Effect of exercise-conditioned human serum on the viability of human cancer cell cultures: A systematic review and meta-analysis

Carlos M. Soares[1,2*], Ana M. Teixeira[1], Hugo Sarmento[1], Fernanda M. Silva[1], Marcio C. Rusenhack[1], Meirielly Furmann[3], Paulo R. Nobre[1], Miguel A. Fachada[1], Ana M. Urbano[4] and José P. Ferreira[1]

[1] University of Coimbra – Research Unit for Sport and Physical Activity (CIDAF, UID/PTD/04213/2019), Faculty of Sport Sciences and Physical Education, 3040-248 Coimbra, Portugal; carlos.soares@student.fcdef.uc.pt (C.M.S.); ateixeira@fcdef.uc.pt (A.M.T.); hugo.sarmento@uc.pt (H.S.); geral.fernandasilva@gmail.com (F.M.S.); marciocascante@gmail.com (M.C.R.); prnobre@fcdef.uc.pt (P.R.N.); miguelfachada@fcdef.uc.pt (M.A.F.); jpferreira@fcdef.uc.pt (J.P.L.)
[2] Higher Institute of Educational Sciences of the Douro, 4560-708 Penafiel, Portugal.
[3] Universidade Estadual do Centro Oeste, 84505-677 Irati Paraná, Brasil; meiry.furmann2@gmail.com (M.F.)
[4] Molecular Physical Chemistry R&D Unit, Center of Investigation in Environment, Genetics and Oncobiology (CIMAGO), Department of Life Sciences, University of Coimbra, 3000-456 Coimbra, Portugal; amurbano@ci.uc.pt (A.M.U.)
[*] Correspondence: soares.cm.researcher@gmail.com;

ABSTRACT:

Numerous epidemiological studies have shown the existence of a relationship between exercise and a reduced risk of different types of cancer. In vitro studies have identified a direct effect of exercise-conditioned human serum on cancer cell lines of the lung, breast, prostate, and colon. The aim of this systematic review with meta-analysis (SRM) was to estimate the magnitude of the effect that exercise-conditioned human serum produced on the viability of human cancer cell cultures. The design followed the PRISMA guidelines and the TREND statement to assess the quality of information (QoI) in each study. Nine in vitro studies were included in the SRM, involving a total of nine cancer cell lines and serum from 244 individuals from different countries, including namely healthy sedentary individuals, at risk of prostate cancer individuals and cancer patients, with ages ranging from 18 to 73 years. The impact of acute exercise-conditioned human serum on the viability of cancer cell cultures was analysed by a variety of assays, using pre-exercise human serum for comparison purposes. Globally, cultures of cancer cell lines exposed to human serum conditioned by acute exercise of various intensities exhibited a reduced viability, when compared with control cultures, with an overall effect size (ES) of -1.126 (95% CI; -1.300 to -0.952; $p < 0.001$). When the analysis only included human serum conditioned by acute high-intensity exercise, the effect became more pronounced (ES -1.350; -1.522 to -1.179 (95% CI); $p < 0.001$). These results are in line with the hypothesis that changes in human serum induced by exercise might play a role in the beneficial effects of physical activity in cancer prevention and management and that these effects depend on exercise intensity.

Keywords: physical activity; anticancer activity; cancer prevention; cancer management; cellular studies; tumor

Corresponding author:
soares.cm.researcher@gmail.com

1. Introduction

Cancer is a major public health problem worldwide, with a significant impact on people's quality of life and health costs (11, 53). Significantly, a large proportion of new cancer cases and cancer deaths are preventable by eliminating or reducing exposure to environmental risk factors and/or modifying lifestyles, as revealed by recent studies (13, 63). In terms of lifestyle factors, consistent data from epidemiological studies suggest that physical activity reduces risk for various cancers and improves cancer survival rates (24, 25, 38, 45, 48). According to the 2018 Physical Activity Guidelines Advisory Committee (PAGAC), there is strong evidence that increased physical activity reduces the risk of several major types of cancer by 10% to 21%, including breast, colon, endometrium, bladder, stomach, oesophagus, and kidney cancers (42), moderate evidence for a reduced risk of lung cancer 21%-25% (25, 42), and limited evidence for a reduced risk of prostate cancer (42). On the contrary, an increased risk of melanoma has been observed in some cases (48). The evidence for an association between physical activity and survival after cancer is more limited, although emerging data suggest a 40% to 50% reduction in mortality from breast, colon and prostate cancer (4, 42), as well as a decrease in disease recurrence (4). Epidemiological evidence also suggests that the benefits of physical activity in terms of cancer prevention, particularly in the case of lung cancer, are also present in smokers (12). Of note, levels of cardiorespiratory fitness and the primary incidence of various forms of cancer have been found to be inversely correlated (3). Unfortunately, the optimal amount of physical activity (namely in terms of frequency, type, intensity, and duration) required to produce the above-mentioned benefits is still unknown (48).

It is believed that the effects of physical activity on prevention are mediated by multiple systemic responses, namely by reducing inflammation and improving immune system function and surveillance (30, 62). In addition, physical activity can also have a direct effect on cancer cells. In order to understand whether and to which extent cancer cells are affected by exercise, i.e., by the subset of physical activity that is planned, structured, repetitive, and performed with the purpose of improving or maintaining physical fitness, several research groups investigated the impact of acute

and/or chronic exercise-conditioned serum from animals or humans in human cancer cell lines (5-7, 15-17, 31, 33, 37, 39, 46, 50, 55, 57). Although using different endpoints, all of these studies ultimately assessed the effects of acute and/or chronic exercise on cell culture viability under a variety of experimental contexts, which were then taken as a measure of transformation status. Endpoints included, but were not restricted to, number of live or viable cells in culture, ability for anchorage-independent growth, clonogenic potential, and levels of apoptosis. These studies differed in various other aspects of their design, such as in number and characteristics of participants recruited, type and intensity of the exercise intervention, time points of serum collection, cell line and exposure regimen (serum concentration and duration of exposure). Also, in some of these studies, the intervention involved not only exercise, but a combination of exercise and other factors (e.g., dietary modification). While most studies showed that exercise-conditioned serum affected cancer cell culture viability, the magnitude of the effects varied across the studies, even for those employing a similar design or the same cancer cell lines (33, 50). Altogether, the results suggest that the observed effects are dependent on exercise intensity, suggesting also that they might be modulated by other factors.

One major aim of this systematic review with meta-analysis was to quantify the magnitude of the effect of acute exercise-conditioned human serum on the viability of human cancer cell cultures and to verify if this effect was dependent on the intensity of the exercise performed. Our hypothesis is that human serum conditioned by acute exercise reduces the viability of cancer cells and that this effect is dependent on exercise intensity, when compared to the corresponding pre-exercise serum.

2. Methods

2.1 Search Strategies

Search strategies followed the PRISMA guidelines (44) and were based on the following descriptor terms and keywords defined by the authors and indexed in the Medical Subject Headings (MeSH, U.S.National Library of Medicine, 8600 Rockville Pike, Bethesda, MD 20894): ((exercise* OR "physical activity" OR sport* OR training OR "resistance training" OR "aerobic training" OR "high intensity interval training" OR "physical exercise") AND (neoplasm* OR tumor* OR malignant* OR cancer* OR carcinoma) AND (cell* OR "cell culture" OR "*in vitro*")). This combination was used to search the following academic journal databases: PubMed, Web of Science, SPORTDiscus and Scopus. The advanced options were carried out using the filter by title into each database. Research procedures were carried out in July 2020.

2.2 Data Extraction

Data from search were imported into EndNote X7 (Thomson Reuters EndNote X7). After this, the following screening procedures were implemented to select the relevant articles for the study: (a) all duplicates were removed; (b) articles whose title and abstract did not provide enough information on the topic were removed; (c) articles whose full texts did not meet the inclusion criteria were removed; (d) quality of information (QoI) from each study was checked using the TREND statement guidelines (14). If the total score for TREND items and sub-items was below 50% of the maximum score, publications were excluded from the study due to lack of QoI. Additionally, if the article's full text did not provide complete data, authors were contacted by e-mail requesting the missing information. If no response was obtained, the article was excluded from the study.

2.3 Criteria for Study Selection

The inclusion criteria used to select the articles for the present study were: a) the effects of exercise-conditioned serum were assessed *in vitro* using human cancer cell lines; b) the exercise-conditioned serum was of human origin; c) exercise was the only intervention, i.e., no combined interventions (e.g., diet and exercise) were involved; d) written in English.

2.4 Methodological Design

The PRISMA Statement (40) positioning guidelines were followed to assist the design of this SRM. These guidelines describe the four stages (identification, screening, eligibility, and final selection) required to search and select manuscripts for a systematic review and feature the option of illustrating procedures in a flowchart (40). The PRISMA presents the PICOS acronym ("patient, problem or population", "intervention", "control or comparison" and "outcomes"), which helps making research questions and systematic searches more effective (47). Qualitative data from the different articles were selected, extracted, and organized in a specific table, following the PRISMA method, i.e., including authors, year and country, number of participants included, their age and gender, cancer type, intervention characteristics, central outcomes, and the existence of a control group. The protocol for this systematic review and meta-analysis has been registered at the International Platform of Registered Systematic Review and Meta-Analysis Protocols (INPLASY) under the registration number: INPLASY2020120096.

2.5 Quality of Information (QoI)

The Quality of Information (QoI) of the articles included in the systematic review was evaluated with application of the TREND statement guidelines (Transparent Evaluation Report with Nonrandomized Designs) (14). The method requires the evaluation of a list of 22 items (general criteria), subdivided into 59 sub-items (specific criteria) to quantitatively assess the QoI (14). One point is assigned to each reported sub-item. All studies with QoI $\geq$50% were included in the meta-analysis, since this qualification considers them as highly relevant for the topic under study.

2.6 Publication Bias

The publication bias was calculated using the software Comprehensive Meta-Analysis (CMA) (Biostat, Englewood, NJ, USA, version 3.3.070) creating a funnel plot by the standard error (y-axis) and the standard difference in means (x-axis) to determine whether the plot was balanced. Funnel plots are either symmetrical or asymmetrical (18). Studies without publication bias are distributed symmetrically around the mean effect size, since the sampling error is random. Studies with publication bias are expected to follow the model with symmetry at the top of the funnel plot, a few studies missing in the middle, and more studies missing near the

bottom of the plot. If the direction of the effect is toward the right, then near the bottom of the funnel plot we expect a gap on the left, where the non-significant studies would have been if we had been able to locate them. Because the interpretation of the funnel plot is sometimes subjective, different tests such as the Begg, and Mazumdar, and the Egger's tests have been proposed to quantify bias and test the relationship between sample size and effect size (8, 19). In the present study, the Egger´s test was used to check publication bias as suggested by Borenstein et al. (10).

2.7 Effect-Size Calculations

This meta-analysis was conducted to quantify the magnitude of the effects of acute exercise-conditioned human serum on the viability of cancer cell cultures. The meta-analysis took into account the intensity of the exercise performed before blood collection. Effect size was calculated using the software Comprehensive Meta-Analysis (CMA) (Biostat, Englewood, NJ, USA, version 3.3.070). The effect-size metric selected was the standardized difference in means (Std diff in means), since all studies evaluated the same outcome variable, but with different criteria. In such circumstances, it is necessary to standardize the results from each study using a uniform scale before they can be combined (20).

Data extracted for effect-size calculations from the different studies included sample size (N), statistical significance (p value) and effect direction. A random-effects model was used for the present meta-analysis, as it combines sampling error and between-study variance to estimate effect size (20). The following thresholds were used to interpret the effect sizes: trivial ($d < 0.20$), small ($0.21 < d < 0.50$), moderate ($0.51 < d < 0.79$), and large ($d > 0.80$) (10).

2.8 Heterogeneity of Variance

We followed the assumption that there would be variability in the true effect sizes between studies due to the expected differences in sampling error and between-study variance. The following statistics were used to quantify between-study heterogeneity: Q-value, I-squared (I^2), tau-squared (τ^2), and tau (τ). The Q Cochran statistic was used as a significance test to verify the null hypothesis and assess if all publications involved in this SRM share common effect sizes. Any variation would be due to the sample error within the studies. If all studies share the same effect size, the expected Q value will be equal to the degrees of freedom (df), e.g., the number of studies minus one. The I^2 statistic corresponds to the ratio between the true heterogeneity and the total variation of the observed effects. It shows the proportion (percentage) of the observed variance that reflects the differences in the true effect size rather than in the sample error (2). The τ^2 is the variance of the true effect sizes (in log units) among studies, while the τ value refers to the standard deviation of the true effects (20).

