Effects of exercise-conditioned serum on cancer cell behaviour *in vitro*: a systematic review and meta-analysis

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Abstract

Background

Physical activity reduces cancer risk but the underlying mechanisms remain unclear. We meta-analysed the effects of acute exercise and exercise training on serum-stimulated cancer cell behaviours *in vitro*.

Methods

Systematic searches of PubMed, Scopus and SPORTDiscus up to February 2021 revealed 8 studies reporting 61 effect sizes eligible for meta-analysis. Standardised mean differences (SMDs) between exercise-conditioned serum and resting serum were pooled using random-effects models.

Results

Acute exercise-conditioned serum significantly reduced cancer cell growth (SMD -1.31, 95% CI -1.77 to -0.86, $p<0.0001$) and colony formation (SMD -2.33, 95% CI -2.66 to -2.00, $p<0.0001$) *in vitro*. These growth-inhibitory effects were sustained at 1 to 4 h (growth: SMD -1.56, 95% CI -2.47 to -0.64, $p=0.0008$; colony formation: -2.57, 95% CI -3.05 to -2.09, $p<0.0001$) and 24 h post-exercise (growth: SMD -2.62, 95% CI -4.18 to -1.05, $p<0.001$; colony formation: SMD -2.80, 95% CI -3.42 to -2.18, $p<0.0001$). In contrast, exercise training-conditioned serum had no effect on cancer cell growth *in vitro* (SMD -0.14, 95% CI -0.47 to 0.19, $p=0.41$).

Conclusion

Acute exercise promotes transient serological changes that suppress cancer cell growth and clonogenicity *in vitro*. Repetitive exposure to these factors may play an important role in reducing cancer risk.

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Key points

- Human serum conditioned by acute exercise, but not exercise training, suppresses cancer cell growth and colony formation *in vitro*.
- There is evidence that the anti-growth effect of acute exercise is sustained in serum collected up to 24 hours into recovery.
- Repetitive exposure to acute-exercise-induced transient alterations in serum factors may be an important mechanism to explain the relationship between regular physical activity and reduced cancer risk.

1. Introduction

Cancer is a leading cause of morbidity and mortality worldwide.¹ The pathophysiology of each cancer is unique and complex, yet it is clear that there is a modifiable component to the risk of developing many site-specific cancers, with accumulating evidence emphasising the key preventative role of regular exercise and physical activity.² Indeed, epidemiological studies indicate that regular physical activity is associated with a lower risk of developing at least 13 cancer types,³ with the strongest evidence for cancer of the colon, breast and endometrium.⁴ Moreover, regular physical activity after a cancer diagnosis is associated with a better prognosis and a reduced risk of disease recurrence in breast, colon, and prostate cancer patients.^{5,6}

The physiological and molecular mechanisms underlying the association between physical activity and reduced cancer risk remain poorly understood. Chronic reductions in systemic levels of biological risk factors for cancer, including sex hormones, metabolic hormones, and pro-inflammatory cytokines, are thought to be involved.^{7,8,9} However, these factors appear to be more heavily impacted by body composition rather than physical activity *per se* and, from this perspective, it is notable that recent evidence clearly demonstrates that physical activity is associated with a reduction in cancer risk independently of adiposity.¹⁰ Other lines of evidence indicate that transient but marked systemic changes that occur in the hours after each acute exercise bout may also play an important role.⁷ For example, acute exercise is known to mobilise, redistribute, and increase the activity of key cytotoxic immune cell populations including natural killer (NK) and T cells.^{11,12} Furthermore, several recent studies have demonstrated that incubation of cancer cells with serum collected after acute exercise can directly impact key hallmarks of cancer cell behaviour *in vitro*, such as cell proliferation.^{13,14} These findings are particularly interesting because they suggest that exercise may stimulate the release of ‘anti-carcinogenic’ proteins and metabolites into the circulation and identifying such factors may, therefore, ultimately lead to novel therapeutic targets for cancer treatment and prevention.

Two recent systematic reviews have attempted to synthesise the evidence for the effects of exercise-conditioned serum on cancer cell behaviour *in vitro*, but both have been limited in scope.^{15,16} For example, Orange et al.¹⁵ only examined the effect of serum collected immediately after acute exercise and limited their outcome measures to cell growth. Brown et al.¹⁶ examined a wider range of *in vitro* outcomes, including viability/growth, colony

formation, and migration, but limited their review to breast cancer cell lines only. Furthermore, they considered studies that had ‘mechanically loaded’ cells, and combined serum stimulated cell behaviour from both acute exercise and exercise training studies, together within the same meta-analysis, meaning their findings are challenging to interpret.¹⁶ In particular, the serological response to acute exercise is incredibly dynamic and complex and any impact of post-exercise serum upon *in vitro* cancer cell behaviour will be time sensitive,¹⁷ yet it remains unknown how long the effect persists for. Furthermore, acute exercise and chronic exercise training elicit two distinct systemic exercise responses, which are likely to have very different impacts on the serological environment and could be anticipated to differentially impact cancer cell behaviour. Therefore, the aim of the current systematic review and meta-analysis was to assess the separate effects of serum conditioned by acute exercise and exercise training on a comprehensive range of cancer cell behaviours *in vitro*, and, importantly, to map the time course of the effects post-exercise.

2. Methods

The current systematic review and meta-analysis was pre-registered with PROSPERO at www.crd.york.ac.uk/prospero/ on 13 August 2020 (registration number: CRD42020197773) and conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.¹⁸

2.1. Literature search and study selection

A literature search using the databases PubMed, Scopus and SPORTDiscus was conducted from inception to 1st February 2021. Title and abstract fields were searched using the following search terms: (*exercise* OR “*physical activity*” OR “*high intensity*” OR “*moderate intensity*” OR “*low intensity*” OR *swimming* OR *running* OR “*aerobic training*” OR “*endurance training*” OR “*anaerobic training*” OR “*resistance training*” OR “*strength training*”) AND (“*cancer cell**” OR “*tumor cell**” OR “*tumour cell**” OR “*malignant cell**” OR “*neoplastic cell**”). All database searches were limited to peer-reviewed articles published in the English language and no limits were set for year of publication. The electronic search was supplemented by a manual review of reference lists from relevant retrieved original articles and reviews to identify any additional studies. All search results were collated using Zotero

reference management software and duplicate studies removed. The remaining studies were then imported into the web-based application Rayyan for screening.¹⁹ Two reviewers (RNK and RSM) independently assessed the studies by title and abstract in phase one and by full text in phase two of screening against the pre-defined inclusion and exclusion criteria (outlined below). Any discrepancies in relation to study eligibility were discussed until consensus was reached.

