (PMD = − 3.18; 95% confidence interval [CI] − 8.32, 1.97; $p = 0.23$), the weight of metastatic tumors (PMD = − 0.03; 95% CI − 0.10, 0.04; $p = 0.41$), or the risk of developing metastasis (OR = 0.64; 95% CI 0.10, 4.12; $p = 0.64$). These findings suggest that exercise has no overall influence on any marker of cancer metastasis incidence or severity in animal models. However, the wide methodological heterogeneity observed between studies might be taken into account and the potential exercise effects on metastasis development remain to be determined in pediatric tumors.

Keywords Dissemination · Physical activity · Metastasis · Exercise · Spontaneous model · Induction model

1 Introduction

Despite significant improvements in diagnosis, treatments, and adjuvant therapies, patients with advanced or metastatic cancer (stages III–IV) have a poor prognosis of survival and metastases are the commonest cause of cancer mortality [1–4]. There is strong evidence to suggest that exercise training is a safe and effective adjuvant strategy for the mitigation of cancer treatment-related side effects in adult cancer (e.g., fatigue and impaired physical fitness) [5–7] as well as in pediatric tumors (e.g., cardiotoxicity, or loss of functional mobility and muscle strength [8–10]). People living with and beyond cancer should in fact be advised to be as physically active as is possible for them [11]. Whether exercise exerts beneficial effects on tumor metastasis, however, is more controversial. Although there are studies suggesting that greater physical activity levels are associated with a lower risk of metastatic-lethal progression in patients with prostate cancer [12] and longer progression-free survival in those with metastatic colon cancer [13], or providing preliminary evidence that an exercise intervention may reduce circulating tumor cells among stage I–III colon cancer patients [14], these studies did not specifically analyze the process of metastasis *per se*. Recently, our group showed no significant effect on risk of metastases of a supervised, pediatric inhospital exercise intervention performed during the neoadjuvant (for solid tumors) or intensive chemotherapy treatment period (for leukemias),

Cecilia Rincón Castanedo and Javier S. Morales contributed equally to this work.

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✉ Carmen Fiuza-Luces
cfiuza.imas12@h12o.es

1 Faculty of Sport Sciences, Universidad Europea de Madrid, Madrid, Spain

2 Department of Systems Biology, University of Alcalá, Madrid, Spain

3 Unidad de Terapias Avanzadas, Servicio de Oncohematología, Hospital Universitario Niño Jesús, Madrid, Spain

4 i+HeALTH, Department of Health Science, European University Miguel de Cervantes, Valladolid, Spain

5 Laboratorio en Actividad Física y Salud, Instituto de Investigación Hospital Universitario 12 de Octubre (i+12), Avenida de Córdoba s/n, 28041 Madrid, Spain

with a median duration of 22 weeks in a cohort of 169 children (mean age 11 years) that was followed up from the start of treatment for up to 5 years [8].

Preclinical models can provide insight into the effects of exercise (whether beneficial or not) on tumor metastasis and can enable the development and testing of cancer therapies—including exercise—that might improve the prognosis of patients with metastatic cancer, as well as contribute to a better understanding of pathobiological metastasis pathways [15]. Along this line, several preclinical studies have analyzed the effects of exercise on tumor metastasis and findings would appear to suggest that it might be a potential therapy for the attenuation of metastasis [16–18], but the evidence to date is inconclusive. Accordingly, the objective of this systematic review and meta-analysis was to assess the effects of voluntary or forced physical exercise on different markers to evaluate metastasis (i.e., number of metastatic foci, the weight of metastasis load, and risk of metastasis development) in murine cancer models, compared with those of control (non-exercise) groups matched for baseline characteristics.

2 Methods

The conduct and reporting of the current systematic review and meta-analysis conform to the Preferred Reporting Items for Systematic Reviews and Meta-analyses [19].

2.1 Systematic search

Two of us (C.R.C. and J.S.M.) independently performed the systematic search and study selection. We conducted a literature search for relevant articles written in English in PubMed and SPORTDiscus (from inception to September 30th, 2019). The search terms used were "exercise," "physical activity," "voluntary wheel," "treadmill," "physical therapy," "running," "mice," "mouse," "rodent," "animal," "preclinical model," "cancer," "tumor," "tumor growth," "tumor progression," "metastasis," "metastasis process," "invasion," "intravenous injection," "metastasis formation," "experimental metastasis," "spontaneous metastasis," "orthotopic injection," "artificial route," and "organ colonization." The search strategy was built by combining the descriptors with each other and with the Boolean connectors "AND" and "OR." The electronic search was supplemented by a thorough manual review of reference lists from relevant publications and reviews to find additional publications on the subject. Gray literature (e.g., abstracts, conference proceedings, and editorials) and reviews were excluded.

2.2 Study selection and data extraction

After removing duplicated studies, citations initially selected by systematic search were first retrieved and preliminarily screened by title and abstract. Studies that met the inclusion criteria were assessed in their entirety. Each author provided a separate list with the studies selected at each stage, as well as with those to be finally included in the review. Disagreements were resolved through discussion with a third reviewer (P.L.V.).

We only included in the systematic review those studies meeting each of the following criteria: (1) analyzing a murine model with spontaneous or induced development of metastasis; (2) including a voluntary or forced exercise training intervention; (3) following a controlled design in which the control group was not subjected to the exercise intervention; and (4) assessing metastasis-related variables after the intervention. In those studies where the animals received pharmacological treatment, both groups (i.e., exercise and non-exercise) should receive the same treatment. We collected the following data from each study, if available: authors' names, year of publication, sample size, animals' characteristics (host, sex, age, cancer type), tumor induction protocol, intervention characteristics, main study outcomes, and results. We contacted the authors when necessary to clarify any uncertainty or to request additional data. In this regard, the authors of one study [20] provided us with the requested data.

2.3 Outcome measures

We assessed metastasis in general—the number of metastatic tumor foci, the weight of the metastatic tissue, the percentage of radioactivity retention in studies using labeled cells (number of radioactive cells in tissue/total among of radioactive cells injected), and the metastases ratio (size of metastatic tumor foci/size of the organ)—as continuous outcomes. The incidence of metastasis was assessed as a dichotomous outcome.

2.4 Quality assessment

The risk of bias of the included studies was assessed using the SYstematic Review Center for Laboratory Animal Experimentation (SYRCLE)'s Risk of Bias (RoB) tool (Cochrane RoB tool adapted for animals) [21]. Two of us (C.R.C. and J.S.M.) independently rated the studies as having "high," "low," and "unclear" risk of bias in six dimensions: sequence generation, baseline characteristics and allocation concealment (selection bias), random housing and blinding (performance bias), random outcome assessment and blinding (detection bias), incomplete outcome data (attrition bias), selective outcome reporting (reporting bias), and other sources of bias (other) (Supplementary File 1). Disagreements in scores were resolved through discussion with a third reviewer (P.L.V.).

2.5 Statistical analysis

A meta-analysis was performed to estimate the overall effect of physical exercise on markers of metastasis incidence and severity. The odds ratio (OR) was computed for dichotomous outcomes using a random-effects model. Calculations were performed on the log of ORs from individual studies, and the resulting pooled values were then back-transformed to the OR scale. For continuous outcomes, the pooled mean differences (PMD) between groups (last evaluation at the end of the follow-up *minus* baseline data) were computed using a random-effects model. For the continuous model, the weight assigned to each study in the meta-analysis was defined by the standard deviation of the variables and the sample size. Begg's test was used to determine the presence of publication bias, and the Q and I^2 statistics were used to assess heterogeneity among studies. In an additional subanalysis, we pooled, whenever feasible, results by type of metastatic tissue, tumor induction technique, or induced tumor cell. The level of significance was set at 0.05. All statistical analyses were performed using MIX 2.0 Pro for Excel software (BiostatXL; Mountain View, CA) [22].

3 Results

A total of 26 studies [20, 23–47] met all inclusion criteria and were included in the systematic review (Fig. 1).

3.1 Studies characteristics

3.1.1 Sample and characteristics

Descriptive data of the included studies are presented in Table 1. The studies included animals ranging in age from 3 [43] to 12 weeks [39]. The studies were also heterogeneous with respect to the seven cancer model and included melanoma [31, 32, 43, 44], liver cancer [37, 38], lung metastasis [24–26, 30, 40, 45], lung cancer [28, 35, 39, 43], mammary cancer [20, 23, 33, 34, 41, 46], colon carcinoma [27], prostate cancer [42, 47], liposarcoma [36], and fibrosarcoma [29]. Regarding the method used for metastasis development, spontaneous models were used in only two studies [20, 41], and induced models were used in the remainder. Among the induced model studies, two used patient-derived tumor xenografts [35, 36], and the remaining studies induced metastasis by injecting animal tumor cells into different injection sites of the mice, such as the tail vein [24–27, 29–32, 34, 35, 37, 38, 40, 43–45], or injecting subcutaneously [28, 37, 39, 43], intraperitoneally [33], or orthotopically [23, 36, 46, 47]. Other studies infused the tumor cells into the internal carotid artery [39] or the skin [42].

3.1.2 Exercise interventions

All studies used an aerobic exercise intervention, which consisted of forced treadmill exercise [26–29, 31, 33, 40, 44, 46], forced swimming exercise [37], voluntary wheel exercise [20, 23–25, 32, 35, 36, 39, 42, 43, 47], voluntary swimming exercise [37, 38], or a combination of both forced and voluntary exercise [30, 34, 41, 45].