3. Results

3.1 Study Selection

A total of 888 publications were identified through an electronic database search and one additional publication was identified through cross-referencing (*Figure 1*). After duplicate removal, 389 publications were screened by title and abstract. Of these, 363 publications were excluded, because they did not reflect the research question or were meeting abstracts. At the eligibility stage, the full texts of the remaining 26 publications were read and we excluded: ten studies that were conducted on animals, rather than on cell lines; five studies where interventions involved not only exercise, but also diet; one study that focused on electrostimulation intervention; and one study that evaluated the methods used, rather than the effects of exercise-conditioned human serum in cancer cells. The TREND methodology guidelines were applied to the nine publications that fulfilled all eligibility criteria. All were accepted and regarded as satisfactory for the inclusion in the SRM, as their QoI scores were higher than 50% (*Table 1*).

3.2 Characteristics of the Studies and Participants

This SRM included nine publications of in vitro studies: four publications on prostate cancer, two publications on breast cancer, one publication on prostate cancer and breast cancer, one publication on lung cancer and one publication on colon cancer. All studies followed the same research hypothesis, testing the effect of acute and/or chronic exercise-conditioned human serum on the viability of human cancer cell cultures. The samples tested were obtained from a total of 244 participants from different countries, with ages varying between 18 to 73 years. Participants' characteristics varied significantly across the studies and included healthy sedentary women, breast cancer patients, women after cancer treatment, healthy sedentary men, men considered to be at risk of prostate cancer, and male colorectal cancer survivors.

Altogether, nine cell lines were used: two prostate cancer cell lines (LNCaP and PC3), two breast cancer cell lines (MCF-7 and MDA-MB-231), three lung cancer cell lines (A549, H460 and H1299) and two colon cancer cell lines (CaCo and LoVo). The different studies employed a variety of complementary viability assays (*Table 2*) and some of them also assessed apoptotic cell death (*Table 3*). Culture medium was supplemented with either 5% (v/v) or 10% (v/v) human serum. The types of exercise (one single session) or training (repeated exercise sessions performed periodically) performed were: (1) integrative (two or more activities by exercise (e.g., strength exercise plus cycling exercise) at intensities between 50% to 95% of their VO_2peak or one maximal repetition at workload resistance); (2) high intensity (cycling intervals with active rest periods at 85% – 95% of their VO_2peak); (3) moderate intensity (ergometer cycling at 50% – 65% of their VO_2peak).

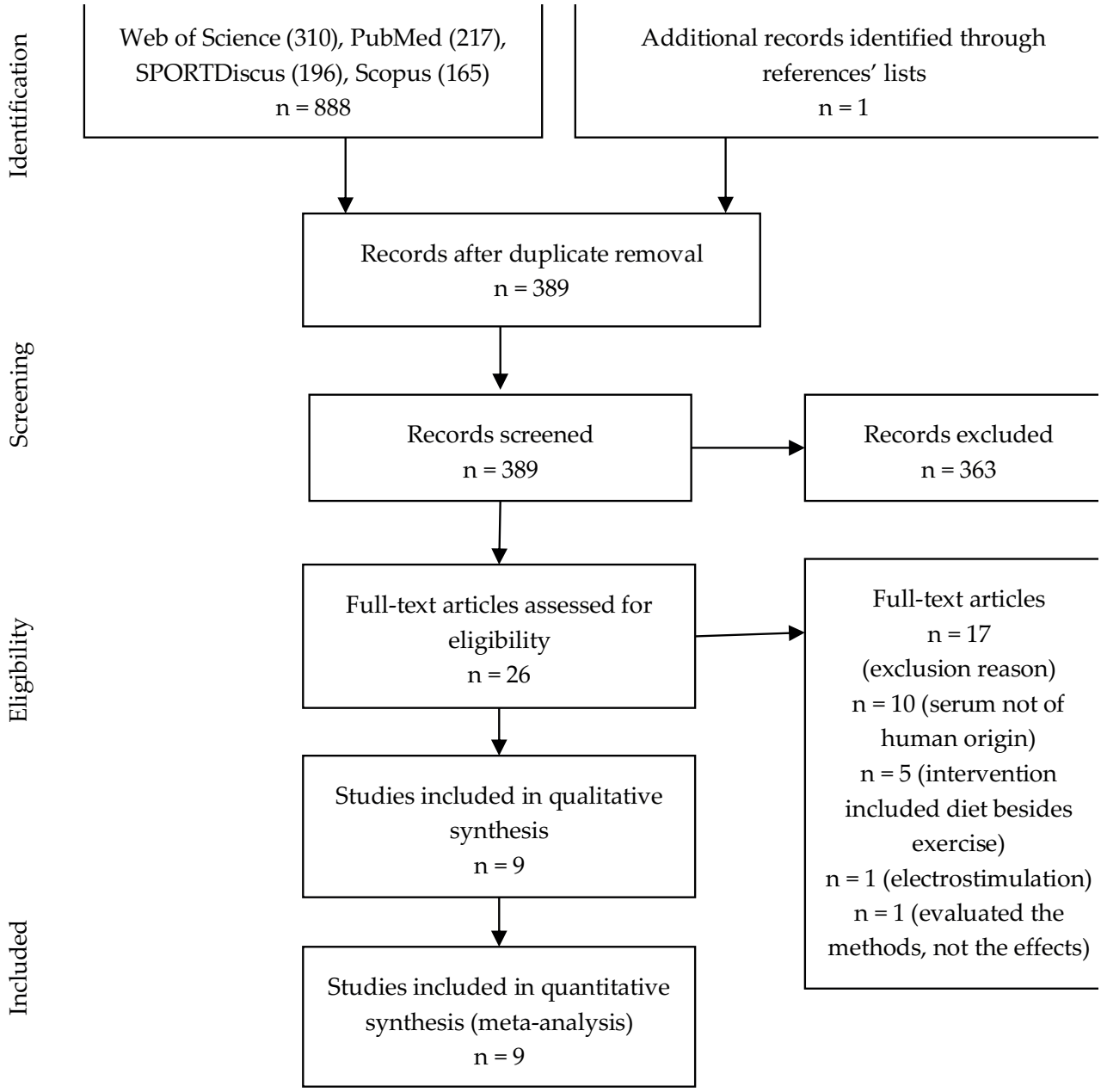

Figure 1. Schematic description of the different phases of the systematic search performed according to the PRISMA statement guidelines.

Study	TREND Assessment Protocol																							
	Title and abstract	Introduction	Participants	Interventions	Objectives	Outcomes	Sample size	Assignment method	Blinding (masking)	Unit of analysis	Statistical methods	Participant flow	Recruitment	Baseline data	Baseline equivalence	Numbers analysed	Outcomes and estimation	Ancillary analyses	Adverse events	Interpretation	Generalizability	Overall evidence	Total item	Total percentage
Items (paper sections)	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX	XX	XXI	XXII	22	100%
Sub-items (descriptor) per items	3	2	4	8	1	3	1	3	1	2	4	7	1	4	1	2	3	1	1	4	1	1	58	100%
Barnard et al. 2003 (7)																								
Item	1	1	1	1	1	1	0	1	0	1	1	1	0	1	1	1	1	1	0	1	1	1	18	81.8%
Sub-item	3	2	2	3	1	3	0	1	0	1	3	2	0	3	1	2	4	1	0	4	1	1	38	65.5%
Leung et al. 2004 (39)																								
Item	1	1	1	1	1	1	0	1	0	1	1	1	0	1	1	1	1	1	0	1	1	1	18	81.8%
Sub-item	2	2	3	5	1	3	0	1	0	2	1	3	0	3	1	2	3	1	0	3	1	1	38	65.5%
Rundqvist et al. 2013 (50)																								
Item	1	1	1	1	1	1	0	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	19	86.4%
Sub-item	2	2	1	5	1	3	0	1	0	2	2	2	0	2	1	2	3	1	1	3	1	1	36	62.1%
Dethlefsen et al. 2016 (16)																								
Item	1	1	1	1	1	1	1	0	0	1	1	1	0	1	1	1	1	1	1	1	1	1	19	86.4%
Sub-item	2	2	4	7	1	3	1	0	0	2	3	4	0	4	1	2	3	1	1	3	1	1	46	79.3%
Dethlefsen et al. 2017 (15)																								
Item	1	1	1	1	1	1	0	0	0	1	1	1	0	1	0	1	1	1	0	1	1	1	16	72.7%
Sub-item	2	2	2	5	1	3	0	0	0	1	3	3	0	2	0	2	3	1	0	3	1	1	35	60.3%
Kurgan, et al. 2017 (37)																								
Item	1	1	1	1	1	1	0	0	0	1	1	1	0	1	0	1	1	1	0	1	1	1	16	72.7%
Sub-item	2	2	1	5	1	3	0	0	0	2	3	2	0	2	0	2	3	1	0	2	1	1	33	56.9%
Devin et al. 2019 (17)																								
Item	1	1	1	1	1	1	0	0	0	1	1	1	0	1	0	1	1	1	0	1	1	1	16	72.7%
Sub-item	3	2	2	5	1	3	0	0	0	2	3	2	0	2	0	2	2	1	0	3	1	1	35	60.3%
Hwang, et al. 2020 (33)																								
Item	1	1	1	1	1	1	0	1	0	1	1	1	0	1	1	1	1	1	0	1	1	1	18	72.7%
Sub-item	1	1	2	3	1	3	0	1	0	1	2	4	0	3	1	2	3	1	0	3	1	1	34	58.6%
Baldelli et al. 2020 (5)																								
Item	1	1	1	1	1	1	0	1	0	1	1	1	0	1	1	1	1	1	0	1	1	1	18	81.8%
Sub-item	3	2	2	5	1	3	0	1	0	1	3	2	0	3	1	2	3	1	0	3	1	1	38	65.5%

Table 1. Categories and subcategories that emerged from the results in the nine studies selected for meta-analysis.

Note: Roman numerals refer to the 22 categories of the TREND assessment protocol. Arabic numerals indicate the number of sub-items reported for each item.

Table 2. Synthesis of the systematic search of articles assessing the effects of acute and chronic exercise-conditioned human serum on the viability[1] of human cancer cell cultures.