2.2. Eligibility criteria

Human and animal experimental trials investigating the effect of serum conditioned by acute exercise or exercise training on key hallmarks of cancer cell behaviour *in vitro* were considered for inclusion. Acute exercise was defined as a single bout of exercise, and consistent with previous systematic reviews on exercise and cancer, exercise training was defined as repeated bouts of exercise for ≥ 4 weeks to evaluate chronic adaptations.²⁰ All types of exercise interventions were eligible (e.g. aerobic, anaerobic, resistance, combined). Studies that comprised of multiple interventions (e.g. study arms with dietary supplementation or restriction), were included, as long as it was possible to compare exercise only and inactive control conditions. Inclusion criteria for human studies required participants to be aged ≥ 18 years, and animal studies conducted in any species of mice or rats. Studies needed to assess the effect of exercise-conditioned serum on at least one cancer cell outcome measure (e.g. growth/viability, colony formation, apoptosis, migration) *in vitro*. Studies in which serum was collected either pre- and post-exercise (non-controlled), or from an exercise and resting control condition/group (controlled) were considered eligible. Studies were excluded if they utilised case, cohort, or cross-sectional designs, as were reviews, editorials, or other non-original articles.

2.3. Data extraction

Data extraction was performed by one reviewer (RNK) using a custom-designed Excel spreadsheet, and verified by a second reviewer (RSM). For all selected studies, the following data items were extracted: (1) Study ID (e.g. authors name, year of publication), (2) sample size, (3) participant characteristics (e.g. sex, age, health and physical activity status), (4) study design, (5) characteristics of the acute exercise / training protocol based on the FITT

(frequency, intensity, type and time) principle, (6) the timepoint of serum collection (post-exercise for acute studies, and following the final training session for chronic studies), (7) type of cancer and cell line(s) studied, (8) cell assay methods, and (9) cancer cell behaviour outcome data (mean and standard deviation). Where studies measured and presented cancer cell behaviour at multiple incubation time-points during an assay (e.g. 24, 48, 72 h), only data from the endpoint of the assay was extracted. If data was unavailable within the published text, in the first instance, corresponding authors of all selected studies were contacted by email to retrieve required data for meta-analysis outcomes. In total, authors from 8 studies were contacted²¹⁻²⁸ and we received raw data from 4 studies.^{21-23,26} If we did not receive a response from authors after 4 weeks, and where data were presented graphically, a reliable and valid digitizing tool (WebPlotDigitizer, v4.3) was used to convert data to numerical format.²⁹

2.4. Risk of bias

Risk of bias within the included studies was assessed using the OHAT Risk of Bias Rating Tool for Human and Animal Studies.³⁰ Two reviewers (RNK and RSM) independently rated the studies as having “definitely low”, “probably low”, “probably high” or “definitely high” risk of bias across the following six domains: selection, confounding, performance, attrition/exclusion, detection, and selective reporting. Any discrepancies were resolved by discussion in consensus meetings.

2.5. Data synthesis

Separate meta-analyses were carried out to quantify the effects of: (1) acute exercise on cancer cell growth; (2) acute exercise on cancer cell colony formation; and (3) exercise training on cancer cell growth. For remaining outcomes, as less than 3 studies were available, a narrative review of the results was presented in accordance with the Synthesis Without Meta-Analysis (SWiM) guidelines.³¹

The majority of studies included in the meta-analyses used a within-group design. Therefore, standardised mean differences (SMDs) between exercise-conditioned serum and resting serum within each study were calculated as Hedges’ g_{rm} to account for the correlation between repeated measures,^{32,33} where resting serum refers to pre-exercise serum in non-controlled studies and seated rest trial serum in controlled studies. To calculate Hedges’ g_{rm} , a

conservative correlation (r value) of 0.7 was assumed in line with guidelines by Rosenthal,³⁴ and sensitivity analyses were performed with $r = 0.5$ and $r = 0.9$. Where studies comprised of a between-group design ($n=1$), the standardiser used to calculate SMD was the pooled pretest standard deviation.³⁵

Negative SMDs reflect a reduction in cancer cell growth / colony formation and thus a beneficial effect of exercise. Effect sizes (g) were categorised as: trivial (< 0.2), small (0.2 to 0.49), moderate (0.5 to 0.79) and large (≥ 0.8).³⁶ Pooled effect size across studies was calculated using a random-effects model fitted with the Sidik-Jonkman estimator³⁷ as the degree of heterogeneity was expected to be large due to methodological variations. Heterogeneity was assessed using the I^2 statistic, whereby values of 0–40% = minimal heterogeneity, 30–60% = moderate heterogeneity, 50–90% = substantial heterogeneity, and 75–100% = considerable heterogeneity.³⁸ Exploration of the sources of heterogeneity was conducted using random-effects meta-regressions with age, health status (cancer survivor vs. apparently healthy), and cancer type as moderators. Publication bias was assessed by the visual inspection of funnel plots of SMD vs. standard error³⁹ and quantified by Egger's test of the intercept when at least 10 SMDs were included in the meta-analysis.⁴⁰

All statistical analyses were conducted using Stata software v.16 (StataCorp LLC, College Station, Texas). Statistical significance was set at $P \leq 0.05$. Data are presented as SMD and 95% CIs.

3. Results

3.1. Study selection

The search identified a total of 1929 articles, of which 744 were duplicates. After screening by title and abstract, 23 potentially relevant articles were identified for full text assessment. A total of 10 studies met the inclusion criteria and were included in the qualitative synthesis. Only two of these studies were conducted in animals,^{41,42} and therefore only human studies were included in the quantitative meta-analysis (Fig. 1). Some studies investigated both acute exercise and chronic exercise training (i.e. two separate studies) in one article^{21,23,25} and/or reported results for more than one of the outcome measures investigated in this review. As a result, some studies were included in multiple meta-analytical computations.