3.1.3 Treatments

Five studies applied pharmacological treatments during the study period, including an anti-natural killer (NK) antibody (anti-asialo monosialotetrahexosylganglioside [ASGM1] in subgroups of trained and control animals—in order to determine if NK cells were, at least partially, responsible for the effects of exercise on lung metastasis development [45]; domperidone and bromocriptine [37]; tamoxifen [46]; and also the natural treatments *Songyou Yin* (a Chinese herbal compound) [38] and dietary oat β-glucan fiber [31].

3.2 Quality assessment and publication bias

All studies were used for data synthesis independently of their risk of bias. The risk of bias in the included studies was overall moderate (Fig. 2) [21].

3.3 Outcomes

3.3.1 Metastasis incidence

Eight studies analyzed the effects of exercise on metastasis incidence [24, 29, 30, 34, 35, 40, 41, 46]. One reported a reduction in metastasis incidence with exercise [24] and six reported no significant differences between groups [30, 34, 35, 40, 41, 46]. In one study, the metastasis incidence was not clearly reported by the authors [29] (Table 2). Seven studies including a total of 479 mice [24, 26, 29, 30, 33, 34, 40] reported the data needed to calculate the pooled OR, which suggested no effects of exercise on metastasis incidence (OR = 0.64; 95% CI 0.10, 4.12; $p = 0.64$) (Fig. 3) with no evidence of publication bias ($p = 0.58$) but with statistical heterogeneity among studies ($I^2 = 83\%$; $Q = 35.73$; $p < 0.001$). It was not possible to perform subanalysis due to the characteristics of the studies.

3.3.2 Number of metastatic foci

Eleven studies analyzed the effects of exercise on the number of metastatic foci [20, 23, 24, 26, 28, 29, 31, 32, 40, 42, 43]. Of these, six found no significant differences between groups [20, 24, 26, 40, 42, 43], three found a lower number of tumor foci in the exercise group [29, 31, 32], and another study

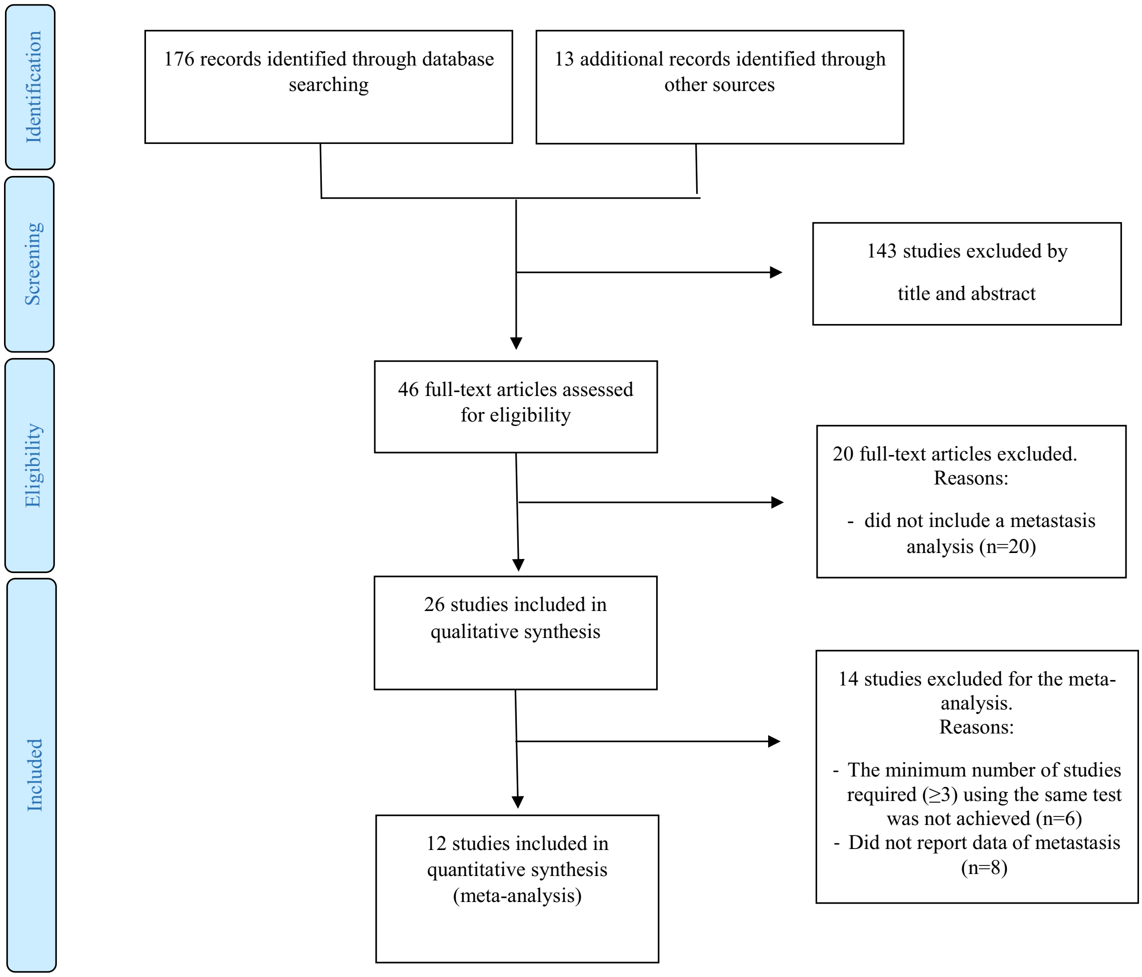

Fig. 1 Flow diagram of literature search until September 2019

reported an increase in the number of tumor foci in the exercise group [23]. In total, six studies (with five subanalyses in MacNeil et al. [24] and two subanalyses in Tsai et al. [28] and Yan et al. [43], respectively) with 464 animals [23, 24, 28, 32, 40, 43] could be included in the meta-analysis, which showed no significant effects (PMD = − 3.18; 95% CI − 8.32, 1.97; $p = 0.23$) of exercise for this outcome (Fig. 4), with no evidence of publication bias ($p = 0.23$) but with statistical heterogeneity among studies ($I^2 = 69\%$; $Q = 37.83$; $p < 0.001$).

Additional subanalysis revealed no significant exercise effects (PMD = − 2.01; 95% CI − 5.64, 1.63; $p = 0.28$; no publication bias [$p = 0.47$] or statistical heterogeneity; $I^2 = 1\%$; $Q = 4.03$; $p = 0.40$) on the number of tumor foci inductioning the tumor (CIRAS 1 cell murine line of C3H fibroblasts) by intravenous injection in the tail vein.

3.3.3 Weight of metastatic tissue

Four studies analyzed the effects of exercise on the weight of metastatic tissue [27, 28, 33, 47] and none of them observed any significant influence, although one observed a tendency toward a lower weight in the exercise group [47]. Three of these studies (Tsai et al. [28] is divided in two subanalyses) (115 animals) [28, 33, 47] could be included in the meta-analysis, which revealed no significant exercise effects (PMD = − 0.03; 95% CI − 0.10, 0.04; $p = 0.41$) on the weight of metastatic tissue (Fig. 5) with no evidence of publication bias ($p = 0.93$) or statistical heterogeneity among studies ($I^2 = 46\%$; $Q = 5.56$; $p = 0.14$). Subanalyses were not done because of the characteristics of the combined studies.

3.3.4 Percentage of radioactivity retention

Four studies assessed the percentage of radioactivity (labeled cells) remaining in lung tissue [25, 26, 30, 45]. All of these studies injected less aggressive prometastatic cells to develop metastasis (radiolabeled H-Ras-transformed fibroblasts, CIRAS 1 cell line) and observed that exercise decreased the percentage of radioactivity retention. One of these studies also injected high aggressive prometastatic cells to develop metastasis (radiolabeled H-Ras-transformed fibroblasts, CIRAS 3 cell line) but did not observe significant differences between groups

Table 1 Main characteristics of controlled studies included in the systematic review