Study	Country	Cancer	Sample N	Subjects	Age	BMI	Design	Exercise	Systemic marker	In vitro assay[1]	Outcomes Main results by cell line	*p value*	Output
Acute													
Baldelli et al. 2020 (5)	Italy	Breast cancer	12	Healthy sedentary women	21 (0.8)	21 (1.3)	Non-randomized *in vitro* study Control Group (5% pre-exercise serum) and Exercise Group (5% serum collected immediately, 4 hours and 24 hours after exercise)	High Intensity Endurance Cycling (HIEC; 5 min warm-up + four 5 min incremental stage (50%, 55%, 60%, 70%) + ten 90 sec sprints at 90% interspersed by a 180 sec recovery) before(I) and after(II) 9 weeks period training	CK levels ↑ In post-HIEC (t1) and then decreased steadily, returning to basal values after 24 hours	Trypan blue dye exclusion assay Three- dimensional (3D) cell culture assay (soft agar) Trypan blue dye exclusion assay Three- dimensional (3D) cell culture assay (soft agar)	a) MDA-MB-231 (I 0 hours) 12.1% b) MDA-MB-231 (I 4 hours) 24.0% c) MDA-MB-231 (I 24 hours) 24.9% d) MDA-MB-231 (II 0 hours) 15.7% e) MDA-MB-231 (II 4 hours) 30.6% f) MDA-MB-231 (II 24 hours) 35.3% g) MDA-MB-231 (I 0 hours) 17.6% h) MDA-MB-231 (I 4 hours) 33.7% i) MDA-MB-231 (I 24 hours) 30.0% j) MDA-MB-231 (II 0 hours) 10.1% k) MDA-MB-231 (II 4 hours) 18.2% l) MDA-MB-231 (II 24hours) 15.6% m) LNCaP (I 0 hours) 13.8% n) LNCaP (I 4 hours) 21.8% o) LNCaP (I 24 hours) 22.8% p) LNCaP (II 0 hours) 14.0% q) LNCaP (II 4 hours) 22.9% r) LNCaP (II 24 hours) 27.2% s) LNCaP (I 0 hours) 15.95% t) LNCaP (I 4 hours) 21.45% u) LNCaP (I 24 hours) 25.94% v) LNCaP (II 0 hours) 14.44% x) LNCaP (II 4 hours) 20.31% z) LNCaP (II 24 hours) 25.79%	p < 0.001 p < 0.001 p < 0.001 p < 0.001 p < 0.001 p < 0.001 p < 0.001 p < 0.001 p < 0.001 p < 0.001 p < 0.001 p < 0.001 p < 0.001 p < 0.001 p < 0.001 p < 0.001 p < 0.001 p < 0.001 p < 0.001 p < 0.001 p < 0.001 p < 0.001 p < 0.001 p < 0.001	Reduction in breast cancer cell viability and prostate cancer cell viability, stimulated with all human serum conditioned by HIEC. Result were remarkably similar between human serum obtained immediately after the HIEC and the Human serum collected after 4hours and 24hours.
		Prostate cancer	18	Healthy sedentary men	21 (1.4)	22 (2.8)							
Hwang et al. 2020 (33)	Australia Australia	Prostate cancer	12	Healthy young men	28.2 (2.6)	22.8 (2.1)	Non-randomized experimental *in vitro* study Control Group (5% serum collected pre-exercise) and Exercise Group (5% serum collected post-exercise)	65 min exercise electro-dynamically loaded cycle ergometer (20 min 50% VO2peak + 40min 65% VO2peak)	Granulocyte ↑ Lymphocyte ↑ Testosterone ↑ (young group) osteonectin (SPARC) ↑ oncostatin M (OSM) ↑ IL-6, IL-15 and Irisin (not consistently detectable or did not change)	DNA quantification assay Alamar blue assay DNA quantification assay Alamar blue assay	a) LNCaP (no sig. difference) b) LNCaP (no sig. difference) c) PC3(no sig. difference) d) PC3 (no sig. difference) e) PC3 (no sig. difference) f) LNCaP (no sig. difference) g) LNCaP (sig. reduction) h) PC3 (no sig. difference)	p > 0.05 p > 0.05 p > 0.05 p > 0.05 p > 0.05 p > 0.05 p < 0.01 p > 0.05	Pre-exercise serum, collected from older men after a single bout of exercise reduced the metabolic activity of (androgen-responsive) LNCaP prostate cancer cells. No such effect was observed after treatment of the cancer cells with pre- versus post-exercise serum collected from young men.
			10	Healthy old man	63.1 (6.9)	24.9 (1.92)							
Devin et al. 2019 (17)	Australia	Colon cancer	10	Male colorectal cancer survivors	66.9 (8.4)	27.7 (3.6)	Non-randomized experimental *in vitro* study Control Group (10% serum collected at rest) and Exercise Group (10% serum collected immediately after exercise or 120 min after exercise)	Acute trial - HIIE 38 min (10 min warm-up; 4x4 minutes bouts of cycling 85-95% Hr peak with 3 minutes active recovery)	*IL-6 ↑ *IL-8 ↑ *TNF-α ↑ *(returned to baseline levels at 120 min post-exercise) **Insulin ↑ ** (significantly lower than baseline at 120 min)	Alamar blue assay	a) CaCo2 (0 hours at 24 hours of incubation) ES= -1.3 b) CaCo2 (0 hours at 48 hours of incubation) ES= -1.7 c) CaCo2 (0 hours at 72 hours of incubation) ES= -1.1 d) LoVo (0 hours at 24 hours of incubation) ES= -1.2 e) LoVo (0 hours at 48 incubation hours) ES=-0.8 f) LoVo (0 hours at 72 hours of incubation) ES= -1.1 g) CaCo2 (2 hours at 24 hours of incubation) h) CaCo2 (2 hours at 48 hours of incubation) i) CaCo2 (2 hours at 72 hours of incubation) j) LoVo (2 hours at 24 hours of incubation) k) LoVo (2 hours at 48 hours of incubation) l) LoVo (2 hours at 72 hours of incubation)	p=0.002 p<0.001 p=0.035 p=0.001 p≥0.05 p=0.032 p≥0.05	The serological changes associated with acute HIIE transiently reduce colon cancer cell number.

Table 2. Synthesis of the systematic search of articles assessing the effects of acute and chronic exercise-conditioned human serum on the viability[1] of human cancer cell cultures (Continuation).

Study	Country	Cancer	Sample N	Subjects	Age	BMI	Design	Exercise	Systemic marker	Outcomes In vitro assay[1]	Main results by cell line	*p value*	Output
							Acute						
Kurgan et al. 2017 (37)	Canada	Lung cancer	23	Recreationally active male university students	21.8 (2.4)	23.9 (2.5)	Non-randomized experimental *in vitro* study Control Group (10% FBS treatment) and Exercise Group (10% serum collected 5min, 1h, 24h after exercise)	High Intensity Exercise Trial 90% (4 min warm-up; 6x(1min High Intensity cycling+1min active rest + 2-3 min cool-down)	Insulin - levels were not significantly changed - (pre-exercise, 5 minutes, 1 hour, or 24 hours post-exercise: 34.9 ± 3.6, 32.5 ± 5.6, 42.4 ± 6.3, 42.2 ± 3.6 pmol/L, respectively)	Crystal violet proliferation assay Clonogenic survival assay	a) A549 (5 minutes) 90.1 (4.2) % b) A549 (1 hour) 91.0 (2.1) % c) A549 (24 hours) 84.1(1.9)% d) A549 (5 minutes) 21.5 (2.9) % e) A549 (1 hour) 33.9 (3.5)% f) A549 (24 hours) 35.8(6.7)% g) H460 (5 minutes) 37.7 % h) H460 (1 hour) 38.4% i) H460 (24 hours) 39.3% j) H1299 (5 minutes) 33.5% k) H1299 (1 hour) 41.9% l) H1299 (24 hours) 37.7%	p < 0.05 p < 0.05 p < 0.05 p < 0.001 p < 0.001 p < 0.001 p < 0.001 p < 0.001 p < 0.001 p < 0.001 p < 0.001 p < 0.001	The treatment of lung cancer cells with serum taken post-exercise leads to significant inhibition of cell survival and proliferation. Post-exercise serum did not have any significant effect on normal lung fibroblasts.
Dethlefsen et al. 2017 (15)	Denmark	Breast cancer	7	Healthy women	24.6 (1.0)	23.3 (2.7)	Non-randomized experimental *in vitro* study Control Group (10% pre-exercise serum) and Exercise Group 1 (10% 1 hour of exercise) or Exercise Group 2 (10% Serum collected after the end of exercise)	2h - 55% VO2peak cycling ergometer	Epinephrine ↑ Norepinephrine ↑ Lactate ↑	CellTiter-Fluor cell viability assay	a) MCF-7 (1 hour) 10%; b) MDA-MB-231 (1 hour) 14%; c) MCF-7 (2 hours) 19%; d) MDA-MB-231 (2 hour) 13%	p < 0.01 p < 0.05 p < 0.001 p < 0.05	Systemic changes occurring during exercise can reduce breast cancer cell viability.
			20	Breast cancer patients	48.5 (7.3)	26.0 (5.2)	Non-randomized experimental *in vitro* study Control Group (pre-exercise serum) and Exercise Group (serum collected immediately after exercise)	2hour (30 min warm-up +60 min whole body training + 30 min High Intensity)	IL6 ↑		e) MCF-7 11%; f) MDA-MB-231 9%;	p < 0.05 p < 0.05	
Dethlefsen et al. 2016 (16)	Denmark	Breast cancer	20	Breast cancer patients	49.5 (7.3)	26.0 (5.2)	Non-randomized experimental *in vitro* study Control Group (10% pre-exercise serum) and Exercise Group (10% serum after 2 hours acute exercise)	2hour (30 min warmup +60 min resistance training + 30 min High Intensity on stationary bicycles)	Insulin ↓ IL-6 ↑ IL-8 ↑ IL-10 ≃ TNF-α ↑ Lactate ↑ Epinephrine ↑ Norepinephrine ↑	CellTiter-Fluor cell viability assay	a) MDA-MB-231 -9.4%; b) MCF-7 -9.2%;	p < 0.001 p = 0.04	The marked systemic response occurring acutely during performance of exercise reduces breast cancer viability.
Rundqvist et al. 2013 (50)	Sweden	Prostate cancer	10	Healthy male	25		Non-randomized experimental *in vitro* study Control Group (5% serum collected pre-exercise) and Exercise Group (5% serum collected post exercise or 120 min after exercise)	65 min exercise electro-dynamically loaded cycle ergometer (20 min 50% VO2peak + 40min 65% VO2peak)	EGF ↓ IGFBP-1 ↑	Click-iT EdU microplate assay kit	a) LNCaP - reduce 9/10 individual serum b) LNCaP -31% (pool of 10 serum)	p < 0.05	Serum obtained directly after a bout of strenuous exercise, despite its mitogenic potential, reduced proliferation of prostate cancer tumor cells.

Table 2. Synthesis of the systematic search of articles assessing the effects of acute- and chronic exercise-conditioned human serum on the viability[1] of human cancer cell cultures (Continuation).

Study	Country	Cancer	Sample N	Sample Subjects	Sample Age	Sample BMI	Design	Exercise	Outcomes Systemic marker	Outcomes In vitro assay[1]	Outcomes Main results by cell line	Output *p value*	Output
Chronic													
Baldelli et al. 2020 (5)	Italy	Breast cancer	12	Healthy sedentary women	21 (0.8)	21 (1.3)	Non-randomized experimental *in vitro* study Control Group (5% serum collected at rest before training period) and Exercise Group (5% serum collected at rest after training period)	9 weeks training period (36 indoor cycling training sessions; 3 sessions per week - first 3 weeks - and 4 sessions per week from de fourth to the sixth week)	-----	Trypan blue dye exclusion assay Three-dimensional (3D) cell culture assay (soft agar)	w) LNCaP (no sig. difference) y) MDA-MB-231 (no sig. difference) aa) LNCaP (no sig. difference) bb)MDA-MB-231 (no sig. difference)	... ...	No significant difference between the proliferative and tumorigenic stimulatory capacity of human serum obtained at rest, before and after the training period.
		Prostate cancer	18	Healthy sedentary men	21 (1.4)	22 (2.8)							
Devin et al. 2019 (17)	Australia	Colon cancer	10	Male colorectal cancer survivors	64.9 (6.0)	30.2 (3.9)	Non-randomized experimental *in vitro* study Control Group (10% serum collected at rest before intervention) and Exercise Group (10% serum collected at rest after 4 weeks intervention).	Short-term training (4 Weeks - 3x HIIE/week)	IL-6 ≈ IL-8 ≈ TNF-α ≈ Insulin ≈ resting serum after 4 weeks of HIIE	Alamar blue assay	o) CaCo (no sig. difference) p) LoVo (no sig. difference)	p= 0.223 p= 0.375	Short-term HIIE training was not associated with changes in cell number or common metabolic factors related to the risk of colorectal cancer when measured at rest.
Dethlefsen et al. 2016 (16)	Denmark	Breast cancer	37	Women after cancer treatment (PA - physical activity group)	46.0 (9.6)	24.0 (3.8)	Non-randomized experimental *in vitro* study Control Group (10% rest serum) and Exercise Group (10% serum collected at rest after 6 months of intervention)	Exercise ≥ 3 hours/week Health evaluation without exercise counselling	Insulin ↑ Leptin ≈ Glucose ≈ LDL/HDL ↓ IL-6 ↓ IL-8 ≈ IL-10 ↓ TNF-α ↓	CellTiter-Fluor cell viability assay	c) MCF-7 no effect d) MDA-MB-231 no effect e) MCF-7 no effect f) MDA-MB-231 no effect	... ...	The systemic adaptations to 6 months of training had no effect on breast cancer cell.
			37	Women after cancer treatment (HE - health evaluation group)	48.2 (7.8)	24.8 (3.7)							
Leung et al. 2004 (39)	EUA	Prostate cancer	10	Men considered to be at risk of (CG)	62 (2)	31.5 (1.6)	Non-randomized experimental *in vitro* study Control Group (10% serum of sedentary lifestyle at rest) and Exercise Group (10% serum collected at rest from adult with Exercise >10 years)	Sedentary lifestyle 5 days/wk for 1 hour (warm-up + 45-50 min of continuous, strenuous exercise	Insulin ↓ IGF-I ↓ IGFBP-1 ↑	CellTiter 96AQ assay	a) LNCaP 27%;	$p < 0.05$	Serum obtained from men after a regular exercise program for >10 years reduced the growth rate.
			12	Adult Fitness Program at least 10 years (EG)	60 (3)	26.5 (1)							
Barnard et al. 2003 (7)	EUA	Prostate cancer	14	Men considered to be risk (CG)	60 (3)	38 (2)	Non-randomized experimental *in vitro* study Control Group (10 % serum of sedentary lifestyle at rest) and Exercise Group (10% serum collected at rest from adult with Exercise >10 years)	Sedentary lifestyle 5 days/wK for 1 hour (warm-up + 45-50 min of continuous, strenuous exercise	Insulin ↓ IGF-I ↓ IGFBP-1 ↑	MTS assay	a) LNCaP 65 (4)%	$p < 0.01$	Intensive exercise training can favourably alter the IGF axis and reduce LNCaP prostate cancer cell growth.
			12	Men considered to be risk (EG)	62 (2)	26.5 (1)							

[1] For the majority of the studies, the viability assays employed do not allow to distinguish between changes in viability due to alterations in proliferation rates and those due to increased/decreased cell death.