3.2. Study characteristics

In total, 10 studies with 240 participants were included in the review. Exercise-conditioned serum was used to stimulate 10 different cancer cell lines (MDA-MB-231, MCF-7, CaCO-2, LoVo, LNCaP, PC-3, AT-1, A459, H460, H1299) across four cancer types (breast, colon, prostate and lung). Details of the included studies investigating the effects of acute exercise ($n = 9$ studies) and exercise training ($n = 4$ studies) are presented in Table 1 and Table 2, respectively.

3.2.1. Acute exercise studies

One study assessed the effects of acute exercise-conditioned serum collected from mice,⁴² while the remaining eight studies were conducted in humans using breast,^{21,22,24,25} prostate,^{21,28} colon²³ and lung²⁷ cancer cell lines. Two studies recruited cancer patients/survivors,^{23,25} whereas the other six studies collected serum from an apparently healthy population.^{21,22,24,26-28} Participants mean age ranged from 21 to 66.9 years (mean = 35.2) with sample sizes ranging from 3 to 20 (43% female).

Acute exercise protocols consisted of moderate-intensity continuous aerobic exercise,^{22,24,26,28} high-intensity interval exercise (HIIE),^{23,27} combined exercise: HIIE with whole-body resistance,²⁵ and a combined session of aerobic incremental exercise, HIIE followed by exercise to exhaustion at a set workload (90% Pmax).²¹ The majority of studies utilised a single-group pre-post study design, with exception of one study which included a separate within-subject seated rest trial; however no 'pre / baseline' sample was taken in either the exercise or control condition.²⁴

3.2.2. Exercise training studies

The majority of studies utilised human serum ($n = 3$ studies),^{21,23,25} while one study assessed the effects of animal serum conditioned by moderate-intensity continuous training (MICT) on human and animal prostate cancer cells.⁴¹ In the human serum studies, five different cell lines across breast,^{21,25} prostate²¹ and colon²³ cancer types were investigated using serum derived from a healthy population²¹ and cancer survivors.^{23,25} Participants mean age ranged from 21 to 64.9 years (mean = 38) with sample sizes in the exercise group ranging from 12 to 37 (64% female).

Studies involved short (4-weeks),²³ short to medium (9-weeks),²¹ and longer term (6-month)²⁵ exercise training interventions. Exercise modalities consisted of high-intensity interval training (HIIT) on a cycle ergometer either alone^{21,23} or combined with whole-body resistance training.²⁵ Exercise frequency ranged from one to five sessions per week, each session lasted between 38 and 90-min. However, only one study reported exercise adherence and low levels of adherence were reported (mean attendance 66%).²⁵

3.3. Effect of acute exercise on cancer cell behaviour

3.3.1. Cell growth and colony formation

Of the eight studies included in the meta-analysis, all reported cell growth as an outcome measure, which generated a total of 32 effect sizes across multiple post-exercise time-points (Fig. 2A). Serum collected immediately post-exercise resulted in a large and statistically significant reduction in cancer cell growth compared with pre-exercise/resting serum (SMD -0.83, 95% CI -1.20 to -0.46, $p < 0.0001$). This growth-inhibitory effect was sustained at 1 to 4 h post-exercise (SMD -1.56, 95% CI -2.47 to -0.64, $p = 0.0008$) and 24 h post-exercise (SMD -2.62, 95% CI -4.18 to -1.05, $p < 0.001$). Overall, the effect of acute exercise-conditioned serum across all timepoints was large and statistically significant (SMD -1.31, 95% CI -1.77 to -0.86, $p < 0.0001$, Fig. 2A), however, considerable between-study heterogeneity was identified (Overall $I^2 = 98.80\%$).

Three studies were included in the meta-analysis on the effects of acute exercise on colony formation, resulting in a total of 23 effect sizes across multiple post-exercise time points (Fig. 2B). Overall, a large and statistically significant effect was found in favour of exercise (SMD -2.33, 95% CI -2.66 to -2.00, $p < 0.0001$, Fig. 2B). In accordance with the cell growth results, the reduction in cancer cell colony formation immediately post-exercise (SMD -1.71, 95% CI -2.06 to -1.36, $p < 0.0001$) was sustained at 1 to 4 h post-exercise (SMD -2.57, 95% CI -3.05 to -2.09, $p < 0.0001$) and 24 h post-exercise (SMD -2.80, 95% CI -3.42 to -2.18, $p < 0.0001$). However, heterogeneity was substantial to considerable across all timepoints (Overall: $I^2 = 89.24\%$; Post: $I^2 = 86.33\%$; 1 – 4 h post: $I^2 = 70.56\%$; 24 h post: $I^2 = 77.48\%$).

3.3.2 Apoptosis

Three studies assessed cancer cell apoptosis (i.e. cell death) utilising serum derived from exercising mice⁴² and humans^{23,28} (Table 1). In a mouse model, serum collected immediately post-exercise increased apoptosis through a 54% increase in caspase activity in the breast cancer cell line MCF-7 compared with resting serum ($P < 0.05$), while serum collected 2 h post-exercise had no significant effect. Conversely, in humans, no apoptotic effect was observed in colon and prostate cancer cell lines stimulated with serum collected at either immediately post-²³ or 2 h post-exercise²⁸ (Table 1).

3.4. Effect of exercise training on cancer cell behaviour

3.4.1. Cell growth

The meta-analysis on the effects of exercise training on cancer cell growth comprised of 6 effect sizes from three studies (Fig. 3). Overall, there was a trivial and non-statistically significant effect of exercise training on serum stimulated cancer cell growth (SMD -0.14, 95% CI -0.47 to 0.19, $p = 0.41$, $I^2 = 73.24\%$).