Authors (year)	Main sample characteristics and study groups	Tumor or metastasis type	Animal model	Exercise intervention	Treatment	Outcomes	Main results
Alvarado et al. [33]	Female 50 days old Study groups: 1. Exercise + MNU $n = 15$ at the start (10 analyzed) 2. Exercise + vehicle $n = 10$ at the start (0 analyzed) 3. Non-exercise + MNU $n = 15$ at the start (11 analyzed) 4. Non-exercise + vehicle $n = 10$ at the start (0 analyzed) (other study groups were used for purposes other than studying metastasis development)	Mammary cancer	Intraperitoneal injection of the carcinogenic agent MNU (50 mg/kg body weight) dissolved in 0.9% saline solution to a concentration of 11 mg/ml or vehicle (0.9% of saline solution) in Sprague–Dawley rats Moment of injection: before exercise/non-exercise conditions	Forced treadmill running at 20 m/min (moderate intensity), 60 min/day, 0° of slope, 5 days/week Duration: starting 24 h post-injection and after an acclimatization period to the treadmill for 5 days, animals exercised for 35 weeks	No	Number and absolute and relative weight of metastasis in the lungs	No differences in the number and weight of the lung metastasis between groups
Assi et al. [36]	Male 4 weeks old Study groups: 1. Exercise + tumor $n = 9$ 2. Exercise + vehicle $n = 9$ at the start (0 analyzed) 3. Non-exercise + tumor $n = 9$ 4. Non-exercise + vehicle $n = 9$ at the start (0 analyzed)	Liposarcoma	Orthotopic intramuscular injection of 2×10^6 human SW872 cells or vehicle in the inner side of the right hindlimb of nude mice Moment of injection: following a 6-week exercise/non-exercise period	Voluntary wheel running Duration: all study groups exercised before tumor injection for 6 weeks. After injection, only exercise groups (groups 1 and 2) exercised for 8 more weeks (total training duration: 14 weeks)	No	Number of lung metastatic nodules and lung metastatic area FABP4 mRNA expression	The data of the number of metastasis and the lung metastatic area were N/A Exercised mice showed higher tendency to express mRNA level of FABP4 in the tumor which was correlated with lung metastasis
Bryner et al. [42]	Male Age: N/A Study groups: 1. Exercise $n = 19$ 2. Non-exercise $n = 18$	Prostate adenocarcinoma	Inoculation of 10^6 PAIII tumor cells under the skin of the right flank of Lobund–Wistar rats Moment of injection: after an 11-week exercise/non-exercise period	Voluntary wheel running Duration: 11 weeks before inoculation + 1 week used for performing a treadmill test and the tumor cell inoculation +6 weeks after inoculation	No	Number of tumor foci in the lungs	No differences in the mean number of lung metastases between groups
Colbert et al. [41]	Female 9 weeks old	Mammary cancer	Heterozygous p53-deficient: MMTV-Wnt-1 Tg mice	Forced treadmill running: after ~ 8 weeks of wheel	No	Incidence of metastasis in the lungs	Incidence of mammary carcinoma metastasis to

Table 1 (continued)

Authors (year)	Main sample characteristics and study groups	Tumor or metastasis type	Animal model	Exercise intervention	Treatment	Outcomes	Main results
	Study groups: Treadmill running: 1. Moderate exercise $n = 20$ 2. Vigorous exercise $n = 21$ 3. Non-exercise $n = 22$ Wheel running: 4. Exercise $n = 20$ 5. Non-exercise $n = 21$			running, treadmill training was performed 5 days/week, 45 min/day, 5° of slope at 20 m/min for moderate intensity training (group 1) or 24 m/min for vigorous intensity training (group 2) Voluntary wheel running (group 4) Duration: from 9 weeks old until mammary tumor reached 1.5 cm in diameter, mice became moribund or there was 1 mouse/group remaining			the lung was similar across the treadmill groups, but was elevated in the wheel running mice compared to their non-exercise controls
Davis et al. [44]	Male 6–8 weeks old Study groups: Low-dose tumor experiment 1. Exercise (30 min run) $n = 11$ 2. Exercise (run to fatigue) $n = 12$ 3. Exercise (run to fatigue 24 h before injection) $n = 12$ 4. Non-exercise $n = 20$ High-dose tumor experiment 5. Exercise (30 min run) $n = 10$ 6. Exercise (run to fatigue) $n = 10$ 7. Non-exercise $n = 11$ Low-dose-radiolabeled cell experiment 8. Exercise (run to fatigue)	Melanoma	Low-dose tumor experiment: intravenous injection of 1×10^5 B16F1 tumor cells into the tail vein of C57BL/6 mice Moment of injection: 30 min (groups 1, 2, and 4) or 24 h (group 3) after the acute exercise/non-exercise condition and 10 days before mice were killed High-dose tumor experiment: intravenous injection of 1×10^6 B16F1 cells into the tail vein of C57BL/6 mice Moment of injection: 30 min (groups 5, 6, and 7) after the acute exercise/non-exercise condition and 7 days before mice were killed Low-dose-radiolabeled cell experiment: intravenous injection of 2×10^5 ^{51}Cr-labeled B16F1 cells into the tail vein of C57BL/6 mice	Forced treadmill running Exercise (30 min run) group: running for 30 min at 18 m/min (5° of slope) Exercise (run to fatigue) group: running the first 30 min at 18 m/min, and thereafter, the speed was increased 3 m/min every 30 min until fatigue (5° of slope) Exercise (run to fatigue 24 h before injection) group: the same as the previous group but the exercise was completed 24 h before tumor cell injection Duration: an acute bout of exercise	No	Low- and high-dose tumor experiments: number of tumor foci on the surface of the lungs Radiolabeled cell experiment: percentage of radiolabeled cells localized in the lungs, spleen, blood, and liver; these organs were also weighted	A bout of exercise until the point of volitional fatigue decreased the number of pulmonary metastases. Regardless of the tumor cell dose, exercise (run to fatigue) groups (groups 2 and 6) had fewer tumors than mice in either the non-exercise (groups 4 and 7) or exercise (30 min run) groups (groups 1 and 5). Mice exercised 24 h before tumor injection (group 3) showed fewer tumor foci than the non-exercise mice (group 4) The majority of B16 cells (75%) were settled in the lung, and alterations in circulating patterns occurring during exercise do not change the pattern of tumor cell arrest in groups (groups 10, 11, and 12) There was less radiolabel in the lungs at both 4 and

Table 1 (continued)

Authors (year)	Main sample characteristics and study groups	Tumor or metastasis type	Animal model	Exercise intervention	Treatment	Outcomes	Main results
	$n = 8$ 9. Non-exercise $n = 24$ High-dose-radiolabeled cell experiment 10. Exercise (30 min run) $n = 8$ 11. Exercise (run to fatigue) $n = 8$ 12. Non-exercise $n = 8$ *Number of mice per sacrifice time point (30 min, 4 h, and 16 h) (other study groups were used for purposes other than studying metastasis development)		Moment of injection: 15 min (groups 8 and 9) after the acute exercise/non-exercise condition and 30 min, 4 h, and 16 h before mice were killed High-dose-radiolabeled cell experiment: intravenous injection of 1×10^6 ^{51}Cr-labeled B16F1 cells into the tail vein of C57BL/6 mice Moment of injection: 15 min (groups 10, 11, and 12) after the acute exercise/non-exercise condition and 30 min before mice were killed				16 h after tumor cell injection compared with that 30 min post-exercise (groups 8 and 9)
Goh et al. [20]	Female 6 weeks old Study groups: 1. Tg exercise $n = 13$ at the start (11 analyzed) 2. WT exercise $n = 5$ at the start (0 analyzed) 3. Tg non-exercise $n = 12$ at the start (11 analyzed) 4. WT non-exercise $n = 5$ at the start (0 analyzed)	Mammary carcinoma	MMTV-PyMT Tg male mice on a 100% FVB background were crossed with C57BL/6 female to produce F1 hybrid females	Voluntary wheel running Duration: 10 weeks	No	Lung metastatic foci Gene expression of the tumor invasion and metastatic markers CXCR4 and CXCL12 in mammary tumors Proliferative index (Ki-67 as a cell proliferation marker) in mammary tumors	No differences in lung metastatic lesions between Tg exercise and Tg non-exercise groups Tg exercise increased gene expression of CXCR4 compare to Tg non-exercise condition in breast tumors No differences in the proliferative index and in the gene expression of CXCL12 in breast tumors between Tg exercise and Tg non-exercise groups
Higgings et al. [35]	Male 6 weeks Study groups: 1. Exercise $n = 10$ 2. Non-exercise $n = 10$	Lung adenocarcinoma	Injection of 5×10^5 A549-luc-C8 (derived from A549 human lung adenocarcinoma cells by stable transfection of the North American firefly luciferase gene) cells into the tail vein of	Voluntary wheel running Duration: 4 weeks of training after lung tumor detection	No	Incidence of metastasis in the lungs	No differences in the development of metastasis between groups because no mouse developed metastasis

Table 1 (continued)

Authors (year)	Main sample characteristics and study groups	Tumor or metastasis type	Animal model	Exercise intervention	Treatment	Outcomes	Main results
			immunodeficiency (SCID)-*beige* mice Moment of injection: before beginning the exercise/non-exercise period				
Hoffman-Goetz et al. [25]	Male 4–5 weeks old Study groups: 1. Exercise *n* = 54 2. Non-exercise *n* = 53	Lung metastasis	Intravenous injection of 5×10^5 ^{51}Cr-labeled CIRAS 1 (H-ras $10T^{1/2}$ transformed murine C3H fibroblast line) cells into the tail vein of C3H/HeNsd mice and (C3H/He × BALB/c) F1 mice Moment of injection: following the 9-week exercise/non-exercise period	Voluntary wheel running Duration: 9 weeks	No	Retention of radioactivity in the lungs, liver, and spleen expressed as the % of injected tumor cells retained in the lungs (the amount of radioactivity remaining in the lungs relative to the total amount of radioactivity injected) It was assessed 5 min, 30 min, 90 min, 3 h and 24 h after tumor cell injection	Exercise reduced % of retention of radioactivity tumor cells in the lungs, liver, spleen, and kidney Exercised mice displayed a lower retention of radioactivity in the lungs relative to non-exercise mice 5 and 30 min post-injection Exercised mice also had a small reduction in recovery of radioactivity from the liver, spleen, and kidney at 30 min and 3 h post-injection
Hoffman-Goezt et al. [34]	Female 3 weeks old Study groups: 1. Treadmill exercise ➔ treadmill exercise *n* = 10 2. Treadmill exercise ➔ non-exercise *n* = 15 3. Wheel exercise ➔ wheel exercise *n* = 10 4. Wheel exercise ➔ non-exercise *n* = 15 5. Non-exercise ➔ treadmill exercise *n* = 10 6. Non-exercise ➔ wheel exercise *n* = 15 7. Non-exercise ➔ non-exercise	Mammary adenocarcino-ma	Intravenous injection of 1×10^4 mouse mammary adenocarcinoma line 66 or heat-killed tumor cells into the tail vein of BALB/c mice Moment of injection: after 8 weeks of exercise or non-exercise period	Forced treadmill running at 18 m/min (moderate intensity), 30 min/day, 0° of slope Voluntary wheel running Duration: Running only before injection: 8 weeks Running only after injection: 3 weeks Running before and after injection: 11 weeks	No	Incidence of lung tumors	No differences in tumor multiplicity due to exercise condition