Abbreviations: BMI, body mass index; EGF, epidermal growth factor; HDL, high-density lipoprotein; HIIE, high-intensity intermittent exercise; HR, heart rate; IGFBP-1, insulin-like growth factor binding protein 1; IGF-I, insulin-like growth factor-1; IL-6, interleukin-6; IL-8, interleukin-8; N, number of subjects; TNF-α, tumor necrosis factor alpha; VO_2peak, peak oxygen uptake.

Study	Country	Cancer	Sample N	Subjects	Age	BMI	Design	Exercise	Systemic marker	Outcomes In vitro assay	Main results by cell line	*p value*	Output
Acute													
Devin et al. 2019 (17)	Australia	Colon cancer	10	Male colorectal cancer survivors	66.9 (8.4)	27.7 (3.6)	Non-randomized experimental *in vitro* study Control Group (10% serum collected at rest) and Exercise Group (10% serum collected immediately after exercise or 120 min after exercise)	Acute trial - HIIE 38 min (10 min warm-up; 4x4 minutes bouts of cycling 85-95% HR peak with 3 minutes active recovery)	IL-6 ↑ IL-8 ↑ TNF-α ↑ Insulin ↑	Annexin-V Kit - flow cytometry (apoptosis)	m) CaCo2 (0 hours)-0.04% n) LoVo (0 hours) 0.64%	p=0.702 p=0.395	Distribution of apoptotic cells incubation with serum post HIIE was not significantly different.
Rundqvist et al. 2013 (50)	Sweden	Prostate cancer	10	Healthy male	25		Non-randomized experimental *in vitro* study Control Group (5% serum collected pre-exercise) and Exercise Group (5% serum collected post exercise or 120 min after exercise)	65 min exercise electro-dynamically loaded cycle ergometer (20 min 50% VO2 peak + 40min 65% VO2 peak)	EGF ↓ IGFBP-1 ↑	Annexin-V Kit (apoptosis)	c) LNCaP fraction of apoptotic cells did not differ between groups	-----	Acute exercise serum had no apoptotic effect on the tumours cells.
Chronic													
Leung et al. 2004 (39)	EUA	Prostate cancer	10	Men considered to be risk (CG)	62 (2)	31.5 (1.6)	Non-randomized experimental *in vitro* study Control Group (10%serum of sedentary lifestyle at rest) and Exercise Group (10%serum collected at rest from adult with Exercise >10 years)	Sedentary lifestyle 5 days/week for 1 hour (warm-up + 45-50 min of continuous, strenuous exercise	Insulin ↓ IGF-I ↓ IGFBP-1 ↑	Cell death detection ELISA plus (apoptosis)	b) LNCaP +371%;	p < 0.01	Serum obtained from men after a regular exercise program for >10 years induced apoptosis of LNCaP prostate cancer cell line.
			12	Adult fitness program at least 10 years (EG)	60 (3)	26.5 (1)							
Barnard et al. 2003 (7)	EUA	Prostate cancer	14	Men considered to be risk (CG)	60 (3)	38 (2)	Non-randomized experimental *in vitro* study Control Group (10% serum of sedentary lifestyle at rest) and Exercise Group (10% serum collected at rest from adult with Exercise >10 years)	Sedentary lifestyle 5 days/week for 1 hour (warm-up + 45-50 min of continuous, strenuous exercise	Insulin ↓ IGF-I ↓ IGFBP-1 ↑	Annexin-V / TUNEL assay (apoptosis)	b) LNCaP apoptotic cells increased significantly at culture with exercise-conditioned serum	--	Intensive exercise training can favourably alter the IGF axis and inducing apoptosis.
			12	Adult fitness program at least 10 years (EG)	62 (2)	26.5 (1)							

Table 3. Synthesis of the systematic search of articles assessing the effects of acute and chronic exercise-conditioned human serum on levels of apoptosis in human cancer cell cultures.

Abbreviations: BMI, body mass index; EGF, epidermal growth factor; HDL, high-density lipoprotein; HIIE, high-intensity intermittent exercise; HR, heart rate; IGFBP-1, insulin-like growth factor binding protein 1; IGF-I, insulin-like growth factor-1; IL-6, interleukin-6; IL-8, interleukin-8; N, number of subjects; TNF-α, tumor necrosis factor alpha; VO_2peak, peak oxygen uptake.

3.3 Meta-Analysis Outcomes

The nine publications under consideration presented separate results for more than one cell line and/or assay. The results of all samples were used in the meta-analysis for the overall effect outcomes, except in the case of a single study, in which cultures were exposed for different times to the same serum, in which case only the result from one incubation time was considered (17). Meta-analysis outcomes were: effect of acute exercise-conditioned human serum on the viability of cancer cell cultures (section 3.3.1); effect of high-intensity acute exercise-conditioned human serum on the viability of cancer cell cultures (section 3.3.2); effect of moderate-intensity exercise-conditioned human serum and of human serum conditioned by an integrative exercise of moderate and high-intensity on the viability of cancer cell cultures (section 3.3.3).

Due to limited data, we did not perform a quantitative analysis on the effects of acute exercise-conditioned human serum on levels of apoptosis and on the effects of chronic exercise-conditioned human serum on the viability of cancer cell cultures.

3.3.1 Effect of Acute Exercise-Conditioned Human Serum on the Viability of Human Cancer Cell Cultures

The meta-analysis on the effect of acute exercise-conditioned human serum on the viability of cancer cell cultures included the data from all samples and assays from seven studies. The high number of entries was due to the high number of samples and assays in each study. A random effects model was used to run the meta-analysis, the results showing, based on the standard difference in means, that acute exercise-conditioned human serum exhibited an overall effect size of -1.126 in reduction of the viability of cancer cell line cultures (*Figure 2*), when compared to the same cultures exposed to at pre-exercise human serum. The confidence interval for the standard difference in means was -1.300 to -0.952 (95% CI) with a corresponding p value < 0.001 (*Figure 2*). Of note, this interval does not include a zero effect. Similarly, z-values obtained to test the null hypothesis, that the standard difference in means is zero, showed a $z = -12.694$ and a corresponding value of $p < 0.001$ (*Figure 2*). Thus, the null hypothesis was rejected and the alternative hypothesis was accepted in all analysed studies, i.e., upon exposure of cancer cells to acute exercise-conditioned human serum there is a reduction in their viability, with a standard difference in means higher than 1 point.

When verifying the homogeneity of the effects, a Q-value of 130.350 was obtained, with 59 degrees of freedom and a $p < 0.001$ indicating a true effect-size, which was not identical in all studies. The I^2 value was 54.737, meaning that about 54.737 % of the variance in the observed effects reflects variance in the true effects. The τ^2 value had a value of 0.244. The τ value, i.e., the standard deviation of the true effects in this SRM was equal to 0.494. The funnel plot (Figure 5a) for the distribution of the observed studies was not entirely symmetrical, with a little trend of the studies distributed towards the left side of the mean effect size. Additionally, the Egger´s test was performed and the intercept value was -3.23286, with a 95% confidence interval between −4.61752 and -1.84819, t value = 4.67354. The recommended p value (2-tailed) was 0.00002. These statistical results show the lack of studies on the right side where the non-significant studies would be if there were any or if we had managed to locate them.

3.3.2 Effect of High-Intensity Acute Exercise-Conditioned Human Serum on the Viability of Human Cancer Cell Cultures

The meta-analysis of the effect of high-intensity acute exercise-conditioned human serum on the viability of cancer cell cultures was restricted to those four studies where the exercise intervention consisted of high-intensity exercise (>80% VO_2peak) and it included data from all samples and assays. A random effects model was used to run the meta-analysis and the results showed, based on the standard difference in means, that cancer cells cultured in the presence of acute high intensity exercise-conditioned human serum exhibited a reduction of -1.350 in their viability (*Figure 3*). The confidence interval for the standard difference in means was -1.522 to -1.179 (95% CI) with a corresponding value of $p < 0.001$ (*Figure 3*). Once again, this interval does not include a zero effect. Similarly, z-values obtained to test the null hypothesis i.e., that the standard difference in means is zero, showed a $z = -15.428$, and a corresponding value of $p < 0.001$ (*Figure 3*). Thus, the null hypothesis, was rejected and the alternative hypothesis accepted in all analysed studies, i.e., cultures of cancer cells exposed to acute high-exercise-conditioned human serum exhibited a lower viability than cultures of cancer cells exposed to at rest human serum.

When verifying the homogeneity of the effects, a Q-value of 59.006 was obtained, with 41 degrees of freedom and a $p < 0.05$ indicating that true effect-size was not identical in all studies. The I^2 value was 30.516, meaning that about 30.516% of the variance in the observed effects reflects variance in the true effects. The τ^2 value was 0.094. The τ value, i.e., the standard deviation of the true effects in this SRM, is equal to 0.306. The funnel plot (Figure 5b) for the distribution of the observed studies is not entirely symmetrical, with a trend towards the distribution of the studies on the left side of the mean effect size. Additionally, the Egger´s test was performed and the intercept value was -2.99967, with a 95% confidence interval between −4.19201 and -1.80734, t value = 5.08461. The recommended p value (2-tailed) was 0.00001. These statistical results show the lack of studies on the right side where the non-significant studies would be if there were any or if we had managed to locate them.