3.4.2. Colony formation and migration

Only one study assessed the effects of exercise training on cancer cell colony formation²¹ and migration⁴¹ (Table 2). In accordance with the cell growth data, serum collected after a period of exercise training (9 wks) had no effect on the clonogenic potential of cancer cells, compared to pre-training serum.²¹ Similarly, cancer cell migration was not significantly different between cells stimulated with serum taken from exercise trained (10-11 wks) versus sedentary rats.⁴¹

3.5. Risk of bias within studies

The risk of bias within individual studies is summarised in Fig. 4. The main risks identified were in the areas of appropriate comparison groups and confounding bias. All acute exercise human studies were considered at high risk of confounding bias as they did not include a control condition, or where a control condition was included,²⁴ the trial order was not counterbalanced with a VO₂max test performed the day prior to the resting control condition, and no ‘pre’

sample was collected meaning day-to-day variation in serological profile may have confounded the results. Similarly, with the exception of one study,²⁵ all training studies in humans were at high risk of confounding bias due to no control group. Three studies did not report reasons for incomplete outcome data or how it was addressed.^{41,27,25} The majority of statistical methods were deemed appropriate, however, two studies scored “probably high risk of bias” due to a very small sample size ($n=3$),²² or the use of pooled serum from all participants, which may have masked inter-individual differences.⁴²

3.6. Publication bias

Visual inspection of funnel plots revealed some asymmetry for acute exercise (Fig. 5) and exercise training studies (Fig. 6). Further analysis using Egger’s test was only performed for the acute exercise and cell growth meta-analysis, as using the test for other meta-analyses (with < 10 SMDs) would have been underpowered to distinguish chance from real asymmetry.³⁹ No significant publication bias was identified ($\beta = -3.02$, $p = 0.137$).

3.7. Sensitivity analyses

Sensitivity analyses demonstrated that calculating the standard deviation of the difference (SD_{diff}) assuming $r = 0.5$ or $r = 0.9$, instead of $r = 0.7$, did not alter the conclusions of any of the aforementioned meta-analyses. Acute exercise-conditioned serum still had a large and statistically significant effect on cancer cell growth using $r = 0.5$ (SMD -1.30, 95% CI -1.76 to -0.85, $p < 0.0001$) and $r = 0.9$ (SMD -1.21, 95% CI -1.61 to -0.80, $p < 0.0001$), and on cancer cell colony formation using $r = 0.5$ (SMD -2.35, 95% CI -2.69 to -2.01, $p < 0.0001$) and $r = 0.9$ (SMD -2.13, 95% CI -2.46 to -1.80, $p < 0.0001$). For exercise training-conditioned serum, a trivial and non-statistically significant effect on cancer cell growth was still observed using $r = 0.5$ (SMD -0.13, 95% CI -0.47 to 0.21, $p = 0.45$) and $r = 0.9$ (SMD -0.13, 95% CI -0.43 to 0.17, $p = 0.40$).

3.8. Meta-regressions

Following the initial meta-analyses, exploratory meta-regressions revealed that incorporating study characteristics (age, health status, cancer type) into the model as covariates had no influence on the degree of statistical heterogeneity identified ($I^2 = 98.80\%$) for the effect of

acute exercise-conditioned serum on cancer cell growth (Table 3). For acute exercise effects on cancer colony formation and training effects on cancer cell growth, there was insufficient data available to explore potential explanatory variables.

4. Discussion

This is the first systematic review to investigate the separate effects of serum conditioned by acute exercise and exercise training on a comprehensive range of cancer cell behaviours *in vitro*. There are several novel and important findings. Firstly, acute exercise-conditioned serum had a large and significant suppressive effect on cancer cell growth and colony formation *in vitro*, which both appear to be sustained for up to 24 hours into the post-exercise recovery period. In contrast, exercise training-conditioned serum had no effect on cancer cell growth *in vitro*. Finally, this review revealed that very limited human studies have examined the effects of exercise-conditioned serum on other key hallmarks of cancer cell behaviour such as cell apoptosis and migration. Collectively, these findings suggest that acute exercise promotes a transient anti-carcinogenic serological environment which, with repeated exposure, may constitute an important mechanism to explain previous epidemiological relationships between regular physical activity and reduced cancer risk.

Recently, a meta-analysis by Orange et al.¹⁵ reported a significant reduction in cancer cell growth *in vitro* with serum collected immediately post-exercise, compared to pre-exercise. The present review extends the scope of these acute exercise findings by demonstrating that the short-term anti-growth effects observed with growth / viability assays are also mirrored in clonogenic assays. The clonogenic assay allows for long-term changes in cell proliferation to be established and is widely accepted as the gold standard *in vitro* measure of cancer cell growth.⁴³ Therefore, the combined reduction in both growth and colony formation reported in the current meta-analysis provides stronger evidence of an anti-growth effect of acute exercise serum. Notably, the use of three-dimensional (3D) models to investigate clonogenic potential in two of the three studies included in this review,^{21,22} better recapitulates the 3D cellular environment *in vivo* and therefore has greater physiological relevance in comparison to conventional 2D cell growth models.⁴⁴ Although Brown et al.¹⁶ also found a suppressive effect of exercise on the clonogenic potential of breast cancer cells, they combined acute exercise and exercise training studies together within the same analysis. This can be considered a major limitation because acute exercise and chronic exercise training represent two distinct

physiological stimuli which promote substantially different serological responses. Indeed, our findings show a suppressive effect of acute exercise, but not exercise training (once the effects of the final training session have subsided), on serum-stimulated cancer cell growth. Thus, the current analysis provides more valid inferences of the effects of exercise-conditioned serum on clonogenicity, in addition to demonstrating that the beneficial effects of acute exercise on breast cancer cells also appear to be apparent in prostate²¹ and lung²⁷ cancer cell lines.

Another novel finding of the current review is that the anti-growth effects observed immediately after acute exercise appear to be maintained during both the short (1-4 h) and longer term (24 h) post-exercise recovery period. Identifying how long the effect persists post-exercise is important for two reasons. Firstly, in these *in vitro* serum incubation studies, cancer cells are continuously exposed to serum representative of a ‘snapshot’ in time for 24–96 hours and 7-18 days in growth and clonogenic assays, respectively. In contrast, in the *in vivo* physiological scenario, tumour cells are never going to be exposed to the same serum for a prolonged period of time, but instead, will be exposed to the highly dynamic serological profile as exercise-induced changes in various active components (e.g. proteins, metabolites, miRNAs) begin to return to baseline levels at various rates post-exercise and exert any downstream effects. Therefore, the physiological relevance of finding an anti-growth effect with serum collected immediately post-exercise only is questionable. However, if the serum remains anti-carcinogenic for at least 24 hours after exercise cessation, this presents a longer exposure window to interact with tumour cells and is therefore more likely to have a meaningful impact on tumour growth and development *in vivo*. Secondly, from a practical perspective, determining the duration of the effect can help to inform the optimal frequency of exercise required to preserve an anti-carcinogenic effect and accumulate to clinically meaningful outcomes. However, the smaller number of effect sizes at the later timepoints in our analysis should be noted. Furthermore, whilst this review provides preliminary evidence to suggest that the anti-growth effect is sustained for up to 24 hours post-exercise, no studies have taken serum samples beyond this timepoint to investigate when the effect subsides. The lack of effect of exercise *training*, with serum samples collected in the days after the final training session, demonstrates that the acute effects will eventually subside (as would be anticipated). Therefore, future research should aim to collect multiple post-exercise serum samples, including beyond the 24 h post-exercise timepoint, to comprehensively investigate the trajectory of the anti-carcinogenic effects of acute exercise.