Table 1 (continued)

Authors (year)	Main sample characteristics and study groups	Tumor or metastasis type	Animal model	Exercise intervention	Treatment	Outcomes	Main results
	$n = 10$ 8. Control (non-exercise + nontumor) $n = 10$						
Hoffman-Goetz et al. [40]	Male Age: N/A 1. Exercise + group housing $n = 30$ 2. Non-exercise + group housing $n = 29$ 3. Exercise + individual housing $n = 30$ 4. Non-exercise + individual housing $n = 30$	Lung metastasis	Intravenous injection of 6×10^4 CIRAS 3 (H-ras $10T^{1/2}$ transformed murine C3H fibroblast line) tumor cells in C3H/HeNCrl IBR mice Moment of injection: after 4 weeks of exercise or non-exercise period	Forced treadmill running at 30 m/min (moderate to severe intensity), 30 min/day, 8° of final slope, 5 days/week Duration: 2 weeks of an acclimation period followed by 8 weeks of training	No	Incidence of metastasis and number of tumor foci in the lungs	No differences in incidence and metastatic tumor foci related to either housing conditions or activity conditions
Jadeski and Hoffman-Goet [26]	Gender: N/A 4–9 weeks old Study groups: Post-3-h injection experiment: 1. WT exercise + CIRAS 1 $n = 10$ 2. WT exercise + CIRAS 3 $n = 9$ 3. WT non-exercise + CIRAS 1 $n = 10$ 4. WT non-exercise + CIRAS 3 $n = 9$ 5. *Beige* exercise + CIRAS 1 $n = 11$ 6. *Beige* non-exercise + CIRAS 1 $n = 9$ Post-3-week injection experiment:	Lung metastasis	Post-3-h injection experiment: injection of 5×10^5 lung metastatic ^{51}Cr-labeled CIRAS 1 or CIRAS 3 cells into the tail vein of C3H/HeJ and C3H/*He-bg*^{2J}/+ (*beige*) mice Moment of injection: after the 9-week exercise or non-exercise period; mice were killed 3 h after the injection Post-3-week injection experiment: injection of 3×10^5 CIRAS 1 or CIRAS 3 cells into the tail vein of C3H/HeJ mice Moment of injection: after the 9-week exercise or non-exercise period; mice were killed 3 weeks after the injection	Forced treadmill running at 20 m/min, 30 min/day, 0° of slope, 5 days/week Duration: 1 week of an acclimation period followed by 9 weeks of training (before tumor cell injection)	No	Post-3-h injection experiment: amount of radioactivity remaining in the lungs relative to the total amount of radioactivity injected Post-3-week injection experiment: number of metastatic foci in the lungs	Post-3-h injection experiment: Irrespective of genotype, exercise mice injected with ^{51}Cr-labeled CIRAS 1 tumor cells retained less radioactivity in the lungs relative to non-exercise mice. WT exercise mice injected with ^{51}Cr-labeled CIRAS 1 retained less radioactivity in the lungs relative to WT non-exercise mice Post-3-week injection experiment: Irrespective of injected tumor cells, no differences in metastatic tumor foci between exercise and non-exercise WT mice groups were found

Table 1 (continued)

Authors (year)	Main sample characteristics and study groups	Tumor or metastasis type	Animal model	Exercise intervention	Treatment	Outcomes	Main results
	7. WT exercise + CIRAS 1 $n = 8$ 8. WT exercise + CIRAS 3 $n = 8$ 9. WT non-exercise + CIRAS 1 $n = 9$ 10. WT non-exercise + CIRAS 3 $n = 10$						
Jee et al. [27]	Male 4 weeks old Study groups: 1. Moderate exercise + tumor $n = 10$ 2. Severe exercise + tumor $n = 10$ 3. Non-exercise + tumor $n = 10$ 4. Non-exercise + no tumor $n = 10$ at the start (0 analyzed)	Colon carcinoma	Intravenous injection of 1×10^5 mouse colon carcinoma cell line-derived CT-26 into the tail vein of CDF1 mice Moment of injection: before beginning the exercise or non-exercise period	Forced treadmill running at moderate (group 1: 0.5 km/h) or severe (group 2: 1 km/h) intensity for 45 min/session with 0° of slope and performing 1 session every 2 days Duration: 4 weeks	No	Lung weight	No differences in the weight of lung metastases among groups
Jones et al. [47]	Male 6–8 weeks old Study groups: 1. Exercise $n = 30$ at the start (24 analyzed) 2. Non-exercise $n = 32$ Four mice per group were serially killed on days 14, 31, and 36 post-injection; the remaining 38 mice (exercise, $n = 18$; non-exercise, $n = 20$) were killed on day 53	Prostate cancer	Orthotopically injection of 5×10^5 TRAMP C-1 cells into the prostate of C57BL/6 mice Moment of injection: 14 days before beginning the exercise or non-exercise period	Voluntary wheel running Duration: 8 weeks	No	Total weight and number of metastasis in noncontiguous external masses that were grossly visible independent from the primary prostate tissue Gene profiling expression of prometastatic genes (*CXCR4* and *HGFR*) and HIF-1α protein levels on the primary tumor	No differences between groups in the weight and number of metastases CXCR4 was higher in the exercise group compared with the non-exercise group HGFR was lower in the exercise group compared with the non-exercise group HIF-1α protein levels were higher in the exercise group relative to the non-exercise group
Khori et al. 2015 [46]	Female 6–8 weeks old		Orthotropic inoculation of 1×10^6 mouse mammary	Forced treadmill running began at 16–18 m/min,	Yes		No mice had metastatic lesions

Table 1 (continued)

Authors (year)	Main sample characteristics and study groups	Tumor or metastasis type	Animal model	Exercise intervention	Treatment	Outcomes	Main results
	Study groups: 1. Exercise + tumor $n = 8$ 2. Exercise + tumor + tamoxifen $n = 8$ 3. Non-exercise + tumor + tamoxifen $n = 8$ 4. Non-exercise + tumor $n = 8$	Mammary adenocarcinoma	adenocarcinoma MC4-L2 cells in the right flank near to the upper part of rear foot of the BALB/c mice Moment of injection: before the exercise/non-exercise period	10–14 min/day at 0° of slope, 5 days/week and followed the training program described by [48] Duration: 5 weeks after tumor establishment	Tamoxifen (5 mg/kg, daily) was given *via* oral gavage for 2 weeks after tumor establishment	Incidence of metastasis in the brain, heart, liver, kidney, and lungs	
Mac Neil and Hoffman-Goetz [45]	Male 6–8 weeks old Study groups: 1. Moderate treadmill exercise $n = 30$ at the start (24 analyzed) 2. Wheel exercise $n = 30$ at the start (29 analyzed) 3. Slow-moving treadmill exercise $n = 30$ at the start (29 analyzed) 4. Non-exercise $n = 30$ at the start (29 analyzed)	Lung metastasis	Intravenous injection of 5×10^5 CIRAS 3 cells into the tail vein of C3H/He mice Moment of injection: after a 9-week exercise/non-exercise period and 3 h before the mice were killed	Moderate treadmill exercise: running at 15 m/min, 30 min/day, 0° of slope, 5 days/week Slow-moving treadmill exercise: running at 5 m/min, 5 min/day, 0° of slope, 5 days/week Voluntary wheel running Duration: 9 weeks	Yes Twenty-four hours before being killed, 10 mice/group (except for treadmill exercise group, $n = 9$) received ASGM1 antiserum (20 µl diluted 1:10 with PBS; final volume 200 µl) *via* the tail vein; 19 mice/group (except for treadmill exercise group $n = 15$) received an equal volume of PBS	Radioactivity retention in the lungs (analyzed by % of injected CIRA3 cells retained in the lungs = the amount of radioactivity remaining relative to the total amount of radioactivity injected)	Radioactivity retention was higher in anti-ASGM1-injected animals compared with PBS-injected mice Exercise training reduced radioactivity retention in the treadmill and wheel exercised mice that received PBS or ASGM1 injections below that of non-exercise or slow-moving treadmill groups The % of ASGM1-positive cells in splenocytes was higher in the exercised-trained mice than in the non-exercise or slow-moving treadmill groups The results supported a role for NK cells against metastasis development, which seems to be potentiated by chronic exercise
Mac Neil and Hoffman-Goetz [30]	Male 4–5 weeks old Study groups: 1. Moderate treadmill exercise $n = 20$ at the start (19 analyzed)	Lung metastasis	Post-9-week injection experiment: intravenous injection of 3×10^5 lung metastatic CIRAS 1 cells into the tail vein of C3H/He mice	Forced treadmill running: Moderate treadmill exercise group: running at 15 m/min, 30 min/day, 0° of slope, 5 days/week Slow-moving treadmill exercise group: running at	No	Post-9 wee-k injection experiment: tumor incidence and number of lung surface tumor colonies per mouse Post-12-week injection experiment: retention of	Exercise did not influence the incidence of pulmonary metastasis relative to non-exercise condition However, exercise increased the multiplicity of metastasis in the lungs: the