Study name	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Baldelli et al 2020 a)	-1,548	0,465	0,217	-2,460	-0,636	-3,326	0,001
Baldelli et al 2020 b)	-1,548	0,465	0,217	-2,460	-0,636	-3,326	0,001
Baldelli et al 2020 c)	-1,548	0,465	0,217	-2,460	-0,636	-3,326	0,001
Baldelli et al 2020 d)	-1,548	0,465	0,217	-2,460	-0,636	-3,326	0,001
Baldelli et al 2020 e)	-1,548	0,465	0,217	-2,460	-0,636	-3,326	0,001
Baldelli et al 2020 f)	-1,548	0,465	0,217	-2,460	-0,636	-3,326	0,001
Baldelli et al 2020 g)	-1,548	0,465	0,217	-2,460	-0,636	-3,326	0,001
Baldelli et al 2020 h)	-1,548	0,465	0,217	-2,460	-0,636	-3,326	0,001
Baldelli et al 2020 i)	-1,548	0,465	0,217	-2,460	-0,636	-3,326	0,001
Baldelli et al 2020 j)	-1,548	0,465	0,217	-2,460	-0,636	-3,326	0,001
Baldelli et al 2020 k)	-1,548	0,465	0,217	-2,460	-0,636	-3,326	0,001
Baldelli et al 2020 l)	-1,548	0,465	0,217	-2,460	-0,636	-3,326	0,001
Baldelli et al 2020 m)	-1,200	0,362	0,131	-1,910	-0,491	-3,315	0,001
Baldelli et al 2020 n)	-1,200	0,362	0,131	-1,910	-0,491	-3,315	0,001
Baldelli et al 2020 o)	-1,200	0,362	0,131	-1,910	-0,491	-3,315	0,001
Baldelli et al 2020 p)	-1,200	0,362	0,131	-1,910	-0,491	-3,315	0,001
Baldelli et al 2020 q)	-1,200	0,362	0,131	-1,910	-0,491	-3,315	0,001
Baldelli et al 2020 r)	-1,200	0,362	0,131	-1,910	-0,491	-3,315	0,001
Baldelli et al 2020 s)	-1,200	0,362	0,131	-1,910	-0,491	-3,315	0,001
Baldelli et al 2020 t)	-1,200	0,362	0,131	-1,910	-0,491	-3,315	0,001
Baldelli et al 2020 u)	-1,200	0,362	0,131	-1,910	-0,491	-3,315	0,001
Baldelli et al 2020 v)	-1,200	0,362	0,131	-1,910	-0,491	-3,315	0,001
Baldelli et al 2020 x)	-1,200	0,362	0,131	-1,910	-0,491	-3,315	0,001
Baldelli et al 2020 z)	-1,200	0,362	0,131	-1,910	-0,491	-3,315	0,001
Devin et al 2019 a)	-1,615	0,515	0,265	-2,624	-0,605	-3,136	0,002
Devin et al 2019 d)	-1,754	0,526	0,277	-2,785	-0,722	-3,333	0,001
Devin et al 2019 g)	0,000	0,447	0,200	-0,877	0,877	0,000	1,000
Devin et al 2019 j)	0,000	0,447	0,200	-0,877	0,877	0,000	1,000
Hwang et al 2020 e)	0,000	0,408	0,167	-0,800	0,800	0,000	1,000
Hwang et al 2020 f)	0,000	0,408	0,167	-0,800	0,800	0,000	1,000
Hwang et al 2020 a)	0,000	0,408	0,167	-0,800	0,800	0,000	1,000
Hwang et al 2020 b)	0,000	0,408	0,167	-0,800	0,800	0,000	1,000
Hwang et al 2020 g)	-1,287	0,491	0,241	-2,250	-0,324	-2,620	0,009
Hwang et al 2020 h)	0,000	0,447	0,200	-0,877	0,877	0,000	1,000
Hwang et al 2020 c)	0,000	0,447	0,200	-0,877	0,877	0,000	1,000
Hwang et al 2020 d)	0,000	0,447	0,200	-0,877	0,877	0,000	1,000
Kurgan et al 2017 a)	-1,165	0,578	0,334	-2,298	-0,032	-2,015	0,044
Kurgan et al 2017 b)	-1,165	0,578	0,334	-2,298	-0,032	-2,015	0,044
Kurgan et al 2017 c)	-1,165	0,578	0,334	-2,298	-0,032	-2,015	0,044
Kurgan et al 2017 d)	-2,648	0,791	0,626	-4,198	-1,098	-3,348	0,001
Kurgan et al 2017 e)	-2,648	0,791	0,626	-4,198	-1,098	-3,348	0,001
Kurgan et al 2017 f)	-2,648	0,791	0,626	-4,198	-1,098	-3,348	0,001
Kurgan et al 2017 g)	-2,648	0,791	0,626	-4,198	-1,098	-3,348	0,001
Kurgan et al 2017 h)	-2,648	0,791	0,626	-4,198	-1,098	-3,348	0,001
Kurgan et al 2017 i)	-2,648	0,791	0,626	-4,198	-1,098	-3,348	0,001
Kurgan et al 2017 j)	-2,648	0,791	0,626	-4,198	-1,098	-3,348	0,001
Kurgan et al 2017 k)	-2,648	0,791	0,626	-4,198	-1,098	-3,348	0,001
Kurgan et al 2017 l)	-2,648	0,791	0,626	-4,198	-1,098	-3,348	0,001
Rundqvist et al 2013 a)	-0,940	0,471	0,222	-1,863	-0,016	-1,994	0,046
Dethlefsen et al 2016 a)	-1,128	0,340	0,116	-1,795	-0,460	-3,312	0,001
Dethlefsen et al 2016 b)	-0,673	0,325	0,106	-1,310	-0,035	-2,069	0,039
Dethlefsen et al 2017 a)	-1,633	0,617	0,381	-2,842	-0,423	-2,645	0,008
Dethlefsen et al 2017 b)	-1,165	0,578	0,334	-2,298	-0,032	-2,015	0,044
Dethlefsen et al 2017 c)	-2,308	0,690	0,476	-3,660	-0,956	-3,345	0,001
Dethlefsen et al 2017 d)	-1,165	0,578	0,334	-2,298	-0,032	-2,015	0,044
Dethlefsen et al 2017 e)	-0,640	0,324	0,105	-1,276	-0,005	-1,974	0,048
Dethlefsen et al 2017 f)	-0,640	0,324	0,105	-1,276	-0,005	-1,974	0,048
Devin et al 2019 m)	-0,174	0,448	0,201	-1,052	0,704	-0,388	0,698
Devin et al 2019 n)	-0,390	0,451	0,204	-1,275	0,495	-0,863	0,388
Rundqvist et al 2013 c)	0,000	0,447	0,200	-0,877	0,877	0,000	1,000
	-1,126	0,089	0,008	-1,300	-0,952	-12,694	0,000

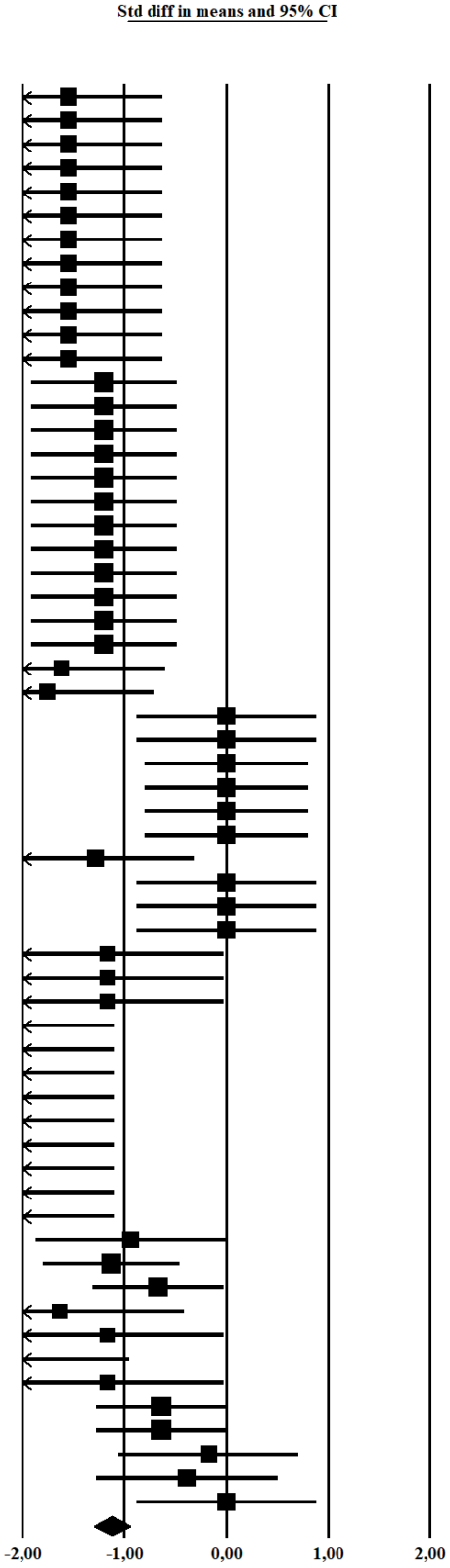

Figure 2. Summary of descriptive and inferential statistics of results for each study and overall effect size of the effects of the acute-exercise-conditioned human serum in human cancer cell viability.

Study name	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Baldelli et al 2020 a)	-1,548	0,465	0,217	-2,460	-0,636	-3,326	0,001
Baldelli et al 2020 b)	-1,548	0,465	0,217	-2,460	-0,636	-3,326	0,001
Baldelli et al 2020 c)	-1,548	0,465	0,217	-2,460	-0,636	-3,326	0,001
Baldelli et al 2020 d)	-1,548	0,465	0,217	-2,460	-0,636	-3,326	0,001
Baldelli et al 2020 e)	-1,548	0,465	0,217	-2,460	-0,636	-3,326	0,001
Baldelli et al 2020 f)	-1,548	0,465	0,217	-2,460	-0,636	-3,326	0,001
Baldelli et al 2020 g)	-1,548	0,465	0,217	-2,460	-0,636	-3,326	0,001
Baldelli et al 2020 h)	-1,548	0,465	0,217	-2,460	-0,636	-3,326	0,001
Baldelli et al 2020 i)	-1,548	0,465	0,217	-2,460	-0,636	-3,326	0,001
Baldelli et al 2020 j)	-1,548	0,465	0,217	-2,460	-0,636	-3,326	0,001
Baldelli et al 2020 k)	-1,548	0,465	0,217	-2,460	-0,636	-3,326	0,001
Baldelli et al 2020 l)	-1,548	0,465	0,217	-2,460	-0,636	-3,326	0,001
Baldelli et al 2020 m)	-1,200	0,362	0,131	-1,910	-0,491	-3,315	0,001
Baldelli et al 2020 n)	-1,200	0,362	0,131	-1,910	-0,491	-3,315	0,001
Baldelli et al 2020 o)	-1,200	0,362	0,131	-1,910	-0,491	-3,315	0,001
Baldelli et al 2020 p)	-1,200	0,362	0,131	-1,910	-0,491	-3,315	0,001
Baldelli et al 2020 q)	-1,200	0,362	0,131	-1,910	-0,491	-3,315	0,001
Baldelli et al 2020 r)	-1,200	0,362	0,131	-1,910	-0,491	-3,315	0,001
Baldelli et al 2020 s)	-1,200	0,362	0,131	-1,910	-0,491	-3,315	0,001
Baldelli et al 2020 t)	-1,200	0,362	0,131	-1,910	-0,491	-3,315	0,001
Baldelli et al 2020 u)	-1,200	0,362	0,131	-1,910	-0,491	-3,315	0,001
Baldelli et al 2020 v)	-1,200	0,362	0,131	-1,910	-0,491	-3,315	0,001
Baldelli et al 2020 x)	-1,200	0,362	0,131	-1,910	-0,491	-3,315	0,001
Baldelli et al 2020 z)	-1,200	0,362	0,131	-1,910	-0,491	-3,315	0,001
Devin et al 2019 a)	-1,615	0,515	0,265	-2,624	-0,605	-3,136	0,002
Devin et al 2019 d)	-1,754	0,526	0,277	-2,785	-0,722	-3,333	0,001
Devin et al 2019 g)	0,000	0,447	0,200	-0,877	0,877	0,000	1,000
Devin et al 2019 j)	0,000	0,447	0,200	-0,877	0,877	0,000	1,000
Kurgan et al 2017 a)	-1,165	0,578	0,334	-2,298	-0,032	-2,015	0,044
Kurgan et al 2017 b)	-1,165	0,578	0,334	-2,298	-0,032	-2,015	0,044
Kurgan et al 2017 c)	-1,165	0,578	0,334	-2,298	-0,032	-2,015	0,044
Kurgan et al 2017 d)	-2,648	0,791	0,626	-4,198	-1,098	-3,348	0,001
Kurgan et al 2017 e)	-2,648	0,791	0,626	-4,198	-1,098	-3,348	0,001
Kurgan et al 2017 f)	-2,648	0,791	0,626	-4,198	-1,098	-3,348	0,001
Kurgan et al 2017 g)	-2,648	0,791	0,626	-4,198	-1,098	-3,348	0,001
Kurgan et al 2017 h)	-2,648	0,791	0,626	-4,198	-1,098	-3,348	0,001
Kurgan et al 2017 i)	-2,648	0,791	0,626	-4,198	-1,098	-3,348	0,001
Kurgan et al 2017 j)	-2,648	0,791	0,626	-4,198	-1,098	-3,348	0,001
Kurgan et al 2017 k)	-2,648	0,791	0,626	-4,198	-1,098	-3,348	0,001
Kurgan et al 2017 l)	-2,648	0,791	0,626	-4,198	-1,098	-3,348	0,001
Devin et al 2019 m)	-0,174	0,448	0,201	-1,052	0,704	-0,388	0,698
Devin et al 2019 n)	-0,390	0,451	0,204	-1,275	0,495	-0,863	0,388
	-1,350	0,088	0,008	-1,522	-1,179	-15,428	0,000

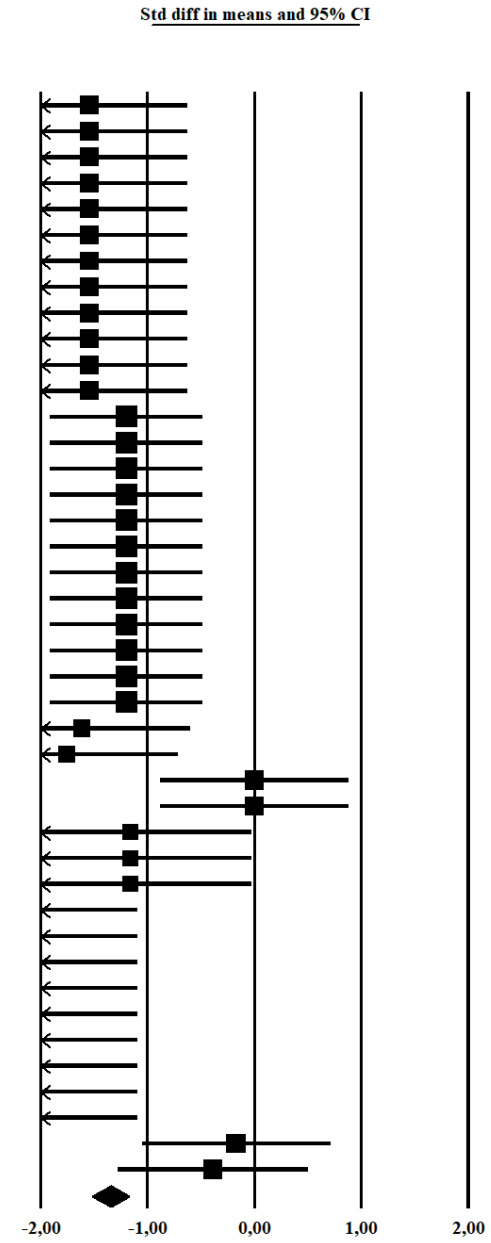

Figure 3. Summary of descriptive and inferential statistics of results for each study and overall effect size of the effect of the acute high-intensity exercise-conditioned human serum in human cancer cell viability.