The anti-growth effect of acute exercise-conditioned serum could be due to an inhibition of cell proliferation and/or the induction of cell apoptosis. The present review revealed that only a very limited number of studies have examined the effects of acute exercise-conditioned serum on cancer cell apoptosis. In a murine model, serum taken immediately post-exercise was found to promote a 54% increase in caspase activity (indicative of an increase in apoptosis), which had abated by 2 h post-exercise.⁴² However, based on the limited studies conducted to date, these findings do not appear to translate to humans, with no effect on apoptosis observed immediately post-²³ or 2 h post-exercise.²⁸ This tentatively suggests that the reductions in cell growth are primarily mediated through the suppression of proliferative signalling pathways, with changes in AKT-mTOR²⁷ and Hippo signalling^{21,24} potentially involved. However, given the paucity of studies identified, and the contrasting evidence from animal and human studies, our review highlights a clear need for future studies to examine cell proliferation, apoptosis and clonogenic potential simultaneously following a range of acute exercise bouts. Additionally, this review revealed that no studies have investigated the effect of acute exercise serum on cancer cell migration and invasiveness. This is an important evidence gap to address for cancer progression as tumor growth rate and metastatic potential do not always correlate.⁴⁵

Contrary to acute exercise, serum conditioned by exercise training had no effect on cancer cell growth *in vitro*. Although only represented with one study each, training-conditioned serum also appeared to have no effect on the clonogenic²¹ or migratory⁴¹ potential of cancer cells *in vitro*. The beneficial effect of acute exercise, but not exercise training, suggests that the anti-carcinogenic properties of serum must be attributed to the release of factors during the acute systemic exercise response. While the exact factors remain incompletely understood, noticeable candidates include muscle-derived myokines,^{46,47,42} catecholamines²⁴ and miRNAs.⁴⁸ Although exercise training does not appear to alter serum-stimulated cancer cell growth, it should be noted that training adaptations are clearly important in other ways. For example, an inverse association between cardiorespiratory fitness and cancer risk and mortality has been well documented.^{49,50} Therefore, the mechanisms underlying the beneficial effects of exercise training may act independently of serological changes. Furthermore, the number of studies included in the meta-analysis on training effects was small (n=3) so this inference should be treated with some caution. Notwithstanding, the findings herein provide support for the notion that repetitive exposure to transient increases in circulating factors after each acute exercise bout may have greater importance in mediating the

anti-carcinogenic effects of regular exercise through their cumulative effects, as opposed to chronic adaptations in basal levels of systemic risk factors at rest.⁷ From this, it can be speculated that a higher frequency of exercise may be advantageous over accumulating the same volume of exercise on fewer days per week.

Although this review provides a coherent picture of the current evidence-base on the effects of exercise-conditioned serum on *in vitro* cancer cell behaviour, the results represent data from low-quality study designs conducted to date. Concerns of risk of bias within individual studies were identified, particularly in the ‘confounding bias’ domain, with the most prominent limitation being a lack of non-exercising control. There is a clear need for future research to include a time-matched control condition and to control for potential confounding variables prior to the trial such as physical activity and diet. It is also important to note that substantial to considerable heterogeneity was identified in each meta-analysis. Investigations into sources of heterogeneity revealed limited influence of study characteristics such as participant age, health status and cancer type. However, these findings should be interpreted with caution given the small number of studies available per moderator and because they were largely based on between-study comparisons, which are less reliable than comparisons of subgroups within studies.⁵¹ Indeed, Hwang et al.²⁶ reported a significant reduction in prostate (LNCaP) cancer cell growth with post-exercise serum taken from older (60 – 73 y) but not younger (20 – 33 y) males, suggesting that age may be an important effect modifier.

Lack of data precluded grouping studies by exercise-level moderators e.g. exercise type and dose (intensity, session duration, frequency and length of training). Exercise is a diverse stimulus, which at different intensities, durations and modes confers very different serological responses.^{52,53,54} Therefore, it can be speculated that the diversity of the exercise protocols included in this review would differentially affect serum-stimulated cancer cell behaviour and explain a great proportion of the heterogeneity. Although a recent meta-analysis by Soares et al.⁵⁵ concluded that acute high-intensity exercise resulted in a larger suppression of serum-stimulated cancer cell growth compared with moderate-intensity exercise, the data presented did not support this conclusion for several important reasons. Firstly, the 95% confidence intervals for the pooled effect of high- and moderate-intensity exercise on cell growth overlapped (i.e., suggesting no difference), moreover, the analysis itself was flawed with identical effect sizes entered into the random-effects model multiple times per study. As shown in our meta-analysis, and in previous meta-analyses,^{15,16} the effect sizes across different assays, cell lines, populations and post-exercise timepoints within each study are not the same.

Secondly, studies included in the analysis of moderate-intensity exercise actually involved a combination of resistance exercise and high-intensity interval exercise (see Dethlefsen et al.²⁵). Finally, the analysis did not appear to control for participant characteristics and the analysis of moderate-intensity exercise included a study where age was suggested to be an important modifier of the effect.²⁶ In other words, the lower pooled effect size with moderate-intensity compared with high-intensity exercise may in part be an artefact of participant characteristics rather than suggesting an effect of exercise intensity. Taken together, it is not yet possible to make conclusions on the effect of exercise intensity on cancer cell growth *in vitro*. Indeed, to date, no studies have directly compared the effects of different exercise characteristics on cancer cell behaviours, making this a key area for future research, both from the perspective of exercise prescription and to elucidate the underlying mechanisms.