Table 1 (continued)

Authors (year)	Main sample characteristics and study groups	Tumor or metastasis type	Animal model	Exercise intervention	Treatment	Outcomes	Main results
	2. Wheel exercise $n = 20$ 3. Slow-moving treadmill exercise $n = 20$ 4. Non-exercise $n = 20$		Moment of injection: after a 9-week exercise/non-exercise period Post-12-week injection experiment: intravenous injection of 5×10^5 ^{51}Cr-labeled CIRAS 1 into the tail vein of C3H/HeJ Moment of injection: 12 weeks after beginning the study; mice were killed 3 h following injection	5 m/min, 5 min/day, 0° of slope, 5 days/week Wheel running Duration: 9 weeks All study groups remained sedentary for 3 weeks after injection		radioactivity in the lungs ➔ % of injected tumor cells retained in the lungs = the amount of radioactivity remaining relative to the total amount of radioactivity injected	median number of tumors for each mouse was greater in exercised (groups 1 and 2) mice than in non-exercising mice (groups 3 and 4) Exercised mice showed a reduction in the retention of labeled tumor cells compared with non-exercising mice
Mac Neil and Hoffmann-Goetz [24]	Male 4–5 weeks old Study groups: 1. Exercise ➔ exercise $n = 30$ at the start (27 analyzed) 2. Exercise ➔ non-exercise $n = 20$ at the start (19 analyzed) 3. Non-exercise ➔ exercise $n = 30$ at the start (28 analyzed) 4. Non-exercise ➔ non-exercise $n = 30$ at the start (29 analyzed)	Lung metastasis	Intravenous injection of 3×10^5 lung metastatic CIRAS 1 cells into the tail vein of C3H/He mice Moment of injection: after a 9-week exercise/non-exercise period	Voluntary wheel running Duration: Running only before injection: 9 weeks Running only after injection: 3 weeks Running before and after injection: 12 weeks	No	Number of tumor foci and incidence in the lungs Incidence of lung tumors	Exercise before tumor cell injection reduced the extent of lung metastases The effect of exercise was not different if exercise was continued or stopped after tumor cell injection Exercise beginning after injection had no effect on tumor growth relative to animals who were sedentary throughout the study
Murphy et al. [31]	Male 7 weeks old Study groups: 1. Exercise + oat β-glucan $n = 8–11$ 2. Exercise + water $n = 8–11$ 3. Non-exercise + oat β-glucan $n = 8–11$ 4. Non-exercise + water $n = 8–11$	Melanoma	Intravenous injection of 2×10^5 melanoma B16F1 cells into the tail vein of C57BL/6 mice Moment of injection: 10 and 9 days before beginning the oat β-glucan/water administration period and/or exercise/non-exercise period. Mice were injected 30 min after the last day of exercise or rest.	Forced treadmill running at 36 m/min (moderate intensity), 60 min/day, 8° of slope Duration: exercise began on the second day of oat β-glucan or water treatment with a 3-day acclimation period followed by 6 days of training. After tumor administration, exercise stopped for 14 days	Yes Groups 1 and 3 received a solution of oat β-glucan (made from an oat bran concentrate enriched to 68% soluble β-glucan) dissolved in the drinking water at a concentration of 0.6 mg/ml for 10 days before tumor administration or death and 14 days after tumor administration	Number of tumor foci on the surface of the lungs	Group 1 (exercise + oat β-glucan) and group 2 (exercise + water) had fewer number of metastatic tumor foci than group 4 (non-exercise + water) Non-exercise + oat β-glucan group had fewer tumors than on-exercise + water group The number of foci in the lungs was not different between the exercise + oat

Table 1 (continued)

Authors (year)	Main sample characteristics and study groups	Tumor or metastasis type	Animal model	Exercise intervention	Treatment	Outcomes	Main results
					Groups 2 and 4 received tap water for 10 days before tumor administration or death		β-glucan group and the exercise + water group or non-exercise + oat β-glucan group, indicating that there were no additive effects of moderate exercise and oat β-glucan solution
Pedersen et al. [32]	Female 8–12 weeks old Study groups: 1. Exercise $n = 10$ 2. Non-exercise $n = 9$ (other study groups were used for purposes other than studying metastasis development)	Melanoma	Intravenous injection of 1×10^5 melanoma B16F1 cells into the tail vein of C57BL/6 mice Moment of injection: following the 4-week exercise/non-exercise period	Voluntary wheel running Duration: 7 weeks (4 weeks prior to tumor cell inoculation + 3 weeks during the tumor challenge)	No	Number of lung tumor foci	Exercise reduced lung metastasis
Smeda et al. [23]	Female Age: N/A Study groups: 1. Exercise $n = 15$ at the start (9 analyzed) 2. Non-exercise $n = 20$ at the start (9 analyzed)	Mammary cancer	Orthotopic injection of 1×10^4 4T1 mammary adenocarcinoma cells into mammary fat pads in BALB/c mice Moment of injection: before beginning the exercise or non-exercise period	Voluntary wheel running Duration: 4 weeks	No	Number of lung tumor foci	Exercise increased the number of metastasis nodules in the lungs
Tsai et al. [28]	Male 8 weeks old Study groups: 1. Exercise $n = 16$ at the start (10 analyzed) 1.1. High interval exercise $n = 8$ (N/R) 1.2. Continuous exercise $n = 8$ (N/R) 2. Non-exercise $n = 16$ at the start (9 analyzed)	Lung cancer	Subcutaneous injection of 5×10^5 Lewis lung carcinoma cells on the back of C57BL/6J Moment of injection: 1 week before beginning the exercise/non-exercise period	Forced treadmill running: High interval exercise group: 5 days/week, 60 min/day consisting of: Weeks 0–2: 6 × 8 min at 20 m/min + 2 min at 10 m/min (10° of slope) Week 3: 6 × 7 min at 20 m/min + 3 min at 10 m/min (10° of slope) Week 4: 6 × 6 min at 20 m/min + 4 min at 10 m/min (10° of slope)	No	Weight of metastasis in liver and lungs = tissue weight/body weight before sacrifice − tumor weight Number of metastatic nodules in the lungs and liver	No differences in lung or liver weight and in the number of metastatic nodules in those tissues between exercise (high interval exercise group + continuous group) and non-exercise groups

Table 1 (continued)

Authors (year)	Main sample characteristics and study groups	Tumor or metastasis type	Animal model	Exercise intervention	Treatment	Outcomes	Main results
				Continuous exercise group: 5 days/week, 45 min/day at 3–4 m/min (0° of slope) Duration: 4 weeks			
Uhlenbruk and Order [29]	Male Age: N/A Study groups: Experiment 1: 1. 2-week exercise $n = 11$ 2. 4-week exercise $n = 12$ 3. 2-week non-exercise $n =$ N/R 4. 4-week non-exercise $n = 10$ Experiment 2: 5. 4-week exercise ➔ 2-week exercise $n =$ N/R 6. 4-week exercise ➔ 2-week non-exercise $n =$ N/R 7. 4-week non-exercise ➔ 2-week non-exercise $n =$ N/R	Fibrosarcoma	Intravenous injection of 1×10^4 or 2×10^4 mouse fibrosarcoma L-1 cells in the experiments 1 and 2, respectively, in BALB/c mice Moment of injection: Experiment 1: 2 or 4 weeks after the exercise/non-exercise period. Experiment 2: 4 weeks after the exercise/non-exercise period	Forced treadmill running Experiment 1: Running for 2 or 4 weeks before tumor cell injection followed by the training program in the same way (N/A more information) Experiment 2: Running for 4 weeks before tumor cell injection followed by a 2-week training/or rest period. Group 5: – 200–400 m at 0.3 m/s, or – 500–750 m at 0.4 m/s, or – 800–850 m at 0.4 m/s, or – 800–850 m at 0.3–0.5 m/s (interval) Group 6: – 200 m at 0.3 m/s, or – 600 m at 0.4 m/s, or – 750 m at 0.4 m/s	No	Relative incidence rate of lung tumor metastasis and number of tumor foci	Experiment 1: 10/11 had lung tumors in the 2-week exercise group but in lower numbers than the 2-week non-exercise group 8/12 mice were free of lung tumors in the 4-week exercise group; 10/10 mice had lung tumors in the 4-week non-exercise group Experiment 2: 71% of the animals of the 4-week non-exercise + 2-week non-exercise group showed more than 100 lung tumors In the animals of the training groups (groups 5 and 6), less than 100 metastases per lung were found In group 6, nearly half of the mice had less than 50 tumors; in group 5, almost 100% or all of the animals Number of tumor foci: N/A
Wolff et al. [39]	Male 12 weeks old Study groups: 1. Exercise + tumor $n = 30$ 2. Non-exercise + tumor $n = 33$ (other study groups were used for purposes other than studying metastasis development)	Lung carcinoma	Infusion of 1×10^6 murine Lewis lung carcinoma D122-luc/GFP cells into the internal carotid artery of C57BL/6 mice Moment of injection: following 5 weeks of exercise/non-exercise conditions	Voluntary wheel running Duration: 5 weeks prior to injection. Exercise stopped for 48 h or 3 weeks post-tumor cell infusion	No	Number of metastatic nodules and size of these lesions in the brain	No differences in the number of metastases and in the metastatic area between study groups 3 weeks post-tumor cell infusion
Yan and Demars [43]	Male 3 weeks old		Experimental metastasis experiment: intravenous	Voluntary wheel running	No	Number and size of tumor foci in the lungs	No differences between exercise and non-exercise