3.3.3 Effect of Serum Conditioned by Moderate-Intensity Acute Exercise or by an Acute Integrative Exercise of Moderate- and High-Intensity on the Viability of Human Cancer Cell Cultures

The meta-analysis on the effects of serum conditioned by moderate intensity acute exercise or by acute integrative exercise of moderate and high-intensity on the viability of cancer cell cultures included four studies. These studies used moderate-intensity exercise or the combination of exercise with different intensities. This meta-analysis, performed with a random effects model, showed, based on the standard difference in means, that cultures of cancer cells exposed to serum conditioned by acute moderate-intensity or by acute integrative exercise of moderate and high-intensity exercise exhibited a reduction of -0.559 in their viability (*Figure 4*). The confidence interval for the standard difference in means was -0.830 to -0.287 (95% CI) with a corresponding value of $p < 0.001$ (*Figure 4*). Once again, this interval does not include a zero effect. Similarly, z-values obtained to test the null hypothesis i.e., that the standard difference in means is zero, showed a $z = -4.036$, and a corresponding value of $p < 0.001$ (*Figure 4*). Thus, the null hypothesis was rejected and the alternative hypothesis accepted in all analysed studies, i.e., after exposure of cancer cell cultures to serum conditioned by acute moderate-intensity or by an acute integrative exercise of moderate and high-intensity exercise, there was a reduction in their viability.

In the analysis of the homogeneity of the effects, the Q-value was 32.234 with 17 degrees of freedom and $p = 0.019$. Although the values of Q and the degrees of freedom show that all studies may not share the same effect size, the value of p indicates that the null hypothesis must be accepted since the true effect-size is identical in all studies. The I^2 value is

Study name	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Hwang et al 2020 e)	0,000	0,408	0,167	-0,800	0,800	0,000	1,000
Hwang et al 2020 f)	0,000	0,408	0,167	-0,800	0,800	0,000	1,000
Hwang et al 2020 a)	0,000	0,408	0,167	-0,800	0,800	0,000	1,000
Hwang et al 2020 b)	0,000	0,408	0,167	-0,800	0,800	0,000	1,000
Hwang et al 2020 g)	-1,287	0,491	0,241	-2,250	-0,324	-2,620	0,009
Hwang et al 2020 h)	0,000	0,447	0,200	-0,877	0,877	0,000	1,000
Hwang et al 2020 c)	0,000	0,447	0,200	-0,877	0,877	0,000	1,000
Hwang et al 2020 d)	0,000	0,447	0,200	-0,877	0,877	0,000	1,000
Rundqvist et al 2013 a)	-0,940	0,471	0,222	-1,863	-0,016	-1,994	0,046
Dethlefsen et al 2016 a)	-1,128	0,340	0,116	-1,795	-0,460	-3,312	0,001
Dethlefsen et al 2016 b)	-0,673	0,325	0,106	-1,310	-0,035	-2,069	0,039
Dethlefsen et al 2017 a)	-1,633	0,617	0,381	-2,842	-0,423	-2,645	0,008
Dethlefsen et al 2017 b)	-1,165	0,578	0,334	-2,298	-0,032	-2,015	0,044
Dethlefsen et al 2017 c)	-2,308	0,690	0,476	-3,660	-0,956	-3,345	0,001
Dethlefsen et al 2017 d)	-1,165	0,578	0,334	-2,298	-0,032	-2,015	0,044
Dethlefsen et al 2017 e)	-0,640	0,324	0,105	-1,276	-0,005	-1,974	0,048
Dethlefsen et al 2017 f)	-0,640	0,324	0,105	-1,276	-0,005	-1,974	0,048
Rundqvist et al 2013 c)	0,000	0,447	0,200	-0,877	0,877	0,000	1,000
	-0,559	0,138	0,019	-0,830	-0,287	-4,036	0,000

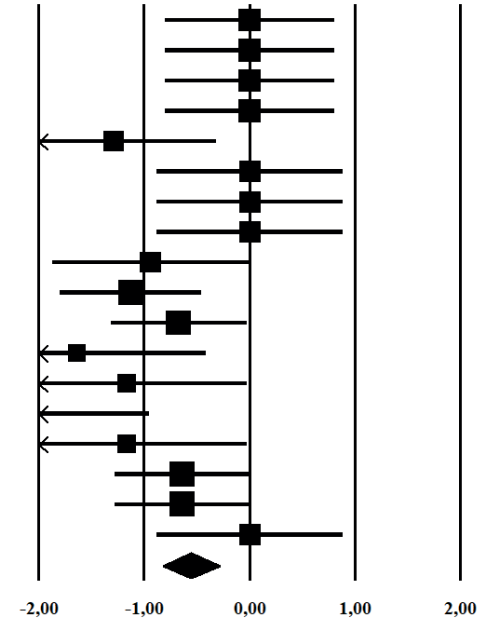

Figure 4. Summary of descriptive and inferential statistics of results for each study and overall effect size of the effect of serum conditioned by moderate-intensity acute exercise or by an integrative exercise of moderate- and high-intensity acute exercise on the viability of human cancer cell cultures.

45.573, meaning that about 45.573% of the variance in the observed effects reflects variance in the true effects. The τ^2 value was 0.152. The τ value, i.e., the standard deviation of the true effects in this SRM was 0.389. The funnel plot (Figure 5c) for the distribution of the observed studies was symmetric, with the majority of the studies distributed symmetrically around the mean effect size, since the sampling error is random, providing no subjective evidence of publication bias. Additionally, the Egger´s test was performed and the intercept value was -2.11767, with a 95% confidence interval between −5.30865 and 1.07331 and a t value = 1.40686. The recommended p value (2-tailed) was 0.17860. Thus, there was also no statistical evidence for publication bias.

4. Discussion

A major aim of the present systematic review and meta-analysis was to determine the magnitude of the effect of exercise-conditioned human serum on the viability of cancer cell cultures. Quantitative analyses could only be performed for the effects of serum conditioned by exercise bouts (acute exercise), due the scarcity the studies and data on the effects of serum conditioned by long-term exercise training (chronic exercise). Our analyses revealed significant effects of acute exercise-conditioned human serum in reducing the viability of cancer cell cultures (ES = -1.126; -1.300 to -0.952 (95% CI); $p < 0.001$). Moreover, it was found that the effect was more pronounced when human serum was conditioned by high-intensity exercise (ES = -1.350; -1.522 to -1.179 (95% CI); $p < 0.001$) than when it was conditioned by moderate-intensity exercise or by an integrative exercise of moderate- and high-intensity exercise (ES = -0.559; -0.830 to -0.287 (95% CI); $p < 0.001$).

Two of the studies simultaneously assessed the effects of exercise-conditioned human serum on the viability of cancer cell cultures and cultures of normal fibroblasts (37, 50). In both cases, no effect was observed in the latter, suggesting that exercise-conditioned human serum does not affect cell culture viability in general, but the viability of cancer cell cultures specifically. It must be noted, though, that in one of these studies, the fibroblasts used (NIH 3T3 cell line) were not of human origin (37, 50).

While the effect of exercise on cancer cell culture viability had the same direction in all studies, regardless of cancer type (breast (5, 15, 16), prostate (5, 33, 39, 50), lung (37), or colon (17)), the magnitude of the effect differed among the studies, likely due to considerable heterogeneity in study designs, namely in terms of type and duration of the exercise intervention (ranging from short exercise bouts to 10 years); population (e.g., healthy sedentary subjects, patients at risk of cancer and cancer patients or survivors); cell line; exposure regimen to exercise-conditioned human serum (5% (v/v) or 10% (v/v) human serum; 24 h to 7 days); endpoints of viability (e.g., total cell numbers, proliferation rates, clonogenic potential). Unfortunately, this considerable heterogeneity in multiple aspects of study design does not allow, at present, the establishment of any trends regarding the influence of any of these parameters. In spite of this limitation, some aspects are worth a brief discussion, as detailed below.

One of the aspect that needs clarification is the duration of the serological changes produced by bouts of exercise. In the study by Devin et al., the marked reduction in the viability of colon cancer cell cultures produced by serum prepared from

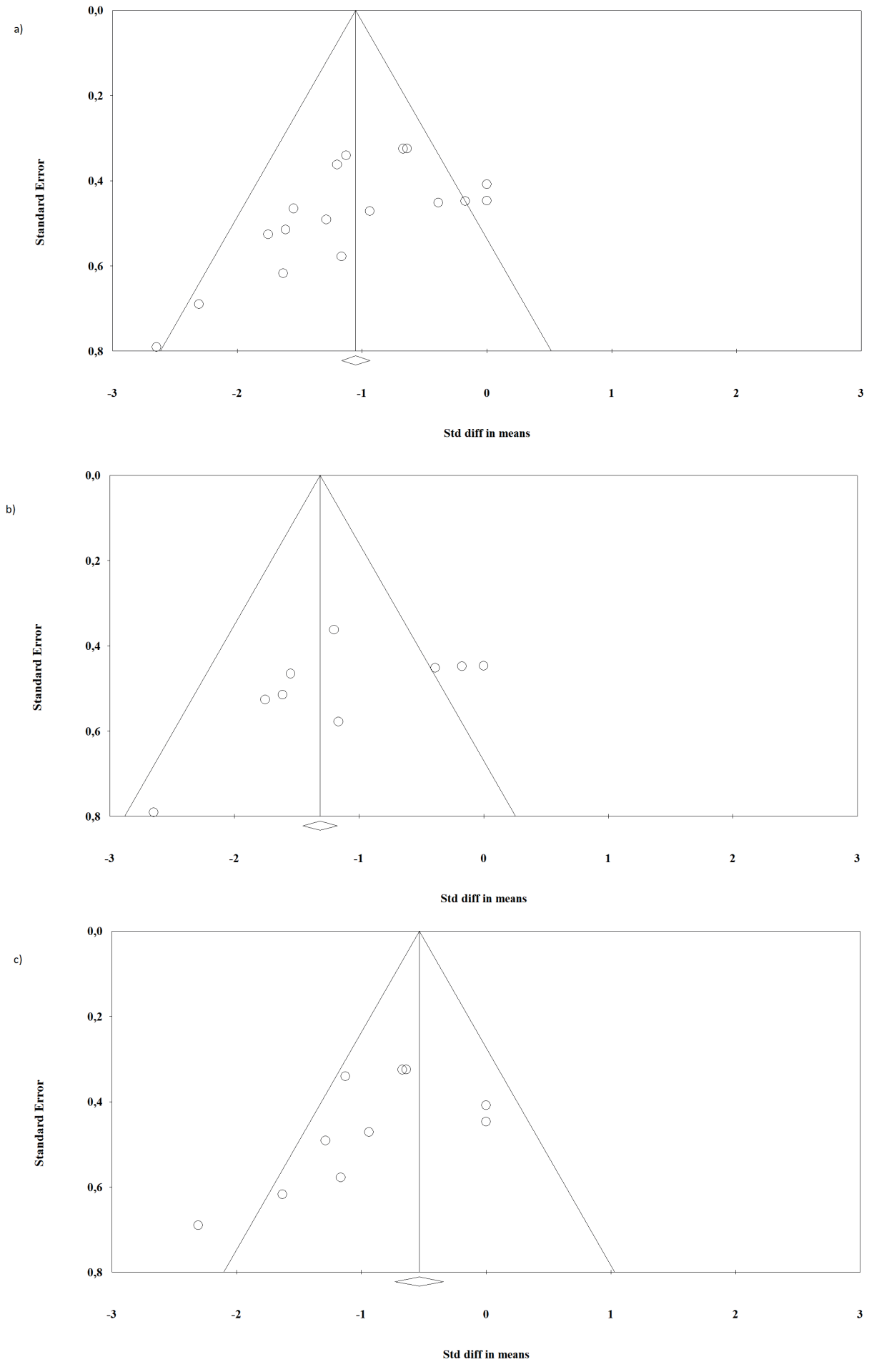

Figure 5. Effect of acute exercise-conditioned human serum in human cancer cell –funnel plot of standard error by std diff in means: a) acute exercise-conditioned human serum; b) acute high-intensity exercise-conditioned human serum; c) acute moderate-intensity or integrative exercise-conditioned human serum.

blood collected immediately after exercise was not replicated by serum prepared from blood collected 2 h after exercise (17). In the study of Rundqvist et al. (17, 50), on the other hand, serum prepared from blood collected 2 h post exercise reduced the viability of prostate cancer cell culture. Unfortunately, as blood was not collected immediately after exercise, so it is not known whether the effect decreased over time post-exercise. In some studies, such as those of Baldelli et al. and Kurgan et al., blood samples were collected several times, up to 24 h post-exercise. In both cases, it was found that the reduction in viability produced in, respectively, prostate and breast cancer cell cultures and lung cancer cell cultures did not decrease over time (5, 37).