The current exercise recommendations to reduce cancer risk are consistent with the broader physical activity guidelines for adults of at least 150 to 300 min·wk⁻¹ of moderate-intensity, or 75 to 100 min·wk⁻¹ of vigorous intensity aerobic activity, or an equivalent combination of the two, in addition to muscle-strengthening exercises on 2 d·wk⁻¹.^{56,57} Notably, all studies identified in this review on moderate-intensity continuous exercise have involved prolonged exercise durations (≥ 1 hour).^{22,24,26,28,41,42} Therefore, an important gap for future research to address is whether lower exercise doses, commensurate to current exercise recommendations and more feasible for cancer survivors and at-risk populations (e.g. 30-min moderate-intensity exercise bouts), have a beneficial effect on cancer cell behaviour *in vitro*. Additionally, while one study reported acute benefits of combined exercise,²⁵ it is currently unknown if aerobic and resistance exercise exert a synergistic effect, and if resistance exercise alone can also have a growth-inhibitory effect on cancer cells.

5. Conclusion

In conclusion, the results of this review demonstrate that the serological changes following acute exercise have a direct suppressive effect on cancer cell growth and colony formation *in vitro*, but there are limited studies on apoptosis and migration. Exploration into the time course of the effects suggests that the anti-growth benefits may persist for up to 24 hours after exercise cessation. Importantly, serum conditioned by exercise training had no effect on cancer cell growth *in vitro*. Collectively, these findings suggest that the acute serological response to exercise may play an important role in reducing cancer risk. However, this review also reveals

a need for further high-quality studies with controlled experimental designs in order to address the high risk of bias present in previous work.

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Figure legends

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram of study selection.

Figure 2. Random-effects model forest plot on the effect of acute exercise-conditioned human serum on (A) cancer cell growth and (B) colony formation. Studies are grouped together by serum collection timepoint: (1) immediately post-exercise; (2) 1 to 4 hours post-exercise; and (3) 24 hours post-exercise. The pooled Hedges' g for each subgroup is presented as a red diamond. The pooled Hedges' g when analysing all exercise-conditioned serum combined (Fig A: 32 SMDs; B: 23 SMDs) is presented as a green diamond.

Figure 3. Random-effects model forest plot on the effect of exercise training-conditioned human serum on cancer cell growth.

Figure 4. Heat map of the Risk of Bias (RoB) within included studies assessed using the Office of Health Assessment and Translation (OHAT) RoB tool. Risk of bias for each question is indicated by colour: definitely low RoB (++ , dark green); probably low RoB (+, light green); probably high risk RoB (-, orange); definitely high RoB (--, red). NR = not reported; N/A = not applicable i.e. question on RoB tool does not apply to study design.

Figure 5. Funnel plot of standardised mean difference (pre to immediately post-exercise) versus standard error for the meta-analysis of acute exercise-conditioned serum on (A) cancer cell growth and (B) colony formation.

Figure 6. Funnel plot for the meta-analysis of exercise-training conditioned serum on cancer cell growth.

Additional information

Authors' contributions

All authors contributed to the design of the review. RNK performed the searches and screening, data extraction and analysis, and wrote the initial draft of the manuscript. RSM also conducted the screening and contributed to the revised manuscript. All authors read and approved the final manuscript.

Data availability

Please contact the corresponding author for data requests.

Competing interests

The authors declare no conflict of interest.

Domains based on OHAT (2015)		Acute exercise studies										Exercise training studies			
		Baldelli et al. [21]	Dethlefsen et al. [25]	Dethlefsen et al. [24]	Devin et al. [23]	De Santi et al. [22]	Hojman et al. [42]	Hwang et al. [26]	Kurgan et al. [27]	Rundqvist et al. [28]		Baldelli et al. [21]	Dethlefsen et al. [25]	Devin et al. [23]	Opoku-Acheampong et al. [41]
Selection bias	Q1: Was administered dose or exposure level adequately randomised?	N/A	N/A	N/A	N/A	N/A	NR	N/A	N/A	N/A		N/A	-	N/A	+
	Q2: Was allocation to study groups adequately concealed?	N/A	N/A	N/A	N/A	N/A	NR	N/A	N/A	N/A		N/A	-	N/A	NR
	Q3: Did selection of study participants result in appropriate comparison groups?	-	-	-	-	-	N/A	-	-	-		-	N/A	-	N/A
Confounding bias	Q4: Did the study design or analysis account for important confounding and modifying variables?	--	--	--	--	--	N/A	--	--	--		--	N/A	--	N/A
Performance bias	Q5: Were experimental conditions identical across study groups?	N/A	N/A	-	N/A	N/A	+	N/A	N/A	N/A		N/A	++	N/A	+
	Q6: Were the research personnel and human subjects blinded to the study group during the study?	N/A	N/A	N/A	N/A	N/A	-	N/A	N/A	N/A		N/A	-	N/A	-
Attrition/Exclusion bias	Q7: Were outcome data complete without attrition or exclusion from analysis?	++	++	+	++	++	NR	++	-	++		++	-	++	-
Detection bias	Q8: Can we be confident in the exposure characterisation?	-	++	++	++	++	++	++	++	++		+	-	++	++
	Q9: Can we be confident in the outcome assessment?	+	+	+	+	+	+	+	+	+		+	+	+	+
Selective reporting bias	Q10: Were all measured outcomes reported?	++	++	++	++	++	++	++	++	++		++	++	++	++

Other bias

Q11: Were statistical methods appropriate?

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Table 1. Characteristics of acute exercise studies included in the systematic review.