Table 1 (continued)

Authors (year)	Main sample characteristics and study groups	Tumor or metastasis type	Animal model	Exercise intervention	Treatment	Outcomes	Main results
	Study groups: Experimental metastasis experiment: 1. Exercise + tumor $n = 30$ 2. Non-exercise + tumor $n = 30$ at the start (28 analyzed) Spontaneous metastasis experiment: 3. Exercise + tumor $n = 30$ 4. Non-exercise + tumor $n = 30$	Melanoma and lung carcinoma	injection of 0.75×10^5 murine melanoma B16BL/6 cells *via* the lateral tail vein of C57BL/6 mice Spontaneous metastasis experiment: subcutaneous injection of 2.5×10^5 Lewis lung cancer cells into the lower dorsal region of C57BL/6 mice Moment of injection: following 9 weeks of exercise/non-exercise conditions	Experimental metastasis experiment duration: 9 weeks prior to injection followed by a 2-week post-injection training Spontaneous metastasis experiment duration: 9 weeks prior to injection. When the tumor reached ~ 1 cm in diameter, it was removed. Then, the mice were maintained on their respective treatments for an additional 2 weeks			mice with either experimental or spontaneous metastasis models in the number and size of metastasis
Zhang et al. [37]	Male 6 weeks old Study groups: Exercise alone: 1. Moderate exercise $n = 12$ at the start (6 analyzed) 2. Vigorous exercise $n = 12$ at the start (6 analyzed) 3. Very vigorous exercise $n = 12$ at the start (6 analyzed) 4. Non-exercise $n = 12$ at the start (6 analyzed) Exercise and domperidone 5. Moderate exercise $n = 6$ 6. Moderate exercise + domperidone $n = 6$ 7. Non-exercise $n = 6$ Exercise and bromocriptine 8. Very vigorous exercise	Hepatocarcinoma	Groups 1 ➔ 4 and 8 ➔ 9: subcutaneous injection of 5×10^6 Hepa1–6 murine liver cancer cells and intravenous injection of 3×10^6 Hepa1–6–GFP cells into the tail vein of C57BL/6 mice Groups 5 ➔ 7: subcutaneous injection of 2.5×10^6 Hepa1–6 tumor cells in C57BL/6 mice Moment of injection: before beginning the 6-week exercise or non-exercise period	Voluntary and forced swimming: there was an adaptation period (trained mice swam 5 min in the first week; 6, 12, or 16 min/day in the second week; and 8, 16, or 32 min/day in the third week) followed by a training period at moderate (8 min/day; groups 1, 5, 6), vigorous (16 min/day; group 2) or very vigorous (32 min/day; groups 3, 8, 9) intensity, 5 days/week Duration: the adaptation period started 3 weeks before tumor inoculation and the training period continued after inoculation for 6 weeks	Yes Domperidon: 10 mg/kg/day, orally, starting 3 days after inoculation Bromocriptine: 10 mg/kg/day, orally, starting 3 days after inoculation	Metastases ratio (relative lung metastasis size, % of non-exercise control) Lung metastases ratio (the ratio of total size of metastatic foci to the size of a whole lung)	The mean lung metastases ratio was reduced in the moderate exercise group (group 1) and increased in the vigorous (group 2) and very vigorous (group 3) exercise groups, compared with the non-exercise group (group 4) Moderate exercise + domperidone (group 6) increased the lung metastasis compared with moderate exercise alone (group 5), indicating that the suppressive effect of moderate swimming on liver cancer is mediated by D2 receptor Very vigorous exercise + bromocriptine (group 9) reduced the lung metastasis compared with very vigorous exercise alone (group 8), showing that D2 receptor agonist canceled the tumor-enhancing effect of very vigorous swimming

Table 1 (continued)

Authors (year)	Main sample characteristics and study groups	Tumor or metastasis type	Animal model	Exercise intervention	Treatment	Outcomes	Main results
	n = 6 9. Very vigorous exercise + bromocriptine *n* = 6 (another mouse model was used for purposes other than studying metastasis development)						
Zhang et al. [38]	Male 7 weeks old Study groups: 1. Exercise *n* = 12 at the start (6 analyzed) 2. Herbal compound *n* = 12 at the start (6 analyzed) 3. Exercise + herbal compound *n* = 12 at the start (6 analyzed) 4. Non-exercise + non-herbal compound *n* = 12 at the start (6 analyzed)	Hepatocarcinoma	Subcutaneous injection of 5×10^6 Hepa1–6 murine liver cancer cells (subcutaneous tumor model) and intravenous injection of 3×10^6 Hepa1–6–GFP tumor cells into the tail vein of C57BL/6 mice (experimental lung metastasis model) Moment of injection: before beginning the 6-week exercise or non-exercise period	Voluntary swimming: there was an adaptation period (trained mice swam 5 min in the first week, 6 min/day in the second week, and 8 min/day in the third week) followed by a training period at 8 min/day (moderate intensity), 5 days/week Duration: Adaptation period: 3 weeks (before injection) Training period: 6 weeks (after injection)	Yes Chinese herbal medicine formula *Songyou Yin*: 0.2 ml (4 g/kg/day per mouse) *via* oral gavage after tumor cell inoculation for 6 weeks	Lung metastases ratio (the ratio of total size of metastatic foci to the size of a whole lung)	Compared with the non-exercise + non-herbal compound group: (*i*) lower lung metastases ratio (− 54.5%) in the exercise + herbal compound group; (*ii*) 35.6% in the herbal compound group; and (*iii*) 34.3% in the exercise group

Abbreviation: *ASGM1*, anti-asialo GM1; *CXCR4*, C-X-C chemokine receptor type 4; *CXCL12*, C-X-C motif ligand 12; *DMEM*, Dulbecco's modified Eagle's medium; *FABP4*, fatty acid-binding protein 4; *GFP*, green fluorescent protein; *HGFR*, hepatocyte growth factor receptor; *HIF-1α*, hypoxia-inducible factor-1 alpha; *luc*, luciferase; *MMTV*, mouse mammary tumor virus promoter/enhancer; *MNU*, 1-methyl-1-nitrosourea; *N/A*, data not available; *N/R*, exact number of mice assessed was not clearly/consistently reported by the authors; *PBS*, phosphate-buffered saline; *PyMT*, polyoma middle T oncoprotein; *TRAMP*, transgenic adenocarcinoma of mouse prostate; *Tg*, transgenic; *WT*, wild type; ➔, followed by

Fig. 2 Analysis of SYRCLE's Tool Risk of bias [20]

[26]. No meta-analysis could be performed for this outcome because studies did not provide data using the same measure.

3.3.5 Metastases ratio

Two studies assessed the metastases ratio [37, 38]. Both studies observed that the combination of exercise and treatment (bromocriptine or herbal compound) decreased the mean of lung metastases ratio compared with the control group, which was also treated. By contrast, one of these studies reported that, whereas moderate-load exercise (8 min of swimming) significantly decreased the metastases ratio, the opposite trend was observed with higher loads of exercise (32 min of swimming) [37]. No meta-analysis could be performed for this outcome because the minimum number of studies required (≥ 3) was not achieved.