Regarding long-term exercise training, five studies reported that it did not induce any changes in baseline serum (i.e., prepared from blood collected at rest) in terms of effects on the viability of the cancer cell cultures (5, 15-17, 33). This contrasts with the findings of Leung et al. and Barnard et al., who did observe higher effects for baseline serum collected after training (7, 39). This apparent contradiction might be due to the fact that the long-term exercise training interventions in the former studies were much shorter than the latter (months versus years).

Differences in outcomes could be found even among studies employing the same cell line, exercise protocol and exposure time. This can be exemplified by the results obtained in two studies employing the prostate cancer cell line LNCaP. In one of those studies, which aimed to determine whether the effects of exercise-conditioned human serum are influenced by aging, Hwang et al. recruited individuals from two distinct age groups (young age group, aged 20 to 33 years; old age group, aged 60 to 73 years) and compared the effects of a 96 h exposure to exercise-conditioned serum from the two groups on cell culture viability. Their results showed that exercise-conditioned serum from the old age group, but not from the young age group, reduced the viability of cancer cell cultures (33). Rundqvist et al., on the other hand, found a reduction in cell culture viability for 9 out of the 10 sera assessed, from participants aged 18 to 37 years, i.e., roughly within the young age group defined by Hwang et al. (17, 50). It must be mentioned that the two studies employed different endpoints of cell culture viability, but it is unlikely that this difference alone could account for the strikingly different outcomes in what concerns the effects of exercise-conditioned serum from young adults.

It is interesting to note that the studies of Devin et al. (17) and of Rundqvist et al. (50) assessed the effects of exercise-conditioned serum on the proportion of apoptotic cells in culture. Considering that this proportion was found unchanged in both studies, the observed reduction in total cell numbers might safely be assigned to lower proliferation rates, suggesting a lower tumorigenicity. Leung et al. and Barnard et al., on the other hand, observed an upregulation on the levels of apoptosis induced by serum collected at rest from regular exercising individuals in prostate cancer cell lines (7, 39). Taking into consideration that the same cell line (LNCaP) was used in three of these studies (7, 17, 39, 50), it is possible that the different outcomes are due, at least partially, to the duration of the exercise intervention.

Our analysis suggests that changes in serum composition promoted by exercise affect the viability of cancer cell cultures and may interfere with some hallmarks of cancer (26, 27), such as: sustentation of proliferative signalling and evasion of growth suppressors.

In the human model, the effects of exercise on tumorigenesis can occur through direct actions on cancer cells and through changes in the immune response (32, 56, 66). These effects may result from changes in serum levels of biomolecules such as epinephrine, norepinephrine, lactate, myokines, cytokines (IL-6, IL-8, TNF-α), SPARC and oncostatin M. Acute high-intensity exercise and integrative exercise (strength and high-intensity) increased IL-6, IL-8, TNF-α, epinephrine, norepinephrine, lactate, but not IL-10 (15-17), while moderate intensity exercise only increased SPARC and oncostatin M levels and did not change IL-6, IL-15 and Irisin levels (33). These changes promoted by high-intensity exercise tended to disappear 120 min post-exercise (17). Long-term exercise training elicited a reduction of IL-6, IL-8 and TNF-α at the end of 6 months (16), but not at the end of four weeks (17). However, the precise mechanisms linking these changes to cancer cell proliferation are still unclear.

Leung et al. (39) and Barnard et al. (7) suggested that exercise promoted changes in the IGF axis, reducing IGF-I (which was directly correlated with tumor cell growth) and increasing IGFBP-1 (which was inversely correlated with tumor cell growth), increasing p53 protein expression, reducing cell growth, and increasing apoptosis, in prostate cancer cell lines (LNCaP) (7, 39). The results obtained by Rundqvist et al. (50) support this hypothesis and showed that reduction of EGF by exercise could be another factor linking it to the inhibition of LNCaP cell growth (50, 51).

To explain the observed reduction in breast cancer cell viability and tumorigenesis, Dethlefsen et al. (15) suggested that exercise regulated the Hippo signalling pathway through the action of catecholamines (epinephrine and norepinephrine). The increase of catecholamine secretion during exercise stimulates Hippo signalling through β-adrenergic receptors inactivating the oncoproteins YAP/ZAP (35, 64, 65) by induction of YAP phosphorylation and YAP cytoplasmic retention (15). Kurgan et al., on the other hand, suggested that serum changes promoted by exercise may target directly Akt, mTOR/p70S6K, PI3K or PDK1/2 (37). This hypothesis was supported by their results, showing inhibition of Akt and ErK1/2 by exercise, ultimately leading to inhibition of cell proliferation and decreased survival (37).

Exercise can also reduce tumorigenesis through the regulation of the immune and metabolic networks (36), with muscle-to-tumor cross-talk playing an important role. Exercise induced myokines may affect immune cell activity through the release of immune regulatory cytokines like IL-6, IL-7 and IL-15 (32). Regular exercise has been shown to mitigate immunosenescence and low-grade inflammation (9, 43). It also has direct effects on cytotoxic NK and T cells, whose mobilization and redistribution into circulation depend on epinephrine and IL-6 release during exercise (49, 62). These changes in the mobilization and infiltration of specific immune cell populations (mostly NK and T cytotoxic cells) and inflammatory cytokines that occur during exercise could directly affect cancer cell formation and progression (30, 34, 49, 61). Exercise can also target the specific energy metabolism of cancer cells, which is highly reliant on lactic acid fermentation, a phenotype often described as the Warburg

effect (1, 58). High-intensity exercise, which can inhibit lactic acid fermentation in places away from the lactate-producing muscles, may neutralize tumor fermentation and affect this metabolic predominance (29, 59, 60), decreasing the inhibitory effect that lactate has on cytotoxic immune cells (22, 52).

This systematic review with meta-analysis has a high methodological value due to the quality of the studies included and the large number of samples tested. However, it is possible to identify some limitations, namely the employment of a search strategy that only included studies with defined terms in English on the title from specific selected databases, thereby potentially overlooking other relevant publications, namely in other languages, even if the included studies were from different countries and continents. Another possible limitation is the reduced number of articles published in the literature that analysed the effect of exercise-conditioned human serum in human cell lines. Lastly, the imbalance on the number of studies between cancer types and exercise types can minimize the inferential power over the real effects of exercise in general cancer cell viability.

Future studies should explore whether the observed effects on cancer cell viability are also observed in other cancer cell lines and for serum conditioned by other activities (e.g., running, swimming, and football). It would also be important to gather more information regarding the influence of exercise intensity on the observed effects and to determine whether there is a causal relationship between serum factors (e.g., cytokines, myokines and catecholamines) modified by exercise (acute and chronic) and type of activities. It is also important to consolidate the research carried out regarding the hypothesized mechanisms here discussed, as well as to investigate other mechanisms that may contribute to the anti-cancer effect of exercise, namely the impact that serum factors may have on oncometabolism, specifically on the "*Warburg effect*", and on immunosurveillance (e.g., energy alterations in NK cell via metabolism) (21).

Exercise can be safely implemented and is feasible in cancer patients (28, 54) with its benefits in preventing cancer and survival rates widely recognized (23, 25, 32, 41). Data on exercise-conditioned human serum effects on the viability of cancer cell cultures support the importance of exercise as a prevention strategy and support therapy in the treatment of cancer patients.

5. Conclusions

This systematic review with meta-analysis provides evidence that exercise promotes a large overall effect in reducing the viability of human culture cancer cells, also showing that this effect is more pronounced for high-intensity exercise-conditioned human serum. This effect is promoted by molecular and cellular mechanisms that can be triggered by acute or systematic changes in serum factors which are possibly dependent on exercise type, regularity and intensity. More research is needed to better understand the mechanisms underlying this effect, as well as the chronic effects of exercise and sport, in order to improve the prescription of exercise as a prevention strategy and/or as an adjuvant to the treatment of cancer.

Author Contributions:
Conceptualization, C.M.S.; J.P.F.; A.M.T.; H.S.; Methodology, C.M.S.; J.P.F.; A.M.T; H.S.; Software, C.M.S.; F.M.S.; M.F.; M.C.R.; P.R.N.; M.A.F.; J.P.F.; Formal Analysis, C.M.S. and J.P.F; Writing – C.M.S; Writing – Review & Editing, J.P.F.; A.M.T.; H.S.; A.M.U.; P.R.N.; M.A.F.; Visualization, J.P.F; A.T; H.S; Supervision, J.P.F; A.M.T.; H.S; All authors approved the final version of the manuscript.

Funding:
This research received no external funding.

Conflicts of Interest:
The authors declare no conflict of interest.

ACKNOWLEDGMENT:
F.M.S acknowledge a grant by Portuguese Foundation for Science and Technology (2020.08759.BD).
Research in A.M.U.'s laboratory is currently funded by Fundação para a Ciência e a Tecnologia (FCT, grant UIDB/00070/2020) and Associação de Apoio ao Centro de Investigação em Meio Ambiente, Genética e Oncobiologia (ACIMAGO, grant 16/12).

REFERENCES

1. Abreu PL, and Urbano AM. Targeting the Warburg effect for cancer therapy: a long and winding road. In: Front Clin Drug Res—Anti-Cancer Agents, 2016, p. 271-324.
2. Alan MB, and William GH. Making meaningful inferences about magnitudes. Int J Sports Physiol Perform 1: 50-57, 2006.
3. Ashcraft KA, Peace RM, Betof AS, Dewhirst MW, and Jones LW. Efficacy and mechanisms of aerobic exercise on cancer initiation, progression, and metastasis: a critical systematic review of in vivo preclinical data. Cancer Res 76: 4032-4050, 2016.
4. Ashcraft KA, Warner AB, Jones LW, and Dewhirst MW. Exercise as adjunct therapy in cancer. Semin Radiat Oncol 29: 16-24, 2019.
5. Baldelli G, De Santi M, Gervasi M, Annibalini G, Sisti D, Højman P, Sestili P, Stocchi V, Barbieri E, and Brandi G. The effects of human sera conditioned by high-intensity exercise sessions and training on the tumorigenic potential of cancer cells. Clin Transl Oncol, 2020.
6. Barnard RJ, Gonzalez JH, Liva ME, and Ngo TH. Effects of a low-fat, high-fiber diet and exercise program on breast cancer risk factors in vivo and tumor cell growth and apoptosis *in vitro*. Nutr Cancer 55: 28-34, 2006.
7. Barnard RJ, Ngo TH, Leung PS, Aronson WJ, and Golding LA. A low-fat diet and/or strenuous exercise alters the IGF axis in vivo and reduces prostate tumor cell growth *in vitro*. The Prostate 56: 201-206, 2003.
8. Begg CB, and Mazumdar M. Operating characteristics of a rank correlation test for publication bias. Biometrics 50: 1088-1101, 1994.
9. Bigley AB, Spielmann G, LaVoy ECP, and Simpson RJ. Can exercise-related improvements in immunity influence cancer prevention and prognosis in the elderly? Maturitas 76: 51-56, 2013.
10. Borenstein MH, L.V.; Higgins, J.P.T.; Rothstein, H.R. Introduction to meta-analysis. Chichester, UK: John Wiley & Sons, Ltd., 2009.

11. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, and Jemal A. Global cancer statistics 2018: globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68: 394-424, 2018.
12. Brenner DR, Yannitsos DH, Farris MS, Johansson M, and Friedenreich CM. Leisure-time physical activity and lung cancer risk: a systematic review and meta-analysis. Lung Cancer 95: 17-27, 2016.
13. Brown KF, Rumgay H, Dunlop C, Ryan M, Quartly F, Cox A, Deas A, Elliss-Brookes L, Gavin A, Hounsome L, Huws D, Ormiston-Smith N, Shelton J, White C, and Parkin DM. The fraction of cancer attributable to modifiable risk factors in England, Wales, Scotland, Northern Ireland, and the United Kingdom in 2015. Br J Cancer 118: 1130-1141, 2018.
14. Des Jarlais DC, Lyles C, and Crepaz N. Improving the reporting quality of nonrandomized evaluations of behavioral and public health interventions: the TREND statement. Am J Public Health 94: 361-366, 2004.
15. Dethlefsen C, Hansen LS, Lillelund C, Andersen C, Gehl J, Christensen JF, Pedersen BK, and Hojman P. Exercise-induced catecholamines activate the hippo tumor suppressor pathway to reduce risks of breast cancer development. Cancer Res 77: 4894-4904, 2017.
16. Dethlefsen C, Lillelund C, Midtgaard J, Andersen C, Pedersen BK, Christensen JF, and Hojman P. Exercise regulates breast cancer cell viability: systemic training adaptations versus acute exercise responses. Breast Cancer Res Treat 159: 469-479, 2016.
17. Devin JL, Hill MM, Mourtzakis M, Quadrilatero J, Jenkins DG, and Skinner TL. Acute high intensity interval exercise reduces colon cancer cell growth. J Physiol 597: 2177-2184, 2019.
18. Duval S, and Tweedie R. A nonparametric "trim and fill" method of accounting for publication bias in meta-analysis. J AM STAT ASSOC 95: 89-98, 2000.
19. Duval S, and Tweedie R. Trim and fill: a simple funnel-plot–based method of testing and adjusting for publication bias in meta-analysis. Biometrics 56: 455-463, 2000.
20. Egger M, Smith GD, Schneider M, and Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ 315: 629, 1997.
21. Ferreira LMR, Li AM, Serafim TL, Sobral MC, Alpoim MC, and Urbano AM. Intermediary metabolism: an intricate network at the crossroads of cell fate and function. Biochim Biophys Acta Mol Basis Dis 1866: 165887, 2020.
22. Fischer K, Hoffmann P, Voelkl S, Meidenbauer N, Ammer J, Edinger M, Gottfried E, Schwarz S, Rothe G, Hoves S, Renner K, Timischl B, Mackensen A, Kunz-Schughart L, Andreesen R, Krause SW, and Kreutz M. Inhibitory effect of tumor cell-derived lactic acid on human T cells. Blood 109: 3812-3819, 2007.
23. Fong DYT, Ho JWC, Hui BPH, Lee AM, Macfarlane DJ, Leung SSK, Cerin E, Chan WYY, Leung IPF, Lam SHS, Taylor AJ, and Cheng K-k. Physical activity for cancer survivors: meta-analysis of randomised controlled trials. BMJ 344: e70, 2012.
24. Friedenreich CM. Physical activity and breast cancer: review of the epidemiologic evidence and biologic mechanisms. Cancer Causes Control 188: 125-139, 2011.
25. Friedenreich CM, Neilson HK, and Lynch BM. State of the epidemiological evidence on physical activity and cancer prevention. Eur J Cancer 46: 2593-2604, 2010.
26. Hanahan D, and Weinberg RA. The hallmarks of cancer. Cell 100: 57-70, 2000.
27. Hanahan D, and Weinberg RA. Hallmarks of cancer: the next generation. Cell 144: 646-674, 2011.
28. Heywood R, McCarthy AL, and Skinner TL. Safety and feasibility of exercise interventions in patients with advanced cancer: a systematic review. Support Care Cancer 25: 3031-3050, 2017.
29. Hofmann P. Cancer and exercise: Warburg hypothesis, tumour metabolism and high-intensity anaerobic exercise. Sports (Basel) 6: 10, 2018.
30. Hojman P. Exercise protects from cancer through regulation of immune function and inflammation. Biochem Soc Trans 45: 905, 2017.
31. Hojman P, Dethlefsen C, Brandt C, Hansen J, Pedersen L, and Pedersen BK. Exercise-induced muscle-derived cytokines inhibit mammary cancer cell growth. Am J Physiol Endocrinol Metab 301: E504-E510, 2011.
32. Hojman P, Gehl J, Christensen JF, and Pedersen BK. Molecular mechanisms linking exercise to cancer prevention and treatment. Cell Metab 27: 10-21, 2018.
33. Hwang JH, McGovern J, Minett GM, Della Gatta PA, Roberts L, Harris JM, Thompson EW, Parker TJ, Peake JM, and Neubauer O. Mobilizing serum factors and immune cells through exercise to counteract age-related changes in cancer risk. Exerc Immunol Rev 26: 80-99, 2020.
34. Idorn M, and Hojman P. Exercise-dependent regulation of nk cells in cancer protection. Trends Mol Med 22: 565-577, 2016.
35. Kim M, Kim M, Lee S, Kuninaka S, Saya H, Lee H, Lee S, and Lim DS. cAMP/PKA signalling reinforces the LAST-YAP pathway to fully suppress YAP in response to actin cytoskeletal changes. Embo J 32: 1543-1555, 2013.
36. Koelwyn GJ, Zhuang X, Tammela T, Schietinger A, and Jones LW. Exercise and immunometabolic regulation in cancer. Nat Metab 2: 849-857, 2020.
37. Kurgan N, Tsakiridis E, Kouvelioti R, Moore J, Klentrou P, and Tsiani E. Inhibition of human lung cancer cell proliferation and survival by post-exercise serum is associated with the inhibition of AKT, mTOR, p70 S6K, and Erk1/2. Cancers 9: 46, 2017.
38. Lee IM. Physical activity and cancer prevention - data from epidemiologic studies. Med Sci Sports Exerc 35: 1823-1827, 2003.
39. Leung PS, Aronson WJ, Ngo TH, Golding LA, and Barnard RJ. Exercise alters the IGF axis in vivo and increases p53 protein in prostate tumor cells *in vitro*. J Appl Physiol 96: 450-454, 2004.
40. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JP, Clarke M, Devereaux PJ, Kleijnen J, and Moher D. The prisma statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. PLoS Med 6: e1000100, 2009.
41. McTiernan A. Mechanisms linking physical activity with cancer. Nat Rev Cancer 8: 205-211, 2008.
42. McTiernan A, Friedenreich CM, Katzmarzyk PT, Powell KE, Macko R, Buchner D, Pescatello LS, Bloodgood B, Tennant B, Vaux-Bjerke A, George SM, Troiano RP, Piercy KL, and Physical Activity Guidelines Advisory C. Physical activity in cancer prevention and survival: a systematic review. Med Sci Sports Exerc 51: 1252-1261, 2019.
43. Minuzzi LG, Rama L, Chupel MU, Rosado F, Dos Santos JV, Simpson R, Martinho A, Paiva A, and Teixeira AM. Effects of lifelong training on senescence and mobilization of T lymphocytes in response to acute exercise. Exerc Immunol Rev 24: 72-84, 2018.

44. Moher D, Liberati A, Tetzlaff J, and Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the prisma statement. PLoS Med 6: e1000097, 2009.
45. Moore SC, Lee IM, Weiderpass E, Campbell PT, Sampson JN, Kitahara CM, Keadle SK, Arem H, Berrington de Gonzalez A, Hartge P, Adami HO, Blair CK, Borch KB, Boyd E, Check DP, Fournier A, Freedman ND, Gunter M, Johannson M, Khaw KT, Linet MS, Orsini N, Park Y, Riboli E, Robien K, Schairer C, Sesso H, Spriggs M, Van Dusen R, Wolk A, Matthews CE, and Patel AV. Association of leisure-time physical activity with risk of 26 types of cancer in 1.44 million adults. JAMA Intern Med 176: 816-825, 2016.
46. Ngo TH, Barnard RJ, Leung P-S, Cohen P, and Aronson WJ. Insulin-like growth factor i (IGF-I) and IGF binding protein-1 modulate prostate cancer cell growth and apoptosis: possible mediators for the effects of diet and exercise on cancer cell survival. Endocrinology 144: 2319-2324, 2003.
47. Panic N, Leoncini E, de Belvis G, Ricciardi W, and Boccia S. Evaluation of the endorsement of the preferred reporting items for systematic reviews and meta-analysis (prisma) statement on the quality of published systematic review and meta-analyses. PLoS One 8: e83138, 2013.
48. Patel AV, Friedenreich CM, Moore SC, Hayes SC, Silver JK, Campbell KL, Winters-Stone K, Gerber LH, George SM, Fulton JE, Denlinger C, Morris GS, Hue T, Schmitz KH, and Matthews CE. American college of sports medicine roundtable report on physical activity, sedentary behavior, and cancer prevention and control. Med Sci Sports Exerc 51: 2391-2402, 2019.
49. Pedersen L, Idorn M, Olofsson GH, Lauenborg B, Nookaew I, Hansen RH, Johannesen HH, Becker JC, Pedersen KS, Dethlefsen C, Nielsen J, Gehl J, Pedersen BK, Straten PT, and Hojman P. Voluntary running suppresses tumor growth through epinephrine- and IL-6-dependent nk cell mobilization and redistribution. Cell Metab 23: 554-562, 2016.
50. Rundqvist H, Augsten M, Strömberg A, Rullman E, Mijwel S, Kharaziha P, Panaretakis T, Gustafsson T, and Östman A. Effect of acute exercise on prostate cancer cell growth. PLoS ONE 8: 1-9, 2013.
51. Schuurmans AL, Bolt J, Veldscholte J, and Mulder E. Regulation of growth of LNCaP human prostate tumor cells by growth factors and steroid hormones. J Steroid Biochem Mol Biol 40: 193-197, 1991.
52. Schwartz L, Supuran CT, and Alfarouk KO. The Warburg effect and the hallmarks of cancer. Anticancer Agents Med Chem 17: 164-170, 2017.
53. Siegel RL, Miller KD, and Jemal A. Cancer statistics, 2020. CA Cancer J Clin 70: 7-30, 2020.
54. Singh B, Spence RR, Steele ML, Sandler CX, Peake JM, and Hayes SC. A systematic review and meta-analysis of the safety, feasibility, and effect of exercise in women with stage II+ breast cancer. Arch Phys Med Rehabil 99: 2621-2636, 2018.
55. Soliman S, Aronson WJ, and Barnard RJ. Analyzing serum-stimulated prostate cancer cell lines after low-fat, high-fiber diet and exercise intervention. Evid Based Complement Alternat Med 2011: 529053, 2011.
56. Thomas RJ, Kenfield SA, and Jimenez A. Exercise-induced biochemical changes and their potential influence on cancer: a scientific review. Br J Sports Med 51: 640-644, 2017.
57. Tymchuk CN, Barnard RJ, Heber D, and Aronson WJ. Evidence of an inhibitory effect of diet and exercise on prostate cancer cell growth. J Urol 166: 1185-1189, 2001.
58. Urbano AM. Otto Warburg: the journey towards the seminal discovery of tumor cell bioenergetic reprogramming. Biochim Biophys Acta Mol Basis Dis 1867: 165965, 2021.
59. Warburg O. On the origin of cancer cells. Science 123: 309-314, 1956.
60. Warburg O, Wind F, and Negelein E. The metabolism of tumors in the body. J Gen Physiol 8: 519-530, 1927.
61. Wennerberg E, Lhuillier C, Rybstein MD, Dannenberg K, Rudqvist N-P, Koelwyn GJ, Jones LW, and Demaria S. Exercise reduces immune suppression and breast cancer progression in a preclinical model. Oncotarget 11: 2020.
62. Weyh C, Krüger K, and Strasser B. Physical Activity and Diet Shape the Immune System during Aging. Nutrients 12: 2020.
63. Wilson LF, Antonsson A, Green AC, Jordan SJ, Kendall BJ, Nagle CM, Neale RE, Olsen CM, Webb PM, and Whiteman DC. How many cancer cases and deaths are potentially preventable? Estimates for Australia in 2013. Int J Cancer 142: 691-701, 2018.
64. Yu FX, Zhang Y, Park HW, Jewell JL, Chen Q, Deng Y, Pan D, Taylor SS, Lai ZC, and Guan KL. Protein kinase a activates the hippo pathway to modulate cell proliferation and differentiation. Genes Dev 27: 1223-1232, 2013.
65. Zanconato F, Cordenonsi M, and Piccolo S. YAP/TAZ at the roots of cancer. Cancer cell 29: 783-803, 2016.
66. Zimmer P, Schenk A, and Elter T. Molecular and biological mechanism of physical exercise on carcinogenesis. Gastroenterologe 14: 332-339, 2019.