No.	Study	n	Participants	Exercise protocol		Cancer type (cell line)	Serum time-point	Cancer cell measure		Results	
				Type	Duration/ Intensity			Measure	Assay, Incubation time	% change compared to resting serum	p value
Human studies											
1	Baldelli et al. [21]	30	Healthy sedentary F's (n = 12), 21 ± 0.8 y and M's (n = 18), 21 ± 1.4 y	Aerobic incremental and HIIE cycling	4 x 5-min @ 50%, 55%, 60%, 70% Pmax. 10 x 90-s @ 90% Pmax interspersed with 180-s @ 55% Pmax. TTE @ 90% Pmax.	Breast (MDA-MB-231) and Prostate (LNCaP)	Pre, post, 4h post, 24h post	Cell growth	Trypan Blue Exclusion, 48 h	MDA-MB-231 Post: -12.1, -15.7* 4h post: -24.0, -30.6 24h post: -24.9, -35.3 LNCaP Post: -13.8, -14.0 4h post: -21.8, -22.9 24h post: -22.8, -27.2	< 0.001 < 0.001 < 0.001 < 0.001 < 0.001 < 0.001
	Baldelli et al. [21]	30	Healthy sedentary F's (n = 12), 21 ± 0.8 y and M's (n = 18), 21 ± 1.4 y	Aerobic incremental and HIIE cycling	4 x 5-min @ 50%, 55%, 60%, 70% Pmax. 10 x 90-s @ 90% Pmax interspersed with 180-s @ 55% Pmax. TTE @ 90% Pmax.	Breast (MDA-MB-231) and Prostate (LNCaP)	Pre, post, 4h post, 24h post	Colony formation	Soft Agar, 18 days	MDA-MB-231 Post: -17.6, -10.1 4h post: -33.7, -18.2 24h post: -30.0, -15.6 LNCaP Post: -16.0, -14.4 4h post: -21.5, -20.3 24h post: -25.9, -25.8	< 0.001 < 0.001 < 0.001 < 0.001 < 0.001 < 0.001
2	Dethlefsen et al [25]	20	Breast cancer patients (F's) receiving adjuvant chemotherapy, 49.5 ± 7.3 y	Combined: resistance and HIIE cycling	2 h total - 30-min warm-up - 60-min whole-body resistance - 30-min HIT @ > 80% HRmax	Breast (MDA-MB-231, MCF-7)	Pre, post	Cell growth	CellTiter-Fluor Cell Viability, 48 h	MDA-MB-231 -13.0 MCF-7 -19.0	< 0.05 < 0.001
3	Dethlefsen et al. [24]	7	Healthy F's, 24.6 ± 1.0 y	MICT cycling	2 h @ 55% VO ₂ max	Breast (MDA-MB-231, MCF-7)	Seated rest trial, post-exercise	Cell growth	CellTiter-Fluor Cell Viability, 48 h	MDA-MB-231 -9.4 MCF-7 -9.2	< 0.001 < 0.05

4	Devin et al. [23]	10	Colon cancer survivors (M's), 66.9 ± 8.4 y	HIIE cycling	38-min total - 10-min warm-up @ 50-70% HRpeak - 4 x 4-min @ 85-95% HRpeak interspersed with 3-min active recovery	Colon (CaCO-2, LoVo)	Pre, post, 2h post	Cell growth	AlamarBlue, 72 h	CaCO-2 Post: -4.6 2h post: -3.3 LoVo Post: -5.1 2h post: +0.2	< 0.05 ≥ 0.05 < 0.05 ≥ 0.05
	Devin et al. [23]	10	Colon cancer survivors (M's), 66.9 ± 8.4 y	HIIE cycling	38-min total - 10-min warm-up @ 50-70% HRpeak - 4 x 4-min @ 85-95% HRpeak interspersed with 3-min active recovery	Colon (CaCO-2, LoVo)	Pre, post	Apoptosis	Annexin V, 72 h	CaCO-2 Post: -0.04 LoVo Post: +0.64	≥ 0.05 ≥ 0.05
5	De Santi et al. [22]	3	Healthy sedentary pre-menopausal F's, 43.3 ± 9.8 y	MICT running	65-min total - 20-min at 50%, 45-min @ 65% VO ₂ max	Breast (MDA-MB-231)	Pre, post, 2h post	Cell growth	CellTiter 96® AQueous Non-Radioactive Cell Proliferation, 72 h	Post: -3.0, -2.0 2h post: -6.0, -5.0	< 0.05 < 0.05
	De Santi et al. [22]	3	Healthy sedentary pre-menopausal F's, 43.3 ± 9.8 y	MICT running	65-min total - 20-min at 50%, 45-min @ 65% VO ₂ max	Breast (MDA-MB-231)	Pre, post, 2h post	Colony formation	Soft Agar, 18 days	Post: -8.46 2h post: -12.26	< 0.01 < 0.001
6	Hwang et al. [26]	22	Healthy M's young (20 - 33 y) (<i>n</i> = 12) and old (60 - 73 y) (<i>n</i> = 12)	MICT cycling	65-min total - 20-min at 50%, 45-min @ 65% VO ₂ max	Prostate (PC3, LNCaP)	Pre, post	Cell growth	AlamarBlue, 96 h	PC3 Young M's: +3.5 Old M's: -7.1 LNCaP Young M's: -0.7 Old M's: -5.7	≥ 0.05 ≥ 0.05 ≥ 0.05 < 0.01
	Kurgan et al. [27]	6	Healthy recreationally active M's, 21.8 ± 2.4 y	HIIE cycling	- 4-min warm up and 2-3 min cool down @ 70W - 6 x 1-min @ 90% WRmax interspersed with 1-min active recovery	Lung (A549)	Pre, post, 1h post, 24h post	Cell growth	Crystal Violet, 72 h	Post: -14.6 1h post: -13.4 24h post: -20.5	< 0.05 < 0.05 < 0.05

	Kurgan et al. [27]	6	Healthy recreationally active M's, 21.8 ± 2.4 y	HIIE cycling	- 4-min warm up and 2-3 min cool down @ 70W - 6 x 1-min @ 90% WRmax interspersed with 1-min active recovery	Lung (A459, H460, H1299)	Pre, post, 1h post, 24h post	Colony formation	Clonogenic, 7 days	A459 Post: -72.3 1h post: -60.2 24h post: -53.5 H460 Post: -57.7 1h post: -56.9 24h post: -56.0 H1299 Post: -60.1 1h post: -50.0 24h post: -55.0	< 0.001 < 0.001 < 0.001 < 0.001 < 0.001 < 0.001 < 0.001 < 0.001 < 0.001
8	Rundqvist et al. [28]	10	Healthy M's, 18 - 37 y	MICT cycling	60-min total - 20-min at 50%, 40-min @ 65% VO ₂ max	Prostate (LNCaP)	Pre, 2h post	Cell growth	Crystal Violet, 48 h	-20.7	≤ 0.05
	Rundqvist et al. [28]	10	Healthy M's, 18 - 37 y	MICT cycling	60-min total - 20-min at 50%, 40-min @ 65% VO ₂ max	Prostate (LNCaP)	Pre, 2h post	Apoptosis	Annexin V, 48 h	-7.3	> 0.05
Animal studies											
9	Hojman et al. [42]	10	Naval Medical Research Institute Mice, 10 wk old	Swimming	60-min	Breast (MCF-7)	Pre, post, 2h post	Cell growth	Unclear, 24 h	Post: -52.0 2h post: -11.0	< 0.001 > 0.05
	Hojman et al. [42]	10	Naval Medical Research Institute Mice, 10 wk old	Swimming	60-min	Breast (MCF-7)	Pre, post, 2h post	Caspase activity	Caspase-3/CPP32 Colorimetric, 24 h	Post: +54.0 2h post: -1.8	< 0.05 > 0.05