4 Discussion

The aim of this systematic review and meta-analysis was to assess the effects of physical exercise on different markers of

Table 2 Outcomes assessed in the present systematic review and meta-analysis

Outcome	Assessment method	Number of studies in the systematic review	Number of mice in the systematic review		Risk of bias			Number of studies in the meta-analysis	Number of mice in meta-analysis		Meta-analysis results
			Exercise	Non-exercise	Low	Unclear	High		Exercise	Non-exercise	
Incidence	Number of mice with metastasis	8	378	217	32%	53%	15%	7	291	188	OR = 0.64 (0.10, 4.12); $p = 0.64$ Publication bias ($p = 0.58$) $I^2 = 83\%$; $Q = 35.73$; $p < 0.001$
Number of metastatic foci	Counting the number of foci	11	311–317	293–299	32%	48%	20%	6	233	231	ES = − 3.18; (− 8.32, 1.97); $p = 0.23$ Publication bias ($p = 0.23$) $I^2 = 69\%$; $Q = 37.83$; $p < 0.001$ Subanalysis: ES = − 2.01 (− 5.64, 1.63); $p = 0.28$ Publication bias ($p = 0.47$) $I^2 = 1\%$; $Q = 4.03$; $p = 0.40$
Metastasis weight	Weighing the metastasis tissue	4	74	71	40%	46%	14%	3	54	61	ES = − 0.03 (− 0.10, 0.04); $p = 0.41$ Publication bias ($p = 0.93$) $I^2 = 46\%$; $Q = 5.56$; $p = 0.14$
Radioactive cell retention	% Radioactivity retention in a tissue	4	199	105	30%	50%	20%	N/A	N/A	N/A	Variable not meta-analyzed (< 3 studies using the same assessment method)
Metastases ratio	The total size of metastasis tumor foci/total size of the whole tissue	2	42	12	33%	38%	29%	N/A	N/A	N/A	Variable not meta-analyzed (< 3 studies using the same assessment method)

Abbreviation: *ES*, effect size; *N/A*, not available; *OR*, odds ratio

	Sample size	Measure (CI)	Weight %	P-value
Alvarado et al. (33)	21	0.18 (0.01; 4.24)	12.35%	0.29
Hoffman-Goetz et al. (40)	119	0.97 (0.46; 2.04)	18.54%	0.94
Hoffman-Goetz et al. (34)	85	44.70 (8.45; 236.51)	16.58%	<0.01
Jadeski & Hoffman-Goetz (26)	38	0.01 (0.01; 0.19)	13.12%	<0.01
MacNeil & Hoffman-Goetz (30)	79	1.51 (0.24; 9.51)	16.10%	0.66
MacNeil & Hoffman-Goetz (24)	94	2.53 (0.05; 130.26)	10.31%	0.64
Uhlenbruck & Order (29)	43	0.04 (0.01; 0.69)	13.01%	0.03
Synthesis	479	0.64 (0.10; 4.12)	100%	0.64

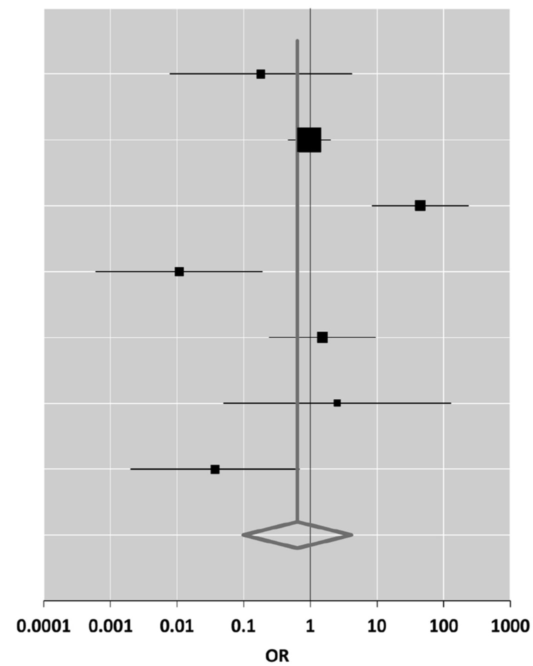

Fig. 3 Effects of physical exercise on metastasis incidence. Abbreviation: CI, confidence interval; OR, odds ratio

metastasis incidence and severity in animal cancer models. Our main finding was that exercise does not have overall effects—beneficial or harmful—on any of the analyzed parameters. However, it must be highlighted that the wide methodological heterogeneity observed between studies (e.g., different tumor models and exercise interventions, and different methods to evaluate tumor metastasis) might have partly confounded these findings.

Although no consistent benefits were observed in the present study, there is evidence suggesting that exercise may exert beneficial effects on cancer [17, 49], reducing tumor volume or attenuating its growth [32, 35, 46]. For instance, Goh et al.

	Sample size	Measure (CI)	Weight %	P-value
Hoffman-Goetz et al. (40)	119	-0.70 (-4.58; 3.18)	14.14%	0.72
MacNeil & Hoffman-Goetz (24)	39	-11.00 (-24.87; 2.87)	7.26%	0.12
MacNeil & Hoffman-Goetz (24)	39	0.57 (-15.24; 15.24)	6.54%	1.00
MacNeil & Hoffman-Goetz (24)	38	-11.00 (-24.90; 2.90)	7.25%	0.12
MacNeil & Hoffman-Goetz (24)	18	15.00 (-44.61; 74.61)	0.71%	0.62
MacNeil & Hoffman-Goetz (24)	18	-52.00 (-107.71; 3.71)	0.81%	0.07
Pedersen et al. (32)	19	-27.04 (-38.43; -15.65)	8.77%	<0.01
Smeda et al. (23)	18	13.20 (1.97; 24.43)	8.87%	0.02
Tsai et al. (28)	19	-2.13 (-7.58; 3.32)	13.11%	0.44
Tsai et al. (28)	19	3.58 (-0.99; 8.15)	13.71%	0.13
Yan & Demars (43)	58	-2.00 (-13.10; 9.10)	8.96%	0.72
Yan & Demars (43)	60	0.09 (-9.80; 9.80)	9.87%	1.00
Synthesis	464	-3.18 (-8.32; 1.97)	100%	0.23

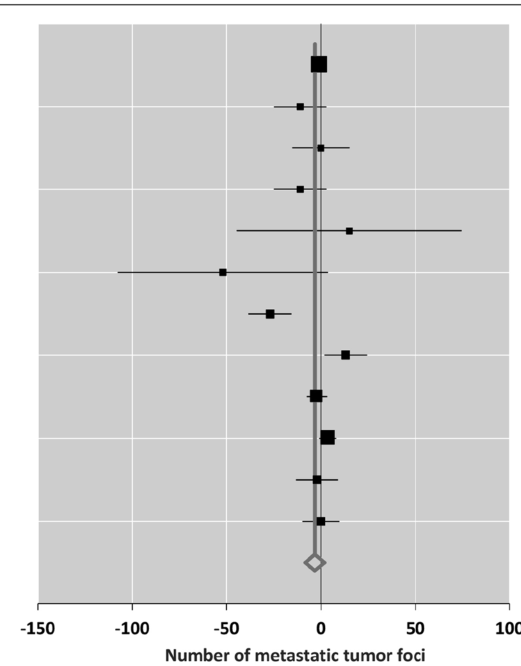

Fig. 4 Effects of physical exercise (i.e., pooled mean differences between groups: last evaluation at the end of the follow-up *minus* baseline data) on the number of metastatic tumor foci per mouse. Abbreviation: CI, confidence interval

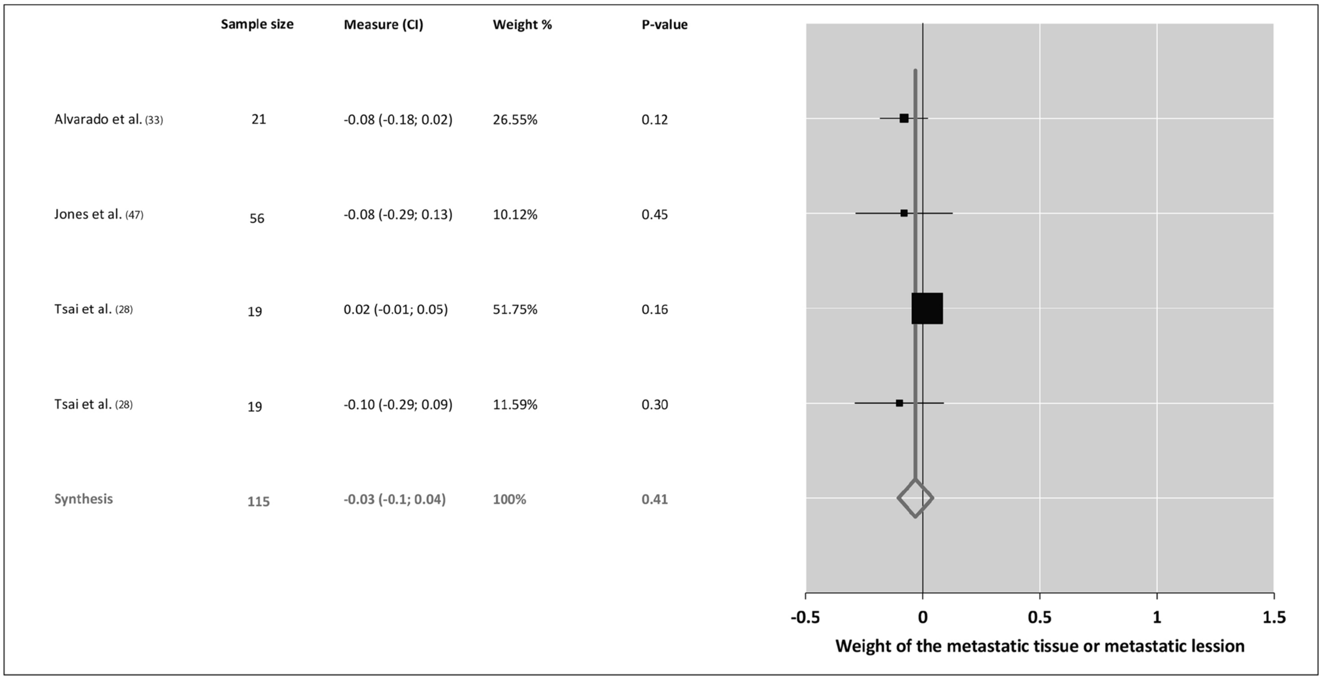

Fig. 5 Effects of physical exercise (i.e., pooled mean differences between groups: last evaluation at the end of the follow-up *minus* baseline data) on the weight (milligrams) of the metastatic tissue or metastatic lesion. Abbreviation: CI, confidence interval