Abbreviations: n, sample size; M's, males; F's, females; HIIE, high-intensity interval exercise; MICT, moderate-intensity continuous exercise; TTE, time to exhaustion. * % change scores for the effects of acute exercise-conditioned serum investigated before and after a period of exercise training.

Table 2. Characteristics of exercise training studies included in the systematic review.

No.	Study	EX <i>n</i> (total <i>n</i>)	Study design	Participants	Training protocol				Cancer (cell line)	Cancer cell measure		Results	
					Type	Freq/week	Duration, Intensity	Length		Measure	Assay, Incubation time	% change compared to pre-training or control group	<i>p</i> value
Human studies													
1	Baldelli et al. [21]	30	NC	Healthy sedentary F's (<i>n</i> =12), 21 ± 0.8 y and M's (<i>n</i> =18), 21 ± 1.4 y	HIIT cycling	3-4	Unclear	9 wks.	Breast (MDA-MB- 231) and Prostate (LNCaP)	Cell growth	Trypan Blue Exclusion, 48 h	MDA-MB-231 -3.6	> 0.05
												LNCaP +4.8	> 0.05
	Baldelli et al. [21]	30	NC	Healthy sedentary F's (<i>n</i> =12), 21 ± 0.8 y and M's (<i>n</i> =18), 21 ± 1.4 y	HIIT cycling	3-4	Unclear	9 wks.	Breast (MDA-MB- 231) and Prostate (LNCaP)	Colony formation	Soft Agar, 18 days	MDA-MB-231 +3.2	> 0.05
												LNCaP -1.3	> 0.05
2	Dethlefsen et al. [25]	37 (74)	C	Breast cancer survivors F's, 46 ± 9.6 y	Combined: resistance, HIIT cycling	1	90-min total HIIT: 30-s @ max to 6-min @ 80-90% HRmax with 1:2 to 3:1 exerc:recovery ratio. Resistance: 3 x 8-10 reps @ 70 - 90% 1RM	6 mos.	Breast (MDA-MB- 231, MCF-7)	Cell growth	CellTiter-Fluor Cell Viability, 48 h	MDA-MB-231 +0.5	> 0.05
												MCF-7 +28.0	> 0.05
3	Devin et al. [23]	10	NC	Colon cancer survivors M's, 64.9 ± 6.0 y	HIIT cycling	3	38-min total - 10-min warm- up @ 50-70% HRpeak, - 4 x 4-min @ 85-95% HRpeak interspersed with 3-min active recovery	4 wks.	Colon (CaCO-2, LoVo)	Cell growth	AlamarBlue, 72 h	CaCO-2 -3.6	> 0.05
												LoVo -2.0	> 0.05

Animal studies

4	Opoku-Acheampong et al. [41]	16 (30)	C	Copenhagen (immune-competent) healthy rats (<i>n</i> =20) Nude (immune-deficient) healthy rats (<i>n</i> =10)	MICT treadmill running	5	60-min moderate-intensity	10-11 wks.	Prostate (AT-1 in Copenhagen rats, PC-3 in Nude rats)	Cell growth	CellTiter 96® AQueous Non-Radioactive Cell Proliferation (MTT), 96 h	AT-1 -37.3 PC-3 +39.5	≤ 0.05 > 0.05
	Opoku-Acheampong et al. [41]	4 (8)	C	Nude (immune-deficient) tumor-bearing rats	MICT treadmill running	5	60-min moderate-intensity	10-11 wks.	Prostate (PC-3)	Cell growth	CellTiter 96® AQueous Non-Radioactive Cell Proliferation (MTT), 96 h	+5.8	> 0.05
	Opoku-Acheampong et al. [41]	16 (26)	C	Copenhagen (immune-competent) healthy rats (<i>n</i> =20) Nude (immune-deficient) healthy rats (<i>n</i> =10)	MICT treadmill running	5	60-min moderate-intensity	10-11 wks.	Prostate (AT-1 in Copenhagen rats, PC-3 in Nude rats)	Cell migration	(1) Cell Exclusion Zone, 96 h (2) Transwell Migration, 24 h	AT-1 (1): +7.0 (2): -7.0 PC-3 (2): -8.0	> 0.05 > 0.05 > 0.05
	Opoku-Acheampong et al. [41]	4 (8)	C	Nude (immune-deficient) tumor-bearing rats	MICT treadmill running	5	60-min moderate-intensity	10-11 wks.	Prostate (PC-3)	Cell migration	Transwell Migration, 24 h	+27.0	> 0.05

Abbreviations: NC, non-controlled; C, controlled; EX, exercise group; *n*, sample size; M's, males; F's, females; HIIT, high-intensity interval training; MICT, moderate-intensity continuous training.

Table 3. Results of meta-regressions performed to identify possible sources of statistical heterogeneity for the effect of acute exercise-conditioned serum on cancer cell growth.

Covariate	Coefficient (95% CI)	<i>p</i> value	<i>I</i> ²	<i>R</i> ²
Age	0.03 (0.01 to 0.06)	0.01	98.52%	16.86%
Health status (Cancer survivor, Apparently healthy)	-0.93 (-2.05 to 0.19)	0.10	98.73%	5.41%
Cancer type (Breast, Prostate, Other)	0.48 (-0.08 to 1.05)	0.09	98.71%	6.27%