[20] observed an inverse relationship between the levels of exercise (distance run) in mice and tumor growth in a spontaneous model of mammary cancer. Theriau et al. [50] postulated that exercise provides a protective effect over the tumor microenvironment, reducing the proliferation of a human breast adenocarcinoma cell line (MCF-7). Exercise can also influence the immune system, regulating Th1 and Th2 responses. The increase in Th1-type cytokine (interleukin [IL]-12 and interferon-γ) production and *Tbet* expression after physical activity might contribute to the increased immune response against tumors [51]. A recent mechanistic study found that exercise training (wheel running) in mice for 6 weeks induced the total pool of natural killer (NK) cells in nontumor-bearing mice (especially in spleen and bone marrow), and showed the specific involvement of these cells in the antitumorigenic effects of exercise [32]. On the other hand, a previous study found that chronic exercise reduced the development of lung metastases, as reflected by lower radioactivity retention (CIRAS 3 cells) in the lungs [45]. A subset of exercise-trained and nontrained (control) mice received treatment with ASGM1—an antibody which is routinely used to deplete NK cells *in vivo*. This treatment reduced radioactivity retention in all ASGM1-treated mice compared with nontreated animals, thereby providing overall support for a role of NK cells against metastasis development in all conditions. Nevertheless, lung radioactivity retention was lower—but the frequency of ASGM1-positive cells in splenocytes higher—in ASGM1-treated, exercise-trained mice than in ASGM1-treated, nontrained animals. Thus, the authors provided mechanistic support for a possible role of NK cell function to explain, at least partly, the potential effects of exercise against metastasis development.

Beyond the immune system, exercise can regulate several metabolic hormones including epinephrine, norepinephrine, glucagon, or insulin, controlling whole-body metabolism through AMP-activated protein kinase (AMPK) signaling pathways, which in turn decrease anabolic pathways and downregulate tumor growth [5]. Clearly, further research is needed to confirm the effects of exercise on tumor growth/metastasis, as well as to elucidate how manipulating training variables (i.e., intensity, volume or frequency) might influence these effects.

The physiological mechanisms by which exercise might shape metastatic processes remain to be fully elucidated [52]. As mentioned above, the tumor microenvironment, which can be influenced by lifestyle factors such as physical exercise, seems to exert an important influence on the metastatic process. In relation to tumor vascularization, exercise responses include changes in several circulating factors that play an essential role in systemic adaptation and promote neo-angiogenesis in other tissues [5]. Physical exercise promotes the release of bone marrow-derived dendritic cells to circulation, which can positively stimulate angiogenesis in the primary tumor and could, ultimately, promote colonization of tumor cells and metastasis [53, 54]. In turn, this increase of angiogenesis might also enhance oxygen supply to the tumor, which would promote the mobilization and the infiltration of immune cells. Together with NK cells, T cells (fundamentally

$CD8^+$ T cells) and to a lesser extent B cells are also mobilized to the circulation during a bout of acute exercise, as well as neutrophils, monocytes, and macrophages, in addition to anti-inflammatory cytokines (IL-1, IL-4, IL-6, IL-10, IL-11, IL-13), and immunosuppressive factors like tumor necrosis factor-α, transforming growth factor-β, and insulin-like growth factor-1, which can facilitate the control of tumor progression [52]. In this line, Hojman et al. [55] suggested that exercise leads to an increased release of catecholamines and myokines that increase tumor perfusion and oxygen supply, reduce intratumoral metabolic stress, and activate different signaling pathways involved in the prevention of metastasis like AKT/mTOR or AMPK. Increases in blood flow have also been proposed as a potential mechanism underlying the purported—but still unclear—benefits of exercise against metastasis. The development of premetastatic niches (i.e., the accumulation of circulating tumoral cells at sites distant to the primary tumor, where they remain quiescent until their activation days or even years later) is one of the most important phases of the metastatic process [4], with tumoral cells accumulating on the vascular wall due to both its narrowness and low blood flow rate. As exercise increases blood flow, it could potentially prevent the accumulation of tumoral cells on vessel walls and their extravasation to other tissues [56]. However, there is still a paucity of evidence on this topic.

While there are several physiological mechanisms supporting a potential influence of physical exercise in the metastasis prevention, no consistent effects on any parameters for evaluation of metastasis were observed in the present study. The spontaneous model—arguably the most realistic cancer model—was only used in two of the included studies, but with no negative or positive conclusions [20, 41]. In this line, Ashcraft et al. [16] propose a more suitable method to induce spontaneous metastasis that consists of making an incision in the primary tumor, liberating the tumor cells into the bloodstream, and analyzing the subsequent true metastasis lesions. Most studies used an induced model, injecting tumor cells directly into the animals and analyzing tumor progression. Although the induced model is a faster method than the spontaneous model, it only allows for the analysis of a single step of the metastatic cascade, for example, extravasation, dissemination, colonization, or overt metastasis. In this regard, it could be argued that the orthotopic induced model, in which tumor cells are injected directly into the target organ, is a robust model, as the formation of primary tumor growth and metastatic spread in a "natural" form might be a more realistic model. We found that the study using an orthotopic model showed/reported an increase in metastasis [23] or in the susceptibility to develop lung metastasis [36] with exercise. The majority of studies induced metastasis by injecting the tumor cells into mouse tail vein. Although this technique was the most popular, the results were unclear. It has been also proposed that exercise effects on metastasis could depend on the exercise load applied (i.e., volume [time] and/or intensity). In line with this hypothesis, Zhang et al. [37] observed that whereas moderate-volume exercise decreased the metastases rate, it was increased when mice trained with a 4-fold higher volume. It must be noted, however, that some authors found no benefits with moderate or intense training [28], whereas another study observed that exercise resulted in a trend to have higher levels of mRNA expression of fatty acid-binding protein-4, involved in tumor growth and metastasis development [36]. On the other hand, there is a need for more studies using *in vivo* bioluminescence assays to determine the kinetics of metastatic burden (as, for instance, measured by a metastasis free survival index). To the best of our knowledge, bioluminiscence methodology has only been used to monitor metastases in three exercise studies [27, 35, 39].

All studies have used interventions based on aerobic (forced or voluntary exercise) and thus whether muscle resistance ("strength") exercise also impacts cancer remains to be determined. This is an important consideration because, together with aerobic exercise, resistance training should form part of the exercise routine of all population groups including cancer patients, particularly for its effects against muscle atrophy, a prevalent side effect of chemotherapy and bed rest [7, 57]. Notably, there is evidence for a benefit on muscle strength in children with hematological tumors, particularly if resistance-based training is conducted in the hospital setting [58].

The findings of the reviewed studies are not easily extrapolable to pediatric cancer owing to the model of tumor or metastases used in the vast majority of them, as well as to the animal's age (i.e., usually several weeks, thereby not corresponding to early phases of life). Childhood cancer is different from that of adults in several biological aspects, and some specific murine models for childhood tumors based on specific molecular alterations have been developed, such as the transgenic models of medulloblastoma [59] and neuroblastoma [60]. An advantage of transgenic murine models of pediatric cancer over exogenous implants is that tumors develop "naturally" in an immunocompetent host, with the appropriate tissue microenvironment, recapitulating embryonic development, and reproducing tumor interactions with stroma and vasculature. In this context, the conditions that allow the interaction of the immune system with the developing tumor are also reproduced. In the case of pediatric tumors, this interaction is different from adult tumors. The latter develop by the accumulation of mutations over several years and are subject to the action of a postnatal immune system. By contrast, pediatric tumors are associated with a relatively small number of mutations that occur in a relatively short period of time. Further, they also occur during a period in which the immune system is acquiring its full potential. This fact is especially critical during the intrauterine period, when the first mutations in pediatric tumors occur. On the other hand, the mechanisms underlying tumor metastasis in pediatric tumors remain poorly understood compared with adult cancer [61], and some metastatic models of childhood cancer have been reproduced by systemic administration of tumor lines in syngeneic mice or xenografts in immunodeficient mice, notably medulloblastoma leptomeningeal metastases [48]. Future research on physical exercise and cancer (and particularly metastasis) development is thus needed using pediatric-specific models. In this regard, our group has recently initiated a study to analyze the impact of exercise on tumor immunology in a neuroblastoma model.

5 Conclusion

The results of the present systematic review and meta-analysis show that physical exercise has no overall influence on any marker of metastasis development in preclinical models. While these results suggest that exercise is not beneficial, they also indicate that it does not seem not to accelerate the metastatic process, providing additional support to the notion that exercise is safe in the context of cancer. It must be noted, however, that these findings might have been partly confounded by the methodological heterogeneity observed among the included studies, as most of them analyzed different tumor models (e.g., spontaneous *vs* induced), applied different exercise interventions (e.g., voluntary *vs* forced, moderate *vs* intense), and assessed different metastasis-related outcomes. More research is needed to understand the effects of physical exercise on the metastatic process (particularly in pediatric cancer), as well as to elucidate which factors (exercise load, subjects' characteristics, type of cancer, pharmacological treatment) can modulate these effects.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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